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APPLICATION NO.	ISSUE DATE	PATENT NO.	ATTORNEY DOCKET NO.	CONFIRMATION NO.
14/096,346	09/22/2015	9138456		2832

210 7590 09/02/2015

MERCK
P O BOX 2000
RAHWAY, NJ 07065-0907

ISSUE NOTIFICATION

The projected patent number and issue date are specified above.

Determination of Patent Term Adjustment under 35 U.S.C. 154 (b)
(application filed on or after May 29, 2000)

The Patent Term Adjustment is 0 day(s). Any patent to issue from the above-identified application will include an indication of the adjustment on the front page.

If a Continued Prosecution Application (CPA) was filed in the above-identified application, the filing date that determines Patent Term Adjustment is the filing date of the most recent CPA.

Applicant will be able to obtain more detailed information by accessing the Patent Application Information Retrieval (PAIR) WEB site (http://pair.uspto.gov).

Any questions regarding the Patent Term Extension or Adjustment determination should be directed to the Office of Patent Legal Administration at (571)-272-7702. Questions relating to issue and publication fee payments should be directed to the Application Assistance Unit (AAU) of the Office of Data Management (ODM) at (571)-272-4200.

APPLICANT(s) (Please see PAIR WEB site http://pair.uspto.gov for additional applicants):

Cubist Pharmaceuticals, Inc., Lexington, MA;
Sandra O'Connor, Hudson, NH;
Sophie Sun, Lexington, MA;
Gaauri Naik, Cambridge, MA;

Receipt date: 01/06/2014	Application Number	14096346 14096346 - GAU: 1676
INFORMATION DISCLOSURE STATEMENT BY APPLICANT	Filing Date	2013-12-04
(Not for submission under 37 CFR 1.99)	First Named Inventor	Sandra O'Connor
	Art Unit	~~1654~~ 1676
	Examiner Name	~~Not Yet Assigned~~ Li Komatsu
	Attorney Docket Number	552815 (CPT-011USDV)

	23	20110207658		2011-08-25	Kelleher, Thomas J.	
Change(s) applied to document: /M.C.E./ 5/29/2015	24	20120270772		2012-10-25	O'Connor et al. ~~CUBIST PHARMACEUTICALS, INC.~~	
	25	20090197799		2009-08-01	Keith et al.	
	26	20020111311		2002-08-01	Govardhan et al.	
	27	20070116729		2007-05-01	Palepu	

If you wish to add additional U.S. Published Application citation information please click the Add button. Add

FOREIGN PATENT DOCUMENTS Remove

Examiner Initial*	Cite No	Foreign Document Number[3]	Country Code[2] i	Kind Code[4]	Publication Date	Name of Patentee or Applicant of cited Document	Pages,Columns,Lines where Relevant Passages or Relevant Figures Appear	T[5]
	1	WO2001/044274 A1	WO		2001-06-21	CUBIST PHARMACEUTICALS, INC		☐
	2	WO2001/053330 A2	WO		2001-07-26	CUBIST PHARMACEUTICALS, INC		☐
	3	WO2002/055537 A1	WO		2002-07-18	INTRABIOTICS PHARMACEUTICALS, INC.		☐
	4	WO2002/056829 A2	WO		2002-07-25	CUBIST PHARMACEUTICALS, INC		☐

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /L.K./

Receipt date: 01/06/2014	Application Number	14096346 14096346 - GAU: 1676
INFORMATION DISCLOSURE STATEMENT BY APPLICANT	Filing Date	2013-12-04
(Not for submission under 37 CFR 1.99)	First Named Inventor	Sandra O'Connor
	Art Unit	~~1654~~ 1676
	Examiner Name	~~Not Yet Assigned~~ Li Komatsu
	Attorney Docket Number	552815 (CPT-011USDV)

	12	20060018934		2006-01-26	Vaya, Navin	
	13	20060024365		2006-02-02	Vaya, Navin	
Change(s) applied to document, /M.C.E./ 5/29/2015	14	20060264513		2006-11-23	Leone-Bay et al. ~~Emisphere Technologies, Inc.~~	
	15	20060269485		2006-11-30	Friedman, Doron	
	16	20070116729		2007-05-24	Palepu, Nageswara R.	
	17	20070191280		2007-08-16	Kelleher, Thomas	
	18	20080220441		2008-09-11	Birnbaum, Eva R.	
	19	20090197799		2009-08-06	Keith, Dennis	
	20	20100041589		2010-02-18	Keith, Dennis	
	21	20110124551		2011-05-26	Palepu et al. ~~EAGLE PHARMACEUTICALS, INC~~	
	22	20110172167		2011-07-14	Palepu et al. ~~EAGLE PHARMACEUTICALS, INC~~	

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /L.K./

Receipt date: 01/06/2014	Application Number	14096346	14096346 - GAU: 1676
INFORMATION DISCLOSURE STATEMENT BY APPLICANT	Filing Date	2013-12-04	
(Not for submission under 37 CFR 1.99)	First Named Inventor	Sandra O'Connor	
	Art Unit	~~4654~~ 1676	
	Examiner Name	~~Not Yet Assigned~~ Li Komatsu	
	Attorney Docket Number	552815 (CPT-011USDV)	

Change(s) applied to document, /M.C.E./ 5/29/2015	1	20020111311		2002-08-15	Govardhan, et al. ~~Cubist Pharmaceuticals, Inc.~~	
	2	20020132762		2002-09-19	Borders, Donald B.	
	3	20030045484		2003-03-06	Keith, Dennis	
	4	20030045678		2003-03-06	Keith, Dennis	
	5	20040067878		2007-04-08	Hill, Jason	
	6	20040077601		2004-04-22	Adams, Sharlene	
	7	20040242467		2004-12-02	Borders, Donald B.	
	8	20050009747		2005-01-13	Kelleher, Thomas	
	9	20050196418		2005-09-08	Yu, Ruey J.	
	10	20060014674		2006-01-19	Keith, Dennis	
	11	20060018933		2006-01-26	Vaya, Navin	

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /L.K./

Receipt date: 01/06/2014

14096346 - GAU: 1676

Doc code: IDS

Doc description: Information Disclosure Statement (IDS) Filed

PTO/SB/08a (07-10)
Approved for use through 07/31/2012. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
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INFORMATION DISCLOSURE STATEMENT BY APPLICANT
(Not for submission under 37 CFR 1.99)

Application Number	14096346
Filing Date	2013-12-04
First Named Inventor	Sandra O'Connor
Art Unit	~~4654~~ 1676
Examiner Name	~~Not Yet Assigned~~ Li Komatsu
Attorney Docket Number	552815 (CPT-011USDV)

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Examiner Initial*	Cite No	Patent Number	Kind Code[1]	Issue Date	Name of Patentee or Applicant of cited Document	Pages,Columns,Lines where Relevant Passages or Relevant Figures Appear
	1	6696412		2004-02-24	Thomas J. Kelleher	
Change(s) applied to document, /M.C.E./ 5/29/2015	2	6716962		2004-04-06	Borders, et al. ~~Micrologix Biotech Inc.~~	
	3	7138487		2006-11-21	Borders, et al. ~~Migenix Inc.~~	
	4	7279597		2007-10-09	Leone-Bay, et al. ~~Emisphere Technologies, Inc.~~	
	5	8058238		2011-11-15	Kelleher, et al. ~~Cubist Pharmaceuticals, Inc.~~	
	6	8003673		2011-08-23	Alder et al.	

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Receipt date: 01/06/2014	Application Number	14096346 14096346 - GAU: 1676
INFORMATION DISCLOSURE STATEMENT BY APPLICANT	Filing Date	2013-12-04
(Not for submission under 37 CFR 1.99)	First Named Inventor	Sandra O'CONNOR
	Art Unit	~~1654~~ 1676
	Examiner Name	~~Not Yet Assigned~~ Li Komatsu
	Attorney Docket Number	552815 (CPT-011USDV)

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Examiner Initial*	Cite No	Publication Number	Kind Code[1]	Publication Date	Name of Patentee or Applicant of cited Document	Pages,Columns,Lines where Relevant Passages or Relevant Figures Appear
	1	20120149062		2012-02-16	Kelleher et al.	
	2	20100041589		2010-02-18	Keith et al.	
	3	20120270772		2012-07-10	O'Conner	
	4	20050027113		2005-02-03	Vivian Pak Woon Miao et al.	
	5	20070128694		2007-06-07	Baltz et al.	
Change(s) applied to document /M.C.E./ 5/29/2015	6	20130280760	A1	2013-10-24	Kelleher et al. ~~Cubist Pharmaceuticals, Inc.~~	

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Examiner Initial*	Cite No	Foreign Document Number[3]	Country Code[2] i	Kind Code[4]	Publication Date	Name of Patentee or Applicant of cited Document	Pages,Columns,Lines where Relevant Passages or Relevant Figures Appear	T[5]
	1	WO 00018419	WO		2000-04-06	Cubist Pharmaceuticals		☐
	2	WO 99027957	WO		1999-06-10	The Immune Response Co.		☐

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ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /L.K./

Receipt date: 01/06/2014	Application Number	14096346 14096346 - GAU: 1676
INFORMATION DISCLOSURE STATEMENT BY APPLICANT (**Not for submission under 37 CFR 1.99)**	Filing Date	2013-12-04
	First Named Inventor	Sandra O'CONNOR
	Art Unit	~~1654~~ 1676
	Examiner Name	~~Not Yet Assigned~~ Li Komatsu
	Attorney Docket Number	552815 (CPT-011USDV)

	9	4482487		1984-11-13	Abbott et al,	
	10	4331594		1982-05-25	Hamill et al.	
	11	4439425		1984-03-27	Tarcsay et al.	
	12	5336756		1994-08-09	Schwartz et al.	
	13	8431539		2013-04-30	Palepu et al.	
	14	6696412		2004-02-24	Kelleher et al. Change(s) applied to document, /R.K.C./ 6/10/2015	
	15	8309061		2012-11-13	Chaudry ~~Tigabu et al.~~	

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Examiner Initial*	Cite No	Publication Number	Kind Code[1]	Publication Date	Name of Patentee or Applicant of cited Document	Pages,Columns,Lines where Relevant Passages or Relevant Figures Appear
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Receipt date: 01/06/2014 14096346 - GAU: 1676

Doc code: IDS

Doc description: Information Disclosure Statement (IDS) Filed

PTO/SB/08a (07-12)
Approved for use through 07/31/2012. OMB 0651-0031
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INFORMATION DISCLOSURE STATEMENT BY APPLICANT (**Not for submission under 37 CFR 1.99)**		
	Application Number	14096346
	Filing Date	2013-12-04
	First Named Inventor	Sandra O'CONNOR
	Art Unit	~~1654~~ 1676
	Examiner Name	~~Not Yet Assigned~~ Li Komatsu
	Attorney Docket Number	552815 (CPT-011USDV)

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Examiner Initial*	Cite No	Patent Number	Kind Code[1]	Issue Date	Name of Patentee or Applicant of cited Document	Pages,Columns,Lines where Relevant Passages or Relevant Figures Appear
	1	6194383		2001-02-27	Hammann et al.	
	2	5955509		1999-09-21	Webber et al.	
	3	5629288		1997-05-13	Lattrell et al.	
	4	5387670		1995-02-07	Roy et al.	
	5	5271935		1993-12-21	Franco et al.	Change(s) applied to document, /R.K.C./ 6/10/2015
	6	4882164		1989-11-21	Ferro	
	7	4331594		1982-05-25	Hamill et al. ~~Alder et al.~~	
	8	8604164		2013-12-10	Kelleher et al.	
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APPLICATION NUMBER	FILING OR 371(C) DATE	FIRST NAMED APPLICANT	ATTY. DOCKET NO./TITLE
14/096,346	12/04/2013	Sandra O'Connor	23961-US-DIV

CONFIRMATION NO. 2832

113613
Lathrop & Gage
28 State Street
Boston, MA 02109-1775

POWER OF ATTORNEY NOTICE

OC000000076859236

Date Mailed: 08/14/2015

NOTICE REGARDING CHANGE OF POWER OF ATTORNEY

This is in response to the Power of Attorney filed 08/10/2015.

- The Power of Attorney to you in this application has been revoked by the assignee who has intervened as provided by 37 CFR 3.71. Future correspondence will be mailed to the new address of record(37 CFR 1.33).

Questions about the contents of this notice and the requirements it sets forth should be directed to the Office of Data Management, Application Assistance Unit, at **(571) 272-4000** or **(571) 272-4200** or **1-888-786-0101**.

/lhill/

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APPLICATION NUMBER	FILING OR 371(C) DATE	FIRST NAMED APPLICANT	ATTY. DOCKET NO./TITLE
14/096,346	12/04/2013	Sandra O'Connor	

CONFIRMATION NO. 2832

210
MERCK
P O BOX 2000
RAHWAY, NJ 07065-0907

POA ACCEPTANCE LETTER

OC000000076859248

Date Mailed: 08/14/2015

NOTICE OF ACCEPTANCE OF POWER OF ATTORNEY

This is in response to the Power of Attorney filed 08/10/2015.

The Power of Attorney in this application is accepted. Correspondence in this application will be mailed to the above address as provided by 37 CFR 1.33.

Questions about the contents of this notice and the requirements it sets forth should be directed to the Office of Data Management, Application Assistance Unit, at **(571) 272-4000** or **(571) 272-4200** or **1-888-786-0101**.

/lhill/

PART B - FEE(S) TRANSMITTAL

Complete and send this form, together with applicable fee(s), to: Mail **Mail Stop ISSUE FEE**
Commissioner for Patents
P.O. Box 1450
Alexandria, Virginia 22313-1450
or Fax (571)-273-2885

INSTRUCTIONS: This form should be used for transmitting the ISSUE FEE and PUBLICATION FEE (if required). Blocks 1 through 5 should be completed where appropriate. All further correspondence including the Patent, advance orders and notification of maintenance fees will be mailed to the current correspondence address as indicated unless corrected below or directed otherwise in Block 1, by (a) specifying a new correspondence address; and/or (b) indicating a separate "FEE ADDRESS" for maintenance fee notifications.

CURRENT CORRESPONDENCE ADDRESS (Note: Use Block 1 for any change of address)

113613 7590 05/13/2015

~~Lathrop & Gage~~
~~28 State Street~~
~~Boston, MA 02109-1775~~

Merck Sharp & Dohme Corp.
126 East Lincoln Ave.
Rahway, New Jersey 07065

Note: A certificate of mailing can only be used for domestic mailings of the Fee(s) Transmittal. This certificate cannot be used for any other accompanying papers. Each additional paper, such as an assignment or formal drawing, must have its own certificate of mailing or transmission.

Certificate of Mailing or Transmission
I hereby certify that this Fee(s) Transmittal is being deposited with the United States Postal Service with sufficient postage for first class mail in an envelope addressed to the Mail Stop ISSUE FEE address above, or being facsimile transmitted to the USPTO (571) 273-2885, on the date indicated below.

(Depositor's name)
(Signature)
(Date)

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
14/096,346	12/04/2013	Sandra O'Connor	552815: CFT-011USDV	2832

TITLE OF INVENTION: LIPOPEPTIDE COMPOSITIONS AND RELATED METHODS

APPLN. TYPE	ENTITY STATUS	ISSUE FEE DUE	PUBLICATION FEE DUE	PREV. PAID ISSUE FEE	TOTAL FEE(S) DUE	DATE DUE
nonprovisional	UNDISCOUNTED	$960	$0	$0	$960	08/13/2015

EXAMINER	ART UNIT	CLASS-SUBCLASS
KOMATSU, LIN	1676	514-021100

1. Change of correspondence address or indication of "Fee Address" (37 CFR 1.363).
☐ Change of correspondence address (or Change of Correspondence Address form PTO/SB/122) attached.
☐ "Fee Address" indication (or "Fee Address" Indication form PTO/SB/47; Rev 03-02 or more recent) attached. **Use of a Customer Number is required.**

2. For printing on the patent front page, list
(1) The names of up to 3 registered patent attorneys or agents OR, alternatively,
(2) The name of a single firm (having as a member a registered attorney or agent) and the names of up to 2 registered patent attorneys or agents. If no name is listed, no name will be printed.

1 Dianne Pecoraro
2 Laura M. Ginkel
3

3. ASSIGNEE NAME AND RESIDENCE DATA TO BE PRINTED ON THE PATENT (print or type)

PLEASE NOTE: Unless an assignee is identified below, no assignee data will appear on the patent. If an assignee is identified below, the document has been filed for recordation as set forth in 37 CFR 3.11. Completion of this form is NOT a substitute for filing an assignment.

(A) NAME OF ASSIGNEE	(B) RESIDENCE: (CITY and STATE OR COUNTRY)	REEL/FRAME
Cubist Pharmaceuticals LLC	Kenilworth, New Jersey	032543/0011
		036283/0189

Please check the appropriate assignee category or categories (will not be printed on the patent): ☐ Individual ☒ Corporation or other private group entity ☐ Government

4a. The following fee(s) are submitted:
☒ Issue Fee
☒ Publication Fee (No small entity discount permitted)
☐ Advance Order - # of Copies .....

4b. Payment of Fee(s): **(Please first reapply any previously paid issue fee shown above)**
☐ A check is enclosed.
☐ Payment by credit card. Form PTO-2038 is attached.
☒ The director is hereby authorized to charge the required fee(s), any deficiency, or credits any overpayment, to Deposit Account Number 13-2755 (enclose an extra copy of this form).

5. **Change in Entity Status** (from status indicated above)
☐ Applicant certifying micro entity status. See 37 CFR 1.29
NOTE: Absent a valid certification of Micro Entity Status (see forms PTO/SB/15A and 15B), issue fee payment in the micro entity amount will not be accepted at the risk of application abandonment.
☐ Applicant asserting small entity status. See 37 CFR 1.27
NOTE: If the application was previously under micro entity status, checking this box will be taken to be a notification of loss of entitlement to micro entity status.
☐ Applicant changing to regular undiscounted fee status.
NOTE: Checking this box will be taken to be a notification of loss of entitlement to small or micro entity status, as applicable.

NOTE: This form must be signed in accordance with 37 CFR 1.31 and 1.33. See 37 CFR 1.4 for signature requirements and certifications.

Authorized Signature [signature] Date 8/12/15
Typed or printed name Dianne Pecoraro Registration No. 42,068

PTOL-85 Part B (10-13) Approved for use through 10/31/2013. OMB 0651-0033 U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

Electronic Patent Application Fee Transmittal

Application Number:	14096346
Filing Date:	04-Dec-2013
Title of Invention:	LIPOPEPTIDE COMPOSITIONS AND RELATED METHODS
First Named Inventor/Applicant Name:	Sandra O'Connor
Filer:	Dianne Pecoraro/Pia Paras-Sanjurjo
Attorney Docket Number:	552815: CPT-011USDV

Filed as Large Entity

Filing Fees for Utility under 35 USC 111(a)

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Basic Filing:				
Pages:				
Claims:				
Miscellaneous-Filing:				
Petition:				
Patent-Appeals-and-Interference:				
Post-Allowance-and-Post-Issuance:				
Utility Appl Issue Fee	1501	1	960	960

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Extension-of-Time:				
Miscellaneous:				
			Total in USD ($)	**960**

Electronic Acknowledgement Receipt

EFS ID:	23188623
Application Number:	14096346
International Application Number:	
Confirmation Number:	2832
Title of Invention:	LIPOPEPTIDE COMPOSITIONS AND RELATED METHODS
First Named Inventor/Applicant Name:	Sandra O'Connor
Customer Number:	113613
Filer:	Dianne Pecoraro/Pia Paras-Sanjurjo
Filer Authorized By:	Dianne Pecoraro
Attorney Docket Number:	552815: CPT-011USDV
Receipt Date:	12-AUG-2015
Filing Date:	04-DEC-2013
Time Stamp:	14:40:19
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	yes
Payment Type	Deposit Account
Payment was successfully received in RAM	$960
RAM confirmation Number	882
Deposit Account	132755
Authorized User	PECORARO, DIANNE
The Director of the USPTO is hereby authorized to charge indicated fees and credit any overpayment as follows:	

Charge any Additional Fees required under 37 C.F.R. Section 1.20 (Post Issuance fees)

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Issue Fee Payment (PTO-85B)	23961-Execlfee-12Aug2015.pdf	416726 d63ab8bde54e026d90965457a9390eb77324e8bc	no	1
Warnings:					
Information:					
2	Fee Worksheet (SB06)	fee-info.pdf	30456 108c93e3fd1ef4ebbc5567172087f764df8e5f06	no	2
Warnings:					
Information:					
		Total Files Size (in bytes):	447182		

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New Applications Under 35 U.S.C. 111
If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371
If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office
If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

PTO/AIA/80 (07-12)
Approved for use through 11/30/2014. OMB 0651-0035
U.S. Patent and Trademark Office; U.S DEPARTMENT OF COMMERCE

POWER OF ATTORNEY TO PROSECUTE APPLICATIONS BEFORE THE USPTO

I hereby revoke all previous powers of attorney given in the application identified in the attached statement under 37 CFR 3.73(c).

I hereby appoint:

■ Practitioners associated with Customer Number: 00210

OR

☐ Practitioner(s) named below (if more than ten patent practitioners are to be named, then a customer number must be used):

Name	Registration Number	Name	Registration Number

As attorney(s) or agent(s) to represent the undersigned before the United States Patent and Trademark Office (USPTO) in connection with any and all patent applications assigned only to the undersigned according to the USPTO assignment records or assignments documents attached to this form in accordance with 37 CFR 3.73(c).

Please change the correspondence address for the application identified in the attached statement under 37 CFR 3.73(c) to:

■ The address associated with Customer Number: 00210

OR

☐

Firm or Individual Name			
Address			
City		State	Zip
Country			
Telephone		Email	

Assignee Name and Address: Cubist Pharmaceuticals LLC
2000 Galloping Hill Road
Kenilworth, New Jersey 07033

A copy of this form, together with a statement under 37 CFR 3.73(c) (Form PTO/AIA/96 or equivalent) is required to be Filed in each application in which this form is used. The statement under 37 CFR 3.73(c) may be completed by one of The practitioners appointed in this form, and must identify the application in which this Power of Attorney is to be filed.

SIGNATURE of Assignee of Record
The individual whose signature and title is supplied below is authorized to act on behalf of the assignee

Signature	/Laura M. Ginkel, Reg. No. 51,737/	Date	August 10, 2015
Name	Laura M. Ginkel	Telephone	732-594-1932
Title	Managing Counsel - Patents		

Privacy Act Statement

The **Privacy Act of 1974 (P.L. 93-579)** requires that you be given certain information in connection with your submission of the attached form related to a patent application or patent. Accordingly, pursuant to the requirements of the Act, please be advised that: (1) the general authority for the collection of this information is 35 U.S.C. 2(b)(2); (2) furnishing of the information solicited is voluntary; and (3) the principal purpose for which the information is used by the U.S. Patent and Trademark Office is to process and/or examine your submission related to a patent application or patent. If you do not furnish the requested information, the U.S. Patent and Trademark Office may not be able to process and/or examine your submission, which may result in termination of proceedings or abandonment of the application or expiration of the patent.

The information provided by you in this form will be subject to the following routine uses:

1. The information on this form will be treated confidentially to the extent allowed under the Freedom of Information Act (5 U.S.C. 552) and the Privacy Act (5 U.S.C 552a). Records from this system of records may be disclosed to the Department of Justice to determine whether disclosure of these records is required by the Freedom of Information Act.
2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.
3. A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.
4. A record in this system of records may be disclosed, as a routine use, to a contractor of the Agency having need for the information in order to perform a contract. Recipients of information shall be required to comply with the requirements of the Privacy Act of 1974, as amended, pursuant to 5 U.S.C. 552a(m).
5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent Cooperation Treaty.
6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).
7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (*i.e.*, GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.
8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspection or an issued patent.
9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

MERCK & CO., INC.

CERTIFICATION

I, Katie E. Fedosz, Senior Assistant Secretary of Merck & Co., Inc. (the "Company"), a corporation duly organized and existing under the laws of the State of New Jersey, United States of America, do hereby certify that the attached, presently in full force and effect, is a true and correct copy of General Corporate Resolution #5, Patent Matters, as amended and readopted the Board of Directors of said Company at a meeting thereof duly called and held on July 22, 2015, at which a quorum of Directors was present.

IN WITNESS WHEREOF, I have hereunto subscribed my signature and affixed the seal of the Company this 22nd day of July, 2015.

Senior Assistant Secretary

(SEAL)

United States of America)
State of New Jersey) SS
County of Hunterdon)

Subscribed and sworn to before me on this 22nd day of July, 2015.

Notary Public

SANDRA MARIE MCFADDEN
NOTARY PUBLIC, STATE OF NEW JERSEY
MY COMMISSION EXPIRES JANUARY 18, 2020

General Corporate Resolution #5

PATENT MATTERS

RESOLVED, that any of the following:

Kenneth C. Frazier – Chairman, President and Chief Executive Officer
Michael J. Holston – Executive Vice President and General Counsel
William Krovatin – Senior Vice President and Assistant General Counsel
Mark R. Daniel – Associate Vice President and Group Managing Counsel
Gerard M. Devlin, Jr. – Managing Counsel, IP Litigation
Catherine D. Fitch – Managing Counsel, Patents
Laura M. Ginkel – Managing Counsel, Patents
Sheldon O. Heber – Senior Counsel, Patents
J.J.L. Mestrom – Managing Counsel, IP Animal Health
Mary J. Morry – Senior Counsel, IP Litigation
Immac Thampoe – Managing Counsel, Biologics
John C. Todaro – Managing Counsel, Patents
Anna L. Cocuzzo – Assistant Managing Counsel, Biologics
Gloria Fuentes – Assistant Managing Counsel, Biologics
James Horgan – Assistant Managing Counsel, European Patents
Cynthia A. Francisco – Director, Asset Management

are authorized to execute and to revoke on behalf of Merck & Co., Inc. and its affiliates (including subsidiaries) the following documents relating to patent matters:

Powers of attorney as fully in law as may be necessary and proper in connection with the acquisition, registration, maintenance and enforcement of patents and applications for patents, including powers of attorney relating to the prosecution or defense of patent rights before courts of law or other governmental tribunals, agencies or departments; affidavits and declarations; and any other documents which are necessary and proper for the acquisition, registration, maintenance, litigation and protection of patents.

PTO/AIA/96 (08-12)
Approved for use through 01/31/2013. OMB 0651-0031
U.S. Patent and Trademark Office;U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of1995, no persons arerequired to respond to a collection of information unless it displays a valid OMB control number.

STATEMENT UNDER 37 CFR 3.73(c)

Applicant/Patent Owner: Cubist Pharmaceuticals LLC

Application No./Patent No.: 14/096,346 Filed/Issue Date: 12/04/2013

Titled: LIPOPEPTIDE COMPOSITIONS AND RELATED METHODS

Cubist Pharmaceuticals LLC, a Limited Liability Company

(Name of Assignee) (Type of Assignee, e.g., corporation, partnership, university, government agency, etc.)

states that, for the patent application/patent identified above, it is (choose **one** of options 1, 2, 3 or 4 below):

1. ☑ The assignee of the entire right, title, and interest.

2. ☐ An assignee of less than the entire right, title, and interest (check applicable box):

   ☐ The extent (by percentage) of its ownership interest is _____%. Additional Statement(s) by the owners holding the balance of the interest must be submitted to account for 100% of the ownership interest.

   ☐ There are unspecified percentages of ownership. The other parties, including inventors, who together own the entire right, title and interest are:

   Additional Statement(s) by the owner(s) holding the balance of the interest must be submitted to account for the entire right, title, and interest.

3. ☐ The assignee of an undivided interest in the entirety (a complete assignment from one of the joint inventors was made). The other parties, including inventors, who together own the entire right, title, and interest are:

   Additional Statement(s) by the owner(s) holding the balance of the interest must be submitted to account for the entire right, title, and interest.

4. ☐ The recipient, via a court proceeding or the like (*e.g.*, bankruptcy, probate), of an undivided interest in the entirety (a complete transfer of ownership interest was made). The certified document(s) showing the transfer is attached.

The interest identified in option 1, 2 or 3 above (not option 4) is evidenced by either (choose **one** of options A or B below):

A. ☐ An assignment from the inventor(s) of the patent application/patent identified above. The assignment was recorded in the United States Patent and Trademark Office at Reel _____, Frame _____, or for which a copy thereof is attached.

B. ☑ A chain of title from the inventor(s), of the patent application/patent identified above, to the current assignee as follows:

1. From: Sandra O' Connor, et al. To: Cubist Pharmaceuticals, Inc.

   The document was recorded in the United States Patent and Trademark Office at

   Reel 032543, Frame 0011, or for which a copy thereof is attached.

2. From: Cubist Pharmaceuticals, Inc. To: Cubist Pharmaceuticals LLC

   The document was recorded in the United States Patent and Trademark Office at

   Reel 36283, Frame 0189, or for which a copy thereof is attached.

PTO/AIA/96 (08-12)
Approved for use through 01/31/2013. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

STATEMENT UNDER 37 CFR 3.73(c)

3. From: _____ To: _____

The document was recorded in the United States Patent and Trademark Office at

Reel _____, Frame _____, or for which a copy thereof is attached.

4. From: _____ To: _____

The document was recorded in the United States Patent and Trademark Office at

Reel _____, Frame _____, or for which a copy thereof is attached.

5. From: _____ To: _____

The document was recorded in the United States Patent and Trademark Office at

Reel _____, Frame _____, or for which a copy thereof is attached.

6. From: _____ To: _____

The document was recorded in the United States Patent and Trademark Office at

Reel _____, Frame _____, or for which a copy thereof is attached.

☐ Additional documents in the chain of title are listed on a supplemental sheet(s).

☑ As required by 37 CFR 3.73(c)(1)(i), the documentary evidence of the chain of title from the original owner to the assignee was, or concurrently is being, submitted for recordation pursuant to 37 CFR 3.11.

[NOTE: A separate copy (i.e., a true copy of the original assignment document(s)) must be submitted to Assignment Division in accordance with 37 CFR Part 3, to record the assignment in the records of the USPTO. See MPEP 302.08]

The undersigned (whose title is supplied below) is authorized to act on behalf of the assignee.

/Laura M. Ginkel, Reg. No. 51,737/	August 10, 2015
Signature	Date
Laura M. Ginkel	Managing Counsel - Patents
Printed or Typed Name	Title or Registration Number

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9. A record from thissystem of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

Electronic Acknowledgement Receipt

EFS ID:	23166622
Application Number:	14096346
International Application Number:	
Confirmation Number:	2832
Title of Invention:	LIPOPEPTIDE COMPOSITIONS AND RELATED METHODS
First Named Inventor/Applicant Name:	Sandra O'Connor
Customer Number:	113613
Filer:	Dianne Pecoraro/Pia Paras-Sanjurjo
Filer Authorized By:	Dianne Pecoraro
Attorney Docket Number:	552815: CPT-011USDV
Receipt Date:	10-AUG-2015
Filing Date:	04-DEC-2013
Time Stamp:	17:27:17
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	no

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Power of Attorney	23961-PostAIAPOA-10Aug2015-2.pdf	142727 750db0cef35eddba0c1e3775e6cff89a04baf83a	no	4

Warnings:

The page size in the PDF is too large. The pages should be 8.5 x 11 or A4. If this PDF is submitted, the pages will be resized upon entry into the Image File Wrapper and may affect subsequent processing					
Information:					
2	Assignee showing of ownership per 37 CFR 3.73	23961DIV-Statement373Csigned.pdf	121670 de01829af37868b23f46644b635ec5b4bbc7a33a	no	3
Warnings:					
Information:					
			Total Files Size (in bytes):	264397	

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

New Applications Under 35 U.S.C. 111
If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371
If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office
If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

NOTICE OF ALLOWANCE AND FEE(S) DUE

113613 7590 05/13/2015

Lathrop & Gage
28 State Street
Boston, MA 02109-1775

EXAMINER
KOMATSU, LI N

ART UNIT	PAPER NUMBER
1676	

DATE MAILED: 05/13/2015

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
14/096,346	12/04/2013	Sandra O'Connor	552815: CPT-011USDV	2832

TITLE OF INVENTION: LIPOPEPTIDE COMPOSITIONS AND RELATED METHODS

APPLN. TYPE	ENTITY STATUS	ISSUE FEE DUE	PUBLICATION FEE DUE	PREV. PAID ISSUE FEE	TOTAL FEE(S) DUE	DATE DUE
nonprovisional	UNDISCOUNTED	$960	$0	$0	$960	08/13/2015

THE APPLICATION IDENTIFIED ABOVE HAS BEEN EXAMINED AND IS ALLOWED FOR ISSUANCE AS A PATENT. PROSECUTION ON THE MERITS IS CLOSED. THIS NOTICE OF ALLOWANCE IS NOT A GRANT OF PATENT RIGHTS. THIS APPLICATION IS SUBJECT TO WITHDRAWAL FROM ISSUE AT THE INITIATIVE OF THE OFFICE OR UPON PETITION BY THE APPLICANT. SEE 37 CFR 1.313 AND MPEP 1308.

THE ISSUE FEE AND PUBLICATION FEE (IF REQUIRED) MUST BE PAID WITHIN THREE MONTHS FROM THE MAILING DATE OF THIS NOTICE OR THIS APPLICATION SHALL BE REGARDED AS ABANDONED. THIS STATUTORY PERIOD CANNOT BE EXTENDED. SEE 35 U.S.C. 151. THE ISSUE FEE DUE INDICATED ABOVE DOES NOT REFLECT A CREDIT FOR ANY PREVIOUSLY PAID ISSUE FEE IN THIS APPLICATION. IF AN ISSUE FEE HAS PREVIOUSLY BEEN PAID IN THIS APPLICATION (AS SHOWN ABOVE), THE RETURN OF PART B OF THIS FORM WILL BE CONSIDERED A REQUEST TO REAPPLY THE PREVIOUSLY PAID ISSUE FEE TOWARD THE ISSUE FEE NOW DUE.

HOW TO REPLY TO THIS NOTICE:

I. Review the ENTITY STATUS shown above. If the ENTITY STATUS is shown as SMALL or MICRO, verify whether entitlement to that entity status still applies.

If the ENTITY STATUS is the same as shown above, pay the TOTAL FEE(S) DUE shown above.

If the ENTITY STATUS is changed from that shown above, on PART B - FEE(S) TRANSMITTAL, complete section number 5 titled "Change in Entity Status (from status indicated above)".

For purposes of this notice, small entity fees are 1/2 the amount of undiscounted fees, and micro entity fees are 1/2 the amount of small entity fees.

II. PART B - FEE(S) TRANSMITTAL, or its equivalent, must be completed and returned to the United States Patent and Trademark Office (USPTO) with your ISSUE FEE and PUBLICATION FEE (if required). If you are charging the fee(s) to your deposit account, section "4b" of Part B - Fee(s) Transmittal should be completed and an extra copy of the form should be submitted. If an equivalent of Part B is filed, a request to reapply a previously paid issue fee must be clearly made, and delays in processing may occur due to the difficulty in recognizing the paper as an equivalent of Part B.

III. All communications regarding this application must give the application number. Please direct all communications prior to issuance to Mail Stop ISSUE FEE unless advised to the contrary.

IMPORTANT REMINDER: Utility patents issuing on applications filed on or after Dec. 12, 1980 may require payment of maintenance fees. It is patentee's responsibility to ensure timely payment of maintenance fees when due.

PART B - FEE(S) TRANSMITTAL

Complete and send this form, together with applicable fee(s), to: Mail **Mail Stop ISSUE FEE**
Commissioner for Patents
P.O. Box 1450
Alexandria, Virginia 22313-1450
or Fax (571)-273-2885

INSTRUCTIONS: This form should be used for transmitting the ISSUE FEE and PUBLICATION FEE (if required). Blocks 1 through 5 should be completed where appropriate. All further correspondence including the Patent, advance orders and notification of maintenance fees will be mailed to the current correspondence address as indicated unless corrected below or directed otherwise in Block 1, by (a) specifying a new correspondence address; and/or (b) indicating a separate "FEE ADDRESS" for maintenance fee notifications.

CURRENT CORRESPONDENCE ADDRESS (Note: Use Block 1 for any change of address)

113613 7590 05/13/2015
Lathrop & Gage
28 State Street
Boston, MA 02109-1775

Note: A certificate of mailing can only be used for domestic mailings of the Fee(s) Transmittal. This certificate cannot be used for any other accompanying papers. Each additional paper, such as an assignment or formal drawing, must have its own certificate of mailing or transmission.

Certificate of Mailing or Transmission
I hereby certify that this Fee(s) Transmittal is being deposited with the United States Postal Service with sufficient postage for first class mail in an envelope addressed to the Mail Stop ISSUE FEE address above, or being facsimile transmitted to the USPTO (571) 273-2885, on the date indicated below.

(Depositor's name)
(Signature)
(Date)

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
14/096,346	12/04/2013	Sandra O'Connor	552815: CPT-011USDV	2832

TITLE OF INVENTION: LIPOPEPTIDE COMPOSITIONS AND RELATED METHODS

APPLN. TYPE	ENTITY STATUS	ISSUE FEE DUE	PUBLICATION FEE DUE	PREV. PAID ISSUE FEE	TOTAL FEE(S) DUE	DATE DUE
nonprovisional	UNDISCOUNTED	$960	$0	$0	$960	08/13/2015

EXAMINER	ART UNIT	CLASS-SUBCLASS
KOMATSU, LI N	1676	514-021100

1. Change of correspondence address or indication of "Fee Address" (37 CFR 1.363).
- ❑ Change of correspondence address (or Change of Correspondence Address form PTO/SB/122) attached.
- ❑ "Fee Address" indication (or "Fee Address" Indication form PTO/SB/47; Rev 03-02 or more recent) attached. **Use of a Customer Number is required.**

2. For printing on the patent front page, list
(1) The names of up to 3 registered patent attorneys or agents OR, alternatively,
(2) The name of a single firm (having as a member a registered attorney or agent) and the names of up to 2 registered patent attorneys or agents. If no name is listed, no name will be printed.
1 _____
2 _____
3 _____

3. ASSIGNEE NAME AND RESIDENCE DATA TO BE PRINTED ON THE PATENT (print or type)

PLEASE NOTE: Unless an assignee is identified below, no assignee data will appear on the patent. If an assignee is identified below, the document has been filed for recordation as set forth in 37 CFR 3.11. Completion of this form is NOT a substitute for filing an assignment.

(A) NAME OF ASSIGNEE (B) RESIDENCE: (CITY and STATE OR COUNTRY)

Please check the appropriate assignee category or categories (will not be printed on the patent) : ❑ Individual ❑ Corporation or other private group entity ❑ Government

4a. The following fee(s) are submitted:
- ❑ Issue Fee
- ❑ Publication Fee (No small entity discount permitted)
- ❑ Advance Order - # of Copies _____

4b. Payment of Fee(s): **(Please first reapply any previously paid issue fee shown above)**
- ❑ A check is enclosed.
- ❑ Payment by credit card. Form PTO-2038 is attached.
- ❑ The director is hereby authorized to charge the required fee(s), any deficiency, or credits any overpayment, to Deposit Account Number _____ (enclose an extra copy of this form).

5. **Change in Entity Status** (from status indicated above)
- ❑ Applicant certifying micro entity status. See 37 CFR 1.29 — NOTE: Absent a valid certification of Micro Entity Status (see forms PTO/SB/15A and 15B), issue fee payment in the micro entity amount will not be accepted at the risk of application abandonment.
- ❑ Applicant asserting small entity status. See 37 CFR 1.27 — NOTE: If the application was previously under micro entity status, checking this box will be taken to be a notification of loss of entitlement to micro entity status.
- ❑ Applicant changing to regular undiscounted fee status. — NOTE: Checking this box will be taken to be a notification of loss of entitlement to small or micro entity status, as applicable.

NOTE: This form must be signed in accordance with 37 CFR 1.31 and 1.33. See 37 CFR 1.4 for signature requirements and certifications.

Authorized Signature _____ Date _____

Typed or printed name _____ Registration No. _____

PTOL-85 Part B (10-13) Approved for use through 10/31/2013. OMB 0651-0033 U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
14/096,346	12/04/2013	Sandra O'Connor	552815: CPT-011USDV	2832

113613 7590 05/13/2015

Lathrop & Gage
28 State Street
Boston, MA 02109-1775

EXAMINER	
KOMATSU, LI N	
ART UNIT	PAPER NUMBER
1676	

DATE MAILED: 05/13/2015

Determination of Patent Term Adjustment under 35 U.S.C. 154 (b)
(Applications filed on or after May 29, 2000)

The Office has discontinued providing a Patent Term Adjustment (PTA) calculation with the Notice of Allowance.

Section 1(h)(2) of the AIA Technical Corrections Act amended 35 U.S.C. 154(b)(3)(B)(i) to eliminate the requirement that the Office provide a patent term adjustment determination with the notice of allowance. See Revisions to Patent Term Adjustment, 78 Fed. Reg. 19416, 19417 (Apr. 1, 2013). Therefore, the Office is no longer providing an initial patent term adjustment determination with the notice of allowance. The Office will continue to provide a patent term adjustment determination with the Issue Notification Letter that is mailed to applicant approximately three weeks prior to the issue date of the patent, and will include the patent term adjustment on the patent. Any request for reconsideration of the patent term adjustment determination (or reinstatement of patent term adjustment) should follow the process outlined in 37 CFR 1.705.

Any questions regarding the Patent Term Extension or Adjustment determination should be directed to the Office of Patent Legal Administration at (571)-272-7702. Questions relating to issue and publication fee payments should be directed to the Customer Service Center of the Office of Patent Publication at 1-(888)-786-0101 or (571)-272-4200.

OMB Clearance and PRA Burden Statement for PTOL-85 Part B

The Paperwork Reduction Act (PRA) of 1995 requires Federal agencies to obtain Office of Management and Budget approval before requesting most types of information from the public. When OMB approves an agency request to collect information from the public, OMB (i) provides a valid OMB Control Number and expiration date for the agency to display on the instrument that will be used to collect the information and (ii) requires the agency to inform the public about the OMB Control Number's legal significance in accordance with 5 CFR 1320.5(b).

The information collected by PTOL-85 Part B is required by 37 CFR 1.311. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 12 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, Virginia 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, Virginia 22313-1450. Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

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1. The information on this form will be treated confidentially to the extent allowed under the Freedom of Information Act (5 U.S.C. 552) and the Privacy Act (5 U.S.C 552a). Records from this system of records may be disclosed to the Department of Justice to determine whether disclosure of these records is required by the Freedom of Information Act.
2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.
3. A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.
4. A record in this system of records may be disclosed, as a routine use, to a contractor of the Agency having need for the information in order to perform a contract. Recipients of information shall be required to comply with the requirements of the Privacy Act of 1974, as amended, pursuant to 5 U.S.C. 552a(m).
5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent Cooperation Treaty.
6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).
7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (i.e., GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.
8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspection or an issued patent.
9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

Examiner-Initiated Interview Summary	**Application No.** 14/096,346	**Applicant(s)** O'CONNOR ET AL.	
	Examiner LI NI KOMATSU	**Art Unit** 1676	

All participants (applicant, applicant's representative, PTO personnel):

(1) *LI NI KOMATSU*. (3)*Jana Lewis*.

(2) *Julie Ha* . (4)_____.

Date of Interview: *22 April 2015*.

Type: ☒ Telephonic ☐ Video Conference
☐ Personal [copy given to: ☐ applicant ☐ applicant's representative]

Exhibit shown or demonstration conducted: ☐ Yes ☒ No.
If Yes, brief description: _____.

Issues Discussed ☐101 ☐112 ☐102 ☐103 ☒Others
(For each of the checked box(es) above, please describe below the issue and detailed description of the discussion)

Claim(s) discussed: *43-45,51 and 52*.

Identification of prior art discussed: _____.

Substance of Interview
(For each issue discussed, provide a detailed description and indicate if agreement was reached. Some topics may include: identification or clarification of a reference or a portion thereof, claim interpretation, proposed amendments, arguments of any applied references etc...)

The Examiner telephoned Applicant's representative, Jana Lewis, to discuss amendments to put this application in condition for allowance. Minor changes are needed for claims 43-45, 51 and 52. Authorization for an examiner's amendment was given on the phone by applicant's representative.

Applicant recordation instructions: It is not necessary for applicant to provide a separate record of the substance of interview.

Examiner recordation instructions: Examiners must summarize the substance of any interview of record. A complete and proper recordation of the substance of an interview should include the items listed in MPEP 713.04 for complete and proper recordation including the identification of the general thrust of each argument or issue discussed, a general indication of any other pertinent matters discussed regarding patentability and the general results or outcome of the interview, to include an indication as to whether or not agreement was reached on the issues raised.

☐ Attachment

/JULIE HA/ Primary Examiner, Art Unit 1675	/LI NI KOMATSU/ Examiner, Art Unit 1676

Notice of Allowability	Application No. 14/096,346	Applicant(s) O'CONNOR ET AL.	
	Examiner LI NI KOMATSU	Art Unit 1676	AIA (First Inventor to File) Status No

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address--

All claims being allowable, PROSECUTION ON THE MERITS IS (OR REMAINS) CLOSED in this application. If not included herewith (or previously mailed), a Notice of Allowance (PTOL-85) or other appropriate communication will be mailed in due course. **THIS NOTICE OF ALLOWABILITY IS NOT A GRANT OF PATENT RIGHTS.** This application is subject to withdrawal from issue at the initiative of the Office or upon petition by the applicant. See 37 CFR 1.313 and MPEP 1308.

1. ☒ This communication is responsive to *amendment filed on 4/10/2015*.
   ☐ A declaration(s)/affidavit(s) under **37 CFR 1.130(b)** was/were filed on_____.
2. ☐ An election was made by the applicant in response to a restriction requirement set forth during the interview on _____; the restriction requirement and election have been incorporated into this action.
3. ☒ The allowed claim(s) is/are *22,31,39 and 42-53*. As a result of the allowed claim(s), you may be eligible to benefit from the **Patent Prosecution Highway** program at a participating intellectual property office for the corresponding application. For more information, please see http://www.uspto.gov/patents/init_events/pph/index.jsp or send an inquiry to PPHfeedback@uspto.gov .
4. ☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

   Certified copies:

   a) ☐ All b) ☐ Some *c) ☐ None of the:
      1. ☐ Certified copies of the priority documents have been received.
      2. ☐ Certified copies of the priority documents have been received in Application No. _____ .
      3. ☐ Copies of the certified copies of the priority documents have been received in this national stage application from the International Bureau (PCT Rule 17.2(a)).

   * Certified copies not received: _____.

Applicant has THREE MONTHS FROM THE "MAILING DATE" of this communication to file a reply complying with the requirements noted below. Failure to timely comply will result in ABANDONMENT of this application.
THIS THREE-MONTH PERIOD IS NOT EXTENDABLE.

5. ☐ CORRECTED DRAWINGS (as "replacement sheets") must be submitted.
   ☐ including changes required by the attached Examiner's Amendment / Comment or in the Office action of Paper No./Mail Date _____.

   Identifying indicia such as the application number (see 37 CFR 1.84(c)) should be written on the drawings in the front (not the back) of each sheet. Replacement sheet(s) should be labeled as such in the header according to 37 CFR 1.121(d).
6. ☐ DEPOSIT OF and/or INFORMATION about the deposit of BIOLOGICAL MATERIAL must be submitted. Note the attached Examiner's comment regarding REQUIREMENT FOR THE DEPOSIT OF BIOLOGICAL MATERIAL.

Attachment(s)

1. ☐ Notice of References Cited (PTO-892)
2. ☒ Information Disclosure Statements (PTO/SB/08), Paper No./Mail Date 4/10/2015
3. ☐ Examiner's Comment Regarding Requirement for Deposit of Biological Material
4. ☒ Interview Summary (PTO-413), Paper No./Mail Date *20150422* .
5. ☒ Examiner's Amendment/Comment
6. ☒ Examiner's Statement of Reasons for Allowance
7. ☐ Other _____.

/JULIE HA/	/LI NI KOMATSU/
Primary Examiner, Art Unit 1675	Examiner, Art Unit 1676

DETAILED ACTION

1. The present application is being examined under the pre-AIA first to invent provisions.

2. Amendment after Non-final office action filed on 4/10/2015 is acknowledged.

3. Claims 1-21, 23-30, 32-38, 40 and 41 have been cancelled.

4. New claims 43-53 have been added.

5. Claims 22, 31, 39 and 42-53 are pending in this application.

6. Applicant elected **without traverse** of sucrose as species of excipient; a molar ratio of daptomycin to the sugar of about 1:1.12 to about 1:21.32 as recited in claim 25 as species of molar ratio of daptomycin to the sugar; a pH of 6.5-7.5 as recited in claim 37 as species of pH; a phosphate buffering agent as species of buffering agent; and converting the aqueous daptomycin solution to a solid pharmaceutical composition by lyophilization as recited in claim 40 as species of way to convert the aqueous daptomycin solution to a solid pharmaceutical composition in the reply filed on 9/24/2014.

Restriction requirement was deemed proper and made FINAL in the previous office action. The instant claims 22, 31, 39 and 42-53 are drawn to a solid pharmaceutical daptomycin composition comprising daptomycin and sucrose. A search was conducted on the elected species, and this appears to be free of prior art. A search was extended to the genus in claims 22, 50 and 53, and this too appears to be free of prior art.

Withdrawn Objections and Rejections

7. Objection to the specification is hereby withdrawn in view of Applicant's amendment to the specification.

8. Objection to the drawings is hereby withdrawn in view of Applicant's amendment to the drawings.

9. Objection to claims 31 and 41 is hereby withdrawn in view of Applicant's amendment to the claim.

10. Rejection to claims 22-42 under 35 U.S.C. 101 is hereby withdrawn in view of Applicant's amendment to the claim and Applicant’s persuasive arguments.

11. Rejection to claims 22-42 under 35 U.S.C. 112(b) or 35 U.S.C. 112 (pre-AIA), second paragraph is hereby withdrawn in view of Applicant's amendment to the claim.

12. Rejection to claim 41 under 35 U.S.C. 112(d) or 35 U.S.C. 112 (pre-AIA), 4th paragraph is hereby withdrawn in view of Applicant's cancellation of claim 41.

13. Rejection to claims 22-25, 27 and 32-42 under pre-AIA 35 U.S.C. 102(b) as being anticipated by Inman et al (EP 0386951 A2, filed with IDS) is hereby withdrawn in view of Applicant's amendment to the claim.

14. Rejection to claims 22-26 and 34-42 under pre-AIA 35 U.S.C. 102(b) as being anticipated by Wei et al (CN 1616083 A, machine translation used, filed with IDS) is hereby withdrawn in view of Applicant's amendment to the claim.

15. Rejection to claims 22-31 and 34-42 under pre-AIA 35 U.S.C. 103(a) as being unpatentable over Wei et al (CN 1616083 A, machine translation used, filed with IDS) in view of Smales et al (Therapeutic proteins, methods and protocols, Humana press, 2005, pages 287-292, filed with IDS) is hereby withdrawn in view of Applicant's amendment to the claim and Applicant's persuasive arguments.

16. Rejection to claims 22-25 and 27-42 under pre-AIA 35 U.S.C. 103(a) as being unpatentable over Inman et al (EP 0386951 A2, filed with IDS) in view of Smales et al (Therapeutic proteins, methods and protocols, Humana press, 2005, pages 287-292, filed with IDS) is hereby withdrawn in view of Applicant's amendment to the claim and Applicant's persuasive arguments.

Examiner's Amendment

17. An examiner's amendment to the record appears below. Should the changes and/or additions be unacceptable to applicant, an amendment may be filed as provided by 37 CFR 1.312. To ensure consideration of such an amendment, it MUST be submitted no later than the payment of the issue fee.

Authorization for this examiner's amendment was given in telephone interviews with Jana Lewis on 4/22/2015.

Claims 43-45, 51 and 52 have been amended as follows:

43. (Currently Amended) The pharmaceutical product according to claim 42, wherein the pharmaceutically acceptable diluent is selected from sterile water for injection, sterile sodium chloride for injection, or bacteriostatic water for injection.

44. (Currently Amended) The solid pharmaceutical daptomycin composition of claim 39, wherein the buffering agent ~~comprises~~ is selected from the group consisting of phosphate, citrate, maleate, carbonate, or a combination thereof.

45. (Currently Amended) The solid pharmaceutical daptomycin composition of claim 39, wherein the buffering agent ~~comprises~~ is selected from the group consisting of sodium phosphate dibasic, sodium citrate, sodium bicarbonate, histidine monohydrochloride, tris(hydroxymethyl)aminomethane, ~~or~~ maleate, or a combination thereof.

51. (Currently Amended) The solid pharmaceutical daptomycin composition of claim 50, wherein the buffering agent ~~comprises~~ is selected from the group consisting of phosphate, citrate, maleate, carbonate, or a combination thereof.

52. (Currently Amended) The solid pharmaceutical daptomycin composition of claim 50, wherein the buffering agent ~~comprises~~ is selected from the group consisting of sodium phosphate dibasic, sodium citrate, sodium bicarbonate, histidine monohydrochloride, tris(hydroxymethyl)aminomethane, ~~or~~ maleate, or a combination thereof.

Claims 22, 31, 39, 42, 46-50 and 53 as filed in the amendment filed on 4/10/2015.

Claims 22, 31, 39 and 42-53 are allowed.

Reasons for Allowance

18. The following is an examiner's statement of reasons for allowance:

A solid pharmaceutical daptomycin composition comprising daptomycin and sucrose recited in instant claims 22, 31, 39 and 42-53 is free of prior art. The closest prior arts are Wei et al (CN 1616083 A, machine translation used, filed with IDS) and Smales et al (Therapeutic proteins, methods and protocols, Humana press, 2005, pages 287-292, filed with IDS). Wei et al teach a solid pharmaceutical daptomycin composition comprising 250 mg daptomycin and 100 mg lactose in a bottle obtained by freeze-drying/lyophilization, and reconstituting the solid pharmaceutical daptomycin preparation comprising 125 to 500 mg daptomycin in a pharmaceutically acceptable diluent such as 3 or 10 ml water to obtain a reconstituted pharmaceutical daptomycin composition for intravenous administration, for example, Abstract; claims 1-5; page 4, the 2nd paragraph; and pages 7-8, Embodiment 2. Smales et al teach that therapeutic proteins/peptides can be stabilized by adding protein-stabilizers, such as sugar at concentration of 10-100 mg/ml, in the process of formulation, and nonreducing disaccharides, such as sucrose and trehalose, are the most potent and useful excipients to protect protein conformation in aqueous solutions and freeze-dried solids, and sucrose has been widely used in various pharmaceutical formulation, for example, page 288, Table 2; page 289, "*3.2. Formulation Design*"; and pages 290-291, "*3.3.2. Sugars*". However, Applicant has presented unexpected results of surprising rapid reconstitution of solid daptomycin compositions comprising sucrose and increased chemical stability of such composition (see pages 15-17 of Applicant's Arguments/Remarks filed on 4/10/2015). These unexpected results rebut any *prima facie* case of obviousness. Therefore, the solid pharmaceutical daptomycin composition comprising daptomycin and sucrose recited in instant claims 22, 31, 39 and 42-53 is both novel and unobvious over the prior arts of record, and the claimed composition is markedly different from what exist in nature.

Any comments considered necessary by applicant must be submitted no later than the payment of the issue fee and, to avoid processing delays, should preferably accompany the issue fee. Such submissions should be clearly labeled "Comments on Statement of Reasons for Allowance."

Conclusion

Claims 22, 31, 39 and 42-53 are allowed.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to LI NI KOMATSU whose telephone number is (571)270-3534. The examiner can normally be reached on Mon-Thurs 8-5pm EST.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Karlheinz Skowronek can be reached on (571)-272-9047. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

/L. K./

Examiner, Art Unit 1676

/JULIE HA/
Primary Examiner, Art Unit 1675

Examiner-Initiated Interview Summary	**Application No.** 14/096,346	**Applicant(s)** O'CONNOR ET AL.	
	Examiner LI NI KOMATSU	**Art Unit** 1676	

All participants (applicant, applicant's representative, PTO personnel):

(1) *LI NI KOMATSU*. (3)*Jana Lewis*.

(2) *Julie Ha* . (4)_____.

Date of Interview: *22 April 2015*.

Type: ☒ Telephonic ☐ Video Conference
☐ Personal [copy given to: ☐ applicant ☐ applicant's representative]

Exhibit shown or demonstration conducted: ☐ Yes ☒ No.
If Yes, brief description: _____.

Issues Discussed ☐101 ☐112 ☐102 ☐103 ☒Others
(For each of the checked box(es) above, please describe below the issue and detailed description of the discussion)

Claim(s) discussed: *43-45,51 and 52*.

Identification of prior art discussed: _____.

Substance of Interview
(For each issue discussed, provide a detailed description and indicate if agreement was reached. Some topics may include: identification or clarification of a reference or a portion thereof, claim interpretation, proposed amendments, arguments of any applied references etc...)

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☐ Attachment

/JULIE HA/ Primary Examiner, Art Unit 1675	/LI NI KOMATSU/ Examiner, Art Unit 1676

Issue Classification	Application/Control No.	Applicant(s)/Patent Under Reexamination
	14096346	O'CONNOR ET AL.
	Examiner	**Art Unit**
	LI NI KOMATSU	1676

CPC						
Symbol					**Type**	**Version**
A61K		38	/	12	F	2013-01-01
A61K		9	/	0019	I	2013-01-01
A61K		9	/	08	A	2013-01-01
A61K		9	/	19	I	2013-01-01
A61K		38	/	00	A	2013-01-01
A61K		47	/	26	I	2013-01-01
A61K		9	/	00	A	2013-01-01
A61K		38	/	10	I	2013-01-01
C07K		11	/	02	A	2013-01-01
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CPC Combination Sets							
Symbol					**Type**	**Set**	**Ranking**	**Version**
			/					
			/					

/LI NI KOMATSU/ Examiner.Art Unit 1676	05/042015	**Total Claims Allowed:**	
(Assistant Examiner)	(Date)	15	
/JULIE HA/ Primary Examiner.Art Unit 1675	05/05/2015	O.G. Print Claim(s)	O.G. Print Figure
(Primary Examiner)	(Date)	1	None

Issue Classification	Application/Control No.	Applicant(s)/Patent Under Reexamination
	14096346	O'CONNOR ET AL.
	Examiner	**Art Unit**
	LI NI KOMATSU	1676

US ORIGINAL CLASSIFICATION		INTERNATIONAL CLASSIFICATION										
CLASS	SUBCLASS	CLAIMED					NON-CLAIMED					
514	21.1	A	6	1	K	38 / 12 (2006.01.01)						
CROSS REFERENCE(S)		C	0	7	K	7 / 50 (2006.01.01)						
CLASS	SUBCLASS (ONE SUBCLASS PER BLOCK)											
530	317											
514	2.3	2.4										

/LI NI KOMATSU/ Examiner.Art Unit 1676	05/042015	**Total Claims Allowed:**	
(Assistant Examiner)	(Date)	15	
/JULIE HA/ Primary Examiner.Art Unit 1675	05/05/2015	O.G. Print Claim(s)	O.G. Print Figure
(Primary Examiner)	(Date)	1	None

Issue Classification	Application/Control No.	Applicant(s)/Patent Under Reexamination
	14096346	O'CONNOR ET AL.
	Examiner	**Art Unit**
	LI NI KOMATSU	1676

☐ Claims renumbered in the same order as presented by applicant ☐ CPA ☐ T.D. ☐ R.1.47

Final	Original	Final	Original	Final	Original	Final	Original	Final	Original	Final	Original	Final	Original	Final	Original
1	22														
2	31														
3	39														
10	42														
11	43														
4	44														
5	45														
6	46														
7	47														
8	48														
9	49														
12	50														
13	51														
14	52														
15	53														

/LI NI KOMATSU/ Examiner.Art Unit 1676	05/042015	**Total Claims Allowed:**	
(Assistant Examiner)	(Date)	15	
/JULIE HA/ Primary Examiner.Art Unit 1675	05/05/2015	O.G. Print Claim(s)	O.G. Print Figure
(Primary Examiner)	(Date)	1	None

EAST Search History (Prior Art)

Ref #	Hits	Search Query	DBs	Default Operator	Plurals	Time Stamp
L1	11	((Sandra) near2 (O'Connor)).INV.	US-PGPUB; USPAT; USOCR	OR	ON	2015/04/13 11:32
L2	4	((Sandra) near2 (O'Connor)).INV.	EPO; JPO; DERWENT	OR	ON	2015/04/13 11:32
L3	5	((Sophie) near2 (Sun)).INV.	US-PGPUB; USPAT; USOCR	OR	ON	2015/04/13 11:32
L4	0	((Sophie) near2 (Sun)).INV.	EPO; JPO; DERWENT	OR	ON	2015/04/13 11:32
L5	0	((Gaauri) near2 (Naik)).INV.	EPO; JPO; DERWENT	OR	ON	2015/04/13 11:32
L6	6	((Gaauri) near2 (Naik)).INV.	US-PGPUB; USPAT; USOCR	OR	ON	2015/04/13 11:32
L8	56	("20070128694"|"4882164"|"20030045678"|"5336756"|"8309061"|"RE39071"|"20120270772"|"5955509"|"20020111311"|"20040242467"|"20050009747"|"20060018934"|"20110172167"|"20120270772"|"20050152979"|"4331594"|"5271935"|"20060264513"|"20060269485"|"20070116729"|"20080220441"|"20090197799"|"20110207658"|"6468967"|"20100041589"|"5387670"|"8604164"|"20040067878"|"20060014674"|"20060018933"|"20110124551"|"6716962"|"7138487"|"7279597"|"4331594"|"8058238"|"20050027113"|"20130280760"|"5629288"|"4439425"|"4537717"|"4874843"|"6696412"|"6194383"|"20050196418"|"20060024365"|"5912226"|"6852689"|"8129342"|"8835382"|"20020132762"|"20030045484"|"20040077601"|"6696412"|"8058238"|"4482487"|"20120149062"|"20070191280"|"20100041589"|"8003673"|"8431539").PN.	US-PGPUB; USPAT; USOCR	OR	ON	2015/04/13 11:34
L9	2	"8835382"	US-PGPUB; USPAT; USOCR	OR	ON	2015/04/13 11:34
L10	2680	daptomycin	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2015/04/13 11:35

EAST Search History (Prior Art)

L11	365006	sucrose	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2015/04/13 11:35
L12	373322	(A61K9/0019 or A61K9/08 or A61K9/19 or A61K38/00 or C07K11/02 or A61K47/26 or A61K9/00 or A61K38/10 or A61K38/12).cpc.	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2015/04/13 11:35
L13	35	l10 same l11	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2015/04/13 11:36
L14	28	l12 and l13	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2015/04/13 11:36

INFORMATION DISCLOSURE STATEMENT BY APPLICANT (**Not for submission under 37 CFR 1.99)**		
	Application Number	14096346
	Filing Date	2013-12-04
	First Named Inventor	O'Connor, Sandra
	Art Unit	1676
	Examiner Name	Komatsu, Li N.
	Attorney Docket Number	552815: CPT-011USDV

U.S.PATENTS Remove

Examiner Initial*	Cite No	Patent Number	Kind Code[1]	Issue Date	Name of Patentee or Applicant of cited Document	Pages,Columns,Lines where Relevant Passages or Relevant Figures Appear
	1	8835382	B2	2014-09-16	O'Connor et al.	

If you wish to add additional U.S. Patent citation information please click the Add button. Add

U.S.PATENT APPLICATION PUBLICATIONS Remove

Examiner Initial*	Cite No	Publication Number	Kind Code[1]	Publication Date	Name of Patentee or Applicant of cited Document	Pages,Columns,Lines where Relevant Passages or Relevant Figures Appear
	1	20050152979	A1	2005-07-14	Besman et al.	

If you wish to add additional U.S. Published Application citation information please click the Add button. Add

FOREIGN PATENT DOCUMENTS Remove

Examiner Initial*	Cite No	Foreign Document Number[3]	Country Code[2] i	Kind Code[4]	Publication Date	Name of Patentee or Applicant of cited Document	Pages,Columns,Lines where Relevant Passages or Relevant Figures Appear	T[5]
	1	2675622	CA	A1	2008-08-28	Adachi et al.		☐
	2	H10-212241	JP	A	1998-08-11	Tanaka et al. Abstract only	English Abstract	☐
	3	H05-194257	JP	A	1993-08-03	Horowitz et al. Abstract only	English Abstract	☐

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /L.K./

Receipt date: 04/10/2015	Application Number	14096346	14096346 - GAU: 1676
INFORMATION DISCLOSURE STATEMENT BY APPLICANT	Filing Date	2013-12-04	
(Not for submission under 37 CFR 1.99)	First Named Inventor	O'Connor, Sandra	
	Art Unit	1676	
	Examiner Name	Komatsu, Li N.	
	Attorney Docket Number	552815: CPT-011USDV	

	4	2005-060377	JP	A	2005-03-10	Kojima et al.	English machine translation	☐
	5	2003-095975	JP	A	2003-04-03	Yamazaki et al.	English machine translation	☐
	6	2008/150479	WO	A2	2008-12-11	Chen et al.		☐
	7	2008/102849	WO	A1	2008-08-28	Adachi et al. Abstract only	English Abstract	☐
	8	1997/045135	WO	A1	1997-12-04	Tanaka et al.		☐
	9	1993/010809	WO	A1	1993-06-10	Horowitz et al.		☐

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NON-PATENT LITERATURE DOCUMENTS **Remove**

Examiner Initials*	Cite No	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc), date, pages(s), volume-issue number(s), publisher, city and/or country where published.	T5
	1	"CUBICIN: EPAR - SCIENTIFIC DISCUSSION", EMEA, 2006. [online]. [Published on Internet 11.08.2006]. <URL: http://www.ema.europa.eu/docs/en_GB/_ibrary/EPAR_-_Scientific_Discussion/human/000637/WC500036046.pdf>	☐
	2	"Protein structure," from http://www.sciencedaily.com/articles/p/protein_structure.htm, pages 1-3, accessed 02/11/2015.	☐
	3	Notice of Reasons for Rejection, mailed November 19, 2014 in Japanese Patent Application No.: 2012-540161, 5 pages (English translation).	☐

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /L.K./

Receipt date: 04/10/2015	Application Number	14096346	14096346 - GAU: 1676
INFORMATION DISCLOSURE STATEMENT BY APPLICANT	Filing Date	2013-12-04	
(Not for submission under 37 CFR 1.99)	First Named Inventor	O'Connor, Sandra	
	Art Unit	1676	
	Examiner Name	Komatsu, Li N.	
	Attorney Docket Number	552815: CPT-011USDV	

	4	English translation of Chinese Patent Application Publication No. 1616083 (published May 18, 2005) as cited in the Japanese Notice of Reasons for Rejection, mailed November 19, 2014 in Japanese Patent Application No.: 2012-540161, 4 pages.	☐

If you wish to add additional non-patent literature document citation information please click the Add button **Add**

EXAMINER SIGNATURE

Examiner Signature	/Li Komatsu/	Date Considered	04/13/2015

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through a citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

[1] See Kind Codes of USPTO Patent Documents at www.USPTO.GOV or MPEP 901.04. [2] Enter office that issued the document, by the two-letter code (WIPO Standard ST.3). [3] For Japanese patent documents, the indication of the year of the reign of the Emperor must precede the serial number of the patent document. [4] Kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST.16 if possible. [5] Applicant is to place a check mark here if English language translation is attached.

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /L.K./

Receipt date: 04/10/2015	Application Number	14096346	14096346 - GAU: 1676
INFORMATION DISCLOSURE STATEMENT BY APPLICANT (**Not for submission under 37 CFR 1.99)**	Filing Date	2013-12-04	
	First Named Inventor	O'Connor, Sandra	
	Art Unit	1676	
	Examiner Name	Komatsu, Li N.	
	Attorney Docket Number	552815: CPT-011USDV	

CERTIFICATION STATEMENT

Please see 37 CFR 1.97 and 1.98 to make the appropriate selection(s):

☐ That each item of information contained in the information disclosure statement was first cited in any communication from a foreign patent office in a counterpart foreign application not more than three months prior to the filing of the information disclosure statement. See 37 CFR 1.97(e)(1).

OR

☐ That no item of information contained in the information disclosure statement was cited in a communication from a foreign patent office in a counterpart foreign application, and, to the knowledge of the person signing the certification after making reasonable inquiry, no item of information contained in the information disclosure statement was known to any individual designated in 37 CFR 1.56(c) more than three months prior to the filing of the information disclosure statement. See 37 CFR 1.97(e)(2).

☐ See attached certification statement.

☒ The fee set forth in 37 CFR 1.17 (p) has been submitted herewith.

☒ A certification statement is not submitted herewith.

SIGNATURE

A signature of the applicant or representative is required in accordance with CFR 1.33, 10.18. Please see CFR 1.4(d) for the form of the signature.

Signature	/Brian C. Trinque/	Date (YYYY-MM-DD)	2015-04-10
Name/Print	Brian C. Trinque	Registration Number	56,593

This collection of information is required by 37 CFR 1.97 and 1.98. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 1 hour to complete, including gathering, preparing and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

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2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.

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6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).

7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (i.e., GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.

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9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /L.K./

Search Notes	Application/Control No.	Applicant(s)/Patent Under Reexamination
	14096346	O'CONNOR ET AL.
	Examiner	Art Unit
	LI NI KOMATSU	1676

CPC- SEARCHED		
Symbol	Date	Examiner
A61K: 9/0019, 9/08, 9/19, 38/00, 47/26, 9/00, 38/10, 38/12	4/13/2015	LNK
C07K: 11/02	4/13/2015	LNK

CPC COMBINATION SETS - SEARCHED		
Symbol	Date	Examiner
None	4/13/2015	LNK

US CLASSIFICATION SEARCHED			
Class	Subclass	Date	Examiner
None		4/13/2015	LNK

SEARCH NOTES		
Search Notes	Date	Examiner
Updated PALM and EAST all inventor name search	4/13/2015	LNK
Updated EAST search: please see attached	4/13/2015	LNK
Re-review STIC search	4/13/2015	LNK
Allowance conference with Primary Examiner Julie Ha	4/13/2015	LNK

INTERFERENCE SEARCH			
US Class/ CPC Symbol	US Subclass / CPC Group	Date	Examiner
EAST search: please see attached		4/13/2015	LNK
STIC search	can be accessed via eDAN and SCORE	9/25/2014	LNK

/LI NI KOMATSU/ Examiner.Art Unit 1676	

EAST Search History (Interference)

Ref #	Hits	Search Query	DBs	Default Operator	Plurals	Time Stamp
L15	1608	A61K38/12 or C07K7/50	US-PGPUB; USPAT; UPAD	OR	ON	2015/04/13 11:40
L16	5	((Sophie) near2 (Sun)).INV.	US-PGPUB; USPAT; UPAD	OR	ON	2015/04/13 11:40
L17	6	((Gaauri) near2 (Naik)).INV.	US-PGPUB; USPAT; UPAD	OR	ON	2015/04/13 11:40
L18	11	((Sandra) near2 (O'Connor)).INV.	US-PGPUB; USPAT; UPAD	OR	ON	2015/04/13 11:40
L19	2155	daptomycin	US-PGPUB; USPAT; UPAD	OR	ON	2015/04/13 11:40
L20	291763	sucrose	US-PGPUB; USPAT; UPAD	OR	ON	2015/04/13 11:40
L21	72519	(A61K9/0019 or A61K9/08 or A61K9/19 or A61K38/00 or C07K11/02 or A61K47/26 or A61K9/00 or A61K38/10 or A61K38/12).cpc.	US-PGPUB; USPAT; UPAD	OR	ON	2015/04/13 11:40
L22	6946	514/21.1;514/2.3;514/2.4;530/317.ccls.	US-PGPUB; USPAT; UPAD	OR	ON	2015/04/13 11:40
L23	12	l16 or l17 or l18	US-PGPUB; USPAT; UPAD	OR	ON	2015/04/13 11:41
L24	20	l19 same l20	US-PGPUB; USPAT; UPAD	OR	ON	2015/04/13 11:41
L25	3	l23 and l24	US-PGPUB; USPAT; UPAD	OR	ON	2015/04/13 11:41
L26	12	l15 and l24	US-PGPUB; USPAT; UPAD	OR	ON	2015/04/13 11:41
L27	15	l21 and l24	US-PGPUB; USPAT; UPAD	OR	ON	2015/04/13 11:42
L28	10	l22 and l24	US-PGPUB; USPAT; UPAD	OR	ON	2015/04/13 11:42

I hereby certify that this paper (along with any paper referred to as being attached or enclosed) is being transmitted via the Office electronic filing system in accordance with § 1.6(a)(4).

Dated: April 10, 2015
Electronic Signature for Brian C. Trinque, Ph.D., Esq.:
/Brian C. Trinque/

PATENT
Attorney Docket No. C111-02/02 US / 552815

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:
Sandra O'Connor et al.

Examiner: Komatsu, Li N.

Application No.: 14/096,346

Art Unit: 1676

Filed: December 4, 2013

Conf. No.: 2832

For: LIPOPEPTIDE COMPOSITIONS AND RELATED METHODS

RESPONSE TO OFFICE ACTION

MS Amendment
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Colleague:

This communication is responsive to the Non-Final Office Action having a mailing date of December 10, 2014 for the referenced application. Applicants herein petition for a one-month extension of time.

Amendments to the Specification begin on page 2 of this paper;

Amendments to the Figures begin on page 6 of this paper;

Amendments to the Claims begin on page 7 of this paper; and

Remarks begin on page 10 of this paper.

AMENDMENTS TO THE SPECIFICATION

Please amend the paragraphs at page 6, line 18 through page 7, line 5 of the application as filed as follows:

~~Figure 1~~FIG. **1** is the chemical structure of daptomycin.

~~Figure 2~~FIG. **2** is the chemical structure of anhydro-daptomycin.

~~Figure 3~~FIG. **3** is the chemical structure of the beta-isomer of daptomycin.

~~Figure 4~~FIG. **4** is the chemical structure of the lactone hydrolysis product of daptomycin.

~~Figure 5 is~~FIGS. **5A-5E** show Table 6, which lists ~~listing~~ examples of preferred daptomycin compositions. These compositions were prepared as liquid solutions, then lyophilized to provide solid pharmaceutical daptomycin preparations that reconstitute in an aqueous pharmaceutical diluent within less than 2 minutes (including compositions that reconstitute in less than 1 minute). In Table 6, "Recon time" refers to the time required for about 500 mg the lyophilized daptomycin composition described in the "Formulation (solid state)" column to dissolve in 10 mL of 0.9% aqueous sodium chloride at room temperature (about 25 degrees C).

~~Figure 6 is~~FIGS. **6A** and **6B** show Table 7, which lists ~~listing~~ examples of other daptomycin compositions. These compositions were prepared as liquid solutions, then lyophilized to provide solid pharmaceutical lipopeptide preparations that reconstitute in an aqueous pharmaceutical diluent within 2 minutes or more. In Table 7, "Recon time" refers to the time required for about 500 mg the lyophilized daptomycin solution to dissolve in 10 mL of 0.9% aqueous sodium chloride at room temperature (about 25 degrees C).

~~Figure 7 is~~FIGS. **7A-7H** show Table 8, which lists ~~listing~~ examples of daptomycin compositions containing a sugar.

~~Figure 8 is~~FIGS. **8A-8C** show Table 9, which shows ~~showing~~ the percent change in total daptomycin purity measured and calculated for various daptomycin formulations according to Example 4.

Please amend the paragraph at page 11, line 21 through page 12, line 2 of the application as filed as follows:

Unexpectedly, combining daptomycin with one or more non-reducing sugars (e.g., ~~sucrose,~~ trehalose, sucrose and mannitol) in a solid pharmaceutical preparation enhanced the chemical stability of daptomycin in both solid and reconstituted liquid phases. Daptomycin chemical stabilities were measured by comparing measurements of total daptomycin purity from multiple solid samples stored under known time periods (e.g., up to 12 months) under known conditions (e.g., constant temperatures). The daptomycin total purity for each sample was measured by high performance liquid chromatography (HPLC) (using parameters in Table 3) according to Example 4. In addition, the amount of daptomycin (Figure 1) in the reconstituted daptomycin solution was measured relative to the amount of substances selected from the group consisting of the anhydro-daptomycin (Figure 2), the beta-isomer of daptomycin (Figure 3) and the lactone hydrolysis product of daptomycin (Figure 4). Similarly, to determine daptomycin chemical stability in the reconstituted daptomycin solution, the HPLC measurement and calculation of daptomycin purity in the reconstituted daptomycin solution was repeated according to Example 4 at various time intervals up to 14 days after preparing the reconstituted daptomycin solution.

Please amend the paragraph at page 16, line 31 to page 17, line 11 of the application as filed as follows:

According to the package insert for daptomycin for injection sold under the trademark ~~CUBICIN®~~ CUBICIN® (i.e., daptomycin without glycine or a sugar):

> "The contents of a ~~CUBICIN~~CUBICIN® 500 mg vial should be reconstituted using aseptic technique as follows:
> Note: To minimize foaming, AVOID vigorous agitation or shaking of the vial during or after reconstitution.
> 1. Remove the polypropylene flip-off cap from the ~~CUBICIN~~CUBICIN® vial to expose the central portion of the rubber stopper.
> 2. Slowly transfer 10 mL of 0.9% sodium chloride injection through the center of the rubber stopper into the ~~CUBICIN~~CUBICIN® vial, pointing the transfer needle toward the wall of the vial.
> 3. Ensure that the entire ~~CUBICIN~~CUBICIN® product is wetted by gently rotating the vial.
> 4. Allow the product to stand undisturbed for 10 minutes.
> 5. Gently rotate or swirl the vial contents for a few minutes, as needed, to obtain a completely reconstituted solution."

Please amend the paragraphs at page 32, line 30 through page 33, line 16 of the application as filed as follows:

Other compositions include a powder, pharmaceutical composition comprising daptomycin and at least one excipient selected from sorbitol, mannitol, sucrose, glycine, trehalose, lactose, maltose, fructose and dextrose.

The composition of [[claim]]specific embodiment 1 comprising:

a. 500 mg daptomycin;

b. 714.3 mg sucrose; and

c. 35.5 mg sodium phosphate dibasic

wherein the composition is compounded at a pH of about 7.

The composition of [[claim]]specific embodiment 1 comprising:

a. 500 mg daptomycin;

b. 476.2 mg sucrose;

c. 142.9 mg mannitol; and

d. 35.5 mg sodium phosphate dibasic

wherein the composition is compounded at a pH of about 7.

The composition of [[claim]]specific embodiment 1 comprising:

a. 500 mg daptomycin;

b. 476.2 mg sucrose;

c. 285.8 mg mannitol; and

d. 35.5 mg sodium phosphate dibasic

wherein the composition is compounded at a pH of about 7.

Please amend the paragraphs at page 36, lines 5-28 of the application as filed as follows:

In another aspect of the invention is provided a method for preparing compositions of [[claim]]specific embodiment 1 that are compounded with a buffer, for example at pH 7. This process comprises the steps of

a. supplying a daptomycin preparation

b. adding a pH adjuster to obtain a solution of about pH 4.7-6.0;

c. adding a buffering agent;

d. adding at least one excipient selected from sorbitol, mannitol, sucrose, glycine, trehalose, lactose, maltose, fructose and dextrose;

e. adding a pH adjuster to obtain a pH of about 7.0

f. diluting the bulk solution with sWFI

g. filtering the solution of step f; and

h. converting the composition to a powder form to obtain the solid daptomycin composition.

In another aspect of the invention is provided a method for preparing compositions of [[claim]]specific embodiment 1 that are compounded with a buffer, for example at pH 7. This process comprises the steps of

a. supplying a daptomycin preparation

b. adding a pH adjuster to obtain a solution of about pH 4.7-6.0;

c. adding a buffering agent;

d. adding at least one excipient selected from sorbitol, mannitol, sucrose, glycine, trehalose, lactose, maltose, fructose and dextrose;

e. adding a pH adjuster to obtain a pH of about 7.0

f. diluting the bulk solution with sWFI

g. filtering the solution of step f; and

h. converting the composition to a powder form to obtain the composition of [[claim]]specific embodiment 1.

AMENDMENT TO THE FIGURES

Applicants respectfully submit herewith Replacement Figures 1-8 in accordance with 37 CFR 1.121 and 1.84(u)(1).

AMENDMENTS TO THE CLAIMS

1-21. (**Canceled**)

22. (**Currently Amended**) A solid pharmaceutical daptomycin composition, wherein said composition is prepared by lyophilizing an aqueous daptomycin solution comprising daptomycin and sucrose ~~at least one excipient selected from glycine and a sugar, wherein an amount of the solid pharmaceutical daptomycin composition comprising 500 mg of daptomycin dissolves in 10 mL of 0.9% aqueous sodium chloride in less than 5 minutes at about 25 degrees C~~.

23-30. (**Canceled**)

31. (**Currently Amended**) [[he]]The solid pharmaceutical daptomycin composition of claim 22[[30]], wherein the molar ratio of daptomycin to sucrose is about 1:1.12 to about 1:8.98.

32-38. (**Canceled**)

39. (**Currently Amended**) The solid pharmaceutical daptomycin composition of claim 22[[34]], wherein the aqueous daptomycin solution further comprises a buffering agent.

40. (**Canceled**)

41. (**Canceled**)

42. (**Previously Presented**) A pharmaceutical product comprising the solid pharmaceutical daptomycin composition of claim 22 and a pharmaceutically acceptable diluent.

43. (**New**) The pharmaceutical product according to claim 42 wherein the pharmaceutically acceptable diluent is selected from sterile water for injection, sterile sodium chloride injection, or bacteriostatic water for injection.

44. (**New**) The solid pharmaceutical daptomycin composition of claim 39, wherein the buffering agent comprises phosphate, citrate, maleate, carbonate, or a combination thereof

45. (**New**) The solid pharmaceutical daptomycin composition of claim 39, wherein the buffering agent comprises sodium phosphate dibasic, sodium citrate, sodium bicarbonate, histidine monohydrochloride, tris(hydroxymethyl)aminomethane, or maleate.

46. (**New**) The solid pharmaceutical daptomycin composition of claim 39, wherein the buffering agent is sodium phosphate dibasic.

47. (**New**) The solid pharmaceutical daptomycin composition of claim 22, wherein the aqueous daptomycin solution has a pH of about 4.5 to about 8.0.

48. (**New**) The solid pharmaceutical daptomycin composition of claim 47, wherein the aqueous daptomycin solution has a pH of about 6.5 to about 7.5.

49. (**New**) The solid pharmaceutical daptomycin composition of claim 48, wherein the aqueous daptomycin solution has a pH of about 7.0.

50. (**New**) A solid pharmaceutical daptomycin composition, wherein the solid pharmaceutical daptomycin composition is prepared by a process comprising:

a. forming an aqueous solution comprising daptomycin, wherein the aqueous solution has a pH of about 4.5 to about 5.0;
b. adding a buffering agent to the aqueous solution of daptomycin;
c. dissolving sucrose in the aqueous solution of daptomycin to form a buffered daptomycin sucrose formulation;
d. adjusting the pH of the buffered daptomycin sucrose formulation to about 6.5 to about 7.5; and
e. converting the buffered daptomycin sugar formulation to the solid pharmaceutical daptomycin composition.

51. (**New**) The solid pharmaceutical daptomycin composition of claim 50, wherein the buffering agent comprises phosphate, citrate, maleate, carbonate, or a combination thereof

52. (**New**) The solid pharmaceutical daptomycin composition of claim 50, wherein the buffering agent comprises sodium phosphate dibasic, sodium citrate, sodium bicarbonate, histidine monohydrochloride, tris(hydroxymethyl)aminomethane, or maleate.

53. (**New**) A solid pharmaceutical daptomycin composition, wherein the solid pharmaceutical daptomycin composition is prepared by a process comprising:

a. dissolving sucrose in an aqueous solution comprising daptomycin to form a daptomycin sucrose formulation;
b. adjusting the pH of the daptomycin sucrose formulation to about 6.5 to about 7.5; and
c. converting the daptomycin sugar formulation to the solid pharmaceutical daptomycin composition.

REMARKS

Status of the claims

Prior to entry of the instant amendments, claims 22-42 were pending in the instant application. Applicants herein amend claims 22, 31, and 39, and cancel claims 23-30, 32-38, 40, and 41. Claims 43-53 have been added. Therefore, upon entry of the instant amendments, claims 22, 31, 39, and 42-53 will be pending in the instant application.

Claim 22 has been amended to specify that the solid pharmaceutical daptomycin composition is prepared by lyophilizing an aqueous daptomycin solution comprising daptomycin and sucrose. Claims 31 and 39 have been amended to depend from claim 22. Support for these amendments to the claims can be found at least, for example, at page 3, line 31 through page 4, line 3; page 5, lines 15-17; and Table 6 (Figure 5) of the application as filed.

New claim 43 specifies diluents for the pharmaceutical product. Support for new claim 43 can be found at least, for example, at page 10, lines 27-28 of the application as filed. New claims 44-46, 51, and 52 specify the buffering agent of the solid pharmaceutical daptomycin composition. Support for new claims 44-46, 51, and 52 can be found at least, for example, at page 8, lines 9-10 and page 8, lines 13-17. New claims 47-49 specify the pH of the aqueous daptomycin solution. Support for new claims 47-49 can be found at least, for example, at page 5, lines 25-28. New claims 50 and 53 specify a process for preparing the solid pharmaceutical daptomycin composition. Support for new claims 50 and 53 can be found at least, for example, at page 4, lines 11-24 and page 5, lines 18-19.

The foregoing claim amendments have been made solely for the purpose of expediting prosecution of the present application. No new matter is added. Applicants reserve the right to pursue the subject matter of the present claims prior to being amended herein in this application or in another related application. Entry and consideration of these amendments are respectfully requested.

Examiner Interview

Applicants thank the Examiner and the Examiner's supervisor for the interview of March 31, 2015, during which this application was discussed.

Objections

According to the Examiner, the use of trademark should be properly referred to in the specification. *Solely for the purpose of expediting prosecution of the present application*, Applicants have amended the specification in accordance with the Examiner's comments. Applicants therefore request reconsideration and withdrawal of this objection.

Applicants here amend the specification to correct minor informalities including updates to the Brief Description of the Drawings, a redundant "sucrose" in the recitation on page 11, lines 21-22, and references to claim 1 in the specification.

Applicants submit herewith a replacement set of drawings. Figures 1, 2 and 4 are objected to as allegedly being unclear as to "what '5' in the figures is referring to" (page 3 of the Office Action). Applicants note that the 5 is merely a line number. *Solely for the purpose of expediting prosecution of the present application*, Applicants have removed the number "5" from the figures 1, 2 and 4. Applicants therefore request reconsideration and withdrawal of this objection.

Amendments to the specification and drawings are in compliance with 37 CFR §121. In particular, Figures 5A-5E, 6A, 6B, 7A-7H, and 8A-8C are properly labeled in accordance with 37 CFR 1.84(u)(1).

Claim 31 is objected to for a typographical error. Applicants have amended the claim to correct this clear clerical error. Applicants therefore request reconsideration and withdrawal of this objection.

Claim 41 is objected to for the use of the term "containing." *Solely for the purpose of expediting prosecution of the present application*, Applicants have canceled claim 41. Applicants therefore request reconsideration and withdrawal of this objection.

Rejection under 35 U.S.C. § 101

Claims 22-42 have been rejected under 35 U.S.C. § 101 as allegedly not being directed to patent-eligible subject-matter. Applicants respectfully disagree. The instant claims are directed toward a solid pharmaceutical daptomycin composition containing daptomycin and sucrose. According to the "2014 Interim Guidance on Patent Subject Matter Eligibility," "the markedly different characteristics analysis should be applied to the resultant nature-based combination, rather than its component parts" (Federal Register, Vol. 79, No. 241, p. 74623, December 2014).

Each individual component of the claimed composition may exist in nature (e.g., daptomycin and sucrose), but a composition comprising a *combination* of these components, let alone a solid pharmaceutical composition, does not exist in nature. Therefore, the combination should be analyzed for markedly different characteristics.

"Markedly different characteristics can be expressed as the product's structure, function, and/or other properties" (Federal Register, Vol. 79, No. 241, p. 74623, December 2014). The physical characteristics of Applicants' claimed composition comprising daptomycin in a solid form are structurally different from the naturally-occurring daptomycin. In nature, daptomycin can be derived from the fermentation product of the microorgainism *Streptomyces roseosporus*, but daptomycin does not exist in a solid form in the microorganism. Furthermore, the claimed solid pharmaceutical daptomycin compositions have different functional characteristics (*e.g.*, increased chemical stability, as described below) as compared to the naturally-occurring daptomycin. These differences rise to the level of a marked difference, and accordingly the claimed daptomycin composition is not a "product of nature" exception as described in the "Nature-Based Products" examples accompanying the "2014 Interim Guidance on Patent Subject Matter Eligibility" (e.g., claim 2 of Example 4 "Purified Proteins"). Thus, the claimed invention qualifies as eligible subject-matter.

Accordingly, Applicants respectfully request reconsideration and withdrawal of the rejection of claims under 35 U.S.C. § 101.

Rejection under 35 U.S.C. § 112 ¶2

Claims 22-42 have been rejected under 35 U.S.C. § 112 ¶2 as allegedly being indefinite. Specifically, the Examiner takes the position that "the speed of dissolving the solid pharmaceutical daptomycin composition depends on many conditions, such as the physical form of the solid, the type of mixing involved and many others" (page 9 of the Office Action). Applicants respectfully disagree, and take the position that dissolution is dependent on the physiochemical properties of the compound and the type of diluent. However, *solely for the purpose of expediting prosecution of the present application*, this phrase has been removed from the claims. Claim 22, as amended, is a product-by-process claim, which specifies a solid pharmaceutical daptomycin composition comprising certain components (sucrose), wherein the composition is prepared by a particular process (lyophilization). Based on the application as

filed, a person of ordinary skill in the art could easily interpret the metes and bounds of claim 22 so as to understand how to avoid infringement (MPEP § 2173.02(II)).

Applicants therefore respectfully request reconsideration and withdrawal of the rejection of claims under 35 U.S.C. § 112 ¶2.

Rejection under 35 U.S.C. § 112 ¶4

Claim 41 is rejected as allegedly being of improper dependent form. Specifically, the Office Action alleges that claim 41 recites an inherent property and fails to further limit the subject matter of claim 22 (page 10 of the Office Action). Applicants respectfully disagree. However, s*olely for the purpose of expediting prosecution of the present application*, claim 41 has been canceled. Applicants therefore request reconsideration and withdrawal of this rejection.

Rejections under 35 U.S.C. § 102(b)

Claims 22-25, 27, and 32-42

Claims 22-25, 27, and 32-42 have been rejected under 35 U.S.C. § 102(b) as being anticipated by Inman et al. (EP 0386951 A2; hereinafter referred to as "Inman"). Applicants respectfully disagree. However, *solely for the purpose of expedited prosecution*, independent claim 22 has been amended to specify a solid pharmaceutical daptomycin composition prepared by lyophilizing an aqueous daptomycin solution comprising sucrose.

In contrast, Inman discloses a *liquid* formulation with greater buffer capacity for daptomycin in order to solve the problem of daptomycin degradation in solution. Inman fails to disclose preparing *solid* pharmaceutical formulations, the subject-matter of Applicants' claims. In addition, Inman discloses buffered solutions of dextrose, not sucrose, as required by the instant claims. Thus, Inman fails to recite each and every limitation of Applicants' claimed invention.

Claims 23-25, 27, 32-38, 40, and 41 have been canceled, and claims 31, 39, 42, and new claims 42-53 also specify these patentable limitations.

In view of the foregoing, Applicants respectfully request withdrawal of the rejection of claims under 35 U.S.C. § 102(b).

Claims 22-26 and 34-42

Claims 22-26 and 34-42 have been rejected under 35 U.S.C. § 102(b) as being anticipated by Wei et al. CN 1616083A; hereinafter referred to as "Wei"). Applicants respectfully disagree. However, *solely for the purpose of expedited prosecution*, independent claim 22 has been amended to specify a solid pharmaceutical daptomycin composition prepared by lyophilizing an aqueous daptomycin solution comprising sucrose.

In contrast, Wei does not teach the use of sucrose, as specified in the instant claims. Thus, Wei fails to recite each and every limitation of Applicants' claimed invention. Claims 23-26, 34-38, 40, and 41 have been canceled, and instant claims 31, 39, and 42 also specify the patentable limitations discussed above.

Applicants therefore respectfully request withdrawal of the rejection of claims under 35 U.S.C. § 102(b).

Rejections under 35 U.S.C. § 103(a)

Claims 22-31 and 34-42

Claims 22-31 and 34-42 have been rejected under 35 U.S.C. § 103(a) as allegedly being obvious in view of Wei and Smales et al. (Therapeutic Proteins: Methods and Protocols, Humana Press, 2005, 287-292; hereinafter referred to as "Smales"). Specifically, the Office Action alleges that Wei teaches "a solid pharmaceutical daptomycin composition comprising 250 mg daptomycin and 100 mg lactose in a bottle obtained by freeze-drying/lyophilization" (page 15 of the Office Action). The Office Action further alleges that Smales teaches that "therapeutic proteins/peptides can be stabilized by adding protein-stabilizers, such as sugar" and that sucrose and trehalose are "the most potent and useful excipients to protect protein conformation in aqueous solutions and freeze-dried solids" (page 16 of the Office Action).

Applicants respectfully disagree. Applicants respectfully point out that, to support an obviousness rejection, MPEP 2141.02 requires consideration of the "invention and prior art references as a whole." According MPEP 2143(I)(A), a proper, post-KSR obviousness determination still requires the Office to show "that all the claimed elements were known in the prior art and one skilled in the art could have combined the elements as claimed by known methods with no change in their respective functions." *KSR,* 550 U.S. at 416, 82 USPQ2d at 1395; *Sakraida v. AG Pro, Inc.,* 425 U.S. 273, 282, 189 USPQ 449, 453 (1976); *Anderson's-*

Black Rock, Inc. v. Pavement Salvage Co., 396 U.S. 57, 62-63, 163 USPQ 673, 675 (1969); *Great Atl. & P. Tea Co. v. Supermarket Equip. Corp.,* 340 U.S. 147, 152, 87 USPQ 303, 306 (1950). Applicants submit that it is well-settled law that an obviousness rejection requires at least a suggestion of all the claim elements. For the reasons set forth below, Applicants respectfully submit that a *prima facie* case of obviousness has not been established by the Office Action for the present claims.

The instant claims specify a solid pharmaceutical daptomycin composition prepared by lyophilizing an aqueous daptomycin solution comprising sucrose. In contrast, the Examiner has stated that Wei teaches "a solid pharmaceutical daptomycin composition comprising 250 mg daptomycin and 100 mg lactose in a bottle obtained by freeze-drying/lyophilization" (page 15 of the Office Action).

As discussed above, Wei does not disclose the use of sucrose, as specified in the instant claims. In addition, Wei does not teach the surprising stabilizing effect of sucrose on solid daptomycin as discovered by the instant inventors, as shown in Table 4 on pages 25-26 and in Table 9 (Figure 8) of the application as filed. For example, as shown in Table 4, combining 15-20% sucrose with daptomycin in a lyophilized composition increases daptomycin chemical stability by about 78-96% (page 26, lines 11-13 of the specification as filed). As noted by the Examiner, Wei teaches a composition comprising daptomycin and lactose (page 15 of the Office Action). In Table 4 of the instant application, Applicants provided data that demonstrated that combining 20% lactose with daptomycin in a lyophilized composition *decreases* daptomycin chemical stability. In addition, Table 9 (Figure 8) shows that sucrose increases the chemical stability of solid daptomycin compositions over time at elevated temperatures. Thus, Wei does not teach or suggest the surprising benefit associated with sucrose. As Wei is silent regarding any rationale for excipient selection, one of ordinary skill in the art would not be motivated to substitute sucrose as an excipient based on Wei.

Smales does not make up for the deficiencies in the teachings of Wei. The Office Action alleges that Smales teaches that "therapeutic proteins/peptides can be stabilized by adding protein-stabilizers" (page 16 of the Office Action). Applicants respectfully submit that Smales relates to preserving a protein's three-dimensional structure in order to retain biological activity. For example, Smales discloses that "various saccharides (sugars) protect the conformation of proteins in aqueous solutions and during freeze-drying" (see page 290). The purpose of sugars

as taught by Smales is to act as stabilizers during lyophilization, but the purpose of sucrose as claimed by Applicants is to act as stabilizers during long term storage. Smales teaches techniques to prevent proteins from denaturing during freezing or lyophilizing. The average protein length is estimated being about 300 amino acids, with the lower limit of about 40-50 amino acids, so that the protein can fold into three-dimensional structures and perform biochemical functions (see, e.g., http://www.sciencedaily.com/articles/p/protein_structure.htm). In contrast, daptomycin is a cyclic peptide of only 13 amino acids, and has a molecular weight of about 1.7 kDa. One of ordinary skill in the art would therefore appreciate that daptomycin is not a protein, and would not be concerned with retaining the "three-dimensional" structure of daptomycin. Daptomycin has no complexed higher-order structure. Thus, one of ordinary skill in the art would not be motivated to apply techniques for retaining three-dimensional protein structures to daptomycin, at least in view of the significant difference between a protein and daptomycin.

In addition, neither Wei nor Smales, alone or in combination, teach or suggest the surprising rapid reconstitution of solid daptomycin compositions comprising sucrose. Prior to the present disclosure, a 500 mg vial of lyophilized daptomycin for injection (CUBICIN®) powder, which is prepared from a daptomycin solution with no sugar, is combined with 10 mL of 0.9% aqueous sodium chloride and allowed to stand for 10 minutes (or more). (See section 2.5 of the CUBICIN® label, as shown on page 16, line 31 through page 17, line 11 of the specification as filed). See also the conference poster and accompanying abstract cited as reference 1 of the Non-Patent Literature Documents in the Information Disclosure Statement submitted on January 6, 2014 [Sun et al., "Development of an Improved Daptomycin Drug Product: Immediate Reconstitution, Room Temperature Product Stability and Reconstitution Stability," AAPS 2011, Poster No. T3328], which discloses that the average reconstitution time for a 500 mg vial of lyophilized daptomycin for injection (CUBICIN®) powder is about 15 minutes. In contrast, the presently claimed compositions, which are "prepared by lyophilizing an aqueous daptomycin solution comprising daptomycin and sucrose," have much shorter reconstitution times. As shown in Tables 6 (Figure 5) and 7 (Figure 6) of the application as filed, compositions comprising 500 mg daptomycin prepared from daptomycin solutions comprising sucrose reconstitute in less than 2 minutes, with most reconstituting in less than 1 minute (e.g.,

compositions 4, 6, 13, 17, and 19). Composition 00 (daptomycin without a sugar) has a reconstitution time of 5 minutes.

For at least the foregoing reasons, the combination of Wei and Smales does not teach or suggest all elements of the instant claims. It is Applicants' position that any arrival at the instant claims using the cited references is the result of improper hindsight reconstruction. ("It is well established that an obvious analysis that relies on the applicant's own disclosure rather than the prior art reference is improper as being based upon an impermissible hindsight reconstruction." *In re Duel*, 51 F.3d 1551, 1558 (Fed. Cir. 1995)). Furthermore, as discussed above, neither Wei nor Smales predict the enhanced daptomycin chemical stability in compositions comprising sucrose, or the rapid reconstitution of daptomycin solid compositions, as demonstrated in the instant application. Applicants therefore request reconsideration and withdrawal of this rejection of the claims.

Claims 22-25 and 27-42

Claims 22-25 and 27-42 are rejected under 35 U.S.C. § 103(a) as allegedly being obvious over Inman in view of Smales. According to the Office Action, Inman teaches "a solid pharmaceutical daptomycin composition comprising 150 mg daptomycin and 50 mg mannitol obtained by freeze-drying/lyophilization" (page 19 of the Office Action). The Office Action also alleges that "[the] difference between [Inman] and the instant claims. . . is that [Inman] does not teach sucrose or trehalose as excipient" (page 20 of the Office Action). The Office Action cites Smales to cure this deficiency, further alleging that Smales teaches that "therapeutic proteins/peptides can be stabilized by adding protein-stabilizers, such as sugar" and that sucrose and trehalose are "the most potent and useful excipients to protect protein conformation in aqueous solutions and freeze-dried solids" (page 20 of the Office Action). Applicants respectfully disagree, at least for the following reasons.

Inman focuses on daptomycin degradation caused by dextrose. For example, Inman discloses that "daptomycin, when dissolved in a 5% dextrose solution, undergoes 15-20% degradation in 24 h at 25°C" (see page 2, lines 37-40). As such, Inman is directed toward stabilizing daptomycin solutions comprising dextrose, not sucrose, with a buffer to allow for storage with less degradation. The buffered dextrose formulations of Inman also may contain excipients such as tonicity modifiers, preservatives, and mannitol.

In contrast, the present claims are based on, *inter alia*, the advantageous combination of daptomycin and sucrose. Sucrose is not taught or suggested by Inman. All of the example liquid daptomycin formulations of Inman contain dextrose because 5% dextrose is a common diluent for antibiotics (Inman, page 3, lines 39-40). Inman provides no motivation to substitute sucrose for dextrose.

In addition, Inman discloses *liquid* daptomycin formulations. Inman does not teach any *solid* daptomycin compositions, let alone solid compositions comprising sucrose, as is currently claimed.

Furthermore, Inman does not teach or suggest the surprising stabilizing effect that sucrose has on daptomycin as shown in Tables 4 and 9 of the application as filed. As described above, Applicants have demonstrated that, surprisingly, the presently claimed solid compositions, which comprise sucrose, provide daptomycin compositions with significantly enhanced chemical stability of solid daptomycin compositions over time. Such a surprising result is not predicted based on the teachings of Inman.

As described above, Smales does not predict, or even relate to, the presently claimed compositions. Smales teaches techniques to prevent proteins from denaturing during freezing or lyophilizing processes. However, one of ordinary skill in the art would not be motivated to apply techniques for retaining three-dimensional protein structures to daptomycin, at least in view of the significant difference between a protein and daptomycin. Accordingly, Smales fails to remedy the deficiencies in the teachings of Inman.

In addition, Smales does not disclose or teach the rapid reconstitution of daptomycin solid compositions, which was discovered by the Applicants. Smales also does not teach or suggest the surprising stabilizing effect of sucrose on daptomycin in solid compositions.

For at least the foregoing reasons, the combination of Inman and Smales does not teach or suggest all elements of the instant claims. Applicants therefore respectfully request reconsideration and withdrawal of this rejection.

CONCLUSION

In view of the remarks herein, reconsideration and withdrawal of all rejections, and allowance of the instant application with all pending claims are respectfully solicited. If a telephone conversation with Applicants' attorney would help expedite the prosecution of the above-identified application, the Examiner is urged to call Applicants' attorney at 857-300-4003.

No fees are believed to be due, other than the fee for the one-month extension of time. However, the Director is hereby authorized to charge the fees which should have been filed herewith (or with any paper hereafter filed in this application by this firm) to our Deposit Account No. 12-0600, under Order No. 552815: CPT-011USDV.

Dated: April 10, 2015

Respectfully submitted,

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Electronic Patent Application Fee Transmittal

Application Number:	14096346
Filing Date:	04-Dec-2013
Title of Invention:	LIPOPEPTIDE COMPOSITIONS AND RELATED METHODS
First Named Inventor/Applicant Name:	Sandra O'Connor
Filer:	Brian C. Trinque
Attorney Docket Number:	552815: CPT-011USDV

Filed as Large Entity

Filing Fees for Utility under 35 USC 111(a)

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Basic Filing:				
Pages:				
Claims:				
Miscellaneous-Filing:				
Petition:				
Patent-Appeals-and-Interference:				
Post-Allowance-and-Post-Issuance:				
Extension-of-Time:				

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Extension - 1 month with $0 paid	1251	1	200	200
Miscellaneous:				
Submission- Information Disclosure Stmt	1806	1	180	180
		Total in USD ($)		**380**

Electronic Acknowledgement Receipt

EFS ID:	22025436
Application Number:	14096346
International Application Number:	
Confirmation Number:	2832
Title of Invention:	LIPOPEPTIDE COMPOSITIONS AND RELATED METHODS
First Named Inventor/Applicant Name:	Sandra O'Connor
Customer Number:	113613
Filer:	Brian C. Trinque
Filer Authorized By:	
Attorney Docket Number:	552815: CPT-011USDV
Receipt Date:	10-APR-2015
Filing Date:	04-DEC-2013
Time Stamp:	17:10:15
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	yes
Payment Type	Deposit Account
Payment was successfully received in RAM	$380
RAM confirmation Number	3696
Deposit Account	120600
Authorized User	
The Director of the USPTO is hereby authorized to charge indicated fees and credit any overpayment as follows:	

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Drawings-only black and white line drawings	Replacement_Drawings.pdf	10152972 b7ebba14ef6b9925fad607bbb1e559adc5b324a8	no	22
Warnings:					
Information:					
2	Foreign Reference	Cited_Document4_EP.pdf	3052239 168df47a50e6456b144e15860154a0bc4fb8fbf7	no	28
Warnings:					
Information:					
3	Non Patent Literature	Translation_Cited_Document5.pdf	104514 69cb640d2c2b47fa62ef511d88197d2c563217f5	no	13
Warnings:					
Information:					
4	Foreign Reference	Cited_Document_6.pdf	2071391 91c92c4fcd816ca7099191588f0b912b04c5e516	no	17
Warnings:					
Information:					
5	Foreign Reference	Cited_Document_6_CA.pdf	2350582 49a974b3dd08e7ed2dd0437c02f2baf07909c430	no	25
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Information:					
6	Foreign Reference	Cited_Document_7_EP.pdf	4763021 4cf013bd820c5004f18f554fc2123a02c7fa04c3	no	42
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7	Foreign Reference	Cited_Document_8.pdf	4061424 652be9e139330dd4ede1f802da32879d5d27b248	no	28
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8	Non Patent Literature	CUBICIN_EuropeanMedicinesAgency.pdf	7362463 572eed2c7540c23a6852038354 52c6311d7babc5	no	41
Warnings:					
Information:					
9	Non Patent Literature	protein_structure.pdf	420215 f7cbbfa4cd585037c414aa55f1ec3b26e3e7aa5e	no	3
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12	Transmittal Letter	552815_IDS_xmittal.pdf	18227 7a15f3547e2962c1a883e2efe0da96d0f089dcfc	no	2
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13	Information Disclosure Statement (IDS) Form (SB08)	552815_IDS_SB08a.pdf	620964 90fbadc6c8e15cad9d9ad1b321eaefcec7ce3033	no	5
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17		552815_Response.pdf	81679 1c0b395576b5d7addaa1235317af68fb6f48f768	yes	19

Multipart Description/PDF files in .zip description		
Document Description	**Start**	**End**
Amendment/Req. Reconsideration-After Non-Final Reject	1	1
Specification	2	5
Drawings-only black and white line drawings	6	6
Claims	7	9
Applicant Arguments/Remarks Made in an Amendment	10	19

Warnings:

Information:

18	Fee Worksheet (SB06)	fee-info.pdf	32746 be2463fb186880526adeed926f27aa3615348df2	no	2

Warnings:

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Total Files Size (in bytes):	37176802

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New Applications Under 35 U.S.C. 111
If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371
If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office
If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

Daptomycin

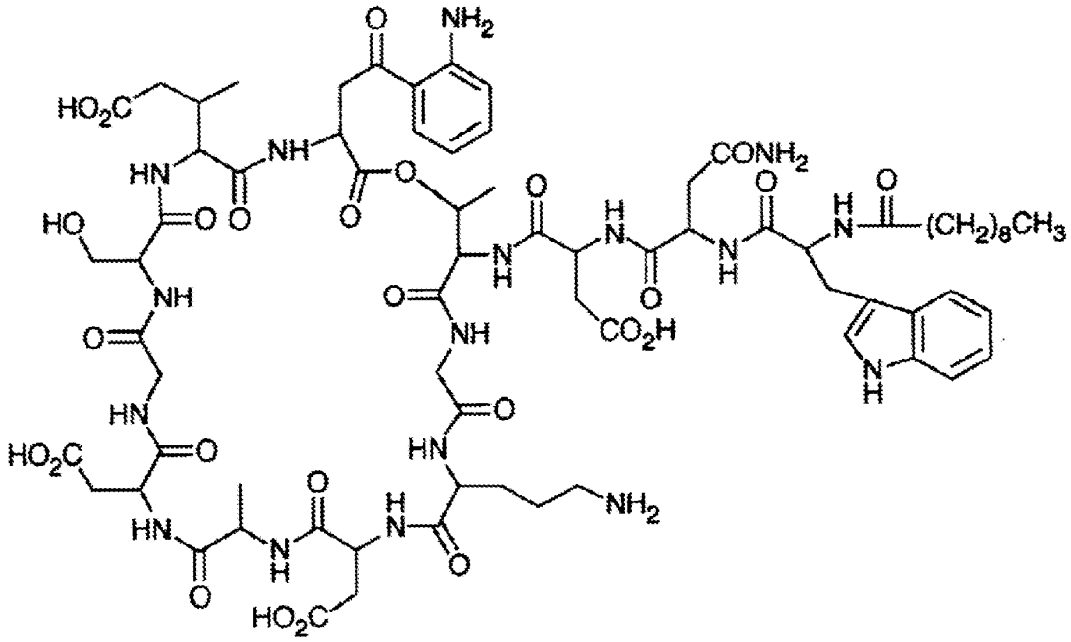

Fig. 1

“anhydro-daptomycin”

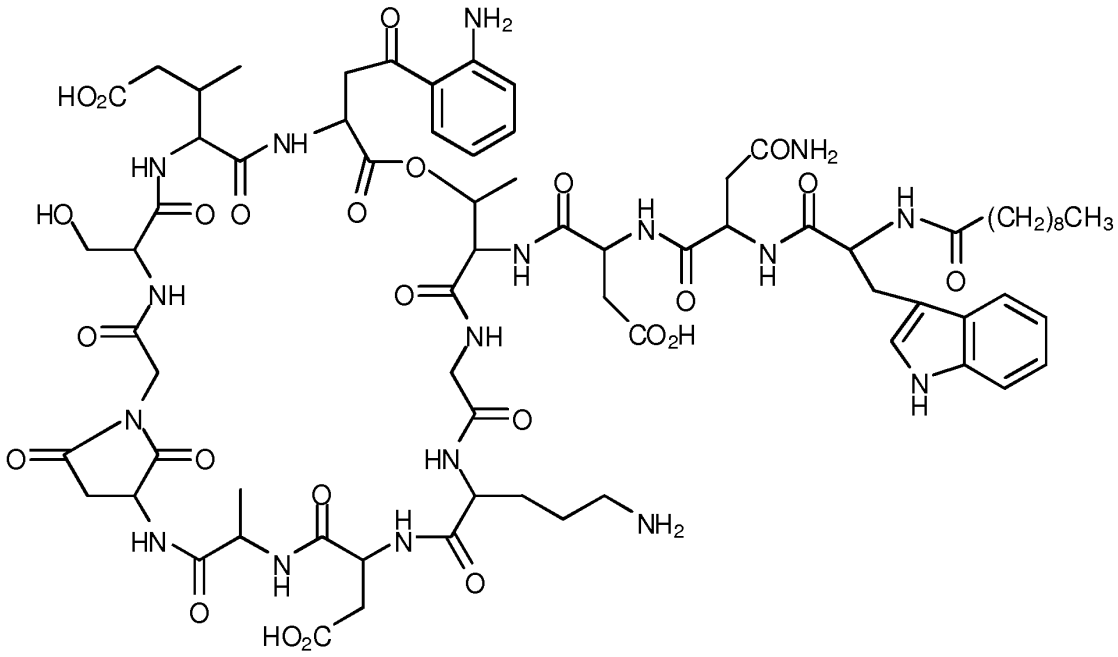

Fig. 2

“β-isomer” or “β-isomer of daptomycin”

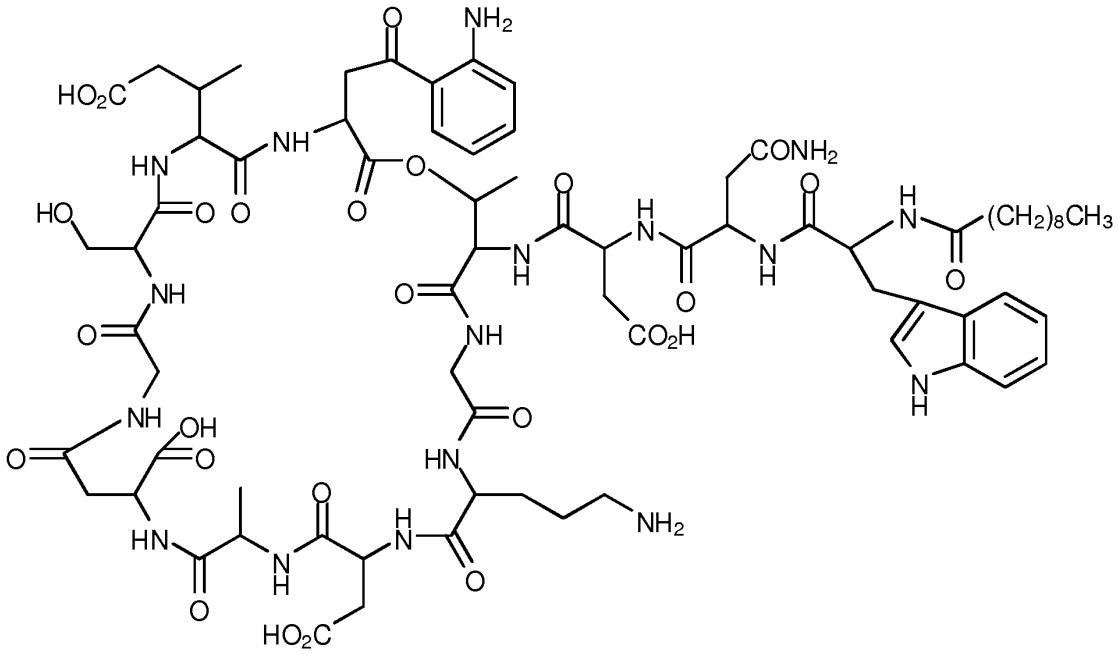

Fig. 3

lactone hydrolysis product

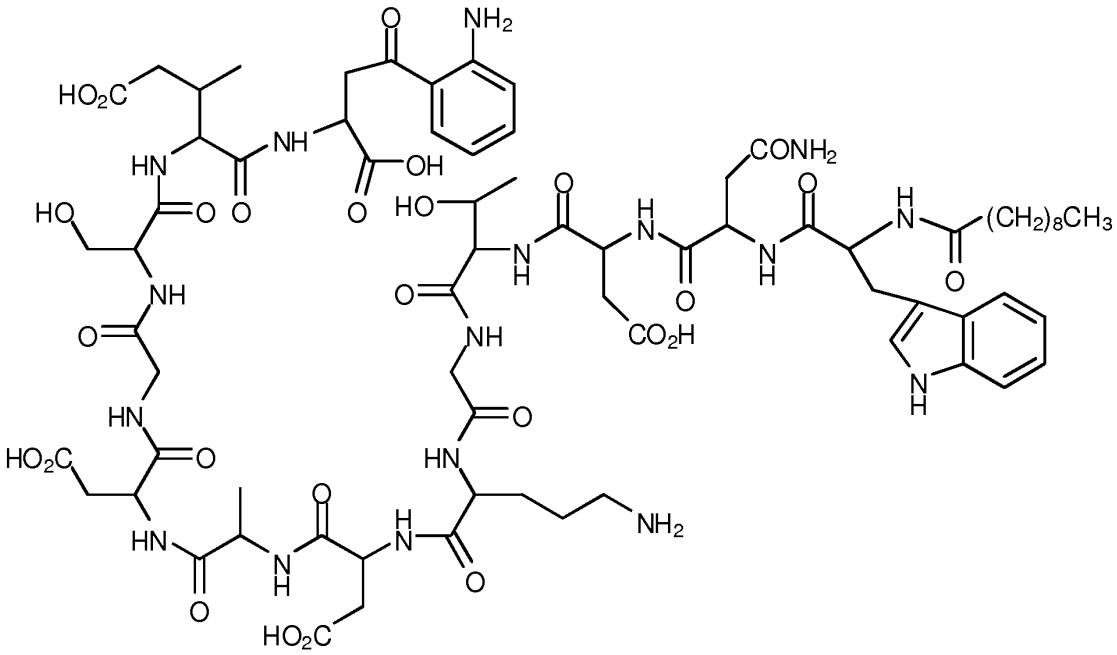

Fig. 4

Table 6

No.	Liquid Formulation Components	Recon Time (min)	Formulation (%w/v in solution)	Formulation (solid state) 500 mg/vial	Ratios Dap : sugar Dap : PO4 Dap : Mannitol	Molar Ratio Dap : Sugar(s)
0	Daptomycin, 50 mm PO4, pH 7.0	1.4 min		500mg Dap		
1	2.5% Trehalose, 50mM PO4, pH 7.0	< 1	10.5% Dap 2.5% Trehalose 0.71% PO4	500mg Dap 119mg Tre 35.5mg PO4	1 : 0.24 1 : 0.071	1 : 2.13 1 : 0.81
2	5 % Trehalose, 50mM PO4, pH 7.0	< 1	10.5% Dap 5% Trehalose 0.71% PO4	500mg Dap 238mg Tre 35.5mg PO4	1 : 0.48 1 : 0.071	1 : 4.26 1 : 0.81
3	10 % Trehalose, 50mM PO4, pH 7.0	< 1	10.5% Dap 10% Trehalose 0.71% PO4	500mg Dap 476.2mg Tre 35.5mg PO4	1 : 0.95 1 : 0.071	1 : 8.52 1 : 0.81
4	2.5% Sucrose, 50mM PO4, pH 7.0	< 1	10.5% Dap 2.5% Sucrose 0.71% PO4	500mg Dap 119mg Sucrose 35.5mg PO4	1 : 0.24 1 : 0.071	1 : 1.12 1 : 0.81
5	5 % Sucrose, 50mM PO4, pH 7.0	< 1	10.5% Dap 5% Sucrose 0.71% PO4	500mg Dap 238mg Sucrose 35.5mg PO4	1 : 0.48 1 : 0.071	1 : 2.24 1 : 0.81
6	10 % Sucrose, 50mM PO4, pH 7.0	< 1	10.5% Dap 10% Sucrose 0.71% PO4	500mg Dap 476.2mg Suc 35.5mg PO4	1 : 0.95 1 : 0.071	1 : 4.48 1 : 0.81
7	2.5% Sucrose, 3% Mannitol, 50mM PO4, pH 7.0	< 1	10.5% Dap 2.5% Sucrose 3% Mannitol 0.71% PO4	500mg Dap 119mg Sucrose 142.9mg Man 35.5mg PO4	1 : 0.24 1 : 0.29 1 : 0.071	1 : 1.12 1 : 2.52 1 : 0.81
8	5 % Sucrose, 3% Mannitol, 50mM PO4, pH 7.0	< 1	10.5% Dap 5% Sucrose	500mg Dap 238mg Sucrose	1 : 0.48	1 : 2.24

Fig. 5A

No.	Liquid Formulation Components	Recon Time (min)	Formulation (%w/v in solution)	Formulation (solid state) 500 mg/vial	Ratios Dap : sugar Dap : PO4 Dap : Mannitol	Molar Ratio Dap : Sugar(s)
			3% Mannitol 0.71% PO4	142.9mg Man 35.5mg PO4	1 : 0.29 1 : 0.071	1 : 2.52 1 : 0.81
9	10 % Sucrose, 3% Mannitol, 50mM PO4, pH 7.0	< 1	10.5% Dap 10% Sucrose 3% Mannitol 0.71% PO4	500mg Dap 476.2mg Suc 142.9mg Man 35.5mg PO4	1 : 0.95 1 : 0.29 1 : 0.071	1 : 4.48 1 : 2.52 1 : 0.81
10	2.5% Sucrose, 6% Mannitol, 50mM PO4, pH 7.0	<1	10.5% Dap 2.5% Sucrose 6% Mannitol 0.71% PO4	500mg Dap 119mg Sucrose 285.8 Man 35.5mg PO4	1 : 0.24 1 : 0.57 1 : 0.071	1 : 1.12 1 : 5.04 1 : 0.81
11	5% Sucrose, 6% Mannitol, 50mM PO4, pH 7.0	< 1	10.5% Dap 5% Sucrose 6% Mannitol 0.71% PO4	500mg Dap 238mg Sucrose 285.8mg Man 35.5mg PO4	1 : 0.48 1 : 0.57 1 : 0.071	1 : 2.24 1 : 5.04 1 : 0.81
12	10% Sucrose, 6% Mannitol, 50mM PO4, pH 7.0	< 1	10.5% Dap 10% Sucrose 6% Mannitol 0.71% PO4	500mg Dap 476.2mg Suc 285.8mg Man 35.5mg PO4	1 : 0.95 1 : 0.57 1 : 0.071	1 : 4.48 1 : 5.04 1 : 0.81
13	20 % Sucrose, 50mM PO4, pH 7.0	< 1	10.5% Dap 20% Sucrose 0.71% PO4	500mg Dap 952.4mg Suc 35.5mg PO4	1 : 1.90 1 : 0.071	1 : 8.96 1 : 0.81
14	25% Trehalose, 50mM PO4, pH 7.0	< 1	10.5% Dap 25% Tre 0.71% PO4	500mg Dap 1190.5mg Tre 35.5mg PO4	1 : 2.38 1 : 0.071	1 : 21.32 1 : 0.81
15	25% Trehalose, pH 4.7	< 1	10.5% Dap 25% Tre	500mg Dap 1190.5mg Tre	1 : 2.38	1 : 21.32
19	20% Sucrose, pH 4.7	< 1	10.5% Dap 20% Sucrose	500mg Dap 952.4mg Suc	1 : 1.90	1 : 8.96

Fig. 5B

No.	Liquid Formulation Components	Recon Time (min)	Formulation (%w/v in solution)	Formulation (solid state) 500 mg/vial	Ratios Dap : sugar Dap : PO4 Dap : Mannitol	Molar Ratio Dap : Sugar(s)
23	15 % Sucrose, 3% Mannitol, pH 4.7	0.3 – 1.5	10.5% Dap 15% Sucrose 3% Mannitol	500mg Dap 750mg Sucrose 142.9mg Man	1 : 1.5 1 : 0.29	1 : 6.73 1 : 2.52
35	20% Lactose, 50mM PO4, pH 7.0	< 1	10.5% Dap 20% Lactose 0.71% PO4	500mg Dap 952.4mg Lact 35.5mg PO4	1 : 1.90 1 : 0.071	1 : 8.80 1 : 0.81
50	2.5% Lactose, 50 mM PO4, pH 7.0	< 1	10.5% Dap 2.5% Lactose 0.71% PO4	500mg Dap 119mg Lac 35.5mg PO4	1 : 0.24 1 : 0.071	1 : 1.10 1 : 0.81
51	2.5% Maltose, 50 mM PO4, pH 7.0	0.5 – 1.2	10.5% Dap 2.5% Maltose 0.71% PO4	500mg Dap 119mg Malt 35.5mg PO4	1 : 0.24 1 : 0.071	1 : 1.12 1 : 0.81
52	2.5% Fructose, 50 mM PO4, pH 7.0	< 1	10.5% Dap 2.5% Fructose 0.71% PO4	500mg Dap 119mg Fruc 35.5mg PO4	1 : 0.24 1 : 0.071	1 : 2.13 1 : 0.81
53	2.5% Dextrose, 50 mM PO4, pH 7.0	0.6 – 1.1	10.5% Dap 2.5% Dextrose 0.71% PO4	500mg Dap 119mg Dex 35.5mg PO4	1 : 0.24 1 : 0.071	1 : 2.13 1 : 0.81
54	2.5%Dextrose/Fructose (1:1), 50mM PO4, pH 7.0	0.5 – 1.2	10.5% Dap 2.5% Dex/Fruc 0.71% PO4	500mg Dap 119mg D/F 35.5mg PO4	1 : 0.24 1 : 0.071	1 : 1.07 : 1.07 1 : 0.81
55	5% Lactose, 50mM PO4, pH 7.0	< 1	10.5% Dap 5% Lactose 0.71% PO4	500mg Dap 238mg Lact 35.5mg PO4	1 : 0.48 1 : 0.071	1 : 2.20 1 : 0.81
56	5% Maltose, 50mM PO4, pH 7.0	< 1	10.5% Dap 5% Maltose 0.71% PO4	500mg Dap 238mg Malt 35.5mg PO4	1 : 0.48 1 : 0.071	1 : 2.24 1 : 0.81
57	5% Fructose, 50mM PO4, pH 7.0	< 1	10.5% Dap	500mg Dap		

Fig. 5C

No.	Liquid Formulation Components	Recon Time (min)	Formulation (%w/v in solution)	Formulation (solid state) 500 mg/vial	Ratios Dap : sugar Dap : PO4 Dap : Mannitol	Molar Ratio Dap : Sugar(s)
			5% Fructose 0.71% PO4	238mg Fruc 35.5mg PO4	1 : 0.48 1 : 0.071	1 : 4.26
58	5% Dextrose, 50 mM PO4, pH 7.0	< 1	10.5% Dap 5% Dextrose 0.71% PO4	500mg Dap 238mg Dex 35.5mg PO4	1 : 0.48 1 : 0.071	1 : 4.26 1 : 0.81
59	5%Dextrose/Fructose (1:1), 50mM PO4, pH 7.0	< 1	10.5% Dap 5% Dex/Fruc 0.71% PO4	500mg Dap 238mg D/F 35.5mg PO4	1 : 0.48 1 : 0.071	1 : 2.13 : 2.13 1 : 0.81
60	2.5% Lactose, pH 4.7	1.1	10.5% Dap 2.5% Lactose	500mg Dap 119mg Lac	1 : 0.24	1 : 1.10
61	2.5% Maltose, pH 4.7	1.1	10.5% Dap 2.5% Maltose	500mg Dap 119mg Malt	1 : 0.24	1 : 1.12
62	2.5% Fructose, pH 4.7	1.2	10.5% Dap 2.5% Fructose	500mg Dap 119mg Fruc	1 : 0.24	1 : 2.13
64	2.5%Dextrose/Fructose (1:1), pH 4.7	1.7	10.5% Dap 2.5% Dex/Fruc	500mg Dap 119mg D/F	1 : 0.24	1 : 1.07 : 1.07
65	5% Lactose, pH 4.7	1.6	10.5% Dap 5% Lactose	500mg Dap 238mg Lact	1 : 0.48	1 : 2.24
71	6% Mannitol, 50 mM PO4, pH 7.0	< 1	10.5% Dap 6% Mannitol 0.71% PO4	500mg Dap 285.6mg Man 35.5mg PO4	1 : 0.57 1 : 0.071	1 : 5.04 1 : 0.81
73	5% Glycine, 50 mM PO4, pH 7.0	< 1	10.5% Dap 5% Glycine 0.71% PO4	500mg Dap 238mg Glycine 35.5mg PO4	1 : 0.48 1 : 0.071	1 : 10.31 1 : 0.81

Fig. 5D

No.	Liquid Formulation Components	Recon Time (min)	Formulation (%w/v in solution)	Formulation (solid state) 500 mg/vial	Ratios Dap : sugar Dap : PO4 Dap : Mannitol	Molar Ratio Dap : Sugar(s)
75	15% Sucrose, 50mM PO4, pH 7.0	< 1	10.5% Dap 15% Sucrose 0.71% PO4	500mg Dap 714.3mg Sucrose 35.5mg PO4	1 : 1.5 1 : 0.071	1 : 6.73 1 : 0.81
76	15% Sucrose, 50mM PO4, pH 7.0	< 1	10.5% Dap 15% Sucrose 0.71% PO4	500mg Dap 714.3mg Sucrose 35.5mg PO4	1 : 1.5 1 : 0.071	1 : 6.73 1 : 0.81

Fig. 5E

Table 7

	Formulation ID	Recon Time (min)	Formulation (% w/v in solution)	Formulation (solid state) 500 mg/vial	Ratios Dap : sugar Dap : PO4 Dap : Mannitol	Molar Ratio Dap : Excipients
00	Daptomycin, pH 4.7	5 min		500mg Dap		
16	2.5% Sucrose, pH 4.7	2 - 4	10.5% Dap 2.5% Sucrose	500mg Dap 119mg Sucrose	1 : 0.24	1 : 1.12
17	5% Sucrose, pH 4.7	0.7 – 2	10.5% Dap 5% Sucrose	500mg Dap 238mg Sucrose	1 : 0.48	1 : 2.24
18	10 % Sucrose, pH 4.7	0.3 – 3	10.5% Dap 10% Sucrose	500mg Dap 476.2mg Suc	1 : 0.95	1 : 4.48
20	2.5% Sucrose, 3% Mannitol, pH 4.7	2 - 8	10.5% Dap 2.5% Sucrose 3% Mannitol	500mg Dap 119mg Sucrose 142.9mg Man	1 : 0.24 1 : 0.29	1 : 1.12 1 : 2.52
21	5% Sucrose, 3% Mannitol, pH 4.7	2 – 6	10.5% Dap 5% Sucrose 3% Mannitol	500mg Dap 238mg Sucrose 142.9mg Man	1 : 0.48 1 : 0.29	1 : 2.24 1 : 2.52
22	10 % Sucrose, 3% Mannitol, pH 4.7	0.5 – 2	10.5% Dap 10% Sucrose 3% Mannitol	500mg Dap 476.2mg Suc 142.9mg Man	1 : 0.95 1 : 0.29	1 : 4.48 1 : 2.52
63	2.5% Dextrose, pH 4.7	2	10.5% Dap 2.5% Dextrose	500mg Dap 119mg Dex	1 : 0.24	1 : 2.13

Fig. 6A

	Formulation ID	Recon Time (min)	Formulation (% w/v in solution)	Formulation (solid state) 500 mg/vial	Ratios Dap : sugar Dap : PO4 Dap : Mannitol	Molar Ratio Dap : Excipients
66	5% Maltose, pH 4.7	2.4	10.5% Dap 5% Maltose	500mg Dap 239mg Malt	1 : 0.48	1 : 2.20
67	5% Fructose, pH 4.7	2.5	10.5% Dap 5% Fructose	500mg Dap 238mg Fruc	1 : 0.48	1 : 4.26
68	5% Dextrose, pH 4.7	2.4	10.5% Dap 5% Dextrose	500mg Dap 238mg Dex	1 : 0.48	1 : 4.26
69	5%Dextrose/Fructose (1:1), pH 4.7	2.0	10.5% Dap 5% Dex/Fruc	500mg Dap 238mg D/F	1 : 0.48	1 : 2.13 : 2.13
77	5 % Trehalose, pH 4.7	3 – 4	10.5% Dap 5% Trehalose	500mg Dap 238mg Tre	1 : 0.48	1 : 4.26
	2.5% Trehalose, pH 4.7	3 – 5	10.5% Dap 2.5% Trehalose	500mg Dap 119mg Tre	1 : 0.24	1 : 2.13

Fig. 6B

Table 8

ID No.	Lipopeptide [A]	Compound [B]	Compound [C]	Buffering Agent [D]	Compounding pH	Molar Ratio of existing components, respectively	Formulation in Solution upon addition of diluent (weight/volume)
1	daptomycin	Trehalose		Sodium phosphate dibasic	about 7.0	1 : 2.13 : 0.77	10.5% Dap 2.5% Trehalose 0.71% Na_2HPO_4
2	daptomycin	Trehalose		Sodium phosphate dibasic	about 7.0	1 : 4.26 : 0.77	10.5% Dap 5% Trehalose 0.71% Na_2HPO_4
3	daptomycin	Trehalose		Sodium phosphate dibasic	about 7.0	1 : 8.53 : 0.77	10.5% Dap 10% Trehalose 0.71% Na_2HPO_4
4	daptomycin	Sucrose		Sodium phosphate dibasic	about 7.0	1 : 1.12 : 0.77	10.5% Dap 2.5% Sucrose 0.71% Na_2HPO_4
5	daptomycin	Sucrose		Sodium phosphate dibasic	about 7.0	1 : 2.24 : 0.77	10.5% Dap 5% Sucrose 0.71% Na_2HPO_4
6	daptomycin	Sucrose		Sodium phosphate dibasic	about 7.0	1 : 4.49 : 0.77	10.5% Dap 10% Sucrose 0.71% Na_2HPO_4
7	daptomycin	Sucrose	Mannitol	Sodium phosphate dibasic	about 7.0	1 : 1.12 : 2.52 : 0.77	10.5% Dap 2.5% Sucrose 3% Mannitol 0.71% Na_2HPO_4
8	daptomycin	Sucrose	Mannitol	Sodium phosphate dibasic	about 7.0	1 : 2.24 : 2.52 : 0.77	10.5% Dap 5% Sucrose 3% Mannitol 0.71% Na_2HPO_4

Fig. 7A

ID No.	Lipopeptide [A]	Compound [B]	Compound [C]	Buffering Agent [D]	Compounding pH	Molar Ratio of existing components, respectively	Formulation in Solution upon addition of diluent (weight/volume)
9	daptomycin	Sucrose	Mannitol	Sodium phosphate dibasic	about 7.0	1 : 4.49 : 2.52 : 0.77	10.5% Dap 10% Sucrose 3% Mannitol 0.71% Na_2HPO_4
10	daptomycin	Sucrose	Mannitol	Sodium phosphate dibasic	about 7.0	1 : 1.12 : 5.04 : 0.77	10.5% Dap 2.5% Sucrose 6% Mannitol 0.71% Na_2HPO_4
11	daptomycin	Sucrose	Mannitol	Sodium phosphate dibasic	about 7.0	1 : 2.24 : 5.04 : 0.77	10.5% Dap 5% Sucrose 6% Mannitol 0.71% Na_2HPO_4
12	daptomycin	Sucrose	Mannitol	Sodium phosphate dibasic	about 7.0	1 : 4.49 : 5.04 : 0.77	10.5% Dap 10% Sucrose 6% Mannitol 0.71% Na_2HPO_4
13	daptomycin	Sucrose		Sodium phosphate dibasic	about 7.0	1 : 8.98 : 0.77	10.5% Dap 20% Sucrose 0.71% Na_2HPO_4
14	daptomycin	Trehalose		Sodium phosphate dibasic	about 7.0	1 : 21.32 : 0.77	10.5% Dap 25% Trehalose 0.71% Na_2HPO_4
15	daptomycin	Trehalose			about 4.7	1 : 21.32	10.5% Dap 25% Trehalose
16	daptomycin	Sucrose			about 4.7	1 : 1.12	10.5% Dap 2.5% Sucrose
17	daptomycin	Sucrose			about 4.7	1 : 2.24	10.5% Dap 5% Sucrose
18	daptomycin	Sucrose			about 4.7	1 : 4.49	10.5% Dap 10% Sucrose
19	daptomycin	Sucrose			about 4.7	1 : 8.98	10.5% Dap 20% Sucrose

Fig. 7B

ID No.	Lipopeptide [A]	Compound [B]	Compound [C]	Buffering Agent [D]	Compounding pH	Molar Ratio of existing components, respectively	Formulation in Solution upon addition of diluent (weight/volume)
20	daptomycin	Sucrose	Mannitol		about 4.7	1 : 1.12 : 2.52	10.5% Dap 2.5% Sucrose 3% Mannitol
21	daptomycin	Sucrose	Mannitol		about 4.7	1 : 2.24 : 2.52	10.5% Dap 5% Sucrose 3% Mannitol
22	daptomycin	Sucrose	Mannitol		about 4.7	1 : 4.49 : 2.52	10.5% Dap 10% Sucrose 3% Mannitol
23	daptomycin	Sucrose	Mannitol		about 4.7	1 : 6.73 : 2.52	10.5% Dap 15% Sucrose 3% Mannitol
24	daptomycin	Sucrose	Mannitol		about 4.7	1 : 1.12: 5.04	10.5% Dap 2.5% Sucrose 6% Mannitol
25	daptomycin	Sucrose	Mannitol		about 4.7	1 : 2.24 : 5.04	10.5% Dap 5% Sucrose 6% Mannitol
26	daptomycin	Sucrose	Mannitol		about 4.7	1 : 4.49 : 5.04	10.5% Dap 10% Sucrose 6% Mannitol
27	daptomycin	Sucrose	Mannitol		about 4.7	1 : 6.73 : 5.04	10.5% Dap 15% Sucrose 6% Mannitol
28	daptomycin	Sucrose	Mannitol	Sodium phosphate dibasic	about 7.0	1 : 6.73 : 2.24 : 0.77	10.5% Dap 15% Sucrose 3% Mannitol 0.71% Na_2HPO_4
29	daptomycin	Sucrose	Mannitol	Sodium phosphate dibasic	about 7.0	1 : 6.73 : 5.04 : 0.77	10.5% Dap 15% Sucrose 6% Mannitol 0.71% Na_2HPO_4

Fig. 7C

ID No.	Lipopeptide [A]	Compound [B]	Compound [C]	Buffering Agent [D]	Compounding pH	Molar Ratio of existing components, respectively	Formulation in Solution upon addition of diluent (weight/volume)
30	daptomycin	Lactose		Sodium phosphate dibasic	about 7.0	1 : 4.49 : 0.77	10.5% Dap 10% Lactose 0.71% Na_2HPO_4
31	daptomycin	Maltose		Sodium phosphate dibasic	about 7.0	1 : 4.49 : 0.77	10.5% Dap 10% Maltose 0.71% Na_2HPO_4
32	daptomycin	Fructose		Sodium phosphate dibasic	about 7.0	1 : 8.52 : 0.77	10.5% Dap 10% Fructose 0.71% Na_2HPO_4
33	daptomycin	Dextrose		Sodium phosphate dibasic	about 7.0	1 : 8.52 : 0.77	10.5% Dap 10% Dextrose 0.71% Na_2HPO_4
34	daptomycin	Dextrose	Fructose	Sodium phosphate dibasic	about 7.0	1 : 4.26 : 4.26 : 0.77	10.5% Dap 5% Dextrose 5% Fructose 0.71% Na_2HPO_4
35	daptomycin	Lactose		Sodium phosphate dibasic	about 7.0	1 : 8.98 : 0.77	10.5% Dap 20% Lactose 0.71% Na_2HPO_4
36	daptomycin	Maltose		Sodium phosphate dibasic	about 7.0	1 : 8.98 : 0.77	10.5% Dap 20% Maltose 0.71% Na_2HPO_4
37	daptomycin	Fructose		Sodium phosphate dibasic	about 7.0	1 : 17.05 : 0.77	10.5% Dap 20% Fructose 0.71% Na_2HPO_4
38	daptomycin	Dextrose		Sodium phosphate dibasic	about 7.0	1 : 17.05 :0.77	10.5% Dap 20% Dextrose 0.71% Na_2HPO_4
39	daptomycin	Dextrose	Fructose	Sodium phosphate dibasic	about 7.0	1: 8.52 : 8.52 : 0.77	10.5% Dap 10% Dextrose 10% Fructose 0.71% Na_2HPO_4

Fig. 7D

ID No	Lipopeptide [A]	Compound [B]	Compound [C]	Buffering Agent [D]	Compounding pH	Molar Ratio of existing components, respectively	Formulation in Solution upon addition of diluent (weight/volume)
40	daptomycin	Lactose			about 4.7	1 : 4.49	10.5% Dap 10% Lactose
41	daptomycin	Maltose			about 4.7	1 : 4.49	10.5% Dap 10% Maltose
42	daptomycin	Fructose			about 4.7	1 : 8.52	10.5% Dap 10% Fructose
43	daptomycin	Dextrose			about 4.7	1 : 8.52	10.5% Dap 10% Dextrose
44	daptomycin	Dextrose	Fructose		about 4.7	1 : 4.26 : 4.26	10.5% Dap 5% Dextrose 5% Fructose
45	daptomycin	Lactose			about 4.7	1 : 8.98	10.5% Dap 20% Lactose
46	daptomycin	Maltose			about 4.7	1 : 8.98	10.5% Dap 20% Maltose
47	daptomycin	Fructose			about 4.7	1 : 17.05	10.5% Dap 20% Fructose
48	daptomycin	Dextrose			about 4.7	1 : 17.05	10.5% Dap 20% Dextrose
49	daptomycin	Dextrose	Fructose		about 4.7	1 : 8.52 : 8.52	10.5% Dap 10% Dextrose 10% Fructose
50	daptomycin	Lactose		Sodium phosphate dibasic	about 7.0	1 : 1.12 : 0.77	10.5% Dap 2.5% Lactose 0.71% Na_2HPO_4
51	daptomycin	Maltose		Sodium phosphate dibasic	about 7.0	1 : 1.12 : 0.77	10.5% Dap 2.5% Maltose 0.71% Na_2HPO_4
52	daptomycin	Fructose		Sodium phosphate dibasic	about 7.0	1 : 2.13 : 0.77	10.5% Dap 2.5% Fructose 0.71% Na_2HPO_4

Fig. 7E

ID No.	Lipopeptide [A]	Compound [B]	Compound [C]	Buffering Agent [D]	Compounding pH	Molar Ratio of existing components, respectively	Formulation in Solution upon addition of diluent (weight/volume)
53	daptomycin	Dextrose		Sodium phosphate dibasic	about 7.0	1 : 2.13 : 0.77	10.5% Dap 2.5% Dextrose 0.71% Na_2HPO_4
54	daptomycin	Dextrose	Fructose	Sodium phosphate dibasic	about 7.0	1 : 1.07 : 1.07 : 0.77	10.5% Dap 2.5% 1.25% Dextrose 1.25% Fructose 0.71% Na_2HPO_4
55	daptomycin	Lactose		Sodium phosphate dibasic	about 7.0	1 : 2.24 : 0.77	10.5% Dap 5% Lactose 0.71% Na_2HPO_4
56	daptomycin	Maltose		Sodium phosphate dibasic	about 7.0	1 : 2.24 : 0.77	10.5% Dap 5% Maltose 0.71% Na_2HPO_4
57	daptomycin	Fructose		Sodium phosphate dibasic	about 7.0	1 : 4.26 : 0.77	10.5% Dap 5% Fructose 0.71% Na_2HPO_4
58	daptomycin	Dextrose		Sodium phosphate dibasic	about 7.0	1 : 4.26 : 0.77	10.5% Dap 5% Dextrose 0.71% Na_2HPO_4
59	daptomycin	Dextrose	Fructose	Sodium phosphate dibasic	about 7.0	1 : 2.13 : 2.13 : 0.77	10.5% Dap 2.5% Dextrose 2.5% Fructose 0.71% Na_2HPO_4
60	daptomycin	Lactose			about 4.7	1 : 1.12	10.5% Dap 2.5% Lactose
61	daptomycin	Maltose			about 4.7	1 : 1.12	10.5% Dap 2.5% Maltose
62	daptomycin	Fructose			about 4.7	1 : 2.13	10.5% Dap 2.5% Fructose
63	daptomycin	Dextrose			about 4.7	1 : 2.13	10.5% Dap 2.5% Dextrose
64	daptomycin	Dextrose	Fructose		about 4.7	1 : 1.07 : 1.07 :	10.5% Dap 1.25% Dextrose 1.25% Fructose

Fig. 7F

ID No.	Lipopeptide [A]	Compound [B]	Compound [C]	Buffering Agent [D]	Compounding pH	Molar Ratio of existing components, respectively	Formulation in Solution upon addition of diluent (weight/volume)
65	daptomycin	Lactose			about 4.7	1 : 2.24	10.5% Dap 5% Lactose
66	daptomycin	Maltose			about 4.7	1 : 2.24	10.5% Dap 5% Maltose
67	daptomycin	Fructose			about 4.7	1 : 4.26	10.5% Dap 5% Fructose
68	daptomycin	Dextrose			about 4.7	1 : 4.26	10.5% Dap 5% Dextrose
69	daptomycin	Dextrose	Fructose		about 4.7	1 : 2.13 : 2.13	10.5% Dap 2.5% Dextrose 2.5% Fructose
70	daptomycin	Mannitol			about 4.7	1 : 5.04	10.5% Dap 6% Mannitol
71	daptomycin	Mannitol		Sodium phosphate dibasic	about 7.0	1 : 5.04 : 0.77	10.5% Dap 6% Mannitol 0.71% Na_2HPO_4
72	daptomycin	Glycine			about 4.7	1 : 10.23	10.5% Dap 5% Glycine
73	daptomycin	Glycine		Sodium phosphate dibasic	about 7.0	1 : 10.23 : 0.77	10.5% Dap 5% Glycine 0.71% Na_2HPO_4
74	daptomycin	Sucrose			about 4.7	1 : 6.73	10.5% Dap 15% Sucrose
75	daptomycin	Sucrose		Sodium phosphate dibasic	about 7.0	1 : 6.73 : 0.77	10.5% Dap 15% Sucrose 0.71% Na_2HPO_4
76	daptomycin	Sucrose		Sodium phosphate dibasic	about 7.0	1 : 6.73 : 0.77	10.5% Dap 15% Sucrose 0.71% Na_2HPO_4

Fig. 7G

ID No.	Lipopeptide [A]	Compound [B]	Compound [C]	Buffering Agent [D]	Compounding pH	Molar Ratio of existing components, respectively	Formulation in Solution upon addition of diluent (weight/volume)
77	daptomycin	Trehalose			about 4.7	1 : 4.26	10.5% Dap 5% Trehalose
78	daptomycin	Trehalose			about 4.7	1 : 8.53	10.5% Dap 10% Trehalose
79	daptomycin	Trehalose			about 4.7	1 : 14.92	10.5% Dap 17.5% Trehalose

Fig. 7H

Table 9

Formulation ID	Formulation Description	Daptomycin Stability Ratio at 40 Degrees C				
		T0	1 month	2 months	3 months	6 months
0	Daptomycin Control with 50mM Phosphate buffer at pH 7.0 (without sugar or glycine)	0.000	1.000	1.000	1.000	1.000
1	2.5% Trehalose, 50mM PO4, pH 7.0	0.000	0.667	0.800	0.667	1.000
2	5 % Trehalose, 50mM PO4, pH 7.0	0.000	0.867	0.867	0.714	0.871
3	10 % Trehalose, 50mM PO4, pH 7.0	0.000	0.400	0.400	0.381	0.613
4	2.5% Sucrose, 50mM PO4, pH 7.0	0.000	0.533	0.467	0.524	0.742
5	5 % Sucrose, 50mM PO4, pH 7.0	0.000	0.467	0.533	0.476	0.645
6	10 % Sucrose, 50mM PO4, pH 7.0	0.000	0.267	0.133	0.238	0.355
7	2.5% Sucrose, 3% Mannitol, 50mM PO4, pH 7.0	0.000	0.267	0.133	0.238	0.387
8	5 % Sucrose, 3% Mannitol, 50mM PO4, pH 7.0	0.000	0.267	0.133	0.190	0.258
9	10 % Sucrose, 3% Mannitol, 50mM PO4, pH 7.0	0.000	-0.200	0.267	0.190	0.226
10	2.5% Sucrose, 6% Mannitol, 50mM PO4, pH 7.0	0.000	-0.067	0.333	0.238	0.355
11	5% Sucrose, 6% Mannitol, 50mM PO4, pH 7.0	0.000	-0.200	0.133	0.238	0.290
12	10% Sucrose, 6% Mannitol, 50mM PO4, pH 7.0	0.000	0.000	0.067	0.190	0.419
13	20 % Sucrose, 50mM PO4, pH 7.0	0.000	-0.267	0.133	0.143	0.226
14	25% Trehalose, 50mM PO4, pH 7.0	0.000	0.133	0.533	0.381	0.484
15	25% Trehalose, pH 4.7	0.000	-0.067	NT	0.286	0.323
16	2.5% Sucrose, PO4, pH 4.7	0.000	0.333	0.600	0.429	0.581
17	5% Sucrose, PO4, pH 4.7	0.000	0.133	0.267	0.190	0.323
18	10 % Sucrose, PO4, pH 4.7	0.000	0.067	0.133	0.095	0.194
19	20% Sucrose, PO4, pH 4.7	0.000	-0.467	-0.067	0.000	0.097
20	2.5% Sucrose, 3% Mannitol, pH 4.7	0.000	0.000	0.200	0.429	0.484
21	5% Sucrose, 3% Mannitol, pH 4.7	0.000	0.000	0.133	0.333	0.387
22	10 % Sucrose, 3% Mannitol, pH 4.7	0.000	0.333	0.200	0.381	0.226

Fig. 8A

Formulation ID	Formulation Description	Daptomycin Stability Ratio at 40 Degrees C				
		T0	1 month	2 months	3 months	6 months
23	15 % Sucrose, 3% Mannitol, pH 4.7	0.000	0.133	0.000	0.190	0.129
24	2.5% Sucrose, 6% Mannitol, pH 4.7	0.000	0.400	0.400	0.571	0.516
25	5% Sucrose, 6% Mannitol, pH 4.7	0.000	0.333	0.333	0.476	0.419
26	10% Sucrose, 6% Mannitol, pH 4.7	0.000	0.200	0.067	0.238	0.226
27	15% Sucrose, 6% Mannitol, pH 4.7	0.000	0.200	0.067	0.286	0.226
35	20%Lactose with 50mM Phosphate buffer at pH 7.0	0.000	2.600	0.800	0.524	0.484
45	20% Lactose at pH 4.7	0.000	2.267	2.867	1.571	2.161
50	2.5%Lactose with 50mM Phosphate buffer at pH 7.0	0.000	2.667	4.733	3.286	2.935
51	2.5%Maltose with 50mM Phosphate buffer at pH 7.0	0.000	2.933	4.467	3.476	3.129
52	2.5% Fructose with 50mM Phosphate buffer at pH 7.0	0.000	3.133	4.800	3.905	4.032
53	2.5%Dextrose with 50mM Phosphate buffer at pH 7.0	0.000	7.467	12.400	9.333	8.516
54	2.5% Dextrose/Fructose (1:1) with 50mM Phosphate buffer at pH 7.0	0.000	5.400	8.267	6.857	6.419
55	5.0% Lactose with 50mM Phosphate buffer at pH 7.0	0.000	3.067	4.800	3.810	3.419
56	5.0% Maltose with 50mM Phosphate buffer at pH 7.0	0.000	3.400	4.800	4.048	3.355
57	5.0%Fructosewith 50mM Phosphate buffer at pH 7.0	0.000	2.533	4.133	3.190	3.355
58	5.0%Dextrosewith 50mM Phosphate buffer at pH 7.0	0.000	7.667	11.133	8.905	8.258
59	5.0% Dextrose/Fructose(1:1)with 50mM Phosphate buffer at pH 7.0	0.000	4.267	7.600	6.524	6.161
60	2.5% Lactose pH 4.7	0.000	2.267	3.533	2.905	2.774
61	2.5% Maltose pH 4.7	0.000	2.133	3.600	2.905	2.645
62	2.5% Fructose pH 4.7	0.000	3.133	4.933	3.905	3.968
63	2.5%Dextrose pH 4.7	0.000	9.267	14.400	10.952	9.903
64	2.5%Dextrose/Fructose(1:1) pH 4.7	0.000	5.000	9.267	7.571	7.645

Fig. 8B

Formulation ID	Formulation Description	Daptomycin Stability Ratio at 40 Degrees C				
		T0	1 month	2 months	3 months	6 months
65	5.0%Lactose pH 4.7	0.000	2.333	3.333	2.571	2.452
66	5.0%MaltosepH 4.7	0.000	2.133	3.600	2.905	2.645
67	5.0%Fructose pH 4.7	0.000	2.200	4.467	3.810	3.581
68	5.0%Dextrose pH 4.7	0.000	4.200	8.867	7.000	7.516
69	5.0%Dextrose/Fructose(1:1) pH 4.7	0.000	3.333	7.200	6.048	6.452
70	6% Mannitol, pH 4.7	0.000	0.533	0.867	0.667	0.903
71	6% Mannitol, 50 mM PO4, pH 7.0	0.000	0.533	0.600	0.524	0.645
72	5% Glycine, pH 4.7	0.000	0.600	1.000	0.667	0.935
73	5% Glycine, 50 mM PO4, pH 7.0	0.000	1.267	1.867	1.524	1.742
74	15 % Sucrose, PO4, pH 4.7	0.000	0.000	0.200	-0.095	0.161
75	15% Sucrose, 50mM PO4, pH 7.0	0.000	0.000	0.200	0.286	0.065
76	15% Sucrose, 50mM PO4, pH 7.0	0.000	0.067	0.267	0.048	0.226
77	5 % Trehalose, pH 4.7	0.000	0.487	NT	0.595	0.639
78	10 % Trehalose, pH 4.7	0.000	0.420	NT	0.490	0.458
79	17.5% Trehalose, pH 4.7	0.000	0.293	NT	0.257	0.313

Fig. 8C

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(57) Abstract

A stable pharmaceutical composition of brain derived neurotrophic factor (BDNF) in the form of an aqueous solution or lyophilized one being suitable for a long-term storage, which contains a surfactant, especially nonionic surfactant (e.g., Tween 80) of 0.001 to 10 %, whereby the polymerization and the denaturation of BDNF are inhibited, and the biological activities of BDNF are maintained for a long time. Said lyophilized composition can be made more stable by addition of a sugar alcohol (e.g., mannitol) and/or an amino acid (e.g., glycine).

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DESCRIPTION

STABLE PHARMACEUTICAL COMPOSITION OF BDNF

TECHNICAL FIELD

The present invention relates to a pharmaceutical composition containing a brain derived neurotrophic factor (BDNF) in the form of an aqueous solution or a lyophilized one, the latter being prepared by lyophilizing said aqueous solution composition.

BACKGROUND ART

Nerve cells of *vertebrata* need a certain group of polypeptides, called a neurotrophic factor, for their survival. As one of the neurotrophic factors, a brain derived neurotrophic factor (BDNF) has been known. BDNF has first been isolated from porcine brain by Barde, Y.E. et al. (cf., The EMBO Journal, **5**, 549-553 (1982)), and thereafter BDNF genes of pig, human and mouse have been cloned in 1989 whereby it has been confirmed that it has a primary structure consisting of 119 amino acids (cf., Leibrock, J. et al., Nature, **341**, 149 (1989)). Recently, a lot of attentions have been given to BDNF because BDNF is considered to act a very important role in the central nervous system.

BDNF is a polypeptide exhibiting various pharmacological activities in the nervous system, and the pharmacological activities thereof have been disclosed in Seitai no Kagaku (Science of Living body), **43** (6), 616-625 (1992). Besides, BDNF has been expected to be useful as an agent for treatment of various diseases such as amyotrophic lateral sclerosis (ALS), anticancer agent-

intoxicated neuropathy, diabetic neuropathy, retinal pigment degeneration, glaucoma, Huntington's chorea, Parkinson disease, Alzheimer's disease, terminal cancer ache, depression, obesity, etc., based on the pharmacological activities thereof (cf., USP 5180820, Seitai no Kagaku, **43** (6), pages 616-625, (1992)).

A compound to be used as a medicament is usually required to be stable without changing of activity thereof with time under conventional storage conditions when formulated in a form of a conventional pharmaceutical composition. Especially, highly purified polypeptides such as BDNF have many problems to be solved in order to be kept stably for a long time. For example, when keeping BDNF in the form of a solution in a conventional physiological saline solution, BDNF has a tendency to aggregate even by storage for several days to dozens of days, which is a very serious problem. The aggregate of BDNF is known to cause immune toxicity to the living body, so that the prevention of the production of BDNF aggregate is very important. Besides, denatured and/or polymerized BDNF are often produced as well. Hitherto, there has been no report on effective ways to solve these serious problems of BDNF.

In general, when a compound of a low molecular weight is unstable in the form of an aqueous solution, the solution is usually tried to be lyophilized for stabilization. However, polypeptides are known not to be stable during the lyophilization procedures (cf., Tanpakushitsu, Kakusan, Koso (i.e., Protein, Nucleic Acid, Enzyme), **37** (9), 1517 (1992)). Besides, the conventional stabilizers exhibit their stabilization effects in an aqueous solution of a polypeptide by supporting hydration between a water molecule and a polypeptide. Therefore, these stabilizers cannot exhibit their stabilization effects

in a lyophilized composition in many cases because no water molecule exists therein (cf., Tanpakushitsu, Kakusan, Koso (i.e., Protein, Nucleic Acid, Enzyme), 37 (9), 1517 (1992)). Hitherto, it has not been known at all a lyophilized pharmaceutical composition of BDNF, and any skilled person in the art cannot imagine the physicochemical and biological stability of the lyophilized pharmaceutical composition of BDNF.

DISCLOSURE OF INVENTION

When keeping BDNF at a low temperature or room temperature for several days to dozens of days, BDNF aggregates, shows varied properties, and denatured and/or polymerized BDNF are produced. Thus, the physicochemical stability of BDNF is low so that BDNF cannot be kept for a long time. The instability has prohibited from the development of BDNF as a medicament for human or for other animals in the form of a pharmaceutical preparation such as an injection preparation.

Under the above-mentioned circumstances, the present inventors have intensively studied to develop a pharmaceutical composition of BDNF, and have found that the addition of a surfactant is quite effective for stabilization of BDNF, and have accomplished the present invention.

That is, the present invention provides a stable pharmaceutical composition of a brain derived neurotrophic factor (BDNF), which comprises as an active ingredient a BDNF and as a stabilizer a surfactant, preferably a nonionic surfactant, and optionally a salt and/or a buffering agent, and further optionally an additional stabilizer such as an amino acid and a sugar alcohol, which may be in the form of an aqueous solution, or in a lyophilized form.

The present invention provides also a method for stabilizing a BDNF in a pharmaceutical composition by incorporating a surfactant as a stabilizer into the composition and optionally further adding a salt and/or a buffering agent and further optionally adding an additional stabilizer such as an amino acid and a sugar alcohol.

Embodiments of the composition of the present invention are exemplified below.

(1) A stable pharmaceutical composition, which comprises a BDNF and a surfactant.

(2) The pharmaceutical composition according to (1), wherein the surfactant is a nonionic surfactant.

(3) The pharmaceutical composition according to (2), wherein the nonionic surfactant is Tween 80.

(4) The pharmaceutical composition according to (3), wherein the Tween 80 is contained in an amount of 0.001 % (w/v) to 10 % (w/v).

(5) The pharmaceutical composition according to (1), which further comprises a salt.

(6) The pharmaceutical composition according to (5), wherein the salt is sodium chloride.

(7) The pharmaceutical composition according to (1), which further comprises a buffering agent.

(8) The pharmaceutical composition according to (7), wherein the buffering agent is a phosphate buffer.

(9) The pharmaceutical composition according to (1), which has a pH

value in the range of 5.5 to 7.5.

(10) The pharmaceutical composition according to (1), which is in the form of a lyophilized composition.

(11) The pharmaceutical composition according to (10), which further comprises an additional stabilizer.

(12) The pharmaceutical composition according to (11), wherein the additional stabilizer is a member selected from an amino acid and a sugar alcohol, or a combination thereof.

(13) The pharmaceutical composition according to (12), wherein the amino acid is glycine, and the sugar alcohol is mannitol.

(14) The pharmaceutical composition according to (11), wherein the additional stabilizer is contained in the range of 0.1 to 10 % by weight to the weight of BDNF.

(15) A lyophilized pharmaceutical composition of BDNF which contains as a stabilizer Tween 80 and as an additional stabilizer mannitol in the total amount of from 0.01 % (w/v) to 10 % (w/v), based on the whole weight of the composition reconstituted.

The BDNF used in the present invention may be any one of any animal origins, such as mouse, pig, or human, and can be prepared by various processes. When a BDNF isolated from animal tissues is used in the present invention, it may be purified to such a degree that it can be used as a medicament (cf., The EMBO Journal, 5, 549-553 (1982)). Alternatively, a BDNF can be obtained by culturing a primary culture cell or an established cell line which can produce BDNF, and isolating from the culture broth thereof (e.g., culture supernatant,

cultured cells). Moreover, there may be used a recombinant BDNF which can be obtained by a conventional gene engineering technique, e.g., by inserting a gene coding for BDNF into a suitable vector, transforming a suitable host with the recombinant vector, and isolating from the culture supernatant of the resulting transformant (cf., Proc. Natl. Acad. Sci. USA, **88**, 961 (1991); Biochem. Biophys. Res. Commun., **186**, 1553 (1992)), which is suitable for production of BDNF of uniform property in a large scale. The host cells to be used in the above process is not critical, and may be any conventional host cells which have been used in gene engineering technique, for example, *Escherichia coli*, *Bacillus subtilis*, yeasts, vegetable cells or animal cells.

A modified protein of BDNF can be obtained by addition, substitution, deletion or removal of a part of amino acid sequence of a natural BDNF by a gene engineering technique. Any modified protein of BDNF thus obtained is also included in the BDNF to be used in the present invention even though a part of the amino acid sequence thereof is deleted, or substituted by other amino acid, or inserted thereto a part of other amino acid sequence, or bonded with one or more amino acids at the N-terminus and/or C-terminus, as long as said modified protein of BDNF shows the biological activities of the same quality as those of BDNF, i.e., the biological activities on the nerve cells, such as an activity of survival of nerve cells, activity of extending neurodendrite, activity of promoting the production of neurotransmitter. That is, in addition to mature BDNFs, Met-BDNF having a methionine at the N-terminus of BDNF, etc. can be used in the present composition as long as it shows the neurotrophic activities of the same quality as those of natural BDNFs.

The "surfactant" used in the present invention means any pharmaceutically acceptable surfactant which is useful in medicaments for human, or for other animals, and includes, for example, a nonionic surfactant. The most preferable surfactant is Tween 80 (Polysorbate 80 = polyoxyethylene sorbitan mono-oleate), or Tween 20 (Polysorbate 20 = polyoxyethylene sorbitan mono-lauratc), Pluronic F-68 (= a polyoxyethylcnc polyoxypropylene glycol), polyethylene glycol, etc. The surfactant is added to the pharmaceutical composition of the present invention in an amount of from 0.001 to 10 % by weight, preferably in an amount of from 0.001 to 0.1 % by weight, to the weight of water in the aqueous composition.

The "salt" may be a pharmaceutically acceptable salt which is useful in medicaments for human, or for other animals, and includes, for example, sodium chloride. Sodium chloride is used in order to keep the osmotic pressure of the present pharmaceutical composition suitable for an injection preparation, especially in an amount of 150 to 300 mM by which the injection preparation shows an osmotic pressure ratio of 1 to 2.

The "buffering agent" means a buffering agent which is added to the composition in order to adjust the pH value in a solution preparation or in a lyophilized preparation, in the latter preparation, the pH when reconstituted. The representative buffering agent is, for example, phosphate buffer, Tris buffer and citrate buffer. The buffering agent adjusts the pH value of the solution so that the stability of BDNF is maintained. The pH value of the present composition is not critical, but it is preferably in the range of 5.5 to 7.5. BDNF is hydrolyzed under acidic conditions to produce many fragments derived from

BDNF, and is further de-amidated or hydrolyzed under basic conditions. The final concentration of the buffering agent in the composition is in the range of 1 mM to 100 mM.

The "additional stabilizer" includes, for example, amino acids such as glycine or sugar alcohols such as mannitol, and these additional stabilizers may be used together. When prepared the pharmaceutical composition of BDNF with adding the additional stabilizer, the storage stability of BDNF in the preparation is further improved. The additional stabilizer, for example, glycine or mannitol, is added in an amount of from 0.01 to 100 times by weight, more preferably 0.1 to 10 times by weight, of the weight of BDNF. Glycine and/or mannitol can be used in a solution composition of the present invention, but can show more excellent stabilization effects in the lyophilized composition of the present invention. These amino acid and sugar alcohol may be used individually, but preferably in combination.

The "lyophilized composition" of the present invention can be prepared by subjecting a solution composition of BDNF to lyophilization by a conventional lyophilization, or freeze-drying technique. For example, BDNF is dissolved in a suitable aqueous solvent such as a distilled water for injection, a buffer solution, a physiological saline solution, etc., and thereto is added a stabilizer, a buffering agent, or a salt, if necessary, and the solution thus obtained is sterilized by filtration through a filter, and then lyophilized to give a lyophilized composition of the present invention.

The compositions of the present invention may additionally contain a conventional additive which is usually used for pharmaceutical preparations, for

example, a solubilizer, antioxidant, anaesthetic agent, isotonic agent, etc. The lyophilizing method is, for example, a method consisting of three steps: a step of freezing a solution under atmospheric pressure, a primary drying step of sublimation of a free water which is not adsorbed by or bound to a solute under reduced pressure, and a secondary drying step of removing water adsorbed by or bound to a solute (cf., Pharm. Tech. Japan, **8** (1), 75-87 (1992)). BDNF to be contained in the composition can be kept very stably during the procedures of preparing the composition of the present invention, such as dissolving in a solvent, freeze-drying thereof, as well as reconstituting a lyophilized composition.

The content of BDNF in the compositions may be varied depending on the kinds of diseases to be cured, or the administration route thereof.

The pharmaceutical composition of BDNF of the present invention may be filled in a vial by putting in a vial, fulfilling with nitrogen gas, and then sealing the vial. When the vial is fulfilled with nitrogen gas, BDNF contained therein is prevented from denature and hence can be kept more stably.

BEST MODE FOR CARRYING OUT THE INVENTION

The present invention is illustrated in more detail by the following Examples, but should not be construed to be limited thereto.

Example 1 (Effects of surfactant 1)

Preparation of a solution composition of BDNF without a surfactant (Reference Solution Composition 1)

BDNF was dissolved in 10 mM phosphate buffer (pH 7.0, 150 mM sodium chloride) to give an aqueous BDNF solution (20 mg/ml). The solution

thus obtained was put into vials aseptically to give a solution composition of BDNF containing no surfactant.

Preparation of a solution composition of BDNF with a surfactant (Present Solution Composition 1)

BDNF was dissolved in 10 mM phosphate buffer (pH 7.0, 150 mM sodium chloride, 0.01 % Tween 80) to give an aqueous BDNF solution (20 mg/ml). The solution thus obtained was put into vials aseptically to give a solution composition of BDNF containing a surfactant.

Experiment 1

Using Reference Solution Composition 1 and Present Solution Composition 1, the inhibitory effect of a surfactant on the production of aggregates was tested. The compositions were kept at 25°C at a vibration of 5 cm x 75 strokes/min. The period (days) till the production of aggregates was determined by visual observation. The results are shown in Table 1. From the resulting data, it is proved that the addition of Tween 80 inhibited the production of aggregates of BDNF in a solution composition.

Table 1

Effects of Tween 80 on the production of aggregates of BDNF (n=5)

	Concentration of Tween 80 (%)	Period for the production of aggregate (days)
Reference Solution Composition 1	0	10
Present Solution Composition 1	0.01	>30

Example 2 (Effects of surfactant 2)

Preparation of a solution composition of BDNF without a surfactant (Reference

Solution Composition 2)

BDNF was dissolved in 10 mM phosphate buffer (pH 7.0, 150 mM sodium chloride) to give an aqueous BDNF solution (0.1 mg/ml). The solution thus obtained was put into vials aseptically to give a solution composition of BDNF containing no surfactant.

Preparation of a solution composition of BDNF with a surfactant (Present Solution Composition 2)

BDNF was dissolved in 10 mM phosphate buffer (pH 7.0, 150 mM sodium chloride, 0.01 % Tween 80) to give an aqueous BDNF solution (0.1 mg/ml). The solution thus obtained was put into vials aseptically to give a solution composition of BDNF containing a surfactant.

Experiment 2

Using Reference Solution Composition 2 and Present Solution Composition 2, the inhibitory effect of a surfactant on the adsorption of BDNF onto the vessel was tested. The concentration of BDNF was determined by absorption spectrophotometry immediately after and before the BDNF solution was put into a glass vial, and the amount of BDNF adsorbed onto the glass vial was calculated. The results are shown in Table 2. From the results, it is proved that the addition of Tween 80 reduced the adsorption amount of BDNF onto the glass vial in a solution composition.

Table 2

Effects of Tween 80 on the adsorption of BDNF onto the glass surface

	Concentration of Tween 80 (%)	Adsorbed BDNF on the surface of glass vial ($\mu g/cm^2$)
Reference Solution Composition 1	0	0.73
Present Solution Composition 2	0.01	0.28

Example 3 (Effects of pH)

Preparation of a solution composition of BDNF (Present Solution Composition 3)

BDNF was dissolved in 10 mM phosphate buffer (pH 7.0, 150 mM sodium chloride, 0.01 % Tween 80) to give an aqueous BDNF solution (5 mg/ml). The pH value of the aqueous BDNF solution thus obtained was adjusted with 1N HCl or 1N NaOH to six degrees of pH 4, 5, 6, 7, 8 or 9. The solutions thus obtained was put into vials aseptically to give a solution composition of BDNF.

Preparation of a lyophilized composition of BDNF (Present Lyophilized Composition 3)

BDNF was dissolved in 10 mM phosphate buffer (pH 7.0, 150 mM sodium chloride, 0.01 % Tween 80) to give an aqueous BDNF solution (20 mg/ml). The solution thus obtained was put into vials aseptically, and lyophilized under the conditions as shown in Table 3 to give a lyophilized BDNF composition. In Table 3, the mark → means that the temperature was changed.

Table 3

Lyophilization conditions

	Freezing step		Primary drying step		Secondary drying step	
Temperature (°C)	5 → −40	−40	−40 → 0	0	0 → 20	20
Period (hr)	1	10	8	24	1	24
Pressure (mmHg)	760	760	<1	<1	<1	<1

Experiment 3

Using Present Solution Composition 3 and Present Lyophilized Composition 3, the effects of pH value on the storage stability of BDNF was studied. The compositions obtained above were kept at 25°C or 40°C for three months, and the contents of BDNF, polymerized BDNF and denatured BDNF were determined by the methods mentioned hereinbelow. As shown in Table 4, the content of BDNF was reduced under basic conditions, but slightly reduced under acidic conditions. The polymerized BDNF was hardly produced under acidic conditions, but increased under basic conditions. On the other hand, the content of the denatured BDNF was more increased under acidic conditions than under basic conditions.

Method for determining the BDNF content:

BDNF was diluted to 2 mg/ml, and the concentration thereof was determined by reverse phase chromatography under the following conditions.

Column: VYDAC214BTPC4

Solvent: Solution A: 0.1 % aqueous trifluoroacetic acid solution

Solution B: 0.1 % trifluoroacetic acid solution in acetonitrile

Graduation conditions:

The concentration (%) of Solution B was 26, 35, 35, 90, 26 and 26 at a time (minutes) of 0, 36, 42, 46, 47, 66, respectively.

Detection: 215 nm

Flow rate: 1.0 ml/min.

Temperature: 60°C

Apply: 25 µl

Method for determining the polymerized:denatured BDNF:

Method for determining the BDNF content:

BDNF was diluted to 2 mg/ml, and the concentration thereof was determined by gel filtration chromatography under the following conditions.

Column: SUPERDEX75HR

Solvent: 300 mM sodium phosphate, 500 mM sodium chloride, 5 % n-propanol, pH 6

Detection: 215 nm

Flow rate: 0.6 ml/min.

Apply: 10 µl

Table 4

Effects of pH value on the BDNF stability

pH	Tem-perature (°C)	Storage period (month)	Content * of BDNF (%)	Content * of polymerized BDNF (%)	Content * of denatured BDNF (%)
7	–	Initial	93.58	0.00	0.0
4	25	3	93.54	0.00	1.19
	40	3	90.06	0.03	2.07
5	25	3	92.98	0.04	0.18
	40	3	87.87	0.05	1.85
6	25	3	92.77	0.05	0.24
	40	3	90.45	0.12	0.84
7	25	3	90.59	0.23	0.11
	40	3	79.78	0.72	0.49
8	25	3	86.69	0.66	0.00
	40	3	60.61	3.01	0.36
9	25	3	83.96	1.07	0.12
	40	3	–	3.45	0.41

*: The ratio to the total peak area.

Example 4 (Stability during the lyophilization procedures, and effects of the composition forms)

Preparation of a solution composition of BDNF (Present Solution Composition 4)

BDNF was dissolved in 10 mM phosphate buffer (pH 7.0, 150 mM sodium chloride, 0.01 % Tween 80) to give an aqueous BDNF solution (5 mg/ml). The solution thus obtained was put into vials aseptically, and the vials were fulfilled with nitrogen gas, and then sealed to give a solution composition of BDNF.

Preparation of a lyophilized composition of BDNF (Present Lyophilized Composition 4)

BDNF was dissolved in 10 mM phosphate buffer (pH 7.0, 150 mM sodium chloride, 0.01 % Tween 80) to give an aqueous BDNF solution (5 mg/ml). The solution thus obtained was put into vials aseptically, and lyophilized under the conditions as shown in Table 3 to give a lyophilized composition of BDNF. The vials were fulfilled with nitrogen gas and were sealed.

Experiment 4

In order to study the stability of BDNF during the lyophilization procedures, the content of BDNF and the biological activities thereof were determined in a solution composition of BDNF before the lyophilization procedures in Example 4, and in re-dissolved aqueous solution of the lyophilized composition by the method as mentioned hereinbelow. The results are shown in Table 5. The significant changes were not recognized before and after the lyophilization procedures, by which it is proved that BDNF is stable during the lyophilization procedures and the re-dissolving step thereafter, and that BDNF can be formulated into a form of a lyophilized composition.

Method for determining the biological activities of BDNF:

The biological activities of BDNF were determined based on the cell proliferation potency of BAF-trkB cells when treated with BDNF. Said BAF-trkB cells were prepared by introducing a trkB gene (a BDNF receptor) into IL3-dependent pre-B cells (cf., Cell, **41**, 727-734, July 1985) in the same manner as described in U.S. Patent 5,622,862.

Table 5

Stability of BDNF during the lyophilization procedures

	Biological activities (specific activity: x 10^4 TU/mg)	Content of BDNF (%)
Present Solution Composition 4	1.33±0.21	93.34
Immediately after re-dissolution of Present Lyophilized Composition 4	1.61±0.30	93.14

Experiment 5

In order to study the differences in the storage stability between the solution composition and the lyophilized composition of BDNF, the BDNF contents in the compositions prepared in Example 4 were determined immediately after the preparation thereof, or after three-month storage at 25°C, or 40°C. The results are shown in Table 6. In the lyophilized composition, the content of polymerized BDNF was slightly higher than that in the solution composition, but the content of BDNF per se is higher, and the content of the denatured BDNF was lower, than that in the solution composition.

Table 6

Effects of the composition forms on the stability of BDNF

Formulation	Temperature (°C)	Storage period (month)	BDNF content (%)	Content of polymerized BDNF (%)	Content of denatured BDNF (%)
Present Solution Composition 4	–	Initial	92.91	0.09	0
	25	3	91.21	0.26	0.24
	40	3	86.21	0.38	0.75
Present Lyophilized composition 4	–	Initial	93.71	0.07	0.0
	25	3	92.82	0.34	0.0
	40	3	88.40	1.66	0.0

Example 5 (Effects of surfactant 5)

Preparation of a lyophilized composition of BDNF with a surfactant (Present Lyophilized Composition 5)

A lyophilized composition of BDNF was prepared by the same method as in Example 4 to give Present Lyophilized Composition 5.

Preparation of a lyophilized composition of BDNF without a surfactant (Reference Lyophilized Composition 5):

BDNF was dissolved in 10 mM phosphate buffer (pH 7.0, 150 mM sodium chloride) to give an aqueous BDNF solution (5 mg/ml). The solution thus obtained was put into vials aseptically, and lyophilized under the same conditions as shown in Table 3 to give a lyophilized composition of BDNF. The vials were fulfilled with nitrogen gas and sealed.

Experiment 6

In order to study the effects of a surfactant on the appearance of the re-dissolved solution of a lyophilized composition, Reference Lyophilized Composition 5 and Present Lyophilized Composition 5 were dissolved in

purified water, and the appearance of these solutions was visually observed. The results are shown in Table 7. When reconstituting Present Lyophilized Composition 5, the solution was clear, while the solution of Reference Lyophilized Composition 5 wherein no surfactant was added was turbid after the dissolution thereof.

Table 7

Effects of surfactant on the appearance of re-dissolved solution of the lyophilized composition

	Tween 80	Appearance of the re-dissolved solution
Present Lyophilized Composition 5	0.01 %	Clear
Reference Lyophilized Composition 5	Not added	Turbid

Example 6 (Effects of stabilizer on the stability of the lyophilized composition of BDNF)

Preparation of a lyophilized composition of BDNF with a surfactant (Present Lyophilized Composition 6A)

A lyophilized composition of BDNF was prepared by the same method as in Example 4 to give Present Lyophilized Composition 6A.

Preparation of a lyophilized composition of BDNF without a surfactant (Present Lyophilized Composition 6B)

BDNF was dissolved in 10 mM phosphate buffer (pH 7.0, 150 mM sodium chloride, 0.01 % Tween 80) to give an aqueous BDNF solution (5 mg/ml). To the solution was added mannitol so that the final concentration of mannitol was 10 mg/ml. The aqueous solution of BDNF thus obtained was put into vials aseptically, and lyophilized under the same conditions as shown in

Table 3 to give a lyophilized composition of BDNF. The vials were fulfilled with nitrogen gas and sealed.

Preparation of a lyophilized composition of BDNF with a surfactant (Present Lyophilized Composition 6C)

BDNF was dissolved in 10 mM phosphate buffer (pH 7.0, 150 mM sodium chloride, 0.01 % Tween 80) to give an aqueous BDNF solution (5 mg/ml). To the solution was added glycine so that the final concentration of glycine is 10 mg/ml. The aqueous solution thus obtained was put into vials aseptically, and lyophilized under the conditions as shown in Table 3 to give a lyophilized composition of BDNF. The vials were fulfilled with nitrogen gas and sealed.

Experiment 7

Using Present Lyophilized Compositions 6A, 6B and 6C, the content of BDNF was determined immediately after the preparation, or after one-month storage at 40°C. The results are shown in Table 8. In addition, using Present Lyophilized Compositions 6A and 6B, the content of BDNF was also determined immediately after the preparation, or after three-month storage at 25°C or 40°C. The results are shown in Table 9. The compositions containing a stabilizer showed a higher stability than the composition containing no stabilizer.

Table 8

Effects of stabilizer in Lyophilized Compositions 1

	Stabilizer	Temperature	Storage period	Content of BDNF
Present Lyophilized Composition 6A	Not added	–	Initial	91.98
		40	1	78.69
Present Lyophilized Composition 6B	Mannitol	–	Initial	92.16
		40	1	86.74
Present Lyophilized Composition 6C	Glycine	–	Initial	92.20
		40	1	83.99

Note: In Composition 6A, 6B and 6C used in this experiment, the vials were not fulfilled with nitrogen gas.

Table 9

Effects of stabilizer in Lyophilized Compositions 2

	Stabilizer	Temperature (°C)	Storage period (month)	Content of BDNF (%)	Content of polymerized BDNF (%)	Content of denatured BDNF (%)
Present Composition 6A	Not added	–	Initial	93.71	0.07	0.0
		25	3	92.82	0.34	0.0
		40	3	88.40	1.66	0.0
Present Composition 6B	Mannitol	–	Initial	93.03	0.16	0.0
		25	3	92.85	0.18	0.02
		40	3	92.55	0.37	0.0

INDUSTRIAL APPLICATION

The pharmaceutical composition of BDNF of the present invention can maintain BDNF stable for a long time by adding thereto a surfactant. The present compositions of BDNF show the following effects:

(1) prevention of the production of turbidness or aggregations of BDNF in a pharmaceutical solution composition of BDNF of the present invention, during the storage thereof;

(2) prevention of the production of turbidness or aggregations of BDNF, when dissolving a lyophilized pharmaceutical composition of BDNF of the present invention;

(3) prevention of the adsorption of BDNF onto a surface of a glass or resin vessel therefor; and

(4) reservation of the biological activities of BDNF.

The pharmaceutical composition of BDNF containing a salt as an isotonic agent, a buffering agent for keeping an optimal pH value, or containing both of these salt and buffering agent may be in the form of a pharmaceutical composition being suitable for clinical use. The stability of BDNF contained in the pharmaceutical solution composition of the present invention is more improved when lyophilized.

The lyophilized pharmaceutical composition of BDNF of the present invention additionally containing as an additional stabilizer an amino acid and/or a sugar alcohol is more stable. Especially, the lyophilized composition of BDNF containing glycine as an amino acid and/or mannitol as a sugar alcohol is the most stable composition.

CLAIMS

1. A stable pharmaceutical composition of a brain derived neurotrophic factor (BDNF), which comprises a BDNF and a surfactant in admixture of a conventional pharmaceutically acceptable carrier or diluent.

2. The pharmaceutical composition according to claim 1, wherein the surfactant is a nonionic surfactant.

3. The pharmaceutical composition according to claim 2, wherein the nonionic surfactant is Tween 80.

4. The pharmaceutical composition according to claim 3, wherein Tween 80 is contained in an amount of 0.001 % (w/v) to 10 % (w/v).

5. The pharmaceutical composition according to claim 1, which further comprises a salt.

6. The pharmaceutical composition according to claim 5, wherein the salt is sodium chloride.

7. The pharmaceutical composition according to claim 1, which further comprises a buffering agent.

8. The pharmaceutical composition according to claim 7, wherein the buffering agent is a phosphate buffer.

9. The pharmaceutical composition according to claim 1, which has a pH value in the range of 5.5 to 7.5.

10. The pharmaceutical composition according to claim 1, which is in the form of a lyophilized composition.

11. The pharmaceutical composition according to claim 10, which

further comprises an additional stabilizer.

12. The pharmaceutical composition according to claim 11, wherein the additional stabilizer is a member selected from an amino acid and a sugar alcohol, or a combination thereof.

13. The pharmaceutical composition according to claim 12, wherein the amino acid is glycine, and the sugar alcohol is mannitol.

14. The pharmaceutical composition according to claim 11, wherein the additional stabilizer is contained in the range of 0.1 to 10 % by weight to the weight of BDNF.

15. A lyophilized pharmaceutical composition of BDNF which contains as a stabilizer Tween 80 and as an additional stabilizer mannitol in the total amount of from 0.01 % (w/v) and 10 % (w/v), based on the whole weight when reconstituted in an aqueous medium.

16. A method for stabilizing a BDNF in a pharmaceutical composition, which comprises incorporating a surfactant into a pharmaceutical composition of a BDNF.

17. The method according to claim 16, wherein the surfactant is a nonionic surfactant.

18. The method according to claim 16, wherein the pharmaceutical composition of a BDNF comprises further a salt and/or a buffering agent.

19. The method according to claim 16, wherein the pharmaceutical composition is in the form of a lyophilized composition.

20. The method according to claim 19, which further comprises adding an additional stabilizer selected from an amino acid, a sugar alcohol, or a combination thereof.

INTERNATIONAL SEARCH REPORT

Int. onal Application No
PCT/JP 97/01746

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K38/18 A61K47/26 A61K9/19

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 91 07947 A (RAMSEY FOUNDATION) 13 June 1991 see claims 1,4 see page 20, line 33 - page 21, line 21 see page 23, line 11 - line 21 see page 32; example 1 ---	1-3,5,7, 9,16-18
X,P	US 5 604 202 A (JOHN A. KESSLER ET AL.) 18 February 1997 see column 2, line 51 - line 63 see column 3, line 56 - column 4, line 25 -----	1-3, 5-13, 15-20

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

1

Date of the actual completion of the international search	Date of mailing of the international search report
12 September 1997	26.09.97

Name and mailing address of the ISA	Authorized officer
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INTERNATIONAL SEARCH REPORT

Information on patent family members

Inter onal Application No
PCT/JP 97/01746

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9107947 A	13-06-91	AU 6909091 A	26-06-91
		CA 2070823 A	06-06-91
		EP 0504263 A	23-09-92
		US 5624898 A	29-04-97
US 5604202 A	18-02-97	US 5585348 A	17-12-96

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(54) **FREEZE-DRIED PREPARATION CONTAINING INTERLEUKIN-11**

(57)Abstract:
PROBLEM TO BE SOLVED: To provide a stable freeze-dried preparation containing IL (interleukin)-11, without having such an anxiety that the freeze-dried preparation becomes cloudy when redissolved.
SOLUTION: A method for preventing the freeze-dried preparation from becoming cloudy when redissolved comprises adding a nonionic surfactant to a preparation solution for the freeze-dried preparation and/or adding saccharides thereto for dealing with various problems of the freeze-dried preparation containing the IL-11, particularly, a problem of becoming cloudy when redissolved, namely, solubility (turbidity) of the preparation when reconstructed. The method solves the problem that the freeze-dried preparation containing the IL-11 becomes cloudy when redissolved, and further is effective for stabilizing the preparation.

* NOTICES *

CLAIMS

[Claim(s)]
[Claim 1]
It is a nebula prevention method at the time of remelting of lyophilized products introducing at least 1 chosen from the following as a means to improve the solubility of the interleukin 11, in a manufacturing process of interleukin 11 content lyophilized products containing the interleukin 11 and a buffer.
1) Add a nonionic surfactant to a prepared solution.
2) Add sugars to a prepared solution.
[Claim 2]
It is a nebula prevention method at the time of remelting of the lyophilized products according to claim 1 whose nonionic surfactant is a polyoxy sorbitan fatty acid ester.
[Claim 3]
It is a nebula prevention method at the time of remelting of the lyophilized products according to claim 1 or 2 whose nonionic surfactant is polysorbate 80.
[Claim 4]
It is a nebula prevention method at the time of remelting of the lyophilized products according to claim 3 whose concentration of polysorbate 80 is about 0.0001 % by weight thru/or 0.01 % by weight among a solution at the time of preparation.
[Claim 5]
It is a nebula prevention method at the time of remelting of the lyophilized products according to claim 1 whose sugars are purified sucrose and/or lactose.
[Claim 6]
It is a nebula prevention method at the time of remelting of the lyophilized products according to claim 1 to 5 whose buffers are sodium phosphate and/or histidine.
[Claim 7]
A manufacturing method of interleukin 11 content lyophilized products which contain a method of a description in any 1 item of Claims 1-6.
[Claim 8]
Interleukin 11 content lyophilized products prepared by a manufacturing method of Claim 7.
[Claim 9]
A freeze-drying medicinal composition for parenteral administration which contains 1 type thru/or 2 type of sugars chosen from the interleukin 11, a glycine, a buffer and polyoxyethylene sorbitan fatty acid ester and/or purified sucrose, and lactose.
[Claim 10]
The freeze-drying medicinal composition for parenteral administration according to claim 9 whose polyalkylene glycol of fatty alcohol is polysorbate 80.

DETAILED DESCRIPTION

[Detailed Description of the Invention]
[Field of the Invention]

[0001]
The present invention relates to the interleukin 11 (it is described as IL-11 below) content lyophilized products in which the nebula prevention method of interleukin 11 content lyophilized products and the prevention from nebula at the time of remelting were attained.
[Background of the Invention]
[0002]
Bioactive protein is used at various places as drugs.
There are a various interferon and hematogenous factor, tPA, urokinase, various CSFs, and various interleukin (IL).
As for pharmaceutical preparation, when these are parenterally prescribed for the patient, it is common to be liquids-and-solutions-ized at the time of administration. Bioactive protein is lacking in preservation stability, and difficulty exists plentifully liquid-preparations-izing. generally the prepared pharmaceutical preparation is freeze-dried -- business -- the time -- a solution -- liquefying -- having -- parenteral administration -- making -- having .
In lyophilized products, it is made by examination of various stabilizing agents and Concomitant use of useful protein and a surfactant (Patent document 2), There is a report of mixing (Patent document 3) of IL-12 and a surfactant, addition (Patent document 4) of the nonreducing sugar of IL-6, addition (Patent document 5) of the various sugars to G-CSF, addition (Patent document 6) of the water-soluble heterocyclic compound to a human growth hormone, etc. A Patent document 2, and 3 and 4 make problem active deterioration evasion of protein of a description to each.
A Patent document 5 and 6 make evasion of irreversible floc generation problem.

[0003]
IL-11 which is bioactive protein is the protein produced by a recombinant gene manipulation method.
Various hemopoiesis and an immune function are stimulated.
Genetics Institute (expression Wyeth). The pharmaceutical preparation "Neumega (product name)" which consists of recombinant Homo sapiens IL-11 (rhIL-11) which is a proline deletion object of an amino terminal is developed, It provides for the medical spot by adaptation of "prevention of the serious thrombocytopenia seen after the bone marrow prevention chemotherapy in a non-myelogenous malignant tumor, and improvement in evasion of a platelet transfusion." This pharmaceutical preparation is the lyophilized products described in a Patent document 1.
IL-11 is contained in 5mg [ml] /, and 10mM (pH 7.0) and a glycine are contained for sodium phosphate 300 mM as concentration after pharmaceutical preparation remelting.

[0004]
[Patent document 1] The International-Publication WO 95/No. 28951 gazette (correspondence U.S. Pat. No. 6,270,757 gazette)
[Patent document 2] JP,2001-192343,A
[Patent document 3] JP,2002-275197,A
[Patent document 4] JP,H8-502722,A
[Patent document 5] JP,H8-504784,A
[Patent document 6] JP,H10-265404,A
[Description of the Invention]
[Problem to be solved by the invention]
[0005]
There is the problem given to the present invention in providing the stable lyophilized products which contain IL-11 which does not cause the nebula prevention method at the time of remelting of IL-11 content lyophilized products, and the prevention from nebula at the time of remelting so that it may explain in full detail below.

As for IL-11, it is desirable to pharmaceutical-preparation-ize in the form of the liquid preparations for injection conventionally made advantageous in various meanings into an aqueous solution since it is comparatively stable in the pH range of neutral vicinity. However, since it hydrolyzes depending on temperature, in liquid preparations, the mothball of a period demanded practically is difficult and cannot but consider it as lyophilized products in practice. In order to consider it as lyophilized products, IL-11 solution was cooled to urgency to less than -30 degree C, the supercooling of the solution was broken and frozen, and nebula was observed in passing away after that by the general freeze drying process to dry at the time of remelting.
Generally, becoming refractory to some freeze-dried cakes, and becoming cloudy in it when remelting, is observed by some bioactive protein content lyophilized products. In order to remelt and prepare the lyophilized products of bioactive protein on the occasion of a clinical use using water for injection, remelting promptly needs mixing of a foreign matter, etc. in that case so that the existence of the abnormalities of pharmaceutical preparation can be checked easily. Therefore, even if transient, lyophilized products which produce the nebula at the time of remelting should be hard to be treated at the clinical spot, and a method of preventing the nebula at the time of such remelting was desired.
[Means for solving problem]
[0006]
The inventor receives the problem of the nebula at the time of remelting of IL-11 content lyophilized products, i.e., the solubility at the time of reconstruction, (turbidity), as a result of repeating examination variously for business solutions, such as the above-mentioned passing away nebula, By adding adding a nonionic surfactant to a prepared solution, and/or sugars, it found out that the problem of nebula is solved at the time of remelting of IL-11 content lyophilized products, that solubility improves, and that the stability of pharmaceutical preparation had an effect, and the present invention was completed.
[0007]
That is, the present invention consists of the followings.
1. It is a nebula prevention method at the time of remelting of the lyophilized products introducing at least 1 chosen from the following as a means to improve the solubility of the interleukin 11, in the manufacturing process of the interleukin 11 content lyophilized products containing the interleukin 11 and a buffer.
1) Add a nonionic surfactant to a prepared solution.
2) Add sugars to a prepared solution.
2. It is a nebula prevention method at the time of remelting of the lyophilized products of the description to the above 1 whose nonionic surfactant is a polyoxy sorbitan fatty acid ester.
3. It is a nebula prevention method at the time of remelting of the lyophilized products of the above 1 whose nonionic surfactant is polysorbate 80, or the description to 2.
4. It is a nebula prevention method at the time of remelting of the lyophilized products of the description to the above 3 whose concentration of polysorbate 80 is about 0.0001 % by weight thru/or 0.01 % by weight among the solution at the time of preparation.
5. It is a nebula prevention method at the time of remelting of the lyophilized products of the description to the above 1 whose sugars are purified sucrose and/or lactose.
6. It is a nebula prevention method at the time of remelting of the lyophilized products of the description to the above 1 thru/or 5 whose buffers are sodium phosphate and/or histidine.
7. Manufacturing method of interleukin 11 content lyophilized products which contain method of description in any 1 of the above 1 thru/or 6.
8. Interleukin 11 content lyophilized products prepared by manufacturing method of the above 7.

9. Freeze-drying medicinal composition for parenteral administration which contains 1 type thru/or 2 type of sugars chosen from INTAROIKIN 11, glycine, buffer and polyoxyethylene sorbitan fatty acid ester and/or purified sucrose, and lactose.
10. The freeze-drying medicinal composition for parenteral administration of nine aforementioned description whose polyalkylene glycol of fatty alcohol is polysorbate 80.
[0008]
The present invention is the IL-11 content lyophilized products and the freeze-drying medicinal composition for parenteral administration adding adding a nonionic surfactant to the prepared solution at the time of the IL-11 content lyophilized-products manufacture which contains IL-11 and a buffer at least, and/or sugars.
As IL-11 in the present invention, it may be the change object acquired by natural origin or gene modification technology, and they may be those modification objects (for example, chemical modification object by a polyethylene glycol etc.). These may be used as a monomer or may be used as a polymer of homo or hetero.
[0009]
As for IL-11 chosen as optimal mode of the present invention, the protein of a description is mentioned, for example to US,5,215,895,B, US,5,270,181,B, and US,5,292,646,B. The protein obtained with the protein or the above-mentioned combination synthesized by the protein produced by the recombinant gene manipulation method, the protein refined from the cell source which produces IL-11, or the chemical method is included, Recombinant Homo sapiens IL-11 (rhIL-11) which is a proline deletion object of an amino terminal can be especially chosen as a preferable thing. If it is IL-11 which can attain the object of this invention, it is also possible to choose other things.
the inside of this Description -- IL-11 -- the arrangement of not only naturally occurring type IL-11 but naturally occurring type IL-11 -- or -- or -- some amino acid sequences include substitution, deletion and/, or the inserted arrangement -- IL-11 -- being active (hematopoiesis) -- the shown protein is meant.
[0010]
By the present invention, as for the concentration at the time of front [freeze-drying] preparation of IL-11, it is preferable to be adjusted to the concentration of 0.1 thru/or 20mg/ml, and it is adjusted [ml] more to optimum in 3 thru/or 8mg /still more preferably 1 thru/or 10mg/ml. It is also possible to choose the stabilizing agent of each protein or/, and a solubilizing agent, and to add in the achievement range of each purpose effect by request. For example, in the case of IL-11, the protein concentration at the time of front [freeze-drying] preparation has a preferable range from 0.1mg/ml to 20.0mg/ml, and is [ml] about 5mg/ml most preferably 10mg /from ml in 1mg /. as a solubilizing agent -- amino acid -- a glycine is added preferably, the optimum concentration has a preferable range from 100mM to 400mM, and 350mM from 150mM and about 300 mM(s) are more preferably the most preferable.
[0011]
By the present invention, a buffer means stabilizer of the pH of an aqueous solution and what is generally used in the field of medicine manufacture can be chosen.
In pharmaceutical-preparation-izing of IL-11, it is also possible to choose the phosphate buffer solution containing sodium phosphate. In this case, addition of a stabilizing agent is required because of the-object-of-this-invention achievement. It is also possible as other buffers histidine, tris buffers, and to pass and to choose Pes buffer solution etc. Using combining sodium phosphate and histidine is also possible.
The concentration at the time of front [freeze-drying] preparation of a suitable buffer is the range of 5mM to 40mM, and is about 10 thru/or 20mM especially preferably 7 thru/or 30 mM more preferably. When choosing IL-11 and using sodium phosphate, it is the range of 5mM to 40mM, and 10mM is preferable, when it is histidine, it is the range of 5mM to 40mM, and about 20 mM(s) are preferable.
[0012]

The present invention makes it main means to add adding a nonionic surfactant to the prepared solution at the time of lyophilized-products manufacture, and/or sugars. Freeze-drying stocks a drug solution filled product in a freeze-drying warehouse, and settles it on a shelf. Next, the shelf of a freeze-drying warehouse is cooled at less than -30 degree C, and a filled product is frozen. Decompress after freezing and the inside of a freeze-drying warehouse, raise a temperature on tray to the temperature which a filled product does not dissolve, moisture is made to sublimate, and primary drying is performed. Then, secondary drying is performed by raising a temperature on tray and removing attached groundwater.
In order to manufacture such lyophilized products, in the present invention, the means chosen from the following technique can be introduced specifically.
1) Add a nonionic surfactant to a prepared solution.
2) Add sugars to a prepared solution.
3) Crystallize a buffer.
4) Pretreat by temperature-conditions-ization (-20 degree C thru/or 0 degree C) before lyophilization treatment.
As mentioned above, introducing at least 1 chosen provides a nebula prevention method at the time of remelting of lyophilized products.
[0013]
Adding a nonionic surfactant to a prepared solution by the present invention means adding a nonionic surfactant in the aqueous solution (prepared solution) used when preparing before the lyophilization treatment of IL-11.
The surfactant in which ionicity, such as polyoxyethylene alkyl ether, polyoxyethylene alkyl phenyl ether, and polyoxyethylene sorbitan fatty acid ester, is not shown can be chosen as the nonionic surfactant used for the present invention. Polyoxyethylene sorbitan fatty acid ester can be chosen preferably, and more preferably, it is polysorbate 80 and the polysorbate 20, is polysorbate 80 still more preferably, and is polysorbate 80 of vegetable origin most preferably. The nonionic surfactant of the present invention can also be blended combining 1 type or 2 type or more.
[0014]
The concentration of a nonionic surfactant in the present invention is about 0.0001 % by weight among a solution. Or 0.01% by weight of ranges are 0.0005 thru/or 0.001 % by weight preferable still more preferably. When this concentration is thinner than 0.0001 % by weight, we are anxious about producing nebula at the time of remelting of a freeze-dried cake. When higher than 0.01 % by weight, we are anxious about the increase in an oxidant in the pharmaceutical preparation resulting from the specific promotion of oxidation of protein with the impurity or change object (degradation thing) in a nonionic surfactant. Therefore, the concentration of the nonionic surfactant in the present invention can be determined by choosing the concentration by which we are not anxious about the increase in the oxidant of IL-11, for example. The nonionic surfactant of the present invention also has the effect of improving the remelting nature of a freeze-dried cake.
[0015]
Adding sugars to a prepared solution by the present invention means adding sugars in the aqueous solution (prepared solution) used when preparing before the lyophilization treatment of IL-11. As these sugars, sugar-alcohol, such as disaccharides, such as monosaccharides, such as glucose, xylose, galactose, and fructose, lactose, malt sugar, purified sucrose, and sucrose, mannitol, sorbitol, xylitol, and inositol, etc. are mentioned specifically. Preferably, they are purified sucrose and lactose. The sugars of the present invention can also be blended combining 1 type or 2 type or more. The sugars of the present invention also have the function of the improvement effect of the stability of the pharmaceutical preparation containing IL-11 and the freeze-dried cake at the time of remelting of a collapsibility improvement.
[0016]

In the present invention, as for the concentration of the sugars to add, 0.1 thru/or 50 % by weight are preferable, and 0.5 thru/or 5 % by weight are still more preferable. This concentration is 0.1. When thinner than the weight %, we are anxious about proteinic potency deterioration and the increase in a related substance. When deeper than 50 % by weight, we are anxious about the deposit of sugars etc.
In the combination of IL-11, by choosing a glycine, a buffer and a nonionic surfactant, and/or sugars, Become possible to attain the the-object-of-this-invention effect, and as a buffer A phosphoric acid buffer, It is polyoxyethylene sorbitan fatty acid ester (preferably) as sodium phosphate and a nonionic surfactant preferably. It is polysorbate 80 and the polysorbate 20, and it is polysorbate 80 still more preferably and it is possible to attain the further effect by choosing purified sucrose and/or lactose as the polysorbate 80 of vegetable origin and sugars most preferably.
[0017]
The present invention is choosing the above technique and a means to avoid that the surface of IL-11 at the time of lyophilization treatment becomes canal-like is provided. As a means to avoid that the surface of IL-11 at the time of lyophilization treatment becomes canal-like besides the present invention, there being the method of controlling freeze-drying conditions, and crystallizing one buffer specifically, or pretreating under temperature conditions (-20 degree C thru/or 0 degree C) before 2 lyophilization treatment -- it is mentioned by carrying out. Of course, it is also possible to adopt these two or more simultaneously and to apply them.
[0018]
The medicinal preparation for parenteral which introduces the means of a nebula prevention method in this way at the time of remelting of lyophilized products, and is manufactured will not be restricted especially if it is dosage forms permitted usually in medicine manufacture. in addition -- the freeze-drying conditions at the time of manufacture of lyophilized products remove the conditions related to pretreatment conditions -- the very thing -- publicly known conditions can be set up suitably.
[0019]
An example of the manufacturing method of IL-11 lyophilized products of the present invention is as follows. Liquid is prepared by mixing the aqueous solution which contains protein at high concentration, and the buffer solution for dilution which mixed and dissolved a glycine, a nonionic surfactant, sugars, etc. so that it might become the last request concentration. A container is filled up with the prepared liquid and it is made to freeze below -30 degrees C. Then, it can decompress and the lyophilized products of the present invention can be prepared by making it dry.
[0020]
The excipient (for example, a solubilizing agent, a preservative, stabilizer, an emulsifier, a soothing agent, an isotonizing agent, a buffer, an excipient, colorant, a thickening agent) usually added by the medicinal composition for parenteral can also be blended with the medicinal composition for parenteral of the IL-11 content lyophilized products of the present invention. For example, L-arginine and cyclodextrin are mentioned as a solubilizing agent. Sodium benzoate, methyl parahydroxybenzoate, etc. are mentioned as a preservative. Lecithin etc. are mentioned as an emulsifier. Benzyl alcohol, chlorobutanol, etc. are mentioned as a soothing agent. Sodium chloride etc. are mentioned as an isotonizing agent. The malt sugar etc. for which an excipient is used also as sugars of the present invention are mentioned. Hyaluronic acid etc. are mentioned as a thickening agent.
[Effect of the Invention]
[0021]
In the present invention, the IL-11 content lyophilized products which have good remelting nature when remelting lyophilized products were provided, and improvement in the convenience in the clinical spot of IL-11 content lyophilized products was attained. The IL-11 content lyophilized products of the present invention show the

outstanding stability in solution states or a freeze-drying state. In particular, in a freeze-drying state, room temperature preservation is possible and lyophilized products show the outstanding remelting nature and the nebula preventive effect at the time of remelting further.

[Best Mode of Carrying Out the Invention]

[0022]

Although the present invention is described in an working example, a reference example, the example of an experiment, etc. below, the present invention is not limited to these.

[0023]

It describes about the test method used in the working example below. The test method 1 was used for measurement of turbidity.

Turbidity evaluation of the remelting liquid by the [test-method 1] spectrometry

Water for injection (1.2 mL) is injected into the lyophilized products as for which 5 mg contains IL-11 as turbidity evaluation of remelting liquid, and the absorbance in OD650 nm of the remelting liquid after 3, 5, and a 7-minute lapse is measured. It referred to two news described below about the turbidity valuation method of the solution by spectrometry.

(1) Drugs research 26 (4) Examination about the turbidity valuation method in the clarity-and-color-of-solution examination of 223-230 "(1955) drugs"

(2) J. Pharm. Sci. Tech. and 48 (2) 64-70 "(1994) A turbidimetric method to determine visual appearance of protein solutions"

[Test method 2] Check of the related substance by SDS-PAGE (argentation)

Let the dimer and decomposition product by a covalent bond be a measuring object as a check of the related substance by SDS-PAGE (argentation). Gel uses the polyacrylamide gel (large gel format) which has the acrylamide concentration inclination of 10 thru/or 20 %. Sample 50 mug is added on each lane, and bottom migration of 45-mA constant current is performed. It dyes according to an attachment description, using an argentation kit (2D-argentation reagent and the "first" and II, Daiichi Pure Chemicals make) in dyeing. Only qualitative evaluation by check visually is performed about evaluation.

[0024]

A fixed quantity (RP-HPLC) of the amount of related substances by the [test-method 3] high-speed liquid chromatography

Use a fixed quantity [the amount of related substances by high-speed liquid chromatography], and let a decomposition product, Met122 oxidant, and the change object of the polymer by a covalent bond be measuring objects. It examines by liquid chromatography about 100 muL of the solution as for which 0.65 mg contains IL-11 on the following conditions. The area percentage of peak areas other than IL-11 is measured for a peak area by automatic integration.

Detector: Ultraviolet absorptiometer

Column: Fill up the stainless steel tube of the about 4.6 inner diameter mm, and length about 10 cm with styrene divinylbenzene copolymer for liquid chromatographs of 10 micrometer.

Column temperature: Constant temperature near 25 degree C.

Mobile phase A: The solution which added water to trifluoroacetic acid 1.0 g, and was set to 1000 mL.

Mobile phase B: The solution which added acetonitrile 800 mL for liquid chromatographs, and water to trifluoroacetic acid 1.0 g, and was set to 1000 mL.

Liquid sending of a mobile phase: The mixture ratio of the mobile phase A and the mobile phase B is changed as follows, and it is concentration gradient control.

[0025]
[Table 1]

注入後からの時間（分）	移動相A（%）	移動相B（%）
0 ～ 2	70	30
2 ～ 12	70 → 50	30 → 50
12 ～ 37	50 → 36	50 → 64
37 ～ 38	36	64

Flow rate: Per minute 0.5 mL
[0026]
A fixed quantity (SE-HPLC) of the abundant body weight by the [test-method 4] high-speed liquid chromatography
Use a fixed quantity [abundant body weight] and let the polymer by the noncovalent bond and a covalent bond be a measuring object. It examines by liquid chromatography about 50 muL of the solution as for which 0.6 mg contains IL-11 on the following conditions. The area percentage of the peak area in which retention time is smaller than IL-11 is measured for a peak area by automatic integration.
Detector: Ultraviolet absorptiometer
Column: Fill up the stainless steel tube of the about 7.8 inner diameter mm, and length about 30 cm with the porous silica gel for liquid chromatographs of 5 micrometer.
Column temperature: Constant temperature near 4 degree C.
Mobile phase: The solution which melted glycine 7.5 mg, sodium chloride 29.2 g, and 2-morpholino ethane-sulfonic-acid 9.75 g in water 750 mL, added the sodium hydroxide test solution, adjusted pH to 6.0, added water, and was set to 1000 mL.
Flow rate: Adjust so that the retention time of IL-11 may become about 9 minutes.
[0027]
As stabilizer, the pharmaceutical preparation of the working examples 1 thru/or 9 and the comparative examples 1 and 2 was prepared, and the nonionic surfactant and the addition effect of sugars were checked.
[Work example 1]
[0028]
The sample solution which added the sugars described in Table 2 on the basis of IL-11 concentration 5 mg/mL, sodium phosphate buffer solution concentration 10 mM, and glycine concentration 300 mM was prepared.
[Work example 2]
[0029]
The sample solution was adjusted like the working example 1 except the sugars described in Table 2.
[Work example 3]
[0030]
The sample solution was adjusted like the working example 1 except the sugars described in Table 2.
[0031]
(Comparative example 1)

The sample solution was adjusted like the working example 1 except not adding sugars.
[Table 2]

	添加糖		結果 上段：力価残存率(%) 下段：多量体量(%)				
	種類	添加濃度（重量%）	初期	5℃／3箇月	5℃／6箇月	40℃／3箇月	40℃／6箇月
実施例1	イノシトール	2.5	100	109	121	89	84
			4.10	4.16	4.11	5.65	6.67
実施例2	ラクトース	5	100	101	89	95	87
			4.12	4.00	4.05	4.05	3.91
実施例3	精製白糖	5	100	121	110	92	97
			4.05	4.04	4.15	4.05	4.09
比較例1	－	－	100	100	85	60	57
			5.56	5.79	5.96	10.10	10.64

[0032]
the sample solution of the working examples 1-3 and the comparative example 1 -- after sterile filtration and the bottom of a non-fairy ring boundary -- every [1 mL] -- the vial bottle which carried out sterilization treatment previously was filled up, a capping blockade was performed after freeze-drying, and present invention pharmaceutical preparation was obtained. Present invention pharmaceutical preparation and comparison pharmaceutical preparation were saved at 5 degrees C and 40 degrees C, and the comparative examination was carried out about stability. A test result is shown in Table 2. So that clearly also from Table 2 in the sugar additive-free pharmaceutical preparation of a comparative example, By the pharmaceutical preparation of sugar addition of the working example 1 thru/or 3, the preventive effect was especially accepted under the elevated-temperature (40 degrees C) condition to the increase in abundant body weight and decline in a potency survival rate having been accepted notably about the upward tendency of abundant body weight, and the deterioration tendency of the potency survival rate. In particular in the pharmaceutical preparation which added purified sucrose of the working example 3, a clear change of abundant body weight and a potency survival rate was not accepted on which preservation conditions. Therefore, it can say that present invention pharmaceutical preparation is very extremely stable pharmaceutical preparation.

[Work example 4]
[0033]
The sample solution which fluctuated the addition amount of the purified sucrose described in Table 3 on the basis of IL-11 concentration 5 mg/mL, sodium phosphate buffer solution concentration 10 mM, and glycine concentration 300 mM and polysorbate 80 was prepared.
[Work example 5]
[0034]
The sample solution was adjusted like the working example 4 except having fluctuated the addition amount of the purified sucrose described in Table 3, and polysorbate 80.
[Work example 6]
[0035]
The sample solution was adjusted like the working example 4 except having fluctuated the addition amount of the purified sucrose described in Table 3, and polysorbate 80.
[Work example 7]
[0036]
The sample solution was adjusted like the working example 4 except having fluctuated the addition amount of the purified sucrose described in Table 3, and polysorbate 80.
[0037]
(Comparative example 2)
The sample solution was adjusted like the working example 4 except not having added purified sucrose and polysorbate 80.
[Table 3]

	精製白糖濃度（重量%）	ポリソルベート80濃度（重量%）	結果	上段：類縁物質量(%) 下段：多量体量(%)	
			初期	40℃／1箇月	40℃／3箇月
実施例 4	1.25 %	0.0010 %	1.58	1.53	1.78
			2.45	2.61	2.55
実施例 5	1.25 %	0.0005 %	1.59	1.56	---
			2.57	2.65	---
比較例 2	–	–	1.57	2.22	3.54
			3.43	4.30	4.86
実施例 6	1.25 %	–	1.57	1.55	---
			2.51	2.63	---
実施例 7	–	0.0010 %	1.59	2.23	---
			3.45	4.49	---

---: Don't measure.
[0038]
the sample solution of the working examples 4-7 and the comparative example 2 -- after sterile filtration and the bottom of a non-fairy ring boundary -- every [1 mL] -- the vial bottle which carried out sterilization treatment previously was filled up, a capping blockade was performed after freeze-drying, and present invention pharmaceutical preparation was obtained. Present invention pharmaceutical preparation and comparison pharmaceutical preparation were saved at 40 degrees C, and the comparative examination was carried out about stability. A test result is shown in Table 3 and Fig.1 - Fig.3.A test sample for chemical analysis is 50 mug/Lane. By the purified sucrose content pharmaceutical preparation (working examples 4 thru/or 6) of the present invention, the increase was not accepted to be also the amount of related substances abundant body weight to the related substance and the polymer increasing under an

elevated-temperature (40 degrees C) condition in purified sucrose of a comparative example, and polysorbate 80 additive-free pharmaceutical preparation (comparative example 2) so that clearly from Table 3. Although the remarkable increase in a covalent bond type dimer (SDS-Stable Dimer) and the increase in a low-molecular-weight decomposition product were accepted by the comparative example also from Fig.1 - Fig.3, in present invention pharmaceutical preparation, the covalent bond type dimer was the degree which only one band increased slightly.Therefore, it can say that present invention pharmaceutical preparation is very extremely stable pharmaceutical preparation. The meaning of each lane in Fig.1 - Fig.3 is as in the following table 4.
[Table 4]

	Lane 1	Lane 2	Lane 3	Lane 4	Lane 5	Lane 6	Lane 7
図1	比較例2 (開始時)	実施例4 (開始時)	実施例6 (開始時)	実施例7 (開始時)	実施例5 (開始時)	IL-11 標準品	-
図2	比較例2 (40℃／1箇月)	実施例4 (40℃／2週)	実施例4 (40℃／1箇月)	実施例6 (40℃／1箇月)	実施例7 (40℃／1箇月)	実施例5 (40℃／1箇月)	IL-11 標準品
図3	比較例2 (開始時)	比較例2 (40℃／3箇月)	実施例4 (開始時)	実施例4 (40℃／3箇月)	-	-	-

[Work example 8]
[0039]
The sample solution which fluctuated the addition amount of the purified sucrose described in Table 5 on the basis of IL-11 concentration 5 mg/mL, sodium phosphate buffer solution concentration 10 mM, and glycine concentration 300 mM and polysorbate 80 was adjusted.
[Work example 9]
[0040]
The sample solution was adjusted like the working example 8 except having fluctuated the addition amount of polysorbate 80 described in Table 5.
[0041]
[Table 5]

	精製白糖濃度 (重量%)	ポリソルベート80濃度 (重量%)	溶状 吸光度(650 nm)		
			3分後	5分後	7分後
実施例4	1.25 %	0.0010 %	0.007	0.005	0.005
実施例5	1.25 %	0.0005 %	0.013	0.008	0.006
実施例8	1.25 %	0.0002 %	0.013	0.008	0.008
実施例9	1.25 %	0.0001 %	0.012	0.009	0.008
比較例2	-	-	0.062	0.038	0.023
実施例6	1.25 %	-	0.033	0.018	0.012
実施例7	-	0.0010 %	0.017	0.010	0.007

the sample solution of the working examples 4-9 and the comparative example 2 -- sterile environmental Shimo after sterile filtration -- every [1 mL] -- the vial bottle which carried out sterilization treatment previously was filled up, an after-freeze-drying capping blockade was performed, and present invention pharmaceutical preparation was obtained. The comparative examination was carried out about the remelting nature by the water for injection of present invention pharmaceutical preparation and comparison pharmaceutical preparation. A test result is shown in Table 5. The pharmaceutical preparation of the comparative example showed the low absorbance (namely, low turbidity or high clarity) by the present invention pharmaceutical preparation prepared in the working example to the comparatively high absorbance (namely, high turbidity or low clarity) having been shown.

[Brief Description of the Drawings]

[0042]

[Drawing 1]It is an analysis result of SDS-PAGE (argentation) of the time of the start of test [of a sample], and a 40 degree-C [/] one-month preservation sample.

[Drawing 2]It is an analysis result of SDS-PAGE (argentation) of the time of the start of test [of a sample], and a 40 degree-C [/] one-month preservation sample.

[Drawing 3]It is an analysis result of SDS-PAGE (argentation) of the time of the start of test [of a sample], and a 40 degree-C [/] one-month preservation sample.

DESCRIPTION OF DRAWINGS

[Brief Description of the Drawings]

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[Drawing 3]It is an analysis result of SDS-PAGE (argentation) of the time of the start of test [of a sample], and a 40 degree-C [/] one-month preservation sample.

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(54) **Title:** HGF PREPARATION

(54) 発明の名称: ＨＧＦ製剤

(57) **Abstract:** Disclosed is an HGF preparation containing HGF and purified sucrose. This HGF preparation is characterized in that it remains stable even when stored for a long time.

(57) 要約: 本発明はＨＧＦおよび精製白糖を含有するＨＧＦ製剤である。本ＨＧＦ製剤は長期間の保存でも安定であるという特長を有する。

WO 2008/102849 A1

明 細 書

HGF製剤

技術分野

[0001] 本発明は、HGF(Hepatic Growth Factor、肝細胞増殖因子)を含有する製剤に関する。

背景技術

[0002] HGFは、中村らにより発見された、成熟肝細胞に対して最も強力な増殖促進活性を持つ生理活性ペプチドであり(例えば、非特許文献1参照)、近年生物工学的手法により量産が可能になった(例えば、非特許文献2参照)。このHGFは、肝炎や肝硬変のみならず、腎炎や癌などに対する治療・予防薬として、また制癌剤の副作用抑制剤や創傷治癒剤などへの適用も期待されている。

HGFの製剤としては、特許文献1に、HGFにアルブミン、ヒト血清、ゼラチン、ソルビトール、マンニトール、キシリトールなどを安定化剤として含有させた水溶液製剤が開示されている。しかしながら、前記HGF水溶液製剤は保存中にHGFが凝集、白濁、ゲル化が進行するという難点があり、また重合体が形成されるなど物理化学的安定性が低く、生物活性が低下するという問題がある。

[0003] この問題を解決するために、特許文献2には、HGFにアルギニン、リジン、ヒスチジン、グルタミン、プロリン、グルタミン酸、アスパラギン酸などを安定化剤として含有させた凍結乾燥製剤が開示されている。また、特許文献3には、HGFにグリシン、アラニン、ソルビトール、マンニトール、デキストラン硫酸などを安定化剤として含有させた凍結乾燥製剤が開示されている。

上記の凍結乾燥製剤は、HGFの安定化をある程度達成することができるが、さらに安定化効果のよいHGF製剤が望まれている。

特許文献1:国際公開WO90／10651号パンフレット

特許文献2:国際公開WO00／72873号パンフレット

特許文献3:特開平9－25241号公報

非特許文献1:Biochem. Biophys. Res. Commun. , 122, 1450, 1984

非特許文献2:Nature, 342, 440, 1989

発明の開示

発明が解決しようとする課題

[0004] 本発明は、従来のHGF製剤に比べて、長期間の保存でもより安定なHGF製剤を提供することを目的とする。

課題を解決するための手段

[0005] 本発明者らは、上記課題を解決するために鋭意研究を重ねた結果、HGFに精製白糖を添加することにより、HGFの重合体生成が阻害され、安定なHGF製剤が得られることを見出し、この知見に基づいてさらに研究を進め、本発明を完成するに至った。

[0006] すなわち、本発明は、

[1]HGFおよび精製白糖を含有するHGF製剤、

[2]精製白糖の含有量が、HGF1重量部に対して0.01～9重量部である前記[1]に記載のHGF製剤、

[3]さらに、中性アミノ酸を含有する前記[1]または[2]に記載のHGF製剤、

[4]中性アミノ酸がアラニンである前記[3]に記載のHGF製剤、

[5]さらに、緩衝剤を含有する前記[1]～[4]のいずれかに記載のHGF製剤、

[6]緩衝剤がクエン酸塩である前記[5]に記載のHGF製剤、

[7]さらに、塩化ナトリウムを含有する前記[1]～[6]のいずれかに記載のHGF製剤、

[8]HGFおよび精製白糖の他に、さらに中性アミノ酸、塩化ナトリウム、緩衝剤および界面活性剤を含有する前記[1]または[2]に記載のHGF製剤、

[9]中性アミノ酸がアラニンであり、緩衝剤がクエン酸塩であり、界面活性剤がポリソルベートである前記[8]に記載のHGF製剤、

[10]凍結乾燥製剤である前記[1]～[9]のいずれかに記載のHGF製剤、

[11]HGFに精製白糖を添加してHGF重合体生成を抑制することを特徴とするHGFの安定化方法、および

[12]精製白糖の添加量がHGF1重量部に対して0.01～9重量部である前記[11]

に記載の安定化方法、

に関する。

発明の効果

[0007] 本発明のHGF製剤は、長期間保存しても、従来のHGF製剤に比べてより安定であるという効果を有する。

発明を実施するための最良の形態

[0008] 本発明は、HGFおよび精製白糖を含有してなるHGF製剤である。

[0009] 本発明の有効成分であるHGFは、医薬として使用できる程度に精製されたものであれば、種々の方法で調製されたものを用いることができる。また、本発明に用いるHGFはアミノ酸5残基が欠失したデリーションタイプ(dLeHGF)であってもよい。

HGFの調製方法としては、各種の方法が知られており、例えば、ラット、ウシ、ウマ、ヒツジなどの哺乳動物の肝臓、脾臓、肺臓、骨髄、脳、腎臓、胎盤などの臓器、血小板、白血球などの血液細胞や血漿、血清などから抽出、精製して得ることができる。また、HGFを産生する初代培養細胞や株化細胞を培養し、培養物(培養上清、培養細胞など)から分離精製してHGFを得ることもできる。あるいは遺伝子工学的手法によりHGFをコードする遺伝子を適切なベクターに組込み、これを適当な宿主に挿入して形質転換し、この形質転換体の培養物から目的とする組換えHGFを得ることができる(例えば、Nature,342,440,1989など参照)。上記の宿主細胞は特に限定されず、従来から遺伝子工学的手法で用いられている各種の宿主細胞、例えば大腸菌、枯草菌、酵母、糸状菌、植物または動物細胞などを用いることができる。

[0010] より具体的には、HGFを生体組織から抽出精製する方法としては、例えば、ラットに四塩化炭素を腹腔内投与し、肝炎状態にしたラットの肝臓を摘出して粉砕し、S－セファロース、ヘパリンセファロースなどのゲルカラムクロマトグラフィー、HPLCなどの通常の蛋白質精製法にて精製することができる。また、遺伝子組換法を用い、ヒトHGFのアミノ酸配列をコードする遺伝子を、ウシパピローマウィルスDNAなどのベクターに組み込んだ発現ベクターによって動物細胞、例えば、チャイニーズハムスター卵巣(CHO)細胞、マウスC127細胞、サルCOS細胞などを形質転換し、その培養上清より得ることができる。

[0011] 本発明の精製白糖は、第十五改正日本薬局方に収載されている精製白糖を安定化剤として好適に使用することができる。精製白糖の添加量は、HGF1重量部に対して、0．01～9重量部が好ましく、特に0．1～5重量部の範囲が好ましいが、下限のより好ましい値は0．5重量部であり、上限のより好ましい値は4重量部、さらに好ましい値は3重量部、特に好ましい値は2重量部である。

[0012] 本発明の製剤は種々の製剤形態（例えば、液剤、固形剤、カプセル剤、クリーム剤、スプレー剤など）をとりうるが、一般的には有効成分であるHGFおよび精製白糖のみまたはそれらと慣用の添加物（担体など）と共に水溶性製剤、凍結乾燥製剤などとするのが好ましく、とりわけ凍結乾燥製剤が好ましい。

[0013] 本発明のHGF製剤は、HGFおよび精製白糖を含有する水溶液とすることで水溶液製剤とすることができ、また該水溶液を通常の凍結乾燥方法で凍結乾燥することでHGF凍結乾燥製剤を製造できる。前記水溶液における精製白糖の含有量は0．1重量％以上、好ましくは0．5重量％以上であって、9重量％以下、好ましくは5重量％以下、より好ましくは4重量％以下、さらに好ましくは3重量％以下、特に好ましくは2重量％以下である。凍結乾燥製剤における精製白糖の含有量は10～80重量％が好ましく、特に20～60重量％が好ましい。例えば、凍結乾燥製剤は、HGFを適切な溶剤（例えば、滅菌水、注射用蒸留水、緩衝液、生理食塩水など）に溶解した後、精製白糖を好ましくは0．1～5重量％、特に好ましくは0．5～2重量％となるように添加し、必要に応じて、精製白糖以外の安定化剤、緩衝剤、界面活性剤、塩化ナトリウムなどを加え、フィルターなどで濾過して滅菌し、バイアルまたはアンプルに注入して凍結乾燥する。フィルターは、ポアサイズ0．22μm以下の滅菌用フィルターを使用するのが好ましい。滅菌用フィルターとしては、例えば、デュラポア（登録商標、日本ミリポア株式会社製）またはザルトポア2（登録商標、ザルトリウス株式会社製）などが挙げられる。凍結乾燥方法としては、例えば、常圧下で冷却凍結する凍結過程、溶質に拘束されない自由水を減圧下で昇華乾燥する一次乾燥過程、溶質固有の吸着水や結晶水を除去する二次乾燥過程の3つの単位操作による方法が挙げられる。凍結過程の冷却温度は－60℃～－40℃が好ましく、一次乾燥過程の温度は－50℃～0℃が好ましく、さらに二次乾燥過程の温度は4℃～40℃が好ましい。真空圧力は0

．1～1．5Paが好ましく、特に0．5～1．2Paが好ましい。凍結乾燥後の乾燥庫内は復圧させる。復圧の方法としては、無菌の空気または不活性ガス（例えば、無菌窒素ガス、無菌ヘリウムガスなど）を庫内に送入して約70～100kPa、好ましくは約80～95kPaまで一次復圧し、次いで大気圧まで復圧（二次復圧）する方法が好ましい。バイアルの打栓は、一次復圧後に行うのが好ましい。

[0014] 安定化剤は精製白糖のみでもよいが、精製白糖と共に従来安定化剤として用いられていたグリシン、アラニン、アルギニン、リジン、ヒスチジンなどのアミノ酸、ヘパリン、デキストラン硫酸などの多糖類、ソルビトール、マンニトールなどの糖アルコールなどを好適に使用できる。これらのうち、アミノ酸が好ましく、とりわけアミノ酸のうちグリシン、アラニンなどの中性アミノ酸が好ましい。これらの精製白糖以外の安定化剤の添加量は特に制限されないが、例えばグリシン、アラニンなどの中性アミノ酸を用いる場合の添加量は、精製白糖1重量部に対して、0．01～50重量部が好ましく、0．1～20重量部がより好ましい。

安定化剤として、精製白糖と共に中性アミノ酸などの従来の安定化剤を併用することにより、精製白糖のみを用いる場合に比べて安定性をより向上させることができる。

[0015] 本発明で用いられる緩衝剤としては、例えばリン酸緩衝液、クエン酸緩衝液などが挙げられる。緩衝剤は、再溶解後の水溶液のpHを調整しHGFの溶解性を保つ作用を有する。緩衝剤は、再溶解後の水溶液のpHが4．5～6．5となるものが好ましい。緩衝剤として好ましいものは、クエン酸緩衝液が挙げられ、特に好ましくはクエン酸ナトリウム緩衝液が挙げられる。このクエン酸緩衝液は、再溶解後の水溶液中でのHGFの安定化にも寄与する。緩衝剤の添加量は、凍結乾燥製剤を製造する際の凍結乾燥直前の水溶液中の濃度が、1～100mMの範囲となるようにするのが好ましい。

[0016] 本発明で用いられる界面活性剤としては、例えばポリソルベート20、ポリソルベート80、プルロニックF－68、ポリエチレングリコールなどが挙げられ、二種以上を併用してもよい。界面活性剤として特に好ましくは、ポリソルベート系界面活性剤が好ましく、とりわけポリソルベート80が好ましい。HGFが容器の材質であるガラスや樹脂などに吸着しやすいため、このような界面活性剤を添加することによって、再溶解後のHGFの容器への吸着を防止することができる。界面活性剤の添加量は、凍結乾燥製

剤を製造する際の凍結乾燥直前の水溶液中の濃度が、0．001～2．0重量%の範囲であるのが好ましい。

[0017] 塩化ナトリウムは、HGFの溶解性を保つ作用を有する。すなわち、例えば実施例で使用した組換HGFの場合、塩化ナトリウムの添加により組換HGFの溶解度が向上し、特に300mM以上では著しく溶解性が向上する。塩化ナトリウムの添加量は浸透圧比により制限を受けるが、一般的に用いられる注射剤の浸透圧比を示す量でよい。特に医療用または動物薬用注射剤の浸透圧比として許容される浸透圧比1～3となる量が好ましい。通常、凍結乾燥製剤を製造する際の凍結乾燥直前の水溶液中の塩化ナトリウム濃度が150～1000mMとすることが好ましい。

[0018] 本発明においては、製剤化に必要な他の添加剤、例えば、溶解補助剤、酸化防止剤、無痛化剤、等張化剤などを含んでもよい。

[0019] 上記の如くして得られる本発明の製剤、例えば凍結乾燥製剤は、使用に当たっては、HGF濃度が0．1～40mg／mLとなるように注射用蒸留水に溶解し、注射液として用いることができる。さらに、凍結乾燥製剤を含有するクリーム剤、スプレー剤などの外用剤とすることもできる。

実施例

[0020] 以下に実施例を用いて本発明を説明するが、本発明はこれらに限定されるものではない。なお、本実施例においては、HGFとして5アミノ酸欠失型HGFを用いた。HGFの重合体の面積百分率(%)(以下、重合体含量(%)という)は高速液体クロマトグラフィー(HPLC)にて定量した値を用いて下記式1により求めた。

式1

[0021]

$$\text{重合体含量（%）} = \frac{A_A}{A_M + A_A} \times 100$$

式中、A_MはHGFピーク面積、A_Aは重合体ピーク面積を示す。

[0022] (HPLC条件)

カラム:ゲルろ過カラム(商品名:Superdex 200 10／300、アマシャムバイオサイエンス社製)

移動相:塩化ナトリウム58．44g、クエン酸三ナトリウム二水和物2．94g、ポリソルベート80　0．1gを水に溶かし、1Lとした液をA液とする。塩化ナトリウム58．44g、クエン酸一水和物2．10g、ポリソルベート80　0．1gを水に溶かし、1Lとした液をB液とする。A液にB液を加え、pH6．0に調整後、0．45μmのフィルター(商品名:Millcup－HV、孔径:0．45μm、ミリポア社製)でろ過し、使用前に脱気する。室温で保存し、2週間以内に使用する。

カラム温度:25℃

流量:0．5mL／分

検液注入量:25μL

分析時間:60分

検出器:吸光光度計

検出波長:280nm

サンプルクーラー:5分

分子量マーカーは、Gel　Filtration　Standard(カタログ番号:151－1901、Bio－Rad社製)バイアル1本に水500μLを加え溶解し、少量試液調整用ろ過フィルター(商品名:Ultrafree－MC、孔径:0．45μm、ミリポア社製)でろ過し、2～8℃で保存し、3ヶ月以内のものを使用する。

[0023]　また、下記実施例および試験例で用いた希釈用緩衝液は下記のように調製した。

(希釈用緩衝液の調製)

塩化ナトリウム1．1688g、クエン酸三ナトリウム二水和物2．94g、ポリソルベート80　0．3gを超純水に溶かし、全量1Lとした液をA液とした。塩化ナトリウム1．1688g、クエン酸一水和物2．10g、ポリソルベート80　0．3gを超純水(超純水製造装置(商品名:MilliQ　Gradient、ミリポア社製)を用いて調製、以下同じ)に溶かし、全量1Lとした液をB液とした。A液にB液を加えてpH6．0に調整し、希釈用緩衝液(1)とした。

塩化ナトリウム17．53g、クエン酸三ナトリウム二水和物2．94g、ポリソルベート80　0．1gを超純水に溶かし、全量1Lとした液をC液とした。塩化ナトリウム17．53g、クエン酸一水和物2．10g、ポリソルベート80　0．1gを超純水に溶かし、全量1Lとした液

をD液とした。C液にD液を加えてpH6．0に調整し、希釈用緩衝液(2)とした。

[0024] [実施例1]

希釈用緩衝液(1)に、5アミノ酸欠失型HGF(以下、単にHGFという)を10mg／mLとなるように添加し、精製白糖を0．5重量%濃度となるように添加することによって下記表1の組成の溶液が得られた。

[表1]

成分	濃度
HGF	10mg／ml
クエン酸三ナトリウム二水和物	10mM
塩化ナトリウム	300mM
ポリソルベート80	0．03重量%
精製白糖	0．5重量%

得られた上記溶液をバイアル(φ23×43mm)に2mLずつ無菌的に分注した。バイアルにゴム栓を半打栓し、トレイに整列させ、凍結乾燥機(トリオマスター;共和真空技術株式会社製)に入れ、下記表2に記載した条件で凍結乾燥を実施した。なお、表中の→は、温度を変化させたことを示す。

[表2]

	凍結過程		一次乾燥過程		二次乾燥過程	
温度(℃)	10→−50	−50	−50→−20	−20	−20→20	20
真空度(Pa)	−	−	1	1	1	1
時間	6	5	8	33.5	8	11

凍結乾燥終了後、トリオマスター庫内に無菌窒素を送入して復圧(庫内圧力:88.0kPa;一次復圧)し、ゴム栓を全打栓してから無菌窒素でトリオマスター庫内を大気圧に戻し(二次復圧)、バイアルを取り出した後、速やかにバイアルをキャップで締めた。このようにして、本発明のHGF凍結乾燥製剤を得た。

本HGF凍結乾燥製剤における精製白糖の含有量は、HGF1重量部に対して0．5重量部であり、HGF凍結乾燥製剤に対して26．3重量%である。

[0025] [実施例2]

精製白糖の添加濃度を1．0重量％とする以外は、実施例1と同様にして、HGF凍結乾燥製剤を得た。

本HGF凍結乾燥製剤における精製白糖の含有量は、HGF1重量部に対して1重量部であり、HGF凍結乾燥製剤に対して41．7重量％である。

[0026] ［実施例3］

精製白糖の添加濃度を2．0重量％とする以外は、実施例1と同様にして、HGF凍結乾燥製剤を得た。

本HGF凍結乾燥製剤における精製白糖の含有量は、HGF1重量部に対して2重量部であり、HGF凍結乾燥製剤に対して58．8重量％である。

[0027] ［実施例4］

精製白糖の添加濃度を1．0重量％とし、さらにアラニンを5mg／mLの濃度で添加する以外は、実施例1と同様にして、HGF凍結乾燥製剤を得た。

本HGF凍結乾燥製剤における精製白糖の含有量は、HGF1重量部に対して1重量部であり、HGF凍結乾燥製剤に対して34．5重量％である。

[0028] ［比較例1］

添加剤を精製白糖の代わりに、アラニンを20mg／mLの濃度で添加する以外は、実施例1と同様にして、HGF凍結乾燥製剤を得た。

[0029] ［比較例2］

精製白糖を添加しない以外は、実施例1と同様（以下、基本処方という）にして、HGF凍結乾燥製剤を得た。

[0030] ［試験例1］

上記実施例および比較例記載の凍結乾燥製剤を50℃で保存し、1週間後にサンプリングし、タンパク質濃度5mg／mLになるように希釈用緩衝液（2）で希釈し、HPLCを用いて定量後、上記式1から重合体含量（％）を算出した。その結果を下記表3に示す。

［表3］

	添加剤（濃度）		重合体含量（%）		
実施例No	精製白糖	アラニン	凍結乾燥前	保存開始時（イニシャル）	50℃－1週間
実施例1	＋（0.5重量%）	－	0．47	0．61	1．90
実施例2	＋（1.0重量%）	－	0．48	0．58	1．29
実施例3	＋（2.0重量%）	－	0．48	0．56	0．92
実施例4	＋（1.0重量%）	＋（5mg/mL）	0．44	0．56	0．90
比較例1	－	＋（20mg/mL）	0．50	0．63	2．32
比較例2	－	－	0．55	0．84	6．12

表3から明らかなように、基本処方に精製白糖または精製白糖とアラニンを添加した本発明のHGF製剤は、基本処方または基本処方にアラニンを添加したHGF製剤に比べて、顕著に重合体含量が抑制されていた。

[0031] [試験例2]

希釈用緩衝液（20mM クエン酸緩衝液，1M塩化ナトリウム,ポリソルベート80 0．01重量%）に、HGFを10mg／mLとなるように添加し、精製白糖を0重量%濃度、1重量%濃度、5重量%濃度、10重量%または20重量%濃度となるように添加した試料溶液1～5を各50μL調製した。各試料溶液は、測定に供するまで、約24時間凍結した。凍結した各試料溶液を再融解し、HGFの分子量（約84kDa）分布を動的光散乱（Dynamic Light Scattering, DLS）法で測定した。測定装置は、蛋白質溶液専用のProtein－Solution社製Dyna－Proを用いた。測定温度は4℃に設定した。バックグランドには、HGFを含まない希釈用緩衝液（各50μL）を用いた。各試料溶液中におけるHGFの分子量の多分散度（Pd%）を表4に示す。

[表4]

	HGF（mg/mL）	精製白糖（重量%）	Pd%
試料溶液1	10	0	22．8
試料溶液2	10	1	21．7
試料溶液3	10	5	13．6
試料溶液4	10	10	32．3
試料溶液5	10	20	28．5

表4から明らかなように、精製白糖を1重量%または5重量%となるように添加した試料溶液2および3では、Pd%値が極めて小さく、HGFの分子量分布が単分散（単一

様相）であることを示した。試料溶液1、4および5では、HGFの分子量分布ピークがブロードであり、異なる分子量を有するものを含むことが示唆された。また試料溶液4および5では高分子側にもう一つピークが現れ、試料溶液5においては、さらに低分子のものが多量に含まれていることを示すピークも現れた。

産業上の利用可能性

[0032] 本発明によれば、医薬として有用な保存安定性の優れたHGF製剤を提供できる。

請求の範囲

[1] HGFおよび精製白糖を含有するHGF製剤。

[2] 精製白糖の含有量がHGF1重量部に対して0．01～9重量部である請求の範囲第1項に記載のHGF製剤

[3] さらに、中性アミノ酸を含有する請求の範囲第1または2項に記載のHGF製剤。

[4] 中性アミノ酸がアラニンである請求の範囲第3項に記載のHGF製剤。

[5] さらに、緩衝剤を含有する請求の範囲第1～4項のいずれかに記載のHGF製剤。

[6] 緩衝剤がクエン酸塩である請求の範囲第5項に記載のHGF製剤。

[7] さらに、塩化ナトリウムを含有する請求の範囲第1～6項のいずれかに記載のHGF製剤。

[8] HGFおよび精製白糖の他に、さらに中性アミノ酸、塩化ナトリウム、緩衝剤および界面活性剤を含有する請求の範囲第1または2項に記載のHGF製剤。

[9] 中性アミノ酸がアラニンであり、緩衝剤がクエン酸塩であり、界面活性剤がポリソルベートである請求の範囲第8項に記載のHGF製剤。

[10] 凍結乾燥製剤である請求の範囲第1～9項のいずれかに記載のHGF製剤。

[11] HGFに精製白糖を添加してHGF重合体生成を抑制することを特徴とするHGFの安定化方法。

[12] 精製白糖の添加量がHGF1重量部に対して0．01～9重量部である請求の範囲第11項に記載の安定化方法。

INTERNATIONAL SEARCH REPORT

International application No.
PCT/JP2008/052979

A. CLASSIFICATION OF SUBJECT MATTER
A61K38/22(2006.01)i, *A61K47/12*(2006.01)i, *A61K47/18*(2006.01)i, *A61K47/26*(2006.01)i, *A61K47/32*(2006.01)i

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61K38/22, A61K47/12, A61K47/18, A61K47/26, A61K47/32

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
Jitsuyo Shinan Koho 1922-1996 Jitsuyo Shinan Toroku Koho 1996-2008
Kokai Jitsuyo Shinan Koho 1971-2008 Toroku Jitsuyo Shinan Koho 1994-2008

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
BIOSIS(STN), CAplus(STN), EMBASE(STN), MEDLINE(STN), JSTPlus(JDreamII), JMEDPlus(JDreamII), JST7580(JDreamII)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	CN 1579544 A (YANG X.), 16 February, 2005 (16.02.05), Example 3 (Family: none)	1,5
Y	JP 09-025241 A (SNOW BRAND MILK PROD. CO. LTD.), 28 January, 1997 (28.01.97), Example 6; test example 3 & WO 97/02832 A1 & EP 838221 A1 & US 2001/051604 A1 & US 2003/069183 A1 & US 2006/229245 A1 & US 7173008 B2	2-4,6-12

☒ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
16 April, 2008 (16.04.08)	01 May, 2008 (01.05.08)
Name and mailing address of the ISA/ Japanese Patent Office Facsimile No.	Authorized officer Telephone No.

Form PCT/ISA/210 (second sheet) (April 2007)

INTERNATIONAL SEARCII REPORT

International application No.
PCT/JP2008/052979

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Tsutomu ARAKAWA, "Toketsu Sosa ni Oite Tenkabutsu wa Donoyoni shite Tanpakushitsu o Anteika surunoka", Protein, Nucleic acid and Enzyme, 1992, Vol.37, No.9, pages 1517 to 1523, page 1518, right column, lines 3 to 5, table 1, page 1522, left column, lines 11 to 15, Fig. 5	2-4,6-12

国際調査報告

国際出願番号　ＰＣＴ／ＪＰ２００８／０５２９７９

Ａ．　発明の属する分野の分類（国際特許分類（ＩＰＣ））

Int.Cl.　A61K38/22(2006.01)i, A61K47/12(2006.01)i, A61K47/18(2006.01)i, A61K47/26(2006.01)i, A61K47/32(2006.01)i

Ｂ．　調査を行った分野

調査を行った最小限資料（国際特許分類（ＩＰＣ））

Int.Cl.　A61K38/22, A61K47/12, A61K47/18, A61K47/26, A61K47/32

最小限資料以外の資料で調査を行った分野に含まれるもの

日本国実用新案公報	１９２２－１９９６年
日本国公開実用新案公報	１９７１－２００８年
日本国実用新案登録公報	１９９６－２００８年
日本国登録実用新案公報	１９９４－２００８年

国際調査で使用した電子データベース（データベースの名称、調査に使用した用語）

BIOSIS(STN), CAplus(STN), EMBASE(STN), MEDLINE(STN), JSTPlus(JDreamII), JMEDPlus(JDreamII), JST7580(JDreamII)

Ｃ．　関連すると認められる文献

引用文献のカテゴリー＊	引用文献名　及び　一部の箇所が関連するときは、その関連する箇所の表示	関連する請求の範囲の番号
X	CN 1579541 A (YANG X.) 2005.02.16, 実施例３, (ファミリーなし)	1,5
Y	JP 09-025241 A (SNOW BRAND MILK PROD. CO. LTD.) 1997.01.28, 実施例６、試験例３, & WO 97/02832 A1 & EP 838221 A1 & US 2001/051604 A1 & US 2003/069183 A1 & US 2006/229245 A1 & US 7173008 B2	2-4,6-12

☑　Ｃ欄の続きにも文献が列挙されている。　　☐　パテントファミリーに関する別紙を参照。

＊　引用文献のカテゴリー
「Ａ」特に関連のある文献ではなく、一般的技術水準を示すもの
「Ｅ」国際出願日前の出願または特許であるが、国際出願日以後に公表されたもの
「Ｌ」優先権主張に疑義を提起する文献又は他の文献の発行日若しくは他の特別な理由を確立するために引用する文献（理由を付す）
「Ｏ」口頭による開示、使用、展示等に言及する文献
「Ｐ」国際出願日前で、かつ優先権の主張の基礎となる出願の日の後に公表された文献
「Ｔ」国際出願日又は優先日後に公表された文献であって出願と矛盾するものではなく、発明の原理又は理論の理解のために引用するもの
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「＆」同一パテントファミリー文献

国際調査を完了した日 １６．０４．２００８	国際調査報告の発送日 ０１．０５．２００８
国際調査機関の名称及びあて先 日本国特許庁（ＩＳＡ／ＪＰ） 郵便番号１００－８９１５ 東京都千代田区霞が関三丁目４番３号	特許庁審査官（権限のある職員）　４Ｐ　３４３６ 鳥居　福代 電話番号　０３－３５８１－１１０１　内線　３４９２

様式ＰＣＴ／ＩＳＡ／２１０（第２ページ）（２００７年４月）

Ｃ（続き）．　関連すると認められる文献

引用文献のカテゴリー＊	引用文献名　及び一部の箇所が関連するときは、その関連する箇所の表示	関連する請求の範囲の番号
Ｙ	荒川　力，凍結操作において添加物はどのようにして蛋白質を安定化するのか，蛋白質核酸酵素，1992，Vol.37，No.9，p.1517-1523，第1518頁 右欄 第3-5行及び表１、第1522頁 左欄 第11-15行及び図５	2-4,6-12

Office de la Propriété Intellectuelle du Canada
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(30) Priorité/Priority: 2007/02/22 (JP PCT/JP2007/053316)

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(54) Titre : PREPARATION DE HGF
(54) Title: HGF PREPARATION

(57) Abrégé/Abstract:
Disclosed is an HGF preparation containing HGF and purified sucrose. This HGF preparation is characterized in that it remains stable even when stored for a long time.

ABSTRACT

The present invention relates to an HGF preparation comprising HGF and purified sucrose. The HGF preparation is characterized by being stable even after a long-term storage.

English translation of the application
PCT/JP2008/052979

DESCRIPTION

HGF PREPARATION

TECHNICAL FIELD

[0001]

The present invention relates to an HGF (Hepatic Growth Factor)-containing preparation.

BACKGROUND ART

[0002]

HGF is a physiologically active peptide discovered by Nakamura et al, having the most potent mitogenic activity against mature hepatocytes (see, for example, Non-patent Document 1), and its mass production has become possible by bioengineering techniques in recent years (see, for example, Non-patent Document 2). This HGF is expected as a therapeutic or preventive agent for not only hepatitis and hepatic cirrhosis but also nephritis, cancers, etc., and is further expected in the application as a suppressant for adverse reactions to anti-cancer agents and as a wound-healing agent.

Among HGF preparations, an aqueous preparation of HGF containing albumin, human serum, gelatin, sorbitol, mannitol, xylitol, etc., as a stabilizer for HGF is disclosed in Patent Document 1. However, the above-mentioned aqueous HGF preparation has a defect that aggregation, turbidity and gelation occur during storage, as well as a problem of low physicochemical stability due to aggregates formation, resulting in reduction of the biological activity of HGF.

[0003]

In order to solve these problems, a freeze-dried preparation wherein arginine, lysine, histidine, glutamine, proline, glutamic acid, aspartic acid, etc. are contained as a stabilizer for HGF is disclosed in Patent Document 2. Further, in Patent Document 3, a freeze-dried preparation wherein glycine, alanine, sorbitol, mannitol, dextran sulfate, etc., are added as a stabilizer to HGF is disclosed.

Although the above-mentioned freeze-dried preparations can attain stabilization of HGF to some extent, an HGF preparation with a still more sufficient stabilization effect has been demanded.

[Patent Document 1] PCT International Publication WO 90/10651 Pamphlet

[Patent Document 2] PCT International Publication WO 00/72873 Pamphlet

[Patent Document 3] Japanese Patent Application Laid-Open (JP-A) No. 9-25241

[Non-patent Document 1] Biochem. Biophys. Res. Commun., 122, 1450, 1984

[Non-patent Document 2] Nature, 342, 440, 1989.

DISCLOSURE OF THE INVENTION

PROBLEMS TO BE SOLVED BY THE INVENTION

[0004]

It is an object of the present invention to provide a more stable HGF preparation when stored for a long term as compared with the conventional HGF preparation.

MEANS FOR SOLVING THE PROBLEMS

[0005]

The inventors of the invention conducted various studies

to achieve the foregoing problems. As a result, they found that a stable HGF preparation was obtained by adding purified sucrose to HGF so that the formation of HGF aggregates was suppressed. Studies based on the above findings were further made to complete the invention.

[0006]

Namely, the invention relates to:

[1] an HGF preparation comprising HGF and purified sucrose,

[2] the HGF preparation according to the above item [1], wherein the purified sucrose content is 0.01 to 9 parts by weight based on 1 part by weight of HGF,

[3] the HGF preparation according to the above item [1] or [2], further comprising a neutral amino acid,

[4] the HGF preparation according to the above item [3], wherein the neutral amino acid is alanine,

[5] the HGF preparation according to any one of the above items [1] to [4], further comprising a buffer,

[6] the HGF preparation according to the above item [5], wherein the buffer is a citric acid salt,

[7] the HGF preparation according to any one of the above items [1] to [6], further comprising sodium chloride,

[8] the HGF preparation according to the above item [1] or [2], further comprising a neutral amino acid, sodium chloride, a buffer and a surfactant, in addition to HGF and purified sucrose,

[9] the HGF preparation according to the above item [8], wherein the neutral amino acid is alanine, the buffer is a citric acid salt, and the surfactant is a Polysorbate,

[10] the HGF preparation according to any one of the above items [1] to [9], which is a freeze-dried preparation,

[11] a stabilization method of HGF, which comprises suppressing the formation of HGF aggregates by adding purified sucrose to HGF, and

[12] the stabilization method according to the above item [11], wherein the addition amount of purified sucrose is 0.01 to 9 parts by weight based on 1 part by weight of HGF.

EFFECT OF THE INVENTION

[0007]

The HGF preparation of the invention has a more stable effect even after a long-term storage as compared with the conventional HGF preparations.

BEST MODE FOR CARRYING OUT THE INVENTION

[0008]

The invention relates to an HGF preparation comprising HGF and purified sucrose.

[0009]

The active ingredient HGF prepared by various processes can be used in the present invention if it is purified enough to be used as a medicine. Further, HGF used in the invention may be a deletion type of HGF, which lacks five amino acid residues (referred to as dLeHGF).

Various methods are known for preparing HGF. For example, HGF can be obtained by extraction and purification from organs (e.g. liver, spleen, lung, bone marrow, brain, kidney, placenta, etc.), blood cells (e.g. platelets, leukocytes, etc.), plasma, and serum of mammals including rat, cow, horse, sheep, and the like. Also, HGF can be obtained by cultivating primary culture

cells or cell lines capable of producing HGF, followed by isolation and purification from the culture (e.g. culture supernatant, cultured cells, etc.). Further, a recombinant HGF can also be obtained according to a gene technology by integrating a gene encoding HGF into an appropriate vector, inserting the vector into a proper host cell to give a transformant, and separating the desired recombinant HGF from the culture of the transformant (see, for example, Nature, 342, 440, 1989). The above-mentioned host cells are not particularly limited, and various host cells conventionally used in gene technologies, such as Escherichia coli, Bacillus subtilis, yeasts, filamentous fungi, and plant or animal cells can be used.

[0010]

More specifically, the method of extracting and purifying HGF from biological tissues comprises, for example, administering carbon tetrachloride to rats intraperitoneally, removing the liver from the rats with hepatitis, grinding it, and purifying HGF by the conventional protein purifying technique, such as gel column chromatography on S-Sepharose or heparin-Sepharose, and HPLC and the like. In addition, by use of a gene recombinant technique, an animal cell (e.g. Chinese hamster ovary (CHO) cells, mouse C127 cells, monkey COS cells, etc.) is transformed by an expression vector, wherein a gene encoding the amino acid sequence of human HGF is inserted into a vector such as bovine papilloma virus DNA, and HGF can be obtained from the culture supernatant of the transformants.

[0011]

The purified sucrose for use in the present invention is

one which is listed in Japanese Pharmacopoeia, Fourteenth Edition, Part II, and it can be used preferably as a stabilizer. The addition amount of the purified sucrose is preferably 0.01 to 9 parts by weight, especially preferably 0.1 to 5 parts by weight, based on 1 part by weight of HGF. The lower limit of the addition amount of the purified sucrose is more preferably 0.5 part by weight based on 1 part by weight of HGF. The upper limit of the addition amount of the purified sucrose is more preferably 4 parts by weight, still more preferably 3 parts by weight, and especially preferably 2 parts by weight, based on 1 part by weight of HGF.

[0012]

Although the preparation of this invention may take various dosage forms (for example, liquid preparations, solid preparations, capsules, creams, sprays, etc.), an aqueous preparation, a freeze-dried preparation and the like containing generally HGF as an active ingredient and purified sucrose alone or a conventional additive (carrier, etc.) in addition to them are preferable, and in particular, a freeze-dried preparation is preferable.

[0013]

Regarding the HGF preparations of the invention, an aqueous preparation can be prepared through the formation of an aqueous solution containing HGF and purified sucrose. Also, a freeze-dried preparation of HGF can be prepared by freeze-drying said aqueous solution in a conventional freeze-drying method. The purified sucrose content in the aforementioned aqueous solution is 0.1% or more by weight, preferably 0.5% or more by weight, and is 9% or less by weight,

preferably 5% or less by weight, more preferably 4% or less by weight, still more preferably 3% or less by weight, especially preferably 2% or less by weight. The purified sucrose content in the freeze-dried preparation is preferably 10 to 80% by weight and especially preferably 20 to 60% by weight. For example, the freeze-dried preparation can be prepared by dissolving HGF in a suitable solvent (e.g. sterilized water, distilled water for injection, buffer, physiological saline, etc.); adding purified sucrose to the solution to a concentration of preferably 0.1 to 5% by weight and especially preferably 0.5 to 2% by weight; optionally adding stabilizers, buffers, surfactants, sodium chloride, etc., other than purified sucrose; sterilizing the solution through filtration with a filter or the like; filling the solution in a vial or ampoule; and freeze-drying the solution. It is preferable to use a sterilization filter with a pore size of 0.22 μm or less. The sterilization filter includes, for example, DURAPORE (Registered trade mark, manufactured by Nihon Millipore K.K.) and SARTOPORE 2 (Registered trade mark, manufactured by Sartorius AG.). An example of the freeze- drying methods includes, for example, a method comprising three unit operations: a freezing step for chilling and freezing under atmospheric pressure, a primary drying step for sublimating and drying free water not restrained by a solute under reduced pressure, and a secondary drying step for removing adsorbed water or crystal water intrinsic to the solute. The chilling temperature in the freezing step is preferably -60 to -40°C, the temperature in the primary drying step is preferably -50 to 0°C, and the temperature in the secondary drying step is

preferably 4 to 40°C. The vacuum pressure is preferably 0.1 to 1.5 Pa, and in particular, preferably 0.5 to 1.2 Pa. After the freeze-drying operation, the pressure in the drying chamber is recovered. The method for the pressure recovery is preferably a method of introducing a sterilized air or an inert gas (e.g. sterile nitrogen gas, sterile helium gas) into the chamber to return the pressure back to about 70 to 100 kPa, preferably about 80 to 95 kPa (primary pressure recovery) and then to the atmospheric pressure (secondary pressure recovery). Capping for vials is preferably carried out after the primary pressure recovery.

[0014]

Purified sucrose alone may be used as the stabilizer. However, purified sucrose may be preferably used in combination with a conventional stabilizer such as amino acids (e.g. glycine, alanine, arginine, lysine, histidine, etc.), polysaccharides (e.g. heparin, dextran sulfate, etc.), and sugar alcohols (e.g. sorbitol, mannitol, etc.). Among these stabilizers, amino acids are preferable, and in particular, neutral amino acids such as glycine and alanine are preferable among the amino acids. The amount of each of these stabilizers to be added is not limited except for purified sucrose, and when the neutral amino acid such as glycine and alanine is used, its addition amount is preferably 0.01 to 50 parts by weight, and more preferably 0.1 to 20 parts by weight based on 1 part by weight of purified sucrose.

Stability of HGF can be more improved by combination use of purified sucrose with a conventional stabilizer including a neutral amino acid, when compared to the case where purified

sucrose alone is used as a stabilizer.

[0015]

The buffer used in the invention includes, for example, a phosphoric acid buffer, a citric acid buffer, and the like. The buffer has an action of adjusting the pH of an aqueous solution after redissolution of the freeze-dried preparation, and maintaining the solubility of HGF. It is preferable to use a buffer which enables to maintain the pH of the aqueous solution at 4.5 to 6.5 after redissolution of the freeze-dried preparation. A preferable buffer is a citric acid buffer and especially sodium citrate buffer. This citric acid buffer also contributes to the stabilization of HGF in the aqueous solution obtained upon redissolution of the freeze-dried preparation. It is desirable to adjust the concentration of the buffer to be added, within the range of 1 to 100 mM in the aqueous solution immediately before the freeze-drying operation for the production of freeze-dried preparations.

[0016]

Surfactants used in the invention include, for example, Polysorbate 20, Polysorbate 80, Pluronic F-68, polyethylene glycols, etc., and two or more kinds thereof may be used in combination. An especially preferred surfactant is Polysorbate-based surfactants, including particularly Polysorbate 80. Although HGF is easy to be adsorbed on the surface of the materials of the container made of glass or resins, the adsorption of HGF onto the container after redissolution of the freeze-dried preparation, can be prevented by the addition of such a surfactant. As for the addition amount of the surfactant, the concentration of the aqueous solution just

before freeze-drying operation in the production of freeze-dried preparations is preferably 0.001 to 2.0% by weight.

[0017]

Sodium chloride has an action to maintain the solubility of HGF. That is, for example, in the case of the recombinant HGF used in the Examples, addition of sodium chloride makes it possible to increase the solubility of HGF. Particularly, a remarkable improvement in the solubility of the recombinant HGF is observed at a concentration of 300 mM or more of sodium chloride. Although the addition amount of sodium chloride receives a restriction by an osmotic pressure ratio, an amount showing the osmotic pressure ratio of injectable solutions generally used may be good. It is desirable to use an addition amount of sodium chloride reaching an osmotic pressure ratio of 1 to 3, which is an acceptable osmotic pressure ratio of injections for medical use in humans or animals. Usually, when a freeze-dried preparation is manufactured, the concentration of sodium chloride in an aqueous solution just prior to its freeze-drying operation is preferably in the range of 150 to 1000 mM.

[0018]

The preparation of the invention may include other additives necessary for formulating preparations, such as solubilizers, antioxidants, soothing agents, isotonic agents, and the like.

[0019]

The preparation obtained above in accordance with the invention, e.g. a freeze-dried preparation, is dissolved in

distilled water for injection so that the concentration of HGF in use is in the range of 0.1 to 40 mg/mL, and the solution can be served as an injectable solution. In addition, an external preparation, such as creams, sprays, etc., containing the freeze-dried preparation may be formulated.

EXAMPLES

[0020]

The following Examples further illustrate the present invention but are not to be construed to limit the scope thereof. In the Examples of the invention, a five amino acid deletion type of HGF was used as HGF. The area percentage (%) (hereinafter referred to as aggregates content (%)) of the HGF aggregates was determined according to the following equation 1 using measured values which were quantitatively analyzed by high performance liquid chromatography (HPLC).

[0021]

Equation 1

$$\text{Aggregates content (\%)} = \frac{A_A}{A_M + A_A} \times 100$$

In the equation, A_M is the peak area of HGF and A_A is the peak area of HGF aggregates.

[0022]

(Conditions for HPLC)

Column: Gel filtration column (Trade name: Superdex 200 10/300, manufactured by Amersham Biosciences)

Mobility phase: sodium chloride 58.44 g, trisodium citrate dihydrate 2.94 g, and Polysorbate 80 0.1 g are dissolved in water to make up to 1L, which is served as Solution A. Sodium chloride 58.44 g, citric acid monohydrate 2.10 g, and

Polysorbate 80 0.1 g are dissolved in water to make up to 1L, which is served as Solution B. Solution B is added to Solution A and the pH is adjusted to 6.0. The mixed solution is filtered with a 0.45 μm filter (Trade name: Millicup-HV, pore size: 0.45 μm, manufactured by Millipore Corp.) and degassed prior to its use. The solution is stored at room temperature and used within two weeks.

Column temperature: 25°C

Flow rate: 0.5 mL/min

Injection amount of sample: 25 μL

Analysis time: 60 minutes

Detector: absorption spectrophotometer

Detection wave length: 280 nm

Sample cooler: 5 minutes

A molecular weight marker is dissolved in one vial of Gel Filtration Standard (Catalogue Number: 151-1901, manufactured by Bio-Rad Laboratories, Inc.) with water (500 μL), and the solution is filtered through a filtration filter (Trade name: Ultrafree-MC, pore size: 0.45 μm, manufactured by Millipore Corp.) for use in clarification of test solutions in small quantities, stored at 2 to 8°C, and used within 3 months.

[0023]

The buffer solution for dilution used in the following Examples and Test Examples was prepared in the following manner.

(Preparation of buffer solution for dilution)

Sodium chloride 1.1688 g, trisodium citrate dihydrate 2.94 g and Polysorbate 80 0.3 g were dissolved in ultra pure water to make up to a total volume of 1L, and the solution was served as Solution A. Sodium chloride 1.1688 g, citric acid

monohydrate 2.10 g, and Polysorbate 80 0.3 g were dissolved in ultra pure water (prepared by using an apparatus for ultra pure water production; Trade name: MilliQ Gradient, manufactured by MilliPore Corp.; hereinafter the same) to make up to 1L, which was served as Solution B. Solution B was added to Solution A and the pH was adjusted to 6.0. The solution was served as a buffer for dilution (1).

Sodium chloride 17.53 g, trisodium citrate dihydrate 2.94 g, and Polysorbate 80 0.1 g were dissolved in ultra pure water to make up to a total volume of 1L, which was served as Solution C. Sodium chloride 17.53 g, citric acid monohydrate 2.10 g, and Polysorbate 80 0.1 g were dissolved in ultra pure water to make up to a total volume of 1L, which was served as Solution D. Solution D was added to Solution C, and the pH of the solution was adjusted to 6.0. This solution was served as a buffer for dilution (2).

[0024]

Example 1

The five amino acids-deleted type of HGF (hereinafter, simply referred to as HGF) was added to a buffer for dilution (1) so that HGF concentration became to be 10 mg/ml, and purified sucrose was then added thereto to a concentration of 0.5% by weight, thereby to obtain solutions with the components as shown in Table 1 below.

Table 1.

Component	Concentration
HGF	10 mg/ml
Trisodium citrate dihydrate	10 mM
Sodium chloride	300 mM
Polysorbate 80	0.03% by weight
Purified sucrose	0.5% by weight

Each (2 mL) of the solutions obtained above was aseptically subdivided into a vial (ϕ23 × 43 mm). The vial was semi-capped with a rubber stopper, arrayed on a tray, placed in a freeze-dryer (Triomaster; manufactured by Kyowa Vacuum Engineering Ltd.) and then freeze-dried under the conditions as shown in Table 2 below. The arrow symbol "→" in the table shows that the temperatures was changed.

Table 2

	Freezing Step		Primary Drying Step		Secondary Drying Step	
Temperature (°C)	10 → -50	-50	-50 → -20	-20	-20 → 20	20
Degree of Vacuum (Pa)	-	-	1	1	1	1
Time (hr)	6	5	8	33.5	8	11

After freeze-drying operation, a sterile nitrogen gas was introduced into the chamber of Triomaster to recover the pressure (chamber pressure: 88.0 kPa; primary pressure recovery), and each of the vials was fully capped with a rubber stopper. After that, the pressure in the chamber of Triomaster was recovered to the atmospheric pressure with a sterile nitrogen gas (secondary pressure recovery), and vials were taken out, immediately followed by capping with a stopper. In this manner, freeze-dried preparations of HGF according to the invention were obtained.

The purified sucrose content in the freeze-dried preparation is 0.5 part by weight based on 1 part by weight of HGF, and is 26.3% by weight to the freeze-dried preparation.

[0025]

Example 2

A freeze-dried preparation of HGF was obtained in a manner similar to Example 1, except that the concentration of purified sucrose to be added was 1.0% by weight.

The purified sucrose content in the freeze-dried preparation of HGF of the invention is 1 part by weight based on 1 part by weight of HGF and is 41.7% by weight to the freeze-dried preparation of HGF.

[0026]

Example 3

A freeze-dried preparation of HGF was obtained in a manner similar to Example 1, except that the concentration of purified sucrose to be added was 2.0% by weight.

The purified sucrose content in the freeze-dried preparation of HGF is 2 parts by weight based on 1 part by weight

of HGF and is 58.8% by weight to the freeze-dried preparation of HGF.

[0027]

Example 4

A freeze-dried preparation of HGF was obtained in a manner similar to Example 1, except that purified sucrose and alanine were added at a concentration of 1.0% by weight and 5 mg/mL, respectively.

The purified sucrose content in the freeze-dried preparation of HGF is 1 part by weight based on 1 part by weight of HGF and is 34.5% by weight to the freeze-dried preparation of HGF.

[0028]

Comparative Example 1

A freeze-dried preparation of HGF was obtained in a manner similar to Example 1, except that alanine was added as an additive at a concentration of 20 mg/mL in place of purified sucrose.

[0029]

Comparative Example 2

A freeze-dried preparation of HGF was obtained in a manner similar to Example 1, except that purified sucrose was not added (hereinafter referred to as a basic formulation).

[0030]

Test Example 1

The freeze-dried preparations described in the above Examples and Comparative Examples were stored at 50°C and sampled after one week. Each of the samples was diluted with a buffer for dilution (2) so that the concentration of the

protein was 5 mg/mL. Each sample was quantified using HPLC, and the content (%) of HGF aggregates was calculated according to the above equation 1. The results are shown in Table 3 below.

Table 3

	Additive (Concentration)		Aggregates content (%)		
Example No.	Purified sucrose	Alanine	Before freeze-drying	At the beginning of storage (Initial)	50°C, 1 week
Example 1	+ (0.5% by weight)	-	0.47	0.61	1.90
Example 2	+ (1.0% by weight)	-	0.48	0.58	1.29
Example 3	+ (2.0% by weight)	-	0.48	0.56	0.92
Example 4	+ (1.0% by weight)	+ (5 mg/mL)	0.44	0.56	0.90
Comparative Example 1	-	+ (20 mg/mL)	0.50	0.63	2.32
Comparative Example 2	-	-	0.55	0.84	6.12

As apparent from Table 3, in an HGF preparation of the basic formulation + purified sucrose and an HGF preparation of the basic formulation + purified sucrose + alanine according to the present invention, the content of HGF aggregates was suppressed more significantly, when compared with an HGF preparation of the basic formulation or the basic formulation + alanine.

[0031]

Test Example 2

HGF was added to a buffer solution for dilution (20mM citric acid buffer solution, 1M sodium chloride, Polysorbate 80 0.01% by weight) to a concentration of 10 mg/L, and sample solutions 1 to 5 (50 μL each) were prepared in such a manner that purified sucrose was added to a concentration of 0% by weight, 1% by weight, 5% by weight, 10% by weight or 20% by weight. Each sample solution was frozen for about 24 hours until measurement. Each of the frozen sample solutions was redissolved, and molecular weight (about 84 kDa) distribution of HGF was then measured by the dynamic light scattering (DLS) method. Dyna-Pro (manufactured by Protein-Solution Co.) for exclusive use of protein solutions was used as the measurement device. The measurement temperature was set to 4°C. A buffer (50 μL each) for dilution not containing HGF was used as the background. The degree of polydispersity (Pd%) of HGF molecular weights in each of the sample solutions is shown in Table 4.

Table 4

	HGF (mg/mL)	Purified sucrose (% by weight)	Pd%
Sample solution 1	10	0	22.8
Sample solution 2	10	1	21.7
Sample solution 3	10	5	13.6
Sample solution 4	10	10	32.3
Sample solution 5	10	20	28.5

As apparent from Table 4, in the sample solutions 2 and 3 wherein purified sucrose was added to a concentration of 1% by weight and 5% by weight, respectively, Pd% value was very small and the molecular weight distribution of HGF was found to be a single distribution (monomodal distribution). In the sample solutions 1, 4, and 5, the peak of the HGF molecular weight was broad, suggesting that HGFs having different molecular weights were contained in such sample solutions. Moreover, in the sample solutions 4 and 5, another peak appeared in the high molecular side, and in the sample solution 5, a peak showing that a large amount of lower molecular weight substances were contained therein was observed.

[0032]

INDUSTRIAL APPLICABILITY

HGF preparations useful as drugs and excellent in storability can be provided in accordance with the invention.

CLAIMS

1. An HGF preparation comprising HGF and purified sucrose.

2. The HGF preparation according to claim 1, wherein the purified sucrose content is 0.01 to 9 parts by weight based on 1 part by weight of HGF.

3. The HGF preparation according to claim 1 or 2, further comprising a neutral amino acid.

4. The HGF preparation according to claim 3, wherein the neutral amino acid is alanine.

5. The HGF preparation according to any one of claims 1 to 4, further comprising a buffer.

6. The HGF preparation according to claim 5, wherein the buffer is a citric acid salt.

7. The HGF preparation according to any one of claims 1 to 6, further comprising sodium chloride.

8. The HGF preparation according to claim 1 or 2, further comprising a neutral amino acid, sodium chloride, a buffer and a surfactant, in addition to HGF and purified sucrose.

9. The HGF preparation according to claim 8, wherein the neutral amino acid is alanine, the buffer is a citric acid salt, and the surfactant is a Polysorbate.

10. The HGF preparation according to any one of claims 1 to 9, which is a freeze-dried preparation.

11. A stabilization method of HGF, which comprises suppressing the formation of HGF aggregates by adding purified sucrose to HGF.

12. The stabilization method according to claim 11,

wherein the addition amount of purified sucrose is 0.01 to 9 parts by weight based on 1 part by weight of HGF.

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(57) Abstract

A stable and soluble multi-dose ophthalmic solution is disclosed. The solution contains fibronectin, an amino acid, a sugar, and a lower alkyl p-hydroxybenzoate. A method of treatment of ophthalmic wounds employing the ophthalmic solution, a process for preparing fibronectin for ophthalmic use, a method of lyophilizing an aqueous solution of fibronectin free of albumin, a method for inhibiting bacterial growth in an ophthalmic solution while preserving the cellular adhesion and wound healing activities of fibronectin, and a method of treatment of ophthalmic wounds administering a wound-healing accelerator solution are also disclosed.

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TITLE

FIBRONECTIN-CONTAINING OPHTHALMIC SOLUTION, METHOD OF MANUFACTURE OF AN OPHTHALMIC SOLUTION, METHOD OF PRESERVING AN OPHTHALMIC SOLUTION, AND METHODS OF TREATMENT OF OPHTHALMIC WOUNDS

FIELD OF THE INVENTION

This invention relates to a stable and soluble multi-dose ophthalmic solution containing fibronectin, an amino acid, a sugar, and a lower alkyl p-hydroxybenzoate preservative and to a method of treatment of ophthalmic wounds employing the ophthalmic solution. This invention further relates to a process for preparing fibronectin for ophthalmic use. This invention further relates to a method for inhibiting bacterial growth in an ophthalmic solution while preserving the cellular adhesion and wound healing properties of fibronectin.

BACKGROUND OF THE INVENTION

Fibronectin is useful in therapeutic treatment because of the role that it plays in cellular adhesion, blood coagulation, malignant transformation, reticuloendothelial system function, and embryonic differentiation. Fibronectin's role in cellular adhesion and in promoting epithelial cell outgrowth makes it desirable for treatment of ophthalmic wounds, particularly corneal disorders. Other growth factors have also been identified as being useful as healing accelerators for treatment of ophthalmic wounds. For example, recombinant human epithelial growth factor has been shown to accelerate corneal re-epithelialization following abrasive injury or alkali burn injury (Stern et al., "The Effects of Human Recombinant Epidermal Growth Factor on Epihelial Wound Healing", in Healing Processes in the Cornea, 69-75 (C.E. Crosson and H.E. Kaufman, eds.) (1989). Similarly, fibroblast growth factor has also been

reported to stimulate corneal healing (Countois, Y. et al., 181 C. R. Soc. Biol., 491 (1987)). Numerous other growth promoting substances have also been identified (e.g., interleukin 6, platelet-derived growth factor, etc.) and may be useful in accelerating ophthalmic wound healing. Ophthalmic wounds can be caused in many ways, for example, by puncture, physical trauma, acid splash, surgical incisions, chemical burns, or lacerations. It is believed that fibronectin promotes the migration of epithelial cells over the wound surface and promotes binding of the epithelial cells to the wound surface to provide a permanent closure of the wound. This process may stimulate the production of endogenous growth factors, such as fibroblast growth factors.

To treat an ophthalmic wound with fibronectin, the fibronectin should be applied by means of an ophthalmic solution. Multi-dose ophthalmic solutions to be used by a single user are the typical mode of applying ophthalmic solutions. One problem in using fibronectin arises from U.S. Federal Food and Drug Agency ("FDA") regulations which require the addition of a preservative to inhibit bacterial growth in a multi-dose ophthalmic solution.

Benzalkonium chloride is the most commonly used preservative in ophthalmic solutions, but it cannot be used with fibronectin because it inhibits the wound healing activity of the fibronectin. Chlorobutanol and phenylethyl alcohol are accepted alternative preservatives in ophthalmic solutions, but they also cannot be used with fibronectin. Chlorobutanol is hydrolyzed in a neutral pH solution. Phenylethyl alcohol cannot be used because it inhibits fibronectin's wound healing

activity. Similarly, preservatives made from sodium dehydroacetate or cetylpyridinium dichloride inhibit the wound healing activity of fibronectin. Thimerosal does not inhibit fibronectin's wound healing activity but thimerosal's mercury content and the toxicity problems associated with mercury make it unsuitable for use as a preservative in an ophthalmic solution.

A second difficulty in using fibronectin in ophthalmic settings are problems related to the poor solubility and stabiity of fibronectin in an aqueous solution. Because of fibronectin's poor storage stability in solution it is a standard practice to lyophilize a solution of fibronectin with a stabilizing agent, usually a neutral amino acid, monosaccharide, disaccharide, or sugar alcohol. A solvent is added to the lyophilized fibronectin just before use. The disadvantage of this method is that the dissolving of the lyophilized preparation in the solvent, typically water, takes a long time and the resulting solution is often turbid because of fibrous insoluble matter.

One method to address this lyophilization problem has been disclosed in Ohmura U.S. Patent No. 4,565,651. In the Ohmura patent, prior to lyophilization, both albumin and at least one stabilizer selected from neutral amino acids, monosaccharides, disaccharides, and sugar alcohols are added to a fibronectin-containing aqueous solution which is then lyophilized. According to Ohmura, when his lyophilized fibronectin is dissolved in water, the dissolution time is rapid, with little or no turbidity. For an ophthalmic

solution, however, the lyophilized fibronectin of Ohmura may prove unacceptable because of the presence of an additional protein, albumin. Albumin renders preservatives less effective and may also interfere with the function of fibronectin. Additionally, the lyophilized fibronectin produced by the method of the Ohmura patent tends to cake up and then does not dissolve easily.

SUMMARY OF THE INVENTION

The present invention provides a stable and readily soluble multi-dose ophthalmic solution containing fibronectin and an anti-microbial preservative.

The present invention further provides a stable and readily soluble single-dose ophthalmic solution containing fibronectin.

The present invention further provides anti-microbial preservatives which do not interfere with the properties of wound healing accelerants.

The present invention also provides a method for treatment of ophthalmic wounds by administering to the wound an ophthalmic solution containing virally sterilized, heterologous fibronectin.

The present invention also provides a process for preparing fibronectin for ophthalmic use comprising lyophilizing an aqueous solution free of albumin and comprising fibronectin as the only protein.

Another advantage of this process is that a lyophilized fibronectin is produced that is free of unnecessary

proteins and that, when dissolved, provides a solution that is stable and soluble.

The present invention provides a multi-dose ophthalmic solution containing fibronectin together with a preservative to inhibit bacterial growth.

The present invention enables one to avail oneself of the wound healing activity of virally sterilized, heterologous fibronectin for the treatment of ophthalmic wounds.

The present invention provides an opthalmic solution containing fibronectin in which most, if not virtually all, of the viruses contained therein are inactivated or removed, and in which the structure, function, and activity of fibronectin are maintained.

The present invention also provides a method of obtaining a non-turbid fibronectin solution from lyophilized fibronectin, comprising adding to an aqueous fibronectin solution a sugar and an amino acid, the amount of sugar and the amount of amino acid being sufficient to prevent turbidity when the solution is lyophilized and thereafter dissolved in an aqueous solvent.

The present invention also provides a method for inhibiting bacterial growth while preserving the cellular adhesion and wound healing properties of fibronectin in an ophthalmic solution comprising adding a lower alkyl p-hydroxybenzoate preservative to an ophthalmic solution comprising fibronectin, an amino acid, and a sugar.

DETAILED DESCRIPTION OF THE INVENTION

In the process of the invention, an aqueous solution free of albumin and containing fibronectin, an amino acid, and a sugar, is lyophilized under vacuum. Prior to lyophilization, the fibronectin is present in an amount from 0.25 to 30 mg/ml, preferably 3 mg/ml.

The amino acid may be a water-soluble hydrophilic amino acid such as serine, histidine, alanine, lysine, or glycine. Glycine is the preferred amino acid. The concentration of amino acid in the aqueous solution to be lyophilized is from 0.005 to 1.5 M, preferably 0.12 M.

The sugar may be a monosaccharide such as glucose, a disaccharide such as sucrose or galactose, a trisaccharide such as a raffinose, a polysaccharide such as dextran, or sugar derivatives such as sorbitol or mannitol, or a combination thereof. Sucrose is the preferred sugar. The concentration of sugar in the aqueous solution to be lyophilized is from 0.005 to 1.5 M, preferably 0.30 M.

It is most preferred to add a combination of glycine and sucrose to the aqueous solution of fibronectin to be lyophilized. The glycine is present in the solution to be lyophilized in a concentration of from 0.005 to 1.5 M, preferably 0.12 M, and the sucrose is present in the solution in a concentration of from 0.005 to 1.5 M, preferably 0.30 M.

It is preferred to utilize an aqueous solution containing fibronectin which has been treated to inactivate the lipid-enveloped viruses present in the starting biological material. U.S. Patent No. 4,841,023, and the references

incorporated therein, describe a suitable method for the disruption of lipid-containing viruses. Additionally, efficient virus removal occurs with gelatin sepharose chromatography (Horowitz and Chang in Fibronectin, 441-455 (Deane F. Mosher ed.) (1989)).

When the lyophilization is complete, the flask is sealed under vacuum. It is preferred when lyophilization is complete to introduce nitrogen and seal the flask under nitrogen or another non-reactive gas. The solubility of the lyophilized fibronectin is improved when it has been sealed in this manner.

The lyophilized fibronectin obtained from this process is employed in making the ophthalmic solution of the present invention. It is understood that fibronectin obtained by other methods may also be used in the ophthalmic solution of the present invention.

In one embodiment of the invention, the ophthalmic solution comprises fibronectin, an amino acid, a sugar and a solvent. The fibronectin is present in a concentration from 0.25 mg/ml to 10 mg/ml, preferably 1 mg/ml. The amino acid is glycine, serine, histidine, alanine, lysine or other water-soluble hydrophilic amino acids, and mixtures thereof, preferably glycine, and is present in a concentration of from 0.005 to 0.5 M, preferably 0.04 M. The sugar is a monosaccharide such as glucose, a disaccharide such as sucrose or galactose, a trisaccharide such as a raffinose, a polysaccharide such as dextran, or sugar derivatives such as sorbitol or mannitol, or a combination thereof, preferably sucrose, and is present in a concentration of from 0.005 to 0.5

M, preferably 0.1 M. It is most preferred that the amino acid be glycine and the sugar be sucrose. The solvent may be sterile water, U.S.P. Grade Purified Water, or a neutral physiological buffer, such as phosphate buffered saline ("PBS"). It is preferred to use U.S.P. water as the solvent.

Sodium chloride may optionally be added to the ophthalmic solution in a concentration from 0.01 to 0.2 M, and is preferably 0.087 M.

In another embodiment, the ophthalmic solution also contains a preservative. The preservative is a lower alkyl p-hydroxybenzoate which is commonly referred to as "Parabens" or by the designation "PB". Preferred lower alkyl p-hydroxybenzoate preservatives are methyl p-hydroxybenzoate ("methyl paraben"), ethyl p-hydroxybenzoate ("ethyl paraben"), propyl p-hydroxybenzoate ("propyl paraben"), butyl p-hydroxybenzoate ("butyl paraben"), and mixtures thereof. The preservative is desirably in the form of an aqueous solution at a concentration from 0.002 to 0.25% (w/v). The water used in the aqueous solution may be U.S.P. Grade Purified Water, sterile water, or water purified by conventional techniques.

It is preferred to add two of the lower alkyl p-hydroxybenzoate preservatives to the ophthalmic solution. The preferred combinations of preservatives are:

1. Ethyl p-hydroxybenzoate in a concentration from 0.005 to 0.17% (w/v), preferably 0.02% (w/v), and butyl p-hydroxybenzoate in a concentration from 0.002 to 0.021% (w/v), preferably 0.01% (w/v); or

2. Methyl p-hydroxybenzoate in a concentration from 0.012 to 0.25% (w/v), preferably 0.038% (w/v), and propyl p-hydroxybenzoate in a concentration from 0.005 to 0.05% (w/v), preferably 0.015% (w/v).

In another embodiment, a potentiating agent is added in order to improve the efficacy of the preservative or preservatives in the ophthalmic solution. The potentiating agent is preferably ethylenediaminetetraacetic acid ("EDTA") or a salt thereof, preferably disodium ethylenediaminetetraacetate or disodium dihydrate ethylenediaminetetraacetate ($Na_2C_{10}H_{14}O_8N_2 \cdot 2H_2O$). The preferred potentiating agent is disodium dihydrate ethylenediaminetetraacetate. The potentiating agent is added to the ophthalmic solution in a concentration from 0.005 to 0.1% (w/v). When disodium dihydrate EDTA is used, the concentration is preferably 0.01% (w/v).

Ophthalmic wounds, and in particular, corneal disorders may be treated by administering the ophthalmic solution of the present invention in an amount effective to treat the wound and to promote wound healing. The amount of the ophthalmic solution that will be required for the treatment will depend upon the nature and scope of the ophthalmic wound. Suggested dosages are one drop applied to the eye four times per day during waking hours up to eight weeks or 56 days.

The invention is further illustrated by the following examples:

Example 1

A. FORMULATION OF FIBRONECTIN EYE DROPS

Virus inactivated purified fibronectin (Horowitz and Chang, in Fibronectin, 441-455 (Deane F. Mosher ed.) (1989)) in PBS is formulated to produce a 1.0 ml solution containing 3.0 mg fibronectin, 0.30 M sucrose, 0.12 M glycine, 0.262 M sodium chloride and 0.03 M sodium phosphate buffer, pH 7.4

An aliquot of purified fibronectin containing 3 mg fibronectin is added to 0.339 gm of a 1.0 M sucrose solution, 0.300 gm of a solution containing 0.09 M sodium phosphate buffer, 0.715 M sodium chloride, 0.4 M glycine, pH 7.4 and sufficient PBS (0.01 M sodium phosphate buffer, 0.12 M sodium chloride, pH 7.4) to bring the mixture to 1.039 gm or 1.0 ml.

The mixture is filtered using a Pall, nylon, 0.2 micron filter (Pall Corp., NY, NY) and 1 ml filled into sterile, 6 ml glass vials. A sterile 20 mm, siliconized, 890 grey butyl lyophilization split stopper (West Corp.) is inserted partway into the vial neck and the vials placed into a stainless steel-covered lyophilization box. The vials are frozen at -50° to -70°C prior to lyophilization.

Following lyophilization, the fibronectin is dissolved with 3 ml of sterile U.S.P. Grade Purified Water containing 0.02% ethyl paraben, 0.01% butyl paraben and 0.01% disodium dihydrate ethylenediaminetetraacetate.

B. LYOPHILIZATION OF FIBRONECTIN EYE DROPS

The formulated, vialed fibronectin is frozen at -50° to -70°C. The lyophilization initiates with the shelf temperature at ≤ -45°C and the chamber at a pressure of ≤ 100

microns mercury. The fibronectin is held at these conditions for approximately 2 hours after which the shelf temperature is raised to between -20° and -10°C with the pressure at < 100 microns. When the product temperature begins to rise, the shelf temperature is raised to 10°C above the product temperature. As the product temperature rises the shelf temperature is raised to maintain a constant 10°C differential between the two. The pressure is maintained at < 100 microns.

After the product temperature reaches a final temperature of 20° to 35°C the shelf temperature is held to maintain the final temperature. The product is held at the final temperature for 20.5 to 45.5 hours at a pressure of < 100 microns.

Lyophilization is terminated by stoppering under a pressure of < 100 microns, or after backfilling with nitrogen gas to a pressure of approximately one inch of water.

The moisture content typically is between 0.3 and 3% (w/v).

Example 2

Preparation of Fibronectin-Containing Ophthalmic Solution

An ophthalmic solution was prepared in accordance with the following procedure. The solution was prepared by combining fibronectin lyophilized according to Example 1 with 3 ml of a sterile solution containing 0.01% butyl p-hydroxybenzoate, 0.02% propyl p-hydroxybenzoate, and 0.01% disodium dihydrate ethylenediaminetetraacetic acid ($Na_2C_{10}H_{14}O_8N_2 \cdot 2H_2O$) in U.S.P. Grade Purified Water. The solution is provided from an eyedropper

bottle. The procedure is as follows: The stopper is removed from the vial containing the lyophilized fibronectin; the eyedropper bottle cap is unscrewed; the vial is snapped onto the top of the eyedropper bottle; the solution is added to the fibronectin vial by inverting; the solution is swirled if necessary; the solution is reinverted into the eyedropper bottle; the fibronectin vial is removed from the top of the eyedropper bottle; the eyedropper bottle cap is screwed on tightly; the final solution is swirled gently to ensure a homogeneous solution. A fully soluble solution is typically reached in ≤ 1 minute. The final ophthalmic solution contains the following components in the quantities indicated:

Component	Quantity
Fibronectin	1 mg/ml
Sodium Phosphate Buffer (pH 7.4)	0.01 M
Sucrose	0.1 M
Glycine	0.04 M
Sodium Chloride	0.087 M
Butyl p-hydroxybenzoate	0.01%
Ethyl p-hydroxybenzoate	0.02%
Disodium dihydrate ethylenediaminetetraacetic acid	0.01%

When the ophthalmic solution is intended for the individual use of one patient, the solution may be packaged in sterile multiple-dose containers which are sealed and made tamper-proof.

Example 3

Effect of Parabens Preservative on Cell Binding Activity of Fibronectin

An ophthalmic solution with a fibronectin concentration of 1.197 mg/ml was prepared in phosphate buffered saline ("PBS") with the Parabens preservative being a combination of 0.05% methyl p-hydroxybenzoate and 0.015% propyl p-hydroxybenzoate (Sample 1). A second ophthalmic solution with a fibronectin concentration of 1.197 mg/ml was prepared in PBS without adding a preservative (Sample 2). Samples 1 and 2 were allowed to stand at room temperature for seven days.

A fibronectin standard of 1.0 mg fibronectin/ml in PBS was diluted in triplicate with PBS (NaCl 8,000 mg, KCl 200 mg, Na_2HP0_4 1,150 mg, KH_2PO_4 200 mg in 1 liter of double distilled water, pH 7.3) to make dilution series of 5,000 to 0.078 ug/ml of fibronectin standard. Sample 1 and 2 were each diluted in triplicate with PBS to make dilution series for each sample of 5.000 to 0.078 ug/ml of fibronectin.

The cell binding activity of fibronectin was measured using a BHK cell attachment assay in accordance with the following procedure. A 96-well microplate was precoated with 200 ul of 3% BSA (30 mg/ml of BSA in PBS) at 37°C for 2 hours and rinsed twice with 100 ul of PBS. Fifty (50) ul of each dilution of the reference fibronectin and the test samples (Sample 1 and Sample 2) were transferred into separate wells of the 96-well microplate. The plate was incubated at 37°C for 60 minutes and the dilutions discarded by aspiration. One hundred (100) ul of 3% BSA were added into each well and the plate

incubated for 60 minutes at 37°C. During this incubation, a BHK cell suspension was prepared as follows: BHK cells, cultured in RPMI-1640 media containing 10% fetal bovine serum, were scraped from a tissue culture plate with a cell scraper and centrifuged at 1,000 rpm for 7 minutes. The cell plate was suspended in serum free RPMI-1640 media (RMPI-1640 supplemented with 20 mM HEPES) and centrifuged at 1,000 rpm for 7 minutes. This step was then repeated, to further wash the BHK cells. The washed BHK cells were resuspended in serum free RPMI-1640 media and a single cell suspension generated by pipetting. The cell number was adjusted to 2 x 10^6 cells/ml with serum free RPMI-1640. The 96-well plate was then rinsed twice with 100 ul PBS. Fifty (50) ul of the BHK cell suspension were added to each separate well of the 96-well plate. The plate was incubated at 37°C in a 5% CO_2 incubator for 90 minutes. The cell suspension was discarded by aspiration and the plate rinsed with 100 ul of saline. Fifty (50) ul of E-MEM medium (Eagle's MEM supplemented with 5% FBS) were added into each well of the assay plate. Fifty (50) ul of Neutral Red solution was added into each well of the assay plate. (The Neutral Red solution was prepared by adding 2 ml of 1m HEPES and 10 ml of 1% neutral to 88 mls of E-MEM medium just prior to use). The plate was incubated at 37° in a 5% CO_2 incubator for 60 minutes. The plate was rinsed twice with 100 ul of saline and 200 ul of Neutral Red Extraction Buffer (0.05 M solution phosphate monobasic in 50% EtOH) was added to each well of the plate. The plate was left standing at room temperature overnight and the absorbance of each well was then read spectrophotometrically at 546 nm.

The fibronectin content in mg/ml of each dilution of the dilution series for Sample 1 and Sample 2 was determined in comparison to the reference fibronectin standard. The data obtained was used to calculate the relative potencies of Sample 1 and Sample 2 against the fibronectin sample by parallel line assay. The cell binding activity for each test, the mean, and the standard deviation (S.D.) are presented below in Table I.

TABLE I

	Test 1	Test 2	Test 3	Mean	S.D.
Sample 1	1.229	1.198	1.257	1.228	0.030
Sample 2	1.182	1.133	1.140	1.152	0.027

There was no significant difference in the cell binding activity of Sample 1 and Sample 2 as shown by the results in Table I. This demonstrates that the Parabens preservative did not affect the cell binding activity of fibronectin in an ophthalmic solution.

Example 4

Effect of Different Parabens Preservatives on Cell Binding Activity of Fibronectin

An ophthalmic solution was prepared according to the procedure of Example 2, except that the Parabens preservative was a combination of 0.02% ethyl p-hydroxybenzoate and 0.01% butyl p-hydroxybenzoate, and the concentration of disodium ethylenediaminetetraacetate (in sterile water) was 0.05% (Sample 1). Sample 1 was divided into four parts (Samples 1A,

1B, 1C, and 1D). Sample 1A was stored at 4°C for 7 days. Sample 1B was stored at 4°C for 14 days. Sample 1C was stored at 37°C for 7 days. Sample 1D was stored at 37°C for 14 days.

A second ophthalmic solution was prepared according to the procedure of Example 2, except that the Parabens preservative was a combination of 0.038% methyl p-hydroxybenzoate and 0.015% propyl p-hydroxybenzoate, and the concentration of disodium ethylenediaminetetraacetate (in sterile water) was 0.05% (Sample 2). Sample 2 was divided into four parts (Samples 2A, 2B, 2C and 2D). Sample 2A was stored at 4°C for 7 days. Sample 2B was stored at 4°C for 14 days. Sample 2C was stored at 37°C for 7 days. Sample 2D was stored at 37°C for 14 days.

The cell binding activity of fibronectin was measured using a standard BHK cell attachment assay in accordance with the procedure described in Example 3. A fibronectin standard of 1.0 mg fibronectin/ml of PBS, stored at -80°C, was diluted with PBS to make a control dilution series of 5.000 to 0.078 ug/ml of fibronectin standard. On day 7, Samples 1A and 1C and Samples 2A and 2C were each diluted with PBS to make a dilution series for each sample of 5.000 to 0.078 ug/ml of sample. The BHK cell attachment assay was performed on each dilution series for Samples 1A and 1C, Samples 2A and 2C, and the fibronectin standard and the fibronectin content in mg/ml of each dilution was determined. On day 14, the dilution series procedures and the BHK cell attachment assays were performed on the dilution series for Samples 1B and 1D, Samples 2B and 2D, and the fibronectin standard. The data obtained was then used to

calculate by parallel line assay the relative potencies of Samples 1A-D and Samples 2A-D against the fibronectin standard. The assay was repeated four more times for each sample. Table II below presents the cell binding activity results of these assays as an average of the five assays and the standard deviation (± S.D.).

TABLE II

Sample #	Storage (°C)	# Days Storage	Fibronectin (mg/ml)	Activity (%)
Control	-80	(-)	1.090±0.72	100.0±6.6
1A	4	7	1.027±0.025	94.2±2.3
1B	4	14	1.131±0.045	103.8±4.1
1C	37	7	1.083±0.053	99.4±4.9
1D	37	14	1.059±0.024	97.2±2.2
2A	4	7	1.094±0.027	100.4±2.5
2B	4	14	1.094±0.036	100.4±3.3
2C	37	7	1.158±0.048	106.2±4.4
2D	37	14	1.090±0.069	100.0±6.3

There was no significant difference in the cell binding activity of Sample 1 and Sample 2, whether stored for 7 days or 14 days, or stored at room temperature or under refrigeration, as shown by the results in Table II. This demonstrates that Parabens preservatives together with disodium ethylenediaminetetratacetic acid did not affect the cell binding activity or stability of fibronectin in ophthalmic solutions.

Example 5

Effect of Parabens Preservative On
Gelatin Binding Activity of Fibronectin

An ophthalmic solution with a fibronectin concentration of 1.0 mg/ml was prepared in PBS, with the Parabens preservative being a combination 0.05% methyl p-hydroxybenzoate and 0.015% propyl p-hydroxybenzoate (Sample 1). A second ophthalmic solution with a fibronectin concentration of 1.0 mg/ml was prepared in PBS without adding a preservative (Sample 2). Samples 1 and 2 were allowed to stand at room temperature for seven days.

The gelatin binding activity of fibronectin was measured by gelatin-Sepharose affinity chromatography. First, Sample 1 was subjected onto a GPC-HPLC system (Asahipak GS 710, BioRad 402T HRLC system), the Parabens preservative eliminated, and protein fractions collected. Sample 2 was similarly subjected onto a GPC-HPLC system and protein fractions collected. The collected protein fractions of Sample 1 and Sample 2 were respectively subjected to gelatin-Sepharose chromatography, specifically the gelatin-Sepharose in HR5/5, BioRad 402T, Affinity Chromatography system. The gelatin binding activity of fibronectin was determined by measuring retention time in minutes and the elution peak area of fibronectin. The elution peak area was measured spectrophotometrically at a wavelength of 280nm. The gelatin binding activity results are presented below in Table III.

TABLE III

(280nm)	Retention Time (min)	Elution Peak Area
Sample 1	42.92	345.357
Sample 2	42.97	342.332

There was no significant difference in the gelatin binding activity of Sample 1 and Sample 2, as shown by the results in Table III. This demonstrates that the Parabens preservative did not affect the gelatin binding activity of fibronectin in an ophthalmic solution.

Example 6

Effect of Parabens Preservative On Bacteria Binding Activity of Fibronectin

An ophthalmic solution with a fibronectin concentration of 1.0 mg/ml was prepared in PBS, with the Parabens preservative being a combination of 0.05% methyl p-hydroxybenzoate and 0.015% propyl p-hydroxybenzoate (Sample 1). A second ophthalmic solution with a fibronectin concentration of 1.0 mg/ml was prepared in PBS without adding a preservative (Sample 2). Samples 1 and 2 were allowed to stand at room temperature for seven days.

The bacteria binding activity of fibronectin was measured by observing the agglutination after incubation of the ophthalmic solution with a heat-treated *Staphylococcus aureus* solution. The *S. aureus* solution was prepared by diluting *S. aureus* in PBS to a concentration of approximately 1 X 10^9

cells/ml, then heating the solution to 100°C for 10 minutes. Samples 1 and 2 were diluted with PBS to make a dilution series for each sample from 1,000 to 0.2 ug/ml. Using a 24-well microtiter cell culture assay plate, 500 ul of each dilution of Sample 1 and Sample 2 were dispensed into individual wells in the assay plate. Subsequently into each well, 50 ul of *S. aureus* solution was added. At room temperature, the solutions were repeatedly mixed by gently shaking the assay plate every 5 minutes up to one hour. The presence or absence of an agglutinating clump of fibronectin and *S. aureus* bacteria was observed and noted for each dilution of each test sample. The bacteria binding activity results are presented below in TABLE IV.

TABLE IV

Concentration of Fibronectin in Sample (ug/ml)	Sample 1	Sample 2
1,000	++	++
500	++	++
200	++	++
100	++	++
50	++	++
20	++	++
10	+	+
5	+	+
2	+	+
1	±	±
0.5	-	-
0.2	-	-
0.1	-	-
0	-	-

++ : Intense clumping
+ : Clumping
± : Weak clumping
- : No clumping

Clumping by fibronectin for both samples was observed when the concentration of fibronectin exceeded 1 ug/ml. No differences in the bacteria-binding activity were observed between Sample 1 and Sample 2 as shown by the results in Table IV. This demonstrates that the Parabens preservative did not affect the bacteria-binding activity of fibronectin in an ophthalmic solution.

Example 7

Minimum Inhibitory Concentration of Parabens Preservatives

Ophthalmic solutions were prepared in accordance with the procedure of Example 2 with the following variables indicated in the tables below. The type and concentration of Parabens preservative was varied. The Parabens preservatives used were methyl paraben ("Mp"), propyl paraben ("Pp"), ethyl paraben ("Ep"), and butyl paraben ("Bp"). Disodium ethylenediaminetetraacetic acid ("EDTA") was added and the concentration of EDTA was varied to test the potentiating effect of EDTA on the Parabens preservatives. These different formulations were separately challenged with an inoculum of *P. aeruginosa* or *C. albicans*. At 6 hours and 24 hours, the inoculated formulations were streaked onto individual culture plates and examined for growth. Growth, indicated by colony forming units, was scored on a scale of 0-4, with 0 indicating no growth to 4 indicating highest growth. The minimum inhibitory concentrations ("MIC") of the Parabens preservatives and the potentiating effects of EDTA on the Parabens preservatives are presented below in Tables V to X.

TABLE V

MIC Results of Ophthalmic Solution Containing Mp, Pp, and EDTA

% Mp	% Pp	%EDTA	*P.aeruginosa* 6 hrs	*P.aeruginosa* 24 hrs	*C.albicans* 6 hrs	*C.albicans* 24 hrs
0.068	0.027	0.089	1	0	2	0
0.051	0.020	0.067	2	0	0	0
0.038	0.015	0.05	2	1	1	1
0.029	0.011	0.038	2	2	1	1
0.021	0.008	0.028	2	2	1	1
0.016	0.006	0.021	2	3	1	1
0.012	0.005	0.016	3	3	1	1

TABLE VI

MIC Results of Ophthalmic Solution Containing Mp, Pp, and 0.05% EDTA

% Mp	% Pp	*P.aeruginosa* 6 hrs	*P.aeruginosa* 24 hrs	*C.albicans* 6 hrs	*C.albicans* 24 hrs
0.068	0.027	1	0	1	0
0.051	0.020	2	0	0	0
0.038	0.015	2	1	0	0
0.029	0.011	3	2	0	0
0.021	0.008	3	2	0	0
0.016	0.006	4	3	2	1
0.012	0.005	4	3	2	1

TABLE VII

MIC Results Of Ophthalmic Solution Containing Mp, Pp, But No EDTA

% Mp	% Pp	*P.aeruginosa*		*C.albicans*	
		6 hrs	24 hrs	6 hrs	24 hrs
0.068	0.027	1	0	0	0
0.051	0.020	3	1	1	0
0.038	0.015	3	2	1	0
0.029	0.011	3	3	1	0
0.021	0.008	3	3	3	1
0.016	0.006	3	3	3	1
0.012	0.005	3	3	3	1

TABLE VIII

MIC Results of Ophthalmic Solution Containing Ep, Bp, and EDTA

% Ep	% Bp	%EDTA	*P.aeruginosa*		*C.albicans*	
			6 hrs	24 hrs	6 hrs	24 hrs
0.027	0.013	0.067	0	0	0	0
0.020	0.01	0.05	1	0	0	0
0.015	0.007	0.038	2	1	0	0
0.011	0.006	0.028	3	2	0	0
0.008	0.004	0.021	3	3	0	0
0.006	0.003	0.016	3	3	2	2
0.005	0.002	0.012	3	4	4	2

TABLE IX

MIC Results of Ophthalmic Solution Containing Ep, Bp, and 0.05% EDTA

% Ep	% Bp	*P.aeruginosa* 6 hrs	*P.aeruginosa* 24 hrs	*C.albicans* 6 hrs	*C.albicans* 24 hrs
0.027	0.013	0	0	0	0
0.020	0.010	1	0	1	0
0.015	0.007	2	1	1	0
0.011	0.006	3	2	1	0
0.008	0.004	3	3	1	0
0.006	0.003	3	3	1	1
0.005	0.002	4	3	1	1

TABLE X

MIC Results of Ophthalmic Solution Containing Ep, Bp, But No EDTA

% Ep	% Mp	*P.aeruginosa* 6 hrs	*P.aeruginosa* 24 hrs	*C.albicans* 6 hrs	*C.albicans* 24 hrs
0.027	0.013	0	0	0	0
0.020	0.010	2	1	1	0
0.015	0.007	2	2	1	0
0.011	0.006	3	2	1	0
0.008	0.004	3	3	1	0
0.006	0.003	4	3	1	0
0.005	0.002	4	3	2	0

A preservative made from a combination of methyl paraben in a concentration from 0.012 to 0.068% and propyl paraben in a concentration from 0.005 to 0.027% inhibited the growth of microbes in the ophthalmic solution, as shown in

Tables V to VII. The efficacy of this preservative in inhibiting microbial growth was improved when a potentiating agent, EDTA, was added, as shown by a comparison of Tables V and VI with Table VII.

A preservative made from a combination of ethyl paraben in a concentration from 0.005 to 0.027% and butyl paraben in a concentration from 0.002 to 0.013% inhibited the growth of microbes in the ophthalmic solutions, as shown in Tables VIII to X. The efficacy of this preservative in inhibiting microbial growth was improved when a potentiating agent, EDTA, was added, as shown by a comparison of Tables VIII and IX with Table X.

This demonstrates that Parabens preservatives inhibit microbial growth in an ophthalmic solution.

Example 8

Effect of Parabens Preservative On Corneal Wound Closing Activity of Fibronectin

An ophthalmic solution with a fibronectin concentration of 1.0 mg/ml was prepared in PBS, with the Parabens preservative being a combination of 0.05% methyl p-hydroxybenzoate and 0.015% propyl p-hydroxybenzoate (Sample 1). A second ophthalmic solution with a fibronectin concentration of 1.0 mg/ml was prepared in PBS without adding a preservative (Sample 2). Samples 1 and 2 were allowed to stand at room temperature for seven days. A control ophthalmic solution free of fibronectin and preservative was also prepared.

The corneal wound closing activity of fibronectin was measured following the procedures described in Moses et al., 18 Invest. Ophthalmol 103-106 (1979), and Nishida et al., 102 Arch. Ophthalmol. 455-456 (1984). Rabbit corneal epithelium was injured by iodine vapor treatment for 3 minutes. Samples 1 and 2 and the control were applied to 27 injured rabbit corneal epithelium samples apiece. One drop of the ophthalmic solution being tested was applied to the injured corneal epithelium at 4, 5, 6, and 7 hours after injury, and at every hour from 16 to 30 hours after injury. At 4, 16, 20, 24, 28, and 32 hours after the iodine treatment, the rabbit corneas were stained with 2% fluorescein and photographed. The stained area of the corneal epithelium was measured by a computerized image analyzer. The healing rate of each corneal wound was calculated by a linear regression of the wound area during the period of 16 to 32 hours after injury by the iodine treatment. The Student's t test was employed. The rabbits that did not have enough corneal epithelium defect at 4 hours after the iodine treatment were excluded by Smirnov's method. The corneal wound healing activity results are presented below in Table XI.

TABLE XI

	Healing Rate 16-32 hr, mm^2/hr	Student's t test (p value)	No. of eyes
Sample 1	1.80 ± 0.07	$p < 0.001$	27
Sample 2	1.66 ± 0.05	$p < 0.005$	27
Control	1.40 ± 0.05	-	27

Healing Rate : Mean ± SEM

There was no significant difference in the corneal wound healing activity of Sample 1 and Sample 2 as shown by the results in Table XI. This test demonstrates that the Parabens preservative did not affect the corneal wound healing activity of fibronectin in an ophthalmic solution.

Example 9

Effect of Different Parabens Preservatives on Corneal Wound Closing Activity of Fibronectin

An ophthalmic solution was prepared in PBS with the Parabens preservative being a combination of 0.02% ethyl p-hydroxybenzoate and 0.01% butyl p-hydroxybenzoate, and the concentration of disodium ethylenediaminetetraacetate was 0.01% (Sample 1).

A second ophthalmic solution with a fibronectin concentration of 0.5 mg/ml was prepared in PBS with the Parabens preservative being a combination of 0.02% ethyl p-hydroxybenzoate and 0.01% butyl p-hydroxybenzoate, and the concentration of disodium ethylenediaminetetraacetate was 0.01% (Sample 2).

A third ophthalmic solution was prepared in PBS, with the Parabens preservative being a combination of 0.038% methyl p-hydroxybenzoate and 0.015% propyl p-hydroxybenzoate, and the concentration of disodium ethylenediaminetetraacetate was 0.05% (Sample 3).

A fourth ophthalmic solution with a fibronectin concentration of 0.5 mg/ml was prepared in PBS, with the Parabens preservative being a combination of 0.038% methyl p-hydroxybenzoate and 0.015% propyl p-hydroxybenzoate, and the concentration of disodium ethylenediaminetetraacetate was 0.05% (Sample 4).

Samples 1-4 were allowed to stand at room temperature for seven days.

The corneal wound closing activity of fibronectin was measured following the procedures described in Moses et al., 18 Invest. Ophthalmol. 103-106 (1979), and Nishida et al., 102 Arch. Ophthalmol. 455-456 (1984). Rabbit corneal epithelium was injured by iodine vapor treatment for 3 minutes. Samples 1-4 and the control were applied to 12 injured rabbit corneal epithelium samples apiece. One drop of the ophthalmic solution being tested was applied to the injured corneal epithelium at 4, 5, 6, and 7 hours after injury, and at every hour from 16 to 30 hours after injury. At 4, 16, 20, 24, 28, and 32 hours after the iodine treatment, the rabbit corneas were stained with 2% fluorescein and photographed. The stained area of the corneal epithelium was measured by a computerized image analyzer. The healing rate of each corneal wound was calculated by a linear regression of

the wound area during the period of 16 to 32 hours after injury by the iodine treatment. The rabbits that did not have enough corneal epithelium defect at 4 hours after the iodine treatment were excluded by Smirnov's method. The corneal wound healing activity results are presented below in Table XII.

TABLE XII

	Concentration of Fibronectin (mg/ml)	Healing Rate 16-32 hr, mm^2/hr
Sample 1	1.0	1.73 ± 0.08
Sample 2	0.5	1.36 ± 0.08
Sample 3	1.0	1.72 ± 0.05
Sample 4	0.5	1.56 ± 0.12

Healing Rate : Mean ± SEM

There was no significant difference in the corneal wound healing activity of Sample 1 versus Sample 3 and Sample 2 versus Sample 4 as shown by the results in Table XII. Moreover, the rate of healing of Samples 1 and 3 was comparable to the rate of healing of Samples 1 and 2 in Example 8. This demonstrates that the different Parabens preservatives did not differentially affect the corneal wound healing activity of fibronectin in an ophthalmic solution.

Example 10

Solubility of Fibronectin Lyophilized in the Presence of Sucrose Without Glycine

Fibronectin at a concentration of 5 mg/ml in PBS was lyophilized with either 0.05 M or 0.1 M sucrose. The degree

of solubilization of the lyophilized fibronectin was determined by absorbance at 280 nm 10 minutes after reconstitution with distilled water. Based on soluble protein, the present solubility was 66% and 71%, respectively.

Example 11

Effect of Sucrose Concentration on the Solubility of Lyophilized Fibronectin in the Presence of Glycine

Fibronectin was lyophilized as in Example 1, except that the sucrose concentration was adjusted such that, after reconstitution, the concentration of sucrose in each of the five samples was as given in Table XIII below. After standing at room temperature for 30 minutes, each sample was dissolved in 3 ml of water. All samples completely dissolved, and the time in seconds to complete dissolution of the fibronectin was measured and is shown in Table XIII.

TABLE XIII

Sucrose Concentration (M)	Time to Complete Dissolution (Seconds)
0.05	75-80
0.075	45-50
0.10	20-25
0.125	20-25
0.107	25-30

When fibronectin is lyophilized in the presence of sucrose and glycine, the fibronectin is completely soluble, whereas when lyophilized in the presence of sucrose only, fibronectin is partially soluble as shown in Example 10. The rate of solubility of fibronectin is dependent upon the

concentration of sucrose as shown by the results in Table XIII.

* * * *

It will be understood that various modifications may be made without departing from the spirit of the present invention.

CLAIMS

1. A stable and soluble multi-dose ophthalmic solution comprising fibronectin, an amino acid selected from the group consisting of water-soluble hydrophilic amino acids and mixtures thereof, a sugar selected from the group consisting of a monosaccharide, a disaccharide, a trisaccharide, a polysaccharide, derivatives thereof, and mixtures thereof, and a lower alkyl p-hydroxybenzoate preservative, said ophthalmic solution being free of albumin.

2. The ophthalmic solution of claim 1 further comprising a potentiating agent selected from the group consisting of ethylenediaminetetraacetic acid and salts thereof.

3. The ophthalmic solution of claim 1, wherein the concentration of fibronectin is from 0.25 to 10.0 mg/ml.

4. The ophthalmic solution of claim 1, wherein the concentration of the amino acid in the solution ranges from 0.005 to 0.5 M.

5. The ophthalmic solution of claim 1, wherein the concentration of the sugar in the solution is from 0.005 to 0.5 M.

6. The ophthalmic solution of claim 1, wherein the amino acid is glycine and the sugar is sucrose.

7. The ophthalmic solution of claim 6, wherein the concentration of glycine 0.04 M and the concentration of sucrose is 0.1 M.

8. The ophthalmic solution of claim 1, wherein the lower alkyl p-hydroxybenzoate preservative is from 0.002 to 0.25% (w/v).

9. The ophthalmic solution of claim 1, wherein the lower alkyl p-hydroxybenzoate preservative comprises methyl p-hydroxybenzoate, ethyl p-hydroxybenzoate, propyl p-hydroxybenzoate, butyl p-hydroxybenzoate, or mixtures thereof.

10. The ophthalmic solution of claim 2, wherein the salts of ethylenediaminetetraacetic acid comprise disodium ethylenediaminetetraacetate and disodium dihydrate ethylenediaminetetraacetate.

11. The ophthalmic solution of claim 10, wherein the preservative comprises a combination of ethyl p-hydroxybenzoate and butyl p-hydroxybenzoate together with the potentiating agent disodium dihydrate ethylenediaminetetraacetate.

12. The ophthalmic solution of claim 11, wherein the concentration of ethyl p-hydroxybenzoate is from 0.005 to 0.17%, the concentration of butyl p-hydroxybenzoate is from 0.002 to 0.02%, and the concentration of disodium dihydrate ethylenediaminetetraacetate is from 0.005 to 0.1%.

13. A stable and soluble single dose ophthalmic solution comprising fibronectin, an amino acid selected from the group consisting of water-soluble hydrophilic amino acids and mixtures thereof, and a sugar selected from the group consisting of a monosaccharide, a disaccharide, a trisaccharide, a polysaccharide, derivatives thereof, and

mixtures thereof, said ophthalmic solution being free of albumin.

14. The ophthalmic solution of claim 13, wherein the concentration of fibronectin is from 0.25 to 10.0 mg/ml.

15. The ophthalmic solution of claim 13, wherein the concentration of the amino acid is from 0.005 to 0.5 M.

16. The ophthalmic solution of claim 13, wherein the concentration of the sugar is from 0.005 to 0.5 M.

17. The ophthalmic solution of claim 13, wherein the amino acid is glycine and the sugar is sucrose.

18. The ophthalmic solution of claim 17, wherein the concentration of glycine is 0.04 M and the concentration of sucrose is 0.1 M.

19. A method of treatment of an ophthalmic wound comprising administering to the wound a wound-healing amount of the ophthalmic solution of claim 1.

20. The method of claim 19, wherein the ophthalmic solution further comprises a potentiating agent selected from the group consisting of ethylenediaminetetraacetic acid and salts thereof.

21. The method of claim 20, wherein the preservative comprises a combination of ethyl p-hydroxybenzoate and butyl p-hydroxybenzoate, together with disodium dihydrate ethylenediaminetetraacetate.

22. A method of treatment of an ophthalmic wound comprising administering to the wound a wound-healing amount of the ophthalmic solution of claim 13.

23. A process for preparing fibronectin for ophthalmic use comprising lyophilizing under vacuum an aqueous solution comprising fibronectin, an amino acid selected from the group consisting of water-soluble hydrophilic amino acids, and mixtures thereof, and a sugar selected from the group consisting of a monosaccharide, a disaccharide, a trisaccharide, a polysaccharide, derivatives thereof, and mixtures thereof, said aqueous solution being free of albumin.

24. The process of claim 23, followed by introducing a non-reactive gas and sealing.

25. The process of claim 23, wherein the non-reactive gas is nitrogen.

26. The process of claim 23, wherein the concentration of fibronectin is from 0.25 to 30.0 mg/ml.

27. The process of claim 23, wherein the concentration of the amino acid is from 0.005 to 1.5 M.

28. The process of claim 23, wherein the concentration of the sugar is from 0.005 to 1.5 M.

29. The process of claim 23, wherein the amino acid is glycine and the sugar is sucrose.

30. The process of claim 29, wherein the glycine is present in an amount of 0.12 M and the sucrose is present in an amount of 0.30 M of the solution.

31. A method for inhibiting bacterial growth in an ophthalmic solution comprising fibronectin, an amino acid selected from the group consisting of water-soluble hydrophilic amino acids and mixtures thereof, and a sugar selected from the group consisting of a monosaccharide, a

disaccharide, a trisaccharide, a polysaccharide, derivatives thereof, and mixtures thereof, while preserving the cellular adhesion and wound healing properties of fibronectin, said method comprising adding to said ophthalmic solution a lower alkyl p-hydroxybenzoate preservative in an amount sufficient to inhibit bacterial growth in said ophthalmic solution.

32. A method of treatment of ophthalmic wounds comprising administering to the wound a wound healing accelerator solution disposed in a multi-use container, wherein said solution comprises a wound healing accelerator and a lower alkyl p-hydroxybenzoate preservative.

33. The method of claim 34, wherein the ophthalmic solution further comprises a potentiating agent selected from the group consisting of ethylenediaminetetraacetic acid and salts thereof.

34. The method of claim 35, wherein the lower alkly p-hydroxybenzoate preservative comprises a combination of ethyl p-hydroxybenzoate and butyl p-hydroxybenzoate, together with disodium dihydrate ethylenediaminetetraacetate.

INTERNATIONAL SEARCH REPORT

International Application No PCT/US 92/10243

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC[5]: A 61 K 37/04,A 61 K 9/08,A 61 K 9/14,A 61 K 47/14, A 61 K 47/00

II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
IPC[5]	A 61 K 37/00,A 61 K 9/00,A 61 K 47/00

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category [10]	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
X,Y	EP, A2, 0 058 993 (THE GREEN CROSS CORPORATION) 01 September 1982 (01.09.82), abstract; page 3, line 23 - page 4, line 9; page 4, lines 19-25. --	1,3-9, 11-18, 23-31
X,Y	US, A, 4 740 498 (Y. HIRAO et al.) 26 April 1988 (26.04.88), column 2, lines 4-8,27-29; column 2, line 57 - column 3, line 31. --	1,3-9, 11-18, 23-31
A	US, A, 4 837 019 (A.C.W. GEORGALAS et al.) 06 June 1989 (06.06.89), claims 1-7,12,14. --	1-18, 31
A	CHEMICAL ABSTRACTS, vol. 101, no. 10, issued September 3, 1984 (Columbus, Ohio, USA)	1,8,9, 11,12, 31

* Special categories of cited documents: [14]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
01 March 1993	18. 03. 93
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	MAZZUCCO e.h.

Form PCT/ISA/210 (second sheet) (January 1985)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 92/10243

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.: 19-22,32-34
because they relate to subject matter not required to be searched by this Authority, namely:
See PCT rule 39.1 (iv)
Methods for treatment of the human or animal body by surgery or therapy, as well as diagnostic methods.

2. [ ] Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

International Application No PCT/US 92/10243

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)		
Category *	Citation of Document, ** with indication, where appropriate, of the relevant passages	Relevant to Claim No.
	SHISEIDO CO., LTD. "Cosmetics containing fibronectins", page 368, column 1, the abstract-no. 78 679b, Jpn. Kokai Tokkyo Koho JP 59-76 007. --	
A	US, A, 4 565 651 (T. OHMURA et al.) 21 January 1986 (21.01.86), abstract; claims 1-8 (cited in the application). ----	1,3-7, 13-18, 23,26-30

ANHANG

zum internationalen Recherchenbericht über die internationale Patentanmeldung Nr.

ANNEX

to the International Search Report to the International Patent Application No.

ANNEXE

au rapport de recherche international relatif à la demande de brevet international n°

PCT/US 92/10243 SAE 67932

In diesem Anhang sind die Mitglieder der Patentfamilien der im obengenannten internationalen Recherchenbericht angeführten Patentdokumente angegeben. Diese Angaben dienen nur zur Unterrichtung und erfolgen ohne Gewähr.

This Annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The Office is in no way liable for these particulars which are given merely for the purpose of information.

La présente annexe indique les membres de la famille de brevets relatifs aux documents de brevets cités dans le rapport de recherche international visée ci-dessus. Les renseignements fournis sont donnés à titre indicatif et n'engagent pas la responsabilité de l'Office.

Im Recherchenbericht angeführtes Patentdokument Patent document cited in search report Document de brevet cité dans le rapport de recherche	Datum der Veröffentlichung Publication date Date de publication	Mitglied(er) der Patentfamilie Patent family member(s) Membre(s) de la famille de brevets	Datum der Veröffentlichung Publication date Date de publication
EP A2 58993	01-09-82	AT E 52413 DE C0 3280166 EP A3 58993 EP B1 58993 ES A1 509833 ES A5 509833 ES A1 8303094 JP A2 57140724 JP B4 3048169 US A 4424206	15-05-90 13-06-90 23-01-85 09-05-90 01-02-83 01-03-83 01-05-83 31-08-82 23-07-91 03-01-84
US A 4740498	26-04-88	CA A1 1254510 DE C0 3585265 EP A2 179477 EP A3 179477 EP B1 179477 JP A2 61103836	23-05-89 05-03-92 30-04-86 04-02-87 22-01-92 22-05-86
US A 4837019	06-06-89	keine - none - rien	
US A 4565651	21-01-86	DE C0 3380924 EP A2 106608 EP A3 106608 EP B1 106608 ES A1 526254 ES A5 526254 ES A1 8600498 JP A2 59067228 JP B4 3078373	11-01-90 25-04-84 26-03-86 06-12-89 01-10-85 30-10-85 01-01-86 16-04-84 13-12-91

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(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, SV, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

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(54) Title: HIGH TEMPERATURE STABLE PEPTIDE FORMULATION

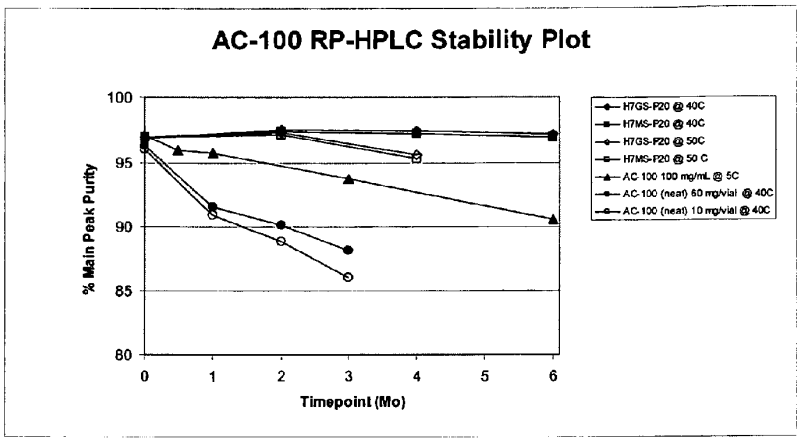

Figure 1. Stability of Various Formulation with AC-100

(57) Abstract: A stabilized pharmaceutical composition in the form of a lyophilized product to be later reconstituted to generate an aqueous drug product is described herein. The therapeutically active ingredient in the form of a peptide of sequence TDLQERGDNDISPFSGDGQPFKD is stabilized with a buffer, carbohydrate stabilizer, a nonionic bulking agent and a surfactant to facilitate reconstitution. The preferred preparation contains a peptide of the sequence TDLQERGDNDISPFSGDGQPFKD, histidine buffer, mannitol or glycine, sucrose and/or Polysorbate 20. This combination of excipients has demonstrated exceptional stability as a lyophilized product when stored at the elevated temperature of 40 °C for at least 6 months and for at least 3 Months at 50 °C. The lyophilized mixture thus formed is reconstituted to a high peptide concentration without apparent loss of stability of the peptide and has also enabled the ability to terminally sterilize the lyophilized product using gamma irradiation without affecting stability.

WO 2008/150479 A2

HIGH TEMPERATURE STABLE PEPTIDE FORMULATION

TECHNICAL FIELD

[0001] The claimed subject matter is directed to a lyophilized peptide formulation. In particular, it relates to a lyophilized peptide formulation that is stable at relatively high temperatures and sterilization by gamma irradiation, which can be reconstituted with a diluent to generate an aqueous drug product which can be administered by injection.

BACKGROUND

[0002] Recent advances in biotechnology have made it possible to produce a variety of peptides for pharmaceutical applications using recombinant DNA and synthetic techniques. AC-100, also known as Dentonin®, is a therapeutically active peptide shown to stimulate proliferation, differentiation, and mineralization of human osteoblasts (Nagel et al. (2004) *J. Cell. Biochem.* 93(6):1107-14; U.S. Patent No. 6,911,425; U.S. Patent No. 7,078,021 and U.S. Patent No. 7,160,862). AC-100 has shown bone formation activities *in vivo* (Hayashibara et al. (2004) *J. Bone and Mineral Res.* 19(3):455-62; Lazarov et al. ((2004) *ASBMR Abs.*); and has also demonstrated stimulation of the proliferation of human dental pulp cells *in vitro* (Liu et al. (2004) *J. of Dental Res.* 83(6):496-99); as well as formation of new dentin in human dental defects in a clinical trial (Lazarov et al. (2006) *IADR Abs.*).

[0003] Accordingly, AC-100 is useful in treating or preventing conditions associated with skeletal loss or weakness, increasing the number and biological activity of osteoblasts, odontoblasts, and other hard tissue forming cells that assist in forming skeletal and dental tissues and promoting regeneration of bones, teeth, and/or cartilage. The described therapeutic peptide may be administered, *inter alia*, in the treatment of bone defects and breakage, cartilage regeneration, and the stimulation of dental pulp cells to promote hard tissue formation.

[0004] Because therapeutic peptides may be larger and more complex than traditional organic and inorganic drugs (*i.e.*, possessing multiple functional groups in addition to possibly complex three-dimensional structures), the formulation of such peptides poses special problems. For a peptide to remain biologically active, a formulation must preserve intact the conformational integrity of at least a core sequence of the peptide's primary structure while at the same time protecting the peptide's multiple functional groups from degradation. Degradation pathways for peptides can involve chemical instability (*i.e.*, any

process which involves modification of the peptide by bond formation or cleavage resulting in a new chemical entity) or physical instability (*i.e.*, changes in the higher order structure of the peptide). Chemical instability can result from deamidation, racemization, hydrolysis, oxidation, beta elimination or disulfide exchange. Physical instability can result from denaturation, aggregation, precipitation or adsorption, for example. The three most common peptide degradation pathways are peptide aggregation, deamidation and oxidation. Cleland et al. (1993) *Critical Rev. in Therapeutic Drug Carrier Sys.* 10(4):307-377.

[0005] Freeze-drying is a commonly employed technique for preserving peptides which serves to remove water from the peptide preparation of interest. Freeze-drying, or lyophilization, is a process by which the material to be dried is first frozen and then the ice or frozen solvent is removed by sublimation in a vacuum environment. An excipient may be included in pre-lyophilized formulations to enhance stability during the freeze-drying process and/or to improve stability of the lyophilized product upon storage. Pikal, M. (1990) *Biopharm.* 3(9):26-30 and Arakawa et al. (1991) *Pharm. Res.* 8(3):285-291.

[0006] It is an object of the present invention to provide a lyophilized peptide formulation, which is stable upon storage and delivery at relatively high temperatures, and which may later be reconstituted to generate an aqueous drug product. It is a further object to provide a stable reconstituted peptide formulation, which is suitable for injectable, such as by intravenous and/or subcutaneous administration, for example. Accordingly, in certain embodiments, it is an object to provide a formulation that is stable as a lyophilized product when stored at elevated temperatures for several months at a time.

[0007] Another object of the present invention is to provide a lyophilized peptide formulation, which can be sterilized by radiation. If the peptide in the formulation is susceptible to degradation or structural modification by radiation, the peptide formulation must be sterilized by filtering through sterile filtration membranes prior to, or following, lyophilization and reconstitution. A formulation that enables the terminal sterilization by radiation, *e.g.*, gamma irradiation, would significantly simplify the final sterilization process of the formulated drug.

SUMMARY

[0008] The claimed subject matter is based on the discovery that a thermally stable lyophilized peptide formulation can be prepared using a buffer (preferably histidine or

phosphate), a lyoprotectant or carbohydrate stabilizer (preferably sucrose), a nonionic bulking agent/tonicity adjuster (preferably mannitol or glycine), and/or a surfactant (preferably Polysorbate 20) to facilitate reconstitution. The lyophilized formulation can be reconstituted to generate a stable reconstituted formulation having a peptide concentration which is significantly higher (*e.g.*, from about 2-80 times higher, preferably 3-20 times higher and most preferably 3-6 times higher) than the peptide concentration in the pre-lyophilized formulation. In particular, while the peptide concentration in the pre-lyophilized formulation may be 10 mg/mL or less, the peptide concentration in the reconstituted formulation is generally 50 mg/mL or more. Such high peptide concentrations in the reconstituted formulation are considered to be particularly useful where the formulation is intended for subcutaneous administration. The lyophilized formulation is stable (*i.e.*, fails to display significant or unacceptable levels of chemical or physical instability of the peptide) at 40 °C for at least about 6 months and is stable at 50 °C for at least about 3 months. Peptides, such as AC-100, in the lyophilized formulation essentially retain their physical and chemical stability and integrity upon lyophilization and storage. In certain embodiments, the reconstituted formulation is isotonic.

[0009] When reconstituted with a diluent comprising a preservative (such as bacteriostatic water for injection ("BWFI")), the reconstituted formulation may be used as a multi-use formulation. Such a formulation is useful, for example, where the patient requires frequent subcutaneous administrations of the peptide to treat a chronic medical condition. The advantage of a multi-use formulation is that it facilitates ease of use for the patient, reduces waste by allowing complete use of vial contents, and results in a significant cost savings for the manufacturer since several doses are packaged in a single vial (lower filling and shipping costs).

[0010] The ratio of lyoprotectant:peptide in the lyophilized formulation of the preceding paragraphs depends, for example, on both the peptide and lyoprotectant of choice, as well as the desired peptide concentration and isotonicity of the reconstituted formulation. In the case of AC-100 (as the peptide of SEQ ID NO:1) and sucrose (as the lyoprotectant) for generating a high peptide concentration isotonic reconstituted formulation, the ratio may, for example, be about 10-100 moles of sucrose:1 mole AC-100 ±20%.

[0011] Generally, the pre-lyophilized formulation of the peptide and lyoprotectant will further include a buffer which provides the formulation at a suitable pH. For this purpose,

it has been found to be desirable to use a histidine buffer in that, as demonstrated below, this appears to have lyoprotective properties.

[0012] The formulation may further include a surfactant (*e.g.*, a polysorbate) in that it has been observed herein that this can reduce aggregation of the reconstituted peptide and/or reduce the formation of particulates in the reconstituted formulation. The surfactant can be added to the pre-lyophilized formulation, the lyophilized formulation and/or the reconstituted formulation (but preferably the pre-lyophilized formulation) as desired.

[0013] In yet a further embodiment, the invention provides a method for preparing a formulation comprising the steps of: (a) lyophilizing a mixture of a peptide and a lyoprotectant; and (b) reconstituting the lyophilized mixture of step (a) in a diluent such that the reconstituted formulation is isotonic and has a peptide concentration of at least about 5 mg/mL. For example, the peptide concentration in the reconstituted formulation may be from about 10 mg/mL to about 400 mg/mL. Generally, the peptide concentration in the reconstituted formulation is about 2-80 times greater than the peptide concentration in the mixture before lyophilization with all numbers being ±20%.

[0014] An article of manufacture is also provided herein which comprises: (a) a container which holds a lyophilized mixture of the peptide and a lyoprotectant; and (b) instructions for reconstituting the lyophilized mixture with a diluent to a peptide concentration in the reconstituted formulation of at least about 50 mg/mL. The article of manufacture may further comprise a second container which holds a diluent (*e.g.*, WFI or BWFI comprising an aromatic alcohol).

[0015] The invention further provides a method for treating a mammal comprising administering a therapeutically effective amount of the reconstituted formulation disclosed herein to a mammal, wherein the mammal has a disorder requiring treatment with the peptide in the formulation. For example, the formulation may be administered intravenously or subcutaneously.

[0016] One useful peptide pre-lyophilized formulation as discovered in the experiment detailed below was found to comprise AC-100 in an amount from about 5-40 mg/mL (*e.g.*, 20-30 mg/mL) and sucrose in an amount from about 10-100 mM (*e.g.*, 40-80 mM) with all numbers being ±20%, glycine from about 50-250 (*e.g.*, 75-150 mM) mM with all numbers being ±20%, a buffer (*e.g.*, histidine, pH 7) and a surfactant (*e.g.*, a polysorbate). The lyophilized formulation was found to be stable at 40 °C for at least 6 months and stable at 50 °C for at least 3 months. This peptide formulation can be reconstituted with a diluent to

generate a formulation suitable for intravenous and/or subcutaneous administration comprising AC-100 in an amount of about 10-30 mg/mL. Where higher concentrations of the peptide are desired (for example, where subcutaneous delivery of the peptide is the intended mode of administration to the patient), the lyophilized formulation may be reconstituted to yield a reconstituted formulation having a peptide concentration of 50 mg/mL or more.

[0017] The invention includes a formulation comprised of a peptide which peptide may be any one of the sequences of the SEQ ID NOS:1-10 shown here. The formulation will include a buffer which may be histidine and a carbohydrate stabilize which may be sucrose along with a bulking agent such as glycine and surfactant such as Polysorbate 20.

[0018] The formulation of the invention may be a lyophilized mixture which is comprised of a non-reducing sugar such as sucrose, a peptide such as the peptide of any of the sequences of SEQ ID NO:1-10, a bulking agent such as glycine and a buffer such as histidine wherein the molar ratio of the non-reducing sugar to the peptide is within a range of from about 20 to 200 moles of non-reducing sugar to 1 mole of peptide with the ratio being an approximation ±20%.

[0019] The invention also includes a sterile reconstituted formulation which includes the lyophilized mixture as described above reconstituted using a diluent such as sterile water or bacteriostatic water which diluent may be isotonic. The resulting reconstituted formulation includes the peptide in an amount in the range of from about 1 mg/ml to about 300 mg/ml ±20%. The lyophilized mixture may be reconstituted using a pH buffered solution, a sterile saline solution, Ringer's solution and a dextrose solution.

[0020] The invention can further include a manufactured article which includes the reconstituted formulation described above along with instructions for reconstituting the lyophilized mixture with a diluent to provide a formulation having the peptide concentrations as described here.

[0021] These and other objects, advantages, and features of the invention will become apparent to those persons skilled in the art upon reading the details of the subject invention, as more fully described below.

BRIEF DESCRIPTION OF THE DRAWINGS

[0022] The invention is best understood from the following detailed description when read in conjunction with the accompanying drawings. It is emphasized that, according to

common practice, the various features of the drawings are not to-scale. On the contrary, the dimensions of the various features are arbitrarily expanded or reduced for clarity. Included in the drawings are the following figures:

[0023] Fig. 1 illustrates the increase in stability of AC-100 lyophilized formulation. The formulations H7GS-P20 (histidine pH 7/glycine/sucrose/ Tween 20) and H7MS-P20 (histidine pH 7/mannitol/sucrose/ Tween 20). The lyophilized cake was incubated at 40°C for 6 mo or 40°C for 3 weeks followed by and additional 3 Mo at 50°C (Noted as 50°C), then reconstituted. The fraction of intact peptide in the reconstituted formulation was measured by reversed-phase chromatography and defined as the peak area of the native peptide relative to the total peak area including degradants. This is compared to lyophilized AC-100 sans excipients (neat) stored at 40°C and the current liquid formulation of AC-100 100 mg/mL (90 mM NaCl, pH 7) stored at the accelerated stability condition of 5°C.

BRIEF DESCRIPTION OF THE SEQUENCES

[0024] SEQ ID NO:1 is set forth as TDLQERGDNDISPFSGDGQPFKD, which corresponds to the amino acid sequence of the therapeutically active ingredient of the invention, AC-100. AC-100 was identified as a small fragment within a large molecule referred to as matrix extracellular phosphoglycoprotein ("MEPE"). AC-100 is characterized by a few unique motifs, such as an RGD integrin-binding motif, and a SGDG glycosaminoglycan motif. These motifs are believed to give the molecule an essential structure resulting in its bioactivity which is preserved over a longer period of time using a formulation of the invention.

[0025] SEQ ID NO:2 is set forth as TDLQEDGRNDISPFSGDGQPFKD, which corresponds to the amino acid sequence of the therapeutically active ingredient of the invention, AC-101. This is a mutant of AC-100 where the RGD integrin-binding motif was scrambled. The RGD sequence was changed to DGR.

[0026] SEQ ID NO:3 is set forth as TDLQERGDNDISPFGDGSQPFKD, which corresponds to the amino acid sequence of the therapeutically active ingredient of the invention, AC-102. This is a mutant of AC-100 where the SGDG glycosaminoglycan motif was scrambled. The SGDG sequence was changed to GDGS.

[0027] SEQ ID NO:4 is set forth as TDLQEDGRNDISPFGDGSQPFKD, which corresponds to the amino acid sequence of the therapeutically active ingredient of the

invention, AC-103. This is a double-mutant of AC-100 where both the integrin-binding and glycosaminoglycan motifs have been modified. The integrin-binding motif sequence was changed from RGD and substituted with DGR and the glycosaminoglycan motif sequence was changed from SGDG to GDGS.

[0028] SEQ ID NO:5 is set forth as TDLQEDRGNDISPFSGDGQPFKD. This is a mutant of AC-100 where the RGD integrin-binding motif was scrambled. The RGD sequence was changed to DRG.

SEQ ID NO:6 is set forth as TDLQERWDNDISPFSGDGQPFKD.

SEQ ID NO:7 is set forth as TDLQERGDNDMSPFSGDGQPFKD.

SEQ ID NO:8 is set forth as PDLQERGDNDISPFSGDGQPFKD.

SEQ ID NO:9 is set forth as PDLQGRGDNDLSPFSGDGPPFKD.

SEQ ID NO:10 is set forth as PDLLVRGDNDVPPFSGDGQHFMH.

[0029] All of the sequences in this invention are amidated at their C-terminal.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

[0030] Before the mixtures, methods, peptides, analogs, and formulations including reconstituted formulations of the present invention are described, it is to be understood that this invention is not limited to any particular embodiment described, as such may, of course, vary. It is also to be understood that the terminology used herein is with the purpose of describing particular embodiments only, and is not intended to limit the scope of the present invention which will be limited only by the appended claims.

[0031] Where a range of values is provided, it is understood that each intervening value, to the tenth of the unit of the lower limit unless the context clearly dictates otherwise, between the upper and lower limits of that range is also specifically disclosed. Each smaller range between any stated value or intervening value in a stated range and any other stated or intervening value in that stated range is encompassed within the invention. The upper and lower limits of these smaller ranges may independently be included or excluded in the range, and each range where either, neither or both limits are included in the smaller ranges is also encompassed within the invention, subject to any specifically excluded limit in the stated range. Where the stated range includes one or both of the limits, ranges excluding either or both of those included limits are also included in the invention.

[0032] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, the preferred methods and materials are now described. All publications mentioned herein are incorporated herein by reference to disclose and describe the methods and/or materials in connection with which the publications are cited. The present disclosure is controlling to the extent there is a contradiction between the present disclosure and a publication incorporated by reference.

[0033] It must be noted that as used herein and in the appended claims, the singular forms "a", "and", and "the" include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to "a peptide" includes a plurality of such peptides and reference to "the method" includes reference to one or more methods and equivalents thereof known to those skilled in the art, and so forth.

[0034] The publications discussed herein are provided solely for their disclosure prior to the filing date of the present application. Nothing herein is to be construed as an admission that the present invention is not entitled to antedate such publication by virtue of prior invention. Further, the dates of publication provided may be different from the actual publication dates which may need to be independently confirmed.

DEFINITIONS

[0035] By "protein" is meant a sequence of amino acids for which the chain length is sufficient to produce the higher levels of tertiary and/or quaternary structure. This is to distinguish from "peptides" or other small molecular weight drugs that do not have such structure. Typically, a protein will have a molecular weight of about 15-20 kD to about 20 kD.

[0036] The terms "peptide" and "peptidic compound" are used interchangeably herein to refer to a polymeric form of amino acids of from about 10 to about 50 amino acids (may consist of at least 10 and not more than 50 amino acids), which can comprise coded and non-coded amino acids, chemically or biochemically modified or derivatized amino acids, L- or D- amino acids, peptides having modified peptide backbones, and peptides comprising amino acid analogs. The amino acid may be limited to only amino acids naturally occurring in humans. The peptidic compounds may be polymers of: (a) naturally

occurring amino acid residues; (b) non-naturally occurring amino acid residues, *e.g.*, N-substituted glycines, amino acid substitutes, *etc.*; or (c) both naturally occurring and non-naturally occurring amino acid residues/substitutes. In other words, the subject peptidic compounds may be peptides or peptoids. Peptoid compounds and methods for their preparation are described in WO 91/19735, the disclosure of which is hereby incorporated in its entirety by reference herein. A peptide compound of the invention may comprise or consist of 23 amino acids or from 18 to 28 amino acids or from 20 to 26 amino acids. The active amino acid sequence of the invention comprises or consists of two characteristic motifs which may be overlapping, which are: an integrin binding motif sequence and a glycosaminoglycan binding motif sequence.

[0037] The terms "treatment", "treating" and the like are used herein to refer to both therapeutic treatment and prophylactic or preventative measures. Those in need of treatment include those already with the disorder as well as those in which the disorder is to be prevented. In general, this encompasses obtaining a desired pharmacologic and/or physiologic effect, *e.g.*, stimulation of angiogenesis. The effect may be prophylactic in terms of completely or partially preventing a disease or symptom thereof and/or may be therapeutic in terms of a partial or complete cure for a disease and/or adverse effect attributable to the disease. The terms as used herein cover any treatment of a disease in a mammal, particularly a human, and include: (a) preventing a disease or condition (*e.g.*, preventing the loss of cartilage) from occurring in a subject who may be predisposed to the disease but has not yet been diagnosed as having it; (b) inhibiting the disease, *e.g.*, arresting loss of cartilage; or (c) relieving the disease (*e.g.*, enhancing the development of cartilage).

[0038] The terms "subject," "individual," "patient," and "host" are used interchangeably herein and refer to any vertebrate, particularly any mammal and most particularly including human subjects, farm animals, and mammalian pets. The subject may be, but is not necessarily under the care of a health care professional such as a doctor.

[0039] The peptide which is formulated is preferably essentially pure and desirably essentially homogeneous (*i.e.*, free from contaminating peptides, *etc.*). "Essentially pure" peptide means a composition comprising at least about 90% by weight of the peptide, based on total weight of the composition, and preferably at least about 95% by weight. "Essentially homogeneous" peptide means a composition comprising at least about 99% by weight of peptide, based on total weight of the composition.

[0040] A "stable" formulation is one in which the peptide therein essentially retains its physical and chemical stability and integrity upon storage and exposure to relatively high temperatures. Various analytical techniques for measuring peptide stability are available in the art and are reviewed in *Peptide and Protein Drug Delivery*, 247-301, Vincent Lee Ed., Marcel Dekker, Inc., New York, N.Y., Pubs. (1991), and Jones, A. (1993) *Adv. Drug Delivery Rev.* 10:29-90. Stability can be measured at a selected temperature for a selected time period. For rapid screening, the formulation may be kept at 40 °C for 2 weeks to 1 month, at which time stability is measured. Where the formulation is to be stored at 2-8 °C, generally the formulation should be stable at 30 °C or 40 °C for at least 1 month and/or stable at 2-8 °C for at least 2 years. Where the formulation is to be stored at 30 °C, generally the formulation should be stable for at least 2 years at 30 °C and/or stable at 40 °C for at least 6 months. For example, the extent of aggregation following lyophilization and storage can be used as an indicator of peptide stability. For example, a "stable" formulation may be one wherein less than about 10% and preferably less than about 5% of the peptide is present as an aggregate in the formulation. In other embodiments, any increase in aggregate formation following lyophilization and storage of the lyophilized formulation can be determined. For example, a "stable" lyophilized formulation may be one wherein the increase in aggregate in the lyophilized formulation is less than about 5% and preferably less than about 3%, when the lyophilized formulation is stored at 2-8 °C for at least one year. In other embodiments, stability of the peptide formulation may be measured using a biological activity assay (*see, e.g.*, Example 1).

[0041] A "reconstituted" formulation is one which has been prepared by dissolving a lyophilized peptide formulation in a diluent such that the peptide is dispersed in the reconstituted formulation. The reconstituted formulation is suitable for administration (*e.g.* parenteral administration) to a patient to be treated with the peptide of interest and, in certain embodiments of the invention, may be one which is suitable for subcutaneous administration.

[0042] By "isotonic" is meant that the formulation of interest has essentially the same osmotic pressure as human blood. Isotonic formulations will generally have an osmotic pressure from about 250 to 350 mOsm (one-thousandth of an osmole, which is a non-SI unit of measurement that defines the number of moles of a chemical compound that contribute to a solution's osmotic pressure). Isotonicity can be measured using a vapor pressure or ice-freezing type osmometer, for example.

[0043] A "lyoprotectant" is a molecule which, when combined with a peptide of interest, significantly prevents or reduces chemical and/or physical instability of the peptide upon lyophilization and subsequent storage. Exemplary lyoprotectants include sugars such as sucrose or trehalose; an amino acid such as monosodium glutamate or histidine; a methylamine such as betaine; a lyotropic salt such as magnesium sulfate; a polyol such as trihydric or higher sugar alcohols, *e.g.*, glycerin, erythritol, glycerol, arabitol, xylitol, sorbitol, and mannitol; propylene glycol; polyethylene glycol; Pluronics; and combinations thereof. The preferred lyoprotectant is a non-reducing sugar, such as trehalose or sucrose.

[0044] The lyoprotectant is added to the pre-lyophilized formulation in a "lyoprotecting amount" which means that, following lyophilization of the peptide in the presence of the lyoprotecting amount of the lyoprotectant, the peptide essentially retains its physical and chemical stability and integrity upon lyophilization and storage.

[0045] The "diluent" of interest herein is one which is pharmaceutically acceptable (safe and non-toxic for administration to a human) and is useful for the preparation of a reconstituted formulation. Exemplary diluents include sterile water, sterile water for injection (WFI), bacteriostatic water for injection ("BWFI"), a pH buffered solution (*e.g.*, phosphate-buffered saline), sterile saline solution, Ringer's solution or dextrose solution.

[0046] A "preservative" is a compound which can be added to the diluent to essentially reduce bacterial action in the reconstituted formulation, thus facilitating the production of a multi-use reconstituted formulation, for example. Examples of potential preservatives include octadecyldimethylbenzyl ammonium chloride, hexamethonium chloride, benzalkonium chloride (a mixture of alkylbenzyldimethylammonium chlorides in which the alkyl groups are long-chain compounds), and benzethonium chloride. Other types of preservatives include aromatic alcohols such as phenol, butyl and benzyl alcohol, alkyl parabens such as methyl or propyl paraben, catechol, resorcinol, cyclohexanol, 3-pentanol, and m-cresol. The most preferred preservative herein is benzyl alcohol.

[0047] A "bulking agent" is a compound which adds mass to the lyophilized mixture and contributes to the physical structure of the lyophilized cake (*e.g.*, facilitates the production of an essentially uniform lyophilized cake which maintains an open pore structure). Exemplary bulking agents include mannitol, glycine and polyethylene glycol.

[0048] "Mammal" for purposes of treatment refers to any animal classified as a mammal, including humans, domestic and farm animals, and zoo, sports, or pet animals, such as dogs, horses, cats, cows, *etc.* Preferably, the mammal is human.

[0049] A "disorder" is any condition that would benefit from treatment with the peptide. This includes chronic and acute disorders or diseases including those pathological conditions which predispose the mammal to the disorder in question. Non-limiting examples of disorders to be treated herein include skeletal loss or weakness and bone defects or breakage.

[0050] "Terminal Sterilization" by Radiation a process for sterilization of drug product using radiation, preferably gamma irradiation

MODES FOR CARRYING OUT THE INVENTION

A. Peptide Preparation

[0051] The peptide to be formulated is prepared using techniques which are well established in the art including synthetic techniques (such as recombinant techniques and peptide synthesis or a combination of these techniques) or may be isolated from an endogenous source of the peptide.

B. Preparation of the Lyophilized Formulation

[0052] After preparation of the peptide of interest as described above, a "pre-lyophilized formulation" is produced. The amount of peptide present in the pre-lyophilized formulation is determined taking into account the desired dose volumes, mode(s) of administration, *etc.* The peptide is generally present in solution. For example, the peptide may be present in a pH-buffered solution at a pH from about 4-8, and preferably from about 5-7. Exemplary buffers include histidine, phosphate, acetate, Tris, citrate, succinate and other organic acids. The buffer concentration can be from about 1 mM to about 20 mM, or from about 3 mM to about 15 mM, depending, for example, on the buffer and the desired tonicity of the formulation (*e.g.*, of the reconstituted formulation). The preferred buffer is histidine in that, as demonstrated below, this can have lyoprotective properties.

[0053] The lyoprotectant is added to the pre-lyophilized formulation. In preferred embodiments, the lyoprotectant is a non-reducing sugar such as sucrose or trehalose. The amount of lyoprotectant in the pre-lyophilized formulation is generally such that, upon reconstitution, the resulting formulation will be isotonic. However, hypertonic reconstituted formulations may also be suitable. In addition, the amount of lyoprotectant must not be too low such that an unacceptable amount of degradation/aggregation of the

peptide occurs upon lyophilization. Where the lyoprotectant is a sugar (such as sucrose or trehalose) and the peptide is AC-100, exemplary lyoprotectant concentrations in the pre-lyophilized formulation are from about 5 mM to about 400 mM, and preferably from about 10 mM to about 200 mM, and most preferably from about 20 mM to about 100 mM.

[0054] The ratio of peptide to lyoprotectant may be selected depending on the peptide and lyoprotectant combination. In the case of AC-100 as the peptide of choice and a sugar (*e.g.*, sucrose or trehalose) as the lyoprotectant for generating an isotonic reconstituted formulation with a high peptide concentration, the molar ratio of lyoprotectant to AC-100 may be from about 10 to about 1500 moles lyoprotectant to 1 mole AC-100, and preferably from about 20 to about 1000 moles of lyoprotectant to 1 mole AC-100, for example from about 200 to about 600 moles of lyoprotectant to 1 mole AC-100.

[0055] In preferred embodiments of the invention, it has been found to be desirable to add a surfactant to the pre-lyophilized formulation. Alternatively, or in addition, the surfactant may be added to the lyophilized formulation and/or the reconstituted formulation. Exemplary surfactants include nonionic surfactants such as polysorbates (*e.g.*, polysorbates 20 or 80); poloxamers (*e.g.*, poloxamer 188); Triton; sodium dodecyl sulfate (SDS); sodium laurel sulfate; sodium octyl glycoside; lauryl-, myristyl-, linoleyl-, or stearyl-sulfobetaine; lauryl-, myristyl-, linoleyl- or stearyl-sarcosine; linoleyl-, myristyl-, or cetyl-betaine; lauroamidopropyl-, cocamidopropyl-, linoleamidopropyl-, myristamidopropyl-, palmidopropyl-, or isostearamidopropyl-betaine (*e.g.*, lauroamidopropyl); myristamidopropyl-, palmidopropyl-, or isostearamidopropyl-dimethylamine; sodium methyl cocoyl-, or disodium methyl oleyl-taurate; and the MONAQUAT™ series (Mona Industries, Inc., Paterson, N.J.), polyethyl glycol, polypropyl glycol, and copolymers of ethylene and propylene glycol (*e.g.*, Pluronics, PF68 etc). The amount of surfactant added is such that it reduces aggregation of the reconstituted peptide and minimizes the formation of particulates after reconstitution. For example, the surfactant may be present in the pre-lyophilized formulation in an amount from about 0.001-0.5%, and preferably from about 0.005-0.05%.

[0056] In certain embodiments of the invention, a mixture of the lyoprotectant (such as sucrose or trehalose) and a bulking agent (*e.g.*, mannitol or glycine) is used in the preparation of the pre-lyophilization formulation. The bulking agent may allow for the production of a uniform lyophilized cake without excessive pockets therein, *etc.*

[0057] Other pharmaceutically acceptable carriers, excipients or stabilizers such as those described in Remington's Pharmaceutical Sciences 16th edition, Osol, A. Ed. (1980) may be included in the pre-lyophilized formulation (and/or the lyophilized formulation and/or the reconstituted formulation) provided that they do not adversely affect the desired characteristics of the formulation. Acceptable carriers, excipients or stabilizers are nontoxic to recipients at the dosages and concentrations employed and include additional buffering agents; preservatives; co-solvents; antioxidants including ascorbic acid and methionine; chelating agents such as EDTA; metal complexes (*e.g.*, Zn-peptide complexes); biodegradable polymers such as polyesters; and/or salt-forming counter-ions such as sodium.

[0058] The formulation herein may also contain more than one peptide as necessary for the particular indication being treated, preferably those with complementary activities that do not adversely affect the other peptide. Such peptides are suitably present in combination in amounts that are effective for the purpose intended.

[0059] The formulations to be used for *in vivo* administration must be sterile. This is readily accomplished by filtration through sterile filtration membranes, prior to, or following, lyophilization and reconstitution. Alternatively, sterility of the entire mixture may be accomplished by autoclaving the ingredients, except for the peptide, at about 120 °C for about 30 minutes, for example. Alternatively, terminal sterilization by radiation of the entire mixture prior to reconstitution may be accomplished by gamma irradiation of the lyophilized product.

[0060] After the peptide, lyoprotectant and other optional components are mixed together, the formulation is lyophilized. Many different freeze-dryers are available for this purpose such as Hull50™ (Hull, USA) or GT20 ™ (Leybold-Heraeus, Germany) freeze-dryers. Freeze-drying is accomplished by freezing the formulation and subsequently subliming ice from the frozen content at a temperature suitable for primary drying. Under this condition, the product temperature is below the eutectic point or the collapse temperature of the formulation. Typically, the shelf temperature for the primary drying will range from about -30 to 25 °C (provided the product remains frozen during primary drying) at a suitable pressure, ranging typically from about 50 to 250 mTorr. The formulation, size and type of the container holding the sample (*e.g.*, glass vial) and the volume of liquid will mainly dictate the time required for drying, which can range from a few hours to several days (*e.g.*, 40-60 hours). A secondary drying stage may be carried out at about -15-40 °C, depending

primarily on the type and size of container and the type of peptide employed. Or, the shelf temperature throughout the entire water removal phase of lyophilization may be from about 15-30 °C (*e.g.*, about 25 °C). The time and pressure required for secondary drying will be that which produces a suitable lyophilized cake, dependent, *e.g.*, on the temperature and other parameters. The secondary drying time is dictated by the desired residual moisture level in the product and typically takes at least about 5 hours (*e.g.*, 10-15 hours). The pressure may be the same as that employed during the primary drying step. Freeze-drying conditions can be varied depending on the formulation and vial size.

[0061] In some instances, it may be desirable to lyophilize the peptide formulation in the container in which reconstitution of the peptide is to be carried out in order to avoid a transfer step. The container in this instance may, for example, be a 1, 2, 3, 5, 10, 20, 50 or 100 cc vial.

[0062] As a general proposition, lyophilization will result in a lyophilized formulation in which the moisture content thereof is less than about 5%, and preferably less than about 2% and most preferably less than about 1%

C. Reconstitution of the Lyophilized Formulation

[0063] At the desired stage, typically when it is time to administer the peptide to the patient, the lyophilized formulation may be reconstituted with a diluent such that the peptide concentration in the reconstituted formulation is at least 50 mg/mL, for example from about 50 mg/mL to about 400 mg/mL, more preferably from about 80 mg/mL to about 300 mg/mL, and most preferably from about 90 mg/mL to about 150 mg/mL. Such high peptide concentrations in the reconstituted formulation are considered to be particularly useful where subcutaneous delivery of the reconstituted formulation is intended. However, for other routes of administration, such as intravenous administration, lower concentrations of the peptide in the reconstituted formulation may be desired (for example from about 5-50 mg/mL, or from about 10-40 mg/mL peptide in the reconstituted formulation). In certain embodiments, the peptide concentration in the reconstituted formulation is significantly higher than that in the pre-lyophilized formulation. For example, the peptide concentration in the reconstituted formulation may be about 2-40 times, preferably 3-10 times and most preferably 3-6 times (*e.g.*, at least three fold or at least four fold) that of the pre-lyophilized formulation.

[0064] Reconstitution generally takes place at a temperature of about 25 °C to ensure complete hydration, although other temperatures maybe employed as desired. The time required for reconstitution will depend, *e.g.*, on the type of diluent, amount of excipient(s) and peptide. Exemplary diluents include sterile water, sterile water for injection (WFI), bacteriostatic water for injection (BWFI), a pH buffered solution (*e.g.*, phosphate-buffered saline), sterile saline solution, Ringer's solution or dextrose solution. The diluent optionally contains a preservative. Exemplary preservatives have been described above, with aromatic alcohols such as benzyl alcohol or phenol being the preferred preservatives. The amount of preservative employed is determined by assessing different preservative concentrations for compatibility with the peptide and preservative efficacy testing. For example, if the preservative is an aromatic alcohol (such as benzyl alcohol), it can be present in an amount from about 0.1-2.0% and preferably from about 0.5-1.5%, but most preferably about 1.0-1.2%. Preferably, the reconstituted formulation has less than 6000 particles per vial which are ≥10 μm in size.

D. Administration of the Reconstituted Formulation

[0065] The reconstituted formulation is administered to a mammal in need of treatment with the peptide, preferably a human, in accord with known methods, such as intravenous administration as a bolus or by continuous infusion over a period of time, by intramuscular, intraperitoneal, intracerobrospinal, subcutaneous, intra-articular, intrasynovial, intrathecal, oral, topical, or inhalation routes.

[0066] The formulation of may be administered to the individual using any available method and route suitable for drug delivery, including *in vivo* and *ex vivo* methods, as well as systemic and localized routes of administration.

[0067] Conventional and pharmaceutically acceptable routes of administration include intranasal, intrapulmonary, intramuscular, intratracheal, subcutaneous, intradermal, intra-articular, topical application, intravenous, rectal, nasal, oral and other parenteral routes of administration. Routes of administration may be combined, if desired, or adjusted depending upon the immunomodulatory nucleic acid molecule and/or the desired effect on the immune response. The peptidic compound formulation for use with the methods of the present invention can be administered in a single dose or in multiple doses.

[0068] The peptidic compound formulation can be administered to a subject using any available conventional methods and routes suitable for delivery of conventional drugs, including systemic or localized routes. In general, routes of administration contemplated by the invention include, but are not necessarily limited to, enteral, parenteral, implantable, or inhalational routes.

[0069] Parenteral routes of administration other than inhalation administration include, but are not necessarily limited to, topical, transdermal, subcutaneous, intramuscular, intraorbital, intracapsular, intraspinal, intrasternal, intra-articular, and intravenous routes, *i.e.*, any route of administration other than through the alimentary canal. Parenteral administration can be carried to effect systemic or local delivery of peptides of the invention. Where systemic delivery is desired, administration typically involves invasive or systemically absorbed topical or mucosal administration of pharmaceutical preparations.

[0070] The peptidic compound formulation of the invention can also be delivered to the subject by enteral administration. Enteral routes of administration include, but are not necessarily limited to, oral and rectal (*e.g.*, using a suppository) delivery.

[0071] Methods of administration of the peptidic compound formulation through the skin or mucosa include, but are not necessarily limited to, topical application of a suitable pharmaceutical preparation with or without a permeation enhancer, transdermal transmission, injection and epidermal administration. Also contemplated for delivery of the peptidic compound formulation of the invention is a patch containing therein a peptide of the invention. A patch can be applied to the skin, or to other tissue, *e.g.*, gum tissue. Any known patch delivery system that is suitable for oral delivery system can be used. *See, e.g.*, U.S. Patent No. 6,146,655.

[0072] In preferred embodiments, the reconstituted formulation is administered to the mammal by subcutaneous (*i.e.*, beneath the skin) administration. For such purposes, the formulation may be injected using a syringe. However, other devices for administration of the formulation are available such as injection devices (*e.g.*, the Inject-ease™ and Genject™ devices); injector pens (such as the GenPen™); needleless devices (*e.g.*, MediJector™ and BioJector™); and subcutaneous patch delivery systems.

[0073] The appropriate dosage ("therapeutically effective amount") of the peptide will depend, for example, on the condition to be treated, the severity and course of the condition, whether the peptide is administered for preventive or therapeutic purposes, previous therapy, the patient's clinical history and response to the peptide, the type of

peptide used, and the discretion of the attending physician. The peptide is suitably administered to the patient at one time or over a series of treatments and may be administered to the patient at any time from diagnosis onwards. The peptide may be administered as the sole treatment or in conjunction with other drugs or therapies useful in treating the condition in question. The progress of this therapy is easily monitored by conventional techniques. Exemplary dosages of AC-100 are in the range 1-50 mg/kg by one or more separate administrations.

E. Articles of Manufacture

[0074] In another embodiment of the invention, an article of manufacture is provided which contains the lyophilized formulation of the present invention and provides instructions for its reconstitution and/or use. The article of manufacture comprises a container. Suitable containers include, for example, bottles, vials (*e.g.*, dual chamber vials), syringes (such as dual chamber syringes) and test tubes. The container may be formed from a variety of materials such as glass or plastic. The container holds the lyophilized formulation and the label on, or associated with, the container may indicate directions for reconstitution and/or use. For example, the label may indicate that the lyophilized formulation is reconstituted to peptide concentrations as described above. The label may further indicate that the formulation is useful or intended for subcutaneous administration. The container holding the formulation may be a multi-use vial, which allows for repeat administrations (*e.g.*, from 2-6 administrations) of the reconstituted formulation. The article of manufacture may further comprise a second container comprising a suitable diluent (*e.g.*, BWFI). Upon mixing of the diluent and the lyophilized formulation, the final peptide concentration in the reconstituted formulation will generally be at least 50 mg/mL. The article of manufacture may further include other materials desirable from a commercial and user standpoint, including other buffers, diluents, filters, needles, syringes, and package inserts with instructions for use.

[0075] The invention will be more fully understood by reference to the following examples. They should not, however, be construed as limiting the scope of the invention. All literature citations are incorporated by reference.

EXAMPLES

[0076] The following examples are put forth so as to provide those of ordinary skill in the art with a complete disclosure and description of how to make and use the present invention, and are not intended to limit the scope of what the inventors regard as their invention nor are they intended to represent that the experiments below are all or the only experiments performed. Efforts have been made to ensure accuracy with respect to numbers used (*e.g.* amounts, temperature, *etc.*) but some experimental errors and deviations should be accounted for. Unless indicated otherwise, parts are parts by weight, molecular weight is weight average molecular weight, temperature is in degrees Centigrade, and pressure is at or near atmospheric.

EXAMPLE 1

A. AC-100 (SEQ ID NO:1) Formulation

[0077] In the development of a lyophilized formulation, excipients and buffers are initially screened by measuring the stability of the peptide after lyophilization and reconstitution. The lyophilized peptide in each formulation is also subjected to accelerated stability studies to determine the potential stability of the peptide over its shelf-life.

[0078] In early screening studies, the stability of several lyophilized AC-100 formulations may be investigated after incubation at 5 °C (proposed storage condition) and 40 °C (accelerated stability condition).

[0079] The stabilizing effects of various lyoprotectant sugars on the lyophilized peptide may be measured.

[0080] The delivery of a high peptide concentration is often required for subcutaneous administration due to the volume limitations (≤1.5 mL) and dosing requirements (≥100 mg). However, high peptide concentrations (≥50 mg/mL) are often difficult to achieve in the manufacturing process since at high concentrations, the peptide has a tendency to aggregate and/or degrade during processing and becomes difficult to manipulate (*e.g.*, pump) and sterile filter. Alternatively, the lyophilization process may provide a method to allow concentration of the peptide. For example, the peptide is filled into vials at a volume (Vf) and then lyophilized. The lyophilized peptide is then reconstituted with a smaller volume (Vr) of water or preservative (*e.g.*, BWFI) than the original volume (*e.g.*, Vr=0.25

Vf) resulting in a higher peptide concentration in the reconstituted solution. This process also results in the concentration of the buffers and excipients. For subcutaneous administration, the solution is desirably isotonic.

[0081] For subcutaneous administration, the formulation was reconstituted to 50 mg/mL (0.2 mL WFI). At this high peptide concentration, the peptide may be more susceptible to aggregation than an intravenous dosage reconstituted to 22 mg/mL peptide (2.2 mL BWFI). This unconstituted formulation maintained the peptide completely intact at the elevated temperature of 40 °C for at least 6 months and at the elevated temperature of 50 °C for at least 3 months, indicating that the lyophilized peptide could be stored at relatively high temperatures.

[0082] The preceding merely illustrates the principles of the invention. It will be appreciated that those skilled in the art will be able to devise various arrangements which, although not explicitly described or shown herein, embody the principles of the invention and are included within its spirit and scope. Furthermore, all examples and conditional language recited herein are principally intended to aid the reader in understanding the principles of the invention and the concepts contributed by the inventors to furthering the art, and are to be construed as being without limitation to such specifically recited examples and conditions. Moreover, all statements herein reciting principles, aspects, and embodiments of the invention as well as specific examples thereof, are intended to encompass both structural and functional equivalents thereof. Additionally, it is intended that such equivalents include both currently known equivalents and equivalents developed in the future, *i.e.*, any elements developed that perform the same function, regardless of structure. The scope of the present invention, therefore, is not intended to be limited to the exemplary embodiments shown and described herein. Rather, the scope and spirit of the present invention is embodied by the appended claims.

CLAIMS

That which is claimed is:

1. A lyophilized mixture, comprising:

a non-reducing sugar;

a peptide;

a bulking agent; and

histidine;

wherein the molar ratio of the non-reducing sugar to the peptide is within a range from 20 to 200 moles of non-reducing sugar:1 mole peptide ± 20%.

2. The lyophilized mixture of claim 1, wherein the peptide is selected from the group consisting of.

TDLQERGDNDISPFSGDGQPFKD (SEQ ID NO:1)

TDLQEDGRNDISPFSGDGQPFKD (SEQ ID NO:2)

TDLQERGDNDISPFGDGSQPFKD (SEQ ID NO:3)

TDLQEDGRNDISPFGDGSQPFKD (SEQ ID NO:4)

TDLQEDRGNDISPFSGDGQPFKD (SEQ ID NO:5)

TDLQERWDNDISPFSGDGQPFKD (SEQ ID NO:6)

TDLQERGDNDMSPFSGDGQPFKD (SEQ ID NO:7)

PDLQERGDNDISPFSGDGQPFKD (SEQ ID NO:8)

PDLQGRGDNDLSPFSGDGPPFKD (SEQ ID NO:9); and

PDLLVRGDNDVPPFSGDGQHFMH (SEQ ID NO:10);

wherein the nonreducing sugar is sucrose and the molar ratio of sucrose to peptide is within the range from 20 to 100 moles sucrose:1 mole peptide ± 20%; and

wherein the bulking agent comprises glycine and wherein less than about 10% of the peptide is present as an aggregate or degraded peptide.

3. The lyophilized mixture of any of claims 1 or 2, wherein any increase in aggregated or degraded peptide in the lyophilized formulation is less than about 5% when the lyophilized formulation is stored at a condition selected from the group consisting of 25 °C for at least one year, 40 °C for at least 6 months and 50 °C for at least 4 months.

4. The lyophilized mixture of any one of claims 1-3, characterized by a moisture content of less than 1.5% and being terminally sterilized via gamma irradiation.

5. A reconstituted formulation, comprising:
the lyophilized mixture of any of claims 1-4 reconstituted in a diluent, wherein the peptide concentration in the reconstituted formulation is within the range of from about 1 mg/mL to 400 mg/mL wherein the diluent is sterile water, or bacteriostatic water for injection (BWFI) which is isotonic.

6. A sterile reconstituted formulation comprising SEQ ID NO:1 in an amount in the range from about 1 mg/mL to about 300 mg/mL ± 20% and a diluent, which reconstituted formulation has been prepared from a lyophilized mixture of any one of claims 1-5 of the SEQ ID NO:1 peptide, sucrose, glycine, histidine buffer and/or a surfactant, wherein the SEQ ID NO:1 peptide concentration in the reconstituted formulation is about 2 to 5 times greater ± 20% than the SEQ ID NO:1 peptide concentration in the mixture before lyophilization.

7. The formulation of claim 6, wherein the diluent is selected from the group consisting of sterile water, bacteriostatic water for injection (BWFI), a pH buffered solution, sterile saline solution, Ringer's solution and dextrose solution.

8. An article of manufacture comprising:
(a) a container which holds the formulation of claim 6; and
(b) instructions for reconstituting the lyophilized formulation with a diluent to a peptide concentration in the reconstituted formulation within the range of from about 1 mg/mL to 300 mg/mL.

9. The article of manufacture of claim 8, further comprising:
a second container which holds the diluent, wherein the diluent is sterile water, or bacteriostatic water for injection (BWFI).

10. A method of preparing an aqueous formulation, comprising the steps of:

providing a lyophilized mixture comprised of a non-reducing sugar, a peptide, a bulking agent and histadine, wherein the non-reducing sugar to peptide molar ratio is within a range of 20 to 200 moles of non-reducing sugar: one molecule peptide ±20%;

storing the lyophilized mixture at 25°C ± 5°C for at least one year ± two months;

adding an aqueous diluent to the lyophilized mixture after being stored; and

obtaining an aqueous formulation wherein aggregated or degraded peptide in the aqueous formulation is less than 5% based on the weight of the peptide.

11. The method of claim 10, further comprising:

sterilizing the lyophilized mixture with radiation prior to storing.

12. The method of claim 11, wherein the radiation is gamma radiation and the mixture is terminally sterilized wherein the lyophilized mixture has a moisture content of less than 1.5% prior to storing.

13. A method of preparing an aqueous formulation, comprising the steps of:

providing a lyophilized mixture comprised of a non-reducing sugar, a peptide, a bulking agent and histadine, wherein the non-reducing sugar to peptide molar ratio is within a range of 20 to 200 moles of non-reducing sugar: one molecule peptide ±20%;

storing the lyophilized mixture at 40°C for at least six months ± two months;

adding an aqueous diluent to the lyophilized mixture after being stored; and

obtaining an aqueous formulation wherein aggregated or degraded peptide in the aqueous formulation is less than 5% based on the weight of the peptide.

14. The method of claim 13, further comprising:

sterilizing the lyophilized mixture with gamma radiation prior to storing wherein the lyophilized mixture has a moisture content of less than 1.5% prior to storing.

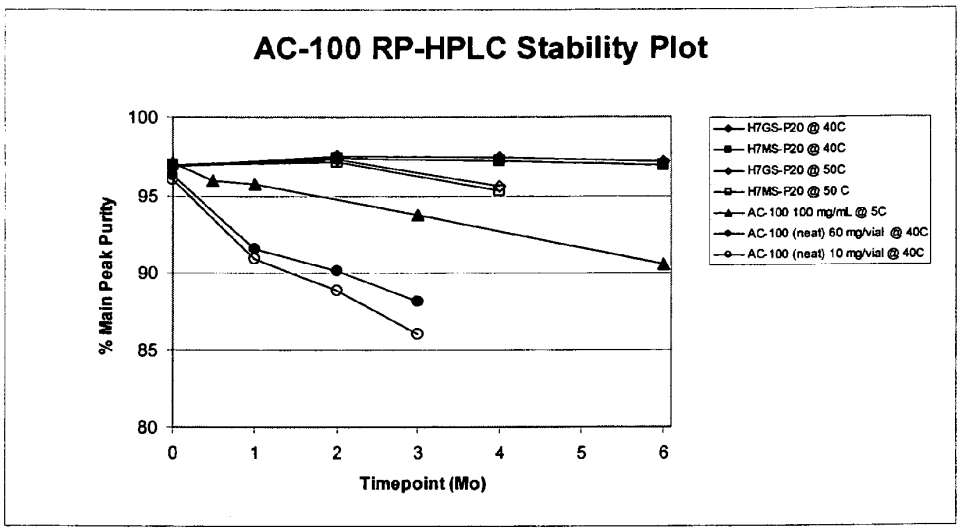

Figure 1. Stability of Various Formulation with AC-100

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19 February 2009

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(54) Title: HIGH TEMPERATURE STABLE PEPTIDE FORMULATION

(57) Abstract: A stabilized pharmaceutical composition in the form of a lyophilized product to be later reconstituted to generate an aqueous drug product is described herein. The therapeutically active ingredient in the form of a peptide of sequence TDLQERGDNDISPFSGDGQPFKD is stabilized with a buffer, carbohydrate stabilizer, a nonionic bulking agent and a surfactant to facilitate reconstitution. The preferred preparation contains a peptide of the sequence TDLQERGDNDISPFSGDGQPFKD, histidine buffer, mannitol or glycine, sucrose and/or Polysorbate 20. This combination of excipients has demonstrated exceptional stability as a lyophilized product when stored at the elevated temperature of 40 °C for at least 6 months and for at least 3 Months at 50 °C. The lyophilized mixture thus formed is reconstituted to a high peptide concentration without apparent loss of stability of the peptide and has also enabled the ability to terminally sterilize the lyophilized product using gamma irradiation without affecting stability.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 08/06898

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - C07D 233/00 (2008.04)
USPC - 548/339.1
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
USPC: 548/339.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
USPC: 424/141.1, 184.1, 484, 486; 514/2; 548/335.5 (see search terms below)

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
PubEAST(USPT,PGPB,EPAB,JPAB); GoogleScholar
Search lyophilized, freeze dried, formulation, peptide, histidine, bulking agent, mannitol, reducing sugar, sucrose, stable, stabilized, gamma radiation

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X ----- Y	US 2006/0008415 A1 (KAISHEVA et al.) 12 January 2006 (12.01.2006) para [0102], [0110]-[0111], [0118], [0036], [0099]	1, 3, 10 and 13 ----- 11-12 and 14
Y	US 2004/0105778 A1 (LEE et al.) 3 June 2004 (03.06.2004) para [0137]	11-12 and 14

☐ Further documents are listed in the continuation of Box C. ☐

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
5 November 2008 (05.11.2008)	20 NOV 2008
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	Authorized officer Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/210 (second sheet) (April 2007)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 08/06898

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.: 2 and 6-9
because they relate to subject matter not required to be searched by this Authority, namely:
Claims 2 and 6-9 are unsearchable as the applicant failed to comply with the ISA/225 mailed on 19 June 2008. Accordingly, the USPTO cannot supply a search for the sequences listed in this application. Claim 3 was searched only to the extent that it depends on other searchable claims.

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 4-5
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

Espacenet

Bibliographic data: JPH10212241 (A) — 1998-08-11

PREPARATION STABLY CONTAINING BDNF

Inventor(s): TANAKA KATSUMI; KUMANO MASAFUMI ± (TANAKA KATSUMI, ; KUMANO MASAFUMI)

Applicant(s): SUMITOMO PHARMA; REGENERON PHARMACEUT INC ± (SUMITOMO PHARMACEUT CO LTD, ; REGENERON PHARMACEUT INC)

Classification: - international: ***A61K38/00; A61K38/18; A61K47/10; A61K47/14; A61K47/18; A61K47/26; A61K9/00; A61K9/08; A61K9/14; A61K9/19; A61P25/00; A61P25/04; A61P25/24; A61P25/26; A61P25/28; A61P27/02; A61P3/04;*** (IPC1-7): A61K38/00; A61K47/10; A61K47/14; A61K47/18; A61K9/08; A61K9/14; A61K9/19

- cooperative: **A61K38/185**; **A61K47/26**; **A61K9/0019**; **A61K9/19**

Application number: JP19960156070 19960527

Priority number(s): JP19960156070 19960527

Also published as: WO9745135 (A1) DE69715414 (T2) US6077829 (A) ES2183170 (T3) AT223728 (T) more

Abstract of JPH10212241 (A)

PROBLEM TO BE SOLVED: To obtain the subject preparation capable of inhibiting the polymerization and degradation of a BDNF(brain-derived neurotrophic factor) and holding the biological activity for a long period and useful as a therapeutic agent for neurological diseases, etc., by adding a specific surfactant to a solution containing the BDNF. SOLUTION: This preparation is obtained by adding a surfactant comprising a nonionic surfactant such as Tween 80 in a concentration of 0.001-10% (w/v) to a 10mM phosphate buffer solution containing a salt such as 150mM sodium chloride and having a pH of 5.5-7.5 and subsequently dissolving BDNF(brain- derived neurotrophic factor) in the obtained solution in a concentration of 20ml/ml. If necessary, the stabilized BDNF preparation may be mixed with an amino acid such as glycine and a sugar alcohol such as mannitol and subsequently lyophilized. The preparation is useful as a medicine for

treating amyotrophic lateral sclerosis, neuropathy, Huntington's disease, Parkinson's disease, Alzheimer disease, etc.

(19)日本国特許庁（ＪＰ）　(12) **公開特許公報（Ａ）**　(11)特許出願公開番号

特開平10－212241

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(51)Int.Cl.[6]	識別記号	ＦＩ		
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	ＡＡＨ		9/08	Ｇ
	ＡＡＫ		47/10	Ｊ
	ＡＡＬ		47/14	Ｊ
	ＡＡＭ		47/18	ＡＢＬＪ

審査請求　未請求　請求項の数15　ＦＤ　（全　９　頁）　最終頁に続く

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ＲＥＧＥＮＥＲＯＮ　ＰＨＡＲＭＡＣＥＵＴＩＣＡＬＳ，　ＩＮＣ.
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(74)代理人　弁理士　青山　葆　（外１名）

最終頁に続く

(54)【発明の名称】　ＢＤＮＦを安定に含有する製剤

(57)【要約】
【課題】長期保存に適した安定化されたＢＤＮＦ製剤を提供する。
【解決手段】界面活性剤、特にＴｗｅｅｎ８０などの非イオン性界面活性剤を０．００１から１０％添加して、ＢＤＮＦ（脳由来神経栄養因子）を製剤化することにより、ＢＤＮＦの重合や分解を抑制し、生物活性を長期間保持できる溶液製剤、凍結乾燥製剤を得た。また、マンニトール等の糖アルコール、グリシン等のアミノ酸と共に用いることにより、特に凍結乾燥製剤の安定性を高めることができる。

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【特許請求の範囲】
【請求項１】界面活性剤を含有することを特徴とするＢＤＮＦ（脳由来神経栄養因子）安定化製剤。
【請求項２】界面活性剤が非イオン性界面活性剤である請求項１記載の製剤。
【請求項３】非イオン界面活性剤がＴｗｅｅｎ８０である請求項２記載の製剤。
【請求項４】Ｔｗｅｅｎ８０の濃度が０．００１％から１０％（ｗ／ｖ）である請求項３記載の製剤。
【請求項５】塩類を含有する請求項１記載の製剤。
【請求項６】塩類が塩化ナトリウムである請求項５記載の製剤。
【請求項７】緩衝剤を含有する請求項１記載の製剤。
【請求項８】緩衝剤がリン酸緩衝液である請求項７記載の製剤。
【請求項９】ｐＨが５．５から７．５である請求項１記載の製剤。
【請求項１０】凍結乾燥された請求項１記載の製剤。
【請求項１１】安定化剤を含有する請求項１０記載の製剤。
【請求項１２】安定化剤としてアミノ酸および糖アルコールの内少なくとも１つを含有する請求項１１記載の製剤。
【請求項１３】アミノ酸がグリシン、糖アルコールがマンニトールである請求項１２記載の製剤。
【請求項１４】安定化剤のＢＤＮＦに対する重量比が０．１から１０である請項１１記載の製剤。
【請求項１５】０．０１％から１０％（ｗ／ｖ）のＴｗｅｅｎ８０および安定化剤としてマンニトールを含有するＢＤＮＦ凍結乾燥製剤。
【発明の詳細な説明】
【０００１】
【発明の属する技術分野】本発明は、ＢＤＮＦを含有する溶液製剤およびその溶液を凍結乾燥することにより得られるＢＤＮＦ凍結乾燥製剤に関する。
【０００２】
【従来の技術】脊椎動物の神経細胞は、その生存に神経栄養因子と呼ばれる一群のポリペプチドを必要とする。これらの一つとして、脳由来神経栄養因子（ＢＤＮＦ）が知られているが、本因子は中枢神経系において重要な作用を果たしていると考えられていることから近年特に注目されている。ＢＤＮＦは神経系において種々の薬理作用を示すポリペプチドであり、その薬理作用については、例えば生体の科学 Vol.43, No.6, 616-625 (1992) に記載されている。ＢＤＮＦはその薬理作用から、筋萎縮性側索硬化症(ALS) 、制癌剤中毒性ニューロパチー、糖尿病性ニューロパチー、網膜色素変性症、緑内障、ハンチントン病、パーキンソン病、アルツハイマー病、末期癌疼痛、鬱病、肥満等の疾患に対する治療剤としての開発が期待されている（例えば、ＵＳ５１８０８２０、

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生体の科学 Vol.43 No.6 (1992) ）。
【０００３】医薬品として応用するためには、通常の医薬品形態及び保存条件下で経時変化することなく安定であることが要求される。殊に、ＢＤＮＦのような高度に精製されたポリペプチドでは、長期間の安定性を保持するためには解決すべき問題が多く存在する。特に、ＢＤＮＦでは、通常の生理食塩水等に溶解して保存する場合、数日から数十日で凝集体が生成する問題がある。凝集体は免疫毒性を惹起することが知られており、凝集生成の防止は非常に重要である。また、ＢＤＮＦの変性体及び重合体が生成する問題もある。ＢＤＮＦについて、これら問題を防止するための有効な手段については何ら報告はなかった。
【０００４】ところで、通常、低分子量の化合物では、溶液中で長期間の安定性が得られない場合、凍結乾燥製剤による安定化を試みる。しかし、ポリペプチドは一般に凍結乾燥操作においてそれほど安定でない（「蛋白質、核酸、酵素」 Vol.37 No.91517 (1992)）。また、水溶液中におけるポリペプチドの安定化剤は、水分子とポリペプチドとの相互作用により安定化させるものであり、したがって、水分子の存在しないポリペプチドの凍結乾燥品においては、水溶液中におけるポリペプチドの安定化剤は、多くの場合、安定化効果を示さない（「蛋白質、核酸、酵素」 Vol.37 No.9 1517 (1992)）。ＢＤＮＦの凍結乾燥製剤については全く知られておらず、またＢＤＮＦの凍結乾燥製剤がどの程度の物理化学的及び生物活性的安定性を示すかは予想することができなかった。
【０００５】
【発明が解決しようとする課題】ＢＤＮＦは、低温又は室温で数日から数十日間保存すると、凝集物が認められ、性状が変化し、変性体、重合体の生成等、物理化学的安定性が低く、長期間の保存に対し安定ではない。このことは、ＢＤＮＦを注射用製剤等とした医薬又は動物薬としての開発に障害となっていた。
【０００６】
【課題を解決するための手段】本発明者らは前記課題を解決するために種々検討を行った結果、ＢＤＮＦの安定化のためには、界面活性剤の添加が極めて有効であることを見いだし、本発明を完成した。すなわち、本発明は以下に示すように、界面活性剤を含有することを特徴とするＢＤＮＦ（脳由来神経栄養因子）安定化製剤である。
（１）界面活性剤を含有することを特徴とするＢＤＮＦ（脳由来神経栄養因子）安定化製剤。
（２）界面活性剤が非イオン性界面活性剤である（１）記載の製剤。
（３）非イオン界面活性剤がＴｗｅｅｎ８０である（２）記載の製剤。
（４）Ｔｗｅｅｎ８０の濃度が０．００１％（ｗ／ｖ）

から１０％である請求項３記載の製剤。
（５）塩類を含有する（１）記載の製剤。
（６）塩類が塩化ナトリウムである（５）記載の製剤。
（７）緩衝剤を含有する（１）記載の製剤。
（８）緩衝剤がリン酸塩である請求項（７）記載の製剤。
（９）ｐＨが５．５から７．５である（１）の製剤。
（１０）凍結乾燥された（１）の製剤。
（１１）安定化剤を含有する（１０）の製剤。
（１２）安定化剤としてアミノ酸および糖アルコールの内少なくとも１つを含有する（１１）の製剤。
（１３）アミノ酸がグリシン、糖アルコールがマンニトールである（１２）の製剤。
（１４）安定化剤のＢＤＮＦに対する重量比が０．１から１０である（１１）の製剤。
（１５）０．０１％から１０％（ｗ／ｖ）のＴｗｅｅｎ８０および安定化剤としてマンニトールを含有するＢＤＮＦ凍結乾燥製剤。
【０００７】本発明に使用されるＢＤＮＦは、Ｂａｒｄｅ，Ｙ．Ｅ（The EMBO Jounal. Vol.5, 549-553 (1982)）らによって、ブタ脳から単離された神経栄養因子であり、その後１９８９年にブタ、ヒト、マウスなどのＢＤＮＦ遺伝子がクローニングされ（Ｌｅｉｂｒｏｃｋ，Ｊ　ｅｔ．　ａｌ．；Ｎａｔｕｒｅ，３４１，１４９（１９８９））、１１９個のアミノ酸から成る一次構造が解析されたものである。
【０００８】ＢＤＮＦの生産方法は種々報告されており、何れの製法によるＢＤＮＦも本発明の製剤に用いることができる。動物組織からの抽出品の場合、医薬として使用できる程度に精製されたものであれば良い（The EMBO Jounal. Vol.5, 549-553 (1982)）。また、ＢＤＮＦを産生する初代培養細胞や株化細胞を培養し、培養物（培養上清、培養細胞）から分離精製してＢＤＮＦを得ることもできる。さらに、遺伝子工学的手法によりＢＤＮＦをコードする遺伝子を適切なベクターに組み込み、これを適切な宿主に挿入して形質転換し、この形質転換体の培養上清から目的とする組み換えＢＤＮＦを得ることができ（例えば、Proc. Natl. Acad. Sci.USA Vol.88 961 (1991)、Biochem. Biophys. Res. Commun. Vol.186 1553 (1992)）、均質かつ大量のＢＤＮＦの生産に好適である。上記宿主細胞は特に限定されず、従来から遺伝子工学的手法で用いられている各種の宿主細胞、例えば大腸菌、枯草菌、酵母、植物又は動物細胞を用いることができる。
【０００９】また、遺伝子工学的手法によると、公知の方法にて、天然型のＢＤＮＦアミノ酸配列の一部を付加、置換、欠失あるいは除去してＢＤＮＦの改変タンパクを製造することができる。かくして得られたＢＤＮＦの改変タンパクの製剤も、その改変タンパクがＢＤＮＦと同質の生物活性、即ち、神経細胞に対する生存維持、

突起伸展、伝達物質合成促進等の生物活性を有しておれば、そのアミノ酸配列の一部が欠失または他のアミノ酸により置換されていたり、他のアミノ酸配列が一部挿入されていたり、Ｎ末端及び／又はＣ末端に１又は２以上のアミノ酸が結合していても、本発明の技術的範囲に包含されるものである。すなわち、マチュアＢＤＮＦの他、Ｎ末端にメチオニンの付加したＭｅｔ－ＢＤＮＦ等も、天然型ＢＤＮＦと同質の神経栄養因子活性を示す限り、本発明の製剤に使用しうる。
【００１０】「界面活性剤」とは医薬または動物薬の配合剤として許容しうる界面活性剤のことを言い、一般的には、非イオン性の界面活性剤が用いられる。最も好適な界面活性剤の一例は、Ｔｗｅｅｎ８０（ポリソルベート８０）である。この他、ポリソルベート２０、プルロニックＦ－６８、ポリエチレングリコール等が挙げられる。界面活性剤の添加量としては、水重量に対して、０．００１～１０％の範囲を用いることができ、特に０．００１～０．１％の重量の範囲が好ましい。
【００１１】「塩類」とは、医薬または動物薬の配合剤として許容しうる塩のことを言い、一般的に、塩化ナトリウムが用いられる。塩化ナトリウムはＢＤＮＦ製剤の浸透圧を保つ作用を有する。塩化ナトリウムの添加量は一般的に用いられる注射剤の浸透圧比を示す量でよい。特に医療用又は動物薬用注射剤の浸透圧比として許容される浸透圧比１～２が好ましく、１５０～３００ｍＭとすることが好ましい。
【００１２】「緩衝剤」とは、溶液製剤または凍結乾燥製剤溶解時のｐＨを調整するために添加する緩衝剤のことを意味する。代表的なものとして、リン酸バッファー、トリスバッファー、クエン酸バッファー等が挙げられる。緩衝剤は、溶液のｐＨを調整し、ＢＤＮＦの安定性を保つ作用を有する。本発明において製剤のｐＨは特に限定されないが、好ましいｐＨの範囲として、５．５～７．５の範囲が挙げられる。すなわち、例えば、酸性条件下ではＢＤＮＦの加水分解が促進され、ＢＤＮＦ由来のフラグメントが生成し、アルカリ条件下では脱アミド化や加水分解が促進される可能性があるからである。また、緩衝剤の添加量として好ましい範囲は、１～１００ｍＭの範囲が挙げられる。
【００１３】「安定化剤」としては、グリシン等のアミノ酸、マンニトール等の糖アルコールが挙げられ、これらを併用してもよい。安定化剤を加えて製造したＢＤＮＦ製剤は、さらにＢＤＮＦの保存安定性を向上させた製剤である。例えば、グリシン、マンニトールの添加量として好ましいのは、ＢＤＮＦの重量に対して、０．０１～１００倍の重量が挙げられ、特に好ましいのは、０．１～１０倍の重量が挙げられる。なお、グリシンおよび／またはマンニトールは、溶液製剤においても用いることができるが、特に本発明のＢＤＮＦ製剤の凍結乾燥製剤において著しい安定化を示すものである。

【００１４】「凍結乾燥された製剤」は、ＢＤＮＦを含有する前記溶液製剤を通常の凍結乾燥方法で凍結乾燥することで製造できる。また、熱処理等の凍結乾燥技術を用いても製造できる。例えば、適切な溶剤（例えば、注射用蒸留水、緩衝液、生理食塩水等）に溶解したＢＤＮＦを必要に応じて、安定化剤、緩衝剤、塩類等を加え、フィルター等でろ過して除菌し、凍結乾燥する。本発明の製剤は製剤化に必要な添加物、例えば、溶解補助剤、酸化防止剤、無痛化剤、等張化剤等を含んでもよい。凍結乾燥方法としては、例えば、常圧下で冷却凍結する凍結工程、溶質に拘束されない自由水を減圧下で昇華乾燥する一次乾燥工程、溶質の吸着水や結晶水を除去する二次乾燥工程の３つの操作による方法が挙げられる（Pharm. Tech. Japan, 8(1), 75-87 (1992)）。ＢＤＮＦは溶液調製時、凍結乾燥時、及びその凍結乾燥製剤を再溶解した水溶液において、非常に安定である。

【００１５】なお、ＢＤＮＦ含量は、適応疾患、適用投与経路などに応じて適宜調整することができる。ＢＤＮＦ製剤は、バイアル内に窒素を封入して密封してもよい。バイアル内に窒素を封入するとＢＤＮＦ変性体等の生成が抑制され、さらに安定な製剤を得ることができる。

【００１６】

【発明の効果】本発明のＢＤＮＦ製剤は、界面活性剤の添加により、ＢＤＮＦを安定化させた長期間の保存が可能なものである。一般的に本発明の製剤は、次の効果を有する。（１）ＢＤＮＦ溶液製剤保存時の白濁および凝集物生成の防止、（２）ＢＤＮＦ凍結乾燥製剤溶解時の白濁、凝集物生成の防止、（３）ＢＤＮＦのガラスまたは樹脂製容器への吸着の防止、及び（４）ＢＤＮＦ生物活性の保持。また、等張化のための塩類、あるいは最適ｐＨを保持するための緩衝剤を含有した製剤、または塩類、緩衝剤の両剤を含有した製剤は臨床応用に適した態様である。上記ＢＤＮＦ含有製剤を凍結乾燥することにより安定性はさらに向上される。安定化剤としてアミノ酸、または糖アルコールを添加したＢＤＮＦ凍結乾燥製剤はさらに安定性を向上した製剤である。特に、アミノ酸としてグリシン、糖アルコールとしてマンニトールを添加した製剤は最も安定である。

【実施例】以下、実施例を挙げて本発明をさらに詳細に説明するが、本発明はこれらの実施例によりなんら限定されるものではない。

【００１７】〔実施例１〕界面活性剤の効果１

・ＢＤＮＦ溶液製剤（対照製剤１）の作製

１５０ｍＭ塩化ナトリウム含有する１０ｍＭリン酸緩衝液（ｐＨ７．０）でＢＤＮＦを２０ｍｇ／ｍｌになるように調製し、ＢＤＮＦ水溶液を得た。無菌的にバイアル充填し、ＢＤＮＦ溶液製剤を得た。

・ＢＤＮＦ溶液製剤（本発明製剤１）の作製

１５０ｍＭ塩化ナトリウム、０．０１％Ｔｗｅｅｎ８０を含有する１０ｍＭリン酸緩衝液（ｐＨ７．０）でＢＤＮＦを２０ｍｇ／ｍｌになるように調製し、ＢＤＮＦ水溶液を得た。無菌的にバイアル充填し、ＢＤＮＦ溶液製剤を得た。

・試験１

対照製剤１および本発明製剤１を用いて、界面活性剤の凝集物の生成防止効果を検討した。製剤を２５℃、５ｃｍ×７５ｓｔｒｏｋｅ／ｍｉｎの振とう条件にて保存し、凝集物の生成が目視にて観察される保存日数を調べた。その結果を表１に示した。Ｔｗｅｅｎ８０の添加により凝集物の生成が抑制された。

【表１】

凝集生成に及ぼすＴｗｅｅｎ８０の効果　　（ｎ＝５）

	Tween80濃度（%）	凝集の生成時間（日）
対照製剤１	０	１０
本発明製剤１	０．０１	＞３０

【００１８】〔実施例２〕界面活性剤の効果２

・ＢＤＮＦ溶液製剤（対照製剤２）の作製

１５０ｍＭ塩化ナトリウム含有する１０ｍＭリン酸緩衝液（ｐＨ７．０）でＢＤＮＦを０．１ｍｇ／ｍｌになるように調製し、ＢＤＮＦ水溶液を得た。無菌的にバイアル充填し、ＢＤＮＦ溶液製剤を得た。

・ＢＤＮＦ溶液製剤（本発明製剤２）の作製

１５０ｍＭ塩化ナトリウム、０．０１％Ｔｗｅｅｎ８０を含有する１０ｍＭリン酸緩衝液（ｐＨ７．０）でＢＤＮＦを０．１ｍｇ／ｍｌになるように調製し、ＢＤＮＦ水溶液を得た。無菌的にバイアル充填し、ＢＤＮＦ溶液製剤を得た。

・試験２

本発明製剤および対照製剤２を用いて、界面活性剤の容器への吸着防止効果を検討した。ガラスバイアル充填前後のＢＤＮＦ濃度を吸光度法を用いて測定し、ガラスバイアルへの吸着量を算出した。その結果を表２に示した。Ｔｗｅｅｎ８０の添加によりＢＤＮＦのガラスバイアルへの吸着量が減少した。

【表２】

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ＢＤＮＦのガラス表面への吸着に及ぼすＴｗｅｅｎ８０の効果

	Tween80 濃度（%）	表面吸着量（μg/cm２）
対照製剤２	０	０．７３
本発明製剤２	０．０１	０．２８

【００１９】〔実施例３〕ｐＨの影響

・ＢＤＮＦ溶液製剤（本発明溶液製剤３）の作製

１５０mM塩化ナトリウム、０．０１％Ｔｗｅｅｎ８０を含有する１０mMリン酸緩衝液（ｐＨ７．０）でＢＤＮＦを５ｍｇ／ｍｌになるように調製し、ＢＤＮＦ水溶液を得た。１ＮＨＣｌ及び１ＮＮａＯＨを用いてＢＤＮＦ水溶液のｐＨを４、５、６、７、８、９の６段階に設定された６つの溶液を調製した。無菌的にバイアル充填し、ＢＤＮＦ溶液製剤を得た。

・ＢＤＮＦ凍結乾燥製剤（本発明凍結乾燥製剤３）の作製

１５０mM塩化ナトリウム、０．０１％Ｔｗｅｅｎ８０を含有する１０mMリン酸緩衝液（ｐＨ７．０）でＢＤＮＦを２０ｍｇ／ｍｌになるように調製し、ＢＤＮＦ水溶液を得た。無菌的にバイアル充填し、表３に示す条件に従って凍結乾燥して、ＢＤＮＦ凍結乾燥製剤を得た。なお、表中の→は温度を変化させたことを示す。

【表３】

凍結乾燥条件

	凍結工程		一次乾燥工程		二次乾燥工程	
温度（℃）	5 →-40	-40	-40 →0	0	0 →20	20
時間（hr）	1	10	8	24	1	24
気圧（mmHg）	760	760	<1	<1	<1	<1

・試験３

本発明溶液製剤３および本発明凍結乾燥製剤３を用いて、保存安定性に対するｐＨの影響を検討した。作製した製剤を２５、４０℃にて３ヶ月保存し、保存後のＢＤＮＦ含量、重合体含量、分解物含量を測定した（各測定法は以下に示す）。表４に示すように、ＢＤＮＦ含量は塩基性条件下にて低下が認められ、酸性条件下では含量低下は僅かであった。また、重合体含量は酸性条件下ではその生成は僅かであったが、塩基性条件下では増大した。一方、分解物含量は塩基性条件下に比べ、酸性条件下にてその生成が高値であった。

【００２０】ＢＤＮＦ含量測定法

ＢＤＮＦ濃度を２ｍｇ／ｍｌに希釈後、逆相クロマトグラフ法を用いて、下記の条件にて測定した。

カラム　　：ＶＹＤＡＣ２１４ＢＴＰＣ４

移動相　　：Ａ液０．１％トリフロロ酢酸水溶液

Ｂ液０．１％トリフロロ酢酸－アセトニトリル溶液

グラジエント条件：時間／０、３６、４２、４６、４７、６６（分）において、Ｂ液濃度／２６、３５、３５、９０、２６、２６（％）

検出　　：２１５ｎｍ

流量　　：１．０ｍｌ／ｍｉｎ

カラム温度：６０℃

アプライ　：２５μｌ

【００２１】重合体・分解物含量測定法

ＢＤＮＦ濃度を2mg/mlに希釈後、ゲルろ過クロマトグラフ法を用いて、下記の条件にて測定した。

カラム　　：ＳＵＰＥＲＤＥＸ７５ＨＲ

移動相　　：３００mMリン酸ナトリウム、５００mM塩化ナトリウム、５％ｎ－プロパノール、ｐＨ６

検出　　：２１５ｎｍ

流量　　：０．６ｍｌ／ｍｉｎ

アプライ　：１０μｌ

【表４】

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ＢＤＮＦの安定性に及ぼすｐＨの影響

ｐＨ	温度（℃）	保存期間（月）	ＢＤＮＦ含量＊（％）	重合体含量＊（％）	分解物含量＊（％）
7	－	イニシャル	９３．５８	０．００	０．０
4	２５	3	９３．５４	０．００	１．１９
	４０	3	９０．０６	０．０３	２．０７
5	２５	3	９２．９８	０．０４	０．１８
	４０	3	８７．８７	０．０５	１．８５
6	２５	3	９２．７７	０．０５	０．２４
	４０	3	９０．４５	０．１２	０．８４
7	２５	3	９０．５９	０．２３	０．１１
	４０	3	７９．７８	０．７２	０．４９
8	２５	3	８６．６９	０．６６	０．０
	４０	3	６０．６１	３．０１	０．３６
9	２５	3	８３．９６	１．０７	０．１２
	４０	3	－	３．４５	０．４１

注）＊：全ピーク面積に対する割合を示す

【００２２】〔実施例４〕凍結乾燥工程中の安定性および剤形の効果

・ＢＤＮＦ溶液製剤（本発明溶液製剤４）の作製

１５０ｍＭ塩化ナトリウム、０．０１％Ｔｗｅｅｎ８０を含有する１０ｍＭリン酸緩衝液（ｐＨ７．０）でＢＤＮＦを５ｍｇ／ｍｌになるように調製し、ＢＤＮＦ水溶液を得た。無菌的にバイアル充填し、窒素をバイアル内に封入後、打栓し、ＢＤＮＦ溶液製剤を得た。

・ＢＤＮＦ凍結乾燥製剤（本発明凍結乾燥製剤４）の作製

１５０ｍＭ塩化ナトリウム、０．０１％Ｔｗｅｅｎ８０を含有する１０ｍＭリン酸緩衝液（ｐＨ７．０）でＢＤＮＦを５ｍｇ／ｍｌになるように調製し、ＢＤＮＦ水溶液を得た。無菌的にバイアル充填し、表３に示す条件に従って凍結乾燥して、ＢＤＮＦ凍結乾燥製剤を得た。バイアル内に窒素を封入し、打栓した。

・試験４

凍結乾燥工程中におけるＢＤＮＦの安定性を確認するため、実施例４において、凍結乾燥前のＢＤＮＦ溶液及び凍結乾燥製剤を再溶解したＢＤＮＦ水溶液を用いてＢＤＮＦ含量の変化及び生物活性変化を測定した（生物活性測定法は以下に示す）。その結果を表５に示す。凍結乾燥前後でＢＤＮＦ含量および生物活性に変化が認められなかったことから、凍結乾燥工程及び再溶解においてＢＤＮＦは安定であり、ＢＤＮＦを凍結乾燥製剤とすることが可能であることが示された。

【００２３】生物活性測定法

ＢＤＮＦレセプターであるｔｒｋＢ遺伝子を導入させたＢＡＦ－ｔｒｋＢ細胞をＢＤＮＦで処理し、その際の細胞増殖性を指標にＢＤＮＦ活性を測定した。

【表５】

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凍結乾燥工程中の安定性

	生物活性（比活性：*10 ４TU/mg）	ＢＤＮＦ含量（％）
本発明溶液製剤４	1.33±0.21	93.34
本発明凍乾製剤４溶解直後	1.61±0.30	93.14

【００２４】・試験５
溶液製剤と凍結乾燥製剤の保存安定性の相違を確認するため、実施例４で作製した製剤を用いて、調製直後、および２５℃、４０℃にて３ヶ月保存後にＢＤＮＦ含量を測定した。その結果を表６に示す。凍結乾燥製剤は、溶液製剤に比べて、重合体含量がやや高かったが、ＢＤＮＦ含量は高く、分解物含量が低かった。
【表６】

安定性に及ぼす剤形の効果

剤形	温度（℃）	保存期間（月）	ＢＤＮＦ含量（％）	重合体含量（％）	分解物量（％）
本発明溶液製剤４	－	イニシャル	９２．９１	０．０９	０
	２５	３	９１．２１	０．２６	０．２４
	４０	３	８６．２１	０．３８	０．７５
本発明凍結乾燥製剤４	－	イニシャル	９３．７１	０．０７	０．０
	２５	３	９２．８２	０．３４	０．０
	４０	３	８８．４０	１．６６	０．０

【００２５】〔実施例５〕界面活性剤の効果３
・ＢＤＮＦ凍結乾燥製剤（本発明凍結乾燥製剤５）の作製
実施例４記載の方法でＢＤＮＦ凍結乾燥製剤を得、本発明凍結乾燥製剤５とした。
・ＢＤＮＦ凍結乾燥製剤（対照凍結乾燥製剤５）の作製
１５０ｍＭ塩化ナトリウムを含有する１０ｍＭリン酸緩衝液（ｐＨ７．０）でＢＤＮＦを５ｍｇ／ｍｌになるように調製し、ＢＤＮＦ水溶液を得た。無菌的にバイアル充填し、表３に示す条件に従って凍結乾燥して、ＢＤＮＦ凍結乾燥製剤を得た。バイアル内に窒素を封入し、打栓した。
・試験６
凍結乾燥製剤の溶解後の性状に及ぼす界面活性剤の効果を確認するため、対照凍結乾燥製剤５および本発明凍結乾燥製剤５を精製水を用いて溶解し、性状を目視にて観察した。その結果を表７に示す。界面活性剤を添加した本発明凍結乾燥製剤５では、溶解後の性状は澄明であったが、界面活性剤の添加していない対照凍結乾燥製剤５では、溶解後白濁した。
【表７】

凍乾製剤溶解後の性状に及ぼす界面活性剤の効果

	Ｔｗｅｅｎ８０	溶解後の性状
本発明凍結乾燥製剤５	０．０１％	澄明
対照凍結乾燥製剤５	無添加	白濁

【００２６】〔実施例６〕凍結乾燥製剤の安定性に及ぼす安定化剤の効果
・ＢＤＮＦ凍結乾燥製剤（本発明凍結乾燥製剤６Ａ）の作製
実施例４記載の方法でＢＤＮＦ凍結乾燥製剤を得、本発明凍結乾燥製剤６Ａとした。
・ＢＤＮＦ凍結乾燥製剤（本発明凍結乾燥製剤６Ｂ）の作製
１５０ｍＭ塩化ナトリウム、０．０１％Ｔｗｅｅｎ８０を含有する１０ｍＭリン酸緩衝液（ｐＨ７．０）でＢＤ

13　　14

ＮＦを５ｍｇ／ｍｌになるように調製した。続いて、マンニトールを１０ｍｇ／ｍｌになるように添加し、ＢＤＮＦ水溶液を得た。無菌的にバイアル充填し、表３に示す条件に従って凍結乾燥して、ＢＤＮＦ凍結乾燥製剤を得た。バイアル内に窒素を封入し、打栓した。

・ＢＤＮＦ凍結乾燥製剤（本発明凍結乾燥製剤６Ｃ）の作製

１５０ｍＭ塩化ナトリウム、０．０１％Ｔｗｅｅｎ８０を含有する１０ｍＭリン酸緩衝液（ｐＨ７．０）でＢＤＮＦを５ｍｇ／ｍｌになるように調製した。続いて、グリシンを１０ｍｇ／ｍｌになるように添加し、ＢＤＮＦ水溶液を得た。無菌的にバイアル充填し、表３に示す条件に従って凍結乾燥して、ＢＤＮＦ凍結乾燥製剤を得た。バイアル内に窒素を封入し、打栓した。

・試験７

本発明凍結乾燥製剤６Ａ、６Ｂおよび６Ｃを用いて、調製直後、及び４０℃保存、１ヶ月後のＢＤＮＦ含量を測定した。その結果を表８に示す。また、製剤６Ａおよび６Ｂを用いて、調製直後、及び２５℃、４０℃、３ヶ月保存後のＢＤＮＦ含量を測定した。その結果を表９に示す。安定化剤を添加した製剤は、無添加の製剤に比べ安定性の向上が認められた。

【表８】

凍結乾燥製剤での安定化剤の効果１

	安定化剤	温度（℃）	保存期間（月）	ＢＤＮＦ含量（％）
本発明凍結乾燥製剤６Ａ	無添加	－ ４０	イニシャル １	９１．９８ ７８．６９
本発明凍結乾燥製剤６Ｂ	マンニトール	－ ４０	イニシャル １	９２．１６ ８６．７４
本発明凍結乾燥製剤６Ｃ	グリシン	－ ４０	イニシャル １	９２．２０ ８３．９９

注）本検討に用いた製剤６Ａ、６Ｂ、６Ｃでは、バイアル内に窒素封入を実施していない。

【表９】

凍結乾燥製剤での安定化剤の効果２

	安定化剤	温度（℃）	保存期間（月）	ＢＤＮＦ含量（％）	重合体含量（％）	分解物含量（％）
本発明製剤６Ａ	無添加	－ ２５ ４０	イニシャル ３ ３	９３．７１ ９２．８２ ８８．４０	０．０７ ０．３４ １．６６	０．０ ０．０ ０．０
本発明製剤６Ｂ	マンニトール	－ ２５ ４０	イニシャル ３ ３	９３．０３ ９２．８５ ９２．５５	０．１６ ０．１８ ０．３７	０．０ ０．０２ ０．０

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47/10	
47/14	
47/18	ＡＢＬ

	Ｅ
37/02	ＡＡＨ
	ＡＡＫ
	ＡＡＬ
	ＡＡＭ
	ＡＣＮ

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PATENT
Attorney Docket No.
552815: CPT-011USDV

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants:	Cubist Pharmaceuticals, Inc.	Examiner:	Komatsu, Li N.
Serial No.:	14/096,346	Group Art No.:	1676
Filed:	December 4, 2013	Confirmation No.:	2832
Title:	Lipopeptide Compositions and Related Methods		

VIA EFS-Web
Commissioner For Patents
P.O. Box 1450
Alexandria, VA 22313-1450

SUPPLEMENTAL INFORMATION DISCLOSURE STATEMENT TRANSMITTAL

Dear Sir:

This Supplemental Information Disclosure Statement is submitted:

☐ under 37 CFR 1.97(b), or
(Within three months of filing national application other than a CPA under §1.53(d); or within three months of date of entry of the national stage in international application; or before mailing date of first Office Action on the merits, or before mailing date of first Office Action after filing RCE under §1.114, whichever occurs last)

☒ under 37 CFR 1.97(c) together with either a:
- ☐ Statement under 37 CFR 1.97(e), or
- ☒ a $180 fee under 37 CFR 1.17(p), or

(After the CFR 1.97(b) time period, but before final action or notice of allowance, whichever occurs first)

☐ under 37 CFR 1.97(d) together with a:
- ☐ Statement under 37 CFR 1.97(e)(1) or (2), and
- ☐ a $180.00 fee set forth in 37 CFR 1.17(p).

(Filed after final action, a notice of allowance, on or before payment of issue fee)

At any time during the pendency of this application, please charge any fees required or credit any overpayment to Deposit Account 12-0600 pursuant to 37 CFR 1.25.

Applicant(s) submit herewith PTO Form SB/08 – Information Disclosure Statement together with copies of non-U.S. patents, publications or other information (if any) of which

applicant(s) are aware, which applicant(s) believe(s) may be material to the examination of the application and for which there may be a duty to disclose in accordance with 37 CFR 1.56.

The Examiner is advised that International Patent Application Publication WO 1997/045135 (reference #8) is believed to be an English language Equivalent of Japanese Patent Application No. H10-212241 (reference #2), International Patent Application Publication WO 1993/010809 (reference #9) is believed to be an English language Equivalent of Japanese Patent Application No. H05-194257 (reference #3) and Canadian Patent Application No. 2675622 (reference #1) is believed to be an English language Equivalent of International Patent Application Publication WO 2008/102849 (reference #7).

In accordance with 37 CFR 1.97(g), the filing of this Information Disclosure Statement shall not be construed to mean that a search has been made or that no other material information as defined in 37 CFR 1.56(a) exists. In accordance with 37 CFR 1.97(h), the filing of this Information Disclosure Statement shall not be construed to be an admission that any patent, publication or other information referred to therein is "prior art" for this invention unless specifically designated as such.

It is submitted that the Information Disclosure Statement is in compliance with 37 CFR 1.98 and the Examiner is respectfully requested to consider the listed references. It is requested that the information disclosed herein be made of record in this application.

Date: April 10, 2015

Respectfully submitted,

Signature: /Brian C. Trinque/

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PTO/SB/08a (01-10)
Approved for use through 07/31/2012. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

INFORMATION DISCLOSURE STATEMENT BY APPLICANT (**Not for submission under 37 CFR 1.99)**		
	Application Number	14096346
	Filing Date	2013-12-04
	First Named Inventor	O'Connor, Sandra
	Art Unit	1676
	Examiner Name	Komatsu, Li N.
	Attorney Docket Number	552815: CPT-011USDV

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Examiner Initial*	Cite No	Patent Number	Kind Code[1]	Issue Date	Name of Patentee or Applicant of cited Document	Pages,Columns,Lines where Relevant Passages or Relevant Figures Appear
	1	8835382	B2	2014-09-16	O'Connor et al.	

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	1	20050152979	A1	2005-07-14	Besman et al.	

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	1	2675622	CA	A1	2008-08-28	Adachi et al.		☐
	2	H10-212241	JP	A	1998-08-11	Tanaka et al.	English Abstract	☐
	3	H05-194257	JP	A	1993-08-03	Horowitz et al.	English Abstract	☐

INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99)		
	Application Number	14096346
	Filing Date	2013-12-04
	First Named Inventor	O'Connor, Sandra
	Art Unit	1676
	Examiner Name	Komatsu, Li N.
	Attorney Docket Number	552815: CPT-011USDV

	4	2005-060377	JP	A	2005-03-10	Kojima et al.	English machine translation	☐
	5	2003-095975	JP	A	2003-04-03	Yamazaki et al.	English machine translation	☐
	6	2008/150479	WO	A2	2008-12-11	Chen et al.		☐
	7	2008/102849	WO	A1	2008-08-28	Adachi et al.	English Abstract	☐
	8	1997/045135	WO	A1	1997-12-04	Tanaka et al.		☐
	9	1993/010809	WO	A1	1993-06-10	Horowitz et al.		☐

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Examiner Initials*	Cite No	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc), date, pages(s), volume-issue number(s), publisher, city and/or country where published.	T5
	1	"CUBICIN: EPAR - SCIENTIFIC DISCUSSION", EMEA, 2006. [online]. [Published on Internet 11.08.2006]. <URL: http://www.ema.europa.eu/docs/en_GB/_ibrary/EPAR_-_Scientific_Discussion/human/000637/WC500036046.pdf>	☐
	2	"Protein structure," from http://www.sciencedaily.com/articles/p/protein_structure.htm, pages 1-3, accessed 02/11/2015.	☐
	3	Notice of Reasons for Rejection, mailed November 19, 2014 in Japanese Patent Application No.: 2012-540161, 5 pages (English translation).	☐

INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99)		
	Application Number	14096346
	Filing Date	2013-12-04
	First Named Inventor	O'Connor, Sandra
	Art Unit	1676
	Examiner Name	Komatsu, Li N.
	Attorney Docket Number	552815: CPT-011USDV

	4	English translation of Chinese Patent Application Publication No. 1616083 (published May 18, 2005) as cited in the Japanese Notice of Reasons for Rejection, mailed November 19, 2014 in Japanese Patent Application No.: 2012-540161, 4 pages.	☐

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EXAMINER SIGNATURE

Examiner Signature		Date Considered	

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through a citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

[1] See Kind Codes of USPTO Patent Documents at www.USPTO.GOV or MPEP 901.04. [2] Enter office that issued the document, by the two-letter code (WIPO Standard ST.3). [3] For Japanese patent documents, the indication of the year of the reign of the Emperor must precede the serial number of the patent document. [4] Kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST.16 if possible. [5] Applicant is to place a check mark here if English language translation is attached.

INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99)		
	Application Number	14096346
	Filing Date	2013-12-04
	First Named Inventor	O'Connor, Sandra
	Art Unit	1676
	Examiner Name	Komatsu, Li N.
	Attorney Docket Number	552815: CPT-011USDV

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Please see 37 CFR 1.97 and 1.98 to make the appropriate selection(s):

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☐ That no item of information contained in the information disclosure statement was cited in a communication from a foreign patent office in a counterpart foreign application, and, to the knowledge of the person signing the certification after making reasonable inquiry, no item of information contained in the information disclosure statement was known to any individual designated in 37 CFR 1.56(c) more than three months prior to the filing of the information disclosure statement. See 37 CFR 1.97(e)(2).

☐ See attached certification statement.

☒ The fee set forth in 37 CFR 1.17 (p) has been submitted herewith.

☒ A certification statement is not submitted herewith.

SIGNATURE

A signature of the applicant or representative is required in accordance with CFR 1.33, 10.18. Please see CFR 1.4(d) for the form of the signature.

Signature	/Brian C. Trinque/	Date (YYYY-MM-DD)	2015-04-10
Name/Print	Brian C. Trinque	Registration Number	56,593

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6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).

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8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspections or an issued patent.

9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

PATENT ABSTRACTS OF JAPAN

(11)Publication number : **2003-095975**

(43)Date of publication of application : **03.04.2003**

(51)Int.Cl. **A61K 39/00**
A61K 9/08
A61K 9/19
A61K 38/00
A61P 11/02
A61P 27/02
A61P 37/08
// C12N 15/09

(21)Application number : **2002-189251**

(22)Date of filing : **28.06.2002**

(71)Applicant : **MEIJI MILK PROD CO LTD**
TAKEDA CHEM IND LTD

(72)Inventor : **YAMAZAKI TETSUYA**
KII KOUSUKE
MATSUHISA YOSHIO
HIROSHIMA TAKASHI

(30)Priority

Priority number : **2001196607** Priority date : **28.06.2001** Priority country : **JP**

(54) **ACETATE COMPOSITION OF MULTIPLE T-CELL EPITOPE POLYPEPTIDE**

(57)Abstract:

PROBLEM TO BE SOLVED: To obtain a multiple T-cell epitope polypeptide having improved solubility and safety.

SOLUTION: This acetate composition of the multiple T-cell epitope polypeptide contains 5-15% acetic acid and has an amino acid sequence represented by a sequence number 1 (reference to the specification). The composition contains about 4-20 wt.% of acetic acid based on 1 wt.% of the multiple T-cell epitope polypeptide containing the amino acid sequence represented by the sequence number 1.

* NOTICES *

JPO and INPIT are not responsible for any damages caused by the use of this translation.

1.This document has been translated by computer. So the translation may not reflect the original precisely.
2.**** shows the word which can not be translated.
3.In the drawings, any words are not translated.

CLAIMS

[Claim(s)]
[Claim 1]Sequence number which 5-15(weight)% Contains acetic acid: A multiplex T cell epitope polypeptide acetate constituent which has an amino acid sequence denoted by one.
[Claim 2]The multiplex T cell epitope polypeptide acetate constituent according to claim 1 which abbreviation 7-13(weight)% Contains acetic acid.
[Claim 3]The multiplex T cell epitope polypeptide acetate constituent according to claim 1 which abbreviation 9-10(weight)% Contains acetic acid.
[Claim 4]Sequence number: A constituent which abbreviation 4-20(weight)% Contains acetic acid to the multiplex T cell epitope polypeptide 1 which has an amino acid sequence denoted by one.
[Claim 5]Sequence number: A constituent which abbreviation 5-18(weight)% Contains acetic acid to the multiplex T cell epitope polypeptide 1 which has an amino acid sequence denoted by one.
[Claim 6]Sequence number: A constituent which abbreviation 7-15(weight)% Contains acetic acid to the multiplex T cell epitope polypeptide 1 which has an amino acid sequence denoted by one.
[Claim 7]Sequence number: A constituent which abbreviation 9-12(weight)% Contains acetic acid to the multiplex T cell epitope polypeptide 1 which has an amino acid sequence denoted by one.
[Claim 8]Lyophilized products which contain a constituent of an any 1 item description of Claims 1-7.

DETAILED DESCRIPTION

[Detailed Description of the Invention]
[0001]
[The technical field to which invention belongs] The present invention relates to the acetate constituent which improved the solubility of prevention of hay fever, or multiplex T cell epitope polypeptide useful as a treating agent, and stability.
[0002]
[Description of the Prior Art]Hay fever is an immediate-type-allergy disease which makes cedar pollen allergen. Since it is not an illness which it dies of, it tends to be made light of, but rhinitis and the conjunctivitis are main condition and it is a very unpleasant condition for a patient. It is said at the scattering term of cedar pollen that twenty percent or more takes ten percent or more of people for this hay fever in a city part, and economic loss is also large.
[0003]Although an antihistaminic agent and not only a steroid but the antiallergic agent has appeared in the therapy of hay fever, these are all symptomaticas. The desensitization therapy which repeats and prescribes a cedar-pollen-allergen extract for the patient is an effective cure which improves allergies clinically. However, since the allergen extract contains the B cell epitope reacted to a patient's allergen specific IgE

antibody, side reactions, such as anaphylaxis, sometimes pose a problem. Since reactivity [as opposed to cedar pollen in the T cell of the patient peripheral blood which received the desensitization therapy at the long period of time] is decreasing, it is thought that the target cell of a desensitization therapy is a T cell. Recently, in the animal model, it was shown clearly that allergen specific T cell epitope peptide derived inactivation to a T cell, and hardly combined with a patient's allergen specific IgE antibody.

[0004]Then, as what is replaced with the desensitization therapy using an old allergen extract, The peptide immunotherapy using the mixture of allergen specific T cell epitope peptide of the main allergen protein Cry j 1 of cedar pollen and Cry j 2 origin is devised (WO 94/01560). Although this method can avoid side reactions, such as the above anaphylaxis, and has the advantage of being easy to standardize artificially since it is producible, When developing such a mixture as drugs, it is necessary to carry out physical properties, a safety test, etc. about each T cell epitope, and there is a problem in respect of product specifications etc.

[0005]In order to solve this problem, from the amino acid sequence of Cry j 1 and Cry j 2, The multiplex T cell epitope polypeptide which combined major and minor T cell epitope peptide of shoes to be chosen based on the difference in an MHC class II restricted molecule with straight chain shape via the peptide bond is devised (WO 97/32600), and the validity is examined.

[0006]since there is generally a problem in stability etc. by solution states when developing protein drugs as injections -- a freeze drying method -- business -- the time -- as dissolved type injections -- producing commercially -- having -- a case -- many . However, during a retention period, protein may show an aggregation and may pose a problem as quality of drugs. About the isoagglutination of such protein, the molecular mobility at the time of adding various sugars is evaluated, and the trial which predicts the stability is reported.

[0007]

[Problem to be solved by the invention]The present invention makes it problem to provide the multiplex T cell epitope polypeptide (it may be hereafter called "epitope polypeptide" or "polypeptide") which improved solubility and stability.

[0008]

[Means for solving problem]The inventors took out this inclusion body from the Escherichia coli which holds multiplex T cell epitope polypeptide as an inclusion body, solubilized it by chloride GUAJININ / urea, and extracted this polypeptide to the supernatant liquid. Copper chelate chromatography, cation exchange chromatography, and the chromatography of the order of reversed phase chromatography refined this polypeptide for this crude extract to the high grade. And the acetic acid content which improves the stability of this refining epitope polypeptide and solubility was determined.

[0009]5-15(weight)% The present invention (1) acetic acid Namely, the multiplex T cell epitope polypeptide acetate constituent which has an amino acid sequence denoted by sequence number:1 to contain, Acetic acid (2) The multiplex T cell epitope polypeptide acetate constituent of about 7 - the 13(weight)% aforementioned (1) description to contain, Acetic acid (3) The multiplex T cell epitope polypeptide acetate constituent of about 9 - the 10(weight)% aforementioned (1) description to contain, Sequence number : (4) To the multiplex T cell epitope polypeptide 1 which has an amino acid sequence denoted by one, To the multiplex T cell epitope polypeptide 1 which has an amino acid sequence denoted by about 4 - constituent [to contain] and 20(weight)%(5) sequence-number: 1 in acetic acid, To the multiplex T cell epitope polypeptide 1 which has an amino acid sequence denoted by about 5 - constituent [to contain] and 18(weight)%(6) sequence-number: 1 in acetic acid, To the multiplex T cell epitope polypeptide 1 which has an amino acid sequence denoted by about 7 - constituent [to contain] and 15(weight)%(7) sequence-number: 1 in acetic acid, Acetic acid is related without the lyophilized products which contain the constituent of an any 1 item

description of about 9 - the constituent to contain and 12(weight)%(8) aforementioned (1) - (7).
[0010]
[Mode for carrying out the invention]Hereafter, the present invention is described in detail. The multiplex T cell epitope polypeptide of the present invention can be synthesized with chemosynthesis or gene modification technology. The chemosynthesis of peptide has been used with rapid vigor since the past several years. The peptide synthesis machine which beginners can also operate spreads in connection with it, and the ordering synthesis of peptide is also briskly performed in and outside. Chemosynthesis also of the long-chain polypeptide which consists of 100 or more amino acid residue is carried out. For example, it is a growth factor of heparin unity and chemosynthesis of the Midkine (midkine) which consists of 121 amino acid residue was carried out recently (2: [J. Peptide Sci. and] T. et al[Inui and].: 28-39, 1996). Therefore, chemosynthesis also of the epitope polypeptide of the present invention can be carried out similarly.
[0011]If gene modification technology is used, it is possible to synthesize polypeptide in large quantities by including the gene which encodes epitope polypeptide in a suitable vector, introducing into a cell, and making this gene reveal. Although an Escherichia coli expression system, a yeast expression system, an insect cell expression system, and an animal cell expression system are mentioned as an expression system of an epitope polypeptide gene, since epitope polypeptide is simple polypeptide of a single strand which does not need posttranslational modification, it is good to use the independent manifestation system of Escherichia coli.
[0012]If the protein constructional system of Escherichia coli is used, it is possible to obtain epitope polypeptide in large quantities and at low lost. Cedar-pollen-allergen Cry j 1 () [Sone and] T. etal.: Biochem. Biophys. Res. Commun., 199 : 619-625, 1994, and Cry j 2 (201: [Biochem. Biophys. Res. Commun. and] N. et al[Komiyama and].: 1021-1028, 1994). The gene to encode is already cloned and its estimated amino acid sequence is clear. Sequence number : Six T cell epitope regions (divided with the Arg dimer) which constitute the epitope polypeptide shown according to the amino acid sequence of one, The part occupied in the amino acid sequence of Cry j 1 and Cry j 2 can be easily checked from the Fig.1 (Cry j 1) and Fig.2 (Cry j 2) of WO97/32600 published unexamined application.And the DNA sequence which encodes these six T cell epitope peptide regions can be known from document of the aforementioned Sone et al., Komiyama and others. Then, chemosynthesis of the PCR primer to the DNA sequence which encodes six T cell epitope peptide is carried out. It connects after amplifying DNA which encodes an epitope polypeptide region by PCR by using as a mold the gene which encodes Cryj 1 and Cry j 2 which were cloned, Operation of furthermore amplifying by PCR is repeated, cloning of the middle and the last arrangement is carried out to a pUC plasmid, and a base sequence is checked suitably. Thus, the gene (sequence number: 2) which encodes the overall length of epitope polypeptide (sequence number: 1) can be built.
[0013]If the foreign gene of eukaryote origin is made to high-reveal with Escherichia coli, production protein will condense within a fungus body and will often form an inertness inclusion body physiologically. This inclusion formation isolates the produced protein from protease in a fungus body, suppresses decomposition by protease, and, moreover, enables separation of the objective gene product from soluble contaminating protein of many fungus body origin. Then, as for polypeptide, it is desirable from the surfaces of subsequent refining to make it generate as an inclusion body in the fungus body of Escherichia coli.
[0014]Although document about the Escherichia coli expression system of a protein gene is too many to mention, For example, It refers to [New Biochemistry Experiment Lectures II, recombinant DNA technology, edited by Japanese Biochemical Society, p126, the Tokyo Kagaku Dojin (1986); new chemical experiment lecture 1, protein VI,

a synthesis and a manifestation, edited by Japanese Biochemical Society, p155, Tokyo Kagaku Dojin (1992), etc.], The person skilled in the art can do building the Escherichia coli expression system of polypeptide easily. The pET system (Novagen, STRATAGENE) by which the independent manifestation system of Escherichia coli is marketed, for example, transcriptional competence uses strong T7 phage RNA polymerase, The pRSET system (Invitrogen) using same T7 phage RNA polymerase, etc. can also be tried.

[0015]Although various derivatives of K-12 of HB101, C600, etc. generally used can be used as host Escherichia coli which introduces an expression plasmid, the difference of the expression amount by a strain is large. K802 which also has strong proliferation potential, and many expression amounts in an working example Although the stock (from ATCC to acquisition) was used as a host, To use other strains, culture conditions (concentration etc. of the tryptophan added culture time) need to be optimized, but the setting range of an experimental condition has such optimization for a person skilled in the art.

[0016]If setting out of the manifestation culture condition of the transformant holding an epitope polypeptide gene is a person skilled in the art, it can carry out document [for example, volume protein experiment protocol 2 and on structural analysis, cell technology separate volume, and Shujunsha (1997)] to reference.

[0017]Centrifugality of the fungus body which holds polypeptide as an inclusion body is carried out, they are collected, it suspends to buffer solution, and a fungus body is crushed by ultrasonication or homogenizer processing. Centrifugality of this crushing liquid is carried out, and an insoluble fraction is obtained. And centrifugality of this insoluble fraction is suspended and carried out to buffer solution, and an inclusion body fraction (or insoluble inclusion body-like fraction) is obtained. In solubilization of this inclusion body, A high-concentration protein modifier (6-8 6 M guanidine hydrochloride and) It is common to use urea of M (Biochemistry, 26: 3129, 1987; J. Biotechnol., 1: 307, 1984;Bio/Technology, 3: 990, 1985). Centrifugality of this solubilization thing is carried out, and polypeptide is extracted to supernatant liquid. Epitope polypeptide can be refined to a high grade by presenting copper chelate chromatography, ion exchange chromatography, and the chromatography of the order of reversed phase chromatography with this polypeptide crude extract. Hereafter, a purification process is described in detail.

[0018]After culture, centrifugality of the Escherichia coli is carried out and it harvests. A fungus body is suspended to buffer solution (pH 5.0), for example, 50 mM trisacetic acid buffer solution, it ultrasonicates or processes [homogenizer] and a fungus body is crushed. Next, centrifugality (for example, for 10,000 x g and 20 minutes) is carried out, and an insoluble fraction is obtained. Centrifugality (for example, for 10,000 x g and 30 minutes) is suspended and carried out to the buffer solution which contains a surfactant for this insoluble fraction, for example, 50mM trisacetic acid buffer solution which contains the triton X-100 2%, (pH 5.0), and an inclusion body fraction is obtained. The extraction buffer solution which contains a protein modifier, for example, guanidine salt, for this inclusion body fraction, For example, with the buffer solution (pH 4.0) containing 6M guanidine hydrochloride or the buffer solution (pH 4.0) containing 0.5M - 1M guanidine hydrochloride, and urea of 5.5M-5M, it stirs at a room temperature for 1.5 to 3 hours, and dissolves. Polypeptide will be extracted by supernatant liquid if centrifugality (for example, for 10,000 x g and 20 minutes) of the solution is carried out.

[0019]If this crude extract is diluted with neutral or weak alkaline buffer solution (for example, 50mM carbonic-acid-buffer-solution pH 9.8) 10 to 20 times and is neglected at around 37 degrees C for 1 hour, epitope polypeptide will precipitate. On the other hand, since most protein of the low molecular weight of Escherichia coli origin (20,000 or less molecular weight) is easily rolled back from a denaturation state and it reproduces higher order structure, it is meltable and it is removed. The precipitation containing epitope polypeptide is suspended to the buffer solution (pH 4.0) containing

the buffer solution (pH 4.0) which contains 6 M guanidine hydrochloride again or 0.5 - 1 M guanidine hydrochloride, and 5.5M - 5M urea, is stirred for 1.5 to 3 hours, and dissolves. Centrifugality (for example, for 10,000xg and 20 minutes) of the solution is carried out, and polypeptide is extracted to supernatant liquid. Even if operation of this dilution and precipitation is omitted and it presents the following chromatography with the crude extract of the above-mentioned beginning directly, it is sufficient degree of refining and epitope polypeptide is obtained.

[0020]Most proteinic separation refinement is based on chromatography today. Ion exchange chromatography is generally used for the stage where separability is high and protein purification is early, in many cases. In ion exchange chromatography, generally, with a pH of seven or less protein is an anion exchanger, and an isoelectric point separates by a cation exchanger by seven or more pH.

[0021]Since isoelectric points are pH 11 and strong base nature, the epitope polypeptide of the present invention can consider cation exchange chromatography first as the 1st step of refining by chromatography. However, the epitope polypeptide under chaotropic-agent (guanidine hydrochloride/urea) nonexistence stuck to the both sides of Hi-trap Q (anion exchange resin) and Hi-trap SP (cation exchange resin) partially. Then, it was judged that the ion exchange chromatography in the state where the chaotropic agent does not exist could not be used for the first process of refining of epitope polypeptide.

[0022]The metal chelate chromatography based on the compatibility of metal ion and amino acid can also apply the solvent which contains a protein modifier like guanidine hydrochloride/urea so much. As for the combination to proteinic copper chelating resin, the intervention of His, Cys, and a Trp residue is known (Trends in Biotechnology, 3: 1-7, 1985). Multiplex epitope polypeptide has chosen the epitope peptide which does not contain a Cys residue in order to avoid formation of the dimer which poses a quality control top problem as drugs, or a polymer. The interaction of a Trp residue and a copper ion is weak as compared with His, and protein containing 1-2 Trp residues cannot be combined with copper chelating resin. On the other hand, the interaction with copper chelating resin of a His residue is strong, and protein containing one His residue can be combined with copper chelating resin. Epitope polypeptide has 2-3 His residues. On the other hand, since almost all Escherichia coli protein is averaged to intramolecular and contains four or more His residues, it sticks to it more strongly [copper chelating resin] than epitope polypeptide. Since it is such, if copper chelate chromatography is used for the 1st step of refining, protein of Escherichia coli origin sticks to a copper chelate column more strongly than epitope polypeptide, and it is expected that the most will be removed.

[0023]The above-mentioned crude extract is mixed with 8M urea / 0.2M sodium chloride / 50mM sodium acetate buffer solution (pH 7.0) buffer solution, and 1:1 (capacity factor), for example, Copper chelate chromatography (Amersham Pharmacia Biotech K.K.), for example, a copper chelate stream line, is presented after adjusting the pH to 7.0. As resin which carries out the chelate of the copper, iminodiacetate agarose and nitrilotriacetic acid agarose are mentioned, for example. Iminodiacetate agarose can be prepared by the method (J. Porath. et al.: Nature, 258: 598, 1975) of the document description. The nitrilotriacetic acid agarose which combined copper can be easily prepared from nickel-NTA agarose (Qiagen). As compared with iminodiacetate agarose, there is little leakage of a copper ion and nitrilotriacetic acid agarose fits refining of epitope polypeptide. It may try POROS MC (Applied Biosystem), KIRETINGU sepharose FF (Amersham Pharmacia Biotech K.K.), chelate SERURO fine (Seikagaku), etc.

[0024]A copper chelate stream line is washed after crude extract addition with 8M urea / 0.2 M sodium chloride / 50mM sodium acetate buffer solution (pH 7.0) 3 capacity of column, for example, and the non-adsorbate is removed. When lowering the pH of elution buffer solution and eluting by the protonation of His, polypeptide is eluted by

pH higher than much Escherichia coli protein with many His residues. By eluting with 8M urea / 0.2M sodium chloride / 50mM sodium acetate buffer solution (pH 5.0), polypeptide is refined to about 70% of purity.
[0025]Since ionic strength is low, the eluate fraction from copper chelate chromatography, The cation exchange chromatography equilibrated after adjusting the pH to 4 with acetic acid with balanced buffer solution (pH 4.0), for example, 8M urea / 0.1 M sodium chloride / 50mM trisacetic acid buffer solution, For example, an eluate fraction is added in an SP-sepharose FF column (Amersham Pharmacia Biotech K.K.), and epitope polypeptide is made to stick to resin. In addition to this as a cation-exchange column, Mono S (Amersham Pharmacia Biotech K.K.), CM sepharose FF (Amersham Pharmacia Biotech K.K.), etc. are mentioned.
[0026]After adding the eluate fraction from copper chelate chromatography in an SP-sepharose FF column, The buffer solution of pH 10, for example, 8M urea / 0.1 M sodium chloride / 50mM sodium carbonate buffer solution, (pH 10.0) washes, Then, the buffer solution of pH 4, for example, 8M urea / 0.2 M sodium chloride / 50mM trisacetic acid buffer solution, (pH 4.0) washes a column. Since an isoelectric point is ten or less most protein of Escherichia coli origin, the purity of epitope polypeptide (that relative polypeptide) becomes 100 % substantially with this cation exchange chromatography. Chemical nature means polypeptide extremely similar to epitope polypeptide, and relative polypeptide means here physical and the polypeptide by which the amino acid of epitope polypeptide was usually embellished or replaced partially. For example, they are the polypeptide in which the Met residue oxidized, the polypeptide by which the Met residue was replaced by the norleucine, the acetylated polypeptide, or the deamidated polypeptide. Next, it elutes with elution buffer solution (pH 4.0), for example, 8M urea / 0.4 M sodium chloride / 50mM trisacetic acid buffer solution. A_{280} of an eluate is monitored and a fraction with absorption is obtained.
[0027]Although ribosome protein with a small molecular weight is slightly mixed in this eluate fraction in some numbers by strong base nature, it is easily removed by the following reversed phase chromatography. In reversed phase chromatography, the relative polypeptide of epitope peptide and most lipopolysaccharide are removed.
[0028]As an opposite phase column, the column C18 (Shiseido) filled up with common octadecyl-ized silica gel for liquid chromatography, for example, a capsule pack, can be used. The column etc. which were filled up with resin with large pore size of a polymer carrier, for example, POROS 50R2, (Applied Biosystems), and SOURCE 15 RPC (Amersham Pharmacia Biotech K.K.) are mentioned. When 50RPOROS2 column (25 x 200 mm) is used, after equilibrating a column with 1 % acetic acid, the eluate fraction of cation exchange chromatography is added. It elutes after washing using an eluate, for example, 22% acetonitrile / 1% acetic acid, with 12% acetonitrile / 1% acetic acid. An eluate can be freeze-dried and the refining polypeptide which has the purity more than 96 to 99% (weight) as purity of polypeptide can be obtained. The freeze-drying article of this polypeptide exists with chromatography as an acetate constituent in which acetic acid is contained eight to 13% by weight. Even if it forms multiplex T cell epitope polypeptide and a salt, it is not necessary to form the acetic acid currently contained into this acetate constituent.
[0029]as an acetic acid content of the multiplex T cell epitope polypeptide acetate constituent of the present invention, about five to 15 (weight) % is preferable -- inside -- about 7-13(weight)% -- about nine to 10 (weight) % is especially preferable.
[0030]The acetic acid content in the multiplex T cell epitope polypeptide acetate constituent of the present invention can be adjusted in accordance with a publicly known method. For example, the acetic acid content in this acetate constituent can be increased by contacting the freeze-drying article of the acetate constituent of the epitope polypeptide obtained above, for example to acetic acid vapor. By exposing the freeze-drying article of the acetate constituent of the epitope polypeptide obtained above under a humidification condition, Or after dissolving the freeze-drying article of an

epitope polypeptide acetate constituent in a suitable solvent (for example, water), the acetic acid content in this acetate constituent can be decreased by giving a solution to freeze-drying. As content of acetic acid in the constituent which contains the multiplex T cell epitope polypeptide and acetic acid of the present invention, to the multiplex T cell epitope polypeptide 1 of the present invention -- acetic acid -- about 4-20(weight)% and inside -- about 5-18(weight)% -- especially -- about 7-15(weight)% -- about nine to 12 (weight) % is especially preferable. Sugars may be added to the multiplex T cell epitope polypeptide acetate constituent of the present invention as a stabilizing agent.
[0031]the multiplex T cell epitope polypeptide acetate which exists in the multiplex T cell epitope polypeptide acetate constituent of the present invention -- the very thing -- salt exchange can be performed by using a publicly known reaction. The salt permitted physiologically is mentioned as this salt. as such a salt -- inorganic acid (for example, chloride, phosphoric acid, and hydrobromic acid --) A salt with sulfuric acid or a salt with organic acid (for example, formic acid, propionic acid, fumaric acid, maleic acid, succinic acid, tartaric acid, citrate, malic acid, oxalic acid, benzoic acid, methanesulfonic acid, benzenesulfonic acid) is used. A salt with chloride is especially preferable.
[0032]As pharmaceutical preparation of the acetate constituent of the epitope polypeptide of the present invention, what is lyophilized products is preferable. These lyophilized products can be considered as the pharmaceutical preparation excellent in stability by adding sugars.
[0033]as ** "sugars" -- monosaccharide (for example, glucose and an erythrose --) xylulose, a ribulose, a sedoheptulose, a ribose, mannose, and those sugar-alcohol (sorbitol --) ribitol, mannitol, etc. -- etc. -- mannitol is especially preferable. Or disaccharide. (For example, maltose, cellobiose, a gentiobiose, a melibiose, milk sugar, turanose, a solo hose, trehalose, isotrehalose, sucrose (purified sucrose), isosaccharose of purified sucrose, milk sugar, and maltose, etc. are preferable, and purified sucrose's are especially the most preferable) is mentioned. Although ** "sugars" may be used independently, it may use also as 2 or more type of mixtures. Especially, it is preferable to use purified sucrose.
[0034]These lyophilized products are shown specifically below. A request performs pH adjustment in the aquosity liquid which melted the acetate constituent of the epitope polypeptide of the present invention, and the both sides of sugars in water or a suitable aqueous solvent (for example, mixture of water and alcohol), and it is considered as sterile preparation by filtering with a 0.22-micrometer filter further, for example. Then, the pharmaceutical preparation made into the solid state is preferable by freeze-drying. In order to inhibit impurity generation of the oxidant in lyophilized products, etc., nitrogen gas etc. may be enclosed into a container.
[0035]the case where aquosity liquid is prepared -- the very thing -- what is necessary is just to dissolve the acetate constituent of the epitope polypeptide of the present invention, and the both sides of sugars in water or an aqueous solvent (for example, mixture of water and alcohol) in accordance with a publicly known method The point may be [order to dissolve] sufficient as whichever. An isotonizing agent may be blended with the acetate constituent of the epitope polypeptide of the above-mentioned present invention, and the aquosity liquid of sugars in order to adjust osmotic pressure. As this isotonizing agent, a thing publicly known as isotonizing agents, such as salts, such as sugar-alcohol, such as monosaccharides, such as glucose, and mannitol, and salt, is mentioned, for example. In order to perform pH adjustment, organic acid, such as inorganic acid, such as chloride, and acetic acid, etc. are used. The lyophilized products of the acetate constituent of the epitope polypeptide of the present invention, usually, this after dissolving the acetate constituent of the epitope polypeptide of the present invention, and the both sides of sugars in water or an aqueous solvent, considering it as aquosity liquid and a request performing pH adjustment -- the very thing -- it can obtain by freeze-drying by a publicly known method. At this time, the concentration of the

epitope polypeptide (acetate) of the present invention in aquosity liquid is usually 0.01 mg/mL - 10 mg/mL, and the concentration of sugars is usually 0.05 mg/mL - 100 mg/mL.
[0036]Thus, the lyophilized products of the present invention obtained can inhibit deterioration of the epitope polypeptide (acetate) of the present invention in a long period of time, and can keep it stable. The lyophilized products of the acetate constituent of the epitope polypeptide of the present invention can be used as the medicinal composition usually mixed with the carrier or excipient which may be permitted independently or pharmacologically, and can be used for taking orally or a parenteral target.
[0037]The lyophilized products of the acetate constituent of the epitope polypeptide of the present invention, tableting this and filling up a tablet at a capsule -- a capsule -- moreover -- it can enclose with a microcapsule and can be considered as a sustained release drug -- business -- the time -- water for injection or infusion solutions (an example, a physiological saline, grape sugar, etc.) -- dissolving -- It can also use as injections, such as injection for intravenous infusion, a subcutaneous injection agent, an intramuscular injection agent, an intravenous drip infusion agent, and non-needle injections, or a nasal drop, and ophthalmic solutions. In this case, the concentration of the epitope polypeptide (acetate) of the present invention in a solution is about 0.01 mg/mL - 10 mg/mL. The concentration of sugars is about 0.05 mg/mL - 100 mg/mL.
[0038]the business for injections -- the time -- dissolution pharmaceutical preparation -- carrying out -- a case -- the very thing -- it is publicly known, for example, it is preferable to prepare the above-mentioned aquosity liquid with the sterile methods of preparation, such as filtration sterilization. Before preparing lyophilized products, depyrogenation processing can be carried out previously and the mixture of sugars or sugars, and other additives can also be used.
[0039]the lyophilized products of the acetate constituent of the epitope polypeptide of the present invention -- the business for injection -- the time -- dissolution pharmaceutical preparation -- it is -- a thing -- it is preferable .
[0040]The acetate constituent of the epitope polypeptide of the present invention, Toxicity is low, for example, as injections, such as freeze-drying injections and liquid for injection, The purpose of hyposensitization can be attained in hypodermic and a vein in leather by prescribing for the patient every week the quantity chosen as intramuscular, intraperitoneal, etc. in abbreviation 1ng-100mg per adult for about one to 12 months one to about twice.
[0041]The acetate constituent of the epitope polypeptide of the present invention, For example, the purpose of the hyposensitization can be advantageously attained by being manufactured also as transderma, such as troches, a sublingual tablet, cataplasms, cream pharmaceuticals, and lotions, and ********, and choosing the dose, administration frequency, etc. suitably. The acetate constituent of the epitope polypeptide of the present invention can be advantageously used also as the preventive of the preventive of hay fever, and not only a treating agent but cypress pollinosis, and a treating agent.
[0042]Although the acetate constituent of the epitope polypeptide of the present invention shows the effective operation as the preventive of the cedar pollen outstanding as single **, a treating agent and the preventive of cypress pollinosis, and a treating agent, it can also be used together with the medicinal components (it is hereafter written as a combined drug) of further others (** agent concomitant use).
[0043]As such a combined drug, for example A chemical mediator isolation depressant. for example, disodium cromoglycate (Intal) and tranilast (Rizaben) -- Amlexanox (Solfa), pemirolast potassium (Alegysal), etc., a chemical mediator receptor antagonist (for example, (1) d-chlorpheniramine maleate (Polaramin) --) Clemastine fumarate (Tavegyl), ketotifen fumarate (ZAJIDEN), azelastine hydrochloride (Azeptin), oxatomide (Celtect), and mequitazine (Zesulan --) Nipolazin, emedastine difumarate (Darren, Remicut), cetirizine hydrochloride (Zyrtec), Levocabastine hydrochloride

(Livostin), fexofenadine (Allegra), Thromboxane-A2 antagonists, such as antihistaminic agents, such as olopatadine hydrochloride (Allelock), and (2) Lamaism TOBAN (Baynas), (3) Leukotriene antagonists, such as pranlukast hydrate (Onon) etc., Th2 cytokine-suppression medicine (for example, suplatast tosilate (IPD) etc.) and steroid medicine (for example, (1) beclometasone dipropionate (Beconase, Aldecin, Rhinocort) and flunisolide (Synaclyn) --) Oral steroid medicine, such as topical steroid medicine, such as fluticasone propionate (Flunase), and (2) celestamines (chlorpheniramine maleate combination drug) etc., an autonomic drug (for example, (1) naphazoline nitrate (Privina) and nitric acid tetrahydrozoline (Narbel) --) alpha stimulants, such as oxymetazoline hydrochloride (Nasivin) and tramazoline hydrochloride (talk), (2) Biologicals (for example, neurotropine, asthremedin, MS antigen, etc.), such as anticholinergic drugs, such as ipratropium bromide (Atrovent) and bromination full TOPIUMU (Flubron), etc. are mentioned.

[0044]Concomitant use with the acetate constituent of the epitope polypeptide of the present invention and a combined drug is faced, The time for administration of the acetate constituent of the epitope polypeptide of the present invention and a combined drug may not be limited, but may prescribe simultaneously the acetate constituent and combined drug of epitope polypeptide of the present invention for the patient to the candidate for administration, and may set and prescribe a time lag for the patient. The dose of a combined drug should just apply to the dose used on clinical, and can be suitably chosen with the route of administration for administration, a disease, combination, etc.

[0045]The dosage form in particular of the acetate constituent of the epitope polypeptide of the present invention and a combined drug is not limited, but the acetate constituent and combined drug of epitope polypeptide of the present invention should just be together put at the time of administration. Administration of the single pharmaceutical preparation produced as such a dosage form by pharmaceutical-preparation-izing simultaneously the acetate constituent and combined drug of epitope polypeptide of (1) present invention, for example, (2) The simultaneous administration by the same route of administration of 2 type of pharmaceutical preparation produced by pharmaceutical-preparation-izing independently the acetate constituent and combined drug of epitope polypeptide of the present invention, (3) The administration which sets the time lag in the same route of administration of 2 type of pharmaceutical preparation produced by pharmaceutical-preparation-izing independently the acetate constituent and combined drug of epitope polypeptide of the present invention, (4) The simultaneous administration by the route of administration from which 2 type of pharmaceutical preparation produced by pharmaceutical-preparation-izing independently the acetate constituent and combined drug of epitope polypeptide of the present invention differ, (5) The administration which sets the time lag in the route of administration from which 2 type of pharmaceutical preparation produced by pharmaceutical-preparation-izing independently the acetate constituent and combined drug of epitope polypeptide of the present invention differ. (For example, administration in an order of the acetate constituent -> combined drug of the epitope polypeptide of the present invention or administration by a reverse order) etc. -- it is mentioned. Hereafter, these dosage forms are summarized and it is written as the concomitant use agent of the present invention.

[0046]the concomitant use agent of the present invention has low toxicity -- for example, the acetate constituent or (and) the above-mentioned combined drug of epitope polypeptide of the present invention -- the very thing -- in accordance with a publicly known method, Mix with the carrier permitted pharmacologically and A medicinal composition, for example, freeze-drying injections, Liquid for injection, troches, a sublingual tablet, ophthalmic solutions, the spray in a nasal cavity, cataplasms, as cream pharmaceuticals, lotions, a tablet (a sugar-coated tablet and a film coated tablet are included), powder medicine, a granule, a capsule (a soft capsule is included), liquids

and solutions, suppositories, a sustained release drug, etc. -- taking orally ---like -- or -- being parenteral (an example, a part, rectum, intravenous administration, etc.) -- a medicine can be safely prescribed for the patient.
[0047]
[Working example]Although the present invention is described below by the reference example, the working example, the example of an examination, the example of pharmaceutical preparation, and the example of an experiment, technical scope of the present invention is not limited to these.
Construction sequence number of DNA which encodes the [reference-example 1] polypeptide: The polypeptide which has an amino acid sequence of one consists of 105 amino acid residue with which six T cell epitope peptide was connected via the Arg dimer. Then, each DNA fragment corresponding to the epitope of Cry j 1 and Cry j 2 is connected after amplification by PCR, The process of furthermore amplifying by PCR was repeated and V-KV-ID-WK-LK-V2 (polypeptide cDNA) which encodes the overall length of polypeptide eventually was built (Fig.1). PCR conditions were ten to 25 cycles about 72 degree-C 90 seconds for 55 degree-C 30 seconds for 96 degree-C 15 seconds using Taq DNA polymerase.
[0048](1) Amplification of c DNA fragment K, and the epitope of cloning pCCI2-2 (199: [Biochem. Biophys. Res. Commun. and] T. et al[Sone and].: 619-625, 1994) to 15 amino acid residue. It is KSMK43S (sequence number: 3) about the cDNA fragment K to encode. KSMK43A (sequence number: 4) It amplified by PCR made into a primer, and the SalI recognition site was simultaneously given to 5' end at the SmaI recognition site and the 3' end. this -- a DNA fragment -- pUC19 -- cloning was turned up and the base sequence was checked (pUC19K#3).
[0049](2) Amplification of the cDNA fragment VF, They are PCVF22S (sequence number: 5) and PCVF22A (sequence number: 6) about the cDNA fragment P which encodes the epitope of cloning pCCI2-2 to 15 amino acid residue of two connected cDNA fragment K-VF. It amplifies by PCR made into a primer, The SalI recognition site was simultaneously given to 5' end at the SmaI recognition site and the 3' end. this -- After digesting a DNA fragment by SmaI, it was made to combine with the cDNA fragment K digested by SalI. It joined together. It is PCVF22A (sequence number: 6) about KSMK43S (sequence number: 3) in a DNA fragment. It amplified by PCR made into a primer. The PCR product was covered over polyacrylamide gel electrophoresis, and DNA fragment K-P of 120 bp was separated and refined. After digesting the K-P fragment by SalI and SmaI, polyacrylamide gel electrophoresis was performed, after refining the DNA fragment, it cloned on the SalI-SmaI arm of pUC19, and pUC19KP#6-1 was obtained. They are VFIK22S2 (sequence number: 7) and PCVF22A (sequence number: 6) about the cDNA fragment VF which enciphers the epitope of pUC19KP#6-1 to 13 amino acid residue. It amplifies by PCR made into a primer, The SalI recognition site was simultaneously given to 5' end at PstI and the 3' end. The PCR product was covered over polyacrylamide gel electrophoresis, and the fragment of 59 bp was separated and refined. this -- After carrying out PstI digestion of the DNA fragment, it mixes with pUC19K#3 which carried out SalI digestion, and it was made to join together after smoothing with a Klenow fragment. KSMK43S (sequence number: 3) and PCVF22A (sequence number: 6) The DNA fragment (111 bp) of K-VF was amplified by PCR made into a primer. The PCR product was covered over polyacrylamide gel electrophoresis, and the DNA fragment was separated and refined. this -- Cloning of the DNA fragment was carried out to pUC19, and it was considered as pUC19 K-VF.
[0050](3) Amplification of the cDNA fragment G, and the cloning pCC. II 1 () Komiyama,N., Sone, T., Shimizu, K., Morikubo, K., and Kino, K.(1994) Biochem. Biophys. Res. Commun. 201, 1021-1028 to 20. They are GIDI37S (sequence number: 8) and GIDI37A (sequence number: 9) about the cDNA fragment G which enciphers the epitope of amino acid residue. It amplified by PCR made into a primer, and the SalI recognition site was simultaneously given to 5' end at the SmaI recognition site and the

3' end. this -- After digesting the DNA fragment by SmaI and SalI, cloning was carried out to pUC19, and it was referred to as pUC19G, and the insertion base sequence of pUC19G#1 was read. It is 1 to the knot of a SmaI end. Although there was deletion (set to ACCGGG) of a base pair, it checked that there was no variation in other portions.
[0051](4) Amplification of the cDNA fragment WK, Connected . WKNN17S (sequence number: 10) and WKNN(not phosphorylated) 17A (sequence number: 11) which phosphorylated the cDNA fragment WK which enciphers the epitope of cloning pCC II 1 to 20 amino acid residue of two cDNA fragment ID-WK It amplified by PCR made into a primer, and the SalI recognition site was simultaneously given to 3' end. The PCR product was covered over polyacrylamide gel electrophoresis, and the DNA fragment of 71bp was separated and refined. this -- A DNA fragment is mixed with pUC19G#1 which carried out SalI digestion, and it was made to join together after smoothing with a Klenow fragment. IDIF37S (sequence number: 12) and WKNN17A (sequence number: 11) The DNA fragment (141 bp) of ID-WK was amplified by PCR made into a primer. The PCR product was covered over polyacrylamide gel electrophoresis, and separated and refined the DNA fragment. this -- Cloning of the DNA fragment was carried out to pUC19, it was considered as pUC19 ID-WK, and that base sequence (pUC19ID-WK#1 and #8) was checked.
[0052](5) It is the cDNA fragment V2 which enciphers amplification of the cDNA fragment V2, and the epitope of cloning pCCII1 to 15 amino acid residue VDGI14S2 (sequence number: 13) and VDGI14A2 (sequence number: 14) By PCR made into a primer. It amplified and the termination codon and the Hind III recognition site were simultaneously given to 5' end at PstI and the 3' end. this -- Cloning of the DNA fragment was carried out to pUC19, it was referred to as pUC19Vph, and the insertion base sequence of pUC19Vph#1 was read. VDGI14A2 (sequence number: 14) Although the place which should serve as the complementary arrangement GCTGGAAGTAA had become a primer with GCTTAAGTAA, there was no variation in other portions.
[0053](6) cDNA fragment LK which enciphers the epitope of 15 amino acid residue from cDNA of Cry j 1 by which cloning cloning of amplification [of cDNA fragment LK] and cDNA fragment LK-V2 was carried out (pCCI-2-2). LKMP17S (sequence number: 15) and LKMP17A (sequence number: 16) It amplified by PCR made into a primer, and the SalI recognition site was simultaneously given to 5' end at KpnI and the 3' end. The PCR product was covered over polyacrylamide gel electrophoresis, and the fragment of 65 bp was separated and refined. this -- After carrying out SalI digestion of the DNA fragment, pUC19Vph#1 which carried out PstI digestion is mixed, and it was made to join together after smoothing with a Klenow fragment. LKMP17S (sequence number: 15) and VDGI14A2 (sequence number: 14) The DNA fragment (119 bp) of LK-V2 was amplified by PCR made into a primer. The PCR product was covered over polyacrylamide gel electrophoresis, and the DNA fragment was separated and refined. this -- carrying out cloning of the DNA fragment to pUC19, and being referred to as pUC19 LK-V2 -- the base sequence of pUC19 LK-V 2#8 -- the right -- things were checked.
[0054](7) An insertion base sequence is cut out by EcoRV/Hind III digestion from cloning pUC19 ID-WK of cDNA fragment K-VF-ID-WK, It is made to combine with the SalI-Hind III arm of pUC19 K-VF#2, and is 3. It checked that the base sequence of a junction was right about a clone (pUC19K-VF-ID-WK#1, #2, and #4).
[0055](8) The six connected cDNA fragments . The insertion base sequence started by KpnI/Hind III digestion from pUC19 LK-V 2#8 is combined with the SalI-Hind III arm of cloning pUC19K-VF-ID-WK#1 of K-VF-ID-WK-LK-V2, and #4, 3 It checked that the base sequence of a junction was right about a clone. Thus, plasmid pUC19F7#2 obtained, #3, and #4 are polypeptides. cDNA is cloned (Fig. 2).
[0056]Thus, built sequence number: Polypeptide which has an amino acid sequence denoted by one The manifestation of the recombinant of cDNA is possible at the host-vector system of various Escherichia coli. Since especially the expression system

in Escherichia coli has abundantly the track record used for various drugs manufactures, it is appropriate that Escherichia coli also performs production of polypeptide.
[0057][Reference example 2] The construction inventors of pQTF7deltacr, As shown in the following reference examples, the Escherichia coli expression system (Bio/Technology, 8: 1036-1040, and 1990) of **** and others who uses a trp promotor is changed, It succeeded in carrying out a remarkable synthesis and storing up epitope polypeptide as an insoluble fraction (inclusion body) into the fungus body of Escherichia coli. This expression system can lessen the amount of a manifestation inducer or the antibiotic used.
(1) trp operon promotor Above-mentioned document for the promotor trp and Shine Dalgarno sequence of Escherichia coli to reference, The oligonucleotides TRPS (sequence number: 17), TRPA (sequence number: 18), and SDSDS (sequence number: 19) and SDSDA (sequence number: 20) were synthesized. TRPA (sequence number: 18) and SDSDS (sequence number: 19) are 5'-ends. It phosphorylated by the polynucleotide kinase of the T4 phage. It is complementary in 11 bases of the 3'-end of TRPS (sequence number: 17) and TRPA (sequence number: 18). It is 50 of the first half by heating, cooling slowly and carrying out an association and performing repair synthesis by a Klenow fragment. Double strand DNA of the base pair was obtained. SDSDS (sequence number: 19) and SDSDA (sequence number: 20) 10 of a 3'-end It is complementary also in a base. The association was heated, cooled slowly and carried out and double strand DNA of 47 base pairs of the second half was obtained by the repair synthesis by a Klenow fragment. These It is [SDSDA which phosphorylated DNA fragment TRP-SDSD which carried out the ligation reaction, and which was connected with the DNA ligase,] a DNA fragment T4 Phage (sequence number: Make 20) and TRPS (it does not phosphorylate) (sequence number: 17) into a primer, and it is 12. It amplified by PCR of the cycle. pUC19F8#10 (plasmid by which DNA which encodes the 5th epitope of pUC19F7 was replaced by DNA which encodes another epitope) to a mold, 15 which made the primer KVTV43S (sequence number: 21) and VDGI(it does not phosphorylate)14A2 which were phosphorylated (sequence number: 14) The cDNA fragment F8 was amplified by PCR of the cycle, and it separated/refined by polyacrylamide gel electrophoresis. F8 is mixed with DNA fragment TRP-SDSD, and it is a Klenow fragment. T4 phage The DNA ligase was made to act and it was made to join together. It is TRPS (sequence number: 17) about united fragment TRP-SDSD-F8. VDGI14A2 (sequence number: 14) was amplified by PCR of 12 cycles made into the primer, and it separated/refined by polyacrylamide gel electrophoresis. DNA fragment TRP-SDSD-F8 was digested by Hind III, and the fragment of about 500 bp was separated / refined by agarose gel electrophoresis. It digested by Hind III. It is EcoRI about DNA fragment TRP-SDSD-F8. After digesting, it is made to combine with the EcoRI-Hind III arm of pUC119, and is Escherichia coli TB1. The stock was transformed. 13 which formed the white colony on the X-gal plate Minute amount preparation of the plasmid was performed about the clone, and two clones (pUC119TF8#6 and #7) by which the fragment of abbreviation 500 bp is cut out by double digestion of EcoRI and Hind III were chosen. When the insertion base sequence of these plasmids was read by the dideoxy method, the recognition site order of Hinc II/HpaI carried out in pUC119TF8#6, and the recognition site of DraI and after that were carrying out 20bp deletion in 32 bp and pUC119TF8#7. the cDNA portion of the insertion base sequence of pUC119TF8#6 -- 5' -- although near 70 bp was read, there was no variation which changes an amino acid sequence in the range. 5' of KVTV43S (sequence number: 21) -- near 18 bp was synthesized as an equal mixture of a degenerate codon -- a sake -- four -- all had changed the 3rd character of the codon to T (Fig.3 and sequence number: 23). A deletion position of pUC119TF8#6 and #7 has deviated.
To between. A recognition site of ClaI exists.
Then, it decided to rearrange these clones and to produce the recombinant target body.

pUC119TF8#6 was digested by ClaI and Hind III, and the DNA fragment of abbreviation 400 bp was separated by agarose gel electrophoresis. After digesting pUC119TF8#7 by alkaline phosphatase of ClaI, Hind III, and a cow small intestine, it applied to agarose gel electrophoresis, and the vector side fragment (abbreviation 3 kbp) was separated. These DNA fragments T4 phage After making it join together with a DNA ligase, it introduced into Escherichia coli GI698, and recombinant was chosen on the plate which added tryptophan of 10microg/mL, and the ampicillin of 100microg/mL. 6 Minute amount preparation of plasmid DNA was performed about the clone (pUC119TF8#6.51-#6.56), and it checked that recombination of DNA intended by the restriction pattern of double digestion of Hae III and EcoRI had occurred.

[0058](2) The DNA fragment of abbreviation 120 bp containing the promotor of construction pUC119TF8#6.54 of intermediate-field plasmid pQTF7 to trp and cDNA of N-end of polypeptide was cut down by EcoRI and Eco47I digestion. From pUC19F7 Abbreviation 290 bp containing cDNA by the side of C-end of polypeptide The DNA fragment was cut down by Hind III and Eco47I digestion. On the EcoRI-Hind III arm of pQE11, these fragments were combined and it introduced into Escherichia coli (GI698 stock). 24 of ampicillin tolerance A little plasmid DNAs are prepared from a clone (pQETF7#1-24), It is the existence of an insertion sequence SDSDS (sequence number: 19) and VDGI14A2 (sequence number: 14) It investigated by PCR and Hind III/EcoRI digestion which were made into the primer, and checked that there was an insertion base sequence of the length expected from pQETF7#12. After performing EcoRI of pQETF7#4, pQETF7#7, and pQETF7#12, and XhoI digestion, it is a vector side at agarose gel electrophoresis. The DNA fragment was separated / refined. It is a Klenow fragment and T4 to the EcoRI-XhoI fragment of pQETF7#12. Phage Make a DNA ligase act and a ring closure is carried out, It introduced into Escherichia coli GI698 and the recombinant body was chosen on LB agar-medium plate which added tryptophan of 10microg/mL, and the ampicillin of 100microg/mL. It checked that prearranged deletion had occurred by restriction enzyme digestion (double digestion of DraI and HindIII) and polyacrylamide gel electrophoresis of plasmid DNA pQTF7 prepared from the recombinant body. pQTF7 Terminator arrangement t_0 of lambda phage has connected downstream from cDNA of polypeptide. the -- further -- downstream -- chloramphenicol acetyltransferase (cat) and ribosome Conclusion signal sequence of RNA transcription T_1 has connected. Since the portion of this cat and T_1 was unnecessary, expression plasmid pQTF7deltacr which removed them was produced.

[0059](3) Construction pQTF7 of pQTF7deltacr. They are WKNN17S (sequence number: 10) and T0XBA (sequence number: 22) to a mold. Terminator t_0 of the second half of cDNA of polypeptide and lambda phage is included in a primer. DNA fragment WK-T0 is amplified by PCR of 20 cycles, The fragment of abbreviation 300 bp was separated by agarose gel electrophoresis. After digesting DNA fragment WK-T0 by XbaI and Hind III, it refined by QIAEX II and was made to join together with the XbaI-Hind III arm and T4 DNA ligase of pUC19. It is plasmid DNA generated by the ligation reaction Escherichia coli MC1061 It introduced into the stock. 4 of ampicillin tolerance The clone (pUC19t_0#1-4) was cultivated and minute amount preparation of plasmid DNA was performed. The band of abbreviation 150 bp was checked by the polyacrylamide gel electrophoresis after restriction enzyme digestion (double digestion of EcoRI and Hind III). The base sequence of pUC19t_0#1 was checked by the dideoxy method. Since the XbaI-Hind III fragment of abbreviation 100 bp of pUC19t_0#1 and the XbaI-Hind III fragment of abbreviation 2.5 kb of pQTF7.12#1 are combined with T4 DNA ligase, it is Escherichia coli K802. It introduced into the stock. The structure of obtained plasmid pQTF7deltacr (Fig.4) was checked by restriction enzyme digestion (double digestion of XbaI and Hind III). The band of DNA of 121 bp was observed by agarose gel electrophoresis.

[0060]Separate an inclusion body fraction from the Escherichia coli which holds the refining epitope polypeptide (sequence number: 1) of the [working-example 1] epitope

polypeptide as an inclusion body, and after extraction with a denaturing agent as follows, 1) In the order of copper chelate column chromatography, 2 cation-exchange column chromatography, and 3 reverse phase column chromatographies, chromatography operation was carried out and it refined. Fur mentor culture of the Escherichia coli stock K 802 transformed by expression plasmid pQTF7**cr (Fig.4) was carried out. Fungus body 45 g (wet fungus body weight) after culture was suspended with 50mM trisacetic acid buffer solution (pH 5.0) of 400 mL, and it crushed with the homogenizer. Centrifugality (for 10,000x g and 20 minutes) of this crushing liquid was carried out, and the insoluble fraction was obtained. Centrifugality (for 10,000 xg and 30 minutes) of the insoluble fraction was suspended and carried out with 50mM trisacetic acid buffer solution (pH 5.0) containing the 2% triton X-100 of 400 mL, and inclusion body fraction 78 g was obtained. After adding 1 M guanidine hydrochloride of 400 mL, 5 M urea, and 0.02% acetic acid to this inclusion body fraction and carrying out the churning dissolution at a room temperature for 1 hour, centrifugality (for 10,000 x g and 20 minutes) was carried out, and supernatant liquid was obtained. After mixing this supernatant liquid with 8M urea / 0.2M sodium chloride / 50mM sodium acetate buffer solution (pH 7.0) by 1:1 and adjusting the pH to 7.0, it added in the copper chelate stream line column (50 x150 mm). After washing the column with the buffer 3 capacity of column same as the above and removing non-adsorbate, it eluted with 8M urea / 0.2M sodium chloride / 50mM sodium acetate buffer solution (pH 5.0), and eluate 980 mL was obtained. It added in the SP-sepharose FF column (50 x 100 mm) which adjusted this eluate to pH 4.0 with acetic acid, and was equilibrated with 8M urea / 0.1M sodium chloride / 50mM trisacetic acid buffer solution (pH 4.0). With 8M urea / 0.1M sodium chloride / 50mM sodium carbonate buffer solution (pH 10.0), and 8M urea / 0.2 M sodium chloride / 50mM trisacetic acid buffer solution (pH 4.0), after washing a column, It eluted with 8M urea / 0.4 M sodium chloride / 50mM trisacetic acid buffer solution (pH 4.0). A_{280} of an eluate is measured and there is absorption -- fraction 720 mL obtained. Load of the eluate fraction was carried out to 50RPOROS2 column (25 x 200 mm) equilibrated with acetic acid 1 more%. It eluted with 22% acetonitrile / 1% acetic acid after washing with 12% acetonitrile / 1% acetic acid. The eluate was freeze-dried and polypeptide 208 mg (dry weight) was obtained. The purity of this refining polypeptide was 99% as polypeptide. purity -- opposite phase HPLC[-- detector: -- it authorized by ultraviolet absorptiometer (measured wavelength: 215 nm) and column:CAPCELLPAK C18, SG 300A 5 micrometer, and 4.6 mm i.d.x 15 cm(Shiseido)]. 12.5% by weight of acetic acid was contained in this polypeptide. The variations in lot-to-lot acetic acid content are about 8 to 13% by weight of within the limits, and idea **.

[0061]

[Table 1]

ロット番号	酢酸含量（%）
1	8.0
2	12.0
3	9.5
4	13.0
5	8.1
6	12.5
7	9.7
8	12.6
9	8.6
10	12.5
11	8.2
12	11.6
13	8.9
14	11.9
15	11.7
16	11.3
17	10.1
18	13.1
19	12.5

[0062]The following processings are performed to the refining epitope polypeptide obtained in preparation working examples 1 of the epitope polypeptide from which the stability comparison 1. acetic acid content of the epitope polypeptide from which the [example 1 of examination] acetic acid content differs differs, Six kinds (samples 1-6) of epitope polypeptides from which an acetic acid content differs were prepared. Since it was thought that the last moisture contents differ, respectively, each prepared sample was saved after controlling the humidity of a sample on the humidity conditions of 25 degree-C50%(relative humidity) RH for 6 hours.
- Sample 1:epitope polypeptide and the sample 2 untaken a measure : - which added water 25mL to about 250 mg of epitope polypeptide, was melted, and was freeze-dried Water 25mL was added to about 250 mg of sample 3:epitope polypeptide, and it melted, and freeze-dried. Water 25mL was added to the freeze-drying article furthermore obtained, and it melted, and freeze-dried. This operation was performed further once again and freeze-drying operation was performed 3 times in total.
- Sample 4 : about 250 mg of epitope polypeptide was saved for five days in the desiccator of 25 degree-C13%RH.
- Sample 5 : after saving about 250 mg of epitope polypeptide in the desiccator of 25 degree-C75%RH for one day, it saved for four days at the desiccator of 25 degree-C13%RH.
- Sample 6 : about 250 mg of epitope polypeptide was saved in the desiccator saturated with acetic acid vapor for 6 hours.
[0063]2. After putting into the transparent airtight glassware (with a screw cap) of about 70 mg of stability preservation each sample (samples 1-6) and carrying out ** by parafilm, 40 degrees C/one week were saved.
[0064]3. Measuring condition 3.1. About 10 mg of the acetic acid above-mentioned samples (samples 1-6) were measured precisely, and water 5mL was added correctly, and it dissolved, and was considered as the sample solution. About 400 mg of acetic acid was measured precisely, and water was added, and it mixed, and was correctly referred to as 20mL. This liquid 2mL was taken correctly, and water was added, and it was correctly referred to as 100mL, and was considered as the standard solution. About sample-solution and standard solution 50muL, it examined by the liquid chromatogram process on the following conditions, the peak area of the acetic acid obtained from each solution was determined, and the acetic acid content was computed from the following formula.
[Formula]
Acetic acid content (%) = (At /As) x (Ws/Wt) x 0.5At : Peak area value As of acetic acid of the sample solution : Peak area value Wt of acetic acid of a standard solution : Weight of a sample (mg)
Ws : weight of acetic acid (mg)
[Test condition]
Detector: Ultraviolet absorptiometer (measured wavelength: 210 nm)
Column: Inertsil ODS-3V 5 micrometer 4.6 mm i.d.x25 cm (GL Sciences Inc.)
Column temperature: Constant temperature mobile phase near 40 degree C: A liquid 0.085% phosphoric acid liquid B liquid acetonitrile / 0.085% phosphoric acid liquid mixture (9:1)
A gradient program (linear) is shown in Table 2.

[0065]
[Table 2]

時間（分）	A液（%）	B液（%）
0（注入）	100	0
10	100	0
12	0	100
18	0	100
20	100	0
30（注入）	100	0

＃10分以降はカラム洗浄と平衡化

[0066]Flow Quantity: It adjusts so that the retention time of acetic acid may become about 6 minutes (usually about 1.0 mL/min).
[0067]3.2. About 10 mg of relative protein samples were measured precisely, and water 5mL was added correctly, and it dissolved, and was considered as the sample solution. About sample-solution 40muL, it examined by liquid chromatography on the following conditions, and the related substance content was computed with area percentage.
[Test condition]
Detector: Ultraviolet absorptiometer (measured wavelength: 215 nm)
Column: CAPCELLPAK C18, SG 300A 5 micrometer, 4.6 mm i.d.x 15 cm (Shiseido)
column temperature: -- constant temperature mobile phase [near 40 degree C]: -- A liquid water / 1 mol/L phosphoric acid and 100 mmol/L sodium perchlorate mixture (9:1) B liquid acetonitrile / 1 mol/L phosphoric acid, and 100 mmol/L sodium perchlorate mixture (9:1)
A gradient program (linear) is shown in Table 3.
[0068]
[Table 3]

時間（分）	A液（%）	B液（%）
0（注入）	65	35
3	65	35
30	58	42
40	50	50
45	0	100
50	65	35
60（注入）	65	35

＃ 45分以降はカラム洗浄と平衡化

[0069]Flow Quantity: It adjusts so that the retention time of polypeptide may become for about about 19 minutes (usually about 1.0 mL/min).
[0070]3.3. The about 10 polymer sample mg was measured, and water 5 mL was added correctly, it dissolved, this liquid was diluted twice with water, and it was considered as the sample solution. About sample-solution 20muL, it examined by liquid

chromatography on the following conditions, and the total polymer content was calculated with area percentage.
[Test condition]
Detector: Ultraviolet absorptiometer (measured wavelength: 215 nm)
Column: TSK-GEL G4000SWXL, 7.8 mm i.d.x30 cm (made by TOSOH CORP.)
column temperature: -- constant temperature mobile phase [near 25 degree C]: -- water / acetonitrile / trifluoroacetic acid mixture (600:400:1)
Flow rate: Adjust so that the retention time of polypeptide may become for about about 18 minutes (usually about 0.5 mL/min).
[0071]3.4. About 10 content this article mg was measured precisely (Wt, mg), and water 5 mL was added correctly, and it dissolved, and was considered as the sample solution. Water 2.5 mL was correctly added to polypeptide standard substance 1 vial, and it dissolved in it, and was considered as the standard solution. About sample-solution and standard solution 40 muL, it examined by liquid chromatography on the following conditions, and the content of this article was computed from the following formula.
[Formula]
content (%) = (At /As) x (Ws /Wt) x 200At: -- polypeptide peak area value As: of the sample solution -- polypeptide content value (mg/vial) of the polypeptide peak area value Ws:polypeptide standard substance of the sample solution
Wt: The weight of a sample (mg)
Polypeptide content Ws=Wpx (1 - 0.01xF)
Wp: The nature content of whole protein of a polypeptide standard substance (mg)
F : the total relative protein content of a polypeptide standard substance (%)
[Test condition] It is the same as the [test condition] (liquid chromatogram process) of 3.2. relative protein.
[0072][A result and consideration] The acetic acid content and description of a sample (samples 1-6) which prepared by performing various operations are shown in Table 4. As for the sample which freeze-dried and decreased the acetic acid content, all showed the property which is rich in electrostatic property, although description was "white floc." The acetic acid contents of the sample prepared by various operations were 6.1% - 17.9%.
[0073]
[Table 4]

表4　各種処理品の品質（酢酸含量と性状）

試料	処理	性状	酢酸（%）
試料1	未処理	白色の綿状の塊	12.5
試料2	凍結乾燥/1回	白色の綿状の塊	7.4
試料3	凍結乾燥/3回	白色の綿状の塊	6.1
試料4	25℃ 13%RH	白色の綿状の塊	9.7
試料5	25℃ 75→13%RH	白色の綿状の塊	9.2
試料6	酢酸蒸気	白色の綿状の塊	17.9

[0074]Change was not observed in 40 degrees C/description when saved 1W in the epitope polypeptide (samples 1-6) from which the acetic acid content shown in Table 4 differs. The relation with the increase of relative polypeptide was shown in (Fig.5), and the relation with the survival rate of (Fig.6) and epitope polypeptide was shown for the relation between the acetic acid content in epitope polypeptide (samples 1-6), and the polymer accepted after 40 degrees C/1W preservation in (Fig.7), respectively.As for the increase of a polymer, the high tendency was accepted with epitope peptide whose acetic acid contents are 6.1% and 17.9%. When the allowable increase of a polymer is

estimated at about 1.5%, an acetic acid content is about 7 to 14% of range. When the increase of relative polypeptide was divided into the component (a part for low fat fusing) eluted before the epitope polypeptide concerned, and the component (a part for high fat fusing) eluted behind and having been evaluated, the increase of the component eluted before and after a main part by the epitope polypeptide whose acetic acid content is 17.9% became high. When the allowable increase of relative polypeptide is estimated to about 3% or less, an acetic acid content is about 11% or less. The acetic acid content of the content (survival rate) in epitope polypeptide was high near 10%, and it became clear that it is deteriorated according to the reduction and the increase in an acetic acid content. When a survival rate is estimated at about 98%, acetic acid content is about 9 to 13% of range. From the above result, when the generated amount of the survival rate, the polymer, and the decomposition product was comprehensively taken into consideration, it was shown that epitope polypeptide has the most stable acetic acid content near 9 to 10%, and an acetic acid content is comparatively stable at 7 to 13%.
[0075]The epitope polypeptide from which an acetic acid content differs by the [example 2 of examination] solubility test freeze-drying operation was prepared, and the solubility in 25 degrees C to the 5% grape sugar solution (Pharmacopoeia of Japan) used for a medication solvent by a GLP toxicity test was measured.
1. Operation information 1.1 Water 50mL was added to about 0.5 g of epitope polypeptide (unsettled sample) obtained by the same method as the preparation working examples 1 of epitope polypeptide which differ in an acetic acid content, and it dissolved and freeze-dried (conditions: 25 degrees C, 1 psi). this operation -- 1 time -- or it carried out 3 times.
1.2 About 50 mg of preparation samples of the sample saturated solution were taken in glass test tubes, and it shook gently and dissolved so that grape sugar solution (Pharmacopoeia of Japan: made by Otsuka Pharmaceutical) 1mL might be added 5% and a bubble might not be stood at 25 degrees C (it is 30 second shaking -> standing at intervals of 5 minutes). In addition, it repeated operation about 25 mg of samples at a time until a sample stopped having melted in shake. When the sample stopped having melted, about 25 mg of samples were added further, and shaking operation (30 second shaking -> standing is repeated 6 times at intervals of 5 minutes) was performed. When it became impossible for the bubble to have stood and stirred by the above-mentioned shaking operation, shaking operation was performed, after carrying out centrifugality and destroying the bubble. The liquid was centrifuged in 25 degrees C and 2000 rpm/5 minutes, the solution layer was filtered with a 0.45-micrometer membrane filter, and the saturated solution of the sample was obtained.
1.3 The sample concentration in the measurement saturated solution of sample concentration was measured with the UV method. After diluting a saturated solution with 0.1 mol/L chloride 200 to 400 times, the absorbance (A_{280}) of 280 nm was measured and sample concentration was computed by the lower type.
sample (mg/mL) = MW sample xFxA_{280}/epsilon sample epsilon sample: -- molar extinction coefficient MW (=20444) sample: at 280 nm of epitope polypeptide -- molecular weight of epitope polypeptide (=12303)
F : according to the measuring method of a description, it measured to the "3.1. acetic acid" of the example 1 of a measurement test of a dilution ratio 1.4 acetic-acid content.
[0076]2. The solubility in 25 degrees C to the 5% of Pharmacopoeia of Japan grape sugar solution of a sample in which the acetic acid contents prepared by result freeze-drying differ is shown in Table 5.

[0077]
[Table 5]

表5　エピトープポリペプチド25℃における日本薬局方5％ブドウ糖溶液に対する溶解度

試　料	酢酸含量(%)	溶解後pH	溶解度(mg/mL)
未処理	13.0	4.54	150
凍結乾燥/1回	8.0	5.80	120
凍結乾燥/3回	6.7	7.01	78 (82)[1)]

1) 日本薬局方注射用水に対する溶解度

[0078]The sample whose acetic acid contents are 13.0% (unsettled) - 6.7% was obtained by freeze-drying. The solubility of the sample was deteriorated with reduction of an acetic acid content, and the solubility of the sample of 6.7% of an acetic acid content was 78 mg/mL. Although it dissolved comparatively easily to the concentration near 50 mg/mL, at high concentration, a sample floats, and the dissolution takes time to each epitope polypeptide whose acetic acid contents prepared this time are 13.0% - 6.7%, and it foamed very easilier than it. It became gel, when the 5% grape sugar solution of 100 or more mg/mL has high viscosity, and epitope polypeptide is difficult for the filtration operation by the membrane filter which is 0.45 micrometer and settled at 25 degrees C on the 1st.
[0079][Example 1 of pharmaceutical preparation] As opposed to the multiplex T cell epitope polypeptide (it is hereafter written as compound A) which has an amino acid sequence denoted by sequence number:1 as shown in Table 6, After preparing the aqueous solution (compound concentration: 0.12 mg/mL, 2 mg/mL) which contains purified sucrose and adjusting pH with chloride, aqueous solution 1mL obtained by disinfection filtration was dispensed, and freeze-drying was performed for GOMUSEN to the vial after half-plugging. After the end of freeze-drying, after nitrogen gas replaced the vial space part, the freeze-drying article was produced by carrying out winding up of GOMUSEN with plugging and a cap.
[0080]
[Table 6]

処方Aおよび処方Bの組成表

	製剤例1	
	処方A	処方B
化合物A	0.12mg	2mg
精製白糖	10mg	10mg
塩酸	適量	適量
薬液pH	4.6	4.5

[0081][Example 1 of an experiment] It saved for six months with 60% of 25 degree-C relative humidity for two months, four months, and six months with 75% of 40 degree-C relative humidity. When the content (survival rate) of pharmaceutical preparation, relative protein, and a polymer were investigated, the result of Table 7 was obtained. Acetic acid was measured about the formula B.
[0082]
[Table 7]

処方Aおよび処方Bの安定性結果

測定項目	時点	処方A	処方B
含量(残存率)	Initial	100.0%	100.0%
	40℃/75%R.H.×2M	100.7%	98.7%
	40℃/75%R.H.×4M	98.6%	97.8%
	40℃/75%R.H.×6M	100.1%	97.2%
	25℃/60%R.H.×6M	99.0%	100.0%
類縁タンパク質	Initial	2.0%	1.7%
	40℃/75%R.H.×2M	2.1%	2.5%
	40℃/75%R.H.×4M	2.5%	2.1%
	40℃/75%R.H.×6M	2.6%	2.6%
	25℃/60%R.H.×6M	2.1%	1.8%
重合体	Initial	0.2%	0.4%
	40℃/75%R.H.×2M	0.3%	0.6%
	40℃/75%R.H.×4M	0.4%	0.7%
	40℃/75%R.H.×6M	0.4%	0.6%
	25℃/60%R.H.×6M	0.4%	0.5%
酢酸	Initial	-	8.9%
	40℃/75%R.H.×2M	-	9.6%
	40℃/75%R.H.×4M	-	8.6%
	40℃/75%R.H.×6M	-	8.7%
	25℃/60%R.H.×6M	-	9.1%

[0083][Example 2 of pharmaceutical preparation] As shown in Table 8, 2 mg of mannitol prepared the aqueous solution (compound concentration: 2 mg/mL) contained 20 mg to 2 mg of compound A, aqueous solution 1mL obtained by disinfection filtration was dispensed, and freeze-drying was performed for GOMUSEN to the vial after half-plugging. Nitrogen gas replaced the vial space part after the end of freeze-drying, and the freeze-drying article was created by carrying out winding up of GOMUSEN with plugging and a cap.
[0084]
[Table 8]

処方Cおよび処方Dの組成表

	製剤例2	
	処方C	処方D
化合物A	2mg	2mg
マンニトール	2mg	20mg

[0085]It saved for one month with 75% of 240 degrees C of example relative humidity of an experiment. When the content (survival rate) of pharmaceutical preparation, relative protein, and a polymer were measured, the result of Table 9 was obtained.
[0086]
[Table 9]

処方Cおよび処方Dの安定性結果

	時点	処方C	処方D
含量(残存率)	Initial	100.0%	100.0%
	40℃/75%R.H.×1M	98.9%	98.4%
類縁タンパク質	Initial	2.2%	2.1%
	40℃/75%R.H.×1M	3.5%	3.0%
重合体	Initial	0.3%	0.3%
	40℃/75%R.H.×1M	1.4%	2.0%

[0087][Comparative example 1] by the formula which dissolves compound A with water for injection, and is shown in Table 10, without adding sugars, After preparing each aqueous solution (compound concentration: 2 mg/mL) and adjusting pH with chloride if needed, each aqueous solution 0.5mL obtained by disinfection filtration was dispensed, and freeze-drying was performed for GOMUSEN to the vial after half-plugging. Nitrogen gas replaced the vial space part after the end of freeze-drying, and the freeze-drying article was produced by carrying out winding up of GOMUSEN with plugging and a cap.
[0088]
[Table 10]

処方Eおよび処方Fの組成表

	比較例1	
	処方E	処方F
化合物A	1mg	1mg
塩酸	−	適量
薬液pH	5.1	4.6

[0089]It saved for one month with 75% of 340 degrees C of example relative humidity of an experiment. When the content (survival rate) of pharmaceutical preparation, relative protein, and a polymer were measured, the result of Table 11 was obtained.
[0090]
[Table 11]

処方Eと処方Fの安定性結果

測定項目	時点	処方E	処方F
含量	Initial	100.0%	100.0%
(残存率)	40℃/75%R.H.×1M	86.4%	91.4%
類縁タンパク	Initial	2.7%	2.8%
質	40℃/75%R.H.×1M	14.2%	11.4%
重合体	Initial	0.3%	0.3%
	40℃/75%R.H.×1M	9.3%	4.5%

[0091]In [compare the example 1 of an experiment, and the example 2 of an experiment with the example 3 of an experiment, and] the lyophilized products of the epitope polypeptide of the present invention, By adding purified sucrose and mannitol showed that generation of relative protein by preservation and a polymer could be lessened, and it could do with the pharmaceutical preparation excellent in stability.
[0092]
[Layout Table]
SEQUENCE LISTING <110> Meiji Dairies Corporation; Takeda Chemical Industries, Ltd.<120> Multiple Epitope Acetylated Polypeptides -- < 130> H14020<150> JP P2001-196607 <151> 2001-06-28<160> 23 <170> PatentIn Ver.2.1<210> 1<211> 105<212> PRT<213> Cryptomeria japonica <400>. 1Met Lys Val Thr Val. Ala Phe Asn Gln Phe. Gly Pro Asn Arg Arg. Val 1 5 10 15 Phe Ile. Lys Arg Val Ser Asn. Val Ile Ile His Gly. Arg Arg Ile Asp 20 25. 30 Ile Phe Ala Ser Lys. Asn Phe His Leu Gln. Lys Asn Thr Ile Gly. Thr 35 40 45 Gly Arg. Arg Trp Lys Asn Asn. Arg Ile Trp Leu Gln Phe Ala Lys Leu 50 55 60 Thr Gly Phe Thr Leu Met Gly Arg Arg Leu Lys Met Pro Met Tyr Ile. 65 70 75 80Ala Gly Tyr. Lys Thr Phe Asp Gly Arg Arg Val Asp Gly Ile Ile Ala 85 90 95 Ala Tyr Gln Asn Pro Ala Ser Trp Lys 100 105 -- < 210> 2<211> 339<212> DNA <213> Cryptomeria japonica<400> 2catcccggga aatccatgaa ggtgacagtg gcgttcaatc aatttggacc taaccgtcga 60 gtgtttatca agagagtgag. caatgttatc atacacggtc. gtcgaatcga catctttgca. 120 tctaaaaact ttcacttaca. aaagaacacg ataggaacag. ggcgtcgatg gaagaacaat. 180 agaatatggt tgcagtttgc. taaacttaca ggttttactc. taatgggtcg tcgactcaaa. 240 atgcctatgt acattgctgg gtataagact tttgatggcc gtcgagtaga tgggataata 300 gctgcgtacc aaaatccagc gagctggaag taagcttgg 339 -- < 210> 3<211> 25<212> DNA<213> Artificial Sequence<220> <223> Description of Artificial Sequence: Primer<400> 3ccatcccggg aaatccatga aggtg 25 <210> 4<211> 26<212> DNA<213>Artificial Sequence<220> <223> Description of Artificial Sequence:Primer <400> 4 ggtagtcgac ggttaggtcc aaattg 26 -- < 210> 5<211> 25<212> DNA<213> Artificial Sequence<220> <223> Description of Artificial Sequence: Primer<400> 5ccatcccggg ccctgtgtgt ttatc 25 <210> 6<211> 26<212> DNA<213>Artificial Sequence<220> <223> Description of Artificial Sequence:Primer <400> 6 ggtagtcgac gaccgtgtat gataac 26 -- < 210> 7<211> 25<212> DNA<213> Artificial Sequence<220> <223> Description of Artificial Sequence: Primer<400> 7ccatctgcag tgtttatcaa gagag 25 <210> 8<211> 27<212> DNA<213>Artificial Sequence<220> <223> Description of Artificial Sequence:Primer <400> 8 ccatcccggg attgatatct ttgcatc 27 -- < 210> 9<211> 26<212> DNA<213> Artificial Sequence<220> <223> Description of Artificial Sequence: Primer<400> 9ggtagtcgac gccctgttcc tatcgt 26 <210> 10<211> 26<212> DNA<213>Artificial Sequence<220> <223> Description of Artificial Sequence:Primer <400> 10 tggaagaaca atagaatatg gttgca 26 -- < 210> 11<211> 26<212> DNA<213> Artificial Sequence<220> <223> Description of Artificial Sequence: Primer<400> 11ggtagtcgac gacccattag agtaaa 26 <210> 12<211> 27<212> DNA<213>Artificial Sequence<220> <223> Description of Artificial Sequence:Primer <400> 12 ccatgatatc gacatctttg catctaa 27 -- < 210> 13<211> 25<212> DNA<213> Artificial Sequence<220> <223> Description of Artificial Sequence: Primer<400> 13gcatctgcag tagatgggat aatag 25 <210> 14<211> 26<212> DNA<213>Artificial Sequence<220> <223> Description of Artificial Sequence:Primer <400> 14 gcataagctt acttccagct cgctgg 26 -- < 210> 15<211> 26<212> DNA<213> Artificial Sequence<220> <223> Description of Artificial Sequence: Primer<400> 15cgatggtacc tcaaaatgcc tatgta 26 <210> 16<211> 26<212> DNA<213>Artificial Sequence<220> <223> Description of Artificial Sequence:Primer <400> 16 ggtagtcgac ggccatcaaa agtctt 26 -- < 210> 17<211> 29<212> DNA<213> Artificial Sequence<220> <223> Description of Artificial Sequence: Primer<400> 17ccagtgaatt cccctgttga caattaatc 29 <210> 18<211> 32<212> DNA< 213> Artificial Sequence<220> <223> Description of Artificial Sequence:Primer<400>18gtactagtta actagttcga tgattaattg tc 32 <210> 19 <211>

26<212> DNA <213> Artificial Sequence -- < 220> <223> Description of Artificial Sequence:Primer<400> 19gcaagttgac gtcaaaaggg tatcga 26 < 210> 20<211> 31<212> DNA<213> Artificial Sequence<220> <223> Description of Artificial Sequence: Primer<400> 20cattttaaac ctccttacta atcgataccc t 31 <210> 21<211> 26<212> DNA< 213> Artificial Sequence<220> <223> Description of Artificial Sequence:Primer<400>21aargtnacng tngcnttyaa tcaatt 26 <210 > 22 <211> 29<212> DNA <213> Artificial Sequence -- < < [220> <223> Description of Artificial Sequence:Primer<400> 22ccattctaga ttctcaccaa taaaaaacg 29] 210> 23<211> 128<212> DNA<213> Artificial Sequence<220> <223> Description of Artificial Sequence< 400> 23aattcccctg ttgacaatta atcatcgaac tagttaacta gtacgcaagt tgacgtcaaa 60 agggtatcga ttagtaagga ggtttaaaat gaaggtgact gttgctttta atcaatttgg 120 acctaac 127 [0093] [Effect of the Invention]The acetate constituent of the multiplex T cell epitope polypeptide which contains 5 to 15% of acetic acid by the present invention in the freeze-drying article which consists of a T cell epitope of the main allergen protein Cry j 1 of cedar pollen and Cry j2 origin, and whose solubility is stable at best for a long period of time was provided. This multiplex T cell epitope polypeptide acetate constituent is useful as injections for prevention of hay fever, or a therapy.

DESCRIPTION OF DRAWINGS

[Brief Description of the Drawings]
[Drawing 1]The construction figure of DNA which encodes multiplex T cell epitope polypeptide is shown.
[Drawing 2]The base sequence which encodes the amino acid sequence of multiplex T cell epitope polypeptide and this polypeptide is shown. The portion of the bold letter of a base sequence shows the region which encodes the amino acid sequence of polypeptide. The base which attached the arrangement Nakashita line is mutated for the Reasons of making construction of a plasmid easy. A small letter shows the base sequence of a plasmid or PCR primer origin.
[Drawing 3]The base sequence (small letter) which encodes the base sequence of a trp promotor periphery of expression plasmid pQTF**cr (capital letter) and the amino acid sequence of the amino terminal of multiplex T cell epitope polypeptide is shown. ** The enclosed portion shows -10 region and -35 region, and an underline portion shows a Shine Dalgarno sequence, and the underline portion of a duplex shows main restriction enzyme recognition sites.
[Drawing 4]Expression plasmid pQTF7**cr is shown. A trp promotor, two Shine Dalgarno sequences, the region that encodes multiplex T cell epitope polypeptide, terminator t_0 of lambda phage origin, main restriction enzyme recognition sites, and an ampicillin resistance gene are shown.
[Drawing 5]The relation between the acetic acid content (%) of multiplex T cell epitope polypeptide and the polymer increase (%) of this polypeptide is shown.
[Drawing 6]The relation between acetic acid content (%) same as the above and the relative polypeptide increase (%) of this polypeptide is shown.
[Drawing 7]The relation between acetic acid content (%) same as the above and residual content (%) is shown.

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(54)【発明の名称】　多重Ｔ細胞エピトープポリペプチドの酢酸塩組成物

(57)【要約】
【課題】　本発明は、溶解性および安定性を向上させた多重T細胞エピトープポリペプチドを提供することを課題とする。
【解決手段】　酢酸を5～15%含有する配列番号：１で表されるアミノ酸配列を有する多重T細胞エピトープポリペプチド酢酸塩組成物。配列番号：１で表されるアミノ酸配列を有する多重T細胞エピトープポリペプチド１に対し、酢酸を約4～20（重量）％含有してなる組成物。

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【特許請求の範囲】
【請求項１】　酢酸を5～15（重量）%含有する配列番号：１で表されるアミノ酸配列を有する多重T細胞エピトープポリペプチド酢酸塩組成物。
【請求項２】　酢酸を約7～13（重量）%含有する、請求項１記載の多重T細胞エピトープポリペプチド酢酸塩組成物。
【請求項３】　酢酸を約9～10（重量）%含有する、請求項１記載の多重T細胞エピトープポリペプチド酢酸塩組成物。
【請求項４】　配列番号：１で表されるアミノ酸配列を有する多重T細胞エピトープポリペプチド１に対し、酢酸を約4～20（重量）%含有してなる組成物。
【請求項５】　配列番号：１で表されるアミノ酸配列を有する多重T細胞エピトープポリペプチド１に対し、酢酸を約5～18（重量）%含有してなる組成物。
【請求項６】　配列番号：１で表されるアミノ酸配列を有する多重T細胞エピトープポリペプチド１に対し、酢酸を約7～15（重量）%含有してなる組成物。
【請求項７】　配列番号：１で表されるアミノ酸配列を有する多重T細胞エピトープポリペプチド１に対し、酢酸を約9～12（重量）%含有してなる組成物。
【請求項８】　請求項１～７のいずれか１項記載の組成物を含有してなる凍結乾燥製剤。
【発明の詳細な説明】
【０００１】
【発明が属する技術分野】本発明は、スギ花粉症の予防または治療剤として有用な多重T細胞エピトープポリペプチドの溶解性および安定性を向上させた酢酸塩組成物に関する。
【０００２】
【従来の技術】スギ花粉症は、スギ花粉をアレルゲンとする即時型アレルギー疾患である。鼻炎、結膜炎が主たる症状であり、死に至る病ではないので軽視されがちであるが、患者にとってはまことに不愉快な症状である。スギ花粉の飛散期には国民の1割以上、都市部においては2割以上がこのスギ花粉症にかかるといわれており、経済的損失も大きい。
【０００３】スギ花粉症の治療には、抗ヒスタミン薬、ステロイド剤だけでなく、抗アレルギー剤も登場しているが、これらはすべて対症薬である。スギ花粉アレルゲン抽出液を繰り返し投与する減感作療法は、臨床的にアレルギー症状を改善する有効な治療法である。しかしながら、アレルゲン抽出液は、患者のアレルゲン特異的IgE抗体と反応するB細胞エピトープを含んでいるために、時としてアナフィラキシーなどの副反応が問題となる。長期に減感作療法を受けた患者末梢血のT細胞はスギ花粉に対する反応性が減弱していることから、減感作療法の標的細胞はT細胞であると考えられている。最近、動物モデルにおいて、アレルゲン特異的T細胞エピトープペプチドが、T細胞に不活性化を誘導し、また、患者のアレルゲン特異的IgE抗体とほとんど結合しないことが明らかにされた。

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【０００４】そこで、これまでのアレルゲン抽出液を用いた減感作療法に代わるものとして、スギ花粉の主要アレルゲンタンパク質Cry j 1およびCry j 2由来のアレルゲン特異的T細胞エピトープペプチドの混合物を用いたペプチド免疫療法が考案されている（WO 94/01560）。この方法は、上記のようなアナフィラキシーなどの副反応を回避でき、また、人工的に作製可能なため標準化しやすい、という利点があるが、このような混合物を医薬品として開発する場合、個々のT細胞エピトープについて物性・安全性試験などを実施する必要があり、製品規格などの点で問題がある。
【０００５】この問題を解決するために、Cry j 1およびCry j 2のアミノ酸配列から、MHCクラスII拘束分子の差異に基づいて選択されたいくつかのメジャーおよびマイナーなT細胞エピトープペプチドを、ペプチド結合を介して直鎖状に結合した多重T細胞エピトープポリペプチドが考案され（WO 97/32600）、その有効性が検討されている。
【０００６】一般に、タンパク質医薬品を注射剤として開発する場合、溶液状態では安定性などに問題があるため、凍結乾燥法により用時溶解型注射剤として製品化される場合が多い。しかし、保存期間中にタンパク質が凝集を示し、医薬品の品質として問題となる場合がある。このようなタンパク質の凝集現象について、種々の糖類を添加した場合の分子運動性を評価し、その安定性を予測する試みが報告されている。
【０００７】
【発明が解決しようとする課題】本発明は、溶解性および安定性を向上させた多重T細胞エピトープポリペプチド（以下、「エピトープポリペプチド」、あるいは「ポリペプチド」ということもある）を提供することを課題とする。
【０００８】
【課題を解決するための手段】本発明者らは、多重T細胞エピトープポリペプチドを封入体として保持する大腸菌から該封入体を取り出し、塩酸グアジニン/尿素で可溶化し、その上清に該ポリペプチドを抽出した。この粗抽出物を、銅キレートクロマトグラフィー、陽イオン交換クロマトグラフィー、および逆相クロマトグラフィーの順のクロマトグラフィーにより、該ポリペプチドを高純度に精製した。そして、この精製エピトープポリペプチドの安定性および溶解性を向上させる酢酸含量を決定した。
【０００９】すなわち、本発明は、（１）　酢酸を5～15（重量）%含有する配列番号：１で表されるアミノ酸配列を有する多重T細胞エピトープポリペプチド酢酸塩組成物、（２）　酢酸を約7～13（重量）%含有する、

前記（１）記載の多重T細胞エピトープポリペプチド酢酸塩組成物、（３）　酢酸を約9～10（重量）%含有する、前記（１）記載の多重T細胞エピトープポリペプチド酢酸塩組成物、（４）　配列番号：１で表されるアミノ酸配列を有する多重T細胞エピトープポリペプチド１に対し、酢酸を約4～20（重量）%含有してなる組成物、（５）　配列番号：１で表されるアミノ酸配列を有する多重T細胞エピトープポリペプチド１に対し、酢酸を約5～18（重量）%含有してなる組成物、（６）　配列番号：１で表されるアミノ酸配列を有する多重T細胞エピトープポリペプチド１に対し、酢酸を約7～15（重量）%含有してなる組成物、（７）　配列番号：１で表されるアミノ酸配列を有する多重T細胞エピトープポリペプチド１に対し、酢酸を約9～12（重量）%含有してなる組成物、（８）　前記（１）～（７）のいずれか１項記載の組成物を含有してなる凍結乾燥製剤、に関する。

【００１０】

【発明の実施の形態】以下、本発明を詳しく説明する。本発明の多重T細胞エピトープポリペプチドは、化学合成あるいは遺伝子組換え技術により合成できる。ペプチドの化学合成は、ここ数年来急激な勢いで利用されている。それに伴い、初心者でも操作できるようなペプチド合成機が普及し、ペプチドの受注合成も国内外で盛んに行われている。100個以上のアミノ酸残基からなる長鎖のポリペプチドも化学合成されている。例えば、最近、ヘパリン結合性の成長因子であり121個のアミノ酸残基からなるミッドカイン（midkine）が化学合成された（Inui, T. et al.: J. Peptide Sci., 2: 28-39, 1996）。したがって、本発明のエピトープポリペプチドも同様にして化学合成することができる。

【００１１】遺伝子組換え技術を用いれば、エピトープポリペプチドをコードする遺伝子を適当なベクターに組み込んで細胞に導入し、該遺伝子を発現させることにより、ポリペプチドを大量に合成することが可能である。エピトープポリペプチド遺伝子の発現系として、大腸菌発現系、酵母発現系、昆虫細胞発現系、および動物細胞発現系が挙げられるが、エピトープポリペプチドは翻訳後修飾を必要としない一本鎖の単純ポリペプチドであるので、大腸菌の単独発現システムを用いるのがよい。

【００１２】大腸菌のタンパク合成系を利用すると、エピトープポリペプチドを大量に、かつ低コストで得ることが可能である。スギ花粉アレルゲンCry j 1（Sone, T. etal.: Biochem. Biophys. Res. Commun., 199: 619-625, 1994）およびCry j 2（Komiyama, N. et al.: Biochem. Biophys. Res. Commun., 201: 1021-1028, 1994）をコードする遺伝子はすでにクローン化され、推定アミノ酸配列が明らかとなっている。配列番号：１のアミノ酸配列で示されるエピトープポリペプチドを構成する６つのT細胞エピトープ領域（Argダイマーで仕切られている）の、Cry j 1およびCry j 2のアミノ酸配列中に占める部位は、WO97/32600公開公報の図１（Cry j 1）および図２（Cry j 2）から容易に確認できる。そして、この６つのT細胞エピトープペプチド領域をコードするDNA配列は、前記Soneら、およびKomiyamaらの文献から知ることができる。そこで、６つのT細胞エピトープペプチドをコードするDNA配列に対するPCRプライマーを化学合成する。クローン化されたCry j 1およびCry j 2をコードする遺伝子を鋳型としてPCRでエピトープポリペプチド領域をコードするDNAを増幅後連結し、さらにPCRで増幅するといった操作を繰り返し、途中および最終の配列をpUCプラスミドにクローニングして塩基配列の確認を適宜行う。このようにしてエピトープポリペプチド（配列番号：１）の全長をコードする遺伝子（配列番号：２）を構築することができる。

【００１３】真核生物由来の外来遺伝子を大腸菌で高発現させると、しばしば、産生タンパク質が菌体内で凝集し、生理的に不活性な封入体を形成する。この封入体形成は、産生したタンパク質を菌体内のプロテアーゼから隔離し、プロテアーゼによる分解を抑え、しかも多くの菌体由来の可溶性夾雑タンパク質からの目的遺伝子産物の分離を可能とする。そこで、ポリペプチドは、大腸菌の菌体内に封入体として生成させるのが、その後の精製の面から望ましい。

【００１４】タンパク質遺伝子の大腸菌発現系に関しての文献は枚挙にいとまがないが、例えば、［続生化学実験講座 II, 組み換えDNA技術, 日本生化学会編, p126, 東京化学同人(1986); 新生化学実験講座１, タンパク質VI, 合成および発現, 日本生化学会編, p155, 東京化学同人(1992)など］などを参考にして、当業者は、ポリペプチドの大腸菌発現系を構築することが容易にできる。また、大腸菌の単独発現システムが市販されており、例えば、転写能力が強いT7ファージRNAポリメラーゼを利用するpETシステム（Novagen, STRATAGENE）や、同様なT7ファージRNAポリメラーゼを利用したpRSETシステム（Invitrogen）などを試みることもできる。

【００１５】発現プラスミドを導入する宿主大腸菌としては、一般に用いられる HB101, C600 などの種々の K-12 の誘導体を用いることができるが、菌株による発現量の差が大きい。実施例では、増殖力が強く、発現量も多い K802 株（ATCC から入手）を宿主として使用したが、他の菌株を使用する場合は培養条件（培養時間、添加するトリプトファンの濃度等）の最適化が必要であるが、そのような最適化は、当業者にとって実験条件の設定範囲にある。

【００１６】エピトープポリペプチド遺伝子を保持する形質転換体の発現培養条件の設定は、当業者であれば、文献［例えば、タンパク実験プロトコール2, 構造解析編,細胞工学別冊, 秀潤社(1997)］を参考に実施することができる。

【００１７】ポリペプチドを封入体として保持する菌体

を遠心して集め、緩衝液に懸濁し、超音波処理あるいはホモジナイザー処理で菌体を破砕する。この破砕液を遠心して不溶性画分を得る。そして、この不溶性画分を緩衝液に懸濁し遠心して封入体画分（あるいは不溶性の封入体状画分）を得る。この封入体の可溶化には、高濃度のタンパク質変性剤（6 M 塩酸グアニジンや6～8 M の尿素）を用いるのが一般的である（Biochemistry, 26: 3129, 1987; J. Biotechnol., 1: 307, 1984;Bio/Technology, 3: 990, 1985 ）。この可溶化物を遠心して上清にポリペプチドを抽出する。このポリペプチド粗抽出液を、銅キレートクロマトグラフィー、イオン交換クロマトグラフィー、および逆相クロマトグラフィーの順のクロマトグラフィーに供することにより、エピトープポリペプチドを高純度に精製することができる。以下、精製工程を詳細に述べる。

【００１８】培養後、大腸菌を遠心して集菌する。菌体を緩衝液、例えば、50 mMトリス酢酸緩衝液（pH 5.0）に懸濁し、超音波処理あるいはホモジナイザー処理して菌体を破砕する。次に、遠心（例えば、10,000 × g、20分間）して不溶性画分を得る。この不溶性画分を、界面活性剤を含む緩衝液、例えば２％トリトンX-100を含む50mM トリス酢酸緩衝液（pH 5.0）に懸濁し、遠心（例えば、10,000 × g、30分間）して封入体画分を得る。この封入体画分を、タンパク質変性剤、例えばグアニジン塩を含む抽出緩衝液、例えば、6M塩酸グアニジンを含む緩衝液（pH4.0）、あるいは0.5M～1M塩酸グアニジンと5.5M～5Mの尿素を含む緩衝液（pH4.0）で1.5～3時間室温で攪拌して溶解する。溶液を遠心（例えば、10,000 × g、20分間）するとポリペプチドは上清に抽出される。

【００１９】さらに、この粗抽出液を、中性あるいは弱アルカリ性の緩衝液（例えば、50 mM炭酸緩衝液 pH 9.8 ）で10～20倍に希釈し、37℃前後で１時間放置すると、エピトープポリペプチドは沈澱する。一方、大腸菌由来の低分子量（分子量2万以下）のタンパク質のほとんどは変性状態から容易に巻き戻しされて高次構造を再生するので、可溶であり、除去される。エピトープポリペプチドを含む沈澱は、再度6 M塩酸グアニジンを含む緩衝液（pH 4.0）、あるいは0.5～1 M塩酸グアニジンおよび5.5M～5M尿素を含む緩衝液（pH 4.0）に懸濁し、1.5～3時間攪拌して溶解する。溶液を遠心（例えば、10,000×g、20分間）して上清にポリペプチドを抽出する。この希釈、沈澱の操作を省略して上記最初の粗抽出液を、直接、以下のクロマトグラフィーに供しても十分な精製度でエピトープポリペプチドが得られる。

【００２０】今日タンパク質の分離精製はほとんどクロマトグラフィーによる。イオン交換クロマトグラフィーは概して分離能が高く、タンパク質精製の早い段階に用いられることが多い。イオン交換クロマトグラフィーでは、一般に等電点がpH 7以下のタンパク質は陰イオン交換体で、pH 7以上では陽イオン交換体で分離する。

【００２１】本発明のエピトープポリペプチドは、等電点がpH 11と強塩基性であることから、クロマトグラフィーによる精製の第１段階として、まず、陽イオン交換クロマトグラフィーが考えられる。しかし、カオトロピック剤（塩酸グアニジン/尿素）非存在下でのエピトープポリペプチドは、Hi-trap Q（陰イオン交換樹脂）およびHi-trap SP（陽イオン交換樹脂）の双方に部分的に吸着した。そこで、カオトロピック剤が存在しない状態でのイオン交換クロマトグラフィーは、エピトープポリペプチドの精製の最初の工程に用いることができないと判断した。

【００２２】金属イオンとアミノ酸の親和性に基づく金属キレートクロマトグラフィーは塩酸グアニジン/尿素のようなタンパク質変性剤を多量に含む溶媒でも適用できる。タンパク質の銅キレート樹脂への結合は、His、Cys、Trp残基の関与が知られている（Trends in Biotechnology, 3: 1-7, 1985 ）。多重エピトープポリペプチドは、医薬品として品質管理上問題となる二量体、あるいは多量体の形成を避けるため、Cys残基を含まないエピトープペプチドを選択している。Trp残基と銅イオンの相互作用はHisに比較して弱く、１～２個のTrp残基を含むタンパク質は銅キレート樹脂に結合することはできない。これに対し、His残基の銅キレート樹脂との相互作用は強く、１個のHis残基を含むタンパク質は銅キレート樹脂に結合することができる。エピトープポリペプチドは2～3個のHis残基を有する。一方、ほとんどの大腸菌タンパク質は分子内に平均して4個以上のHis残基を含んでいるため銅キレート樹脂にエピトープポリペプチドより強く吸着する。このようなことから、精製の第１段階に、銅キレートクロマトグラフィーを用いれば、大腸菌由来のタンパク質はエピトープポリペプチドよりも強く銅キレートカラムに吸着し、その大部分が除かれることが期待される。

【００２３】上記粗抽出液を、例えば、8M 尿素/ 0.2M 塩化ナトリウム/ 50mM 酢酸ナトリウム緩衝液（pH 7.0）緩衝液と１：１（容量比）と混合し、pH7.0に調整後、銅キレートクロマトグラフィー、例えば銅キレートストリームライン（アマシャム・ファルマシア・バイオテク社）に供する。銅をキレートする樹脂としては、例えば、イミノジ酢酸アガロースやニトリロトリ酢酸アガロースが挙げられる。イミノジ酢酸アガロースは文献記載の方法（J. Porath. et al.: Nature, 258: 598, 1975）により調製できる。銅を結合したニトリロトリ酢酸アガロースは、Ni-NTAアガロース（Qiagen）から容易に調製できる。ニトリロトリ酢酸アガロースは、イミノジ酢酸アガロースに比較して銅イオンの漏れが少なくエピトープポリペプチドの精製に適している。POROS MC（アプライドバイオシステム社）、キレーティングセファロースFF（アマシャム・ファルマシア・バイオテク社）、

キレートセルロファイン（生化学工業）などを試みてもよい。

【００２４】粗抽出液添加後、銅キレートストリームラインを、例えば、8M 尿素/ 0.2 M塩化ナトリウム / 50mM 酢酸ナトリウム緩衝液（pH 7.0）3カラム容量で洗浄し、非吸着物を除去する。溶離緩衝液のpHを下げてHisのプロトン化により溶出する場合、ポリペプチドは多数のHis残基をもつ多くの大腸菌タンパク質よりも高いpHで溶出する。8M 尿素/ 0.2 M 塩化ナトリウム / 50mM 酢酸ナトリウム緩衝液（pH 5.0）で溶出することにより、ポリペプチドは純度約70%程度まで精製される。

【００２５】銅キレートクロマトグラフィーからの溶出画分はイオン強度が低いので、酢酸でpH4に調整後、平衡緩衝液、例えば、8M 尿素/ 0.1 M 塩化ナトリウム / 50mMトリス酢酸緩衝液（pH 4.0）で平衡化した陽イオン交換クロマトグラフィー、例えば、SP-セファロース FFカラム（アマシャム・ファルマシア・バイオテク社）に溶出画分を添加してエピトープポリペプチドを樹脂に吸着させる。陽イオン交換カラムとしては、この他Mono S（アマシャム・ファルマシア・バイオテク社）、CMセファロースFF（アマシャム・ファルマシア・バイオテク社）などが挙げられる。

【００２６】銅キレートクロマトグラフィーからの溶出画分をSP-セファロース FFカラムに添加後、pH 10 の緩衝液、例えば8M 尿素/ 0.1 M 塩化ナトリウム / 50mM 炭酸ナトリウム緩衝液（pH 10.0）で洗浄し、続いてpH 4 の緩衝液、例えば8M 尿素/0.2 M 塩化ナトリウム / 50mM トリス酢酸緩衝液（pH 4.0）でカラムを洗浄する。大腸菌由来のタンパク質のほとんどは等電点が 10 以下であるため、この陽イオン交換クロマトグラフィーによりエピトープポリペプチド（とその類縁ポリペプチド）の純度は、ほぼ 100 % となる。ここで類縁ポリペプチドとは、物理的、化学的性質が極めてエピトープポリペプチドに類似しているポリペプチドを意味し、通常、エピトープポリペプチドのアミノ酸が部分的に修飾あるいは置換されたポリペプチドを意味する。例えば、Met残基が酸化されたポリペプチド、Met残基がノルロイシンに置換されたポリペプチド、アセチル化されたポリペプチド、あるいは脱アミド化されたポリペプチドである。次に溶離緩衝液、例えば、8M 尿素/ 0.4 M塩化ナトリウム / 50mM トリス酢酸緩衝液（pH 4.0）で溶出する。溶出液のA_{280} をモニターし、吸収のある画分を得る。

【００２７】この溶出画分には僅かに強塩基性で分子量が小さいリボソームタンパクが数種類混入しているが、つぎの逆相クロマトグラフィーで容易に除去される。逆相クロマトグラフィーでは、またエピトープペプチドの類縁ポリペプチド、リポポリサッカライドのほとんどが除去される。

【００２８】逆相カラムとしては、一般的な液体クロマトグラフィー用オクタデシル化シリカゲルを充填したカラム、例えば、カプセルパックC18（資生堂）が使用できる。また、ポリマー担体のポアサイズが大きい樹脂、例えばPOROS 50R2（アプライドバイオシステムズ社）、SOURCE 15 RPC（アマシャム・ファルマシア・バイオテク社）を充填したカラムなどが挙げられる。POROS 50R2カラム（ 25 × 200 mm)を使用した場合、カラムを1 %酢酸で平衡化した後、陽イオン交換クロマトグラフィーの溶出画分を添加する。12%　アセトニトリル／１%酢酸で洗浄後、溶離液、例えば22%アセトニトリル／１%酢酸を用いて溶出する。溶出液を凍結乾燥して、ポリペプチドの純度として96～99%（重量）以上の純度を有する精製ポリペプチドを得ることができる。このポリペプチドの凍結乾燥品は、クロマトグラフィーにより、酢酸が8～13重量%含まれる酢酸塩組成物として存在する。該酢酸塩組成物中に含有されている酢酸は、多重Ｔ細胞エピトープポリペプチドと塩を形成していても、形成していなくてもよい。

【００２９】本発明の多重Ｔ細胞エピトープポリペプチド酢酸塩組成物の酢酸含量としては、約５～１５（重量）%が好ましく、なかでも約７～１３（重量）%、特に約９～１０（重量）%が好ましい。

【００３０】また、本発明の多重Ｔ細胞エピトープポリペプチド酢酸塩組成物中の酢酸含量は、公知の方法に従って調節することができる。例えば、上記で得られたエピトープポリペプチドの酢酸塩組成物の凍結乾燥品を、例えば酢酸蒸気に接触させることによって、該酢酸塩組成物中の酢酸含量を増大させることができる。また、上記で得られたエピトープポリペプチドの酢酸塩組成物の凍結乾燥品を加湿条件下に暴露することにより、あるいはエピトープポリペプチド酢酸塩組成物の凍結乾燥品を適当な溶媒（例えば、水）に溶解させた後、溶液を凍結乾燥に付すことによって、該酢酸塩組成物中の酢酸含量を減少させることができる。本発明の多重T細胞エピトープポリペプチドと酢酸を含有してなる組成物中の酢酸の含有量としては、本発明の多重T細胞エピトープポリペプチド１に対し、酢酸を約4～20（重量）%、なかでも約5～18（重量）%、とりわけ約7～15（重量）%、特に約9～12（重量）%が好ましい。本発明の多重T細胞エピトープポリペプチド酢酸塩組成物には安定化剤として糖類を加えてもよい。

【００３１】また、本発明の多重T細胞エピトープポリペプチド酢酸塩組成物中に存在する多重T細胞エピトープポリペプチド酢酸塩は、自体公知の反応を用いることにより、塩交換を行うことができる。該塩としては、生理学的に許容される塩が挙げられる。この様な塩としては、例えば、無機酸（例えば、塩酸、リン酸、臭化水素酸、硫酸）との塩、あるいは有機酸（例えば、ギ酸、プロピオン酸、フマル酸、マレイン酸、コハク酸、酒石酸、クエン酸、リンゴ酸、蓚酸、安息香酸、メタンスルホン酸、ベンゼンスルホン酸）との塩などが用いられ

る。なかでも塩酸との塩が好ましい。

【００３２】本発明のエピトープポリペプチドの酢酸塩組成物の製剤としては、凍結乾燥製剤であるものが好ましい。該凍結乾燥製剤は、糖類を添加することにより、安定性に優れた製剤とすることができる。

【００３３】該「糖類」としては、単糖類（例えば、グルコース、エリトロース、キシルロース、リブロース、セドヘプツロース、リボース、マンノースおよびそれらの糖アルコール（ソルビトール、リビトール、マンニトールなど）など、なかでもマンニトールが好ましい。）または二糖類（例えば、麦芽糖、セロビオース、ゲンチオビオース、メリビオース、乳糖、ツラノース、ソロホース、トレハロース、イソトレハロース、ショ糖（精製白糖）、イソサッカロースなど、なかでも精製白糖、乳糖、麦芽糖が好ましく、精製白糖が最も好ましい）が挙げられる。該「糖類」は単独で用いてもよいが、２種以上の混合物としても用いてもよい。なかでも、精製白糖を用いるのが好ましい。

【００３４】以下に該凍結乾燥製剤について具体的に示す。本発明のエピトープポリペプチドの酢酸塩組成物と糖類の双方を水または適当な水性溶媒（たとえば、水とアルコールの混合物）に溶かした水性液に、所望によりｐＨ調整を行い、さらに、たとえば０．２２μｍのフィルターでろ過することにより無菌製剤とする。その後、凍結乾燥を行うことによって固体状とした製剤が好ましい。また凍結乾燥製剤中の酸化体などの不純物生成を抑制するため、容器内へ窒素ガスなどを封入してもよい。

【００３５】水性液を調製する場合、自体公知の方法に従って、本発明のエピトープポリペプチドの酢酸塩組成物及び糖類の双方を水または水性溶媒（たとえば、水とアルコールの混合物）に溶解すればよい。溶解させる順序はどちらが先でもよい。浸透圧を調節するために、上記の本発明のエピトープポリペプチドの酢酸塩組成物及び糖類の水性液に等張化剤を配合してもよい。該等張化剤としては、例えばグルコースなどの単糖類、マンニトールなどの糖アルコール類、食塩などの塩類など等張化剤として公知のものが挙げられる。pH調整を行うために、塩酸などの無機酸、酢酸などの有機酸などが用いられる。本発明のエピトープポリペプチドの酢酸塩組成物の凍結乾燥製剤は、通常、本発明のエピトープポリペプチドの酢酸塩組成物及び糖類の双方を水または水性溶媒に溶解して水性液とし、所望によりpH調整を行ったのち、これを自体公知の方法により凍結乾燥することにより得ることができる。このとき水性液中における本発明のエピトープポリペプチド（の酢酸塩）の濃度は、通常0.01mg/mL～10mg/mLであり、糖類の濃度は、通常0.05mg/mL～100mg/mLである。

【００３６】このようにして得られる本発明の凍結乾燥製剤は、長期間において本発明のエピトープポリペプチド（の酢酸塩）の変質を抑制し、安定に保つことができる。本発明のエピトープポリペプチドの酢酸塩組成物の凍結乾燥製剤は、通常、単独あるいは薬理学的に許容され得る担体もしくは賦形剤と混合してなる医薬組成物とし、経口または非経口的に用いることができる。

【００３７】本発明のエピトープポリペプチドの酢酸塩組成物の凍結乾燥製剤は、これを打錠して錠剤に、カプセルに充填してカプセル剤に、またマイクロカプセルに封入し徐放剤とすることができ、また、用時注射用水あるいは輸液（例、生理食塩水、ブドウ糖など）で溶解して、静脈注射剤、皮下注射剤、筋肉注射剤、点滴注射剤、無針注射剤などの注射剤または点鼻剤、点眼剤として用いることもできる。この場合、溶解液中における本発明のエピトープポリペプチド（の酢酸塩）の濃度は例えば約0.01mg／mL～10mg／mLである。糖類の濃度は約0.05mg／mL～100mg／mLである。

【００３８】注射剤用の用時溶解製剤とする場合、自体公知の、例えば濾過滅菌などの無菌調製法により上記水性液を調製するのが好ましい。また凍結乾燥製剤を調製する前に、糖類、あるいは糖類とその他の添加物との混合物を予め脱パイロジエン処理して用いることもできる。

【００３９】本発明のエピトープポリペプチドの酢酸塩組成物の凍結乾燥製剤は、注射用の用時溶解製剤であるものが好ましい。

【００４０】本発明のエピトープポリペプチドの酢酸塩組成物は、毒性が低く、例えば、凍結乾燥注射剤、溶液注射剤などの注射剤として、皮内、皮下、静脈内、筋肉内、腹腔内などに成人１回当たり約1ng～100mgの範囲で選ばれる量を、毎週1～2回程度約1～12ヶ月間投与することによって、減感作の目的を達成することができる。

【００４１】さらに、本発明のエピトープポリペプチドの酢酸塩組成物は、例えば、トローチ、舌下錠、パップ剤、クリーム剤、ローション剤などの経皮、経粘皮薬としても製造され、その投与量、投与頻度などを適宜選択することにより、その減感作の目的を有利に達成することができる。また、本発明のエピトープポリペプチドの酢酸塩組成物は、スギ花粉症の予防剤、治療剤のみならず、ヒノキ花粉症の予防剤、治療剤としても有利に使用できる。

【００４２】本発明のエピトープポリペプチドの酢酸塩組成物は、単剤として優れたスギ花粉の予防剤、治療剤およびヒノキ花粉症の予防剤、治療剤として有効な作用を示すが、さらに他の医薬成分（以下、併用薬物と略記する）と併用（多剤併用）することもできる。

【００４３】このような併用薬物としては、例えば、ケミカルメディエーター遊離抑制剤（例えば、クロモグリク酸ナトリウム（インタール）、トラニラスト（リザベン）、アンレキサノクス（ソルファ）、ペミロラストカリウム（アレギサール）等）、ケミカルメディエーター受容体拮抗薬（例えば、(1)d-マレイン酸クロルフェニ

ラミン（ポララミン）、フマル酸クレマスチン（タベジール）、フマル酸ケトチフェン（ザジデン）、塩酸アゼラスチン（アゼプチン）、オキサトミド（セルテクト）、メキタジン（ゼスラン、ニポラジン）、フマル酸エメダスチン（ダレン、レミカット）、塩酸セチリジン（ジルテック）、塩酸レボカバスチン（リボスチン）、フェキソフェナジン（アレグラ）、塩酸オロパタジン（アレロック）等の抗ヒスタミン薬、(2)ラマトバン（バイナス）等のトロンボキサンA2拮抗薬、(3)プランルカスト水和物（オノン）等のロイコトリエン拮抗薬等）、Th2サイトカイン抑制薬（例えば、トシル酸スプラタスト（アイピーディー）等）、ステロイド薬（例えば、(1)プロピオン酸ベクロメタゾン（ベコナーゼ、アルデシン、リノコート）、フルニソリド（シナクリン）、プロピオン酸フルチカゾン（フルナーゼ）等の局所ステロイド薬、(2)セレスタミン（マレイン酸クロルフェニラミン配合剤）等の経口ステロイド薬等）、自律神経作用薬（例えば、(1)硝酸ナファゾリン（プリビナ）、硝酸テトラヒドロゾリン（ナーベル）、塩酸オキシメタゾリン（ナシビン）、塩酸トラマゾリン（トーク）等のα刺激薬、(2)臭化イプラトロピウム（アトロベント）、臭化フルトロピウム（フルブロン）等の抗コリン薬等）、生物製剤（例えば、ノイロトロピン、アストレメジン、MSアンチゲン等）等が挙げられる。

【００４４】本発明のエピトープポリペプチドの酢酸塩組成物と併用薬物との併用に際しては、本発明のエピトープポリペプチドの酢酸塩組成物と併用薬物の投与時期は限定されず、本発明のエピトープポリペプチドの酢酸塩組成物と併用薬物とを、投与対象に対し、同時に投与してもよいし、時間差をおいて投与してもよい。併用薬物の投与量は、臨床上用いられている投与量に準ずればよく、投与対象、投与ルート、疾患、組み合わせ等により適宜選択することができる。

【００４５】本発明のエピトープポリペプチドの酢酸塩組成物と併用薬物の投与形態は、特に限定されず、投与時に、本発明のエピトープポリペプチドの酢酸塩組成物と併用薬物とが組み合わされていればよい。このような投与形態としては、例えば、（１）本発明のエピトープポリペプチドの酢酸塩組成物と併用薬物とを同時に製剤化して得られる単一の製剤の投与、（２）本発明のエピトープポリペプチドの酢酸塩組成物と併用薬物とを別々に製剤化して得られる２種の製剤の同一投与経路での同時投与、（３）本発明のエピトープポリペプチドの酢酸塩組成物と併用薬物とを別々に製剤化して得られる２種の製剤の同一投与経路での時間差をおいての投与、（４）本発明のエピトープポリペプチドの酢酸塩組成物と併用薬物とを別々に製剤化して得られる２種の製剤の異なる投与経路での同時投与、（５）本発明のエピトープポリペプチドの酢酸塩組成物と併用薬物とを別々に製剤化して得られる２種の製剤の異なる投与経路での時間差をおいての投与（例えば、本発明のエピトープポリペプチドの酢酸塩組成物→併用薬物の順序での投与、あるいは逆の順序での投与）などが挙げられる。以下、これらの投与形態をまとめて、本発明の併用剤と略記する。

【００４６】本発明の併用剤は、毒性が低く、例えば、本発明のエピトープポリペプチドの酢酸塩組成物または（および）上記併用薬物を自体公知の方法に従って、薬理学的に許容される担体と混合して医薬組成物、例えば、凍結乾燥注射剤、溶液注射剤、トローチ、舌下錠、点眼剤、鼻腔内噴霧剤、パップ剤、クリーム剤、ローション剤、錠剤（糖衣錠、フィルムコーティング錠を含む）、散剤、顆粒剤、カプセル剤（ソフトカプセルを含む）、液剤、坐剤、徐放剤等として、経口的又は非経口的（例、局所、直腸、静脈投与等）に安全に投与することができる。

【００４７】

【実施例】以下に、本発明を参考例、実施例、試験例、製剤例および実験例により説明するが、本発明の技術的範囲は、これらに限定されるものではない。

［参考例１］　ポリペプチドをコードするDNAの構築

配列番号：１のアミノ酸配列を有するポリペプチドは６つのT細胞エピトープペプチドがArgダイマーを介して連結された105アミノ酸残基からなる。そこで、Cry j 1およびCry j 2のエピトープに対応する各DNA 断片を PCRで増幅後連結し、さらにPCRで増幅するといった工程を繰り返し、最終的にポリペプチドの全長をコードするV-KV-ID-WK-LK-V2（ポリペプチドcDNA）を構築した（図１）。PCR条件は、Taq DNAポリメラーゼを使用して96℃15秒、55℃30秒、72℃90秒を10～25サイクルであった。

【００４８】(1)　c DNA 断片 K の増幅とクローン化

pCCI2-2 （Sone, T. et al.: Biochem. Biophys. Res. Commun., 199: 619-625, 1994）から 15 アミノ酸残基のエピトープをコードする cDNA 断片 K を KSMK43S（配列番号：３） と KSMK43A（配列番号： ４） をプライマーとする PCRにより増幅し、同時に 5′末端に SmaI 認識部位、3′末端に SalI 認識部位を付与した。この DNA 断片を pUC19 上にクローニングし、塩基配列を確認した（pUC19K#3）。

【００４９】(2)　cDNA 断片 VF の増幅と、連結した 2 つのcDNA 断片 K-VF のクローン化

pCCI2-2 から 15 アミノ酸残基のエピトープをコードする cDNA 断片 P を PCVF22S （配列番号：５）と PCVF22A（配列番号：６） をプライマーとする PCRにより増幅し、同時に 5′末端に SmaI 認識部位、3′末端に Sa1I 認識部位を付与した。この DNA 断片を SmaI で消化してから SalI で消化した cDNA 断片K と結合させた。結合した DNA 断片を KSMK43S（配列番号：３） を PCVF22A（配列番号：６） をプライマーとする PCR により増幅した。PCR 産物をポリアクリルアミドゲル電気泳動にかけ 120 bp の DNA 断片 K-P を分離、精製した。

K-P 断片を SalI と SmaI で消化してからポリアクリルアミドゲル電気泳動を行い、DNA 断片を精製してから pUC19 の SalI-SmaI アーム上にクローン化し、pUC19KP#6-1 を得た。pUC19KP#6-1 から 13 アミノ酸残基のエピトープを暗号化する cDNA 断片 VFを VFIK22S2 （配列番号:７）と PCVF22A（配列番号：６） をプライマーとするPCR により増幅し、同時に 5′ 末端に PstI, 3′ 末端に SalI 認識部位を付与した。PCR 産物をポリアクリルアミドゲル電気泳動にかけ 59 bp の断片を分離、精製した。この DNA 断片を PstI 消化してから SalI 消化した pUC19K#3 と混合し、クレノウ断片で平滑化してから結合させた。KSMK43S（配列番号：３） と PCVF22A（配列番号：６） をプライマーとする PCR で K-VF の DNA 断片（111 bp）を増幅した。PCR 産物をポリアクリルアミドゲル電気泳動にかけ、DNA 断片を分離、精製した。この DNA 断片を pUC19 にクローニングし、pUC19K-VF とした。

【００５０】(3)　cDNA 断片 G の増幅とクローン化

pCC II 1 （Komiyama, N., Sone, T., Shimizu, K., Morikubo, K., and Kino, K.(1994) Biochem. Biophys. Res. Commun. 201, 1021-1028）から 20 アミノ酸残基のエピトープを暗号化する cDNA 断片 G を GIDI37S （配列番号：８）とGIDI37A（配列番号：９） をプライマーとする PCR により増幅し、同時に 5′ 末端に SmaI 認識部位、3′ 末端に SalI 認識部位を付与した。この DNA 断片をSmaI と SalI で消化してから pUC19 にクローニングし、pUC19G とし、pUC19G#1 の挿入塩基配列を読んだ。SmaI 末端の繋ぎ目に 1 塩基対の欠失（ACCGGG となっていた）があるが、その他の部分に変異がないことを確認した。

【００５１】(4)　cDNA 断片 WK の増幅と、連結した 2 つの cDNA 断片 ID-WK のクローン化

pCC II 1 から 20 アミノ酸残基のエピトープを暗号化する cDNA 断片 WK をリン酸化した WKNN17S （配列番号:10）と（リン酸化されていない）WKNN17A（配列番号:11） をプライマーとする PCR により増幅し、同時に 3′ 末端に SalI認識部位を付与した。PCR 産物をポリアクリルアミドゲル電気泳動にかけ、71bp のDNA 断片を分離、精製した。この DNA 断片を SalI 消化した pUC19G#1 と混合し、クレノウ断片で平滑化してから結合させた。IDIF37S （配列番号:12）と WKNN17A（配列番号:11） をプライマーとする PCR で ID-WK の DNA 断片（141 bp）を増幅した。PCR 産物はポリアクリルアミドゲル電気泳動にかけ DNA 断片を分離、精製した。この DNA 断片を pUC19 にクローニングし、pUC19ID-WK とし、その塩基配列（pUC19ID-WK#1 および #8）を確認した。

【００５２】(5)　cDNA 断片 V2 の増幅とクローン化

pCCII1 から 15 アミノ酸残基のエピトープを暗号化する cDNA 断片 V2 を VDGI14S2 （配列番号:13）と VDGI14A2（配列番号：14） をプライマーとする PCRにより増幅し、同時に 5′ 末端にPstI, 3′ 末端に終止コドンと Hind III 認識部位を付与した。この DNA 断片を pUC19 にクローニングし、pUC19Vph とし、pUC19Vph#1 の挿入塩基配列を読んだ。VDGI14A2（配列番号：14） プライマーに相補的な配列 GCTGGAAGTAA となるべきところが GCTTAAGTAA となっていたが、その他の部分には変異がなかった。

【００５３】(6)　cDNA 断片 LK の増幅と cDNA 断片 LK-V2 のクローン化

クローン化された Cry j 1 の cDNA （pCCI-2-2）から 15 アミノ酸残基のエピトープを暗号化する cDNA 断片 LK を LKMP17S（配列番号：15）と LKMP17A（配列番号：16） をプライマーとする PCR により増幅し、同時に 5′ 末端に KpnI, 3′ 末端に SalI 認識部位を付与した。PCR 産物をポリアクリルアミドゲル電気泳動にかけ 65 bp の断片を分離、精製した。この DNA 断片を SalI 消化してから PstI 消化した pUC19Vph#1 とを混合し、クレノウ断片で平滑化してから結合させた。LKMP17S（配列番号:15）と VDGI14A2（配列番号：14） をプライマーとする PCR で LK-V2 の DNA 断片（119 bp）を増幅した。PCR 産物をポリアクリルアミドゲル電気泳動にかけ、DNA 断片を分離、精製した。この DNA 断片をpUC19 にクローニングし、pUC19LK-V2 とし、pUC19LK-V2#8 の塩基配列が正しいことを確認した。

【００５４】(7)　cDNA 断片 K-VF-ID-WK のクローン化

pUC19ID-WK から挿入塩基配列を EcoRV/Hind III 消化で切り出し、pUC19K-VF#2の SalI-Hind III アームと結合させ、3 クローン（pUC19K-VF-ID-WK#1, #2,および #4）について接合部の塩基配列が正しいことを確認した。

【００５５】(8)　連結した 6 つの cDNA 断片 K-VF-ID-WK-LK-V2 のクローン化

pUC19K-VF-ID-WK#1, #4 の SalI-Hind III アームに pUC19LK-V2#8 から KpnI/Hind III 消化で切りだした挿入塩基配列を結合させ、3 クローンについて接合部の塩基配列が正しいことを確認した。このようにして得られたプラスミド pUC19F7#2, #3, #4 はポリペプチド の cDNA をクローン化している（図２）。

【００５６】このようにして構築した配列番号：１で表されるアミノ酸配列を有するポリペプチド の cDNA の組換え体の発現は、種々の大腸菌の宿主ベクター系で可能である。特に大腸菌での発現系は種々の医薬品製造に使用された実績が豊富にあるので、ポリペプチドの生産も大腸菌で行うのが適当である。

【００５７】［参考例２］　pQTF7Δcr の構築

本発明者らは、以下の参考例に示すように、trpプロモーターを使用した安枝らの大腸菌発現系（Bio/Technology, 8: 1036-1040, 1990 ）を改変し、エピトープポリ

ペプチドを大腸菌の菌体内に著量合成せしめ、不溶性画分（封入体）として蓄積させることに成功した。この発現系は発現誘導剤や抗生物質の使用量を少なくすることが可能である。

(1)　trp オペロンプロモーター

大腸菌のプロモーター trp と SD 配列を上記文献を参考に、オリゴヌクレオチドTRPS（配列番号：17）、 TRPA（配列番号：18）、 SDSDS（配列番号：19）、 および SDSDA（配列番号：20）を合成した。TRPA（配列番号：18）とSDSDS（配列番号：19） は 5′-末端を T4 ファージのポリヌクレオチドキナーゼでリン酸化した。TRPS（配列番号:17） と TRPA（配列番号:18） の 3′-末端の 11 塩基は相補的である。加熱、徐冷して対合させ、クレノウ断片による修復合成を行うことにより前半の 50 塩基対の二本鎖 DNA を得た。SDSDS（配列番号:19）と SDSDA（配列番号:20） の 3′-末端の 10 塩基も相補的である。加熱、徐冷して対合させ、クレノウ断片による修復合成により後半の 47 塩基対の二本鎖 DNA を得た。これらの DNA 断片を T4 ファージの DNA リガーゼで結合反応させ連結したDNA断片TRP-SDSDをリン酸化した SDSDA、（配列番号:20）と（リン酸化しない）TRPS （配列番号：17）をプライマーとして12 サイクルの PCR で増幅した。pUC19F8#10（pUC19F7の5番目のエピトープをコードするDNAが別のエピトープをコードするDNAで置換されたプラスミド）を鋳型に、リン酸化した KVTV43S （配列番号：21）と（リン酸化しない）VDGI14A2（配列番号：14）をプライマーとした 15 サイクルの PCR でcDNA 断片 F8 を増幅し、ポリアクリルアミドゲル電気泳動で分離／精製した。DNA 断片 TRP-SDSD と F8 を混合し、クレノウ断片と T4 ファージの DNA リガーゼを作用させて結合させた。結合した断片 TRP-SDSD-F8 を TRPS（配列番号：17） と VDGI14A2 （配列番号：14）をプライマーとした 12 サイクルの PCRで増幅し、ポリアクリルアミドゲル電気泳動で分離／精製した。DNA 断片 TRP-SDSD-F8 を Hind III で消化し、アガロースゲル電気泳動で約500 bp の断片を分離／精製した。Hind III で消化した DNA 断片 TRP-SDSD-F8 を EcoRIで 消化してから pUC119 のEcoRI-Hind III アームに結合させ、大腸菌 TB1 株の形質転換を行った。X-gal プレートで白色のコロニーを形成した 13 クローンについてプラスミドの微量調製を行い、EcoRI, Hind III の二重消化で約 500 bp の断片が切り出される 2 クローン（pUC119TF8#6 および #7）を選択した。これらのプラスミドの挿入塩基配列をダイデオキシ法で読んだところ、pUC119TF8#6 では Hinc II/HpaIの認識部位の前後が 32 bp, pUC119TF8#7 では DraI の認識部位とその後が 20bp 欠失していた。pUC119TF8#6 の挿入塩基配列のcDNA 部分は 5´側の 70 bpを読んだが、その範囲にはアミノ酸配列を変えるような変異はなかった。KVTV43S（配列番号：２１） の 5´側の 18 bp は縮退コドンの均等な混合物として合成したため 4 つのコドンの 3 文字目がいずれも T に変わっていた（図３および配列番号：23）。　pUC119TF8#6 と #7 の欠失位置は、ずれており、間に ClaI の認識部位が存在する。そこで、これらのクローンを組み換えて目的とする組み換え体を作製することにした。pUC119TF8#6 を ClaI と Hind III で消化し、約 400 bp の DNA断片をアガロースゲル電気泳動で分離した。pUC119TF8#7 を ClaI, Hind III,ウシ小腸のアルカリフォスファターゼで消化してからアガロースゲル電気泳動にかけ、ベクター側断片（約 3 kbp）を分離した。これらの DNA 断片を T4 ファージの DNA リガーゼで結合させてから大腸菌 GI698 に導入し、10μg/mL のトリプトファンと 100μg/mL のアンピシリンを加えたプレートで組換え体を選択した。6 クローン（pUC119TF8#6.51-#6.56）についてプラスミド DNA の微量調製を行い、Hae III, EcoRI の二重消化の制限パターンで、意図した DNA の組み換えが起きていることを確認した。

【００５８】(2)　中間体プラスミドpQTF7 の構築

pUC119TF8#6.54 から trp のプロモーターとポリペプチドの N-末端の cDNAを含む約 120 bp の DNA 断片を EcoRI, Eco47I 消化で切りだした。また pUC19F7 から ポリペプチドの C-末端側の cDNA を含む約 290 bp の DNA 断片を Hind III, Eco47I 消化で切りだした。pQE11 の EcoRI-Hind III アーム上で、これらの断片を結合させて大腸菌（GI698 株）に導入した。アンピシリン耐性の 24 クローン（pQETF7#1-24）からプラスミド DNA を微量調製し、挿入配列の有無を SDSDS（配列番号：19）、 VDGI14A2（配列番号：14） をプライマーとした PCR と Hind III/EcoRI 消化で調べ、pQETF7#12 に期待する長さの挿入塩基配列があることを確認した。pQETF7#4, pQETF7#7, pQETF7#12 の EcoRI, XhoI 消化を行ってからアガロースゲル電気泳動でベクター側 DNA断片を分離／精製した。pQETF7#12 の EcoRI-XhoI 断片にクレノウ断片と T4 ファージの DNA リガーゼを作用させて閉環し、大腸菌 GI698 に導入し、10μg/mL のトリプトファンと100μg/mL のアンピシリンを加えたLB寒天培地プレートで組み換え体を選択した。組み換え体から調製したプラスミド DNA pQTF7 の制限酵素消化（DraI, HindIII の二重消化）とポリアクリルアミドゲル電気泳動で予定通りの欠失が起きていることを確認した。pQTF7 では ポリペプチドのcDNA の下流にλファージの転写終結信号配列 t_0が連結している。その更に下流にはクロラムフェニコールアセチル基転移酵素（cat）とリボソーム RNA 転写終結信号配列 T_1 が連結している。このcatとT_1の部分は不要であるので、それらを除去した発現プラスミド pQTF7Δcr を作製した。

【００５９】(3)　pQTF7Δcr の構築

pQTF7 を鋳型に WKNN17S（配列番号：10）と TOXBA（配列番号：22） をプライマーにポリペプチドの cDNA の

後半とλファージの転写終結信号 to を含む DNA 断片 WK-T0 を 20サイクルの PCR で増幅し、アガロースゲル電気泳動で約 300 bp の断片を分離した。DNA 断片 WK-T0 を XbaI と Hind III で消化してから QIAEX II で精製し pUC19 の XbaI-Hind III アームと T4 DNA リガーゼで結合させた。結合反応により生成したプラスミド DNA を大腸菌 MC1061 株に導入した。アンピシリン耐性の 4 クローン（pUC19to#1-4）を培養し、プラスミド DNAの微量調製を行った。制限酵素消化（EcoRI, Hind III の二重消化）後のポリアクリルアミドゲル電気泳動で約 150 bp のバンドを確認した。 pUC19to#1 の塩基配列をダイデオキシ法で確認した。pUC19to#1 の約 100 bp の XbaI-Hind III 断片と pQTF7.12#1 の約 2.5 kb の XbaI-Hind III 断片を T4 DNA リガーゼで結合させてから大腸菌 K802 株に導入した。得られたプラスミド pQTF7Δcr（図４）の構造を制限酵素消化（XbaI, Hind III の二重消化）で確認した。アガロースゲル電気泳動で 121 bp の DNA のバンドが観察された。

【００６０】［実施例１］　エピトープポリペプチドの精製

エピトープポリペプチド（配列番号：１）を封入体として保持する大腸菌から封入体画分を分離し、変性剤で抽出後、以下のように、1)　銅キレートカラムクロマトグラフィー、2)　陽イオン交換カラムクロマトグラフィー、そして3)　逆相カラムクロマトグラフィーの順にクロマト操作して精製した。発現プラスミドpQTF7△cr（図４）で形質転換した大腸菌株K 802をファーメンター培養した。培養後の菌体45 g（湿菌体重量）を400 mLの50mMトリス酢酸緩衝液（pH 5.0）で懸濁しホモゲナイザーで破砕した。この破砕液を遠心（10,000× g、20分間）して不溶性画分を得た。不溶性画分を400 mLの２％トリトンX-100を含む50mM トリス酢酸緩衝液（pH 5.0）で懸濁し、遠心（10,000 ×g、30分間）して封入体画分78 gを得た。この封入体画分に400 mLの1 M 塩酸グアニジン、5 M 尿素、0.02%酢酸を加え、室温で１時間撹拌溶解した後、遠心（10,000 × g、20分間）して上清を得た。この上清を8M 尿素/ 0.2M 塩化ナトリウム/ 50mM 酢酸ナトリウム緩衝液（pH7.0）と１：１で混合しpH ７.0に調整した後、銅キレートストリームラインカラム（50 ×150 mm）に添加した。同上のバッファー３カラム容量でカラムを洗浄し非吸着物質を除いた後、8M 尿素/ 0.2M 塩化ナトリウム / 50mM 酢酸ナトリウム緩衝液（pH ５.0）で溶出し、溶出液980 mLを得た。この溶出液を酢酸でpH 4.0に調整し、8M 尿素/ 0.1M 塩化ナトリウム / 50mMトリス酢酸緩衝液（pH ４.0）で平衡化したSP-セファロースFFカラム(50 × 100 mm)に添加した。8M 尿素/ 0.1M 塩化ナトリウム / 50mM 炭酸ナトリウム緩衝液（pH 10.0)と8M 尿素/ 0.2 M 塩化ナトリウム / 50mM トリス酢酸緩衝液（pH4.0）でカラムを洗浄後、8M 尿素/ 0.4 M 塩化ナトリウム / 50mM トリス酢酸緩衝液（pH 4.0）で溶出した。溶出液のA_{280} を測定し、吸収のある画分720 mL得た。溶出画分をさらに１％酢酸で平衡化したPOROS 50R2カラム (25 × 200 mm)に負荷した。12%　アセトニトリル／１％酢酸で洗浄後、22%アセトニトリル／１％酢酸で溶出した。溶出液を凍結乾燥してポリペプチド208 mg（乾燥重量）を得た。この精製ポリペプチドの純度はポリペプチドとして99%だった。純度は逆相HPLC［検出器：紫外吸光光度計（測定波長：215nm）、カラム：CAPCELLPAK C18,SG 300Å 5 μm, 4.6 mm i.d.× 15 cm（資生堂）］で検定した。このポリペプチドには、12.5重量%の酢酸が含まれていた。ロット間の酢酸含有量のバラツキは、およそ8～13重量%の範囲内と考えらる。

【００６１】

【表１】

ロット番号	酢酸含量（%）
1	8．0
2	12．0
3	9．5
4	13．0
5	8．1
6	12．5
7	9．7
8	12．6
9	8．6
10	12．5
11	8．2
12	11．6
13	8．9
14	11．9
15	11．7
16	11．3
17	10．1
18	13．1
19	12．5

【００６２】［試験例１］　酢酸含有量の異なるエピトープポリペプチドの安定性比較

１．酢酸含量の異なるエピトープポリペプチドの調製

実施例１で得られた精製エピトープポリペプチドに以下の処理を施して、酢酸含量の異なる６種類（試料1～6）のエピトープポリペプチドを調製した。調製した各試料は、それぞれ最終の水分含量が異なると考えられるため、25℃50%（相対湿度）RHの湿度条件で6時間試料を調湿した後、保存した。

・　試料１：無処置のエピトープポリペプチド

・　試料２：エピトープポリペプチド約250mgに水25mLを加えて溶かし、凍結乾燥した

・　試料３：エピトープポリペプチド約250mgに水25mLを加えて溶かし、凍結乾燥した。さらに得られた凍結乾燥品に水25mLを加えて溶かし、凍結乾燥した。この操作をさらにもう一度行い、凍結乾燥操作を合計で3回行った。

・　試料４：エピトープポリペプチド約250mgを25℃13%RHのデシケーター中に5日間保存した。
・　試料５：エピトープポリペプチド約250mgを25℃75%RHのデシケーター中に1日保存した後、25℃13%RHのデシケーターに4日間保存した。
・　試料６：エピトープポリペプチド約250mgを酢酸蒸気で飽和したデシケーター中に6時間保存した。

【００６３】２．安定性保存
各試料（試料1～6）約70mgの透明の気密性ガラス容器（ネジ蓋付き）に入れ、パラフィルムで封をした後、40℃/1週間保存した。

【００６４】３．測定条件
3.1. 酢酸
上記試料（試料1～6）約10mgを精密に量り、水5mLを正確に加えて溶解し、試料溶液とした。また、酢酸約400mgを精密に量り、水を加えて混和し、正確に20mLとした。この液2mLを正確にとり、水を加えて正確に100mLとし、標準溶液とした。試料溶液及び標準溶液50μLにつき、次の条件で液体クロマトグラム法により試験を行い、それぞれの溶液から得られる酢酸のピーク面積を求め、次式より酢酸含量を算出した。

［計算式］
酢酸含量（%）＝（At /As）×（Ws/ Wt）× 0.5
At　：試料溶液の酢酸のピーク面積値
As　：標準溶液の酢酸のピーク面積値
Wt　：試料の秤量値（mg）
Ws　：酢酸の秤量値（mg）

［試験条件］
検出器：　紫外吸光光度計（測定波長：210nm）
カラム：　Inertsil ODS-3V 5 μm 4.6 mm i.d.×25 cm（GL Sciences Inc.）
カラム温度：40℃付近の一定温度
移動相：　A液）0.085%リン酸液
B液）アセトニトリル/0.085%リン酸液混液（9：1）
グラジエントプログラム（リニア）を表２に示す。

【００６５】
【表２】

時間（分）	A液（%）	B液（%）
0（注入）	100	0
10	100	0
12	0	100
18	0	100
20	100	0
30（注入）	100	0

＃10分以降はカラム洗浄と平衡化

【００６６】流　量：　酢酸の保持時間が約6分になるように調整する（通常約1.0mL/min）

【００６７】3.2. 類縁タンパク質

試料約10mgを精密に量り、水5mLを正確に加えて溶解し、試料溶液とした。試料溶液40μLにつき、次の条件で液体クロマトグラフ法により試験を行い、面積百分率により類縁物質含量を算出した。

［試験条件］
検出器：紫外吸光光度計（測定波長：215nm）
カラム：CAPCELLPAK C18, SG 300Å 5 μm, 4.6 mm i.d.× 15 cm（資生堂）
カラム温度：40℃付近の一定温度
移動相：A液）水/1 mol/Lリン酸・100 mmol/L過塩素酸ナトリウム混液(9：1)
B液）アセトニトリル/1 mol/Lリン酸・100mmol/L過塩素酸ナトリウム混液（9：1）
グラジェントプログラム（リニア）を表３に示す。

【００６８】
【表３】

時間（分）	A液（%）	B液（%）
0（注入）	65	35
3	65	35
30	58	42
40	50	50
45	0	100
50	65	35
60（注入）	65	35

＃　45分以降はカラム洗浄と平衡化

【００６９】流　量：　ポリペプチドの保持時間が約19分付近になるように調整する（通常約1.0 mL/min）

【００７０】3.3. 重合体試料約10 mgを量り、水5 mLを正確に加え溶解し、この液を水で２倍に希釈し、試料溶液とした。試料溶液20μLにつき、次の条件で液体クロマトグラフ法により試験を行い、面積百分率により総重合体含量を求めた。

［試験条件］
検出器：紫外吸光光度計（測定波長：215 nm）
カラム：TSK-GEL　G4000SWＸＬ, 7.8 mm i.d. ×30 cm（東ソー社製）
カラム温度：25℃付近の一定温度
移動相：水/アセトニトリル/トリフルオロ酢酸混液（600：400：1）
流量：ポリペプチドの保持時間が約18分付近になるように調整する（通常約0.5 mL/min）

【００７１】3.4. 含量
本品約10 mgを精密に量り（Wt, mg）、水5 mLを正確に加えて溶解し、試料溶液とした。また、ポリペプチド標準物質1バイアルに水2.5 mLを正確に加えて溶解し、標準溶液とした。試料溶液及び標準溶液40 μLにつき、次の条件で液体クロマトグラフ法により試験を行い、次式より本品の含量を算出した。

［計算式］
含量(%) ＝ (At / As) × (Ws / Wt) × 200
At：試料溶液のポリペプチドピーク面積値
As：試料溶液のポリペプチドピーク面積値
Ws：ポリペプチド標準物質のポリペプチド含量値（mg/vial）
Wt：試料の秤量値（mg）
ポリペプチド含量　Ws＝Wp×（1 - 0.01×F ）
Wp：ポリペプチド標準物質の総タンパク質含量（mg）
F ：ポリペプチド標準物質の総類縁タンパク質含量（%）

［試験条件］3.2. 類縁タンパク質の［試験条件］（液体クロマトグラム法）と同じ。
【００７２】［結果および考察］各種操作を行って調製した試料（試料1～6）の酢酸含量及び性状を表４に示す。いずれも性状は「白色の綿状の塊」であったが、凍結乾燥を行って酢酸含量を減少させた試料は、帯電性に富む性質を示した。各種操作により、調製した試料の酢酸含量は6.1%～17.9%であった。
【００７３】
【表４】

表４　各種処理品の品質（酢酸含量と性状）

試料	処理	性状	酢酸（%）
試料１	未処理	白色の綿状の塊	12.5
試料２	凍結乾燥/1 回	白色の綿状の塊	7.4
試料３	凍結乾燥/3 回	白色の綿状の塊	6.1
試料４	25 ℃ 13%RH	白色の綿状の塊	9.7
試料５	25 ℃ 75 → 13%RH	白色の綿状の塊	9.2
試料６	酢酸蒸気	白色の綿状の塊	17.9

【００７４】表４に示した酢酸含量の異なるエピトープポリペプチド（試料1～6）を40℃/1W保存したときの性状には変化が認められなかった。エピトープポリペプチド（試料1～6）中の酢酸含量と、40℃/1W保存後に認められた重合体との関係を（図５）に、類縁ポリペプチドの増加量との関係を（図６）に、そしてエピトープポリペプチドの残存率との関係を（図７）にそれぞれ示した。重合体の増加量は酢酸含量が6.1%及び17.9%のエピトープペプチドで高い傾向が認められた。重合体の許容増加量を約1.5%と見積もると酢酸含量はおよそ7～14%の範囲である。類縁ポリペプチドの増加量は、当該エピトープポリペプチドの前に溶出する成分（低脂溶成分）と後に溶出する成分（高脂溶成分）に分けて評価したところ、酢酸含量が17.9%のエピトープポリペプチドで本体の前後に溶出する成分の増加量が高くなった。類縁ポリペプチドの許容増加量をおよそ3%以下に見積もると、酢酸含量はおよそ11%以下である。含量(残存率)は、エピトープポリペプチド中の酢酸含量が10%付近で高く、酢酸含量の減少及び増加にしたがって低下することが明らかになった。残存率をおよそ98%と見積もると、酢酸含有量は、およそ9～13%の範囲である。以上の結果から、残存率、重合体及び分解生成物の生成量を総合的に考慮すると、エピトープポリペプチドは酢酸含量が9～10%付近で最も安定であり、酢酸含量が7～13％では比較的安定であることが示された。
【００７５】［試験例２］　溶解性試験
凍結乾燥操作で酢酸含量の異なるエピトープポリペプチドを調製し、GLP毒性試験で投薬溶媒に用いられる5%ブドウ糖溶液（日本薬局方）に対する25℃での溶解度を測定した。

１．操作法
1.1　酢酸含量の異なるエピトープポリペプチドの調製
実施例１と同一の方法で得られたエピトープポリペプチド（未処理試料）約0.5gに水50mLを加えて溶解し、凍結乾燥した（条件：25℃、1psi）。この操作を1回あるいは3回行った。
1.2　試料飽和溶液の調製
試料約50mgをガラス製試験管にとり、5%ブドウ糖溶液（日本薬局方：大塚製薬製）1mLを加えて25℃で泡を立てないように緩やかに振とうして溶解した（5分間隔で30秒振とう→静置）。さらに、試料約25mgずつ加え、振とうで試料が溶けなくなるまで操作を繰返した。試料が溶けなくなったら、さらに試料約25mgを加えて振とう操作（5分間隔で30秒振とう→静置を6回繰返す）を行った。上記の振とう操作で泡が立ち攪拌できなくなったら、遠心して泡を破壊してから振とう操作を行った。その液を25℃、2000rpm/5分で遠心分離し、液層を0.45μmのメンブランフィルターでろ過して試料の飽和溶液を得た。
1.3　試料濃度の測定
飽和溶液中の試料濃度をUV法により測定した。飽和溶液を0.1mol/L塩酸で200～400倍に希釈した後、280nmの吸光度（A_{280} ）を測定し、下式により試料濃度を算出した。
試料(mg/mL)＝　MW試料×F×A_{280} ／ε試料
ε試料　：エピトープポリペプチドの280nmにおけるモル吸光係数 (=20444)
MW試料：エピトープポリペプチドの分子量 (=12303)
F　　：希釈率
1.4　酢酸含量の測定

試験例１の「3.1. 酢酸」に記載の測定方法に準じて測定した。

【００７６】２．結果

凍結乾燥により調製した酢酸含量の異なる試料の日本薬局方5%ブドウ糖溶液に対する25℃での溶解度を表５に示す。

【００７７】

【表５】

表５　エピトープポリペプチド 25 ℃における日本薬局方 5 %ブドウ糖溶液に対する溶解度

試　料	酢酸含量(%)	溶解後pH	溶解度(mg/mL)
未処理	13.0	4.54	150
凍結乾燥/1回	8.0	5.80	120
凍結乾燥/3回	6.7	7.01	78 (82)[1)]

1) 日本薬局方注射用水に対する溶解度

【００７８】凍結乾燥により酢酸含量が13.0％（未処理）～6.7%の試料が得られた。試料の溶解度は、酢酸含量の減少に伴って低下し、酢酸含量6.7%の試料の溶解度は78mg/mLであった。今回調製した酢酸含量が13.0%～6.7%のエピトープポリペプチドはいずれも50mg/mL付近の濃度まで比較的容易に溶解したが、それより高濃度では試料が浮遊して溶解に時間を要し、極めて泡立ち易かった。また、エピトープポリペプチドが100mg/mL以上の5%ブドウ糖溶液は粘性が高く、0.45μmのメンブランフィルターによるろ過操作は困難であり、25℃で1日静置するとゲル状になった。

【００７９】[製剤例１]表６に示すように、配列番号：１で表されるアミノ酸配列を有する多重T細胞エピトープポリペプチド（以下、化合物Ａと略記する）に対して、精製白糖を含有する水溶液(化合物濃度：0.12mg/mL、2mg/mL)を調製し、塩酸によりpHを調整したのち、除菌ろ過により得られた水溶液1mLをバイアルに分注、ゴムセンを半施栓後、凍結乾燥を行った。凍結乾燥終了後、バイアル空間部を窒素ガスで置換した後、ゴムセンを施栓、キャップで巻締することにより凍結乾燥品を作製した。

【００８０】

【表６】

処方Ａおよび処方Ｂの組成表

	製剤例１	
	処方Ａ	処方Ｂ
化合物Ａ	0.12mg	2mg
精製白糖	10mg	10mg
塩酸	適量	適量
薬液pH	4.6	4.5

【００８１】[実験例１]４０℃相対湿度７５％で２箇月、４箇月および６箇月、２５℃相対湿度６０％で６箇月保存した。製剤の含量(残存率)、類縁タンパク質、重合体を調べたところ、表７の結果を得た。処方Ｂについては酢酸を測定した。

【００８２】

【表７】

処方Ａおよび処方Ｂの安定性結果

測定項目	時点	処方Ａ	処方Ｂ
含量(残存率)	Initial	100.0%	100.0%
	40℃/75%R.H.×2M	100.7%	98.7%
	40℃/75%R.H.×4M	98.6%	97.8%
	40℃/75%R.H.×6M	100.1%	97.2%
	25℃/60%R.H.×6M	99.0%	100.0%
類縁タンパク質	Initial	2.0%	1.7%
	40℃/75%R.H.×2M	2.1%	2.5%
	40℃/75%R.H.×4M	2.5%	2.1%
	40℃/75%R.H.×6M	2.6%	2.6%
	25℃/60%R.H.×6M	2.1%	1.8%
重合体	Initial	0.2%	0.4%
	40℃/75%R.H.×2M	0.3%	0.6%
	40℃/75%R.H.×4M	0.4%	0.7%
	40℃/75%R.H.×6M	0.4%	0.6%
	25℃/60%R.H.×6M	0.4%	0.5%
酢酸	Initial	-	8.9%
	40℃/75%R.H.×2M	-	9.6%
	40℃/75%R.H.×4M	-	8.6%
	40℃/75%R.H.×６M	-	8.7%
	25℃/60%R.H.×６M	-	9.1%

【００８３】[製剤例２]表８に示すように、化合物Ａ 2mgに対して、マンニトール2mg、20mg含有する水溶液(化合物濃度：2mg/mL)を調製し、除菌ろ過により得られた水溶液1mLをバイアルに分注、ゴムセンを半施栓後、凍結乾燥を行った。凍結乾燥終了後、バイアル空間部を窒素ガスで置換し、ゴムセンを施栓、キャップで巻締することにより凍結乾燥品を作成した。

【００８４】

【表８】

処方Ｃおよび処方Ｄの組成表

	製剤例２	
	処方Ｃ	処方Ｄ
化合物Ａ	2mg	2mg
マンニトール	2mg	20mg

【００８５】実験例２４０℃相対湿度７５％で１箇月保存した。製剤の含量(残存率)、類縁タンパク質、重合体を測定したところ、表９の結果を得た。

【００８６】

【表９】

処方Ｃおよび処方Ｄの安定性結果

	時点	処方Ｃ	処方Ｄ
含量(残存率)	Initial	100.0%	100.0%
	40℃/75%R.H.×１M	98.9%	98.4%
類縁タンパク質	Initial	2.2%	2.1%
	40℃/75%R.H.×１M	3.5%	3.0%
重合体	Initial	0.3%	0.3%
	40℃/75%R.H.×１M	1.4%	2.0%

【００８７】[比較例１]糖類を加えずに、化合物Ａを注射用水で溶解し、表１０に示す処方により、各水溶液(化合物濃度：2mg/mL)を調製し、必要に応じて塩酸によりpHを調整したのち、除菌ろ過により得られた各水溶液0.5mLをバイアルに分注、ゴムセンを半施栓後、凍結乾燥を行った。凍結乾燥終了後、バイアル空間部を窒素ガスで置換し、ゴムセンを施栓、キャップで巻締することにより凍結乾燥品を作製した。

【００８８】

【表１０】

処方Ｅおよび処方Ｆの組成表

	比較例１	
	処方Ｅ	処方Ｆ
化合物Ａ	1mg	1mg
塩酸	－	適量
薬液pH	5.1	4.6

【００８９】実験例３
４０℃相対湿度７５％で１箇月保存した。製剤の含量(残存率)、類縁タンパク質、重合体を測定したところ、表１１の結果を得た。

【００９０】
【表１１】

処方Ｅと処方Ｆの安定性結果

測定項目	時点	処方Ｅ	処方Ｆ
含量	Initial	100.0%	100.0%
(残存率)	40℃/75%R.H.×１Ｍ	86.4%	91.4%
類縁タンパク	Initial	2.7%	2.8%
質	40℃/75%R.H.×１Ｍ	14.2%	11.4%
重合体	Initial	0.3%	0.3%
	40℃/75%R.H.×１Ｍ	9.3%	4.5%

【００９１】実験例１および実験例２と実験例３を比較し、本発明のエピトープポリペプチドの凍結乾燥製剤において、精製白糖およびマンニトールを添加することにより、保存による類縁タンパク質および重合体の生成を少なくすることができ、安定性に優れた製剤とできることが分かった。

【００９２】
【配列表】

SEQUENCE LISTING

<110> Meiji Dairies Corporation; Takeda Chemical Industries, Ltd.
<120> Multiple Epitope Acetylated Polypeptides
<130> H14020
<150> JP P2001-196607
<151> 2001-06-28
<160> 23
<170> PatentIn Ver. 2.1
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Ile Phe Ala Ser Lys Asn Phe His Leu Gln Lys Asn Thr Ile Gly Thr
35 40 45
Gly Arg Arg Trp Lys Asn Asn Arg Ile Trp Leu Gln Phe Ala Lys Leu
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Thr Gly Phe Thr Leu Met Gly Arg Arg Leu Lys Met Pro Met Tyr Ile

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agggtatcga ttagtaagga ggtttaaaat gaaggtgact gttgctttta atcaatttgg 120
acctaac 127

【００９３】
【発明の効果】本発明により、スギ花粉の主要アレルゲンタンパク質Cry ｊ 1およびCry ｊ 2由来のT細胞エピトープからなる、溶解性がよく長期間安定な凍結乾燥品で、5～15%の酢酸を含む多重T細胞エピトープポリペプチドの酢酸塩組成物が提供された。該多重T細胞エピトープポリペプチド酢酸塩組成物は、スギ花粉症の予防または治療のための注射剤として有用である。
【図面の簡単な説明】
【図１】　多重T細胞エピトープポリペプチドをコード

するDNAの構築図を示す。

【図２】　多重T細胞エピトープポリペプチドのアミノ酸配列および該ポリペプチドをコードする塩基配列を示す。塩基配列の太字の部分はポリペプチドのアミノ酸配列をコードする領域を示す。配列中下線を付した塩基はプラスミドの構築を容易にする等の理由で変異させてある。小文字はプラスミドあるいはPCRプライマー由来の塩基配列を示す。

【図３】　発現プラスミドpQTF△crのtrpプロモーター周辺の塩基配列（大文字）および多重T細胞エピトープポリペプチドのN末端のアミノ酸配列をコードする塩基配列（小文字）を示す。□で囲った部分は－10領域および－35領域を示し、下線部分はSD配列を示し、そして二重の下線部分は主要な制限酵素認識部位を示す。

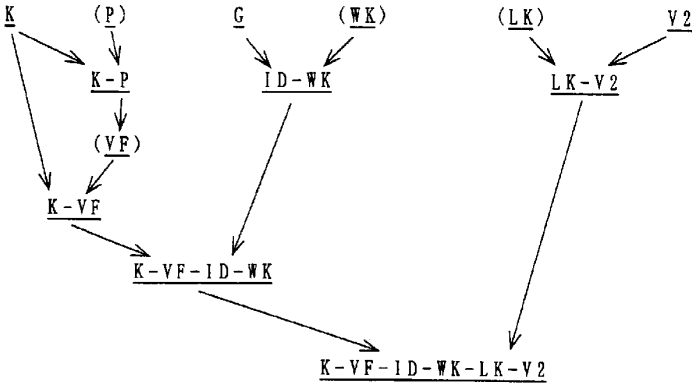
【図４】　発現プラスミドpQTF７△crを示す。trpプロモーター、２つのSD配列、多重T細胞エピトープポリペプチドをコードする領域、λファージ由来のターミネーターto、主要な制限酵素認識部位、およびアンピシリン耐性遺伝子が示されている。

【図５】　多重T細胞エピトープポリペプチドの酢酸含有量（%）と該ポリペプチドの重合体増加量（%）との関係を示す。

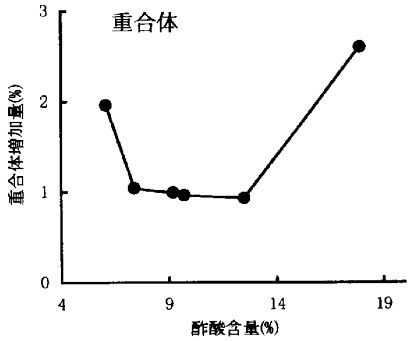
【図６】　同上酢酸含有量（%）と該ポリペプチドの類縁ポリペプチド増加量（%）との関係を示す。

【図７】　同上酢酸含有量（%）と残存含有量（%）との関係を示す。

【図１】



【図５】



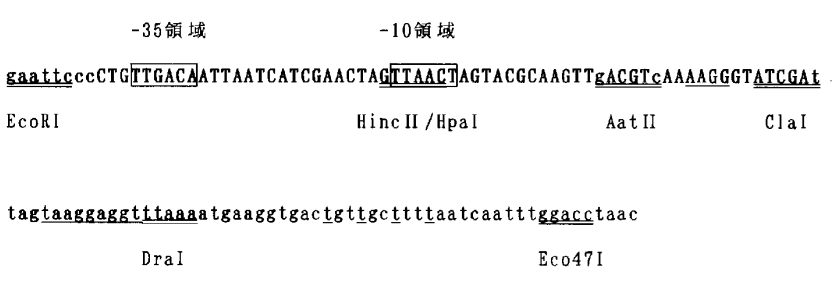
【図２】

pUC19F7#2, 3, 4　　K-VF-ID-WK-LK-V2　　（ポリペプチドをコードする塩基配列）

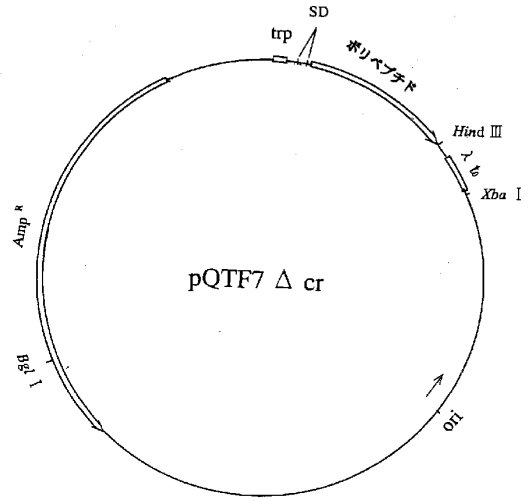
...catcccgggAAATCCATGAAGGTGACAGTGGCGTTCAATCAATTTGGACCTAAC
CGTCGAGTGTTTATCAAGAGAGTGAGCAATGTTATCATACACGGT
CGTCGAATCGACATCTTTGCATCTAAAAACTTTCACTTACAAAAGAACACGATAGGAACAGGG
CGTCGATGGAAGAACAATAGAATATGGTTGCAGTTTGCTAAACTTACAGGTTTTACTCTAATGGGT
CGTCGACTCAAAATGCCTATGTACATTGCTGGGTATAAGACTTTTGATGGC
CGTCGAGTAGATGGGATAATAGCTGCGTACCAAAATCCAGCGAGCTGGAAGtaagcttgg...

ポリペプチドのアミノ酸配列：MKVTVAFNQFGPNrrVFIKRVSNVIIHGrrIDIFASKNFHLQKNTIGTGrrW
KNNRIWLQFAKLTGFTLMGrrLKMPMYIAGYKTFDGrrVDGIIAAYQNPASWK

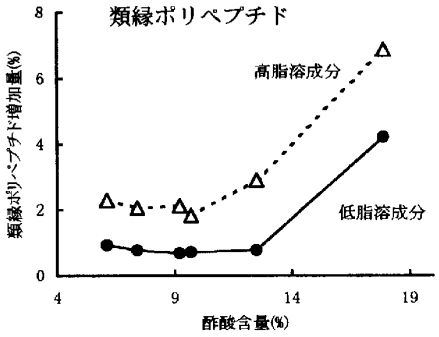
【図３】



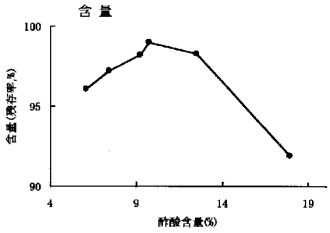
【図４】



【図６】



【図７】



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FIBRONECTIN-CONTAINING EYE LOTION, METHOD FOR ITS PREPARATION AND CONSERVATION AND THERAPEUTIC AGENT FOR TREATING EYE DAMAGE

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Applicant(s): NEW YORK BLOOD CENTER INC; JAPAN CHEM RES; SANTEN PHARMA CO LTD ± (NEW YORK BLOOD CENTER INC, ; NIPPON CHEM RES KK, ; SANTEN PHARMACEUT CO LTD, ; NYUUYOOKU BURATSUDO SENTAA INC, ; NIPPON KEMIKARU RISAACHI KK, ; SANTEN SEIYAKU KK)

Classification: - international: ***A61K38/16; A61K38/17; A61K38/39; A61K47/14; A61K47/18; A61K47/36; A61K9/00; A61K9/08; A61P27/02;*** (IPC1-7): A61K37/04; A61K47/36; A61K9/08

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Abstract of JPH05194257 (A)

PURPOSE: To obtain the subject stable, highly water-miscible instillation containing fibronectin, an amino acid, saccharide and p-hydroxybenzoic acid lower alkyl ester-based antiseptic. CONSTITUTION: This eye drops contain, as active ingredients, 0.25-10mg/mL of fibronectin, 0.005-0.5M of a hydrophilic amino acid (e.g. glycine), 0.005-0.5M of a saccharide such as mono-, di-, tri- or polysaccharide (e.g. sucrose) and

0.002-0.25% (W/V) of a p-hydroxybenzoic acid lower alkyl ester-based antiseptic (e.g. methyl p-hydroxybenzoate), and also an antiseptic effect enhancer (e.g. ethylenediaminetetraacetic acid). This instillation is of multiple dose type, being capable of suppressing/inhibiting bacterial growth while retaining the cell adhesivity and wound-curing nature inherent in fibronectin.

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(54)【発明の名称】　フィブロネクチン含有点眼液、点眼液の製造方法、点眼液の保存方法および眼創傷の治療剤

(57)【要約】
【目的】　安定でかつ水溶性に優れたフィブロネクチン点眼液を得る。
【構成】　本点眼液は、フィブロネクチン、アミノ酸、糖類およびパラヒドロキシ安息香酸低級アルキルエステルとを含有する。また、該点眼液を用いた眼創傷治療剤、フィブロネクチンを眼科用途に製剤化する方法、アルブミンを含まないフィブロネクチンの水溶液を凍結乾燥する方法および細菌の成育をフィブロネクチンの細胞接着活性および創傷治療活性とを保持しつつ阻止する点眼剤も含まれる。

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【特許請求の範囲】

【請求項１】　フィブロネクチン、一種又は複数の水溶性の親水性アミノ酸、単糖類、二糖類、三糖類、多糖類若しくはそれらの誘導体又はそれらの混合物から選択される糖類およびパラヒドロキシ安息香酸低級アルキルエステル防腐剤から構成される安定でかつ可溶性の多数回使用型点眼液。

【請求項２】　エチレンジアミン四酢酸又はその塩類から選択された防腐効果増強剤をさらに含んでなる請求項第１項に記載された点眼液。

【請求項３】　フィブロネクチンの濃度が０．２５ないし１０．０ｍｇ／ｍｌである、請求項第１項に記載された点眼液。

【請求項４】　点眼液中の該アミノ酸の濃度が０．００５ないし０．５Ｍである、請求項第１項に記載された点眼液。

【請求項５】　点眼液中の該糖の濃度が０．００５から０．５Ｍである、請求項第１項に記載された点眼液。

【請求項６】　該アミノ酸がグリシンでありまた該糖類がショ糖である、請求項第１項に記載された点眼液。

【請求項７】　グリシンの濃度が０．０４Ｍでありまたショ糖の濃度が０．１　Ｍである、請求項第６項に記載された点眼液。

【請求項８】　該パラヒドロキシ安息香酸低級アルキルエステル防腐剤の濃度が０．００２ないし０．２５％（ｗ／ｖ）である、請求項第１項に記載された点眼液。

【請求項９】　該パラヒドロキシ安息香酸低級アルキルエステル防腐剤が、パラヒドロキシ安息香酸メチルエステル、パラヒドロキシ安息香酸エチルエステル、パラヒドロキシ安息香酸プロピルエステル、パラヒドロキシ安息香酸ブチルエステルまたはそれらの混合物である、請求項第１項に記載された点眼液。

【請求項１０】　エチレンジアミン四酢酸の該塩類がエチレンジアミン四酢酸二ナトリウム又はエチレンジアミン四酢酸二ナトリウム二水和物とからなる、請求項第２項に記載された点眼液。

【請求項１１】　該防腐剤がパラヒドロキシ安息香酸エチルエステルとパラヒドロキシ安息香酸ブチルエステルおよびさらに効果増強剤であるエチレンジアミン四酢酸二ナトリウム二水和物との組合せから構成される、請求項第１０項に記載された点眼液。

【請求項１２】　パラヒドロキシ安息香酸エチルエステルの濃度が０．００５ないし０．１７　％であり、パラヒドロキシ安息香酸ブチルエステルの濃度が０．００２ないし０．０２％でありかつエチレンジアミン四酢酸二ナトリウム二水和物の濃度が０．００５ないし０．１％である、請求項第１１項に記載された点眼液。

【請求項１３】　フィブロネクチン、一種又は複数の水溶性の親水性アミノ酸、単糖類、二糖類、三糖類、多糖類若しくはそれらの誘導体又はそれらの混合物から選択

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される糖類から構成される安定でかつ可溶性の一回使用型点眼液。

【請求項１４】　フィブロネクチンの濃度が０．２５ないし１０．０ｍｇ／ｍｌである、請求項第１３項に記載された点眼液。

【請求項１５】　該アミノ酸の濃度が０．００５ないし０．５Ｍである、請求項第１３項に記載された点眼液。

【請求項１６】　該糖の濃度が０．００５ないし０．５Ｍである、請求項第１３項に記載された点眼液。

【請求項１７】　該アミノ酸がグリシンでありまた該糖類がショ糖である、請求項第１３項に記載された点眼液。

【請求項１８】　グリシンの濃度が０．０４Ｍでありまたショ糖の濃度が０．１Ｍである、請求項第１７項に記載された点眼液。

【請求項１９】　請求項第１項に記載された成分を含有する眼創傷治療点眼液。

【請求項２０】　エチレンジアミン四酢酸又はその塩類とから選択された防腐効果増強剤をさらに含んでなる請求項第１９項に記載された眼創傷治療点眼液。

【請求項２１】　該防腐剤がパラヒドロキシ安息香酸エチルエステルとパラヒドロキシ安息香酸ブチルエステルおよびさらに効果増強剤であるエチレンジアミン四酢酸二ナトリウム二水和物との組合せから構成される、請求項第２０項に記載された眼創傷治療点眼液。

【請求項２２】　フィブロネクチン、一種又は複数の水溶性の親水性アミノ酸、単糖類、二糖類、三糖類、多糖類若しくはそれらの誘導体又はそれらの混合物から選択される糖類から構成される水溶液であって、アルブミンを含有しない該水溶液を減圧下で凍結乾燥させることからなる、点眼用フィブロネクチン製剤の調製方法。

【請求項２３】　引き続いて不活性気体を封入し次いで溶封する、請求項第２２項に記載された方法。

【請求項２４】　該不活性気体が窒素である、請求項第２２項に記載された方法。

【請求項２５】　フィブロネクチンの濃度が０．２５ないし３０．０ｍｇ／ｍｌである、請求項第２４項に記載された方法。

【請求項２６】　該アミノ酸の濃度が０．００５ないし１．５Ｍである、請求項第２２項に記載された方法。

【請求項２７】　該糖の濃度が０．００５ないし１．５Ｍである、請求項第２２項に記載された方法。

【請求項２８】　該アミノ酸がグリシンでありまた該糖類がショ糖である、請求項第２２項に記載された方法。

【請求項２９】　点眼液中に、グリシンが０．１２Ｍの量で存在し、またショ糖が０．１Ｍの量で存在する、請求項第２８項に記載された方法。

【請求項３０】　凍結乾燥フィブロネクチンから濁りのないフィブロネクチン溶液を得る方法であって、フィブロネクチン水性溶液に一種又は複数の水溶性の親水性ア

ミノ酸、単糖類、二糖類、三糖類、多糖類若しくはそれらの誘導体又はそれらの混合物から選択される糖類を加え、次いで凍結乾燥して凍結乾燥フィブロネクチンを得、さらに該凍結乾燥フィブロネクチンを水性溶媒に再溶解することを特徴とし、加える糖類およびアミノ酸の量が、フィブロネクチン水性溶液を凍結乾燥し次いで水性溶媒に再溶解するとき、濁りを防ぐのに十分な量である方法。

【請求項３１】　該アミノ酸がグリシンであり、また該糖類がショ糖である、請求項第３０項に記載された方法。

【請求項３２】　フィブロネクチン、一種又は複数の水溶性の親水性アミノ酸、単糖類、二糖類、三糖類、多糖類若しくはそれらの誘導体又はそれらの混合物から選択される糖類から構成される点眼液において、フィブロネクチンの持つ細胞接着性および創傷治癒性を保ちつつ細菌成育を阻止できる様、前記点眼液中における細菌成育を阻止するに充分な量のパラヒドロキシ安息香酸低級アルキル系防腐剤を前記点眼液に添加することからなる点眼液。

【発明の詳細な説明】

【０００１】

【産業上の利用分野】本発明は、フィブロネクチン、アミノ酸、糖類およびパラヒドロキシ安息香酸低級アルキルエステル系防腐剤を含有する、安定でしかも可溶性の、多数回使用型点眼液ならびにかかる点眼液を用いる眼創傷の治療剤に関する。本発明はさらに眼科用フィブロネクチン製剤の製造方法に関する。本発明はさらに、フィブロネクチンの細胞接着性および創傷治癒特性を保持しつつ、点眼液中のバクテリア増殖を阻止する点眼液に関する。

【０００２】

【従来の技術】フィブロネクチンは、細胞接着、血液凝固、悪性トランスフォーメーション、細網内皮系機能および胚分化に関与しており、治療処置において有用である。フィブロネクチンが細胞接着や上皮細胞伸長を促進する役割を果たすため、眼創傷、特に種々の角膜障害の治療に使用することが望まれている。その他の増殖因子も、眼創傷の治療のための治癒促進剤として有用であることが判っている。例えば、組み換え型ヒト上皮増殖因子は、擦過傷またはアルカリ熱傷受傷後に角膜上皮の再形成を促進することが明らかになっている（Ｓｔｅｒｎ　ｅｔ　ａｌ．、”Ｔｈｅ　Ｅｆｆｅｃｔｓ　ｏｆ　Ｈｕｍａｎ　Ｒｅｃｏｍｂｉｎａｎｔ　Ｅｐｉｄｅｒｍａｌ　Ｇｒｏｗｔｈ　Ｆａｃｔｏｒ　ｏｎ　ＥｐｉｔｈｅｌｉａｌＷｏｕｎｄ　Ｈｅａｌｉｎｇ”、ｉｎ　Ｈｅａｌｉｎｇ　Ｐｒｏｃｅｓｓｅｓｉｎ　ｔｈｅ　Ｃｏｒｎｅａ、６９（Ｃ．　Ｅ．　Ｃｒｏｓｓｏｎ　ａｎｄ　Ｈ．　Ｅ．　Ｋａｕｆｍａｎ、　ｅｄｓ．）、１９８９）。同様に、繊維牙細胞増殖因子も、角膜治癒を刺激促進するものと報告されている（Ｃｏｕｎｔｏｉｓ、Ｙ．　ｅｔ　ａｌ．、１８１　Ｃ．　Ｒ．　Ｓｏｃ．　Ｂｉｏｌ．、４９１（１９８７））。その他の多くの増殖促進物質も認められており（例えば、インター－ロイキン－６、血小板由来増殖因子など）、眼創傷治癒を促進するうえで有用であるかもしれない。眼創傷は、例えば穿刺、物理的損傷、酸の飛沫、手術による切開、薬品による熱傷または裂傷など多くの態様で起こり得る。フィブロネクチンは、上皮細胞が創傷面全体にわたって遊走するのを促進するとともに、上皮細胞が創傷面に結合して、創傷を永続的に閉塞するのを促進するものと信じられている。このような過程は、線維芽細胞増殖因子などのような多くの内因性増殖因子の産生を刺激・促進する可能性がある。

【０００３】眼創傷をフィブロネクチンで治療するためには、フィブロネクチンを点眼液として適用・投与するべきである。多数回使用型(multi-dose)点眼液を一人で使用するのが点眼液を適用・投与する典型的な型式である。フィブロネクチンを使用するうえでの問題の一つは、米合衆国食品医薬局（”ＦＤＡ”）による規制で、多数回使用型点眼液中においてはバクテリア増殖を抑制・阻止するため防腐剤の添加が要求されていることから生じる。

【０００４】

【発明が解決しようとする課題】塩化ベンザルコニウムは、点眼液に最も多く用いられている防腐剤であるが、フィブロネクチンの創傷治癒作用を阻害するため、フィブロネクチンと一緒に使用することが不可能である。クロロブタノールやフェニルエチルアルコールは、点眼液に適用できる別の防腐剤であるが、これらもフィブロネクチンと共に使用することは出来ない。クロロブタノールは、中性のｐＨ溶液において加水分解される。フェニルエチルアルコールは、フィブロネクチンの創傷治癒作用を阻害するため使用することは出来ない。同様に、デヒドロ酢酸ナトリウムまたは二塩化セチルピリジニウムから調製された防腐剤は、フィブロネクチンの創傷治癒作用を阻害する。チメロサールは、フィブロネクチンの創傷治癒作用を阻害しないが、チメロサールが水銀を含有しておりまた水銀に関連して毒性の問題があるため、点眼液に防腐剤として使用するには適当ではない。

【０００５】点眼製剤にフィブロネクチンを使用するうえで遭遇するもう一つの困難は、水溶液に対するフィブロネクチンの溶解性と安定性の低さに関連して発生する問題である。フィブロネクチンは溶液中での保存安定性が悪いため、フィブロネクチン溶液を安定剤、通常は中性アミノ酸、単糖類、二糖類または糖アルコールと共に凍結乾燥するのが常法である。そして、使用直前に、溶剤を凍結乾燥処理したフィブロネクチンに添加するのである。この方法の欠点は、凍結乾燥処理した製剤を溶剤－典型的には水－に溶解するには長時間を要することお

よび得られた溶液が線維性の不溶物のためしばしば濁りを生ずることである。

【０００６】このような凍結乾燥に係る問題に対処する一つの方法は、Ｏｈｍｕｒａの米合衆国特許第４，５６５，６５１号において開示されている。このＯｈｍｕｒａの特許においては、凍結乾燥に先立って、アルブミンおよび中性のアミノ酸、単糖類、二糖類と糖アルコールから選択された少なくとも一種の安定剤とを、フィブロネクチンを含有する水溶液に添加し、次いでこの溶液を凍結乾燥するのである。Ｏｈｍｕｒａに従えば、得られた凍結乾燥フィブロネクチンを水に溶解した場合、その溶解時間は早く、濁りも殆どまたは生じないのである。しかしながら、点眼薬については、Ｏｈｍｕｒａによる凍結乾燥フィブロネクチンは、もう一つ別のタンパク質であるアルブミンが存在するために許容出来ない。アルブミンは、防腐剤の効果を低くし、またフィブロネクチンの機能を妨害する可能性がある。そのほか、Ｏｈｍｕｒａの特許の方法によって製造された凍結乾燥フィブロネクチンは、かたまって凝集する傾向があり、そのため容易に溶解しない。

【０００７】

【課題を解決するための手段】本発明は、フィブロネクチンと抗菌性防腐剤とを含有する安定でかつ容易に溶解する、多数回使用型点眼液を提供する。

【０００８】本発明はさらには、フィブロネクチンを含有する安定でかつ容易に可溶な、一回使用型(single-use)点眼液を提供する。

【０００９】本発明はさらには、創傷治癒促進剤の諸特性を妨害しない抗菌性防腐剤を提供する。

【００１０】本発明はまた、殺ウイルス滅菌したフィブロネクチンを含有する点眼液を眼創傷に投与することによる眼創傷を治療剤をも提供する。

【００１１】本発明はまた、アルブミンを含まず、唯一のタンパク質としてフィブロネクチンを含有する水溶液を凍結乾燥することからなる、点眼液用のフィブロネクチンを調製する方法を提供する。

【００１２】この方法のもう一つの利点は、不必要なタンパク質を一切含まず、また溶解した場合に、安定で溶解性のよい溶液を生成する、凍結乾燥フィブロネクチンが製造されることである。

【００１３】本発明は、フィブロネクチンをバクテリア増殖を抑制する防腐剤と共に含有する多数回使用型点眼液を提供する。

【００１４】本発明によれば、眼創傷を治療するためウイルス滅菌した、フィブロネクチンの持つ創傷治癒作用を利用することが可能となる。

【００１５】本発明は、フィブロネクチンを含有する点眼液であって、点眼液中に含まれるウイルスが、実質的に全てではないにしてもその大半が不活性化または除去されており、またフィブロネクチンの構造、機能および活性が維持されているフィブロネクチン含有点眼液を提供する。

【００１６】本発明はまた、フィブロネクチンの水溶液に糖とアミノ酸とを添加することからなる、凍結乾燥フィブロネクチンから濁りのないフィブロネクチン溶液を得る方法において、糖の量とアミノ酸の量とが、該溶液を凍結乾燥し、次いで水性溶媒に溶解した場合に濁りを防止するに充分な量である方法を適用するのである。

【００１７】本発明はまた、フィブロネクチン、アミノ酸および糖を含有する点眼液にパラヒドロキシ安息香酸低級アルキルエステル系防腐剤を添加することからなる、フィブロネクチンの持つ細胞接着性と創傷治癒特性を保持しつつ、バクテリア増殖を抑制・阻害する点眼液を提供する。

【００１８】本発明の方法において、フィブロネクチン、アミノ酸および糖を含有するアルブミンフリーの水溶液は、真空中で凍結乾燥される。凍結乾燥を行う前の時点で、このようなフィブロネクチンは、０．２５ないし３０ｍｇ／ｍｌ、好ましくは３ｍｇ／ｍｌの量含まれる。

【００１９】このようなアミノ酸は、たとえばセリン、ヒスチジン、アラニン、リジンやグリシンなど水溶性の、親水性アミノ酸であればよい。グリシンが好ましいアミノ酸である。凍結乾燥されるべき水溶液におけるアミノ酸の濃度は、０．００５から１．５Ｍまでであり、好ましくは０．１２Ｍである。

【００２０】このような糖は、たとえばグルコースなどの単糖類、たとえばショ糖やガラクトースなどの二糖類、たとえばラフィノースなどの三糖類、たとえばデキストランなどの多糖類、もしくはソルビトールやマンニトールなどの糖誘導体、またはこれらの組合せであればよい。ショ糖が好ましい糖類である。凍結乾燥されるべき水溶液における糖の濃度は、０．００５から１．５Ｍまでであり、好ましくは０．３０Ｍである。

【００２１】凍結乾燥されるべきフィブロネクチンの水溶液には、グリシンとショ糖の組合せを添加するのがもっとも好ましい。このようなグリシンは、凍結乾燥されるべき水溶液に０．００５から１．５Ｍまで、好ましくは０．１２Ｍの濃度で含まれ、またショ糖は、該水溶液に０．００５から１．５Ｍまで、好ましくは０．３０Ｍの濃度で含まれる。

【００２２】生物学的出発材料には脂質エンベロープの外殻構造を有するウィルスが存在しており、これを不活化処理したフィブロネクチン含有水溶液を使用するのが好ましい。米合衆国特許第４、８４１、０２３号ならびに該特許に記載された参考文献には、脂質包含ウィルスを死滅させる適当な方法が記載されている。そのほかに、効率的なウィルス除去は、ゼラチンセファロースクロマトグラフィを用いて行われる（ＨｏｒｏｗｉｔｚおよびＣｈａｎｇ、”Ｆｉｂｒｏｎｅｃｔｉｎ”、４４１

（Ｄｅａｎｅ　Ｆ．　Ｍｏｓｃｈｅｒ編集）（１９８９））。

【００２３】凍結乾燥が完了すると、フラスコを真空中で密封する。窒素を導入し、次いでフラスコを窒素またはその他の不活性ガス雰囲気下で密封して凍結乾燥を完了させるのが好ましい。凍結乾燥フィブロネクチンの溶解性は、フラスコをこのような態様で密封した場合に改善される。

【００２４】このような方法で得られたフィブロネクチン凍結乾燥品は、本発明の点眼液を調製するのに用いられる。他の方法で得られたフィブロネクチンも、本発明の点眼液に使用してもよいと理解される。

【００２５】本発明の一つの実施態様において、点眼液は、フィブロネクチン、アミノ酸、糖および溶媒から構成される。該フィブロネクチンは、０．２５ｍｇ／ｍｌから１０ｍｇ／ｍｌまで、好ましくは１　ｍｇ／ｍｌの濃度で含まれる。該アミノ酸は、グリシン、セリン、ヒスチジン、アラニン、リジンもしくはその他水溶性、親水性アミノ酸類またはこれらの混合物、好ましくはグリシンであって、０．００５から０．５Ｍまで、好ましくは０．０４Ｍの濃度で含まれる。該糖は、たとえばグルコースなどの単糖類、たとえばショ糖やガラクトースなどの二糖類、たとえばラフィノースなどの三糖類、たとえばデキストランなどの多糖類、もしくはソルビトールやマンニトールなどの糖誘導体、またはこれらの組合せ、好ましくはショ糖であって、０．００５から０．５Ｍまで、好ましくは０．１Ｍの濃度で含まれる。該アミノ酸がグリシンであり、また該糖がショ糖であるのが最も好ましい。該溶媒は、滅菌水、即ちＵ．Ｓ．Ｐ．グレード精製水であるか、またはたとえばリン酸緩衝食塩水（”ＰＢＳ”）などの中性の緩衝生理食塩水であればよい。溶媒としてはＵ．Ｐ．Ｓ．水を使用するのが好ましい。塩化ナトリウムを、０．０１ないし０．２Ｍ、好ましくは０．０８７Ｍの濃度においてこのような点眼液に随意に添加してもよい。

【００２６】また別の実施態様においては、このような点眼液はまた、防腐剤を含有していてもよい。このような防腐剤は、一般的に“パラベン”または“ＰＢ”という名称で称されるパラヒドロキシ安息香酸の低級アルキルエステルである。好ましいパラヒドロキシ安息香酸低級アルキルエステル防腐剤は、パラヒドロキシ安息香酸メチルエステル（“メチルパラベン”と称する）、パラヒドロキシ安息香酸エチルエステル（“エチルパラベン”と称する）、パラヒドロキシ安息香酸プロピルエステル（“プロピルパラベン”と称する）、パラヒドロキシ安息香酸ブチルエステル（“ブチルパラベン”と称する）やこれらの混合物である。このような防腐剤は好ましくは、濃度が０．００２ないし０．２５％（Ｗ／Ｖ）である水溶液の形状である。このような水溶液に用いられる水は、Ｕ．Ｓ．Ｐ．グレード精製水、滅菌水、または常法により精製された水である。

【００２７】点眼液には、このようなパラヒドロキシ安息香酸低級アルキルエステルの防腐剤を二種添加するのが好ましい。このような防腐剤の好ましい組み合わせとしては、以下のものが挙げられる。

【００２８】１．　濃度が０．００５ないし０．１７％（ｗ／ｖ）、好ましくは０．０２％（ｗ／ｖ）であるパラヒドロキシ安息香酸エチルエステルと濃度が０．００２ないし０。０２１％（ｗ／ｖ）、好ましくは０．０１％（ｗ／ｖ）であるパラヒドロキシ安息香酸ブチルエステル、または

２．　濃度が０．０１２ないし０．２５％（ｗ／ｖ）、好ましくは０．０３８％（ｗ／ｖ）であるパラヒドロキシ安息香酸メチルエステルと濃度が０．００５ないし０．０５％（ｗ／ｖ）、好ましくは０．０１５％（ｗ／ｖ）であるパラヒドロキシ安息香酸プロピルエステル。

【００２９】またもう一つの実施態様においては、点眼液におけるこのような単一または複数の防腐剤の効果を改善するために、効果増強剤を添加する。このような効果増強剤は好ましくは、エチレンジアミン四酢酸（ＥＤＴＡ）またはその塩、好ましくはエチレンジアミン四酢酸二ナトリウムまたはエチレンジアミン四酢酸二ナトリウム二水和物（$Na_2C_{10}H_{14}O_8N_2 \cdot 2H_2O$）．このような好ましい効果増強剤は、エチレンジアミン四酢酸二ナトリウム二水和物である。このような効果増強剤は、かかる点眼液には０．００５ないし０．１％（ｗ／ｖ）の濃度で添加する。ＥＤＴＡ二ナトリウム二水和物を用いる場合は、その濃度は好ましくは０．０１％（ｗ／ｖ）である。

【００３０】眼創傷、および特に角膜障害は、本発明に係る点眼液を、このような創傷を治療しかつ創傷治癒を促進するに有効な量だけ投与することによって治療することができる。このような治療に必要とされる点眼液の量は、眼創傷の性質と範囲・規模に依存して異なる。投与量としては、起きている時間帯に一日当たり四回、４週間ないし５６日間一滴を点眼するのが望ましい。

【００３１】

【実施例】本発明を以下に記載する実施例によってさらに詳しく説明する。

実施例　１

Ａ．　フィブロネクチン点眼液の処方

ＰＢＳ中においてウイルス不活化、精製フィブロネクチン（ＨｏｒｗｉｔｚおよびＣｈａｎｇ、Ｆｉｂｒｏｎｅｃｔｉｎ、４４１（Ｄｅａｎｅ　Ｆ．　Ｍｏｓｈｒｔ編集）（１９８９））を用いて、３．０ｍｇのフィブロネクチン、０．３０Ｍショ糖、０．１２Ｍグリシン、０．２６２Ｍ塩化ナトリウムおよび０．０３Ｍりん酸ナトリウム緩衝液ｐＨ７．４、を含有する溶液１．０ｍｌを製造する。３　ｍｇのフィブロネクチンを含む精製フィブロネクチンの分画を、０．３３９グラムの１．０Ｍショ

糖溶液、０．０９Ｍりん酸ナトリウム緩衝液、０．７１５Ｍ塩化ナトリウム、および０．４Ｍグリシンを含むｐＨ７．４の溶液０．３００ｍｇ、および混合溶液全体が１．０３９グラム即ち１．０ｍｌとなるに充分な量の、ＰＢＳ（０．０１Ｍりん酸ナトリウム緩衝液、０．１２Ｍ塩化ナトリウム、ｐＨ７．４）に加える。

【００３２】混合溶液を、Ｐａｌｌ社製のナイロン０．２ミクロンフィルター（ＰａｌｌＣｏｒｐ．、ＮＹ、ＮＹ）を用いてろ過し、１ｍｌを滅菌した６ｍｌガラスバイアルに充填する。滅菌した、２０ｍｍのシリコーン処理した８９０グレーブチル凍結乾燥スプリットストッパー（Ｗｅｓｔ　Ｃｏｒｐ．）を一部このバイアル首部に挿入し、次いでバイアルをステンレススチール製凍結乾燥ボックスの中に入れる。バイアルは、凍結乾燥に先立って－５０ないし－７０℃に凍結する。凍結乾燥後、フィブロネクチンを、０．０２％エチルパラベン、０．０１％ブチルパラベンおよび０．０１％エチレンジアミン四酢酸二ナトリウム二水和物を含有する滅菌Ｕ．Ｓ．Ｐ．グレード精製水３ｍｌを用いて溶解する。

【００３３】Ｂ．フィブロネクチン点眼液の凍結乾燥調合製剤し、バイアルに充填したフィブロネクチンを－５０°ないし－７０℃に凍結する。凍結乾燥は、棚温度を－４５℃以下としかつチャンバーを水銀柱１００ミクロン以下の圧力として開始する。フィブロネクチンをこのような条件にほぼ２時間保持し、その後、圧力を１００ミクロン以下にしたまま棚温度を－２０°ないし－１０℃に上げる。製品温度が上がり始めると、棚温度を製品温度よりも１０℃高い温度に上げる。製品温度が上がるのに応じて、棚温度は両者の温度差が一定の１０℃に保持されるように上げる。圧力は、１００ミクロン以下に保持しておく。

【００３４】製品温度が２０°ないし３５℃の最終温度に到達した後、棚温度をそのままに保持して最終温度を維持する。製品は、１００ミクロン以下の圧力で、この最終温度に２０．５ないし４５．５時間保持する。

【００３５】凍結乾燥は、１００ミクロン以下の圧力でストッパーするか、またはほぼ１インチの水柱圧力にまで窒素ガスを注入した後でストッパーすることによって終了させる。水分含量は典型的には、０．３と３％（ｗ／ｖ）との間である。

【００３６】実施例　２

フィブロネクチン含有点眼液の調製

点眼液を以下の方法に従って調製した。この点眼液は、実施例１の方法で凍結乾燥したフィブロネクチンを、０．０１％パラヒドロキシ安息香酸プロピルエステル、０．０２％パラヒドロキシ安息香酸ブチルエステルおよび０．０１％エチレンジアミン四酢酸二ナトリウム二水和物（$Na_2C_{10}H_{14}O_8N_2 \cdot 2H_2O$）をＵ．Ｓ．Ｐ．グレード精製水中に含有する滅菌溶液の３ｍｌと調合することによって調製した。この点眼液を点眼用容器に充填する。この方法は、以下の通りである。ストッパーを凍結乾燥したフィブロネクチンを含むバイアルから取りはずす。点眼容器のキャップのねじを回してはずす。バイアルを、点眼容器の先端部にあてがう。転倒させて、溶液をフィブロネクチンのバイアルに移す。この溶液を必要に応じてうず巻かして、混ぜる。これを再び倒立させて、点眼容器に移す。フィブロネクチンのバイアルを点眼容器の先端部から離す。点眼容器のキャップのねじを回して、密栓する。この最終溶液を緩やかにうず巻かして混ぜて、均質な溶液を確保する。完全に溶解した溶液は、典型的には１分以内に得られる。この最終の点眼液は、以下の成分を、表示した量含有する：

【００３７】

成　分	量
フィブロネクチン	１ｍｇ／ｍｌ
りん酸ナトリウム緩衝液（ｐＨ　７．４）	０．０１Ｍ
ショ糖	０．１Ｍ
グリシン	０．０４Ｍ
塩化ナトリウム	０．０８７Ｍ
パラヒドロキシ安息香酸ブチルエステル	０．０１％
パラヒドロキシ安息香酸エチルエステル	０．０２％
エチレンジアミン四酢酸二ナトリウム水和物	０．０１％

このような点眼液は、一人の患者の個別使用を意図する場合は、滅菌した、多数回使用型容器に充填し、次いで容器を密栓し、不正に触れられないないようにする。

【００３８】実施例　３

パラベン系防腐剤がフィブロネクチンの細胞接合活性に及ぼす影響

フィブロネクチン濃度が１．１９７ｍｇ／ｍｌである点眼液を、０．０５％パラヒドロキシ安息香酸メチルエステルと０．０１５％パラヒドロキシ安息香酸プロピルエステルと組み合わせたパラベン系防腐剤を用い、りん酸緩衝生理食塩水（”ＰＢＳ”）中で調製した（試料１）。フィブロネクチン濃度が１．１９７ｍｇ／ｍｌである点眼液を、防腐剤を用いることなくＰＢＳ中で調製した（試料２）。試料１および２を室温で７日間放置した。

【００３９】ＰＢＳ中フィブロネクチン１．０ｍｇ／ｍｌを含むフィブロネクチン標準液をＰＢＳ（二度蒸留水１リットル中にＮａＣｌ　８，０００ｍｇ、ＫＣｌ　２

００mg、Na_2HPO_4　１，１５０mgおよびKH_2PO_4　２００mgを含む、pH　７．３）で希釈して、フィブロネクチンが５．０００から０．０７８μg／mlまでの希釈系列を調製した。試料１および２はそれぞれ、ＰＢＳで三倍に希釈して、各試料についてフィブロネクチンが５．０００から０．０７８μg／mlまでの希釈系列を調製した。

【００４０】フィブロネクチンの細胞結合活性を、ＢＨＫ細胞吸着測定法を用いて以下の方法に従い測定した。９６個のウエルを持つマイクロプレートを３７℃で２時間、３％ＢＳＡ（ＰＢＳ中ＢＳＡが３０mg／ml）２００μlでプレコートし、１００μlのＰＢＳで二度すすいだ。標準フィブロネクチンの各希釈液と試験試料（試料１および２）をそれぞれ５０μlずつ、９６個のウエルを持つマイクロプレートの別々のウエルの中に入れた。このプレートを３７℃で６０分間培養し、希釈液は吸引して、捨てた。３％ＢＳＡを１００μl、それぞれのウエルに加え、プレートを３７℃で６０分間培養した。培養をしている間に、ＢＨＫ細胞分散液を以下のように調製した：即ち、１０％のウシ胎仔血清を含むＲＰＭＩ－１６４０培地で培養したＢＨＫ細胞を、組織培養プレートからセルスクレーパーで掻き取り、１０００回転／分で７分間遠心分離した。この細胞プレートを血清を含まないＲＰＭＩ－１６４０培地（ＲＭＰＩ－１６４０に２０mMのＨＥＰＥＳを添加したもの）に分散させ、１０００回転／分で７分間遠心分離した。ついで、この工程を繰り返しして、さらにＢＨＫ細胞を洗浄した。洗浄したＢＨＫ細胞を、血清を含まないＲＰＭＩ－１６４０培地にもう一度分散させ、ピペットで採ることによって単細胞分散液を得た。血清を含まないＲＰＭＩ－１６４０を用いて、細胞数を２　x　10^6 個／ml

に調製した。９６個のウエルを持つプレートを１００μlのＰＢＳで二度すすいだ。このＢＨＫ細胞分散液を５０μlずつ、９６個のウエルを持つプレートのそれぞれのウエルに加えた。このプレートを５％ＣＯ２の培養器の中で３７℃で９０分間培養した。細胞分散液は、吸引することによって捨て、プレートを１００μlの生理食塩水ですすいだ。Ｅ－ＭＥＭ培地（ＥａｇｌｅのＭＥＭに５％ＦＢＳを添加したもの）を５０μlずつ、この測定用プレートのそれぞれのウエルに加えた。ニュートラルレッド溶液を５０μlずつ、この測定用プレートのそれぞれのウエルに加えた（このニュートラルレッド溶液は、使用直前に２mlの１Ｍ　ＨＥＰＥＳと１０mlの１％中性赤とを８８mlのＥ－ＭＥＭ培地に加えることによって調製した）。このプレートを５％ＣＯ２の培養器の中で３７℃で６０分間培養した。プレートを１００μlの生理食塩水で二度すすぎ、次にニュートラルレッド抽出緩衝液（５０％エタノール中一塩基性りん酸塩の０．０５M溶液）を２００μlずつプレートのそれぞれのウエルに加えた。プレートを一晩室温で放置し、各ウエルの吸光度を分光硬度計を用いて５４６nmで測定した。

【００４１】試料１および試料２の希釈系列の各希釈溶液中のフィブロネクチン含量、mg／ml、はフィブロネクチン標準溶液と比較して決定した。得られたデータを用い、平行線測定法によってフィブロネクチン試料を基準とした試料１および試料２の相対効力を算出した。各試験での細胞結合活性の平均値と標準偏差を下記表１に示す。

【００４２】

【表１】

	試験　１	試験　２	試験　３	平均値	Ｓ．Ｄ．
試料１	1.229	1.198	1.257	1.228	0.030
試料２	1.182	1.133	1.140	1.152	0.027

【００４３】表１の結果から明かなように、試料１と試料２の細胞結合活性に有意差はなかった。この結果から、パラベン系の防腐剤は、点眼液中のフィブロネクチンの細胞結合活性には影響を与えないことが判った。

【００４４】実施例　４

フィブロネクチンの細胞結合活性に及ぼす種々のパラベン系防腐剤の影響

パラベン系防腐剤を０．０２％のパラヒドロキシ安息香酸エチルエステルと０．０１％のパラヒドロキシ安息香酸ブチルエステルとの組み合わせとしたことおよびエチレンジアミン四酢酸二ナトリウム二水和物の濃度（滅菌水中）を０．０５％としたこと以外は、実施例２の方法に従って点眼液を調製した（試料１）。試料１を四つに分割した（試料１Ａ、１Ｂ、１Ｃおよび１Ｄ）。試料１Ａを４℃で７日間保存し、試料１Ｂは４℃で１４日間保存した。試料１Ｃは３７℃で７日間また試料１Ｄは３７℃で１４日間保存した。

【００４５】パラベン系防腐剤を０．０３８％のパラヒドロキシ安息香酸メチルエステルと０．０１５％のパラヒドロキシ安息香酸プロピルエステルとの組み合わせとしたことおよびエチレンジアミン四酢酸二ナトリウム二水和物の濃度（滅菌水中）を０．０５％としたこと以外は、実施例２の方法に従って第二の点眼液を調製した（試料２）。試料２を四つに分割した（試料２Ａ、２Ｂ、２Ｃおよび２Ｄ）。試料２Ａを４℃で７日間保存し、試料２Ｂは４℃で１４日間保存した。試料２Ｃは３７℃で７日間また試料２Ｄは３７℃で１４日間保存した。

【００４６】フィブロネクチンの細胞結合活性を、標準ＢＨＫ細胞吸着測定法を用いて実施例３に記載した方法に従って測定した。－８０℃で保存していた、ＰＢＳ１ml当たりフィブロネクチン１mgを含むフィブロネク

チン標準溶液をＰＢＳで希釈して、フィブロネクチン標準溶液５．０００から０．０７８ｇ／ｍｌまでの対照希釈系列を調製した。７日目に、試料１Ａと１Ｃおよび試料２Ａと２ＣとをそれぞれＰＢＳで希釈して、試料の５．０００から０．０７８μｇ／ｍｌの各試料希釈系列を調製した。ＢＨＫ細胞吸着試験を、試料１Ａと１Ｃおよび試料２Ａと２Ｃのそれぞれの希釈系列とフィブロネクチン標準溶液について行い、各希釈溶液のフィブロネクチン含量、ｍｇ／ｍｌ、を決定した。１４日目に、希釈系列調製方法とＢＨＫ細胞吸着測定法を、試料１Ｂと１Ｄおよび試料２Ｂと２Ｄのそれぞれの希釈系列ならびにフィブロネクチン標準溶液について行った。次いで、得られたデータを使用して、平行線測定方法によってフィブロネクチン標準溶液を基準とした試料１Ａ－Ｄおよび試料２Ａ－Ｄの相対効力を算出した。この測定は夫々の試料について、さらに４回繰り返して行なった。下記の表２には、これら測定による細胞結合活性を五回の測定の平均値として、標準偏差（±Ｓ．Ｄ．）とともに示してある。

【００４７】

【表２】

試料	保存（℃）	保存日数	フィブロネクチン（ｍｇ／ｍｌ）	活性（％）
対照	－８０	（－）	１．０９０±０．７２	１００．０±６．６
１Ａ	４	７	１．０２７±０．０２５	９４．２±２．３
１Ｂ	４	１４	１．１３１±０．０４５	１０３．８±４．１
１Ｃ	３７	７	１．０８３±０．０５３	９９．４±４．９
１Ｄ	３７	１４	１．０５９±０．０２４	９７．２±２．２
２Ａ	４	７	１．０９４±０．０２７	１００．４±２．５
２Ｂ	４	１４	１．０９４±０．０３６	１００．４±３．３
２Ｃ	３７	７	１．１５８±０．０４８	１０６．２±４．４
２Ｄ	３７	１４	１．０９０±０．０６９	１００．０±６．３

【００４８】表２の結果から明らかなように試料１と試料２とには、保存日数が７日であろうと１４日であろうと、また保存温度が室温であろうと冷蔵下であろうと、細胞結合活性には有意差はなかった。この結果、パラベン系防腐剤は、エチレンジアミン四酢酸二ナトリウムとともに用いても、点眼液中のフィブロネクチンの細胞結合活性または安定性に影響を及ぼさないことが判った。

【００４９】実施例５

フィブロネクチンのゼラチン結合活性に及ぼすパラベン系防腐剤の影響

フィブロネクチン濃度が１．０ｍｇ／ｍｌである点眼液をＰＢＳを用いて調製した。パラベン系防腐剤は、パラヒドロキシ安息香酸メチルエステル０．０５％とパラヒドロキシ安息香酸プロピルエステル０．０１５％の組合せとした（試料１）。フィブロネクチン濃度が１．０ｍｇ／ｍｌである第二の点眼液を、防腐剤を使用することなくＰＢＳを用いて調製した（試料２）。試料１および２を室温で７日間放置した。

【００５０】フィブロネクチンの細胞結合活性は、ゼラチン－セファロース　アフィニティクロマトグラフィによって測定した。先ず、試料１をＧＰＣ－ＨＰＬＣ系（Ａｓａｈｉｐａｋ　ＧＳ　７１０、ＢｉｏＲａｄ　４０２Ｔ　ＨＰＬＣ系）に供し、パラベン系防腐剤を除去し、タンパク質分画を集めた。試料２を同様にＧＰＣ－ＨＰＬＣ系に供し、タンパク質分画を集めた。集めた試料１および試料２のタンパク質分画をそれぞれゼラチン－セファロース　クロマトグラフィ、具体的に言えばゼラチン－セファロース、ＨＲ５／５、Ｂｉｏｒａｄ　４０２Ｔ、アフィニティクロマトグラフィにかけた。フィブロネクチンのゼラチン結合活性を、保持時間を分単位で測定しまたフィブロネクチンの溶出ピーク面積を測定することによって求めた。なお溶出ピーク面積は、分光光度計を用いて波長２８０ｎｍにおいて測定したものである。ゼラチン結合活性の測定結果は、以下の表３に示す。

【００５１】

【表３】

	保持時間（分）	溶出ピーク面積（２８０ｎｍ）
試料１	４２．９２	３４５．３５７
試料２	４２．９７	３４２．３３２

試料１および試料２との間には、表３の結果が示すようにゼラチン結合活性には有意差は認められなかった。このことから、パラベン系防腐剤は、点眼液中のフィブロネクチンのゼラチン結合活性に影響を与えなかったことが判る。

【００５２】実施例　６

パラベン系防腐剤がフィブロネクチンのバクテリア結合活性に及ぼす影響

フィブロネクチン濃度が１．０ｍｇ／ｍｌである点眼液をＰＢＳを用いて調製した。パラベン系防腐剤は、０．０５％のパラヒドロキシ安息香酸メチルエステルと０．０１５％のパラヒドロキシ安息香酸プロピルエステルの

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組合せとした（試料１）。フィブロネクチンの濃度が１．０ｍｇ／ｍｌである第二の点眼液をＰＢＳを用いて調製したが、防腐剤の添加は行わなかった（試料２）。これら試料１および試料２とを室温で７日間放置した。

【００５３】フィブロネクチンのバクテリア結合活性は、点眼液を熱処理した黄色ブドウ球菌（Ｓｔａｐｈｙｌｏｃｏｃｃｕｓ　ａｕｒｅｕｓ）溶液と共に培養した後生成する凝集を観察することによって測定した。なお黄色ブドウ球菌溶液は、黄色ブドウ球菌をほぼ菌体が１　ｘ　10^9 ／ｍｌの濃度になるようＰＢＳ中に希釈し、次いでこの溶液を１００℃に１０分間加熱することによって調製した。試料１および試料２とはＰＢＳで希釈して、それぞれの試料について１，０００から０．１

試料中のフィブロネクチンの濃度、μｇ／ｍｌ	試料１	試料２
１，０００	＋＋	＋＋
５００	＋＋	＋＋
２００	＋＋	＋＋
１００	＋＋	＋＋
５０	＋＋	＋＋
２０	＋＋	＋＋
１０	＋	＋
５	＋	＋
２	＋	＋
１	±	±
０．５	－	－
０．２	－	－
０．１	－	－
０	－	－

＋＋：強度の塊状形成
＋：塊状形成
±：若干の塊状形成
－：塊状形成なし

【００５５】フィブロネクチンによる塊状形成は、いずれの試料についてもフィブロネクチンの濃度が１μｇ／ｍｌを越えた場合に認められた。バクテリア結合活性の差異は、表４の結果から判るように、試料１と試料２との間には認められなかった。このことは、パラベン系防腐剤が点眼液中のフィブロネクチンのバクテリア結合活性には影響を及ぼさなかったことを示すものである。

【００５６】実施例　７

パラベン系防腐剤の最小阻止濃度

実施例２の方法に従い、下記変数を以下の表に記載の通りにして種々の点眼液を調製した。パラベン系防腐剤の種類と濃度は変えた。用いたパラベン系防腐剤は、メチルパラベン（“Ｍｐ”）、プロピルパラベン（“Ｐ

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μｇ／ｍｌまで希釈系列を調製した。２４個のウエルのマイクロタイター細胞培養測定プレートを用いて、試料１および試料２の各希釈液を５００μｌずつ、測定プレートのウエルの中にいれた。その後黄色ブドウ球菌溶液を５０μｌ各ウエルの中に加えた。室温で、５分ごとに測定プレートをゆっくりと振とうすることによって、１時間これらの溶液を繰り返し混合した。フィブロネクチンと黄色ブドウ球菌との凝集塊の存在の有無を、各試験試料のそれぞれの希釈液について観察し、記録した。バクテリア結合活性の測定結果は、以下の表４に示す。

【００５４】

【表４】

ｐ”）、エチルパラベン（“Ｅｐ”）およびブチルパラベン（“Ｂｐ”）であった。エチレンジアミン四酢酸二ナトリウム（“ＥＤＴＡ”）を添加し、ＥＤＴＡの濃度を変えて、パラベン系防腐剤に対するＥＤＴＡの増強効果を試験した。これら相互に異なる処方の効果を別々に、Ｐ．ａｅｒｕｇｉｎｏｓａ（緑膿菌）またはＣ．ａｌｂｉｃａｎｓを用いて試験した。６時間および２４時間において、細菌接種した処方を、別個の培養プレートで画線培養し、細菌発育増殖の有無を調べた。細菌増殖をコロニー形成単位で表し、０を増殖なしとし、４を最高増殖とする０－４のスケールで採点・評価した。パラベン系防腐剤の最小発育阻止濃度（“ＭＩＣ”）およびパラベン系防腐剤に対するＥＤＴＡの増強効果を、下記の表５ないし表１０に示す。

【００５７】

【表５】

Ｍｐ、ＰｐおよびＥＤＴＡを含有する点眼液のＭＩＣの結果

％　Ｍｐ	％　Ｐｐ	％　EDTA	P.aeruginosa		c.albicans	
			６時間	２４時間	６時間	２４時間
0.068	0.027	0.089	1	0	2	0

0.051	0.020	0.067	2	0	0	0
0.038	0.015	0.05	2	1	1	1
0.029	0.011	0.038	2	2	1	1
0.021	0.008	0.028	2	2	1	1
0.016	0.006	0.021	2	3	1	1
0.012	0.005	0.016	3	3	1	1

【００５８】 【表６】

Ｍｐ、ＰｐおよびＯ．０５％ＥＤＴＡを含有する点眼液のＭＩＣの結果

％ Ｍｐ	％ Ｐｐ	P.aeruginosa		C.albicans	
		６時間	２４時間	６時間	２４時間
0.068	0.027	1	0	1	0
0.051	0.020	2	0	0	0
0.038	0.015	2	1	0	0
0.029	0.011	3	2	0	0
0.021	0.008	3	2	0	0
0.016	0.006	4	3	2	1
0.012	0.005	4	3	2	1

【００５９】 【表７】

Ｍｐ、Ｐｐを含有するがＥＤＴＡを含まない点眼液のＭＩＣの結果

％ Ｍｐ	％ Ｐｐ	P.aeruginosa		C.albicans	
		６時間	２４時間	６時間	２４時間
0.068	0.027	1	0	0	0
0.051	0.020	3	1	1	0
0.038	0.015	3	2	1	0
0.029	0.011	3	3	1	0
0.021	0.008	3	3	3	1
0.016	0.006	3	3	3	1
0.012	0.005	3	3	3	1

【００６０】 【表８】

Ｅｐ、ＢｐおよびＥＤＴＡを含有する点眼液のＭＩＣの結果

％ Ｅｐ	％ Ｂｐ	％ EDTA	P.aeruginosa		C.albicans	
			６時間	２４時間	６時間	２４時間
0.027	0.013	0.067	0	0	0	0
0.020	0.01	0.05	1	0	0	0
0.015	0.007	0.038	2	1	0	0
0.011	0.006	0.028	3	2	0	0
0.008	0.004	0.021	3	3	0	0
0.006	0.003	0.016	3	3	2	2
0.005	0.002	0.012	3	4	4	2

【００６１】 【表９】

Ｅｐ、ＢｐおよびＯ．０５％ＥＤＴＡを含有する点眼液のＭＩＣの結果

％ Ｅｐ	％ Ｂｐ	P.aeruginosa		C.albicans	
		６時間	２４時間	６時間	２４時間
0.027	0.013	0	0	0	0
0.020	0.010	1	0	1	0
0.015	0.007	2	1	1	0
0.011	0.006	3	2	1	0
0.008	0.004	3	3	1	0
0.006	0.003	3	3	1	1
0.005	0.002	4	3	1	1

【００６２】

【表１０】

Ｅｐ、Ｂｐを含有するがＥＤＴＡを含まない点眼液のＭＩＣの結果

％　Ｅｐ	％　Ｂｐ	P.aeruginosa		C.albicans	
		６時間	２４時間	６時間	２４時間
0.027	0.013	0	0	0	0
0.020	0.010	2	1	1	0
0.015	0.007	3	2	1	0
0.011	0.006	3	2	1	0
0.008	0.004	3	3	1	0
0.006	0.003	4	3	1	0
0.005	0.002	4	3	2	0

【００６３】濃度が０．０１２から０．０６８％であるメチルパラベンと濃度が０．００５から０．０２７％であるプロピルパラベンとの組合せからなる防腐材は、表５から表７にまで示すように点眼液中での微生物の増殖を阻止した。この防腐材が持つ微生物の増殖を阻止する効果は、表５と表６を表７と比較したものから判るように、防腐効果増強剤すなわちＥＤＴＡを添加した場合に高くなった。

【００６４】濃度が０．００５から０．０２７％であるエチルパラベンと濃度が０．００２から０．０１３％であるブチルパラベンとの組合せからなる防腐材は、表８から表１０にまで示すように点眼液中での微生物の増殖を阻止した。この防腐材が持つ微生物の増殖を阻止する効果は、表８と表９を表１０と比較したものから判るように、防腐効果増強剤、すなわちＥＤＴＡを添加した場合に高くなった。このことは、パラベン系防腐剤が点眼液中での微生物の増殖を阻止したことを示している。

【００６５】実施例　８

フィブロネクチンの角膜創傷閉止活性に及ぼすパラベン系防腐剤の影響

フィブロネクチン濃度が１．０ｍｇ／ｍｌである点眼液をＰＢＳを用いて調製した。パラベン系防腐剤は、０．０５％のパラヒドロキシ安息香酸メチルエステルと０．０１５％のパラヒドロキシ安息香酸プロピルエステルとの組合せとした（試料１）。フィブロネクチン濃度が１．０ｍｇ／ｍｌである第二の点眼液をＰＢＳを用いて調製したが、防腐剤は添加しなかった（試料２）。試料１および試料２を室温で７日間放置した。フィブロネクチンと防腐剤とを含まない対照点眼液も調製した。フィブロネクチンの角膜創傷閉止活性は、Ｍｏｓｅｓら、１８　Ｉｎｖｅｓｔ．　Ｏｐｈｔｈａｌｍｏｌ．　１０３－１０６（１９７９）、およびＮｉｓｈｉｄａら、１０２、Ａｒｃｈ．　Ｏｐｈｔｈａｌｍｏｌ．　４５５－４５６（１９８４）に記載された方法に従って測定した。ウサギの角膜上皮を３分間ヨウ素蒸気で処理することによって損傷させた。試料１と試料２および対照を、２７個の損傷したウサギの角膜上皮に別々に適用した。試験する点眼液を一滴ずつ、損傷した角膜上皮に損傷後４時間、５時間、６時間および７時間ならびに損傷後１６時間から３０時間までは１時間ごとに加えた。ヨウ素処理後４時間、１６時間、２０時間、２４時間、２８時間および３２時間に、ウサギの角膜を２％フルオレセインで染色し、写真撮影した。こ角膜上皮の染色面積をコンピュータ画像解析装置で測定し、それぞれの角膜損傷の治癒速度を、ヨウ素処理による損傷後１６時間から３２時間までの期間における創傷面積の直線回帰によって算定した。Ｓｔｕｄｅｎｔのｔ検定を用いた。ヨウ素処理４時間後において充分な角膜上皮損傷の見られなかったウサギは、Ｓｍｉｒｎｏｖの方法によって除外した。角膜創傷治癒速度の結果は、下記表１１に示す。

【００６６】

【表１１】

	治癒速度　１６－３２時間、mm^2／ｈｒ	Studentのｔ検定（ｐ値）	眼の数
試料１	１．８０±０．０７	ｐ＜０．００１	２７
試料２	１．６６±０．０５	ｐ＜０．００５	２７
対照	１．４０±０．０５	－	２７

治癒速度：平均±ＳＥＭ

【００６７】表１１の結果が示すように、試料１と試料２との間では角膜治癒活性に有意差はなかった。この試験の結果、パラベン系防腐剤は点眼液中のフィブロネクチンの角膜治癒活性に影響しなかったことが判る。

【００６８】実施例　９

フィブロネクチンの角膜創傷閉止活性に及ぼす種々のパラベン系防腐剤の影響

ＰＢＳを用いて、点眼液を調製したが、パラベン系防腐剤は、０．０２％のパラヒドロキシ安息香酸エチルエステルと０．０１％のパラヒドロキシ安息香酸ブチルエステルの組合せとした。またエチレンジアミン四酢酸二ナトリウムの濃度は０．０１％とした（試料１）。フィブロネクチンの濃度が０．５ｍｇ／ｍｌである第二の点眼液を、ＰＢＳを用いて調製したが、パラベン系防腐剤

は、０．０２％のパラヒドロキシ安息香酸エチルエステルと０．０１％のパラヒドロキシ安息香酸ブチルエステルの組合せとし、またエチレンジアミン四酢酸二ナトリウムの濃度は０．０１％とした（試料２）。

【００６９】第三の点眼液をＰＢＳを用いて調製したが、パラベン系防腐剤は、０．０３８％のパラヒドロキシ安息香酸メチルエステルと０．０１５％のパラヒドロキシ安息香酸プロピルエステルの組合せとし、またエチレンジアミン四酢酸二ナトリウムの濃度は０．０５％とした（試料３）。

【００７０】フィブロネクチンの濃度が０．５ｍｇ／ｍｌである第四の点眼液をＰＢＳを用いて調製したが、、０．０３８％のパラヒドロキシ安息香酸メチルエステルと０．０１５％のパラヒドロキシ安息香酸プロピルエステルの組合せとし、またエチレンジアミン四酢酸二ナトリウムの濃度は０．０５％とした（試料４）。試料１～４を室温で７日間放置した。

【００７１】フィブロネクチンの角膜創傷閉止活性を、、Ｍｏｓｅｓら、１８　Ｉｎｖｅｓｔ．　Ｏｐｈｔｈａｌｍｏｌ．　１０３－１０６（１９７９）、およびＮｉｓｈｉｄａら、１０２、Ａｒｃｈ．　Ｏｐｈｔｈａｌｍｏｌ．　４５５－４５６（１９８４）に記載された方法に従って測定した。ウサギの角膜上皮を３分間ヨウ素蒸気処理することによって損傷した。試料１～４および対照を、１２個のウサギ損傷角膜上皮試料に別々に適用した。試験する点眼液を一滴ずつ、損傷した角膜上皮に損傷後４時間、５時間、６時間および７時間ならびに損傷後１６時間から３０時間までは１時間ごとに加えた。ヨウ素処理後４時間、１６時間、２０時間、２４時間、２８時間および３２時間に、ウサギの角膜を２％フルオレセインで染色し、写真撮影した。こ角膜上皮の染色面積をコンピュータ画像解析装置で測定し、それぞれの角膜損傷の治癒速度を、ヨウ素処理による損傷後１６時間から３２時間までの期間における創傷面積の直線回帰によって算定した。ヨウ素処理４時間後において充分な角膜上皮損傷の見られなかったウサギは、Ｓｍｉｒｎｏｖの方法によって除外した。角膜創傷治癒活性の結果は、下記表１２に示す。

【００７２】

【表１２】

フィブロネクチン濃度	治癒速度　１６－３２時間、mm^2／ｈｒ
試料１　１．０	１．７３±０．０８
試料２　０．５	１．３６±０．０８
試料３　１．０	１．７２±０．０５
試料４　０．５	１．５６±０．１２

治癒速度：平均±ＳＥＭ

【００７３】表７の結果が示すように、試料１と試料３との間および試料２と試料４との間では角膜創傷治癒活性に有意差はなかった。さらに、試料１と試料３の治癒速度は、実施例８における試料１と試料２の治癒速度に相当し、比肩し得るものであった。このことは、種々にパラベン系防腐剤を変えても、点眼液中におけるフィブロネクチンの角膜創傷治癒速度は影響を受けなかったことを示している。

【００７４】実施例　１０

グリシンを添加せずショ糖の存在下凍結乾燥したフィブロネクチンの溶解性

ＰＢＳ中での濃度が５　ｍｇ／ｍｌのフィブロネクチンを、０．０５Ｍまたは０．１Ｍの蔗糖とともに凍結乾燥した。この凍結乾燥したフィブロネクチンの可溶化度は、蒸留水を加え１０分後波数２８０ｎｍでの吸光度により測定した。可溶性タンパク質に基づいて、これらフィブロネクチンの溶解性は、それぞれ６６％と７１％であった。

【００７５】実施例　１１

グリシンの存在下凍結乾燥したフィブロネクチンの溶解性に及ぼすショ糖濃度の影響

フィブロネクチンを実施例１に記載と同様にして凍結乾燥した。ただし、５つの試料のそれぞれのショ糖濃度が下記表１３に示すようになるように、ショ糖濃度を調節した。室温で３０分間放置した後、それぞれの試料を３ｍｌの水に溶解した。全ての試料を完全に溶解させ、フィブロネクチンの溶解を完結させるのに要した時間を秒単位で測定し、表１３に示す。

【００７６】

【表１３】

ショ糖濃度　（Ｍ）	溶解完結に要した時間　（秒）
０．０５	７５－８０
０．０７５	４５－５０
０．１０	２０－２５
０．１２５	２０－２５
０．１０７	２５－３０

【００７７】フィブロネクチンをショ糖とグリシンの存在下凍結乾燥した場合、フィブロネクチンは完全に可溶性となるが、これに対してショ糖のみの存在下凍結乾燥した場合は、フィブロネクチンは実施例１０に示したように一部分的にしか可溶性とならない。表１３の結果に示されるように、フィブロネクチンの溶解速度はショ糖

の濃度に依存している。種々の改良・修正が、本発明の精神から逸脱することなく実施可能であることが理解されるであろう。

フロントページの続き

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PATENT APPLICATION FEE DETERMINATION RECORD Substitute for Form PTO-875	Application or Docket Number 14/096,346	Filing Date 12/04/2013	☐ To be Mailed

ENTITY: ☒ LARGE ☐ SMALL ☐ MICRO

APPLICATION AS FILED – PART I

	(Column 1)	(Column 2)		
FOR	NUMBER FILED	NUMBER EXTRA	RATE ($)	FEE ($)
☐ BASIC FEE (37 CFR 1.16(a), (b), or (c))	N/A	N/A	N/A	
☐ SEARCH FEE (37 CFR 1.16(k), (i), or (m))	N/A	N/A	N/A	
☐ EXAMINATION FEE (37 CFR 1.16(o), (p), or (q))	N/A	N/A	N/A	
TOTAL CLAIMS (37 CFR 1.16(i))	minus 20 =	*	X $ =	
INDEPENDENT CLAIMS (37 CFR 1.16(h))	minus 3 =	*	X $ =	
☐ APPLICATION SIZE FEE (37 CFR 1.16(s))	If the specification and drawings exceed 100 sheets of paper, the application size fee due is $310 ($155 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s).			
☐ MULTIPLE DEPENDENT CLAIM PRESENT (37 CFR 1.16(j))				
* If the difference in column 1 is less than zero, enter "0" in column 2.			TOTAL	

APPLICATION AS AMENDED – PART II

AMENDMENT	04/10/2015	(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA	RATE ($)	ADDITIONAL FEE ($)
	Total (37 CFR 1.16(i))	* 15	Minus	** 20	= 0	X $80 =	0
	Independent (37 CFR 1.16(h))	* 3	Minus	***3	= 0	X $420 =	0
	☐ Application Size Fee (37 CFR 1.16(s))						
	☐ FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))						
						TOTAL ADD'L FEE	0

AMENDMENT		(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA	RATE ($)	ADDITIONAL FEE ($)
	Total (37 CFR 1.16(i))	*	Minus	**	=	X $ =	
	Independent (37 CFR 1.16(h))	*	Minus	***	=	X $ =	
	☐ Application Size Fee (37 CFR 1.16(s))						
	☐ FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))						
						TOTAL ADD'L FEE	

* If the entry in column 1 is less than the entry in column 2, write "0" in column 3.
** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 20, enter "20".
*** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 3, enter "3".
The "Highest Number Previously Paid For" (Total or Independent) is the highest number found in the appropriate box in column 1.

LIE
/DEANNA RORIE/

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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
14/096,346	12/04/2013	Sandra O'Connor	552815: CPT-011USDV	2832

113613 7590 04/06/2015

Lathrop & Gage
28 State Street
Boston, MA 02109-1775

EXAMINER	
KOMATSU, LI N	

ART UNIT	PAPER NUMBER
1676	

NOTIFICATION DATE	DELIVERY MODE
04/06/2015	ELECTRONIC

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

bostonpatent@lathropgage.com
cubist_docketing@cardinal-ip.com

Applicant-Initiated Interview Summary	**Application No.** 14/096,346	**Applicant(s)** O'CONNOR ET AL.	
	Examiner LI NI KOMATSU	**Art Unit** 1676	

All participants (applicant, applicant's representative, PTO personnel):

(1) *LI NI KOMATSU*.

(2) *Julie Ha*.

(3) *Brian C. Trinque, Sandra O'Connor*.

(4) *Dianne Pecoraro, Laura Ginkel*.

Date of Interview: *3/31/2015*.

Type: ☐ Telephonic ☐ Video Conference
☒ Personal [copy given to: ☐ applicant ☐ applicant's representative]

Exhibit shown or demonstration conducted: ☐ Yes ☒ No.
If Yes, brief description: _____.

Issues Discussed ☒101 ☒112 ☒102 ☒103 ☐Others
(For each of the checked box(es) above, please describe below the issue and detailed description of the discussion)

Claim(s) discussed: _____.

Identification of prior art discussed: _____.

Substance of Interview
(For each issue discussed, provide a detailed description and indicate if agreement was reached. Some topics may include: identification or clarification of a reference or a portion thereof, claim interpretation, proposed amendments, arguments of any applied references etc...)

Applicant's representative, Brian C. Trinque, requested an interview to discuss the rejections set forth in the non-final office action mailed on 12/10/2014. Possible claim amendments to overcome the rejections have been discussed. Applicant will file response to the office action.

Applicant recordation instructions: The formal written reply to the last Office action must include the substance of the interview. (See MPEP section 713.04). If a reply to the last Office action has already been filed, applicant is given a non-extendable period of the longer of one month or thirty days from this interview date, or the mailing date of this interview summary form, whichever is later, to file a statement of the substance of the interview

Examiner recordation instructions: Examiners must summarize the substance of any interview of record. A complete and proper recordation of the substance of an interview should include the items listed in MPEP 713.04 for complete and proper recordation including the identification of the general thrust of each argument or issue discussed, a general indication of any other pertinent matters discussed regarding patentability and the general results or outcome of the interview, to include an indication as to whether or not agreement was reached on the issues raised.

☐ Attachment

/JULIE HA/ Primary Examiner, Art Unit 1675	/LI NI KOMATSU/ Examiner, Art Unit 1676

Summary of Record of Interview Requirements

Manual of Patent Examining Procedure (MPEP), Section 713.04, Substance of Interview Must be Made of Record
A complete written statement as to the substance of any face-to-face, video conference, or telephone interview with regard to an application must be made of record in the application whether or not an agreement with the examiner was reached at the interview.

Title 37 Code of Federal Regulations (CFR) § 1.133 Interviews
Paragraph (b)

In every instance where reconsideration is requested in view of an interview with an examiner, a complete written statement of the reasons presented at the interview as warranting favorable action must be filed by the applicant. An interview does not remove the necessity for reply to Office action as specified in §§ 1.111, 1.135. (35 U.S.C. 132)

37 CFR §1.2 Business to be transacted in writing.
All business with the Patent or Trademark Office should be transacted in writing. The personal attendance of applicants or their attorneys or agents at the Patent and Trademark Office is unnecessary. The action of the Patent and Trademark Office will be based exclusively on the written record in the Office. No attention will be paid to any alleged oral promise, stipulation, or understanding in relation to which there is disagreement or doubt.

———

The action of the Patent and Trademark Office cannot be based exclusively on the written record in the Office if that record is itself incomplete through the failure to record the substance of interviews.

It is the responsibility of the applicant or the attorney or agent to make the substance of an interview of record in the application file, unless the examiner indicates he or she will do so. It is the examiner's responsibility to see that such a record is made and to correct material inaccuracies which bear directly on the question of patentability.

Examiners must complete an Interview Summary Form for each interview held where a matter of substance has been discussed during the interview by checking the appropriate boxes and filling in the blanks. Discussions regarding only procedural matters, directed solely to restriction requirements for which interview recordation is otherwise provided for in Section 812.01 of the Manual of Patent Examining Procedure, or pointing out typographical errors or unreadable script in Office actions or the like, are excluded from the interview recordation procedures below. Where the substance of an interview is completely recorded in an Examiners Amendment, no separate Interview Summary Record is required.

The Interview Summary Form shall be given an appropriate Paper No., placed in the right hand portion of the file, and listed on the "Contents" section of the file wrapper. In a personal interview, a duplicate of the Form is given to the applicant (or attorney or agent) at the conclusion of the interview. In the case of a telephone or video-conference interview, the copy is mailed to the applicant's correspondence address either with or prior to the next official communication. If additional correspondence from the examiner is not likely before an allowance or if other circumstances dictate, the Form should be mailed promptly after the interview rather than with the next official communication.

The Form provides for recordation of the following information:
- Application Number (Series Code and Serial Number)
- Name of applicant
- Name of examiner
- Date of interview
- Type of interview (telephonic, video-conference, or personal)
- Name of participant(s) (applicant, attorney or agent, examiner, other PTO personnel, etc.)
- An indication whether or not an exhibit was shown or a demonstration conducted
- An identification of the specific prior art discussed
- An indication whether an agreement was reached and if so, a description of the general nature of the agreement (may be by attachment of a copy of amendments or claims agreed as being allowable). Note: Agreement as to allowability is tentative and does not restrict further action by the examiner to the contrary.
- The signature of the examiner who conducted the interview (if Form is not an attachment to a signed Office action)

It is desirable that the examiner orally remind the applicant of his or her obligation to record the substance of the interview of each case. It should be noted, however, that the Interview Summary Form will not normally be considered a complete and proper recordation of the interview unless it includes, or is supplemented by the applicant or the examiner to include, all of the applicable items required below concerning the substance of the interview.

A complete and proper recordation of the substance of any interview should include at least the following applicable items:
1) A brief description of the nature of any exhibit shown or any demonstration conducted,
2) an identification of the claims discussed,
3) an identification of the specific prior art discussed,
4) an identification of the principal proposed amendments of a substantive nature discussed, unless these are already described on the Interview Summary Form completed by the Examiner,
5) a brief identification of the general thrust of the principal arguments presented to the examiner,
   (The identification of arguments need not be lengthy or elaborate. A verbatim or highly detailed description of the arguments is not required. The identification of the arguments is sufficient if the general nature or thrust of the principal arguments made to the examiner can be understood in the context of the application file. Of course, the applicant may desire to emphasize and fully describe those arguments which he or she feels were or might be persuasive to the examiner.)
6) a general indication of any other pertinent matters discussed, and
7) if appropriate, the general results or outcome of the interview unless already described in the Interview Summary Form completed by the examiner.

Examiners are expected to carefully review the applicant's record of the substance of an interview. If the record is not complete and accurate, the examiner will give the applicant an extendable one month time period to correct the record.

Examiner to Check for Accuracy

If the claims are allowable for other reasons of record, the examiner should send a letter setting forth the examiner's version of the statement attributed to him or her. If the record is complete and accurate, the examiner should place the indication, "Interview Record OK" on the paper recording the substance of the interview along with the date and the examiner's initials.

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APPLICATION NUMBER	FILING OR 371(C) DATE	FIRST NAMED APPLICANT	ATTY. DOCKET NO./TITLE
14/096,346	12/04/2013	Sandra O'Connor	552815: CPT-011USDV

CONFIRMATION NO. 2832

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Lathrop & Gage
28 State Street
Boston, MA 02109-1775

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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
14/096,346	12/04/2013	Sandra O'Connor	552815: CPT-011USDV	2832

113613 7590 12/10/2014

Lathrop & Gage
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EXAMINER	
KOMATSU, LI N	

ART UNIT	PAPER NUMBER
1676	

NOTIFICATION DATE	DELIVERY MODE
12/10/2014	ELECTRONIC

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

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Office Action Summary	Application No. 14/096,346	Applicant(s) O'CONNOR ET AL.	
	Examiner LI NI KOMATSU	Art Unit 1676	AIA (First Inventor to File) Status No

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE *3* MONTHS FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *9/24/2014*.

☐ A declaration(s)/affidavit(s) under **37 CFR 1.130(b)** was/were filed on _____.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ An election was made by the applicant in response to a restriction requirement set forth during the interview on _____; the restriction requirement and election have been incorporated into this action.

4)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims*

5)☒ Claim(s) *22-42* is/are pending in the application.

5a) Of the above claim(s) _____ is/are withdrawn from consideration.

6)☐ Claim(s) _____ is/are allowed.

7)☒ Claim(s) *22-42* is/are rejected.

8)☒ Claim(s) *31 and 41* is/are objected to.

9)☐ Claim(s) _____ are subject to restriction and/or election requirement.

* If any claims have been determined allowable, you may be eligible to benefit from the **Patent Prosecution Highway** program at a participating intellectual property office for the corresponding application. For more information, please see http://www.uspto.gov/patents/init_events/pph/index.jsp or send an inquiry to PPHfeedback@uspto.gov.

Application Papers

10)☒ The specification is objected to by the Examiner.

11)☒ The drawing(s) filed on *12/4/2013* is/are: a)☐ accepted or b)☒ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

Certified copies:

a)☐ All b)☐ Some** c)☐ None of the:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

** See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)

2) ☒ Information Disclosure Statement(s) (PTO/SB/08a and/or PTO/SB/08b) Paper No(s)/Mail Date *4/1/2014 and 4 IDS on 1/6/2014*.

3) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .

4) ☐ Other: _____.

DETAILED ACTION

1. The present application is being examined under the pre-AIA first to invent provisions.

2. Response to Election/Restriction filed on 9/24/2014 is acknowledged.

3. Claims 1-21 have been cancelled.

4. New claims 22-42 have been added.

5. Claims 22-42 are pending in this application.

Election/Restrictions

6. Applicant's election **without traverse** of sucrose as species of excipient; a molar ratio of daptomycin to the sugar of about 1:1.12 to about 1:21.32 as recited in claim 25 as species of molar ratio of daptomycin to the sugar; a pH of 6.5-7.5 as recited in claim 37 as species of pH; a phosphate buffering agent as species of buffering agent; and converting the aqueous daptomycin solution to a solid pharmaceutical composition by lyophilization as recited in claim 40 as species of way to convert the aqueous daptomycin solution to a solid pharmaceutical composition in the reply filed on 9/24/2014 is acknowledged.

The instant claims 22-42 are drawn to a solid pharmaceutical daptomycin composition comprising daptomycin and at least one excipient selected from glycine and a sugar, wherein an amount of the solid pharmaceutical daptomycin composition comprising 500 mg of daptomycin dissolves in 10 mL of 0.9% aqueous sodium chloride in less than 5 minutes at about 25 degrees C; and a pharmaceutical product comprising such solid daptomycin composition and a pharmaceutically acceptable diluent. A search was conducted on the elected species and prior art was found. Claims 22-42 are examined on the merits in this office action.

Objections

7. The use of trademarks has been noted in this application, for example, CUBICIN® (see page 1, line 20 and many others in instant specification). Each letter of the trademarks should be capitalized wherever it appears and be accompanied by the generic terminology. Although the use of trademarks is

permissible in patent applications, the proprietary nature of the marks should be respected and every effort made to prevent their use in any manner which might adversely affect their validity as trademarks.

8. The specification is objected to for the following minor informality: The specification recites "Unexpectedly, combining daptomycin with one or more non-reducing sugars (e.g., **sucrose**, trehalose, **sucrose** and mannitol) in a solid pharmaceutical preparation…" on page 11, lines 21-22 of instant specification. There appears to be an extra "sucrose" in the recitation. Applicant is required to correct this error.

Please note, the specification has not been checked to the extent necessary to determine the presence of all possible error. Applicant's cooperation is required in correcting any errors of which applicant may become aware in the specification. MPEP § 608.01.

9. The drawings are objected to for the following minor informality:

Figures 1, 2 and 4: It is unclear what "5" in the figures is referring to.

Corrected drawing sheets in compliance with 37 CFR 1.121(d) are required in reply to the Office action to avoid abandonment of the application. Any amended replacement drawing sheet should include all of the figures appearing on the immediate prior version of the sheet, even if only one figure is being amended. The figure or figure number of an amended drawing should not be labeled as "amended." If a drawing figure is to be canceled, the appropriate figure must be removed from the replacement sheet, and where necessary, the remaining figures must be renumbered and appropriate changes made to the brief description of the several views of the drawings for consistency. Additional replacement sheets may be necessary to show the renumbering of the remaining figures. Each drawing sheet submitted after the filing date of an application must be labeled in the top margin as either "Replacement Sheet" or "New Sheet" pursuant to 37 CFR 1.121(d). If the changes are not accepted by the examiner, the applicant will be notified and informed of any required corrective action in the next Office action. The objection to the drawings will not be held in abeyance.

10. Claim 31 is objected to for the following minor informality: Claim 31 recites "he solid composition…". This appears to be a typo. The recitation should be "The solid composition... ". Applicant is required to correct this error.

11. Claim 41 is objected to for the following minor informality: Claim 41 recites the term "containing". Applicant is suggested to amend the recitation to "comprising".

Rejections

Claim Rejections - 35 USC § 101

12. 35 U.S.C. 101 reads as follows:

> Whoever invents or discovers any new and useful process, machine, manufacture, or composition of matter, or any new and useful improvement thereof, may obtain a patent therefor, subject to the conditions and requirements of this title.

13. Claims 22-42 are rejected under 35 U.S.C. 101 because the claimed invention is not directed to patent eligible subject matter. Based upon an analysis with respect to the claim as a whole, claims 22-42 are determined to be directed to a law of nature/natural principle. The rationale for this determination is explained below: The claimed solid pharmaceutical daptomycin composition and a pharmaceutical product comprising such solid daptomycin composition and a pharmaceutically acceptable diluent in instant claims 22-42 recite multiple natural products. For example, daptomycin is a naturally occurring peptide, as evidenced by instant specification. Lactose is a natural product, as evidenced by Food source of lactose (from Dietitians of Canada, enclosed pages 1-3). Sucrose is a natural product, as evidenced by Source of sucrose (from http://www.ehow.com/about_5376127_sources-sucrose.html, enclosed pages 1-2). Mannitol is a natural product, as evidenced by Mannitol (enclosed pages 1-2, from http://www.drugs.com/inactive/mannitol-142.html?printable=1). Trehalose is a natural product, as evidenced by Richards et al (Food and Chemical Toxicology, 2002, 40, pages 871-898). In the broadest reasonable interpretation, water is a pharmaceutically acceptable diluent; and water is a natural product, as evidenced by Water (from http://www.biology-online.org/dictionary/Water, enclosed pages 1-3). Detailed analysis of all the factors is as follows:

Factors that weigh toward eligibility (significantly different):

a) Claim is a product claim reciting something that initially appears to be a natural product, but after analysis is determined to be non-naturally occurring and markedly different in structure from naturally occurring products.

Factor a) is not satisfied, because the claimed solid pharmaceutical daptomycin composition and a pharmaceutical product comprising such solid daptomycin composition and a pharmaceutically acceptable diluent in instant claims 22-42 do not recite features or steps demonstrating a marked difference from what exists in nature; and the claimed solid pharmaceutical daptomycin composition and a pharmaceutical product comprising such solid daptomycin composition and a pharmaceutically acceptable diluent in instant claims 22-42 do not recite meaningful limitations that add something of significance to the judicial exception.

b) Claim recites elements/steps in addition to the judicial exception(s) that impose meaningful limits on claim scope, i.e., the elements/steps narrow the scope of the claim so that others are not substantially foreclosed from using the judicial exception(s).

Factor b) is not relevant, because the claimed solid pharmaceutical daptomycin composition and a pharmaceutical product comprising such solid daptomycin composition and a pharmaceutically acceptable diluent in instant claims 22-42 do not include any elements/steps in addition to the natural products.

c) Claim recites elements/steps in addition to the judicial exception(s) that relate to the judicial exception in a significant way, i.e., the elements/steps are more than nominally, insignificantly, or tangentially related to the judicial exception(s).

Factor c) is not relevant, because the claimed solid pharmaceutical daptomycin composition and a pharmaceutical product comprising such solid daptomycin composition and a pharmaceutically acceptable diluent in instant claims 22-42 do not include any elements/steps in addition to the natural products.

d) Claim recites elements/steps in addition to the judicial exception(s) that do more than describe the judicial exception(s) with general instructions to apply or use the judicial exception(s).

Factor d) is not relevant, because the claimed solid pharmaceutical daptomycin composition and a pharmaceutical product comprising such solid daptomycin composition and a pharmaceutically acceptable diluent in instant claims 22-42 do not include any elements/steps in addition to the natural products.

e) Claim recites elements/steps in addition to the judicial exception(s) that include a particular machine or transformation of a particular article, where the particular machine/transformation implements one or more judicial exception(s) or integrates the judicial exception(s) into a particular practical application. (See MPEP 2106(II)(B)(1) for an explanation of the machine or transformation factors).

Factor e) is not relevant, because the claimed solid pharmaceutical daptomycin composition and a pharmaceutical product comprising such solid daptomycin composition and a pharmaceutically acceptable diluent in instant claims 22-42 do not include any elements/steps in addition to the natural products.

f) Claim recites one or more elements/steps in addition to the judicial exception(s) that add a feature that is more than well-understood, purely conventional or routine in the relevant field.

Factor f) is not relevant, because the claimed solid pharmaceutical daptomycin composition and a pharmaceutical product comprising such solid daptomycin composition and a pharmaceutically acceptable diluent in instant claims 22-42 do not include any elements/steps in addition to the natural products.

Factors that weigh against eligibility (not significantly different):

g) Claim is a product claim reciting something that appears to be a natural product that is not markedly different in structure from naturally occurring products.

Factor g) is satisfied, because the claimed solid pharmaceutical daptomycin composition and a pharmaceutical product comprising such solid daptomycin composition and a pharmaceutically acceptable diluent in instant claims 22-42 do not recite features or steps demonstrating a marked difference from what exists in nature; and the claimed solid pharmaceutical daptomycin composition and a pharmaceutical product comprising such solid daptomycin composition and a pharmaceutically acceptable diluent in instant claims 22-42 do not recite meaningful limitations that add something of significance to the judicial exception.

h) Claim recites elements/steps in addition to the judicial exception(s) at a high level of generality such that substantially all practical applications of the judicial exception(s) are covered.

Factor h) is not relevant, because the claimed solid pharmaceutical daptomycin composition and a pharmaceutical product comprising such solid daptomycin composition and a pharmaceutically acceptable diluent in instant claims 22-42 do not include any elements/steps in addition to the natural products.

i) Claim recites elements/steps in addition to the judicial exception(s) that must be used/taken by others to apply the judicial exception(s).

Factor i) is not relevant, because the claimed solid pharmaceutical daptomycin composition and a pharmaceutical product comprising such solid daptomycin composition and a pharmaceutically acceptable diluent in instant claims 22-42 do not include any elements/steps in addition to the natural products.

j) Claim recites elements/steps in addition to the judicial exception(s) that are well-understood, purely conventional or routine in the relevant field.

Factor j) is not relevant, because the claimed solid pharmaceutical daptomycin composition and a pharmaceutical product comprising such solid daptomycin composition and a pharmaceutically

acceptable diluent in instant claims 22-42 do not include any elements/steps in addition to the natural products.

k) Claim recites elements/steps in addition to the judicial exception(s) that are insignificant extra-solution activity, e.g., are merely appended to the judicial exception(s).

Factor k) is not relevant, because the claimed solid pharmaceutical daptomycin composition and a pharmaceutical product comprising such solid daptomycin composition and a pharmaceutically acceptable diluent in instant claims 22-42 do not include any elements/steps in addition to the natural products.

l) Claim recites elements/steps in addition to the judicial exception(s) that amount to nothing more than a mere field of use.

Factor l) is not relevant, because the claimed solid pharmaceutical daptomycin composition and a pharmaceutical product comprising such solid daptomycin composition and a pharmaceutically acceptable diluent in instant claims 22-42 do not include any elements/steps in addition to the natural products.

Considering all the relevant factors, the claimed solid pharmaceutical daptomycin composition and a pharmaceutical product comprising such solid daptomycin composition and a pharmaceutically acceptable diluent in instant claims 22-42 are not significantly different than a judicial exception (natural product).

Claim Rejections - 35 USC § 112 second paragraph

14. The following is a quotation of 35 U.S.C. 112(b):
(B) CONCLUSION.—The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the inventor or a joint inventor regards as the invention.

The following is a quotation of 35 U.S.C. 112 (pre-AIA), second paragraph:
The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

15. Claims 22-42 are rejected under 35 U.S.C. 112(b) or 35 U.S.C. 112 (pre-AIA), second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which the inventor or a joint inventor, or for pre-AIA the applicant regards as the invention.

16. Claims 22 and 41 are indefinite, because claim 22 recites "wherein an amount of the solid pharmaceutical daptomycin composition comprising 500 mg of daptomycin dissolves in 10 mL of 0.9% aqueous sodium chloride in less than 5 minutes at about 25 degrees C"; and claim 41 recites "wherein an amount of the solid pharmaceutical daptomycin composition containing 500 mg of daptomycin dissolves in 10 mL of 0.9% aqueous sodium chloride in less than 2 minutes at 25 degrees C". However, the speed of dissolving the solid pharmaceutical daptomycin composition depends on many conditions, such as the physical form of the solid, the type of mixing involved and many others. For example, a loose powder would dissolve faster than a solid chunk. Because claims 23-40 and 42 depend from indefinite claim 22, and do not clarify the point of confusion, they must also be rejected under 35 U.S.C. 112(b) or 35 U.S.C. 112 (pre-AIA), second paragraph.

Claim Rejections - 35 USC § 112 fourth paragraph

17. The following is a quotation of 35 U.S.C. 112(d):

> (d) REFERENCE IN DEPENDENT FORMS.—Subject to subsection (e), a claim in dependent form shall contain a reference to a claim previously set forth and then specify a further limitation of the subject matter claimed. A claim in dependent form shall be construed to incorporate by reference all the limitations of the claim to which it refers.
>
> The following is a quotation of 35 U.S.C. 112 (pre-AIA), fourth paragraph:
> Subject to the [fifth paragraph of 35 U.S.C. 112 (pre-AIA)], a claim in dependent form shall contain a reference to a claim previously set forth and then specify a further limitation of the subject matter claimed. A claim in dependent form shall be construed to incorporate by reference all the limitations of the claim to which it refers.

18. Claim 41 is rejected under 35 U.S.C. 112(d) or 35 U.S.C. 112 (pre-AIA), 4th paragraph, as being of improper dependent form for failing to further limit the subject matter of the claim upon which it depends, or for failing to include all the limitations of the claim upon which it depends. Applicant may cancel the claim(s), amend the claim(s) to place the claim(s) in proper dependent form, rewrite the

claim(s) in independent form, or present a sufficient showing that the dependent claim(s) complies with the statutory requirements.

19. Claim 41 depends on claim 22; and claim 41 recites "The solid pharmaceutical daptomycin composition of claim 22, wherein an amount of the solid pharmaceutical daptomycin composition containing 500 mg of daptomycin dissolves in 10 mL of 0.9% aqueous sodium chloride in less than 2 minutes at about 25 degrees C.". Claim 41 recites inherent property of the solid pharmaceutical daptomycin composition of claim 22. Claim 41 does not further limit the structure of the solid pharmaceutical daptomycin composition of claim 22; therefore, claim 41 is improper dependent form for failing to further limit the subject matter of claim 22.

Claim Rejections - 35 USC § 102

20. The following is a quotation of the appropriate paragraphs of pre-AIA 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

> A person shall be entitled to a patent unless –
> (b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

21. Please note, during the search for the elected species, prior art was found for the non-elected species of excipient.

Claims 22-25, 27 and 32-42 are rejected under pre-AIA 35 U.S.C. 102(b) as being anticipated by Inman et al (EP 0386951 A2, filed with IDS).

The instant claims 22-25, 27 and 32-42 are drawn to a solid pharmaceutical daptomycin composition comprising daptomycin and at least one excipient selected from glycine and a sugar, wherein an amount of the solid pharmaceutical daptomycin composition comprising 500 mg of daptomycin dissolves in 10 mL of 0.9% aqueous sodium chloride in less than 5 minutes at about 25 degrees C; and a pharmaceutical product comprising such solid daptomycin composition and a pharmaceutically acceptable diluent.

Inman et al teach a solid pharmaceutical daptomycin composition comprising 150 mg daptomycin and 50 mg mannitol obtained by freeze-drying/lyophilization, and reconstituting such solid pharmaceutical daptomycin composition in a pharmaceutically acceptable diluent, such as isotonic sodium phosphate dibasic solution, for example, page 3, line 50 to page 4, line13. The molar ratio of 150 mg daptomycin to 50 mg mannitol is about 1:2.96. It reads on a molar ratio of daptomycin to the sugar of about 1:1.12 to about 1:21.32 as the elected species of molar ratio of daptomycin to the sugar. It meets the limitation of instant claims 22-25, 27, 32, 33 and 42.

The MPEP § 2112 states: "Once a reference teaching product appearing to be substantially identical is made the basis of a rejection, and the Examiner presents evidence or reasoning tending to show inherency, the burden shifts to the Applicant to show an unobvious difference '[t]he PTO can require an Applicant to prove that the prior art products do not necessarily or inherently possess the characteristics of his [or her] claimed product. Whether the rejection is based on inherency' under 35 U.S.C. 102, on *prima facie* obviousness' under 35 U.S.C. 103, jointly or alternatively, the burden of proof is the same...[footnote omitted]." The burden of proof is similar to that required with respect to product-by-process claims. *In re Fitzgerald*, 619 F.2d 67, 70, 205 USPQ 594, 596 (CCPA 1980) (quoting *In re Best*, 562 F.2d 1252, 1255, 195 USPQ 430, 433-34 (CCPA 1977))." Since the solid pharmaceutical daptomycin composition comprising 150 mg daptomycin and 50 mg mannitol in Inman et al meets all the limitation of the solid pharmaceutical daptomycin composition in instant claim 22, the solid pharmaceutical daptomycin composition comprising 150 mg daptomycin and 50 mg mannitol in Inman et al would necessarily have the same properties and functionality of the solid pharmaceutical daptomycin composition in instant claim 22. Therefore, the solid pharmaceutical daptomycin composition comprising 150 mg daptomycin and 50 mg mannitol in Inman et al has the property that an amount of the solid pharmaceutical daptomycin composition comprising 500 mg of daptomycin dissolves in 10 mL of 0.9% aqueous sodium chloride in less than 5 minutes at about 25 degrees C; and an amount of the solid pharmaceutical daptomycin composition containing 500 mg of daptomycin dissolves in 10 mL of 0.9% aqueous sodium chloride in less than 2 minutes at 25 degrees C.

Claims 34-40 recite product by process claim for the solid pharmaceutical daptomycin composition preparation. The MPEP states the following: "[E]ven though product-by-process claims are limited by and defined by the process determination of patentability is based on the product itself. The patentability of a product does not depend on its method of production. If the product in the product-by-process claim is the same as or obvious from a product of the prior art, the claim is unpatentable even though the prior product was made by a different process...The product-by-process claim was rejected because the end product, in both the prior art and the allowed process, ends up containing metal carboxylate. The fact that the metal carboxylate is not directly added, but is instead produced in-situ does not change the end product" (see MPEP § 2113 [R-I]).

Since the reference teaches all the limitation of instant claims 22-25, 27 and 32-42, the reference anticipates instant claims 22-25, 27 and 32-42.

22. Please note, during the search for the elected species, prior art was found for the non-elected species of excipient.

Claims 22-26 and 34-42 are rejected under pre-AIA 35 U.S.C. 102(b) as being anticipated by Wei et al (CN 1616083 A, machine translation used, filed with IDS).

The instant claims 22-26 and 34-42 are drawn to a solid pharmaceutical daptomycin composition comprising daptomycin and at least one excipient selected from glycine and a sugar, wherein an amount of the solid pharmaceutical daptomycin composition comprising 500 mg of daptomycin dissolves in 10 mL of 0.9% aqueous sodium chloride in less than 5 minutes at about 25 degrees C; and a pharmaceutical product comprising such solid daptomycin composition and a pharmaceutically acceptable diluent.

Wei et al teach a solid pharmaceutical daptomycin composition comprising 250 mg daptomycin and 100 mg lactose in a bottle obtained by freeze-drying/lyophilization, and reconstituting the solid pharmaceutical daptomycin preparation comprising 125 to 500 mg daptomycin in a pharmaceutically acceptable diluent such as 3 or 10 ml water to obtain a reconstituted pharmaceutical daptomycin composition for intravenous administration, for example, Abstract; claims 1-5; page 4, the 2nd paragraph; and pages 7-8, Embodiment 2. The molar ratio of 250 mg daptomycin to 100 mg lactose is about 1:189.

It reads on a molar ratio of daptomycin to the sugar of about 1:1.12 to about 1:21.32 as the elected species of molar ratio of daptomycin to the sugar. It meets the limitation of instant claims 22-26 and 42. Wei et al further teach the solid pharmaceutical daptomycin preparation is a powder preparation that can dissolve rapidly, for example, page 4, the 2nd paragraph.

The MPEP § 2112 states: "Once a reference teaching product appearing to be substantially identical is made the basis of a rejection, and the Examiner presents evidence or reasoning tending to show inherency, the burden shifts to the Applicant to show an unobvious difference '[t]he PTO can require an Applicant to prove that the prior art products do not necessarily or inherently possess the characteristics of his [or her] claimed product. Whether the rejection is based on inherency' under 35 U.S.C. 102, on *prima facie* obviousness' under 35 U.S.C. 103, jointly or alternatively, the burden of proof is the same...[footnote omitted]." The burden of proof is similar to that required with respect to product-by-process claims. *In re Fitzgerald*, 619 F.2d 67, 70, 205 USPQ 594, 596 (CCPA 1980) (quoting *In re Best*, 562 F.2d 1252, 1255, 195 USPQ 430, 433-34 (CCPA 1977))." Since the solid pharmaceutical daptomycin composition comprising 250 mg daptomycin and 100 mg lactose in Wei et al meets all the limitation of the solid pharmaceutical daptomycin composition in instant claim 22, the solid pharmaceutical daptomycin composition comprising 250 mg daptomycin and 100 mg lactose in Wei et al would necessarily have the same properties and functionality of the solid pharmaceutical daptomycin composition in instant claim 22. Therefore, the solid pharmaceutical daptomycin composition comprising 250 mg daptomycin and 100 mg lactose in Wei et al has the property that an amount of the solid pharmaceutical daptomycin composition comprising 500 mg of daptomycin dissolves in 10 mL of 0.9% aqueous sodium chloride in less than 5 minutes at about 25 degrees C; and an amount of the solid pharmaceutical daptomycin composition containing 500 mg of daptomycin dissolves in 10 mL of 0.9% aqueous sodium chloride in less than 2 minutes at 25 degrees C.

Claims 34-40 recite product by process claim for the solid pharmaceutical daptomycin composition preparation. The MPEP states the following: "[E]ven though product-by-process claims are limited by and defined by the process determination of patentability is based on the product itself. The patentability of a product does not depend on its method of production. If the product in the product-by-

process claim is the same as or obvious from a product of the prior art, the claim is unpatentable even though the prior product was made by a different process...The product-by-process claim was rejected because the end product, in both the prior art and the allowed process, ends up containing metal carboxylate. The fact that the metal carboxylate is not directly added, but is instead produced in-situ does not change the end product" (see MPEP § 2113 [R-I]).

Since the reference teaches all the limitation of instant claims 22-26 and 34-42, the reference anticipates instant claims 22-26 and 34-42.

Claim Rejections - 35 USC § 103

23. The following is a quotation of pre-AIA 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

24. The factual inquiries set forth in *Graham v. John Deere Co.*, 383 U.S. 1, 148 USPQ 459 (1966), that are applied for establishing a background for determining obviousness under pre-AIA 35 U.S.C. 103(a) are summarized as follows:

1. Determining the scope and contents of the prior art.

2. Ascertaining the differences between the prior art and the claims at issue.

3. Resolving the level of ordinary skill in the pertinent art.

4. Considering objective evidence present in the application indicating obviousness or nonobviousness.

25. Claims 22-31 and 34-42 are rejected under pre-AIA 35 U.S.C. 103(a) as being unpatentable over Wei et al (CN 1616083 A, machine translation used, filed with IDS) in view of Smales et al (Therapeutic proteins, methods and protocols, Humana press, 2005, pages 287-292, filed with IDS).

The instant claims 22-31 and 34-42 are drawn to a solid pharmaceutical daptomycin composition comprising daptomycin and at least one excipient selected from glycine and a sugar, wherein an amount of the solid pharmaceutical daptomycin composition comprising 500 mg of daptomycin dissolves in 10 mL of 0.9% aqueous sodium chloride in less than 5 minutes at about 25 degrees C; and a pharmaceutical product comprising such solid daptomycin composition and a pharmaceutically acceptable diluent.

Wei et al teach a solid pharmaceutical daptomycin composition comprising 250 mg daptomycin and 100 mg lactose in a bottle obtained by freeze-drying/lyophilization, and reconstituting the solid pharmaceutical daptomycin preparation comprising 125 to 500 mg daptomycin in a pharmaceutically acceptable diluent such as 3 or 10 ml water to obtain a reconstituted pharmaceutical daptomycin composition for intravenous administration, for example, Abstract; claims 1-5; page 4, the 2nd paragraph; and pages 7-8, Embodiment 2. The molar ratio of 250 mg daptomycin to 100 mg lactose is about 1:189. It reads on a molar ratio of daptomycin to the sugar of about 1:1.12 to about 1:21.32 as the elected species of molar ratio of daptomycin to the sugar. It meets the limitation of instant claims 22-26 and 42. Wei et al further teach the solid pharmaceutical daptomycin preparation is a powder preparation that can dissolve rapidly, for example, page 4, the 2nd paragraph.

The MPEP § 2112 states: "Once a reference teaching product appearing to be substantially identical is made the basis of a rejection, and the Examiner presents evidence or reasoning tending to show inherency, the burden shifts to the Applicant to show an unobvious difference '[t]he PTO can require an Applicant to prove that the prior art products do not necessarily or inherently possess the characteristics of his [or her] claimed product. Whether the rejection is based on inherency' under 35 U.S.C. 102, on *prima facie* obviousness' under 35 U.S.C. 103, jointly or alternatively, the burden of proof is the same...[footnote omitted]." The burden of proof is similar to that required with respect to product-by-process claims. *In re Fitzgerald*, 619 F.2d 67, 70, 205 USPQ 594, 596 (CCPA 1980) (quoting *In re Best*, 562 F.2d 1252, 1255, 195 USPQ 430, 433-34 (CCPA 1977))." Since the solid pharmaceutical daptomycin composition comprising 250 mg daptomycin and 100 mg lactose in Wei et al meets all the limitation of the solid pharmaceutical daptomycin composition in instant claim 22, the solid pharmaceutical daptomycin composition comprising 250 mg daptomycin and 100 mg lactose in Wei et al would

necessarily have the same properties and functionality of the solid pharmaceutical daptomycin composition in instant claim 22. Therefore, the solid pharmaceutical daptomycin composition comprising 250 mg daptomycin and 100 mg lactose in Wei et al has the property that an amount of the solid pharmaceutical daptomycin composition comprising 500 mg of daptomycin dissolves in 10 mL of 0.9% aqueous sodium chloride in less than 5 minutes at about 25 degrees C; and an amount of the solid pharmaceutical daptomycin composition containing 500 mg of daptomycin dissolves in 10 mL of 0.9% aqueous sodium chloride in less than 2 minutes at 25 degrees C.

Claims 34-40 recite product by process claim for the solid pharmaceutical daptomycin composition preparation. The MPEP states the following: "[E]ven though product-by-process claims are limited by and defined by the process determination of patentability is based on the product itself. The patentability of a product does not depend on its method of production. If the product in the product-by-process claim is the same as or obvious from a product of the prior art, the claim is unpatentable even though the prior product was made by a different process...The product-by-process claim was rejected because the end product, in both the prior art and the allowed process, ends up containing metal carboxylate. The fact that the metal carboxylate is not directly added, but is instead produced in-situ does not change the end product" (see MPEP § 2113 [R-I]).

The difference between the reference and the instant claims 22-31 and 34-42 is that the reference does not teach sucrose or trehalose as excipient recited in instant claims 27-31; and sucrose as the elected species of excipient.

However, Smales et al, throughout the literature, teach that therapeutic proteins/peptides can be stabilized by adding protein-stabilizers, such as sugar at concentration of 10-100 mg/ml, in the process of formulation, and nonreducing disaccharides, such as sucrose and trehalose, are the most potent and useful excipients to protect protein conformation in aqueous solutions and freeze-dried solids, and sucrose has been widely used in various pharmaceutical formulation, for example, page 288, Table 2; page 289, "*3.2. Formulation Design*"; and pages 290-291, "*3.3.2. Sugars*". It reads on sucrose as the elected species of excipient. Smales et al further teach effective stabilization of protein conformation in aqueous solutions requires relatively high concentrations (approx. > 0.3 M) of disaccharide, and an

approx. 1:1 weight-concentration ratio of disaccharides is needed to freeze-dry proteins without structural changes, and the sugars also stabilize protein conformation in other dehydrating formulation (spray-drying), for example, page 291, paragraph 1. A 1:1 weight ratio of daptomycin to sucrose is a molar ratio of daptomycin to the sugar of about 1:4.79. A 1:1 weight ratio of daptomycin to trehalose is a molar ratio of daptomycin to the sugar of about 1:9.10.

Therefore, it would have been obvious to one of ordinary skilled in the art to combine the teachings of Wei et al and Smales et al to develp a solid pharmaceutical daptomycin composition comprising daptomycin and sucrose or trehalose, wherein an amount of the solid pharmaceutical daptomycin composition comprising 500 mg of daptomycin dissolves in 10 mL of 0.9% aqueous sodium chloride in less than 5 minutes at about 25 degrees C; and a pharmaceutical product comprising such solid daptomycin composition and a pharmaceutically acceptable diluent. One of ordinary skilled in the art would have been motivated to combine the teachings of Wei et al and Smales et al to develp a solid pharmaceutical daptomycin composition comprising daptomycin and sucrose or trehalose, wherein an amount of the solid pharmaceutical daptomycin composition comprising 500 mg of daptomycin dissolves in 10 mL of 0.9% aqueous sodium chloride in less than 5 minutes at about 25 degrees C; and a pharmaceutical product comprising such solid daptomycin composition and a pharmaceutically acceptable diluent, since Smales et al teach that therapeutic proteins/peptides can be stabilized by adding protein-stabilizers, such as sugar at concentration of 10-100 mg/ml, in the process of formulation, and sucrose and trehalose are the most potent and useful excipients to protect protein conformation in aqueous solutions and freeze-dried solids.

In addition, one of ordinary skilled in the art would have been motivated to optimize the molar ratio of daptomycin to the sugar, since it "it is the normal desire of scientists or artisans to improve upon what is already generally known". The MPEP states the following: Generally, differences in concentration or temperature will not support the patentability of subject matter encompassed by the prior art unless there is evidence indicating such concentration or temperature is critical. "[W]here the general conditions of a claim are disclosed in the prior art, it is not inventive to discover the optimum or workable ranges by routine experimentation." *In re Aller*, 220 F.2d 454, 456, 105 USPQ 233, 235 (CCPA 1955) (Claimed

process which was performed at a temperature between 40°C and 80°C and an acid concentration between 25% and 70% was held to be prima facie obvious over a reference process which differed from the claims only in that the reference process was performed at a temperature of 100°C and an acid concentration of 10%.); see also *Peterson*, 315 F.3d at 1330, 65 USPQ2d at 1382 ("The normal desire of scientists or artisans to improve upon what is already generally known provides the motivation to determine where in a disclosed set of percentage ranges is the optimum combination of percentages."); *In re Hoeschele*, 406 F.2d 1403, 160 USPQ 809 (CCPA 1969) (Claimed elastomeric polyurethanes which fell within the broad scope of the references were held to be unpatentable thereover because, among other reasons, there was no evidence of the criticality of the claimed ranges of molecular weight or molar proportions.). For more recent cases applying this principle, see *Merck & Co. Inc. v. Biocraft Laboratories Inc.*, 874 F.2d 804, 10 USPQ2d 1843 (Fed. Cir.), *cert. denied*, 493 U.S. 975 (1989); *In re Kulling*, 897 F.2d 1147, 14 USPQ2d 1056 (Fed. Cir. 1990); and *In re Geisler*, 116 F.3d 1465, 43 USPQ2d 1362 (Fed. Cir. 1997).

A person of ordinary skilled in the art would have reasonable expectation of success in combining the teachings of Wei et al and Smales et al to develp a solid pharmaceutical daptomycin composition comprising daptomycin and sucrose or trehalose, wherein an amount of the solid pharmaceutical daptomycin composition comprising 500 mg of daptomycin dissolves in 10 mL of 0.9% aqueous sodium chloride in less than 5 minutes at about 25 degrees C; and a pharmaceutical product comprising such solid daptomycin composition and a pharmaceutically acceptable diluent.

25. Claims 22-25 and 27-42 are rejected under pre-AIA 35 U.S.C. 103(a) as being unpatentable over Inman et al (EP 0386951 A2, filed with IDS) in view of Smales et al (Therapeutic proteins, methods and protocols, Humana press, 2005, pages 287-292, filed with IDS).

The instant claims 22-25 and 27-42 are drawn to a solid pharmaceutical daptomycin composition comprising daptomycin and at least one excipient selected from glycine and a sugar, wherein an amount of the solid pharmaceutical daptomycin composition comprising 500 mg of daptomycin dissolves in 10 mL

of 0.9% aqueous sodium chloride in less than 5 minutes at about 25 degrees C; and a pharmaceutical product comprising such solid daptomycin composition and a pharmaceutically acceptable diluent.

Inman et al teach a solid pharmaceutical daptomycin composition comprising 150 mg daptomycin and 50 mg mannitol obtained by freeze-drying/lyophilization, and reconstituting such solid pharmaceutical daptomycin composition in a pharmaceutically acceptable diluent, such as isotonic sodium phosphate dibasic solution, for example, page 3, line 50 to page 4, line13. The molar ratio of 150 mg daptomycin to 50 mg mannitol is about 1:2.96. It reads on a molar ratio of daptomycin to the sugar of about 1:1.12 to about 1:21.32 as the elected species of molar ratio of daptomycin to the sugar. It meets the limitation of instant claims 22-25, 27, 32, 33 and 42.

The MPEP § 2112 states: "Once a reference teaching product appearing to be substantially identical is made the basis of a rejection, and the Examiner presents evidence or reasoning tending to show inherency, the burden shifts to the Applicant to show an unobvious difference '[t]he PTO can require an Applicant to prove that the prior art products do not necessarily or inherently possess the characteristics of his [or her] claimed product. Whether the rejection is based on inherency' under 35 U.S.C. 102, on *prima facie* obviousness' under 35 U.S.C. 103, jointly or alternatively, the burden of proof is the same...[footnote omitted]." The burden of proof is similar to that required with respect to product-by-process claims. *In re Fitzgerald*, 619 F.2d 67, 70, 205 USPQ 594, 596 (CCPA 1980) (quoting *In re Best*, 562 F.2d 1252, 1255, 195 USPQ 430, 433-34 (CCPA 1977))." Since the solid pharmaceutical daptomycin composition comprising 150 mg daptomycin and 50 mg mannitol in Inman et al meets all the limitation of the solid pharmaceutical daptomycin composition in instant claim 22, the solid pharmaceutical daptomycin composition comprising 150 mg daptomycin and 50 mg mannitol in Inman et al would necessarily have the same properties and functionality of the solid pharmaceutical daptomycin composition in instant claim 22. Therefore, the solid pharmaceutical daptomycin composition comprising 150 mg daptomycin and 50 mg mannitol in Inman et al has the property that an amount of the solid pharmaceutical daptomycin composition comprising 500 mg of daptomycin dissolves in 10 mL of 0.9% aqueous sodium chloride in less than 5 minutes at about 25 degrees C; and an amount of the solid

pharmaceutical daptomycin composition containing 500 mg of daptomycin dissolves in 10 mL of 0.9% aqueous sodium chloride in less than 2 minutes at 25 degrees C.

Claims 34-40 recite product by process claim for the solid pharmaceutical daptomycin composition preparation. The MPEP states the following: "[E]ven though product-by-process claims are limited by and defined by the process determination of patentability is based on the product itself. The patentability of a product does not depend on its method of production. If the product in the product-by-process claim is the same as or obvious from a product of the prior art, the claim is unpatentable even though the prior product was made by a different process...The product-by-process claim was rejected because the end product, in both the prior art and the allowed process, ends up containing metal carboxylate. The fact that the metal carboxylate is not directly added, but is instead produced in-situ does not change the end product" (see § MPEP 2113 [R-I]).

The difference between the reference and the instant claims 22-25 and 27-42 is that the reference does not teach sucrose or trehalose as excipient recited in instant claims 27-31; and sucrose as the elected species of excipient.

However, Smales et al, throughout the literature, teach that therapeutic proteins/peptides can be stabilized by adding protein-stabilizers, such as sugar at concentration of 10-100 mg/ml, in the process of formulation, and nonreducing disaccharides, such as sucrose and trehalose, are the most potent and useful excipients to protect protein conformation in aqueous solutions and freeze-dried solids, and sucrose has been widely used in various pharmaceutical formulation, for example, page 288, Table 2; page 289, "*3.2. Formulation Design*"; and pages 290-291, "*3.3.2. Sugars*". It reads on sucrose as the elected species of excipient. Smales et al further teach effective stabilization of protein conformation in aqueous solutions requires relatively high concentrations (approx. > 0.3 M) of disaccharide, and an approx. 1:1 weight-concentration ratio of disaccharides is needed to freeze-dry proteins without structural changes, and the sugars also stabilize protein conformation in other dehydrating formulation (spray-drying), for example, page 291, paragraph 1. A 1:1 weight ratio of daptomycin to sucrose is a molar ratio of daptomycin to the sugar of about 1:4.79. A 1:1 weight ratio of daptomycin to trehalose is a molar ratio of daptomycin to the sugar of about 1:9.10.

Therefore, it would have been obvious to one of ordinary skilled in the art to combine the teachings of Inman et al and Smales et al to develp a solid pharmaceutical daptomycin composition comprising daptomycin and sucrose or trehalose, wherein an amount of the solid pharmaceutical daptomycin composition comprising 500 mg of daptomycin dissolves in 10 mL of 0.9% aqueous sodium chloride in less than 5 minutes at about 25 degrees C; and a pharmaceutical product comprising such solid daptomycin composition and a pharmaceutically acceptable diluent. One of ordinary skilled in the art would have been motivated to combine the teachings Inman et al and Smales et al to develp a solid pharmaceutical daptomycin composition comprising daptomycin and sucrose or trehalose, wherein an amount of the solid pharmaceutical daptomycin composition comprising 500 mg of daptomycin dissolves in 10 mL of 0.9% aqueous sodium chloride in less than 5 minutes at about 25 degrees C; and a pharmaceutical product comprising such solid daptomycin composition and a pharmaceutically acceptable diluent, since Smales et al teach that therapeutic proteins/peptides can be stabilized by adding protein-stabilizers, such as sugar at concentration of 10-100 mg/ml, in the process of formulation, and sucrose and trehalose are the most potent and useful excipients to protect protein conformation in aqueous solutions and freeze-dried solids.

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determine where in a disclosed set of percentage ranges is the optimum combination of percentages."); *In re Hoeschele*, 406 F.2d 1403, 160 USPQ 809 (CCPA 1969) (Claimed elastomeric polyurethanes which fell within the broad scope of the references were held to be unpatentable thereover because, among other reasons, there was no evidence of the criticality of the claimed ranges of molecular weight or molar proportions.). For more recent cases applying this principle, see *Merck & Co. Inc. v. Biocraft Laboratories Inc.*, 874 F.2d 804, 10 USPQ2d 1843 (Fed. Cir.), *cert. denied*, 493 U.S. 975 (1989); *In re Kulling*, 897 F.2d 1147, 14 USPQ2d 1056 (Fed. Cir. 1990); and *In re Geisler*, 116 F.3d 1465, 43 USPQ2d 1362 (Fed. Cir. 1997).

A person of ordinary skilled in the art would have reasonable expectation of success in combining the teachings of Inman et al and Smales et al to develp a solid pharmaceutical daptomycin composition comprising daptomycin and sucrose or trehalose, wherein an amount of the solid pharmaceutical daptomycin composition comprising 500 mg of daptomycin dissolves in 10 mL of 0.9% aqueous sodium chloride in less than 5 minutes at about 25 degrees C; and a pharmaceutical product comprising such solid daptomycin composition and a pharmaceutically acceptable diluent.

Conclusion

No claim is allowed.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to LI NI KOMATSU whose telephone number is (571)270-3534. The examiner can normally be reached on Mon-Thurs 8-5pm EST.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Karlheinz Skowronek can be reached on (571)-272-9047. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

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/L. K./
Examiner, Art Unit 1676

/JULIE HA/
Primary Examiner, Art Unit 1675

Notice of References Cited	Application/Control No. 14/096,346	Applicant(s)/Patent Under Reexamination O'CONNOR ET AL.	
	Examiner LI NI KOMATSU	Art Unit 1676	Page 1 of 2

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*		Include as applicable: Author, Title Date, Publisher, Edition or Volume, Pertinent Pages)
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	V	Source of sucrose, from http://www.ehow.com/about_5376127_sources-sucrose.html, pages 1-2, accessed 11/7/2014.
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	U	Water, from http://www.biology-online.org/dictionary/Water, pages 1-3, accessed 4/24/2014.
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Doc code: IDS

Doc description: Information Disclosure Statement (IDS) Filed

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	Application Number	14096346
	Filing Date	2013-12-04
	First Named Inventor	Sandra O'CONNOR
	Art Unit	~~1654~~ 1676
	Examiner Name	~~Not Yet Assigned~~ Li Komatsu
	Attorney Docket Number	552815 (CPT-011USDV)

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Examiner Initial*	Cite No	Patent Number	Kind Code[1]	Issue Date	Name of Patentee or Applicant of cited Document	Pages,Columns,Lines where Relevant Passages or Relevant Figures Appear
	1	6194383		2001-02-27	Hammann et al.	
	2	5955509		1999-09-21	Webber et al.	
	3	5629288		1997-05-13	Lattrell et al.	
	4	5387670		1995-02-07	Roy et al.	
	5	5271935		1993-12-21	Franco et al.	
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	2	20100041589		2010-02-18	Keith et al.	
	3	20120270772		2012-07-10	O'Conner	
	4	20050027113		2005-02-03	Vivian Pak Woon Miao et al.	
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	6	20130280760	A1	2013-10-24	Cubist Pharmaceuticals, Inc.	

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	1	WO 00018419	WO		2000-04-06	Cubist Pharmaceuticals		☐
	2	WO 99027957	WO		1999-06-10	The Immune Response Co.		☐

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	Examiner Name	~~Not Yet Assigned~~ Li Komatsu
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	3	WO 99040113	WO		1999-08-12	Thiam et al.		☐
	4	WO99043700	WO		1999-09-02	Aventis Pharma Deutschland GMBH		☐
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	7	CN101330905A	CN	A	2008-12-24	SCIDOSE LLC		☒
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	1	Sun et al., "Development of an Improved Daptomycin Drug Product: Immediate Reconstitution, Room Temperature Product Stability and Reconstitution Stability", AAPS 2011, Abstract for Poster No. T3328 Published Abstract: http://abstracts.aaps.org/SecureView/AAPSJournal/vmqutdm9e488ov6bh0qy.pdf	☐
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	5	Desai et al., Microbial Production of Surfactants and Their Commercial Potential; Microbiology and Molecular Biology Reviews 1997, Vol 61, pp. 47-64	☐
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	7	AMORIJ: Development of Stable Influenza Vaccine Powder Formulations: challenges and possibilities, Pharmaceutical Research, 2008, 25:1256-1273	☐

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Receipt date: 01/06/2014	Application Number	14096346 14096346 - GAU: 1676
INFORMATION DISCLOSURE STATEMENT BY APPLICANT (**Not for submission under 37 CFR 1.99)**	Filing Date	2013-12-04
	First Named Inventor	Sandra O'CONNOR
	Art Unit	~~1654~~ 1676
	Examiner Name	~~Not Yet Assigned~~ Li Komatsu
	Attorney Docket Number	552815 (CPT-011USDV)

CERTIFICATION STATEMENT

Please see 37 CFR 1.97 and 1.98 to make the appropriate selection(s):

☐ That each item of information contained in the information disclosure statement was first cited in any communication from a foreign patent office in a counterpart foreign application not more than three months prior to the filing of the information disclosure statement. See 37 CFR 1.97(e)(1).

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☐ See attached certification statement.

☐ The fee set forth in 37 CFR 1.17 (p) has been submitted herewith.

☒ A certification statement is not submitted herewith.

SIGNATURE

A signature of the applicant or representative is required in accordance with CFR 1.33, 10.18. Please see CFR 1.4(d) for the form of the signature.

Signature	/Brian C. Trinque/	Date (YYYY-MM-DD)	2014-01-06
Name/Print	Brian C. Trinque, Ph.D.	Registration Number	56,593

This collection of information is required by 37 CFR 1.97 and 1.98. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 1 hour to complete, including gathering, preparing and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

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8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspections or an issued patent.

9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

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Receipt date: 01/06/2014

14096346 - GAU: 1676

Doc code: IDS

PTO/SB/08a (01-10)

Doc description: Information Disclosure Statement (IDS) Filed

Approved for use through 07/31/2012. OMB 0651-0031

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	Attorney Docket Number	552815 (CPT-011USDV)

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Examiner Initial*	Cite No	Patent Number	Kind Code[1]	Issue Date	Name of Patentee or Applicant of cited Document	Pages,Columns,Lines where Relevant Passages or Relevant Figures Appear
	1	6696412		2004-02-24	Thomas J. Kelleher	
	2	6716962		2004-04-06	Micrologix Biotech Inc.	
	3	7138487		2006-11-21	Migenix Inc.	
	4	7279597		2007-10-09	Emisphere Technologies, Inc.	
	5	8058238		2011-11-15	Cubist Pharmaceuticals, Inc.	
	6	8003673		2011-08-23	Alder et al.	
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	Attorney Docket Number	552815 (CPT-011USDV)

	1	20020111311		2002-08-15	Cubist Pharmaceuticals, Inc.	
	2	20020132762		2002-09-19	Borders, Donald B.	
	3	20030045484		2003-03-06	Keith, Dennis	
	4	20030045678		2003-03-06	Keith, Dennis	
	5	20040067878		2007-04-08	Hill, Jason	
	6	20040077601		2004-04-22	Adams, Sharlene	
	7	20040242467		2004-12-02	Borders, Donald B.	
	8	20050009747		2005-01-13	Kelleher, Thomas	
	9	20050196418		2005-09-08	Yu, Ruey J.	
	10	20060014674		2006-01-19	Keith, Dennis	
	11	20060018933		2006-01-26	Vaya, Navin	

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	12	20060018934		2006-01-26	Vaya, Navin	
	13	20060024365		2006-02-02	Vaya, Navin	
	14	20060264513		2006-11-23	Emisphere Technologies, Inc.	
	15	20060269485		2006-11-30	Friedman, Doron	
	16	20070116729		2007-05-24	Palepu, Nageswara R.	
	17	20070191280		2007-08-16	Kelleher, Thomas	
	18	20080220441		2008-09-11	Birnbaum, Eva R.	
	19	20090197799		2009-08-06	Keith, Dennis	
	20	20100041589		2010-02-18	Keith, Dennis	
	21	20110124551		2011-05-26	EAGLE PHARMACEUTICALS, INC	
	22	20110172167		2011-07-14	EAGLE PHARMACEUTICALS, INC	

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	Art Unit	~~4654~~ 1676
	Examiner Name	~~Not Yet Assigned~~ Li Komatsu
	Attorney Docket Number	552815 (CPT-011USDV)

	23	20110207658		2011-08-25	Kelleher, Thomas J.	
	24	20120270772		2012-10-25	CUBIST PHARMACEUTICALS, INC.	
	25	20090197799		2009-08-01	Keith et al.	
	26	20020111311		2002-08-01	Govardhan et al.	
	27	20070116729		2007-05-01	Palepu	

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	1	WO2001/044274 A1	WO		2001-06-21	CUBIST PHARMACEUTICALS, INC		☐
	2	WO2001/053330 A2	WO		2001-07-26	CUBIST PHARMACEUTICALS, INC		☐
	3	WO2002/055537 A1	WO		2002-07-18	INTRABIOTICS PHARMACEUTICALS, INC.		☐
	4	WO2002/056829 A2	WO		2002-07-25	CUBIST PHARMACEUTICALS, INC		☐

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	5	WO2002/059145 A1	WO		2002-08-01	CUBIST PHARMACEUTICALS, INC		☐
	6	WO2002/096936 A2	WO		2002-12-05	ALTUS BIOLOGICS, INC.		☐
	7	WO2004/004658 A2	WO		2004-01-15	POINT THERAPEUTICS, INC.		☐
	8	WO2006/084174 A2	WO		2006-08-10	YU, Ruey, J.		☐
	9	WO2006/084174 A3	WO		2006-08-10	YU, Ruey, J.		☐
	10	WO2007/061529 A1	WO		2007-05-31	SCIDOSE PHARMA INC.		☐
	11	WO2007/099396 A2	WO		2007-09-07	FOAMIX LTD.		☐
	12	WO2007/099396 A3	WO		2007-09-07	FOAMIX LTD.		☐
	13	WO2008/127291 A2	WO		2008-10-23	LOS ALAMOS NATIONAL SECURITY, LLC		☐
	14	WO2008/127291 A3	WO		2008-10-23	LOS ALAMOS NATIONAL SECURITY, LLC		☐
	15	WO2011/019839 A2	WO		2011-02-17	RAFAI FAR, Adel		☐

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	16	WO2011/035108 A1	WO		2011-03-24	EAGLE PHARMACEUTICALS, INC.		☐
	17	WO2011/062676 A1	WO		2011-05-26	EAGLE PHARMACEUTICALS, INC.		☐
	18	WO2011/063419 A2	WO		2011-05-26	CUBIST PHARMACEUTICALS INC.		☐
	19	WO2012/061360 A2	WO		2012-05-10	RIB-X PHARMACEUTICALS, INC.		☐
	20	WO2012/088441 A1	WO		2012-06-28	ALKERMES, INC.		☐
	21	WO2012/112319 A1	WO		2012-08-23	AEGIS THERAPEUTICS, LLC		☐
	22	WO 01/53330	WO		2001-07-01			☐
	23	CN 1616083 A	CN		2005-05-01	SHEN et al.		☐

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	1	Cubicin label August, 2010	☐

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	2	Notification of Transmittal of International Preliminary Report on Patentability in International Application No. PCT/ US2010/057910, mailed May 24, 2012, 7 pages	☐
	3	International Search Report and Written Opinion dated August 22, 2011 in international application no. PCT/ US2010/057910, 15 pages	☐
	4	pH-water quality, from http://extension.usu.edu/waterquality/htm/whats-in-your-water/ph, pages 1-2, accessed 7/23/2013	☐
	5	Smales et al, Therapeutic proteins, methods and protocols, Humana press, 2005, pages 287-292	☐
	6	Wang, Lyophilization and development of solid protein pharmaceuticals, International Journal of Pharmaceutics, 2000, 203, pages 1-60	☐
	7	Machine translation of CN 1616083 A, pages 1-9, publication date 5/18/2005	☐

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Signature	/Brian C. Trinque/	Date (YYYY-MM-DD)	2014-01-06
Name/Print	Brian C. Trinque, Ph.D., Esq.	Registration Number	56593

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CONFIRMATION NO. 2832

SERIAL NUMBER	FILING or 371(c) DATE RULE	CLASS	GROUP ART UNIT	ATTORNEY DOCKET NO.
14/096,346	12/04/2013	514	1676	552815: CPT-011USDV

APPLICANTS

Cubist Pharmaceuticals, Inc., Lexington, MA, Assignee (with 37 CFR 1.172 Interest);

INVENTORS

Sandra O'Connor, Hudson, NH;
Sophie Sun, Lexington, MA;
Gaauri Naik, Cambridge, MA;

**** CONTINUING DATA ***************************

This application is a DIV of 13/511,246 07/10/2012 PAT 8835382
which is a 371 of PCT/US2010/057910 11/23/2010
which claims benefit of 61/263,784 11/23/2009
(*)Data provided by applicant is not consistent with PTO records.

* The Continuing data is corrected. The PCT NO. on the Bib of 13/511,246 is incorrect.

**** FOREIGN APPLICATIONS ***************************

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Foreign Priority claimed ☐ Yes ☑ No 35 USC 119(a-d) conditions met ☐ Yes ☐ No Verified and Acknowledged /LI NI N KOMATSU/ Examiner's Signature	☐ Met after Allowance Initials	STATE OR COUNTRY	SHEETS DRAWINGS	TOTAL CLAIMS	INDEPENDENT CLAIMS
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14096346 - GAU: 1676

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Receipt date: 01/06/2014	Application Number	14096346 14096346 - GAU: 1676
INFORMATION DISCLOSURE STATEMENT BY APPLICANT	Filing Date	2013-12-04
	First Named Inventor	Sandra O'CONNOR
	Art Unit	~~1654~~ 1676
(Not for submission under 37 CFR 1.99)	Examiner Name	~~Not Yet Assigned~~ Li Komatsu
	Attorney Docket Number	552815 (CPT-011USDV)

	1	Haworth et al.; Staphylococcus aureus ventriculitis treated with single-dose intraventricular vancomycin or daptomycin (LY146032): bacterial and antibiotic kinetics in hydrocephalic rabbits; Antimicrobial Agents and Chemotherapy 1990, Vol 34, pp. 245-51	☐
	2	Jancarik et al., Sparse matrix sampling: a screening method for crystallization of proteins, J Appl. Cryst., 1991, Vol 24, pp. 409-11	☐
	3	Johnson et al., ICAA 1987, poster 161, 1 page	☐
	4	Katz et al., A pilot study of high-dose short duration daptomycin for the treatment of patients with complicated skin and skin structure infections caused by gram-positive bacteria, International Journal of Clinical Practice, 2008, pp. 1-10	☐
	5	LeClercq et al., Effects of Combinations of Beta-Lactams, Daptomycin, Gentamicin and Glycopeptides against Glycopeptide-Resistant Enterococci; Antimicrobial Agents and Chemotherapy, 1991, Vol 35, pp. 92-98	☐
	6	Louie et al., Comparison of in vitro inhibitory and Bactericial Activities of Daptomycin (LY 146032) and Four Reference Antibiotics, Singly and in Combination, against Gentamicin-Susceptible and High-Level-Gentamicin-Resistant Enterococci; Chemotherapy; 1993, Vol 39, pp. 302-10	☐
	7	Luu et al., Treatment of Chronic Experimental Staphylococcus aureus Osteomyelitis with LY 146032 and Vancomycin; Eur. J. Clin. Microbiol. Infect. Dis. 1989, Vol 8, pp. 562-63	☐
	8	Mader et al., Comparative Evalulation of Daptomycin (LY146032) and comycin in the Treatment of Experimental Methicillin-Resistant Staphylococcus aureus Osteomyelitis in Rabbits; Comparative Evaluation of DaptomycinAntimicrobial Agents and Chemotherapy, 1989, Vol 33, pp. 689-92	☐
	9	Mariani et al., Development of decreased susceptibility to daptomycin and vancomycin in a Staphylococcus aureus strain during prolonged therapy; Journal of Antimicrobial Chemotherapy 2013, p. 481-83	☐
	10	Mathews et al., IDSA poster, 2001	☐
	11	Mchenney et al., Molecular Cloning and Physical Mapping of the Daptomycin Gene Cluster from Streptomyces roseosporus; Journal of Bacteriology, 1998, Vol 180, pp. 143-51	☐

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	12	Mobarakai et al., Bactericidal Activities of Peptide Antibiotics against Multidrug-Resistant Enterococcus faecium; Antimicrobial Agents and Chemotherapy; 1994, Vol 38, pp. 385-87	☐
	13	Moise et al., susceptibility relationship between vancomycin and daptomycin in Staphylococcus aureus: facts and assumptions; Lancet Infect. Dis. 2009, Vol 9, pp. 617-24	☐
	14	Patel et al., An Association bettween Reduced Susceptibility to Daptomycin and Reduced Susceptibility to Vancomycin in Staphylococcus aureus; Clinical Infectious Diseases: Correspondence to the Editor; 1 June 2006, Vol 42, pp. 1652-53	☐
	15	Sader et al., Nine-Hospital Study Comparing Broth Microdilution and Etest method Results for Vaqncomycin and Daptomycin against Methicillin-Resistant Staphylococcus aureus; Antimicrobial Agents and Chemotherapy, 2009, Vol 53, pp. 3162-65	☐
	16	Sader et al., Update on the In Vitro Activity of Daptomycin Tested against 17,193 Gram-positive Bacteria Isolated from European Medical Centers (2005-2007); Journal of Chemotherapy 2009, Vol 21, pp. 500-506	☐
	17	Sakoulas et al., Clinical Outcomes of Patients Receiving Daptomycin for the Treatment of Staphylococcus aureus Infections and Assessment of Clinical Factors for Daptomycin Failure: A Retrospective Cohort Study Tuilizing the Cubicin Outcomes Registry and Experience; Clinical Therapeutics, 2009, Vol 31, pp.1936-45	☐
	18	Sapico et al., 146032, Alone nad in Combination with Gentamicin, for the Treatment of Enterococcal Pyelonephritis in the Rat Model; Antimicrobial Agents and Chemotherapy 1988, Vol 32, pp. 81-83	☐
	19	Silva et al., In Vitro Activity of LY146032 Against Gram-Positive Bacteria; Diagn. Microbiol. Infect. Dis., 1988, Vol 9, pp. 79-85	☐
	20	Silverman et al., Inhibition of Daptomycin by Pulmonary Surfactant: In Vitro Modeling and Clinical Impact; The Journal of Infectious Disease; 2005, Vol 191, pp. 2149-52	☐
	21	Silverman et al., Resistance Studies with Daptomycin; Antimicrobial Agents and Chemotherapy; 2001, Vol 45, pp. 1799-1802	☐
	22	Snydman et al., Comparative In Virro Activities of Daptomycin and Vancomycin against Resistant Gram-Positive Pathogens; Antimicrobial Agents and Chemotherapy; 2000, Vol 44, pp. 3447-50	☐

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	23	Steenbergen et al., Daptomycin: a lipopeptide antibiotic for the treatment of serious Gram-positive infections; Journal of Antimicrobial Chemotherapy, 2005, Vol 55, pp. 283-88	☐
	24	Stratton et al., Bactericidal Activity of Deptomycin (LY146032) Compared with Those of Ciprofloxacin, Vancomycin, and Ampicillin against Enterococci as Determined by Kill-Kinetic Studies; Antimicrobial Agents and Chemotherapy 1987, Vol 31, pp. 1014-16	☐
	25	U.S. Patent Application No. 10/024,405 (Abandoned) filed on 12/18/2001	☐
	26	Tenover et al., Characterisation of a Staphylococcus aureus strain with progressive loss of susceptibility to vancomycin and daptomycin during therapy; International Journal of Antimicrobial Agents; 2009, p. 564-68	☐
	27	Thibault et al., Attenuation by Daptomycin of Gentamicin-Induced Experimental Nephrotoxicity; Antimicrobial Agents and Chemotherapy; 1994, Vol 38, pp. 1027-35	☐
	28	Woodworth et al., Tobramycin and daptomycin disposition when co-adninistered to healthy volunteers; Journal of Antimicrobial Chemotherapy, 1994, Vol 33 pp. 655-59	☐
	29	U.S. Provisional Application No. 61/243,402, filed September 17, 2009 (Priority Document for WO2011035108)	☐
	30	U.S. Provisional Application No. 61/263,695, filed November 23, 2009 (Priority Document for WO2011035108 and WO2011062676)	☐
	31	U.S. Provisional Application No. 61/371,802, filed August 9, 2010 (Priority Document for WO2011062676)	☐
	32	Cubist Pharmaceutical, Inc. v. Hospira, Inc., No. 1:12cv367 (D. Mass. Filed Mar. 21, 2012) (Def. Hospira, Inc. Preliminary Invalidity Contentions)	☐
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	Attorney Docket Number	552815 (CPT-011USDV)

EXAMINER SIGNATURE			
Examiner Signature	/Li Komatsu/	Date Considered	11/07/2014

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	Art Unit	~~1654~~ 1676
	Examiner Name	~~Not Yet Assigned~~ Li Komatsu
	Attorney Docket Number	552815 (CPT-011USDV)

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OR

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☐ See attached certification statement.

☐ The fee set forth in 37 CFR 1.17 (p) has been submitted herewith.

☒ A certification statement is not submitted herewith.

SIGNATURE

A signature of the applicant or representative is required in accordance with CFR 1.33, 10.18. Please see CFR 1.4(d) for the form of the signature.

Signature	/Brian C. Trinque/	Date (YYYY-MM-DD)	2014-01-06
Name/Print	Brian C. Trinque, Ph.D.	Registration Number	56,593

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9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

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Search Notes	Application/Control No.	Applicant(s)/Patent Under Reexamination
	14096346	O'CONNOR ET AL.
	Examiner	Art Unit
	LI NI KOMATSU	1676

CPC- SEARCHED		
Symbol	Date	Examiner
None	11/7/2014	LNK

CPC COMBINATION SETS - SEARCHED		
Symbol	Date	Examiner
None	11/7/2014	LNK

US CLASSIFICATION SEARCHED			
Class	Subclass	Date	Examiner
None		11/7/2014	LNK

SEARCH NOTES		
Search Notes	Date	Examiner
PALM and EAST all inventor name search	11/7/2014	LNK
EAST search: please see attached	11/7/2014	LNK
STIC search	9/25/2014	LNK

INTERFERENCE SEARCH			
US Class/ CPC Symbol	US Subclass / CPC Group	Date	Examiner
None		11/7/2014	LNK

/LI NI KOMATSU/ Examiner.Art Unit 1676	

EAST Search History (Prior Art)

Ref #	Hits	Search Query	DBs	Default Operator	Plurals	Time Stamp
L1	55970	trehalose	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2014/11/07 12:18
L2	2548	daptomycin$3 or cubicin$2 or LY146032 or LY-146032 or (LY adj "146032")	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2014/11/07 12:18
L3	5	l1 with L2	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2014/11/07 12:18
L4	6	l1 same l2	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2014/11/07 12:18
S1	9	((Sandra) near2 (O'Connor)).INV.	US-PGPUB; USPAT; USOCR	OR	ON	2014/11/07 10:49
S2	4	((Sandra) near2 (O'Connor)).INV.	EPO; JPO; DERWENT	OR	ON	2014/11/07 10:49
S3	4	((Sophie) near2 (Sun)).INV.	US-PGPUB; USPAT; USOCR	OR	ON	2014/11/07 10:49
S4	0	((Sophie) near2 (Sun)).INV.	EPO; JPO; DERWENT	OR	ON	2014/11/07 10:49
S5	5	((Gaauri) near2 (Naik)).INV.	US-PGPUB; USPAT; USOCR	OR	ON	2014/11/07 10:49
S6	0	((Gaauri) near2 (Naik)).INV.	EPO; JPO; DERWENT	OR	ON	2014/11/07 10:49

EAST Search History (Prior Art)

S7	54	("20070128694"|"4882164"|"20030045678"|"5336756"|"8309061"|"RE39071"|"20120270772"|"5955509"|"20020111311"|"20040242467"|"20050009747"|"20060018934"|"20110172167"|"20120270772"|"4331594"|"5271935"|"20060264513"|"20060269485"|"20070116729"|"20080220441"|"20090197799"|"20110207658"|"6468967"|"20100041589"|"5387670"|"8604164"|"20040067878"|"20060014674"|"20060018933"|"20110124551"|"6716962"|"7138487"|"7279597"|"4331594"|"8058238"|"20050027113"|"20130280760"|"5629288"|"4439425"|"4537717"|"4874843"|"6696412"|"6194383"|"20050196418"|"20060024365"|"5912226"|"6852689"|"8129342"|"20020132762"|"20030045484"|"20040077601"|"6696412"|"8058238"|"4482487"|"20120149062"|"20070191280"|"20100041589"|"8003673"|"8431539").PN.	US-PGPUB; USPAT	OR	ON	2014/11/07 10:49
S8	2548	daptomycin$3 or cubicin$2 or LY146032 or LY-146032 or (LY adj "146032")	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2014/11/07 10:51
S9	369307	sucrose or saccharose	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2014/11/07 10:51
S10	15	S8 with S9	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2014/11/07 10:51
S11	32	S8 same S9	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2014/11/07 10:52
S12	17	S11 not S10	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2014/11/07 10:52

EAST Search History (Prior Art)

S13	2342471	dissolv$4 or reconstitut$4	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2014/11/07 10:54
S14	82	S8 with S13	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2014/11/07 10:54

Receipt date: 04/01/2014 14096346 - GAU: 1676

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Doc description: Information Disclosure Statement (IDS) Filed

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	1	WO2009144739	WO	A1	2009-12-03	Biocon Limited		☐

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	1	Supplementary European Search Report PCT/US2010057910 Dated February 28, 2014. 8 Pages	☐
	2	DEBONO: Enzymatic and Chemical Modifications of Lipopeptide Antibiotic A21978C: The Synthesis and Evaluation of Daptomycin (LY146032), The Journal of Antibiotics, 1988, 41(8):1093-1105	☐

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Signature	/Brian C. Trinque/	Date (YYYY-MM-DD)	2014-04-01
Name/Print	Brian C. Trinque, Ph.D., Esq.	Registration Number	56,593

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4. A record in this system of records may be disclosed, as a routine use, to a contractor of the Agency having need for the information in order to perform a contract. Recipients of information shall be required to comply with the requirements of the Privacy Act of 1974, as amended, pursuant to 5 U.S.C. 552a(m).

5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent Cooperation Treaty.

6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).

7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (i.e., GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.

8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspections or an issued patent.

9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

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Receipt date: 01/06/2014

14096346 - GAU: 1676

Doc code: IDS

Doc description: Information Disclosure Statement (IDS) Filed

PTO/SB/08a (07-10)
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(Not for submission under 37 CFR 1.99)

Application Number	14096346
Filing Date	2013-12-04
First Named Inventor	Sandra O'CONNOR
Art Unit	~~1654~~ 1676
Examiner Name	~~Not Yet Assigned~~ Li Komatsu
Attorney Docket Number	552815 (CPT-011USDV)

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Examiner Initial*	Cite No	Patent Number	Kind Code[1]	Issue Date	Name of Patentee or Applicant of cited Document	Pages,Columns,Lines where Relevant Passages or Relevant Figures Appear
	1	6468967		2002-10-22	Oleson et al.	
	2	6852689		2005-02-08	Oleson et al.	
	3	RE39071		2006-04-18	Baker et al.	
	4	8058238		2011-11-15	Kelleher et al.	
	5	8129342		2012-03-06	Kelleher	
	6	4537717		1985-08-27	Abbott et al.	
	7	5912226		1999-06-15	Baker et al.	
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	Attorney Docket Number	552815 (CPT-011USDV)	

	9	4482487		1984-11-13	Abbott et al,	
	10	4331594		1982-05-25	Hamill et al.	
	11	4439425		1984-03-27	Tarcsay et al.	
	12	5336756		1994-08-09	Schwartz et al.	
	13	8431539		2013-04-30	Palepu et al.	
	14	6696412		2004-02-24	Kelleher et al.	
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Examiner Initial*	Cite No	Foreign Document Number[3]	Country Code[2] ¡	Kind Code[4]	Publication Date	Name of Patentee or Applicant of cited Document	Pages,Columns,Lines where Relevant Passages or Relevant Figures Appear	T[5]
	1	EP0294990	EP		1988-12-14	Eli Lilly and Company		☐
	2	JP04224197	JP		1992-08-13	Fujitsu LTD	Abstract only	☒
	3	JP05239090	JP		1993-09-17	Merck and Co. INC.	Abstract only	☒
	4	JP05271284	JP		1993-10-19	Hoechst AG	Abstract only	☒
	5	WO0153330	WO		2001-07-26	Cubist Pharmaceuticals		☐
	6	WO02059145	WO		2002-08-01	Cubist Pharmaceuticals		☐
	7	WO02096936	WO		2002-12-05	Altus Biologics Inc.		☐
	8	EP0511866	EP		1992-11-04	Merck and Co. Inc.	Abstract only	☐
	9	EP0521408	EP		1993-01-07	Hoechst AG in German	Abstract only	☒
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	11	EP1252179	EP		2002-10-30	Cubist Pharmaceuticals		☐
	12	WO9321207	WO		1993-10-28	Abbot Lab		☐
	13	JP64047388	JP		1989-02-21	Eli Lilly and Co.	Abstract only	☐
	14	EP0178152	EP		1986-04-16	Eli Lilly and Co.		☐

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	1	Miao et al., "Daptomycin biosynthesis in Streptomyces roseosporus: cloning and analysis of the gene cluster and revision of peptide stereochemistry," Microbiology 2005, Vol 151 (5), 1507-23	☐
	2	Debono, et al., "A21978C, A Complex of New Acidic Peptide Antibiotics: Isolation, Chemistry, and Mass Spectral Structure Elucidation," The Journal of Antibiotics 1987, Vol XL (6), p. 761-77	☐
	3	Lodish et al., Molecular Cell Biology (ed. by J. Darnell, H. Lodish, and D. Baltimore, Scientific American Books, Inc., New York: 1986), Chapter 3, p. 53	☐
	4	Woodworth et al., "Single-Dose Pharmacokinetics and Antibacterial Activity of Daptomycin, a New Lipopeptide Antibiotic, in Healthy Volunteers," Antimicrobial Agent and Chemotherapy 1992, Vol 36, p. 318-25	☐
	5	McKindley et al., "Drug Use in the Critically Ill Patient with Renal Dysfunction-Application of the DREM System," INFECTIOUS DISEASES IN CRITICAL CARE MEDICINE BIOTECHNOLOGY OF ANTIBIOTICS (ed. B.A. Cunha, New York: Marcel Dekker, Inc., 1998) Chapter 41, pp. 781-801	☐

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	Art Unit	~~4654~~ 1676
	Examiner Name	~~Not Yet Assigned~~ Li Komatsu
	Attorney Docket Number	552815 (CPT-011USDV)

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EXAMINER SIGNATURE

Examiner Signature	/Li Komatsu/	Date Considered	11/07/2014

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	Art Unit	~~1654.~~ 1676
	Examiner Name	~~Not Yet Assigned~~ Li Komatsu
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CERTIFICATION STATEMENT

Please see 37 CFR 1.97 and 1.98 to make the appropriate selection(s):

☐ That each item of information contained in the information disclosure statement was first cited in any communication from a foreign patent office in a counterpart foreign application not more than three months prior to the filing of the information disclosure statement. See 37 CFR 1.97(e)(1).

OR

☐ That no item of information contained in the information disclosure statement was cited in a communication from a foreign patent office in a counterpart foreign application, and, to the knowledge of the person signing the certification after making reasonable inquiry, no item of information contained in the information disclosure statement was known to any individual designated in 37 CFR 1.56(c) more than three months prior to the filing of the information disclosure statement. See 37 CFR 1.97(e)(2).

☐ See attached certification statement.

☐ The fee set forth in 37 CFR 1.17 (p) has been submitted herewith.

☒ A certification statement is not submitted herewith.

SIGNATURE

A signature of the applicant or representative is required in accordance with CFR 1.33, 10.18. Please see CFR 1.4(d) for the form of the signature.

Signature	/Brian C. Trinque/	Date (YYYY-MM-DD)	2014-01-06
Name/Print	Brian C. Trinque, Ph.D.	Registration Number	56,593

This collection of information is required by 37 CFR 1.97 and 1.98. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 1 hour to complete, including gathering, preparing and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

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The Privacy Act of 1974 (P.L. 93-579) requires that you be given certain information in connection with your submission of the attached form related to a patent application or patent. Accordingly, pursuant to the requirements of the Act, please be advised that: (1) the general authority for the collection of this information is 35 U.S.C. 2(b)(2); (2) furnishing of the information solicited is voluntary; and (3) the principal purpose for which the information is used by the U.S. Patent and Trademark Office is to process and/or examine your submission related to a patent application or patent. If you do not furnish the requested information, the U.S. Patent and Trademark Office may not be able to process and/or examine your submission, which may result in termination of proceedings or abandonment of the application or expiration of the patent.

The information provided by you in this form will be subject to the following routine uses:

1. The information on this form will be treated confidentially to the extent allowed under the Freedom of Information Act (5 U.S.C. 552) and the Privacy Act (5 U.S.C. 552a). Records from this system of records may be disclosed to the Department of Justice to determine whether the Freedom of Information Act requires disclosure of these record s.

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PATENT
Attorney Docket No. 552815: CPT-011DIV
Cubist Reference No. C111-02/02 US

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants:	Sandra O'Connor *et al.*	Examiner:	Lin Komatsu
Serial No:	14/096,346	Group Art No:	1676
Filed:	December 4, 2013	Confirmation No:	2832
For	LIPOPEPTIDE COMPOSITIONS AND RELATED METHODS		

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P.O. Box 1450
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RESPONSE TO RESTRICTION REQUIREMENT

Dear Colleagues:

This communication is responsive to the Restriction Requirement having a mailing date of June 25, 2014.

Remarks begin on page 2 of this paper.

REMARKS

According to the Restriction Requirement set forth in the Office Action mailed June 25, 2014, Applicants are required under 35 U.S.C. §121 to elect a single disclosed species for prosecution search and examination purposes:

i. A specific excipient: due to different variables from claims 22, 26 and 27;

ii. A specific molar ratio of daptomycin to the sugar: due to different variables;

iii. A specific pH of aqueous daptomycin solution: from claims 34-38;

iv. A specific buffering agent: from claim 39 and see page 4, lines 14-15 of the instant specification; and

v. A specific way to convert the aqueous daptomycin solution to a solid pharmaceutical composition from claim 40.

As described on page 2 of the instant office action, the Examiner considers claims 22-25 and 34-42 to be generic to the above-disclosed patentably distinct species. Accordingly, Applicants elect the following species:

i. An excipient of sucrose as recited in claim 27;

ii. A molar ratio of daptomycin to sugar of about 1:1.12 to about 1:21.32 as recited in claim 25;

iii. A pH of 6.5-7.5 as recited in claim 37;

iv. A phosphate buffering agent; and

v. Converting the aqueous daptomycin solution to a solid pharmaceutical composition by lyophilization as recited in claim 40.

This election is made without traverse. Claims 22-25, 27, 30, 31 and 34-42 cover the elected species.

It is the Applicants' understanding that this species election is for search purposes only, and that, upon an indication of allowance, Applicants will be entitled to consideration of the claims to additional species that depend from or otherwise include all the limitations of an allowable generic claim as provided by 37 C.F.R. §1.141 and MPEP §809.02(a) (See Office

Action, page 3, 4th paragraph). Applicants request examination of all pending claims in the event the generic claims and/or the elected species claims are held allowable.

CONCLUSION

If a telephone conversation with Applicants' attorney would expedite prosecution of the above-identified application, the Examiner is urged to call the undersigned at the telephone number below.

Applicant requests herewith a one month extension of time under 35 CFR 1.17(a)(1). Applicant is a non-small entity. Applicant believes no additional fee is due at this time; however, the Commissioner is authorized to charge any fees that may be due, or credit any over payment, to the undersigned's Deposit Account No. 12-0600, under Order No. 552815: CPT-011DIV.

Date: September 24, 2014

Respectfully submitted,

Electronic Signature: /Brian C. Trinque/
Brian C. Trinque, Ph.D., Esq.
Reg. No. 56,593
LATHROP & GAGE LLP
28 State Street
Boston, Massachusetts 02109
(857) 300-4003 (Tel)
(857) 300-4001 (Fax)
Attorney for Applicant

Electronic Patent Application Fee Transmittal

Application Number:	14096346
Filing Date:	04-Dec-2013
Title of Invention:	LIPOPEPTIDE COMPOSITIONS AND RELATED METHODS
First Named Inventor/Applicant Name:	Sandra O'Connor
Filer:	Brian C. Trinque
Attorney Docket Number:	552815: CPT-011USDV

Filed as Large Entity

Utility under 35 USC 111(a) Filing Fees

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Basic Filing:				
Pages:				
Claims:				
Miscellaneous-Filing:				
Petition:				
Patent-Appeals-and-Interference:				
Post-Allowance-and-Post-Issuance:				
Extension-of-Time:				
Extension - 1 month with $0 paid	1251	1	200	200

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Miscellaneous:				
			Total in USD ($)	**200**

Electronic Acknowledgement Receipt

EFS ID:	20226244
Application Number:	14096346
International Application Number:	
Confirmation Number:	2832
Title of Invention:	LIPOPEPTIDE COMPOSITIONS AND RELATED METHODS
First Named Inventor/Applicant Name:	Sandra O'Connor
Customer Number:	113613
Filer:	Brian C. Trinque
Filer Authorized By:	
Attorney Docket Number:	552815: CPT-011USDV
Receipt Date:	24-SEP-2014
Filing Date:	04-DEC-2013
Time Stamp:	14:56:55
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	yes
Payment Type	Deposit Account
Payment was successfully received in RAM	$200
RAM confirmation Number	1274
Deposit Account	120600
Authorized User	
The Director of the USPTO is hereby authorized to charge indicated fees and credit any overpayment as follows: Charge any Additional Fees required under 37 C.F.R. Section 1.16 (National application filing, search, and examination fees) Charge any Additional Fees required under 37 C.F.R. Section 1.17 (Patent application and reexamination processing fees)	

Charge any Additional Fees required under 37 C.F.R. Section 1.19 (Document supply fees) Charge any Additional Fees required under 37 C.F.R. Section 1.20 (Post Issuance fees) Charge any Additional Fees required under 37 C.F.R. Section 1.21 (Miscellaneous fees and charges)

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1		552815_Response.pdf	21967 b100d7a0e75ccb2954d06c2f6bc494278da3d033	yes	3

Multipart Description/PDF files in .zip description		
Document Description	**Start**	**End**
Response to Election / Restriction Filed	1	1
Applicant Arguments/Remarks Made in an Amendment	2	3

Warnings:

Information:

2	Fee Worksheet (SB06)	fee-info.pdf	30625 1025187146e7ba8ae3edf65f7e69fd142ca1c153	no	2

Warnings:

Information:

Total Files Size (in bytes):	52592

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

<u>New Applications Under 35 U.S.C. 111</u>
If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

<u>National Stage of an International Application under 35 U.S.C. 371</u>
If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

<u>New International Application Filed with the USPTO as a Receiving Office</u>
If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
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Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NUMBER	FILING or 371(c) DATE	GRP ART UNIT	FIL FEE REC'D	ATTY.DOCKET.NO	TOT CLAIMS	IND CLAIMS
14/096,346	12/04/2013	1676	1820	552815: CPT-011USDV	21	1

CONFIRMATION NO. 2832

CORRECTED FILING RECEIPT

113613
Lathrop & Gage
28 State Street
Boston, MA 02109-1775

OC000000070497452

Date Mailed: 09/05/2014

Receipt is acknowledged of this non-provisional patent application. The application will be taken up for examination in due course. Applicant will be notified as to the results of the examination. Any correspondence concerning the application must include the following identification information: the U.S. APPLICATION NUMBER, FILING DATE, NAME OF APPLICANT, and TITLE OF INVENTION. Fees transmitted by check or draft are subject to collection. Please verify the accuracy of the data presented on this receipt. **If an error is noted on this Filing Receipt, please submit a written request for a Filing Receipt Correction. Please provide a copy of this Filing Receipt with the changes noted thereon. If you received a "Notice to File Missing Parts" for this application, please submit any corrections to this Filing Receipt with your reply to the Notice. When the USPTO processes the reply to the Notice, the USPTO will generate another Filing Receipt incorporating the requested corrections**

Inventor(s)

Sandra O'Connor, Hudson, NH;
Sophie Sun, Lexington, MA;
Gaauri Naik, Cambridge, MA;

Applicant(s)

Cubist Pharmaceuticals, Inc., Lexington, MA

Assignment For Published Patent Application

Cubist Pharmaceuticals, Inc., Lexington, MA

Power of Attorney: The patent practitioners associated with Customer Number 113613

Domestic Priority data as claimed by applicant

This application is a DIV of 13/511,246 07/10/2012 PAT 8835382 *
which is a 371 of PCT/US2010/057910 11/23/2010
which claims benefit of 61/263,784 11/23/2009
(*)Data provided by applicant is not consistent with PTO records.

Foreign Applications for which priority is claimed (You may be eligible to benefit from the **Patent Prosecution Highway** program at the USPTO. Please see http://www.uspto.gov for more information.) - None.
Foreign application information must be provided in an Application Data Sheet in order to constitute a claim to foreign priority. See 37 CFR 1.55 and 1.76.

If Required, Foreign Filing License Granted: 12/23/2013
The country code and number of your priority application, to be used for filing abroad under the Paris Convention, is **US 14/096,346**
Projected Publication Date: 12/11/2014

Non-Publication Request: No

Early Publication Request: No

Title

LIPOPEPTIDE COMPOSITIONS AND RELATED METHODS

Preliminary Class

514

Statement under 37 CFR 1.55 or 1.78 for AIA (First Inventor to File) Transition Applications: No

PROTECTING YOUR INVENTION OUTSIDE THE UNITED STATES

Since the rights granted by a U.S. patent extend only throughout the territory of the United States and have no effect in a foreign country, an inventor who wishes patent protection in another country must apply for a patent in a specific country or in regional patent offices. Applicants may wish to consider the filing of an international application under the Patent Cooperation Treaty (PCT). An international (PCT) application generally has the same effect as a regular national patent application in each PCT-member country. The PCT process **simplifies** the filing of patent applications on the same invention in member countries, but **does not result** in a grant of "an international patent" and does not eliminate the need of applicants to file additional documents and fees in countries where patent protection is desired.

Almost every country has its own patent law, and a person desiring a patent in a particular country must make an application for patent in that country in accordance with its particular laws. Since the laws of many countries differ in various respects from the patent law of the United States, applicants are advised to seek guidance from specific foreign countries to ensure that patent rights are not lost prematurely.

Applicants also are advised that in the case of inventions made in the United States, the Director of the USPTO must issue a license before applicants can apply for a patent in a foreign country. The filing of a U.S. patent application serves as a request for a foreign filing license. The application's filing receipt contains further information and guidance as to the status of applicant's license for foreign filing.

Applicants may wish to consult the USPTO booklet, "General Information Concerning Patents" (specifically, the section entitled "Treaties and Foreign Patents") for more information on timeframes and deadlines for filing foreign patent applications. The guide is available either by contacting the USPTO Contact Center at 800-786-9199, or it can be viewed on the USPTO website at http://www.uspto.gov/web/offices/pac/doc/general/index.html.

For information on preventing theft of your intellectual property (patents, trademarks and copyrights), you may wish to consult the U.S. Government website, http://www.stopfakes.gov. Part of a Department of Commerce initiative, this website includes self-help "toolkits" giving innovators guidance on how to protect intellectual property in specific countries such as China, Korea and Mexico. For questions regarding patent enforcement issues, applicants may call the U.S. Government hotline at 1-866-999-HALT (1-866-999-4258).

LICENSE FOR FOREIGN FILING UNDER

Title 35, United States Code, Section 184

Title 37, Code of Federal Regulations, 5.11 & 5.15

GRANTED

The applicant has been granted a license under 35 U.S.C. 184, if the phrase "IF REQUIRED, FOREIGN FILING LICENSE GRANTED" followed by a date appears on this form. Such licenses are issued in all applications where the conditions for issuance of a license have been met, regardless of whether or not a license may be required as set forth in 37 CFR 5.15. The scope and limitations of this license are set forth in 37 CFR 5.15(a) unless an earlier license has been issued under 37 CFR 5.15(b). The license is subject to revocation upon written notification. The date indicated is the effective date of the license, unless an earlier license of similar scope has been granted under 37 CFR 5.13 or 5.14.

This license is to be retained by the licensee and may be used at any time on or after the effective date thereof unless it is revoked. This license is automatically transferred to any related applications(s) filed under 37 CFR 1.53(d). This license is not retroactive.

The grant of a license does not in any way lessen the responsibility of a licensee for the security of the subject matter as imposed by any Government contract or the provisions of existing laws relating to espionage and the national security or the export of technical data. Licensees should apprise themselves of current regulations especially with respect to certain countries, of other agencies, particularly the Office of Defense Trade Controls, Department of State (with respect to Arms, Munitions and Implements of War (22 CFR 121-128)); the Bureau of Industry and Security, Department of Commerce (15 CFR parts 730-774); the Office of Foreign AssetsControl, Department of Treasury (31 CFR Parts 500+) and the Department of Energy.

NOT GRANTED

No license under 35 U.S.C. 184 has been granted at this time, if the phrase "IF REQUIRED, FOREIGN FILING LICENSE GRANTED" DOES NOT appear on this form. Applicant may still petition for a license under 37 CFR 5.12, if a license is desired before the expiration of 6 months from the filing date of the application. If 6 months has lapsed from the filing date of this application and the licensee has not received any indication of a secrecy order under 35 U.S.C. 181, the licensee may foreign file the application pursuant to 37 CFR 5.15(b).

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
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Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NUMBER	FILING OR 371(C) DATE	FIRST NAMED APPLICANT	ATTY. DOCKET NO./TITLE
14/096,346	12/04/2013	Sandra O'Connor	552815: CPT-011USDV

CONFIRMATION NO. 2832

113613
Lathrop & Gage
28 State Street
Boston, MA 02109-1775

POA ACCEPTANCE LETTER

OC000000070497451

Date Mailed: 09/05/2014

NOTICE OF ACCEPTANCE OF POWER OF ATTORNEY

This is in response to the Power of Attorney filed 08/22/2014.

The Power of Attorney in this application is accepted. Correspondence in this application will be mailed to the above address as provided by 37 CFR 1.33.

/hsarwari/

Office of Data Management, Application Assistance Unit (571) 272-4000, or (571) 272-4200, or 1-888-786-0101

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
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APPLICATION NUMBER	FILING OR 371(C) DATE	FIRST NAMED APPLICANT	ATTY. DOCKET NO./TITLE
14/096,346	12/04/2013	Sandra O'Connor	552815: CPT-011USDV

CONFIRMATION NO. 2832

113613
Lathrop & Gage
28 State Street
Boston, MA 02109-1775

NEW OR REVISED PPD NOTICE

OC000000070554251

NOTICE OF NEW OR REVISED PROJECTED PUBLICATION DATE

The above-identified application has a new or revised projected publication date. The current projected publication date for this application is 12/11/2014. If this is a new projected publication date (there was no previous projected publication date), the application has been cleared by Licensing & Review or a secrecy order has been rescinded and the application is now in the publication queue.

If this is a revised projected publication date (one that is different from a previously communicated projected publication date), the publication date has been revised due to processing delays in the USPTO or the abandonment and subsequent revival of an application. The application is anticipated to be published on a date that is more than six weeks different from the originally-projected publication date.

More detailed publication information is available through the private side of Patent Application Information Retrieval (PAIR) System. The direct link to access PAIR is currently http://pair.uspto.gov. Further assistance in electronically accessing the publication, or about PAIR, is available by calling the Patent Electronic Business Center at 1-866-217-9197.

Questions relating to this Notice should be directed to the Office of Data Management, Application Assistance Unit at (571) 272-4000, or (571) 272-4200, or 1-888-786-0101.

PTO/AIA/80 (07-12)
Approved for use through 11/30/2014. OMB 0651-0035
U.S. Patent and Trademark Office; U.S DEPARTMENT OF COMMERCE

POWER OF ATTORNEY TO PROSECUTE APPLICATIONS BEFORE THE USPTO

I hereby revoke all previous powers of attorney given in the application identified in the attached statement under 37 CFR 3.73(c).

I hereby appoint:

☒ Practitioners associated with Customer Number: 113613

OR

☐ Practitioner(s) named below (if more than ten patent practitioners are to be named, then a customer number must be used):

Name	Registration Number	Name	Registration Number

As attorney(s) or agent(s) to represent the undersigned before the United States Patent and Trademark Office (USPTO) in connection with any and all patent applications assigned only to the undersigned according to the USPTO assignment records or assignments documents attached to this form in accordance with 37 CFR 3.73(c).

Please change the correspondence address for the application identified in the attached statement under 37 CFR 3.73(c) to:

☒ The address associated with Customer Number: 113613

OR

☐	Firm or Individual Name			
	Address			
	City		State	Zip
	Country			
	Telephone		Email	

Assignee Name and Address: Cubist Pharmaceuticals, Inc.
65 Hayden Avenue
Lexington, MA 02421

A copy of this form, together with a statement under 37 CFR 3.73(c) (Form PTO/AIA/96 or equivalent) is required to be Filed in each application in which this form is used. The statement under 37 CFR 3.73(c) may be completed by one of The practitioners appointed in this form, and must identify the application in which this Power of Attorney is to be filed.

SIGNATURE of Assignee of Record

The individual whose signature and title is supplied below is authorized to act on behalf of the assignee

Signature		Date	04/17/14
Name	Thomas J. DesRosier	Telephone	781-860-8660
Title	Secretary		

Privacy Act Statement

The **Privacy Act of 1974 (P.L. 93-579)** requires that you be given certain information in connection with your submission of the attached form related to a patent application or patent. Accordingly, pursuant to the requirements of the Act, please be advised that: (1) the general authority for the collection of this information is 35 U.S.C. 2(b)(2); (2) furnishing of the information solicited is voluntary; and (3) the principal purpose for which the information is used by the U.S. Patent and Trademark Office is to process and/or examine your submission related to a patent application or patent. If you do not furnish the requested information, the U.S. Patent and Trademark Office may not be able to process and/or examine your submission, which may result in termination of proceedings or abandonment of the application or expiration of the patent.

The information provided by you in this form will be subject to the following routine uses:

1. The information on this form will be treated confidentially to the extent allowed under the Freedom of Information Act (5 U.S.C. 552) and the Privacy Act (5 U.S.C 552a). Records from this system of records may be disclosed to the Department of Justice to determine whether disclosure of these records is required by the Freedom of Information Act.
2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.
3. A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.
4. A record in this system of records may be disclosed, as a routine use, to a contractor of the Agency having need for the information in order to perform a contract. Recipients of information shall be required to comply with the requirements of the Privacy Act of 1974, as amended, pursuant to 5 U.S.C. 552a(m).
5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent Cooperation Treaty.
6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).
7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (*i.e.*, GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.
8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspection or an issued patent.
9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

Electronic Patent Application Fee Transmittal

Application Number:	14096346
Filing Date:	04-Dec-2013
Title of Invention:	LIPOPEPTIDE COMPOSITIONS AND RELATED METHODS
First Named Inventor/Applicant Name:	Sandra O'Connor
Filer:	Brian C. Trinque/Denise Vincent
Attorney Docket Number:	552815: CPT-011USDV

Filed as Large Entity

Utility under 35 USC 111(a) Filing Fees

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Basic Filing:				
Pages:				
Claims:				
Miscellaneous-Filing:				
Petition:				
Pet. Revive Abandon App, Delay Pymt-Resp	1453	1	1700	1700
Patent-Appeals-and-Interference:				
Post-Allowance-and-Post-Issuance:				
Extension-of-Time:				

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Miscellaneous:				
			Total in USD ($)	**1700**

Electronic Acknowledgement Receipt

EFS ID:	19937884
Application Number:	14096346
International Application Number:	
Confirmation Number:	2832
Title of Invention:	LIPOPEPTIDE COMPOSITIONS AND RELATED METHODS
First Named Inventor/Applicant Name:	Sandra O'Connor
Customer Number:	113613
Filer:	Brian C. Trinque
Filer Authorized By:	
Attorney Docket Number:	552815: CPT-011USDV
Receipt Date:	22-AUG-2014
Filing Date:	04-DEC-2013
Time Stamp:	14:34:15
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	yes
Payment Type	Deposit Account
Payment was successfully received in RAM	$1700
RAM confirmation Number	830
Deposit Account	120600
Authorized User	
The Director of the USPTO is hereby authorized to charge indicated fees and credit any overpayment as follows: Charge any Additional Fees required under 37 C.F.R. Section 1.16 (National application filing, search, and examination fees) Charge any Additional Fees required under 37 C.F.R. Section 1.17 (Patent application and reexamination processing fees)	

Charge any Additional Fees required under 37 C.F.R. Section 1.19 (Document supply fees)	
Charge any Additional Fees required under 37 C.F.R. Section 1.20 (Post Issuance fees)	
Charge any Additional Fees required under 37 C.F.R. Section 1.21 (Miscellaneous fees and charges)	

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Request for Corrected Filing Receipt	552815_CPT-011USDV_PETITION_CORRECT_FILING_RECEIPT_8-22-14_21987376_.pdf	21134 4d0f6430ead99afa30de17f569622dedbc10f166	no	2
Warnings:					
Information:					
2	Assignee showing of ownership per 37 CFR 3.73.	552815_CPT-011USDV_STATEMENT_UNDER_CFR_373C_AS_FILED_8-22-14.pdf	118081 7bc6544393cb5118266e29c4470e4729e91c1afb	no	3
Warnings:					
Information:					
3	Application Data Sheet	552815_CPT-011USDV_CORRECTED_ADS_8-22-14.pdf	578418 39a37584d25a7668bb1c051d628722f70e726a52	no	7
Warnings:					
Information:					
This is not an USPTO supplied ADS fillable form					
4	Power of Attorney	552815_CPT-011USDV_POA_8-22-14.pdf	3149979 9077ea1959390644461a9c8bff381f47b91aea8ea	no	2
Warnings:					
Information:					
5	Fee Worksheet (SB06)	fee-info.pdf	30504 59246cc84137e47e9825dfb7e6154efea63e83c3	no	2
Warnings:					
Information:					
		Total Files Size (in bytes):	3898116		

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

New Applications Under 35 U.S.C. 111
If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371
If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office
If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

I hereby certify that this paper (along with any paper referred to as being attached or enclosed) is being transmitted via the Office electronic filing system in accordance with § 1.6(a)(4).

Dated: August 22, 2014
Electronic Signature for Brian C. Trinque, Ph.D., Esq.: /Brian C. Trinque/

PATENT
Attorney Docket No.
552815: CPT-011USDV

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of:	
Sandra O'Connor et al.	Confirmation No. 2832
Application No. 14/096,346	Art Unit: 1654
Filed: December 4, 2013	Examiner: Li N. Komatsu

For: LIPOPEPTIDE COMPOSITIONS AND RELATED METHODS

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

PETITION UNDER 37 CFR 1.78
RESPONSE TO RESPONSE TO REQUEST FOR CORRECTION OF FILING RECEIPT

Dear Colleagues,

Further to the Response to Response to Request for Correction of Filing Receipt dated May 5, 2014, Applicants submit herewith a Corrected Application Data Sheet containing changes in inventor addresses and domestic benefit/national stage information in accordance with 37 CFR 1.76(c)(2).

Applicant notes that any delay that may be associated with this Petition is unintentional as indicated by Applicant's numerous requests for a corrected Filing Receipt as previously filed with the Office in the above-referenced application.

Applicants requests that a Corrected Filing Receipt be issued reflecting the above changes.

Applicant hereby submits under 37 CFR 1.78 and 1.17(m) all applicable fees due. The Commissioner is authorized to charge any fees that are due to our Deposit Account No. 12-0600, under Docket Number 552815 CPT-011USDV.

Dated: August 22, 2014

Respectfully submitted,

/Brian C. Trinque/

Brian C. Trinque, Ph.D., Esq.
Registration No.: 56,593
LATHROP & GAGE LLP
28 State Street, 7th Floor
Boston, Massachusetts 02109
857-300-4003
857-300-4001 (Fax)
Attorney/Agent for Applicants

PTO/AIA/96 (08-12)
Approved for use through 01/31/2013. OMB 0651-0031
U.S. Patent and Trademark Office;U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of1995, no persons arerequired to respond to a collection of information unless it displays a valid OMB control number.

STATEMENT UNDER 37 CFR 3.73(c)

Applicant/Patent Owner: Cubist Pharmaceuticals, Inc.

Application No./Patent No.: 14/096,346 Filed/Issue Date: December 4, 2013

Titled: LIPOPEPTIDE COMPOSITIONS AND RELATED METHODS

Cubist Pharmaceuticals, Inc., a Corporation

(Name of Assignee) (Type of Assignee, e.g., corporation, partnership, university, government agency, etc.)

states that, for the patent application/patent identified above, it is (choose **one** of options 1, 2, 3 or 4 below):

1. ☑ The assignee of the entire right, title, and interest.

2. ☐ An assignee of less than the entire right, title, and interest (check applicable box):

   ☐ The extent (by percentage) of its ownership interest is _____%. Additional Statement(s) by the owners holding the balance of the interest must be submitted to account for 100% of the ownership interest.

   ☐ There are unspecified percentages of ownership. The other parties, including inventors, who together own the entire right, title and interest are:

   Additional Statement(s) by the owner(s) holding the balance of the interest must be submitted to account for the entire right, title, and interest.

3. ☐ The assignee of an undivided interest in the entirety (a complete assignment from one of the joint inventors was made). The other parties, including inventors, who together own the entire right, title, and interest are:

   Additional Statement(s) by the owner(s) holding the balance of the interest must be submitted to account for the entire right, title, and interest.

4. ☐ The recipient, via a court proceeding or the like (*e.g.*, bankruptcy, probate), of an undivided interest in the entirety (a complete transfer of ownership interest was made). The certified document(s) showing the transfer is attached.

The interest identified in option 1, 2 or 3 above (not option 4) is evidenced by either (choose **one** of options A or B below):

A. ☑ An assignment from the inventor(s) of the patent application/patent identified above. The assignment was recorded in the United States Patent and Trademark Office at Reel 032543, Frame 0011, or for which a copy thereof is attached.

B. ☐ A chain of title from the inventor(s), of the patent application/patent identified above, to the current assignee as follows:

   1. From: _____ To: _____

      The document was recorded in the United States Patent and Trademark Office at

      Reel _____, Frame _____, or for which a copy thereof is attached.

   2. From: _____ To: _____

      The document was recorded in the United States Patent and Trademark Office at

      Reel _____, Frame _____, or for which a copy thereof is attached.

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STATEMENT UNDER 37 CFR 3.73(c)

3. From: _____ To: _____

The document was recorded in the United States Patent and Trademark Office at

Reel _____, Frame _____, or for which a copy thereof is attached.

4. From: _____ To: _____

The document was recorded in the United States Patent and Trademark Office at

Reel _____, Frame _____, or for which a copy thereof is attached.

5. From: _____ To: _____

The document was recorded in the United States Patent and Trademark Office at

Reel _____, Frame _____, or for which a copy thereof is attached.

6. From: _____ To: _____

The document was recorded in the United States Patent and Trademark Office at

Reel _____, Frame _____, or for which a copy thereof is attached.

☐ Additional documents in the chain of title are listed on a supplemental sheet(s).

☐ As required by 37 CFR 3.73(c)(1)(i), the documentary evidence of the chain of title from the original owner to the assignee was, or concurrently is being, submitted for recordation pursuant to 37 CFR 3.11.

[NOTE: A separate copy (i.e., a true copy of the original assignment document(s)) must be submitted to Assignment Division in accordance with 37 CFR Part 3, to record the assignment in the records of the USPTO. See MPEP 302.08]

The undersigned (whose title is supplied below) is authorized to act on behalf of the assignee.

/Brian C. Trinque/	08/22/2014
Signature	Date
Brian C. Trinque, Ph.D., Esq.	56,593
Printed or Typed Name	Title or Registration Number

Privacy Act Statement

The **Privacy Act of 1974 (P.L. 93-579)** requires that yoube given certain informationin connection with your submission of the attached form related to a patent application or patent. Accordingly, pursuant to the requirements of the Act, pleasebe advised that: (1) the general authority forthe collection of thisinformation is 35 U.S.C. 2(b)(2); (2) furnishing of the information solicited is voluntary; and(3) the principal purpose forwhich the information isused by the U.S. Patent and Trademark Office is to process and/or examine your submission related to a patent applicationor patent. If you do not furnish the requested information,the U.S. Patent and Trademark Office may not be able to process and/or examineyour submission,which may result in termination of proceedings or abandonment of the applicationor expiration of the patent.

The informationprovided by you in this form will be subject to the following routine uses:

1. The information on this form will be treated confidentially to the extent allowed under the Freedom of Information Act (5 U.S.C. 552) and the Privacy Act (5 U.S.C 552a). Records from this system of records may be disclosed to the Department of Justice to determine whether disclosure of these records is required by the Freedom of Information Act.
2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.
3. A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.
4. A record in this system of records may be disclosed, as a routine use, to a contractor of the Agency having need for the informationin order to perform a contract. Recipients of information shall be required to comply with the requirements of the Privacy Act of 1974, as amended, pursuant to 5 U.S.C. 552a(m).
5. A record related to an InternationalApplication filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent Cooperation Treaty.
6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).
7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (*i.e.*, GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.
8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, arecord may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspection or an issued patent.
9. A record from thissystem of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

PTO/AIA/14 (11-13)
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Corrected Application Data Sheet 37 CFR 1.76	Attorney Docket Number	552815 (CPT-011USDV)
	Application Number	14/096,346
Title of Invention	LIPOPEPTIDE COMPOSITIONS AND RELATED METHODS	

The application data sheet is part of the provisional or nonprovisional application for which it is being submitted. The following form contains the bibliographic data arranged in a format specified by the United States Patent and Trademark Office as outlined in 37 CFR 1.76.
This document may be completed electronically and submitted to the Office in electronic format using the Electronic Filing System (EFS) or the document may be printed and included in a paper filed application.

Secrecy Order 37 CFR 5.2

☐ Portions or all of the application associated with this Application Data Sheet may fall under a Secrecy Order pursuant to 37 CFR 5.2 (Paper filers only. Applications that fall under Secrecy Order may not be filed electronically.)

Inventor Information:

Inventor 1 — Remove

Legal Name

Prefix	Given Name	Middle Name	Family Name	Suffix
	Sandra		O'Connor	

Residence Information (Select One) ◉ US Residency ○ Non US Residency ○ Active US Military Service

City	Hudson	State/Province	NH	Country of Residence	US

Mailing Address of Inventor:

Address 1	12 Copper Hill Road		
Address 2			
City	Hudson	State/Province	NH
Postal Code	03051	Country	US

Inventor 2 — Remove

Legal Name

Prefix	Given Name	Middle Name	Family Name	Suffix
	Sophie		Sun	

Residence Information (Select One) ◉ US Residency ○ Non US Residency ○ Active US Military Service

City	Lexington	State/Province	MA	Country of Residence	US

Mailing Address of Inventor:

Address 1	20 Woodcliffe Road		
Address 2			
City	Lexington	State/Province	MA
Postal Code	02421	Country	US

Inventor 3 — Remove

Legal Name

Prefix	Given Name	Middle Name	Family Name	Suffix
	Gaauri		Naik	

Residence Information (Select One) ◉ US Residency ○ Non US Residency ○ Active US Military Service

PTO/AIA/14 (11-13)
Approved for use through 01/31/2014. OMB 0651-0032
U.S. Patent and Trademark Office: U.S. DEPARTMENT OF COMMERCE

Application Data Sheet 37 CFR 1.76	Attorney Docket Number	552815 (CPT-011USDV)
	Application Number	14/096,346
Title of Invention	LIPOPEPTIDE COMPOSITIONS AND RELATED METHODS	

City	Cambridge	State/Province	MA	Country of Residence	US

Mailing Address of Inventor:

Address 1	4 Trowbridge Place, Unit # 2D, Harvard Square		
Address 2			
City	Cambridge	State/Province	MA
Postal Code	02138	Country	US

All Inventors Must Be Listed - Additional Inventor Information blocks may be generated within this form by selecting the **Add** button. Add

Correspondence Information:

Enter either Customer Number or complete the Correspondence Information section below.
For further information see 37 CFR 1.33(a).

☐ **An Address is being provided for the correspondence Information of this application.**

Customer Number	113613		
Email Address	bostonpatent@lathropgage.com	Add Email	Remove Email

Application Information:

Title of the Invention	LIPOPEPTIDE COMPOSITIONS AND RELATED METHODS		
Attorney Docket Number	552815 (CPT-011USDV)	Small Entity Status Claimed	☐
Application Type	Nonprovisional		
Subject Matter	Utility		
Total Number of Drawing Sheets (if any)	22	Suggested Figure for Publication (if any)	

Publication Information:

☐ Request Early Publication (Fee required at time of Request 37 CFR 1.219)

☐ **Request Not to Publish.** I hereby request that the attached application not be published under 35 U.S.C. 122(b) and certify that the invention disclosed in the attached application **has not and will not** be the subject of an application filed in another country, or under a multilateral international agreement, that requires publication at eighteen months after filing.

Representative Information:

Representative information should be provided for all practitioners having a power of attorney in the application. Providing this information in the Application Data Sheet does not constitute a power of attorney in the application (see 37 CFR 1.32).
Either enter Customer Number or complete the Representative Name section below. If both sections are completed the customer Number will be used for the Representative Information during processing.

Please Select One:	◉ Customer Number	○ US Patent Practitioner	○ Limited Recognition (37 CFR 11.9)

PTO/AIA/14 (11-13)
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Application Data Sheet 37 CFR 1.76	Attorney Docket Number	552815 (CPT-011USDV)
	Application Number	14/096,346
Title of Invention	LIPOPEPTIDE COMPOSITIONS AND RELATED METHODS	

Customer Number	113613

Domestic Benefit/National Stage Information:

This section allows for the applicant to either claim benefit under 35 U.S.C. 119(e), 120, 121, or 365(c) or indicate National Stage entry from a PCT application. Providing this information in the application data sheet constitutes the specific reference required by 35 U.S.C. 119(e) or 120, and 37 CFR 1.78.
When referring to the current application, please leave the application number blank.

Prior Application Status	Pending		Remove
Application Number	Continuity Type	Prior Application Number	Filing Date (YYYY-MM-DD)
	Division of	13511246	2012-05-22
Prior Application Status			Remove
Application Number	Continuity Type	Prior Application Number	Filing Date (YYYY-MM-DD)
13511246	a 371 of international	PCT/US2010/057910	2010-11-23
Prior Application Status			Remove
Application Number	Continuity Type	Prior Application Number	Filing Date (YYYY-MM-DD)
PCT/US2010/057910	Claims benefit of provisional	61263784	2009-11-23

Additional Domestic Benefit/National Stage Data may be generated within this form by selecting the **Add** button.

Foreign Priority Information:

This section allows for the applicant to claim priority to a foreign application. Providing this information in the application data sheet constitutes the claim for priority as required by 35 U.S.C. 119(b) and 37 CFR 1.55(d). When priority is claimed to a foreign application that is eligible for retrieval under the priority document exchange program (PDX)[1] the information will be used by the Office to automatically attempt retrieval pursuant to 37 CFR 1.55(h)(1) and (2). Under the PDX program, applicant bears the ultimate responsibility for ensuring that a copy of the foreign application is received by the Office from the participating foreign intellectual property office, or a certified copy of the foreign priority application is filed, within the time period specified in 37 CFR 1.55(g)(1).

Remove

Application Number	Country[1]	Filing Date (YYYY-MM-DD)	Access Code[1] (if applicable)

Additional Foreign Priority Data may be generated within this form by selecting the **Add** button.

PTO/AIA/14 (11-13)
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Application Data Sheet 37 CFR 1.76	Attorney Docket Number	552815 (CPT-011USDV)
	Application Number	14/096,346
Title of Invention	LIPOPEPTIDE COMPOSITIONS AND RELATED METHODS	

Statement under 37 CFR 1.55 or 1.78 for AIA (First Inventor to File) Transition Applications

☐ This application (1) claims priority to or the benefit of an application filed before March 16, 2013 and (2) also contains, or contained at any time, a claim to a claimed invention that has an effective filing date on or after March 16, 2013.
NOTE: By providing this statement under 37 CFR 1.55 or 1.78, this application, with a filing date on or after March 16, 2013, will be examined under the first inventor to file provisions of the AIA.

Authorization to Permit Access:

☐ Authorization to Permit Access to the Instant Application by the Participating Offices

If checked, the undersigned hereby grants the USPTO authority to provide the European Patent Office (EPO), the Japan Patent Office (JPO), the Korean Intellectual Property Office (KIPO), the World Intellectual Property Office (WIPO), and any other intellectual property offices in which a foreign application claiming priority to the instant patent application is filed access to the instant patent application. See 37 CFR 1.14(c) and (h). This box should not be checked if the applicant does not wish the EPO, JPO, KIPO, WIPO, or other intellectual property office in which a foreign application claiming priority to the instant patent application is filed to have access to the instant patent application.

In accordance with 37 CFR 1.14(h)(3), access will be provided to a copy of the instant patent application with respect to: 1) the instant patent application-as-filed; 2) any foreign application to which the instant patent application claims priority under 35 U.S.C. 119(a)-(d) if a copy of the foreign application that satisfies the certified copy requirement of 37 CFR 1.55 has been filed in the instant patent application; and 3) any U.S. application-as-filed from which benefit is sought in the instant patent application.

In accordance with 37 CFR 1.14(c), access may be provided to information concerning the date o f filing this Authorization.

Applicant Information:

Providing assignment information in this section does not substitute for compliance with any requirement of part 3 of Title 37 of CFR to have an assignment recorded by the Office.

PTO/AIA/14 (11-13)
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Application Data Sheet 37 CFR 1.76	Attorney Docket Number	552815 (CPT-011USDV)
	Application Number	14/096,346
Title of Invention	LIPOPEPTIDE COMPOSITIONS AND RELATED METHODS	

Applicant 1

If the applicant is the inventor (or the remaining joint inventor or inventors under 37 CFR 1.45), this section should not be completed. The information to be provided in this section is the name and address of the legal representative who is the applicant under 37 CFR 1.43; or the name and address of the assignee, person to whom the inventor is under an obligation to assign the invention, or person who otherwise shows sufficient proprietary interest in the matter who is the applicant under 37 CFR 1.46. If the applicant is an applicant under 37 CFR 1.46 (assignee, person to whom the inventor is obligated to assign, or person who otherwise shows sufficient proprietary interest) together with one or more joint inventors, then the joint inventor or inventors who are also the applicant should be identified in this section.

Clear

◉ Assignee	○ Legal Representative under 35 U.S.C. 117	○ Joint Inventor
○ Person to whom the inventor is obligated to assign.	○ Person who shows sufficient proprietary interest	

If applicant is the legal representative, indicate the authority to file the patent application, the inventor is:

Name of the Deceased or Legally Incapacitated Inventor :	
If the Applicant is an Organization check here.	☒
Organization Name	Cubist Pharmaceuticals, Inc.

Mailing Address Information For Applicant:

Address 1	65 Hayden Avenue		
Address 2			
City	Lexington	**State/Province**	MA
Country	US	Postal Code	02421
Phone Number		Fax Number	
Email Address			

Additional Applicant Data may be generated within this form by selecting the Add button.

Assignee Information including Non-Applicant Assignee Information:

Providing assignment information in this section does not subsitute for compliance with any requirement of part 3 of Title 37 of CFR to have an assignment recorded by the Office.

Assignee 1

Complete this section if assignee information, including non-applicant assignee information, is desired to be included on the patent application publication . An assignee-applicant identified in the "Applicant Information" section will appear on the patent application publication as an applicant. For an assignee-applicant, complete this section only if identification as an assignee is also desired on the patent application publication.

If the Assignee or Non-Applicant Assignee is an Organization check here. ☒

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Application Data Sheet 37 CFR 1.76	Attorney Docket Number	552815 (CPT-011USDV)
	Application Number	14/096,346
Title of Invention	LIPOPEPTIDE COMPOSITIONS AND RELATED METHODS	

Organization Name	Cubist Pharmaceuticals, Inc.		
Mailing Address Information For Assignee including Non-Applicant Assignee:			
Address 1	65 Hayden Avenue		
Address 2			
City	Lexington	**State/Province**	MA
Country i	US	Postal Code	02421
Phone Number		Fax Number	
Email Address			

Additional Assignee or Non-Applicant Assignee Data may be generated within this form by selecting the Add button.

Signature:

NOTE: This form must be signed in accordance with 37 CFR 1.33. See 37 CFR 1.4 for signature requirements and certifications.

Signature	/Brian C. Trinque/			Date (YYYY-MM-DD)	2014-08-22
First Name	Brian C.	Last Name	Trinque	Registration Number	56593

Additional Signature may be generated within this form by selecting the Add button.

This collection of information is required by 37 CFR 1.76. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 23 minutes to complete, including gathering, preparing, and submitting the completed application data sheet form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

Privacy Act Statement

The Privacy Act of 1974 (P.L. 93-579) requires that you be given certain information in connection with your submission of the attached form related to a patent application or patent. Accordingly, pursuant to the requirements of the Act, please be advised that: (1) the general authority for the collection of this information is 35 U.S.C. 2(b)(2); (2) furnishing of the information solicited is voluntary; and (3) the principal purpose for which the information is used by the U.S. Patent and Trademark Office is to process and/or examine your submission related to a patent application or patent. If you do not furnish the requested information, the U.S. Patent and Trademark Office may not be able to process and/or examine your submission, which may result in termination of proceedings or abandonment of the application or expiration of the patent.

The information provided by you in this form will be subject to the following routine uses:

1. The information on this form will be treated confidentially to the extent allowed under the Freedom of Information Act (5 U.S.C. 552) and the Privacy Act (5 U.S.C. 552a). Records from this system of records may be disclosed to the Department of Justice to determine whether the Freedom of Information Act requires disclosure of these records.

2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.

3. A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.

4. A record in this system of records may be disclosed, as a routine use, to a contractor of the Agency having need for the information in order to perform a contract. Recipients of information shall be required to comply with the requirements of the Privacy Act of 1974, as amended, pursuant to 5 U.S.C. 552a(m).

5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent C o o p eration Treaty.

6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).

7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (i.e., GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.

8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspections or an issued patent.

9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
14/096,346	12/04/2013	Sandra O'Connor	552815 (CPT-011USDV)	2832

113613 7590 06/25/2014

Lathrop & Gage
28 State Street
Boston, MA 02109-1775

EXAMINER	
KOMATSU, LI N	
ART UNIT	PAPER NUMBER
1676	

NOTIFICATION DATE	DELIVERY MODE
06/25/2014	ELECTRONIC

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

bostonpatent@lathropgage.com
cubist_docketing@cardinal-ip.com

Office Action Summary	Application No. 14/096,346	Applicant(s) O'CONNOR ET AL.	
	Examiner LI NI KOMATSU	Art Unit 1676	AIA (First Inventor to File) Status Yes

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 2 MONTHS FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *12/4/2013*.

☐ A declaration(s)/affidavit(s) under **37 CFR 1.130(b)** was/were filed on _____.

2a)☐ This action is **FINAL**. 2b)☐ This action is non-final.

3)☐ An election was made by the applicant in response to a restriction requirement set forth during the interview on _____; the restriction requirement and election have been incorporated into this action.

4)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims*

5)☒ Claim(s) *22-42* is/are pending in the application.

5a) Of the above claim(s) _____ is/are withdrawn from consideration.

6)☐ Claim(s) _____ is/are allowed.

7)☐ Claim(s) _____ is/are rejected.

8)☐ Claim(s) _____ is/are objected to.

9)☒ Claim(s) *22-42* are subject to restriction and/or election requirement.

* If any claims have been determined allowable, you may be eligible to benefit from the **Patent Prosecution Highway** program at a participating intellectual property office for the corresponding application. For more information, please see http://www.uspto.gov/patents/init_events/pph/index.jsp or send an inquiry to PPHfeedback@uspto.gov.

Application Papers

10)☐ The specification is objected to by the Examiner.

11)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

Certified copies:

a)☐ All b)☐ Some** c)☐ None of the:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

** See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☐ Notice of References Cited (PTO-892)

2) ☐ Information Disclosure Statement(s) (PTO/SB/08a and/or PTO/SB/08b) Paper No(s)/Mail Date _____.

3) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .

4) ☐ Other: _____.

DETAILED ACTION

1. The present application, filed on or after March 16, 2013, is being examined under the first inventor to file provisions of the AIA.

2. Claims filed on 12/4/2013 are acknowledged. Claims 1-21 have been cancelled. New claims 22-42 have been added. Claims 22-42 are pending in this application.

Election of Species

3. This application contains claims directed to the following patentably distinct species:

(Please elect a single disclosed species of EACH)

A specific solid pharmaceutical daptomycin composition: due to different variables;

A specific excipient: due to different variables from claims 22, 26 and 27;

A specific molar ratio of daptomycin to the sugar: due to different variables;

A specific pH of an aqueous daptomycin solution: from claims 34-38;

A specific buffering agent: from claim 39 and see page 4, lines 14-15 of instant specification;

A specific way to convert the aqueous daptomycin solution to a solid pharmaceutical composition: from claim 40.

The species are independent or distinct because claims to the different species recite the mutually exclusive characteristics of such species. In addition, these species are not obvious variants of each other based on the current record.

Applicant is required under 35 U.S.C. 121 to elect a single disclosed species, or a single grouping of patentably indistinct specie, for prosecution on the merits to which the claims shall be restricted if no generic claim is finally held to be allowable. Applicant is required to elect a single disclosed species of a solid pharmaceutical daptomycin composition wherein ALL the variables are elected to arrive at a single disclosed species of a solid pharmaceutical daptomycin composition. Currently, claims 22-25 and 34-42 are generic.

There is a search and/or examination burden for the patentably distinct species as set forth above due to their mutually exclusive characteristics. The species require a different field of search (for

example, searching different classes/subclasses or electronic resources, or employing different search queries); and/or prior art applicable to one species would not likely be applicable to another species, and/or the species are likely to raise different non-prior art issues under 35 U.S.C. 101 and/or 35 U.S.C. 112, first paragraph.

Applicant is advised that the reply to this requirement to be complete must include (i) an election of a species or a grouping of patentably indistinct species to be examined even though the requirement may be traversed (37 CFR 1.143) **and (ii) identification of the claims encompassing the elected species or grouping of patentably indistinct species**, including any claims subsequently added. An argument that a claim is allowable or that all claims are generic is considered nonresponsive unless accompanied by an election.

The election of species may be made with or without traverse. To preserve a right to petition, the election must be made with traverse. If the reply does not distinctly and specifically point out supposed errors in the election of species requirement, the election shall be treated as an election without traverse. Traversal must be presented at the time of election in order to be considered timely. Failure to timely traverse the requirement will result in the loss of right to petition under 37 CFR 1.144. If claims are added after the election, applicant must indicate which of these claims are readable on the elected species or grouping of patentably indistinct species.

Should applicant traverse on the ground that the species, or groupings of patentably indistinct species from which election is required, are not patentably distinct, applicant should submit evidence or identify such evidence now of record showing them to be obvious variants or clearly admit on the record that this is the case. In either instance, if the examiner finds one of the species unpatentable over the prior art, the evidence or admission may be used in a rejection under 35 U.S.C. 103(a) of the other species.

Upon the allowance of a generic claim, applicant will be entitled to consideration of claims to additional species which depend from or otherwise require all the limitations of an allowable generic claim as provided by 37 CFR 1.141.

Conclusion

Any inquiry concerning this communication or earlier communications from the examiner should be directed to LI NI KOMATSU whose telephone number is (571)270-3534. The examiner can normally be reached on Mon-Thurs 8-5pm EST.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Karlheinz Skowronek can be reached on (571)-272-9047. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

/L. K./

Examiner, Art Unit 1676

/JULIE HA/

Primary Examiner, Art Unit 1675

I hereby certify that this paper (along with any paper referred to as being attached or enclosed) is being transmitted via the Office electronic filing system in accordance with § 1.6(a)(4).

Dated: May 5, 2014
Electronic Signature for Brian C. Trinque, Ph.D., Esq.: /Brian C. Trinque/

PATENT
Attorney Docket No.
552815 (CPT-011USDV)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of:	
Sandra O'Connor et al.	Confirmation No. 2832
Application No. 14/096,346	Art Unit: 1654
Filed: December 4, 2013	Examiner: Li N. Komatsu

For: LIPOPEPTIDE COMPOSITIONS AND RELATED METHODS

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

RESPONSE TO RESPONSE TO REQUEST FOR CORRECTION OF FILING RECEIPT

Dear Sir/Madam:

In response to the Response to Request for Corrected Filing Receipt issued by the Office on April 1, 2014 in which Applicant's claim for priority was not accepted allegedly due to the fact that the priority claim was not filed during the required time period and was not accompanied by the necessary Application Data Sheet (ADS) with the proper underlines and strike-outs, Applicant herein states as follows:

1) This application was filed on December 4, 2013 with an ADS containing all applicable, correct priority information;
2) The Applicant learned from the Notice to File Missing Parts issued on January 2, 2014 that the substantively-correct ADS was not accepted because a back slash (/) was missing from the electronic signature, and was, therefore, considered unsigned and the priority information unentered;
3) On January 24, 2014, a Response to Notice to File Missing Parts was filed containing a new, properly-signed, ADS still containing the correct priority information; and
4) As the priority information was properly filed within four months of the date of the application as required by 37 C.F.R. 1.78 by filing a complete, properly-signed ADS

containing the priority information, and as no underlines or strike-outs were necessary since there were no changes to the ADS originally-filed with the application, except that it was signed, Applicant respectfully requests that the priority information properly filed with the January 24, 2014 ADS be entered.

Applicant requests that a Corrected Filing Receipt be issued containing the correct priority information.

Although no fees are believed to be due, the Commissioner is authorized to charge any fees that are due to our Deposit Account No. 12-0600, under Docket Number 552815 CPT-011USDV.

Dated: May 5, 2014

Respectfully submitted,

/Brian C. Trinque/

Brian C. Trinque, Ph.D., Esq.
Registration No.: 56,593
LATHROP & GAGE LLP
28 State Street, 7th Floor
Boston, Massachusetts 02109
857-300-4003
857-300-4001 (Fax)
Attorney/Agent for Applicant

Electronic Acknowledgement Receipt

EFS ID:	18936222
Application Number:	14096346
International Application Number:	
Confirmation Number:	2832
Title of Invention:	LIPOPEPTIDE COMPOSITIONS AND RELATED METHODS
First Named Inventor/Applicant Name:	Sandra O'Connor
Customer Number:	113613
Filer:	Brian C. Trinque
Filer Authorized By:	
Attorney Docket Number:	552815 (CPT-011USDV)
Receipt Date:	05-MAY-2014
Filing Date:	04-DEC-2013
Time Stamp:	09:38:10
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	no

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Request for Corrected Filing Receipt	552815Response.pdf	21840 4b104cda5d3086e96bbb00e919959e2cd78ad298	no	2

Warnings:

Information:

Total Files Size (in bytes):	21840

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

New Applications Under 35 U.S.C. 111
If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371
If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office
If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NUMBER	FILING OR 371(C) DATE	FIRST NAMED APPLICANT	ATTY. DOCKET NO./TITLE
14/096,346	12/04/2013	Sandra O'Connor	552815 (CPT-011USDV)

CONFIRMATION NO. 2832

113613
Cubist Pharmaceuticals, Inc.
Lathrop & Gage
65 Hayden Avenue
Lexington, MA 02421

NEW OR REVISED PPD NOTICE

OC000000067549298

NOTICE OF NEW OR REVISED PROJECTED PUBLICATION DATE

The above-identified application has a new or revised projected publication date. The current projected publication date for this application is 06/04/2015. If this is a new projected publication date (there was no previous projected publication date), the application has been cleared by Licensing & Review or a secrecy order has been rescinded and the application is now in the publication queue.

If this is a revised projected publication date (one that is different from a previously communicated projected publication date), the publication date has been revised due to processing delays in the USPTO or the abandonment and subsequent revival of an application. The application is anticipated to be published on a date that is more than six weeks different from the originally-projected publication date.

More detailed publication information is available through the private side of Patent Application Information Retrieval (PAIR) System. The direct link to access PAIR is currently http://pair.uspto.gov. Further assistance in electronically accessing the publication, or about PAIR, is available by calling the Patent Electronic Business Center at 1-866-217-9197.

Questions relating to this Notice should be directed to the Office of Data Management, Application Assistance Unit at (571) 272-4000, or (571) 272-4200, or 1-888-786-0101.

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NUMBER	FILING OR 371(C) DATE	FIRST NAMED APPLICANT	ATTY. DOCKET NO./TITLE
14/096,346	12/04/2013	Sandra O'Connor	552815 (CPT-011USDV)

CONFIRMATION NO. 2832

113613
Cubist Pharmaceuticals, Inc.
Lathrop & Gage
65 Hayden Avenue
Lexington, MA 02421

IMPROPER CFR REQUEST

OC000000067460043

Date Mailed: 04/01/2014

RESPONSE TO REQUEST FOR CORRECTED FILING RECEIPT

Continuity, Priority Claims, Petitions, and Non-Publication Requests

In response to your request for a corrected Filing Receipt, the Office is unable to comply with your request because:

- The priority or continuity claim has not been entered because it was not filed during the required time period. Applicant may wish to consider filing a petition to accept an unintentionally delayed claim for priority. See 37 CFR 1.55 or 1.78.
- To add or correct a benefit claim under 35 U.S.C. 119(e), 120, 121, or 365(c) to a prior-filed national application or international application designating the United States, applicant must submit a new application data sheet (ADS) with the desired benefit claim. For information being changed relative to the information already of record, additions should be shown with underlining, and deletions should be shown with strikeouts. A domestic benefit claim that is presented after the time period set forth in 37 CFR 1.78 must be accompanied by a petition under 37 CFR 1.78.

/byemane/

Office of Data Management, Application Assistance Unit (571) 272-4000, or (571) 272-4200, or 1-888-786-0101

Doc code: IDS
Doc description: Information Disclosure Statement (IDS) Filed

PTO/SB/08a (01-10)
Approved for use through 07/31/2012. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it contains a valid OMB control number.

INFORMATION DISCLOSURE STATEMENT BY APPLICANT (**Not for submission under 37 CFR 1.99)**		
	Application Number	14096346
	Filing Date	2013-12-04
	First Named Inventor	Sandra O'Connor
	Art Unit	1676
	Examiner Name	Li N. Komatsu
	Attorney Docket Number	552815 (CPT-011USDV)

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Examiner Initial*	Cite No	Publication Number	Kind Code[1]	Publication Date	Name of Patentee or Applicant of cited Document	Pages,Columns,Lines where Relevant Passages or Relevant Figures Appear
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Examiner Initial*	Cite No	Foreign Document Number[3]	Country Code[2] ¡	Kind Code[4]	Publication Date	Name of Patentee or Applicant of cited Document	Pages,Columns,Lines where Relevant Passages or Relevant Figures Appear	T[5]
	1	WO2009144739	WO	A1	2009-12-03	Biocon Limited		☐
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NON-PATENT LITERATURE DOCUMENTS			Remove
Examiner Initials*	Cite No	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc), date, pages(s), volume-issue number(s), publisher, city and/or country where published.	T[5]

INFORMATION DISCLOSURE STATEMENT BY APPLICANT (**Not for submission under 37 CFR 1.99)**		
	Application Number	14096346
	Filing Date	2013-12-04
	First Named Inventor	Sandra O'Connor
	Art Unit	1676
	Examiner Name	Li N. Komatsu
	Attorney Docket Number	552815 (CPT-011USDV)

	1	Supplementary European Search Report PCT/US2010057910 Dated February 28, 2014. 8 Pages	☐
	2	DEBONO: Enzymatic and Chemical Modifications of Lipopeptide Antibiotic A21978C: The Synthesis and Evaluation of Daptomycin (LY146032), The Journal of Antibiotics, 1988, 41(8):1093-1105	☐

If you wish to add additional non-patent literature document citation information please click the Add button Add

EXAMINER SIGNATURE

Examiner Signature		Date Considered	

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through a citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

[1] See Kind Codes of USPTO Patent Documents at www.USPTO.GOV or MPEP 901.04. [2] Enter office that issued the document, by the two-letter code (WIPO Standard ST.3). [3] For Japanese patent documents, the indication of the year of the reign of the Emperor must precede the serial number of the patent document. [4] Kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST.16 if possible. [5] Applicant is to place a check mark here if English language translation is attached.

INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99)		
	Application Number	14096346
	Filing Date	2013-12-04
	First Named Inventor	Sandra O'Connor
	Art Unit	1676
	Examiner Name	Li N. Komatsu
	Attorney Docket Number	552815 (CPT-011USDV)

CERTIFICATION STATEMENT

Please see 37 CFR 1.97 and 1.98 to make the appropriate selection(s):

☐ That each item of information contained in the information disclosure statement was first cited in any communication from a foreign patent office in a counterpart foreign application not more than three months prior to the filing of the information disclosure statement. See 37 CFR 1.97(e)(1).

OR

☐ That no item of information contained in the information disclosure statement was cited in a communication from a foreign patent office in a counterpart foreign application, and, to the knowledge of the person signing the certification after making reasonable inquiry, no item of information contained in the information disclosure statement was known to any individual designated in 37 CFR 1.56(c) more than three months prior to the filing of the information disclosure statement. See 37 CFR 1.97(e)(2).

☐ See attached certification statement.

☐ The fee set forth in 37 CFR 1.17 (p) has been submitted herewith.

☒ A certification statement is not submitted herewith.

SIGNATURE

A signature of the applicant or representative is required in accordance with CFR 1.33, 10.18. Please see CFR 1.4(d) for the form of the signature.

Signature	/Brian C. Trinque/	Date (YYYY-MM-DD)	2014-04-01
Name/Print	Brian C. Trinque, Ph.D., Esq.	Registration Number	56,593

This collection of information is required by 37 CFR 1.97 and 1.98. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 1 hour to complete, including gathering, preparing and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

Privacy Act Statement

The Privacy Act of 1974 (P.L. 93-579) requires that you be given certain information in connection with your submission of the attached form related to a patent application or patent. Accordingly, pursuant to the requirements of the Act, please be advised that: (1) the general authority for the collection of this information is 35 U.S.C. 2(b)(2); (2) furnishing of the information solicited is voluntary; and (3) the principal purpose for which the information is used by the U.S. Patent and Trademark Office is to process and/or examine your submission related to a patent application or patent. If you do not furnish the requested information, the U.S. Patent and Trademark Office may not be able to process and/or examine your submission, which may result in termination of proceedings or abandonment of the application or expiration of the patent.

The information provided by you in this form will be subject to the following routine uses:

1. The information on this form will be treated confidentially to the extent allowed under the Freedom of Information Act (5 U.S.C. 552) and the Privacy Act (5 U.S.C. 552a). Records from this system of records may be disclosed to the Department of Justice to determine whether the Freedom of Information Act requires disclosure of these record s.

2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.

3. A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.

4. A record in this system of records may be disclosed, as a routine use, to a contractor of the Agency having need for the information in order to perform a contract. Recipients of information shall be required to comply with the requirements of the Privacy Act of 1974, as amended, pursuant to 5 U.S.C. 552a(m).

5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent Cooperation Treaty.

6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).

7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (i.e., GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.

8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspections or an issued patent.

9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants:	Sandra O'Connor et al.	Examiner:	Li N. Komatsu
Serial No.:	14/096,346	Group Art No.:	1676
Filed:	December 4, 2013	Confirmation No.:	2832
Title:	LIPOPEPTIDE COMPOSITIONS AND RELATED METHODS		

VIA EFS-Web
Commissioner For Patents
P.O. Box 1450
Alexandria, VA 22313-1450

INFORMATION DISCLOSURE STATEMENT TRANSMITTAL

Dear Sir:

This Information Disclosure Statement is submitted:

☒ under 37 CFR 1.97(b), or
(Within three months of filing national application other than a CPA under §1.53(d); or within three months of date of entry of the national stage in international application; or before mailing date of first Office Action on the merits, or before mailing date of first Office Action after filing RCE under §1.114, whichever occurs last)

☐ under 37 CFR 1.97(c) together with either a:
- ☐ Statement under 37 CFR 1.97(e), or
- ☐ a $180 fee under 37 CFR 1.17(p), or

(After the CFR 1.97(b) time period, but before final action or notice of allowance, whichever occurs first)

☐ under 37 CFR 1.97(d) together with a:
- ☐ Statement under 37 CFR 1.97(e)(1) or (2), and
- ☐ a $180.00 fee set forth in 37 CFR 1.17(p).

(Filed after final action, a notice of allowance, on or before payment of issue fee)

At any time during the pendency of this application, please charge any fees required or credit any overpayment to Deposit Account 12-0600 pursuant to 37 CFR 1.25.

Applicant(s) submit herewith PTO Form SB/08 – Information Disclosure Statement together with copies of non-U.S. patents, publications or other information (if any) of which

applicant(s) are aware, which applicant(s) believe(s) may be material to the examination of the application and for which there may be a duty to disclose in accordance with 37 CFR 1.56.

In accordance with 37 CFR 1.97(g), the filing of this Information Disclosure Statement shall not be construed to mean that a search has been made or that no other material information as defined in 37 CFR 1.56(a) exists. In accordance with 37 CFR 1.97(h), the filing of this Information Disclosure Statement shall not be construed to be an admission that any patent, publication or other information referred to therein is "prior art" for this invention unless specifically designated as such.

It is submitted that the Information Disclosure Statement is in compliance with 37 CFR 1.98 and the Examiner is respectfully requested to consider the listed references. It is requested that the information disclosed herein be made of record in this application.

Date: April 1, 2014

Respectfully submitted,

Signature: / Brian C. Trinque /
LATHROP & GAGE LLP
Brian C. Trinque, Ph.D., Esq.
Reg. No. 56,593
28 State Street, Suite 0700
Boston, Massachusetts 02109
Telephone: (857) 300-4003
Facsimile: (857) 300-4001
Customer No: 113613

Electronic Acknowledgement Receipt

EFS ID:	18627217
Application Number:	14096346
International Application Number:	
Confirmation Number:	2832
Title of Invention:	LIPOPEPTIDE COMPOSITIONS AND RELATED METHODS
First Named Inventor/Applicant Name:	Sandra O'Connor
Customer Number:	113613
Filer:	Brian C. Trinque/Ian Clark
Filer Authorized By:	Brian C. Trinque
Attorney Docket Number:	552815 (CPT-011USDV)
Receipt Date:	01-APR-2014
Filing Date:	04-DEC-2013
Time Stamp:	15:09:19
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	no

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Non Patent Literature	Debono_1988.pdf	1746711 a4de33af2fa14d3548bb2a089a583c5a81d2d162	no	13

Warnings:	
Information:	

2	Other Reference-Patent/App/Search documents	EuroSearchReport.pdf	340200 0a5ed85f9a37396556f56102317406f1fe16df0a	no	8
Warnings:					
Information:					
3	Foreign Reference	WO2009144739_A1.pdf	1311980 d732739a30131d504298bd3d61115e29b09fb31e	no	28
Warnings:					
Information:					
4	Information Disclosure Statement (IDS) Form (SB08)	552815CPT011DV_SB08.pdf	612395 731dae29ee0729744c05767c5edd7ed85ea36392	no	4
Warnings:					
Information:					
A U.S. Patent Number Citation or a U.S. Publication Number Citation is required in the Information Disclosure Statement (IDS) form for autoloading of data into USPTO systems. You may remove the form to add the required data in order to correct the Informational Message if you are citing U.S. References. If you chose not to include U.S. References, the image of the form will be processed and be made available within the Image File Wrapper (IFW) system. However, no data will be extracted from this form. Any additional data such as Foreign Patent Documents or Non Patent Literature will be manually reviewed and keyed into USPTO systems.					
5	Transmittal Letter	552815CPT011DV_Transmittal.pdf	60819 09d701ef7ac682a0dda17029d1389c097087a83e	no	2
Warnings:					
Information:					
		Total Files Size (in bytes):	4072105		

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

New Applications Under 35 U.S.C. 111
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National Stage of an International Application under 35 U.S.C. 371
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New International Application Filed with the USPTO as a Receiving Office
If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
3 December 2009 (03.12.2009)

PCT

(10) International Publication Number
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(54) **Title**: AMORPHOUS DAPTOMYCIN AND A METHOD OF PURIFICATION THEREOF

(57) **Abstract**: The invention relates to the purification of daptomycin. More specifically, the invention relates to a simplified and optimized purification process of daptomycin from a fermentation broth by chromatographic procedures enabling high yields, selectivity and purity of the desired end product. The daptomycin is prepared in high purity of at least about 98%, and preferably at least about 96%. The present invention also relates to a process for preparing daptomycin that is scalable for commercial production.

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AMORPHOUS DAPTOMYCIN AND A METHOD OF PURIFICATION THEREOF

FIELD OF THE INVENTION

The invention relates to the purification of daptomycin. More specifically, the invention relates to a simplified and optimized purification process of daptomycin from a fermentation broth by chromatographic procedures enabling high yields, selectivity and purity of the desired end product. The daptomycin is prepared in high purity of at least about 98%, and preferably at least about 96%. The present invention also relates to a process for preparing daptomycin that is scalable for commercial production.

BACKGROUND AND PRIOR ART OF THE INVENTION

Daptomycin, also known as LY 146032, is a cyclic lipopeptide antibiotic that can be derived from the fermentation of *Streptomyces roseosporus*. Daptomycin is a member of the factor A-21978C type antibiotics of *S. roseosporus* and is comprised of a decanoyl side chain linked to the N-terminal tryptophan of a cyclic 13-amino acid peptide. Daptomycin has an excellent profile of activity because it is highly effective against most gram-positive bacteria; it is highly bactericidal and fast-acting; it has a low resistance rate and is effective against antibiotic-resistant organisms.

A number of United States Patents describe A-21978C antibiotics and derivatives thereof including daptomycin (LY 146032) as well as methods of producing and isolating the A-21978C antibiotics and derivatives thereof.

United States Patent No. RE32,333, RE32,455 and 4,800,157 describe a method of synthesizing daptomycin by cultivating *Streptomyces roseosporus* NRL1 5998 under submerged aerobic fermentation conditions.

United States Patent. No. 4,885,243 describes an improved method of synthesizing daptomycin by feeding a fermentation culture a decanoic fatty acid or ester or salt thereof.

United States Patent No. 4,874,843 describes a daptomycin purification method which is incorporated herein by reference.

U.S. Patent. No. 5,912,226 describes the identification and isolation of two impurities produced during the manufacture of daptomycin which is incorporated herein by reference.

US patent No. 6696412 describes commercially feasible methods to produce high levels of purified daptomycin at a purity level of 95-98%. The process chromatography method comprises sequentially using anion exchange chromatography, hydrophobic interaction chromatography (HIC) and anion exchange chromatography to purify a preparation containing daptomycin. WO02056829 disclose the 92% pure amorphous form of daptomycin which is incorporated herein by reference

A number of different chromatographic procedures are applied to obtain the desired end result with respect to purity and yield as discussed above. There is a need in the art to develop a simple chromatographic purification procedure that may be operational on a large scale with minimal steps to isolate the purified daptomycin from a fermentation broth.

The present invention has steps novel with respect to any of the above known methods and uses RP-HPLC on commercial scale to prepare highly pure daptomycin at purity levels of 96-99%. The invention further addresses the long process times as seen with anion exchange method of purification by replacing 2 rounds of ion exchange chromatography with single RP-HPLC purification. The present purification process of daptomycin comprises sequentially carrying out hydrophobic interaction chromatography of microfiltered broth followed by RP-HPLC and subsequent liquid-liquid extraction, HIC or anion exchange chromatography. Finally, solvent wash for removal of endotoxin prior to ultrafiltration, nanofiltration or reverse osmosis followed by lyophilization is carried out to give formulated daptomycin.

Another important advantage of the purification process according to the present invention is that they may be scaled up in a reproducible and consistent manner. Further, the process of the present invention affords products which are superior to those obtained by purification methods hitherto known and give higher yields.

OBJECTIVES OF THE INVENTION

The main objective of the present invention is to obtain an amorphous daptomycin having at least 98% purity.

Another main objective of the present invention is to obtain an amorphous daptomycin having at least 97% purity.

Yet another main objective of the present invention is to obtain an amorphous daptomycin having at least 96% purity.

Still another main objective of the present invention is to obtain an amorphous daptomycin having powder XRD pattern represented in fig 2.

Still another main objective of the present invention is to obtain a method of purification of daptomycin.

STATEMENT OF THE INVENTION

Accordingly, the present invention relates to an amorphous daptomycin having at least 98% purity; an amorphous daptomycin having at least 97% purity; an amorphous daptomycin having at least 96% purity; an amorphous daptomycin having powder XRD pattern represented in fig 2; and a method of purification of daptomycin comprising the steps of a) filtration of the fermentation broth, b) optional concentration of the filtrate from step (a) by hydrophobic interaction chromatography, ultrafiltration or nanofiltration, c) purification of daptomycin by loading the filtrate from step (a) or the concentrate from step (b) on reverse phase resin followed by elution, d) recovery of purified daptomycin from the elute of reverse phase chromatography, and e) depyrogenation and lyophilization of the recovered daptomycin to give highly pure formulated product.

BRIEF DESCRIPTION OF THE ACCOMPANYING FIGURES

Figure1: XRD of Amorphous Daptomycin

Figure 2: Chromatogram of Daptomycin

DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to an amorphous daptomycin having at least 98% purity.

The present invention also relates to an amorphous daptomycin having at least 97% purity.

The present invention also relates to an amorphous daptomycin having at least 96% purity.

The present invention also relates to an amorphous daptomycin having powder XRD pattern represented in fig 2.

The present invention also relates to a method of purification of daptomycin comprising the steps of:

a. filtration of the fermentation broth,
b. optional concentration of the filtrate from step (a) by hydrophobic interaction chromatography, ultrafiltration or nanofiltration,
c. purification of daptomycin by loading the filtrate from step (a) or the concentrate from step (b) on reverse phase resin followed by elution,
d. recovery of purified daptomycin from the elute of reverse phase chromatography, and
e. depyrogenation and lyophilization of the recovered daptomycin to give highly pure formulated product.

In another embodiment of the present invention, the recovery in step (d) is carried out using the steps of:

a. optional extraction of the elute of reverse phase chromatography with a water immiscible solvent or treatment of the elute from reverse phase chromatography by ion exchange chromatography or hydrophobic interaction chromatography,
b. optional concentration of the elute of reverse phase chromatography or the elute of hydrophobic interaction chromatography from step (a) by evaporation,
c. subjecting the elute of reverse phase chromatography, the daptomycin-containing raffinate from step (a), the elute of ion exchange chromatography or hydrophobic interaction chromatography from step (a), or the concentrate from

step (b) to endotoxin removal followed by ultrafiltration, nanofiltration or reverse osmosis.

In yet another embodiment of the present invention, the hydrophobic interaction chromatography is performed on resin selected from the group comprising HP2MG, HP20, HP21, HP20SS, SP20, SP20SS, SP825, SP850, SP207, XAD16, XAD1600, XAD18, XAD761 and XAD7HP .

In still another embodiment of the present invention, the ultrafiltration or nanofiltration is performed using membrane selected from the group comprising polysulfone, polyether sulfone, polypropylene, polyacrylonitrile, cellulose esters, mixed cellulose esters, regenerated cellulose, polyvinylidene difluoride, nylon, teflon (PTFE) and ceramic membranes.

In still another embodiment of the present invention, the reverse phase resin is selected from C4, C8 and C18 reverse phase resin.

In still another embodiment of the present invention, the elution from the reverse phase resin is carried out with mixture of organic solvent and water or mixture of organic solvent and buffer at pH 2.5 to 8.

In still another embodiment of the present invention, the organic solvent is selected from methanol, ethanol, n-propanol, isopropanol, n-butanol, t-butanol, acetonitrile, acetone, tetrahydrofuran or mixture thereof.

In still another embodiment of the present invention, the reverse phase chromatography elution is carried out in isocratic or gradient manner.

In still another embodiment of the present invention, the gradient elution is achieved by changing the content of organic solvent, pH and/or buffer molarity during elution.

In still another embodiment of the present invention, the water-immiscible solvent is selected from ethyl acetate, propyl acetate, butyl acetate, amyl acetate, chloroform, dichloromethane, methyl-t-butyl ether, diethyl ether, butanol, hexane, heptane, cyclohexane or mixture thereof.

In still another embodiment of the present invention, the ion exchange chromatography is anion exchange chromatography or cation exchange chromatography.

In still another embodiment of the present invention, the ion exchange chromatography is anion exchange chromatography.

In still another embodiment of the present invention, the ion exchange chromatography is performed on resin selected from the group comprising diethyl aminoethyl,

quaternary aminoethyl, quaternary ammonium, polyethyleneimine and quaternized polyethyleneimine type resins.

In still another embodiment of the present invention, the ion exchange chromatography elution is carried out using a salt solution.

In still another embodiment of the present invention, the salt is a halogenide.

In still another embodiment of the present invention, the salt is an alkali or alkaline earth halogenide.

In still another embodiment of the present invention, the evaporation is done using a thin or wiped film evaporator, falling film evaporator, forced circulation evaporator, short tube evaporator or long tube evaporator.

In still another embodiment of the present invention, the ultrafiltration or nanofiltration is performed at pH 2.5 to 8.

The term "**chromatography**" refers to the process by which a solute of interest in a mixture is separated from other solutes in a mixture as a result of differences in rates at which the individual solutes of the mixture migrate through a stationary medium under the influence of a moving phase, or in bind and elute processes.

The term "**High Performance liquid chromatography**", as used herein, refers to that chromatographic procedure in which the particles (stationary phase) used in the column packing are small (between 3 and 50 microns) and regular with little variation from the selected size. Such chromatography typically employs relatively high (around 500-3500 psi) inlet pressures.

The term "**ion-exchange**" and "**ion-exchange chromatography**" refers to the chromatographic process in which a solute of interest (such as a protein) in a mixture interacts with a charged compound linked (such as by covalent attachment) to a solid phase ion exchange material such that the solute of interest interacts non-specifically with the charged compound more or less than solute impurities or contaminants in the mixture. The contaminating solutes in the mixture elute from a column of the ion exchange material faster or slower than the solute of interest or are bound to or excluded from the resin relative to the solute of interest. "Ion-exchange chromatography" specifically includes cation exchange, anion exchange, and mixed mode chromatography.

The object of the present invention is provided in a specifically delineated process for purification of daptomycin from fermentation broth by using chromatographic process.

In a broad aspect, the present invention relates to a chromatography process for purifying a daptomycin from fermentation broth comprising the steps of:

a) filtration of the fermentation broth,
b) optional concentration of the filtrate from step (a) by hydrophobic interaction chromatography, ultrafiltration or nanofiltration,
c) purification of daptomycin by loading the filtrate from step (a) or the concentrate from step (b) on reverse phase resin followed by elution,
d) recovery of purified daptomycin from the elute of reverse phase chromatography, and
e) Lyophilization of the recovered daptomycin to give highly pure formulated product.

The present invention also relates to the above process, wherein the recovery of purified daptomycin from the elute of reverse phase chromatography is carried out using the following steps:

a) optional extraction of the elute of reverse phase chromatography with a water immiscible solvent or treatment of the elute from reverse phase chromatography by ion exchange chromatography or hydrophobic interaction chromatography,
b) optional concentration of the elute of reverse phase chromatography or the elute of hydrophobic interaction chromatography from step (a) by evaporation,
c) subjecting the elute of reverse phase chromatography, the daptomycin-containing raffinate from step (a), the elute of ion exchange chromatography from step (a), the elute of hydrophobic interaction chromatography from step (a), or the concentrate from step (b) to endotoxin removal by solvent wash followed by ultrafiltration, nanofiltration or reverse osmosis.

In another aspect of the invention the final purified product daptomycin is amorphous in nature and 98% pure.

Those skilled in the art will recognize that there are various variables which can be adjusted during the chromatographic procedures of the present invention. Such variables include resin selection; loading, wash and eluting conditions, such as ionic strength, buffer composition, pH, temperature, addition of one or more organic solvents, etc. However, such variables are routinely adjusted in this field and those skilled in the art can readily establish optimum conditions.

The present invention relates to a chromatography process for purifying a daptomycin from fermentation broth comprising the steps of:

a) filtration of the fermentation broth,
b) optional concentration of the filtrate from step (a) by hydrophobic interaction chromatography, ultrafiltration or nanofiltration,
c) purification of daptomycin by loading the filtrate from step (a) or the concentrate from step (b) on reverse phase resin followed by elution,
d) recovery of purified daptomycin from the elute of reverse phase chromatography, and
e) depyrogenation and lyophilization of the recovered daptomycin to give highly pure formulated product.

The present invention also relates to the above process, wherein the recovery of purified daptomycin from the elute of reverse phase chromatography is carried out using the following steps:

a) optional extraction of the elute of reverse phase chromatography with a water immiscible solvent or treatment of the elute from reverse phase chromatography by ion exchange chromatography or hydrophobic interaction chromatography,
b) optional concentration of the elute of reverse phase chromatography or the elute of hydrophobic interaction chromatography from step (a) by evaporation,
c) subjecting the elute of reverse phase chromatography, the daptomycin-containing raffinate from step (a), the elute of ion exchange chromatography from step (a), the elute of hydrophobic interaction chromatography from step (a), or the concentrate from step (b) to endotoxin removal followed by ultrafiltration, nanofiltration or reverse osmosis.

In another aspect of the invention the final purified product daptomycin is amorphous in nature and 98% pure.The daptomycin of the present invention can be produced by fermentation. After fermentation, the daptomycin-containing extracellular solution is clarified by removing the mycelia from the fermentation broth. This clarification is performed by any standard solid-liquid separation technique, such as centrifugation or filtration. Preferably, the fermentation broth clarification is carried out using a microfiltration system, filter press, rotary drum filter, depth filter or industrial centrifuge.

The clarified solution may be directly subjected to a reverse phase chromatography. Alternately, the clarified solution is concentrated and the concentrate is subjected to reverse phase chromatography. The concentration of clarified solution can be carried out by ultrafiltration, nanofiltration, or hydrophobic interaction chromatography or combinations thereof. The ultrafiltration and nanofiltration membrane can be selected from polysulfone, polyether sulfone, polypropylene, polyacrylonitrile, cellulose esters, mixed cellulose esters, regenerated cellulose, polyvinylidene difluoride, nylon, teflon (PTFE), or ceramic membranes. The pH during ultrafiltration or nanofiltration is selected in the range 2.5 to 8. The retentate of ultrafiltration or nanofiltration gives concentrated clarified solution. Optionally, the feed for the reverse phase chromatography may be treated with activated charcoal, alumina, silica gel or may be passed through a guard column before passing it through the reverse phase chromatography column.

In hydrophobic interaction chromatography, the resin can be selected from HP2MG, HP20, HP21, HP20SS, SP20, SP20SS, SP825, SP850, SP207, XAD16, XAD1600, XAD18, XAD761, XAD7HP, etc. The clarified solution is contacted with the hydrophobic interaction chromatography resin under conditions such that daptomycin binds to the resin. This contact is carried out at pH in the range from 2.5 to 8. Preferably, the contact is carried out at pH in the range from 3.5 to 5.0. Optionally, additives including salts and/or surfactants are added to the clarified solution. Surfactants are added at small concentrations to prevent precipitation during pH adjustment and column blockage in hydrophobic interaction chromatography. The surfactant can be selected from Triton X, Tween, SDS, etc. The surfactant concentration is selected in the range from 0.001% to 1.0%. Additive salts are selected from sodium chloride, potassium chloride, sodium dihydrogen phosphate, disodium hydrogen phosphate, potassium

dihydrogen phosphate, dipotassium hydrogen phosphate, ammonium dihydrogen phosphate, di-ammonium hydrogen phosphate, sodium acetate, ammonium acetate, ammonium chloride, sodium carbonate, sodium bicarbonate and sodium citrate. The resin is washed and eluted with water, buffer, mixture of organic solvent and water or mixture of organic solvent and buffer. The organic solvent is selected from methanol, ethanol, n-propanol, isopropanol, n-butanol, t-butanol, acetonitrile, acetone, tetrahydrofuran or mixture thereof. The pH during elution is selected in the range from 2.5 to 8. The product-containing elute from hydrophobic interaction chromatography gives concentrated clarified solution.

The clarified solution or the concentrated clarified solution is subjected to a reverse phase chromatography resin. Optionally, the feed for the reverse phase chromatography column is diluted with water. The feed is loaded onto the column at pH in the range from 2.5 to 8. Preferably, the feed pH is in the range from 3.5 to 5.0. The reverse phase resin is selected from C4, C8, C18 silica or polystyrene-divinylbenzene. Daptomycin from the column is eluted with mixture of organic solvent and water or mixture of organic solvent and buffer. The organic solvent is selected from methanol, ethanol, n-propanol, isopropanol, n-butanol, t-butanol, acetonitrile, acetone, tetrahydrofuran or mixture thereof. The pH during elution is selected in the range from 2.5 to 8. The elution is carried out in isocratic or gradient manner. In gradient elution, the content of organic solvent, pH and/or buffer molarity is changed during elution. During product elution, fractions are collected. Fractions containing product with desired purity are combined to obtain elute from reverse phase chromatography. The buffer can be prepared using phosphates of sodium, potassium or ammonium salts, acetates of sodium, potassium or ammonium salts, citrates of sodium, potassium or ammonium salts, oxalates sodium, potassium or ammonium salts, acetic acid, phosphoric acid, citric acid, oxalic acid, hydrochloric acid, sodium hydroxide, potassium hydroxide and ammonium hydroxide or mixtures thereof.

Purified daptomycin in the elute from reverse phase chromatography is then recovered. The elute from reverse phase chromatography is optionally processed by extraction, ion exchange chromatography or hydrophobic interaction chromatography. In extraction, the elute from reverse phase chromatography is extracted with a water-immiscible organic solvent. The water-immiscible solvent is selected from ethyl acetate, propyl

acetate, butyl acetate, amyl acetate, chloroform, dichloromethane, methyl-t-butyl ether, diethyl ether, butanol, hexane, heptane, cyclohexane or mixture thereof. The extraction is carried out in batch manner in single or multiple stages. Alternately, the extraction is carried out in a co-current or counter-current continuous manner. Daptomycin remains in the aqueous layer to give raffinate.

In ion exchange chromatography, the elute from reverse phase chromatography is passed through ion exchange resin column. The ion exchange resin is selected from diethyl aminoethyl, quaternary aminoethyl, quaternary ammonium, polyethyleneimine and quaternized polyethyleneimine type resins. Optionally, the elute from reverse phase chromatography is diluted with water before passing through the ion exchange column. The resin is washed and eluted with water and salt solution in water. The elution is carried out in isocratic or gradient manner. The gradient elution is carried out using step or continuous gradient. During washing and elution, the salt concentration is selected in the range from 0 to 1000 mM. Preferably, this concentration is selected in the range from 0 to 500 mM. The salt is selected from NaCl or KCl.

In hydrophobic interaction chromatography, the elute from reverse phase chromatography is passed through a hydrophobic interaction resin column. The hydrophobic interaction resin is selected from HP2MG, HP20, HP21, HP20SS, SP20, SP20SS, SP825, SP850, SP207, XAD16, XAD1600, XAD18, XAD761, XAD7HP, etc. Optionally, the elute from reverse phase chromatography is diluted with water before passing through the hydrophobic interaction resin column. The loading is carried out at pH in the range from 2.5 to 8. Preferably, the loading is carried out at pH in the range from 3.5 to 5.0. The resin is washed and eluted with water, buffer, mixture of organic solvent and water or mixture of organic solvent and buffer. The organic solvent is selected from methanol, ethanol, n-propanol, isopropanol, n-butanol, t-butanol, acetonitrile, acetone, tetrahydrofuran or mixture thereof. The pH during elution is selected in the range from 2.5 to 8.

The elute from reverse phase chromatography or the elute from hydrophobic interaction chromatography is optionally concentrated by evaporation. Various evaporators including

thin or wiped film evaporator, falling film evaporator, forced circulation evaporator, short tube evaporator or long tube evaporator can be used for this concentration.

The elute of reverse phase chromatography, the daptomycin-containing raffinate from extraction, the elute of ion exchange chromatography, the elute hydrophobic interaction chromatography or the concentrate after evaporation is then subjected to depyrogenation by employing solvent wash. The organic solvent is selected from n-butanol, ethyl acetate, butyl acetate, hexane, heptane, petroleum ether or mixture thereof. The pH during solvent wash is normally selected in the range from 5 to 8. Daptomycin remains in the aqueous layer to give raffinate which is subjected to ultrafiltration or nanofiltration. Optionally, the feed to the ultrafiltration or nanofiltration is diluted with water. The filtration is carried out at a pH in the range from 2.5 to 8. The ultrafiltration and nanofiltration membrane is selected from polysulfone, polyether sulfone, polypropylene, polyacrylonitrile, cellulose esters, mixed cellulose esters, regenerated cellulose, polyvinylidene difluoride, nylon, teflon (PTFE), or ceramic membranes. The daptomycin containing retentate is diafiltered against water.

Finally, the daptomycin solution is sterile filtered and lyophilized to obtain amorphous pure daptomycin. The purity of final product has at least 98% as measured by HPLC.

The disclosures of the prior art references referred to in this patent application are incorporated herein by reference. The invention is further defined by reference to the following examples describing in detail the process and compositions of the invention. It will be apparent to those skilled in the art that many modifications, both to materials and methods, may be practiced without departing from the scope of the invention.

EXAMPLE 1

10 L fermentation broth containing daptomycin was microfiltered through 0.1 μm filter. The filtrate was mixed with 0.05% triton X100 and pH of the solution was adjusted to 4.5. The solution was passed through an HP20ss column, which was pre-equilibrated with 5 % isopropanol in sodium acetate buffer at pH 4.5. The column was washed with 10 % isopronanol in sodium acetate buffer and eluted with 25% isopropanol in sodium

acetate buffer at pH 6.5. The product containing fractions were combined, diluted with water and loaded on a C8 reverse phase chromatography column. The column was eluted under isocratic conditions with acetonitrile and buffer in the ratio of 30:70. During elution, fractions were collected. The fractions with desired purity were combined, diluted with water, pH-adjusted to 6.5 and passed over weak anion exchange resin to remove organic solvent. The resin was pre-equilibrated with 30 mM Tris HCl buffer at pH 6.5. The column was eluted with 0.5 M NaCl solution. The product containing elute was concentrated and diafiltered using an ultrafiltration membrane. The concentrate was sterile filtered, filled in vials and lyophilized. The purity of daptomycin was 97.5%.

EXAMPLE 2

10 L fermentation broth containing daptomycin was microfiltered through 0.1 μm filter. The filtrate was mixed with 0.05% triton X100 and pH of the solution was adjusted to 4.5. The solution was passed through an HP20ss column, which was pre-equilibrated with 5 % isopropanol in sodium acetate buffer at pH 4.5. The column was washed with 10 % isopronanol in sodium acetate buffer and eluted with 25% isopropanol in sodium acetate buffer at pH 6.5. The product containing fractions were combined, diluted with water and loaded on a C8 reverse phase chromatography column. The column was eluted under isocratic conditions with acetonitrile and buffer in the ratio of 30:70. During elution, fractions were collected. The fractions with desired purity were combined, and subjected to liquid-liquid extraction using n-butyl acetate. Post-extraction, the aqueous layer containing daptomycin was concentrated and diafiltered by ultrafiltration. The concentrate was sterile filtered, filled in vials and lyophilized to give daptomycin of purity more than 96%.

EXAMPLE 3

10 L fermentation broth containing daptomycin was microfiltered through 0.1 μm filter. The filtrate was mixed with 0.05% triton X100 and pH of the solution was adjusted to 4.5. The solution was passed through an HP20ss column, which was pre-equilibrated with 5 % isopropanol in sodium acetate buffer at pH 4.5. The column was washed with 10 % isopronanol in sodium acetate buffer and eluted with 25% isopropanol in sodium acetate buffer at pH 6.5. The product containing fractions were combined, diluted with water and loaded on a C8 reverse phase chromatography column. The column was eluted

under isocratic conditions with acetonitrile and buffer in the ratio of 30:70. During elution, fractions were collected. The fractions with desired purity were combined, diluted with water, pH-adjusted to 4.5. This solution was passed through an HP20ss column, which was pre-equilibrated with 5% isopropanol in sodium acetate buffer at pH 4.5. The resin was washed with water and eluted with 70% methanol in buffer. The product-containing elute of purity 97.2% was diluted with water and pH of this solution was adjusted to 4.5. The pH-adjusted solution was concentrated and diafiltered using an ultrafiltration membrane. The concentrate was sterile filtered, filled in vials and lyophilized.

EXAMPLE 4

50 L broth was microfiltered through 0.1 μm and the permeate was concentrated 10 fold on a 0.6KDa nanofiltration membrane. The concentrate was mixed with activated charcoal and filtered to remove the charcoal. The filtrate was loaded on a C8 reverse phase chromatography column. The product was eluted using a linear gradient of 30-50% acetonitrile over 90 minutes. The fractions containing product with desired purity were combined and extracted with n-butyl acetate. Post-extraction, the aqueous layer containing daptomycin was concentrated and diafiltered using an ultrafiltration membrane. The concentrate was sterile filtered, filled in vials and lyophilized.

EXAMPLE 5

50 L fermentation broth containing daptomycin was microfiltered through 0.1 μm filter. The filtrate was mixed with 0.05% triton X100 and pH of the solution was adjusted to 4.5. The solution was passed through an HP20ss column, which was pre-equilibrated with 5 % isopropanol in sodium acetate buffer at pH 4.5. The column was washed with 10 % isopronanol in sodium acetate buffer and eluted with 25% isopropanol in sodium acetate buffer at pH 6.5. The product containing fractions were combined, diluted with water and loaded on a C8 reverse phase chromatography column. The product containing fractions were combined, diluted with water and purified on a C8 reverse phase chromatography column. The product containing fractions were combined, diluted with water and loaded on a C8 reverse phase chromatography column. The column was eluted using a linear gradient from 30 to 50% acetonitrile over 90 minutes. The fractions containing product with purities ~98% were combined, pH was adjusted to 6.5 and

extracted with n-butyl acetate in multiple stages. Post-extraction, the aqueous layer containing daptomycin was concentrated and diafiltered at pH 6.5 using a nanofiltration membrane. The concentrate was sterile filtered, filled in vials and lyophilized.

EXAMPLE 6

pH of 50 L fermentation broth was adjusted to 4.5. The pH-adjusted broth was extracted with n-butanol. The butanol extract was concentrated partially and extracted with sodium phosphate buffer at pH 6.5. pH of this extract was adjusted to 4.5 and loaded onto an HP20ss column, which was pre-equilibrated with 5% isopropanol in sodium acetate buffer at pH 4.5. The column was washed with 10% isopropanol in sodium acetate buffer and eluted with 25% isopropanol in sodium acetate buffer. The product-containing elute was diluted with water and loaded on reverse phase C-8 resin, elution was carried as described in example 1. The fractions containing product with desired purity were combined and diluted with water. pH of the diluted solution was adjusted to 4.5 and passed through an HP20ss column. The column was washed with water and eluted with 70% acetonitrile. The elute was sterile filtered and lyophilized to give the daptomycin. The purity of daptomycin obtained was 98.8%.

EXAMPLE 7

10 L fermentation broth containing daptomycin was microfiltered through 0.1 μm filter. The filtrate was mixed with 0.05% triton X100 and pH of the solution was adjusted to 4.5. The solution was passed through an HP20ss column, which was pre-equilibrated with 5 % isopropanol in sodium acetate buffer at pH 4.5. The column was washed with 10 % isopronanol in sodium acetate buffer and eluted with 25% isopropanol in sodium acetate buffer at pH 6.5. The product containing fractions were combined, diluted with water and loaded on a C8 reverse phase chromatography column. The column was eluted using a linear gradient from 30 to 50% acetonitrile over 90 minutes. During elution, fractions were collected. The fractions with desired purity were combined, diluted with water, pH-adjusted to 4.5. This solution was passed through an HP20ss column, which was pre-equilibrated with 5% isopropanol in sodium acetate buffer at pH 4.5. The resin was washed with water and eluted with aqueous acetonitrile. The product-containing elute of ~98% purity was concentrated by solvent evaporation. The concentrated solution

was diluted with water and diafiltered using an ultrafiltration membrane. The diafiltered solution was sterile filtered and lyophilized.

EXAMPLE 8

10 L fermentation broth containing daptomycin was microfiltered through 0.1 μm filter. The filtrate was mixed with 0.05% triton X100 and pH of the solution was adjusted to 4.5. The solution was passed through an HP20ss column, which was pre-equilibrated with 5 % isopropanol in sodium acetate buffer at pH 4.5. The column was washed with 10 % isopronanol in sodium acetate buffer and eluted with 25% isopropanol in sodium acetate buffer at pH 6.5. The product containing fractions were combined, diluted with water and loaded on a C8 reverse phase chromatography column. The column was eluted under isocratic conditions with acetonitrile and buffer in the ratio of 35:65. During elution, the fractions with desired purity were combined, diluted with water, pH-adjusted to 6.5 and passed over weak anion exchange resin to remove organic solvent. The resin was pre-equilibrated with 30 mM Tris HCl buffer at pH 6.5. The column was eluted with 0.5 M NaCl solution. The product containing elute was depyrogenated by contacting with n-butanol in the ratio of 1: 0.2 for 30 mins. The aqueous layer containing the product was further contacted with n-heptane in the ratio of 1: 0.4 for 30 mins. Finally the aqueous 'product containing layer' was concentrated and diafiltered using an ultrafiltration membrane. The concentrate was sterile filtered, filled in vials and lyophilized. The purity of daptomycin obtained was 96.6%.

EXAMPLE 9

10 L fermentation broth containing daptomycin was microfiltered through 0.1 μm filter. The filtrate was mixed with 0.05% triton X100 and pH of the solution was adjusted to 4.5. The solution was passed through an HP20ss column, which was pre-equilibrated with 5 % isopropanol in sodium acetate buffer at pH 4.5. The column was washed with 10 % isopronanol in sodium acetate buffer and eluted with 25% isopropanol in sodium acetate buffer at pH 6.5. The product containing fractions were combined, diluted with water and loaded on a C8 reverse phase chromatography column. The column was eluted under isocratic conditions with acetone and buffer in the ratio of 37:63. During elution, fractions were collected. The fractions with 97% purity were combined, diluted with water, pH-adjusted to 4.5. This solution was passed through an HP20ss column, which

was pre-equilibrated with 5% isopropanol in sodium acetate buffer at pH 4.5. The resin was washed with water and eluted with 70% methanol in buffer. The product-containing elute was diluted with water and pH of this solution was adjusted to 4.5. The pH-adjusted solution was concentrated and diafiltered using an ultrafiltration membrane. The concentrate was sterile filtered, filled in vials and lyophilized.

EXAMPLE 10

5 L fermentation broth containing daptomycin was microfiltered through 0.1 μm filter. The filtrate was mixed with 0.05% triton X100 and pH of the solution was adjusted to 4.5. The solution was passed through an HP20ss column, which was pre-equilibrated with 5 % isopropanol in sodium acetate buffer at pH 4.5. The column was washed with 10 % isopropanol in sodium acetate buffer and eluted with 25% isopropanol in sodium acetate buffer at pH 6.5. The product containing fractions were combined, diluted with water and loaded on a C8 reverse phase chromatography column. The column was eluted using 40% acetone over 300 minutes. During elution, fractions were collected. The fractions with desired purity were combined, diluted with water, pH-adjusted to 6.5 and passed over weak anion exchange resin to remove organic solvent. The resin was pre-equilibrated with 30 mM Tris HCl buffer at pH 6.5. The column was eluted with 0.5 M NaCl solution. The product containing elute was depyrogenated by contacting with n-butanol in the ratio of 1: 0.2 for 30 mins. The aqueous layer containing the product was further contacted with n-heptane in the ratio of 1: 0.4 for 30 mins. Finally the aqueous 'product containing layer' was concentrated and diafiltered using an ultrafiltration membrane. The concentrate was sterile filtered, filled in vials and lyophilized to give daptomycin of 96%.

We Claim

1. Amorphous daptomycin having at least 98% purity.
2. Amorphous daptomycin having at least 97% purity.
3. Amorphous daptomycin having at least 96% purity.
4. Amorphous daptomycin having powder XRD pattern represented in fig 2
5. A method of purification of daptomycin comprising the steps of:
    a. filtration of the fermentation broth,
    b. optional concentration of the filtrate from step (a) by hydrophobic interaction chromatography, ultrafiltration or nanofiltration,
    c. purification of daptomycin by loading the filtrate from step (a) or the concentrate from step (b) on reverse phase resin followed by elution,
    d. recovery of purified daptomycin from the elute of reverse phase chromatography, and
    e. depyrogenation and lyophilization of the recovered daptomycin to give highly pure formulated product.
6. The method of purification as claimed in claim 5, wherein the recovery in step (d) is carried out using the steps of:

    a. optional extraction of the elute of reverse phase chromatography with a water immiscible solvent or treatment of the elute from reverse phase chromatography by ion exchange chromatography or hydrophobic interaction chromatography,
    b. optional concentration of the elute of reverse phase chromatography or the elute of hydrophobic interaction chromatography from step (a) by evaporation,
    c. subjecting the elute of reverse phase chromatography, the daptomycin-containing raffinate from step (a), the elute of ion exchange chromatography or hydrophobic interaction chromatography from step (a), or the concentrate from step (b) to endotoxin removal followed by ultrafiltration, nanofiltration or reverse osmosis.
7. The method of purification as claimed in claim 5 or 6, wherein the hydrophobic interaction chromatography is performed on resin selected from the group

comprising HP2MG, HP20, HP21, HP20SS, SP20, SP20SS, SP825, SP850, SP207, XAD16, XAD1600, XAD18, XAD761 and XAD7HP .

8. The method of purification as claimed in claim 5 or 6, wherein ultrafiltration or nanofiltration is performed using membrane selected from the group comprising polysulfone, polyether sulfone, polypropylene, polyacrylonitrile, cellulose esters, mixed cellulose esters, regenerated cellulose, polyvinylidene difluoride, nylon, teflon (PTFE) and ceramic membranes.

9. The method of purification as claimed in claim 5, wherein the reverse phase resin is selected from C4, C8 and C18 reverse phase resin.

10. The method of purification according to claim 5, wherein elution from the reverse phase resin is carried out with mixture of organic solvent and water or mixture of organic solvent and buffer at pH 2.5 to 8.

11. The method of purification as claimed in claim 10, wherein the organic solvent is selected from methanol, ethanol, n-propanol, isopropanol, n-butanol, t-butanol, acetonitrile, acetone, tetrahydrofuran or mixture thereof.

12. The method of purification as claimed in claim 5, wherein the reverse phase chromatography elution is carried out in isocratic or gradient manner.

13. The method of purification as claimed in claim 12, wherein the gradient elution is achieved by changing the content of organic solvent, pH and/or buffer molarity during elution.

14. The method of purification as claimed in claim 6, wherein the water-immiscible solvent is selected from ethyl acetate, propyl acetate, butyl acetate, amyl acetate, chloroform, dichloromethane, methyl-t-butyl ether, diethyl ether, butanol, hexane, heptane, cyclohexane or mixture thereof.

15. The method of purification as claimed in claim 6, wherein the ion exchange chromatography is anion exchange chromatography or cation exchange chromatography.

16. The method of purification as claimed in claim 15, wherein the ion exchange chromatography is anion exchange chromatography.

17. The method of purification as claimed in claim 6, wherein the ion exchange chromatography is performed on resin selected from the group comprising diethyl aminoethyl, quaternary aminoethyl, quaternary ammonium, polyethyleneimine and quaternized polyethyleneimine type resins.

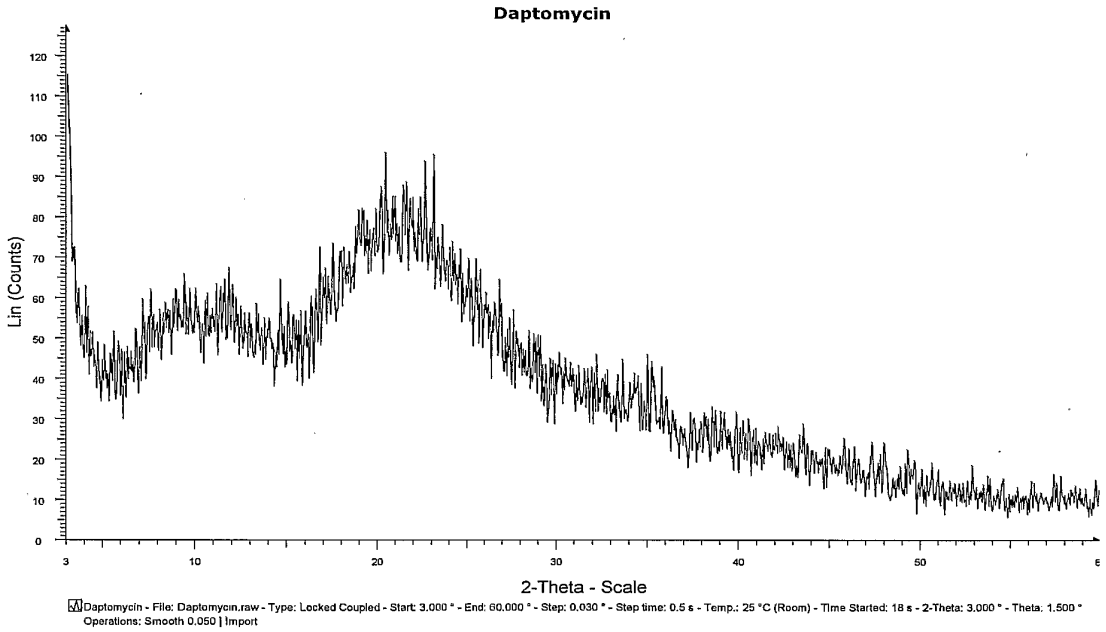
18. The method of purification as claimed in claim 6, wherein the ion exchange chromatography elution is carried out using a salt solution.

19. The method of purification as claimed in claim 18, wherein the salt is a halogenide.

20. The method of purification as claimed in 18, wherein the salt is an alkali or alkaline earth halogenide.

21. The method of purification as claimed in claim 6, wherein the evaporation is done using a thin or wiped film evaporator, falling film evaporator, forced circulation evaporator, short tube evaporator or long tube evaporator.

22. The method of purification as claimed in claim 5 or 6, wherein the ultrafiltration or nanofiltration is performed at pH 2.5 to 8.


Daptomycin
Lin (Counts)
2-Theta - Scale
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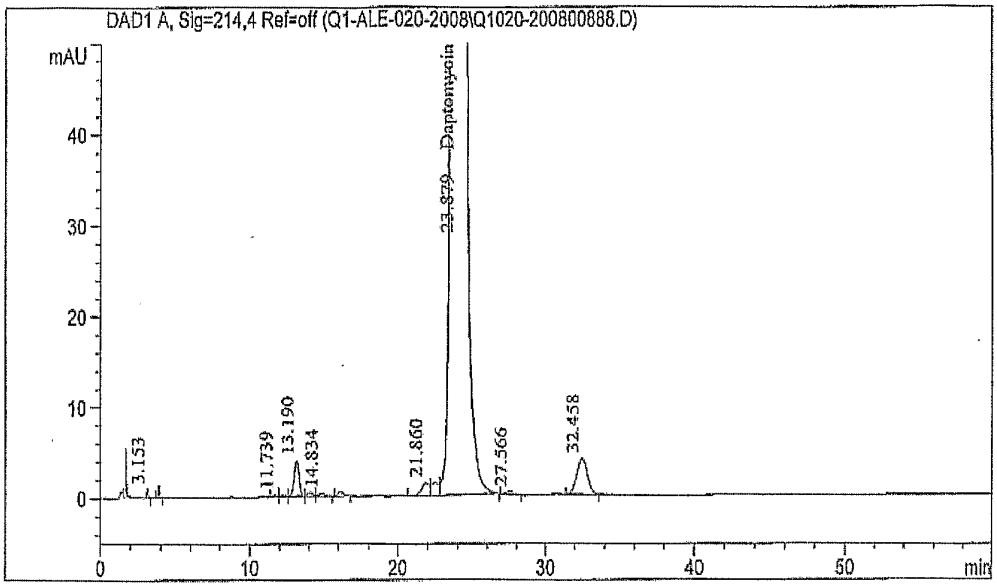
Operations: Smooth 0.050 | Import

Fig. 1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mAU
40
30
20
10
0
3.153
11.739
13.190
14.834
21.860
23.879
Daptomycin
27.566
32.458
0
10
20
30
40
50
min

Fig. 2

INTERNATIONAL SEARCH REPORT

International application No.
PCT/IN2009/000265

A. CLASSIFICATION OF SUBJECT MATTER

Int. Cl.

C07K 1/20 (2006.01) *C07K 1/16* (2006.01)
A61K 38/12 (2006.01) *C12P 21/04* (2006.01)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPODOC, WPI, Medline, CAPLUS (keywords - daptomycin, LY 146302, cubicin, purity, purify, amorphous, chromatography, column, reverse phase)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 6696412 B1 (KELLEHER et al.) 24 February 2004 See abstract, column 9 line 10 – column 12 line 5 and column 14, lines 12-64	1-3, 5-9, 14-16, and 17-22
X	US 2003/0045678 A1 (KEITH et al.) 6 March 2003 See Figure 6	4
X	US 4874843 A (BAKER) 17 October 1989 See abstract, column 2, line 40 – column 3, line 68 and column 4, lines 1-3	5 and 10-13

[X] Further documents are listed in the continuation of Box C [X] See patent family annex

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
17 September 2009	29 SEP 2009

Name and mailing address of the ISA/AU	Authorized officer
AUSTRALIAN PATENT OFFICE PO BOX 200, WODEN ACT 2606, AUSTRALIA E-mail address: pct@ipaustralia.gov.au Facsimile No. +61 2 6283 7999	**MARYKA GAUDIO** AUSTRALIAN PATENT OFFICE (ISO 9001 Quality Certified Service) Telephone No : +61 2 6225 6128

Form PCT/ISA/210 (second sheet) (July 2009)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/IN2009/000265

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US Re. 32333 (HAMILL et al.) 20 January 1987 See examples 1-6	5-22

INTERNATIONAL SEARCH REPORT

International application No.
PCT/IN2009/000265

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a)

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

[See Supplemental Box]

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☒ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

Supplemental Box
(To be used when the space in any of Boxes I to IV is not sufficient)

Continuation of Box No: III

This International Application does not comply with the requirements of unity of invention because it does not relate to one invention or to a group of inventions so linked as to form a single general inventive concept.

In assessing whether there is more than one invention claimed, I have given consideration to those features which can be considered to potentially distinguish the claimed combination of features from the prior art. Where different claims have different distinguishing features they define different inventions.

This International Searching Authority has found that there are different inventions as follows:

- Claims 1-3 are directed to amorphous daptomycin having at least 96% purity. It is considered that amorphous daptomycin having at least 96% purity comprises a first distinguishing feature.

- Claim 4 is directed to amorphous daptomycin having a particular XRD pattern. It is considered that amorphous daptomycin having this XRD pattern comprises a second distinguishing feature.

- Claims 5-22 are directed to a method of purification of daptomycin comprising the steps of:
  a. filtration of a fermentation broth,
  b. optional concentration of the filtrate from step (a) by hydrophobic interaction chromatography, ultrafiltration or nanofiltration,
  c. purification of daptomycin by loading the filtrate from step (a) or the concentrate from step (b) on reverse resin followed by elution,
  d. recovery of purified daptomycin from the elute of the reverse phase chromatography and
  e. depyrogenation and lyphilization of the recovered daptomycin to give highly pure formulated product.

  It is considered that this method comprises a third distinguishing feature.

PCT Rule 13.2, first sentence, states that unity of invention is only fulfilled when there is a technical relationship among the claimed inventions involving one or more of the same or corresponding special technical features. PCT Rule 13.2, second sentence, defines a special technical feature as a feature which makes a contribution over the prior art.

The only feature common to all of the claims is daptomycin. However daptomycin is well known in the art. This means that the common feature can not constitute a special technical feature within the meaning of PCT Rule 13.2, second sentence, since it makes no contribution over the prior art.

Because the common feature does not satisfy the requirement for being a special technical feature it follows that it cannot provide the necessary technical relationship between the identified inventions. Therefore the claims do not satisfy the requirement of unity of invention *a posteriori*.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.
PCT/IN2009/000265

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document Cited in Search Report		Patent Family Member					
US	6696412	AU	30978/01	BR	0107731	CA	2398726
		CN	1404487	CN	101240013	CZ	20022830
		EP	1252179	EP	1586580	HU	0203969
		IS	6470	KR	20070044079	MX	PA02007132
		NO	20023476	NZ	520324	PL	356898
		RU	2002122114	US	2005009747	US	2007191280
		WO	0153330	ZA	200205763		
US	2003045678	AU	2008207496	CA	2432096	CA	2432187
		CN	1592753	CN	1982330	EP	1343811
		EP	1383794	EP	1908770	JP	2008214348
		KR	20080036661	NZ	554405	US	2002111311
		US	2003045484	US	2006014674	US	2009197799
		WO	02056829	WO	02059145	WO	02096936
US	4874843	AU	17493/88	BG	47349	EP	0294990
		HU	47154	IL	86601	JP	1047388
		NZ	224873	US	5912226	ZA	8803887

Due to data integration issues this family listing may not include 10 digit Australian applications filed since May 2001.

END OF ANNEX

I hereby certify that this paper (along with any paper referred to as being attached or enclosed) is being transmitted via the Office electronic filing system in accordance with § 1.6(a)(4).

Dated: March 25, 2014
Electronic Signature for Brian C. Trinque, Ph.D.: /Brian C. Trinque/

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s):	Sandra O'Connor et al.	Examiner:	N/A
Serial No.:	14/096,346	Group Art No.	1654
Filed:	December 4, 2013	Confirmation No.	2832
For:	LIPOPEPTIDE COMPOSITIONS AND RELATED METHODS		

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

REQUEST FOR CORRECTION OF FILING RECEIPT

Dear Sir/Madam:

Applicant respectfully requests that the official Updated Filing Receipt issued in the above-identified application be corrected as follows:

Incorrect Domestic Applications for which benefit is claimed:	~~None~~
Correct Domestic Applications for which benefit is claimed:	This application is a divisional application of US 13/511,246 (05-22-2012), which is a '371 application of PCT/US2010/57910 (11-23-2010), which claims benefit of US 61/263,784 (11-23-2009).

In support hereof, Applicant states that a Second Preliminary Amendment, along with an updated Application Data Sheet, was filed with the correct priority information on January 24, 2014. Applicant further submits a marked-up Updated Filing Receipt noting the corrections thereon.

In view of the above, Applicant hereby requests that a Corrected Filing Receipt be issued in the above-identified patent application.

Applicant hereby authorizes any fees due to be charged to Deposit Account No. 12-0600 under Order No. 552815 (CPT-011USDV).

Date: March 25, 2014

Respectfully submitted,

Electronic Signature: /Brian C. Trinque/
Brian C. Trinque, Ph.D., Esq.
Registration No. 56,593
LATHROP & GAGE LLP
28 State Street, Suite 0700
Boston, Massachusetts 02109
Telephone: (857) 300-4003
Facsimile: (857) 300-4001
Customer No: 113613

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APPLICATION NUMBER	FILING or 371(c) DATE	GRP ART UNIT	FIL FEE REC'D	ATTY.DOCKET.NO	TOT CLAIMS	IND CLAIMS
14/096,346	12/04/2013	1654	1820	552815 (CPT-011USDV)	21	1

CONFIRMATION NO. 2832

UPDATED FILING RECEIPT

113613
Cubist Pharmaceuticals, Inc.
Lathrop & Gage
65 Hayden Avenue
Lexington, MA 02421

Date Mailed: 02/04/2014

Receipt is acknowledged of this non-provisional patent application. The application will be taken up for examination in due course. Applicant will be notified as to the results of the examination. Any correspondence concerning the application must include the following identification information: the U.S. APPLICATION NUMBER, FILING DATE, NAME OF APPLICANT, and TITLE OF INVENTION. Fees transmitted by check or draft are subject to collection. Please verify the accuracy of the data presented on this receipt. **If an error is noted on this Filing Receipt, please submit a written request for a Filing Receipt Correction. Please provide a copy of this Filing Receipt with the changes noted thereon. If you received a "Notice to File Missing Parts" for this application, please submit any corrections to this Filing Receipt with your reply to the Notice. When the USPTO processes the reply to the Notice, the USPTO will generate another Filing Receipt incorporating the requested corrections**

Inventor(s)

Sandra O'Connor, Hudson, NH;
Sophie Sun, Littleton, MA;
Gaauri Naik, Cambridge, MA;

Applicant(s)

Cubist Pharmaceuticals, Inc., Lexington, MA

Assignment For Published Patent Application

Cubist Pharmaceuticals, Inc., Lexington, MA

Power of Attorney: None

Domestic Applications for which benefit is claimed - ~~None.~~
A proper domestic benefit claim must be provided in an Application Data Sheet in order to constitute a claim for domestic benefit. See 37 CFR 1.76 and 1.78.

This application is a divisional of US 13/501,246 (05-22-2012), which is a 371 of PCT/US2010/057910 (11-23-2010) which claims priority to US 61/263,764 (11-23-2009)

Foreign Applications for which priority is claimed (You may be eligible to benefit from the **Patent Prosecution Highway** program at the USPTO. Please see http://www.uspto.gov for more information.) - None.
Foreign application information must be provided in an Application Data Sheet in order to constitute a claim to foreign priority. See 37 CFR 1.55 and 1.76.

If Required, Foreign Filing License Granted: 12/23/2013
The country code and number of your priority application, to be used for filing abroad under the Paris Convention, is **US 14/096,346**
Projected Publication Date: 06/04/2015
Non-Publication Request: No
Early Publication Request: No

Title

LIPOPEPTIDE COMPOSITIONS AND RELATED METHODS

Preliminary Class

530

Statement under 37 CFR 1.55 or 1.78 for AIA (First Inventor to File) Transition Applications: No

PROTECTING YOUR INVENTION OUTSIDE THE UNITED STATES

Since the rights granted by a U.S. patent extend only throughout the territory of the United States and have no effect in a foreign country, an inventor who wishes patent protection in another country must apply for a patent in a specific country or in regional patent offices. Applicants may wish to consider the filing of an international application under the Patent Cooperation Treaty (PCT). An international (PCT) application generally has the same effect as a regular national patent application in each PCT-member country. The PCT process **simplifies** the filing of patent applications on the same invention in member countries, but **does not result** in a grant of "an international patent" and does not eliminate the need of applicants to file additional documents and fees in countries where patent protection is desired.

Almost every country has its own patent law, and a person desiring a patent in a particular country must make an application for patent in that country in accordance with its particular laws. Since the laws of many countries differ in various respects from the patent law of the United States, applicants are advised to seek guidance from specific foreign countries to ensure that patent rights are not lost prematurely.

Applicants also are advised that in the case of inventions made in the United States, the Director of the USPTO must issue a license before applicants can apply for a patent in a foreign country. The filing of a U.S. patent application serves as a request for a foreign filing license. The application's filing receipt contains further information and guidance as to the status of applicant's license for foreign filing.

Applicants may wish to consult the USPTO booklet, "General Information Concerning Patents" (specifically, the section entitled "Treaties and Foreign Patents") for more information on timeframes and deadlines for filing foreign patent applications. The guide is available either by contacting the USPTO Contact Center at 800-786-9199, or it can be viewed on the USPTO website at http://www.uspto.gov/web/offices/pac/doc/general/index.html.

For information on preventing theft of your intellectual property (patents, trademarks and copyrights), you may wish to consult the U.S. Government website, http://www.stopfakes.gov. Part of a Department of Commerce initiative, this website includes self-help "toolkits" giving innovators guidance on how to protect intellectual property in specific countries such as China, Korea and Mexico. For questions regarding patent enforcement issues, applicants may call the U.S. Government hotline at 1-866-999-HALT (1-866-999-4258).

LICENSE FOR FOREIGN FILING UNDER

Title 35, United States Code, Section 184

Title 37, Code of Federal Regulations, 5.11 & 5.15

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Electronic Acknowledgement Receipt

EFS ID:	18573745
Application Number:	14096346
International Application Number:	
Confirmation Number:	2832
Title of Invention:	LIPOPEPTIDE COMPOSITIONS AND RELATED METHODS
First Named Inventor/Applicant Name:	Sandra O'Connor
Customer Number:	113613
Filer:	Brian C. Trinque
Filer Authorized By:	
Attorney Docket Number:	552815 (CPT-011USDV)
Receipt Date:	25-MAR-2014
Filing Date:	04-DEC-2013
Time Stamp:	22:15:08
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	no

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Request for Corrected Filing Receipt	552815ReqCorrectFR.pdf	275395 9e5e1f0c6942f8cdb5d71f86b103015022e9d232	no	5

Warnings:	
Information:	

Total Files Size (in bytes):	275395

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

New Applications Under 35 U.S.C. 111
If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371
If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office
If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

PATENT APPLICATION FEE DETERMINATION RECORD

Substitute for Form PTO-875

Application or Docket Number: 14/096,346

APPLICATION AS FILED - PART I

FOR	(Column 1) NUMBER FILED	(Column 2) NUMBER EXTRA	SMALL ENTITY RATE($)	SMALL ENTITY FEE($)	OR	OTHER THAN SMALL ENTITY RATE($)	OTHER THAN SMALL ENTITY FEE($)
BASIC FEE (37 CFR 1.16(a), (b), or (c))	N/A	N/A	N/A			N/A	280
SEARCH FEE (37 CFR 1.16(k), (i), or (m))	N/A	N/A	N/A			N/A	600
EXAMINATION FEE (37 CFR 1.16(o), (p), or (q))	N/A	N/A	N/A			N/A	720
TOTAL CLAIMS (37 CFR 1.16(i))	21 minus 20 =	* 1			OR	x 80 =	80
INDEPENDENT CLAIMS (37 CFR 1.16(h))	1 minus 3 =	*				x 420 =	0.00
APPLICATION SIZE FEE (37 CFR 1.16(s))	If the specification and drawings exceed 100 sheets of paper, the application size fee due is $310 ($155 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s).						0.00
MULTIPLE DEPENDENT CLAIM PRESENT (37 CFR 1.16(j))							0.00
* If the difference in column 1 is less than zero, enter "0" in column 2.			TOTAL			TOTAL	1680

APPLICATION AS AMENDED - PART II

		(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA	SMALL ENTITY RATE($)	SMALL ENTITY ADDITIONAL FEE($)	OR	OTHER THAN SMALL ENTITY RATE($)	OTHER THAN SMALL ENTITY ADDITIONAL FEE($)
AMENDMENT A	Total (37 CFR 1.16(i))	*	Minus	**	=	x =		OR	x =	
	Independent (37 CFR 1.16(h))	*	Minus	***	=	x =		OR	x =	
	Application Size Fee (37 CFR 1.16(s))									
	FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))							OR		
						TOTAL ADD'L FEE		OR	TOTAL ADD'L FEE	

		(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA	RATE($)	ADDITIONAL FEE($)	OR	RATE($)	ADDITIONAL FEE($)
AMENDMENT B	Total (37 CFR 1.16(i))	*	Minus	**	=	x =		OR	x =	
	Independent (37 CFR 1.16(h))	*	Minus	***	=	x =		OR	x =	
	Application Size Fee (37 CFR 1.16(s))									
	FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))							OR		
						TOTAL ADD'L FEE		OR	TOTAL ADD'L FEE	

* If the entry in column 1 is less than the entry in column 2, write "0" in column 3.

** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 20, enter "20".

*** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 3, enter "3".

The "Highest Number Previously Paid For" (Total or Independent) is the highest found in the appropriate box in column 1.

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NUMBER	FILING or 371(c) DATE	GRP ART UNIT	FIL FEE REC'D	ATTY.DOCKET.NO	TOT CLAIMS	IND CLAIMS
14/096,346	12/04/2013	1654	1820	552815 (CPT-011USDV)	21	1

CONFIRMATION NO. 2832

UPDATED FILING RECEIPT

113613
Cubist Pharmaceuticals, Inc.
Lathrop & Gage
65 Hayden Avenue
Lexington, MA 02421

OC000000066371654

Date Mailed: 02/04/2014

Receipt is acknowledged of this non-provisional patent application. The application will be taken up for examination in due course. Applicant will be notified as to the results of the examination. Any correspondence concerning the application must include the following identification information: the U.S. APPLICATION NUMBER, FILING DATE, NAME OF APPLICANT, and TITLE OF INVENTION. Fees transmitted by check or draft are subject to collection. Please verify the accuracy of the data presented on this receipt. **If an error is noted on this Filing Receipt, please submit a written request for a Filing Receipt Correction. Please provide a copy of this Filing Receipt with the changes noted thereon. If you received a "Notice to File Missing Parts" for this application, please submit any corrections to this Filing Receipt with your reply to the Notice. When the USPTO processes the reply to the Notice, the USPTO will generate another Filing Receipt incorporating the requested corrections**

Inventor(s)

Sandra O'Connor, Hudson, NH;
Sophie Sun, Littleton, MA;
Gaauri Naik, Cambridge, MA;

Applicant(s)

Cubist Pharmaceuticals, Inc., Lexington, MA

Assignment For Published Patent Application

Cubist Pharmaceuticals, Inc., Lexington, MA

Power of Attorney: None

Domestic Applications for which benefit is claimed - None.
A proper domestic benefit claim must be provided in an Application Data Sheet in order to constitute a claim for domestic benefit. See 37 CFR 1.76 and 1.78.

Foreign Applications for which priority is claimed (You may be eligible to benefit from the **Patent Prosecution Highway** program at the USPTO. Please see http://www.uspto.gov for more information.) - None.
Foreign application information must be provided in an Application Data Sheet in order to constitute a claim to foreign priority. See 37 CFR 1.55 and 1.76.

If Required, Foreign Filing License Granted: 12/23/2013
The country code and number of your priority application, to be used for filing abroad under the Paris Convention, is **US 14/096,346**
Projected Publication Date: 06/04/2015
Non-Publication Request: No
Early Publication Request: No

Title

LIPOPEPTIDE COMPOSITIONS AND RELATED METHODS

Preliminary Class

530

Statement under 37 CFR 1.55 or 1.78 for AIA (First Inventor to File) Transition Applications: No

PROTECTING YOUR INVENTION OUTSIDE THE UNITED STATES

Since the rights granted by a U.S. patent extend only throughout the territory of the United States and have no effect in a foreign country, an inventor who wishes patent protection in another country must apply for a patent in a specific country or in regional patent offices. Applicants may wish to consider the filing of an international application under the Patent Cooperation Treaty (PCT). An international (PCT) application generally has the same effect as a regular national patent application in each PCT-member country. The PCT process **simplifies** the filing of patent applications on the same invention in member countries, but **does not result** in a grant of "an international patent" and does not eliminate the need of applicants to file additional documents and fees in countries where patent protection is desired.

Almost every country has its own patent law, and a person desiring a patent in a particular country must make an application for patent in that country in accordance with its particular laws. Since the laws of many countries differ in various respects from the patent law of the United States, applicants are advised to seek guidance from specific foreign countries to ensure that patent rights are not lost prematurely.

Applicants also are advised that in the case of inventions made in the United States, the Director of the USPTO must issue a license before applicants can apply for a patent in a foreign country. The filing of a U.S. patent application serves as a request for a foreign filing license. The application's filing receipt contains further information and guidance as to the status of applicant's license for foreign filing.

Applicants may wish to consult the USPTO booklet, "General Information Concerning Patents" (specifically, the section entitled "Treaties and Foreign Patents") for more information on timeframes and deadlines for filing foreign patent applications. The guide is available either by contacting the USPTO Contact Center at 800-786-9199, or it can be viewed on the USPTO website at http://www.uspto.gov/web/offices/pac/doc/general/index.html.

For information on preventing theft of your intellectual property (patents, trademarks and copyrights), you may wish to consult the U.S. Government website, http://www.stopfakes.gov. Part of a Department of Commerce initiative, this website includes self-help "toolkits" giving innovators guidance on how to protect intellectual property in specific countries such as China, Korea and Mexico. For questions regarding patent enforcement issues, applicants may call the U.S. Government hotline at 1-866-999-HALT (1-866-999-4258).

LICENSE FOR FOREIGN FILING UNDER

Title 35, United States Code, Section 184

Title 37, Code of Federal Regulations, 5.11 & 5.15

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The applicant has been granted a license under 35 U.S.C. 184, if the phrase "IF REQUIRED, FOREIGN FILING LICENSE GRANTED" followed by a date appears on this form. Such licenses are issued in all applications where the conditions for issuance of a license have been met, regardless of whether or not a license may be required as set forth in 37 CFR 5.15. The scope and limitations of this license are set forth in 37 CFR 5.15(a) unless an earlier license has been issued under 37 CFR 5.15(b). The license is subject to revocation upon written notification. The date indicated is the effective date of the license, unless an earlier license of similar scope has been granted under 37 CFR 5.13 or 5.14.

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The grant of a license does not in any way lessen the responsibility of a licensee for the security of the subject matter as imposed by any Government contract or the provisions of existing laws relating to espionage and the national security or the export of technical data. Licensees should apprise themselves of current regulations especially with respect to certain countries, of other agencies, particularly the Office of Defense Trade Controls, Department of State (with respect to Arms, Munitions and Implements of War (22 CFR 121-128)); the Bureau of Industry and Security, Department of Commerce (15 CFR parts 730-774); the Office of Foreign AssetsControl, Department of Treasury (31 CFR Parts 500+) and the Department of Energy.

NOT GRANTED

No license under 35 U.S.C. 184 has been granted at this time, if the phrase "IF REQUIRED, FOREIGN FILING LICENSE GRANTED" DOES NOT appear on this form. Applicant may still petition for a license under 37 CFR 5.12, if a license is desired before the expiration of 6 months from the filing date of the application. If 6 months has lapsed from the filing date of this application and the licensee has not received any indication of a secrecy order under 35 U.S.C. 181, the licensee may foreign file the application pursuant to 37 CFR 5.15(b).

I hereby certify that this paper (along with any paper referred to as being attached or enclosed) is being transmitted via the Office electronic filing system in accordance with § 1.6(a)(4).

Dated: January 24, 2014
Electronic Signature for Brian C. Trinque, Ph.D., Esq.: /Brian C. Trinque/

PATENT
Attorney Docket No.
552815 (CPT-011USDV)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of:
Sandra O'Connor et al.

Examiner: To Be Assigned

Application No.: 14/096,346

Art Unit: 1654

Filed: December 4, 2013

Conf. No.: 2832

For: LIPOPEPTIDE COMPOSITIONS AND RELATED METHODS

RESPONSE TO NOTICE TO FILE MISSING PARTS OF NONPROVISIONAL APPLICATION AND SECOND PRELIMINARY AMENDMENT

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Colleague:

This communication is responsive to the Notice to File Missing Parts of Nonprovisional Application having a mailing date of January 2, 2014, accompanied by a new application data sheet (ADS), an executed inventor's declaration, and payment of the required fee.

This communication also includes a preliminary amendment to the specification under 37 CFR 1.115.

Amendments to the Specification begin on page 2 of this paper;

Remarks begin on page 3 of this paper.

AMENDMENTS TO THE SPECIFICATION

Please amend the first paragraph at page 1, lines 4-5, as follows:

-- This application is a divisional of U.S. Application Ser. No. 13/511,246, filed ~~July 10~~ May 22, 2012, which is a national stage application of PCT Application No. PCT/US2010/057910, filed November 23, 2010, which claims the benefit of U.S. provisional patent application 61/263,784, filed on November 23, 2009, the entire contents of which ~~is~~ are incorporated herein by reference ~~in its entirety~~.--

REMARKS

Prior to examination of this application, please amend the specification as set forth above. Applicants have amended the specification to update the paragraph entitled "Related Applications". *No new matter has been added.*

In response to the Notice to File Missing Parts of Nonprovisional Application, a new application data sheet and an executed inventor's declaration are submitted to the Office along with this paper.

CONCLUSION

Entry of the foregoing Preliminary Amendment is in order and requested. If there are any questions regarding the proposed amendment to the application, we invite the Examiner to call Applicants' representative at the telephone number below.

Dated: January 24, 2014

Respectfully submitted,

Electronic signature: /Brian C. Trinque/
Brian C. Trinque, Ph.D., Esq.
Registration No.: 56,593
LATHROP & GAGE LLP
28 State Street, Suite 0700
Boston, Massachusetts 02109
857-300-4000 tel
857-300-4001 fax
Attorney/Agent for Applicant
Customer No: 12779

PTO/AIA/01 (06-12)
Approved for use through 01/31/2014. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

DECLARATION (37 CFR 1.63) FOR UTILITY OR DESIGN APPLICATION USING AN APPLICATION DATA SHEET (37 CFR 1.76)

Title of Invention	LIPOPEPTIDE COMPOSITIONS AND RELATED METHODS

As the below named inventor, I hereby declare that:

This declaration is directed to:

☐ The attached application, or

☒ United States application or PCT international application number 14/096,346

filed on December 4, 2013.

The above-identified application was made or authorized to be made by me.

I believe that I am the original inventor or an original joint inventor of a claimed invention in the application.

I hereby acknowledge that any willful false statement made in this declaration is punishable under 18 U.S.C. 1001 by fine or imprisonment of not more than five (5) years, or both.

WARNING:

Petitioner/applicant is cautioned to avoid submitting personal information in documents filed in a patent application that may contribute to identity theft. Personal information such as social security numbers, bank account numbers, or credit card numbers (other than a check or credit card authorization form PTO-2038 submitted for payment purposes) is never required by the USPTO to support a petition or an application. If this type of personal information is included in documents submitted to the USPTO, petitioners/applicants should consider redacting such personal information from the documents before submitting them to the USPTO. Petitioner/applicant is advised that the record of a patent application is available to the public after publication of the application (unless a non-publication request in compliance with 37 CFR 1.213(a) is made in the application) or issuance of a patent. Furthermore, the record from an abandoned application may also be available to the public if the application is referenced in a published application or an issued patent (see 37 CFR 1.14). Checks and credit card authorization forms PTO-2038 submitted for payment purposes are not retained in the application file and therefore are not publicly available.

LEGAL NAME OF INVENTOR

Inventor: Gaauri Naik Date (Optional): 10 Jan 2014

Signature: Gaauri Naik

Note: An application data sheet (PTO/SB/14 or equivalent), including naming the entire inventive entity, must accompany this form or must have been previously filed. Use an additional PTO/AIA/01 form for each additional inventor.

PTO/AIA/01 (06-12)
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U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

DECLARATION (37 CFR 1.63) FOR UTILITY OR DESIGN APPLICATION USING AN APPLICATION DATA SHEET (37 CFR 1.76)

Title of Invention	LIPOPEPTIDE COMPOSITIONS AND RELATED METHODS

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This declaration is directed to:

☐ The attached application, or

☒ United States application or PCT international application number 14/096,346 filed on December 4, 2013.

The above-identified application was made or authorized to be made by me.

I believe that I am the original inventor or an original joint inventor of a claimed invention in the application.

I hereby acknowledge that any willful false statement made in this declaration is punishable under 18 U.S.C. 1001 by fine or imprisonment of not more than five (5) years, or both.

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LEGAL NAME OF INVENTOR

Inventor: Sophie Sun Date (Optional): 10 Jan 2014

Signature: Sophie Sun

Note: An application data sheet (PTO/SB/14 or equivalent), including naming the entire inventive entity, must accompany this form or must have been previously filed. Use an additional PTO/AIA/01 form for each additional inventor.

(11-22 for US

PTO/AIA/01 (06-12)
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U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

DECLARATION (37 CFR 1.63) FOR UTILITY OR DESIGN APPLICATION USING AN APPLICATION DATA SHEET (37 CFR 1.76)

Title of Invention	LIPOPEPTIDE COMPOSITIONS AND RELATED METHODS

As the below named inventor, I hereby declare that:

This declaration is directed to:

☐ The attached application, or

☒ United States application or PCT international application number 14/096,346

filed on December 4, 2013.

The above-identified application was made or authorized to be made by me.

I believe that I am the original inventor or an original joint inventor of a claimed invention in the application.

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LEGAL NAME OF INVENTOR

Inventor: Sandra O'Connor Date (Optional): Jan 10, 2014

Signature:

Note: An application data sheet (PTO/SB/14 or equivalent), including naming the entire inventive entity, must accompany this form or must have been previously filed. Use an additional PTO/AIA/01 form for each additional inventor.

PTO/AIA/14 (11-13)
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Application Data Sheet 37 CFR 1.76	Attorney Docket Number	552815 (CPT-011USDV)
	Application Number	14/096,346
Title of Invention	LIPOPEPTIDE COMPOSITIONS AND RELATED METHODS	

The application data sheet is part of the provisional or nonprovisional application for which it is being submitted. The following form contains the bibliographic data arranged in a format specified by the United States Patent and Trademark Office as outlined in 37 CFR 1.76.
This document may be completed electronically and submitted to the Office in electronic format using the Electronic Filing System (EFS) or the document may be printed and included in a paper filed application.

Secrecy Order 37 CFR 5.2

☐ Portions or all of the application associated with this Application Data Sheet may fall under a Secrecy Order pursuant to 37 CFR 5.2 (Paper filers only. Applications that fall under Secrecy Order may not be filed electronically.)

Inventor Information:

Inventor 1 Remove

Legal Name

Prefix	Given Name	Middle Name	Family Name	Suffix
	Sandra		O'Connor	

Residence Information (Select One) ◉ US Residency ○ Non US Residency ○ Active US Military Service

City	Hudson	State/Province	NH	Country of Residence	US

Mailing Address of Inventor:

Address 1	12 Copper Hill Road
Address 2	

City	Hudson	State/Province	NH
Postal Code	03051	Country	US

Inventor 2 Remove

Legal Name

Prefix	Given Name	Middle Name	Family Name	Suffix
	Sophie		Sun	

Residence Information (Select One) ◉ US Residency ○ Non US Residency ○ Active US Military Service

City	Lexington	State/Province	MA	Country of Residence	US

Mailing Address of Inventor:

Address 1	20 Woodcliffe Road
Address 2	

City	Lexington	State/Province	MA
Postal Code	02421	Country	US

Inventor 3 Remove

Legal Name

Prefix	Given Name	Middle Name	Family Name	Suffix
	Gaauri		Naik	

Residence Information (Select One) ◉ US Residency ○ Non US Residency ○ Active US Military Service

PTO/AIA/14 (11-13)
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Application Data Sheet 37 CFR 1.76	Attorney Docket Number	552815 (CPT-011USDV)
	Application Number	14/096,346
Title of Invention	LIPOPEPTIDE COMPOSITIONS AND RELATED METHODS	

City	Cambridge	State/Province	MA	Country of Residence	US

Mailing Address of Inventor:

Address 1	4 Trowbridge Place, Unit # 2D, Harvard Square		
Address 2			
City	Cambridge	State/Province	MA
Postal Code	02138	Country	US

All Inventors Must Be Listed - Additional Inventor Information blocks may be generated within this form by selecting the **Add** button. Add

Correspondence Information:

Enter either Customer Number or complete the Correspondence Information section below.
For further information see 37 CFR 1.33(a).

☐ **An Address is being provided for the correspondence Information of this application.**

Customer Number	113613		
Email Address	bostonpatent@lathropgage.com	Add Email	Remove Email

Application Information:

Title of the Invention	LIPOPEPTIDE COMPOSITIONS AND RELATED METHODS		
Attorney Docket Number	552815 (CPT-011USDV)	Small Entity Status Claimed	☐
Application Type	Nonprovisional		
Subject Matter	Utility		
Total Number of Drawing Sheets (if any)	22	Suggested Figure for Publication (if any)	

Publication Information:

☐ Request Early Publication (Fee required at time of Request 37 CFR 1.219)

☐ **Request Not to Publish.** I hereby request that the attached application not be published under 35 U.S.C. 122(b) and certify that the invention disclosed in the attached application **has not and will not** be the subject of an application filed in another country, or under a multilateral international agreement, that requires publication at eighteen months after filing.

Representative Information:

Representative information should be provided for all practitioners having a power of attorney in the application. Providing this information in the Application Data Sheet does not constitute a power of attorney in the application (see 37 CFR 1.32).
Either enter Customer Number or complete the Representative Name section below. If both sections are completed the customer Number will be used for the Representative Information during processing.

Please Select One:	◉ Customer Number	○ US Patent Practitioner	○ Limited Recognition (37 CFR 11.9)

PTO/AIA/14 (11-13)
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U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
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Application Data Sheet 37 CFR 1.76	Attorney Docket Number	552815 (CPT-011USDV)
	Application Number	14/096,346
Title of Invention	LIPOPEPTIDE COMPOSITIONS AND RELATED METHODS	

Customer Number	113613

Domestic Benefit/National Stage Information:

This section allows for the applicant to either claim benefit under 35 U.S.C. 119(e), 120, 121, or 365(c) or indicate National Stage entry from a PCT application. Providing this information in the application data sheet constitutes the specific reference required by 35 U.S.C. 119(e) or 120, and 37 CFR 1.78.
When referring to the current application, please leave the application number blank.

Prior Application Status	Pending		Remove
Application Number	Continuity Type	Prior Application Number	Filing Date (YYYY-MM-DD)
	Division of	13511246	2012-05-22
Prior Application Status			Remove
Application Number	Continuity Type	Prior Application Number	Filing Date (YYYY-MM-DD)
13511246	a 371 of international	PCT/US2010/057910	2010-11-23
Prior Application Status			Remove
Application Number	Continuity Type	Prior Application Number	Filing Date (YYYY-MM-DD)
PCT/US2010/057910	Claims benefit of provisional	61263784	2009-11-23

Additional Domestic Benefit/National Stage Data may be generated within this form by selecting the **Add** button.

Foreign Priority Information:

This section allows for the applicant to claim priority to a foreign application. Providing this information in the application data sheet constitutes the claim for priority as required by 35 U.S.C. 119(b) and 37 CFR 1.55(d). When priority is claimed to a foreign application that is eligible for retrieval under the priority document exchange program (PDX)[i] the information will be used by the Office to automatically attempt retrieval pursuant to 37 CFR 1.55(h)(1) and (2). Under the PDX program, applicant bears the ultimate responsibility for ensuring that a copy of the foreign application is received by the Office from the participating foreign intellectual property office, or a certified copy of the foreign priority application is filed, within the time period specified in 37 CFR 1.55(g)(1).

			Remove
Application Number	Country[i]	Filing Date (YYYY-MM-DD)	Access Code[i] (if applicable)

Additional Foreign Priority Data may be generated within this form by selecting the **Add** button.

PTO/AIA/14 (11-13)
Approved for use through 01/31/2014. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

Application Data Sheet 37 CFR 1.76	Attorney Docket Number	552815 (CPT-011USDV)
	Application Number	14/096,346
Title of Invention	LIPOPEPTIDE COMPOSITIONS AND RELATED METHODS	

Statement under 37 CFR 1.55 or 1.78 for AIA (First Inventor to File) Transition Applications

☐ This application (1) claims priority to or the benefit of an application filed before March 16, 2013 and (2) also contains, or contained at any time, a claim to a claimed invention that has an effective filing date on or after March 16, 2013.
NOTE: By providing this statement under 37 CFR 1.55 or 1.78, this application, with a filing date on or after March 16, 2013, will be examined under the first inventor to file provisions of the AIA.

Authorization to Permit Access:

☐ Authorization to Permit Access to the Instant Application by the Participating Offices

If checked, the undersigned hereby grants the USPTO authority to provide the European Patent Office (EPO), the Japan Patent Office (JPO), the Korean Intellectual Property Office (KIPO), the World Intellectual Property Office (WIPO), and any other intellectual property offices in which a foreign application claiming priority to the instant patent application is filed access to the instant patent application. See 37 CFR 1.14(c) and (h). This box should not be checked if the applicant does not wish the EPO, JPO, KIPO, WIPO, or other intellectual property office in which a foreign application claiming priority to the instant patent application is filed to have access to the instant patent application.

In accordance with 37 CFR 1.14(h)(3), access will be provided to a copy of the instant patent application with respect to: 1) the instant patent application-as-filed; 2) any foreign application to which the instant patent application claims priority under 35 U.S.C. 119(a)-(d) if a copy of the foreign application that satisfies the certified copy requirement of 37 CFR 1.55 has been filed in the instant patent application; and 3) any U.S. application-as-filed from which benefit is sought in the instant patent application.

In accordance with 37 CFR 1.14(c), access may be provided to information concerning the date o f filing this Authorization.

Applicant Information:

Providing assignment information in this section does not substitute for compliance with any requirement of part 3 of Title 37 of CFR to have an assignment recorded by the Office.

PTO/AIA/14 (11-13)
Approved for use through 01/31/2014. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

Application Data Sheet 37 CFR 1.76	Attorney Docket Number	552815 (CPT-011USDV)
	Application Number	14/096,346
Title of Invention	LIPOPEPTIDE COMPOSITIONS AND RELATED METHODS	

Applicant 1

If the applicant is the inventor (or the remaining joint inventor or inventors under 37 CFR 1.45), this section should not be completed. The information to be provided in this section is the name and address of the legal representative who is the applicant under 37 CFR 1.43; or the name and address of the assignee, person to whom the inventor is under an obligation to assign the invention, or person who otherwise shows sufficient proprietary interest in the matter who is the applicant under 37 CFR 1.46. If the applicant is an applicant under 37 CFR 1.46 (assignee, person to whom the inventor is obligated to assign, or person who otherwise shows sufficient proprietary interest) together with one or more joint inventors, then the joint inventor or inventors who are also the applicant should be identified in this section.

Clear

◉ Assignee	○ Legal Representative under 35 U.S.C. 117	○ Joint Inventor
○ Person to whom the inventor is obligated to assign.	○ Person who shows sufficient proprietary interest	

If applicant is the legal representative, indicate the authority to file the patent application, the inventor is:

Name of the Deceased or Legally Incapacitated Inventor :

If the Applicant is an Organization check here. ☒

Organization Name	Cubist Pharmaceuticals, Inc.

Mailing Address Information For Applicant:

Address 1	65 Hayden Avenue		
Address 2			
City	Lexington	**State/Province**	MA
Country	US	Postal Code	02421
Phone Number		Fax Number	
Email Address			

Additional Applicant Data may be generated within this form by selecting the Add button.

Assignee Information including Non-Applicant Assignee Information:

Providing assignment information in this section does not subsitute for compliance with any requirement of part 3 of Title 37 of CFR to have an assignment recorded by the Office.

Assignee 1

Complete this section if assignee information, including non-applicant assignee information, is desired to be included on the patent application publication . An assignee-applicant identified in the "Applicant Information" section will appear on the patent application publication as an applicant. For an assignee-applicant, complete this section only if identification as an assignee is also desired on the patent application publication.

If the Assignee or Non-Applicant Assignee is an Organization check here. ☒

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Application Data Sheet 37 CFR 1.76	Attorney Docket Number	552815 (CPT-011USDV)
	Application Number	14/096,346
Title of Invention	LIPOPEPTIDE COMPOSITIONS AND RELATED METHODS	

Organization Name	Cubist Pharmaceuticals, Inc.		
Mailing Address Information For Assignee including Non-Applicant Assignee:			
Address 1	65 Hayden Avenue		
Address 2			
City	Lexington	**State/Province**	MA
Country i	US	Postal Code	02421
Phone Number		Fax Number	
Email Address			

Additional Assignee or Non-Applicant Assignee Data may be generated within this form by selecting the Add button.

Signature:

NOTE: This form must be signed in accordance with 37 CFR 1.33. See 37 CFR 1.4 for signature requirements and certifications.

Signature	/Brian C. Trinque/			Date (YYYY-MM-DD)	2014-01-24
First Name	Brian C.	Last Name	Trinque	Registration Number	56593

Additional Signature may be generated within this form by selecting the Add button.

This collection of information is required by 37 CFR 1.76. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 23 minutes to complete, including gathering, preparing, and submitting the completed application data sheet form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

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The information provided by you in this form will be subject to the following routine uses:

1. The information on this form will be treated confidentially to the extent allowed under the Freedom of Information Act (5 U.S.C. 552) and the Privacy Act (5 U.S.C. 552a). Records from this system of records may be disclosed to the Department of Justice to determine whether the Freedom of Information Act requires disclosure of these records.

2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.

3. A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.

4. A record in this system of records may be disclosed, as a routine use, to a contractor of the Agency having need for the information in order to perform a contract. Recipients of information shall be required to comply with the requirements of the Privacy Act of 1974, as amended, pursuant to 5 U.S.C. 552a(m).

5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent C o o p eration Treaty.

6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).

7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (i.e., GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.

8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspections or an issued patent.

9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

Electronic Patent Application Fee Transmittal

Application Number:	14096346
Filing Date:	04-Dec-2013
Title of Invention:	LIPOPEPTIDE COMPOSITIONS AND RELATED METHODS
First Named Inventor/Applicant Name:	Sandra O'Connor
Filer:	Brian C. Trinque/Gang Wang
Attorney Docket Number:	552815 (CPT-011USDV)

Filed as Large Entity

Utility under 35 USC 111(a) Filing Fees

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Basic Filing:				
Pages:				
Claims:				
Miscellaneous-Filing:				
PROCESSING FEE, EXCEPT PROV. APPLS.	1830	1	140	140
Petition:				
Patent-Appeals-and-Interference:				
Post-Allowance-and-Post-Issuance:				
Extension-of-Time:				

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Miscellaneous:				
			Total in USD ($)	**140**

Electronic Acknowledgement Receipt

EFS ID:	18018094
Application Number:	14096346
International Application Number:	
Confirmation Number:	2832
Title of Invention:	LIPOPEPTIDE COMPOSITIONS AND RELATED METHODS
First Named Inventor/Applicant Name:	Sandra O'Connor
Customer Number:	113613
Filer:	Brian C. Trinque/Gang Wang
Filer Authorized By:	Brian C. Trinque
Attorney Docket Number:	552815 (CPT-011USDV)
Receipt Date:	24-JAN-2014
Filing Date:	04-DEC-2013
Time Stamp:	16:24:59
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	yes
Payment Type	Deposit Account
Payment was successfully received in RAM	$140
RAM confirmation Number	3282
Deposit Account	120600
Authorized User	

The Director of the USPTO is hereby authorized to charge indicated fees and credit any overpayment as follows:

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File Listing:					
Document Number	**Document Description**	**File Name**	**File Size(Bytes)/ Message Digest**	**Multi Part /.zip**	**Pages (if appl.)**
1		response.pdf	27941 cfaff852d4e3b86cb6c0b01bfad1602a3971fe5b	yes	4
	Multipart Description/PDF files in .zip description				
	Document Description		**Start**	**End**	
	Applicant Response to Pre-Exam Formalities Notice		1	1	
	Specification		2	2	
	Applicant Arguments/Remarks Made in an Amendment		3	4	
Warnings:					
Information:					
2	Oath or Declaration filed	dec.pdf	1387212 3173f7e83f864719084304b3c7d1e197b386c0af	no	3
Warnings:					
Information:					
3	Application Data Sheet	ads.pdf	942430 f8bf2b9ac3e0790896d00dc11a009ddda80b6667	no	7
Warnings:					
Information:					
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4	Fee Worksheet (SB06)	fee-info.pdf	30470 a509f2a149f9812772766f5e049bcced87dffc80	no	2
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New Applications Under 35 U.S.C. 111
If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371
If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office
If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

Document code: WFEE

United States Patent and Trademark Office
Sales Receipt for Accounting Date: 01/30/2014

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01 FC : 1051 140.00 DA

Document code: WFEE

United States Patent and Trademark Office
Sales Receipt for Accounting Date: 01/30/2014

VVAN11 ADJ #00000009 Mailroom Dt: 01/24/2014
Seq No: 3282 Sales Acctg Dt: 01/27/2014 120600 14096346
01 FC : 1830 140.00 CR

Doc code: IDS
Doc description: Information Disclosure Statement (IDS) Filed

PTO/SB/08a (01-10)
Approved for use through 07/31/2012. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
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INFORMATION DISCLOSURE STATEMENT BY APPLICANT (**Not for submission under 37 CFR 1.99)**		
	Application Number	14096346
	Filing Date	2013-12-04
	First Named Inventor	Sandra O'CONNOR
	Art Unit	1654
	Examiner Name	Not Yet Assigned
	Attorney Docket Number	552815 (CPT-011USDV)

U.S.PATENTS Remove

Examiner Initial*	Cite No	Patent Number	Kind Code[1]	Issue Date	Name of Patentee or Applicant of cited Document	Pages,Columns,Lines where Relevant Passages or Relevant Figures Appear
	1	6194383		2001-02-27	Hammann et al.	
	2	5955509		1999-09-21	Webber et al.	
	3	5629288		1997-05-13	Lattrell et al.	
	4	5387670		1995-02-07	Roy et al.	
	5	5271935		1993-12-21	Franco et al.	
	6	4882164		1989-11-21	Ferro	
	7	4331594		1982-05-25	Alder et al.	
	8	8604164		2013-12-10	Kelleher et al.	

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INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99)		
	Application Number	14096346
	Filing Date	2013-12-04
	First Named Inventor	Sandra O'CONNOR
	Art Unit	1654
	Examiner Name	Not Yet Assigned
	Attorney Docket Number	552815 (CPT-011USDV)

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Examiner Initial*	Cite No	Publication Number	Kind Code[1]	Publication Date	Name of Patentee or Applicant of cited Document	Pages,Columns,Lines where Relevant Passages or Relevant Figures Appear
	1	20120149062		2012-02-16	Kelleher et al.	
	2	20100041589		2010-02-18	Keith et al.	
	3	20120270772		2012-07-10	O'Conner	
	4	20050027113		2005-02-03	Vivian Pak Woon Miao et al.	
	5	20070128694		2007-06-07	Baltz et al.	
	6	20130280760	A1	2013-10-24	Cubist Pharmaceuticals, Inc.	

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FOREIGN PATENT DOCUMENTS Remove

Examiner Initial*	Cite No	Foreign Document Number[3]	Country Code[2] i	Kind Code[4]	Publication Date	Name of Patentee or Applicant of cited Document	Pages,Columns,Lines where Relevant Passages or Relevant Figures Appear	T[5]
	1	WO 00018419	WO		2000-04-06	Cubist Pharmaceuticals		☐
	2	WO 99027957	WO		1999-06-10	The Immune Response Co.		☐

INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99)		
	Application Number	14096346
	Filing Date	2013-12-04
	First Named Inventor	Sandra O'CONNOR
	Art Unit	1654
	Examiner Name	Not Yet Assigned
	Attorney Docket Number	552815 (CPT-011USDV)

	3	WO 99040113	WO		1999-08-12	Thiam et al.		☐
	4	WO99043700	WO		1999-09-02	Aventis Pharma Deutschland GMBH		☐
	5	WO99027954	WO		1999-06-10	Gras-Masse et al.		☐
	6	CN1592753A	CN	A	2005-03-09	CUBIST PHARM INC, US		☒
	7	CN101330905A	CN	A	2008-12-24	SCIDOSE LLC		☒
	8	EP0386951	EP	A2	1989-03-06	ELI LILLY & COMPANY		☐

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NON-PATENT LITERATURE DOCUMENTS Remove

Examiner Initials*	Cite No	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc), date, pages(s), volume-issue number(s), publisher, city and/or country where published.	T5
	1	Sun et al., "Development of an Improved Daptomycin Drug Product: Immediate Reconstitution, Room Temperature Product Stability and Reconstitution Stability", AAPS 2011, Abstract for Poster No. T3328 Published Abstract: http://abstracts.aaps.org/SecureView/AAPSJournal/vmqutdm9e488ov6bh0qy.pdf	☐
	2	Horowitz et al., Isolation and Characterization of a Surfactant Produced by Bacillus Licheniformis 86; Journal of Industrial Microbiology 1990, Vol 6, pp. 243-248	☐
	3	Fostel, et al., "Emerging Novel Antifungal Agents," DDT; vol. 5; No. 1; Jan. 2000; pp. 25-32	☐

INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99)		
	Application Number	14096346
	Filing Date	2013-12-04
	First Named Inventor	Sandra O'CONNOR
	Art Unit	1654
	Examiner Name	Not Yet Assigned
	Attorney Docket Number	552815 (CPT-011USDV)

	4	Caballero Granado et al.; Case-control Study of Risk Factors for the Development of Enterococcal Bacteremia; Eur. J. Clin. Microbiol. Infect. Dis. 2001, Vol 20, p. 83-90	☐
	5	Desai et al., Microbial Production of Surfactants and Their Commercial Potential; Microbiology and Molecular Biology Reviews 1997, Vol 61, pp. 47-64	☐
	6	DEBRUIN. MICHAEL F., Efficacy and safety of daptomycin for the treatment of bacteremia and serious infections due to gram-positive bacteria; 4th Decennial International Conference on Nosocomial and Healthcare-Associated Infections; Poster #594 P-S2-37 (March 5-9, 2000), 14 pages.	☐
	7	AMORIJ: Development of Stable Influenza Vaccine Powder Formulations: challenges and possibilities, Pharmaceutical Research, 2008, 25:1256-1273	☐

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EXAMINER SIGNATURE

Examiner Signature		Date Considered	

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through a citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

[1] See Kind Codes of USPTO Patent Documents at www.USPTO.GOV or MPEP 901.04. [2] Enter office that issued the document, by the two-letter code (WIPO Standard ST.3). [3] For Japanese patent documents, the indication of the year of the reign of the Emperor must precede the serial number of the patent document. [4] Kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST.16 if possible. [5] Applicant is to place a check mark here if English language translation is attached.

INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99)		
	Application Number	14096346
	Filing Date	2013-12-04
	First Named Inventor	Sandra O'CONNOR
	Art Unit	1654
	Examiner Name	Not Yet Assigned
	Attorney Docket Number	552815 (CPT-011USDV)

CERTIFICATION STATEMENT

Please see 37 CFR 1.97 and 1.98 to make the appropriate selection(s):

☐ That each item of information contained in the information disclosure statement was first cited in any communication from a foreign patent office in a counterpart foreign application not more than three months prior to the filing of the information disclosure statement. See 37 CFR 1.97(e)(1).

OR

☐ That no item of information contained in the information disclosure statement was cited in a communication from a foreign patent office in a counterpart foreign application, and, to the knowledge of the person signing the certification after making reasonable inquiry, no item of information contained in the information disclosure statement was known to any individual designated in 37 CFR 1.56(c) more than three months prior to the filing of the information disclosure statement. See 37 CFR 1.97(e)(2).

☐ See attached certification statement.

☐ The fee set forth in 37 CFR 1.17 (p) has been submitted herewith.

☒ A certification statement is not submitted herewith.

SIGNATURE

A signature of the applicant or representative is required in accordance with CFR 1.33, 10.18. Please see CFR 1.4(d) for the form of the signature.

Signature	/Brian C. Trinque/	Date (YYYY-MM-DD)	2014-01-06
Name/Print	Brian C. Trinque, Ph.D.	Registration Number	56,593

This collection of information is required by 37 CFR 1.97 and 1.98. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 1 hour to complete, including gathering, preparing and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

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7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (i.e., GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.

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Doc code: IDS
Doc description: Information Disclosure Statement (IDS) Filed

PTO/SB/08a (01-10)
Approved for use through 07/31/2012. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
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	First Named Inventor	Sandra O'CONNOR
	Art Unit	1654
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	Attorney Docket Number	552815 (CPT-011USDV)

U.S.PATENTS						Remove
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INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99)		
	Application Number	14096346
	Filing Date	2013-12-04
	First Named Inventor	Sandra O'CONNOR
	Art Unit	1654
	Examiner Name	Not Yet Assigned
	Attorney Docket Number	552815 (CPT-011USDV)

	1	Haworth et al.; Staphylococcus aureus ventriculitis treated with single-dose intraventricular vancomycin or daptomycin (LY146032): bacterial and antibiotic kinetics in hydrocephalic rabbits; Antimicrobial Agents and Chemotherapy 1990, Vol 34, pp. 245-51	☐
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	Art Unit	1654
	Examiner Name	Not Yet Assigned
	Attorney Docket Number	552815 (CPT-011USDV)

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SIGNATURE

A signature of the applicant or representative is required in accordance with CFR 1.33, 10.18. Please see CFR 1.4(d) for the form of the signature.

Signature	/Brian C. Trinque/	Date (YYYY-MM-DD)	2014-01-06
Name/Print	Brian C. Trinque, Ph.D.	Registration Number	56,593

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(Not for submission under 37 CFR 1.99)

Application Number	14096346
Filing Date	2013-12-04
First Named Inventor	Sandra O'Connor
Art Unit	1654
Examiner Name	Not Yet Assigned
Attorney Docket Number	552815 (CPT-011USDV)

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	3	7138487		2006-11-21	Migenix Inc.	
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	Art Unit	1654
	Examiner Name	Not Yet Assigned
	Attorney Docket Number	552815 (CPT-011USDV)

	1	20020111311		2002-08-15	Cubist Pharmaceuticals, Inc.	
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	16	20070116729		2007-05-24	Palepu, Nageswara R.	
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	21	20110124551		2011-05-26	EAGLE PHARMACEUTICALS, INC	
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	24	20120270772		2012-10-25	CUBIST PHARMACEUTICALS, INC.	
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	1	WO2001/044274 A1	WO		2001-06-21	CUBIST PHARMACEUTICALS, INC		☐
	2	WO2001/053330 A2	WO		2001-07-26	CUBIST PHARMACEUTICALS, INC		☐
	3	WO2002/055537 A1	WO		2002-07-18	INTRABIOTICS PHARMACEUTICALS, INC.		☐
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	5	WO2002/059145 A1	WO		2002-08-01	CUBIST PHARMACEUTICALS, INC		☐
	6	WO2002/096936 A2	WO		2002-12-05	ALTUS BIOLOGICS, INC.		☐
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	16	WO2011/035108 A1	WO		2011-03-24	EAGLE PHARMACEUTICALS, INC.		☐
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	18	WO2011/063419 A2	WO		2011-05-26	CUBIST PHARMACEUTICALS INC.		☐
	19	WO2012/061360 A2	WO		2012-05-10	RIB-X PHARMACEUTICALS, INC.		☐
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	2	Notification of Transmittal of International Preliminary Report on Patentability in International Application No. PCT/ US2010/057910, mailed May 24, 2012, 7 pages	☐
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	5	Smales et al, Therapeutic proteins, methods and protocols, Humana press, 2005, pages 287-292	☐
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☐ The fee set forth in 37 CFR 1.17 (p) has been submitted herewith.

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Signature	/Brian C. Trinque/	Date (YYYY-MM-DD)	2014-01-06
Name/Print	Brian C. Trinque, Ph.D., Esq.	Registration Number	56593

This collection of information is required by 37 CFR 1.97 and 1.98. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 1 hour to complete, including gathering, preparing and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

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8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspections or an issued patent.

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s):	Sandra O'CONNOR et al.	Examiner:	Not Yet Assigned
Serial No.:	14/096,346	Group Art No.:	1654
Filed:	December 4, 2013	Confirmation No.:	2832
For:	LIPOPEPTIDE COMPOSITIONS AND RELATED METHODS		

VIA EFS-Web
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

INFORMATION DISCLOSURE STATEMENT TRANSMITTAL

Dear Sir:

This Information Disclosure Statement is submitted:

☒ under 37 CFR 1.97(b), or
(Within three months of filing national application, or date of entry of national application, or before mailing date of first office action on the merits, whichever occurs last)

☐ under 37 CFR 1.97(c) together with either a:
- ☐ Statement under 37 CFR 1.97(e), or
- ☐ a $180 fee under 37 CFR 1.17(p), or

(After the CFR 1.97(b) time period, but before final action or notice of allowance, whichever occurs first)

☐ under 37 CFR 1.97(d) together with a:
- ☐ Statement under 37 CFR 1.97(e)(1) or (2), and
- ☐ a $180.00 fee set forth in 37 CFR 1.17(p).

(Filed after final action, a notice of allowance, on or before payment of issue fee)

At any time during the pendency of this application, please charge any fees required or credit any overpayment to Deposit Account 12-0600 pursuant to 37 CFR 1.25.

Applicant submits herewith Form PTO SB/08 – Information Disclosure Statement citing all references for which Applicant is aware, which Applicant believes may be material to the examination of the application and for which there may be a duty to disclose in accordance with 37 CFR 1.56. Applicant is not submitting copies of the listed U.S. patents and U.S. patent application publications.

For the Examiner's convenience in reviewing this divisional application, Applicants submit a consolidated PTO Form SB/08, listing all references cited during prosecution of the parent application. The present application is a divisional of U.S. Serial No. 13/511,246, filed June 10, 2012, (Atty. Docket No. 533264 CPT011US). All references listed on the enclosed PTO Form SB/08 have been previously cited by or submitted to the Office in the prior application, and, in accordance with 37 CFR §1.98(d), copies of the references are not enclosed but will be provided upon request.

Applicants also call to the attention of the Examiner the following co-owned patents and applications:

Commonly Owned Patents/Applications			
Patent/Application No.	Filing Date	First Named Inventor	Docket No.
6,696,412	11-28-2000	Thomas J. Kelleher	C062-02 US
8,058,238	04-24-2007	Thomas J. Kelleher	C062-02/03 US
8,129,342	09-22-2010	Thomas J. Kelleher	C062-02/04 US
8,003,673	11-30-2007	Jeffrey Alder	C083-02/01 US
13/511,246	07-10-2012	Sandra O'Connor	533264 (CPT-011US)
13/398,219	02-16-2012	Thomas J. Kelleher	548263 (CPT-062DVCON3)
13/918,083	06-14-2013	Thomas J. Kelleher	548194 (CPT-062DVCON4)
12/198,666	08-26-2008	Dennis Keith	C070/02US 548420 (CPT-070US)
13/928,505	06-27-2013	Dennis Keith	548355 (CPT-070CONDV)
13/955,495	07-31-2013	Dennis Keith	549116 (CPT-070CON2)

It is respectfully requested that the information be expressly considered during the prosecution of this application, and that the references be made of record therein and appear among the "References Cited" on any patent to issue therefrom.

In accordance with 37 CFR 1.97(g), the filing of this Information Disclosure Statement shall not be construed to mean that a search has been made or that no other material information as defined in 37 CFR 1.56(a) exists. In accordance with 37 CFR 1.97(h), the filing of this Information Disclosure Statement shall not be construed to be an admission that any patent, publication or other information referred to therein is "prior art" for this invention unless specifically designated as such.

It is submitted that the Information Disclosure Statement is in compliance with 37 CFR 1.98 and the Examiner is respectfully requested to consider the listed references.

It is requested that the information disclosed herein be made of record in this application.

Dated: January 6, 2014

Respectfully submitted,

Electronic signature: /Brian C. Trinque/
Brian C. Trinque, Ph. D., Esq.
Registration No.: 56,593
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Attorney/Agent For Applicant
Customer No: 113613

Electronic Acknowledgement Receipt

EFS ID:	17785749
Application Number:	14096346
International Application Number:	
Confirmation Number:	2832
Title of Invention:	LIPOPEPTIDE COMPOSITIONS AND RELATED METHODS
First Named Inventor/Applicant Name:	Sandra O'Connor
Customer Number:	113613
Filer:	Brian C. Trinque/Gang Wang
Filer Authorized By:	Brian C. Trinque
Attorney Docket Number:	552815 (CPT-011USDV)
Receipt Date:	06-JAN-2014
Filing Date:	04-DEC-2013
Time Stamp:	17:14:38
Application Type:	Utility under 35 USC 111(a)

Payment information:

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File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
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If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371
If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

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If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

PTO/SB/08a (01-10)
Approved for use through 07/31/2012. OMB 0651-0031
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INFORMATION DISCLOSURE STATEMENT BY APPLICANT
(Not for submission under 37 CFR 1.99)

Application Number	14096346
Filing Date	2013-12-04
First Named Inventor	Sandra O'CONNOR
Art Unit	1654
Examiner Name	Not Yet Assigned
Attorney Docket Number	552815 (CPT-011USDV)

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Examiner Initial*	Cite No	Patent Number	Kind Code[1]	Issue Date	Name of Patentee or Applicant of cited Document	Pages,Columns,Lines where Relevant Passages or Relevant Figures Appear
	1	6468967		2002-10-22	Oleson et al.	
	2	6852689		2005-02-08	Oleson et al.	
	3	RE39071		2006-04-18	Baker et al.	
	4	8058238		2011-11-15	Kelleher et al.	
	5	8129342		2012-03-06	Kelleher	
	6	4537717		1985-08-27	Abbott et al.	
	7	5912226		1999-06-15	Baker et al.	
	8	4874843		1989-10-17	Baker et al.	

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	9	4482487		1984-11-13	Abbott et al,	
	10	4331594		1982-05-25	Hamill et al.	
	11	4439425		1984-03-27	Tarcsay et al.	
	12	5336756		1994-08-09	Schwartz et al.	
	13	8431539		2013-04-30	Palepu et al.	
	14	6696412		2004-02-24	Kelleher et al.	
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Examiner Initial*	Cite No	Foreign Document Number[3]	Country Code[2] ¡	Kind Code[4]	Publication Date	Name of Patentee or Applicant of cited Document	Pages,Columns,Lines where Relevant Passages or Relevant Figures Appear	T[5]
	1	EP0294990	EP		1988-12-14	Eli Lilly and Company		☐
	2	JP04224197	JP		1992-08-13	Fujitsu LTD		☒
	3	JP05239090	JP		1993-09-17	Merck and Co. INC.		☒
	4	JP05271284	JP		1993-10-19	Hoechst AG		☒
	5	WO0153330	WO		2001-07-26	Cubist Pharmaceuticals		☐
	6	WO02059145	WO		2002-08-01	Cubist Pharmaceuticals		☐
	7	WO02096936	WO		2002-12-05	Altus Biologics Inc.		☐
	8	EP0511866	EP		1992-11-04	Merck and Co. Inc.		☐
	9	EP0521408	EP		1993-01-07	Hoechst AG in German		☒
	10	EP0629636	EP		1994-12-21	Hoechst AG in German		☐

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	Attorney Docket Number	552815 (CPT-011USDV)

	11	EP1252179	EP		2002-10-30	Cubist Pharmaceuticals		☐
	12	WO9321207	WO		1993-10-28	Abbot Lab		☐
	13	JP64047388	JP		1989-02-21	Eli Lilly and Co.		☐
	14	EP0178152	EP		1986-04-16	Eli Lilly and Co.		☐

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Examiner Initials*	Cite No	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc), date, pages(s), volume-issue number(s), publisher, city and/or country where published.	T5
	1	Miao et al., "Daptomycin biosynthesis in Streptomyces roseosporus: cloning and analysis of the gene cluster and revision of peptide stereochemistry," Microbiology 2005, Vol 151 (5), 1507-23	☐
	2	Debono, et al., "A21978C, A Complex of New Acidic Peptide Antibiotics: Isolation, Chemistry, and Mass Spectral Structure Elucidation," The Journal of Antibiotics 1987, Vol XL (6), p. 761-77	☐
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	4	Woodworth et al., "Single-Dose Pharmacokinetics and Antibacterial Activity of Daptomycin, a New Lipopeptide Antibiotic, in Healthy Volunteers," Antimicrobial Agent and Chemotherapy 1992, Vol 36, p. 318-25	☐
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	6	Tally et al., Daptomycin: a novel agent for Gram-positive infections; Expert Opinion. on Investigational Drugs; 1999, Vol 8, pp. 1223-1238	☐
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	8	Lin et al., "Recovery and Purification of the Lipopeptide Biosurfactant of Bacillus subtilis by Ultrafiltration," Biotechnology Techniques, 1997, Vol 11, p. 413-16	☐
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	17	Freeman et al., Once-daily Dosing of Aminoglycosides: Review and Recommendations For Clinical Practice; J. Antimicr. Chemother. 1997, Vol 39, p. 677-86	☐
	18	Ebert et al., Pharmacodynamics Properties of Antibiotics: Application to Drug Monitoring and Dosage Regimen Design; Infection Control and Hospital Epidemiology; 1990, 11(6), pp. 319-26	☐
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	28	Arbeit et al., The Safety and Efficacy of Daptomycin for the Treatment of Complicated Skin and Skin-Structure Infections; Clinical Infectious Diseases; 2004, Vol. 38, pp. 1673-81	☐
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	30	Barry et al., In vitro activities of daptomycin against 2,789 clinical isolates from 11 North American Medical Centers; Antimicrobial Agents and Chemotherapy; 2001, Vol. 45, pp. 1919-22	☐
	31	Benvenuto et al., Pharmacokinetics and Tolerability of Daptomycin at Doses up to 12 Milligrams per Kilogram of Body Weight Once Daily in Healthy Volunteers; Antimicrobial Agents and Chemotherapy; 2006, Vol. 50, pp. 3245-49	☐
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	33	Bingen et al., Bactericidal activity of Fancomycin, Daptomycin, Ampicillin and Aminoglycosides against Vancomycin-resistant Enterecoccus; J of Antimicrobial Chemotherapy; 1990, Vol 26, pp. 619-26	☐
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	35	Caballero_Granado et al.; Case-control Study of Risk Factors for the Development of Enterococcal Bacteremia; Eur. J. Clin. Microbiol. Infect. Dis. 2001, Vol 20, p. 83-90	☐
	36	Caron et al.; Daptomycin or teicoplanin in combination with gentamicin for treatment of experiemental endocarditis due to highly glycopeptide-resistant isolate of Enterococcus faecium; Antimicrobial Agents and Chemotherapy; 1992, Vol. 36, pp. 2611-16	☐
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	39	Chayen et al.; Recent advances in methodology for the crystallization of biological macromolecules; Journal of Crystal Growth; 1999, pp. 649-55	☐
	40	Crompton et al., Outocmes with daptomycin in the treatment of Staphylococcus aureus infections with a range of vancomycin MICs; Journal of Antimicrobial Chemotherapy; 2010, Vol 65, pp. 1784-91	☐
	41	Cubicin label, November 2011; 34 pages	☐
	42	Cui et al., Correlation between Reduced Daptomycin Susceptibility and Vancomycin Resistance in Vancomycin-Intermediate Staphylococcus aureus; Antimicrobial Agents and Chemotherapy; 2006, Vol 50, pp. 1079-82	☐
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	47	Evdokimov et al., Overproduction, purification, crystallization and preliminary X-ray diffraction analysis of YopM, an essential virulence factor extruded by the plague bacterium Yersinia pestis, Acta Crystallographica, 2000, Vol 56, pp. 1676-79	☐
	48	DuCruix, et al., Crystallization of Nucleic acids and Proteins, A Practical Approach, 2d ed., 1999, pp. 92-95, 4 pages	☐
	49	Forward et al., Comparative activity of daptomycin and teicoplanin against enterococci isolated from blood and urine, Can. J. Infect. Dis., 1992, Vol. 3, pp. 173-78	☐

INFORMATION DISCLOSURE STATEMENT BY APPLICANT (**Not for submission under 37 CFR 1.99)**		
	Application Number	14096346
	Filing Date	2013-12-04
	First Named Inventor	Sandra O'CONNOR
	Art Unit	1654
	Examiner Name	Not Yet Assigned
	Attorney Docket Number	552815 (CPT-011USDV)

	50	Fowler et al., Daptomycin versus Standard Therapy for Bacteremia and Endocarditis Caused by Staphylococcus Aureus, The New England Journal of Medicine, 2006, Vol. 355, pp. 653-65	☐

If you wish to add additional non-patent literature document citation information please click the Add button Add

EXAMINER SIGNATURE

Examiner Signature		Date Considered	

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through a citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

[1] See Kind Codes of USPTO Patent Documents at www.USPTO.GOV or MPEP 901.04. [2] Enter office that issued the document, by the two-letter code (WIPO Standard ST.3). [3] For Japanese patent documents, the indication of the year of the reign of the Emperor must precede the serial number of the patent document. [4] Kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST.16 if possible. [5] Applicant is to place a check mark here if English language translation is attached.

INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99)		
	Application Number	14096346
	Filing Date	2013-12-04
	First Named Inventor	Sandra O'CONNOR
	Art Unit	1654
	Examiner Name	Not Yet Assigned
	Attorney Docket Number	552815 (CPT-011USDV)

CERTIFICATION STATEMENT

Please see 37 CFR 1.97 and 1.98 to make the appropriate selection(s):

☐ That each item of information contained in the information disclosure statement was first cited in any communication from a foreign patent office in a counterpart foreign application not more than three months prior to the filing of the information disclosure statement. See 37 CFR 1.97(e)(1).

OR

☐ That no item of information contained in the information disclosure statement was cited in a communication from a foreign patent office in a counterpart foreign application, and, to the knowledge of the person signing the certification after making reasonable inquiry, no item of information contained in the information disclosure statement was known to any individual designated in 37 CFR 1.56(c) more than three months prior to the filing of the information disclosure statement. See 37 CFR 1.97(e)(2).

☐ See attached certification statement.

☐ The fee set forth in 37 CFR 1.17 (p) has been submitted herewith.

☒ A certification statement is not submitted herewith.

SIGNATURE

A signature of the applicant or representative is required in accordance with CFR 1.33, 10.18. Please see CFR 1.4(d) for the form of the signature.

Signature	/Brian C. Trinque/	Date (YYYY-MM-DD)	2014-01-06
Name/Print	Brian C. Trinque, Ph.D.	Registration Number	56,593

This collection of information is required by 37 CFR 1.97 and 1.98. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 1 hour to complete, including gathering, preparing and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

Privacy Act Statement

The Privacy Act of 1974 (P.L. 93-579) requires that you be given certain information in connection with your submission of the attached form related to a patent application or patent. Accordingly, pursuant to the requirements of the Act, please be advised that: (1) the general authority for the collection of this information is 35 U.S.C. 2(b)(2); (2) furnishing of the information solicited is voluntary; and (3) the principal purpose for which the information is used by the U.S. Patent and Trademark Office is to process and/or examine your submission related to a patent application or patent. If you do not furnish the requested information, the U.S. Patent and Trademark Office may not be able to process and/or examine your submission, which may result in termination of proceedings or abandonment of the application or expiration of the patent.

The information provided by you in this form will be subject to the following routine uses:

1. The information on this form will be treated confidentially to the extent allowed under the Freedom of Information Act (5 U.S.C. 552) and the Privacy Act (5 U.S.C. 552a). Records from this system of records may be disclosed to the Department of Justice to determine whether the Freedom of Information Act requires disclosure of these record s.

2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.

3. A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.

4. A record in this system of records may be disclosed, as a routine use, to a contractor of the Agency having need for the information in order to perform a contract. Recipients of information shall be required to comply with the requirements of the Privacy Act of 1974, as amended, pursuant to 5 U.S.C. 552a(m).

5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent Cooperation Treaty.

6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).

7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (i.e., GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.

8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspections or an issued patent.

9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

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APPLICATION NUMBER	FILING or 371(c) DATE	GRP ART UNIT	FIL FEE REC'D	ATTY.DOCKET.NO	TOT CLAIMS	IND CLAIMS
14/096,346	12/04/2013	1654	1680	552815 (CPT-011USDV)	21	1

CONFIRMATION NO. 2832

113613
Cubist Pharmaceuticals, Inc.
Lathrop & Gage
65 Hayden Avenue
Lexington, MA 02421

FILING RECEIPT

OC000000065714834

Date Mailed: 01/02/2014

Receipt is acknowledged of this non-provisional patent application. The application will be taken up for examination in due course. Applicant will be notified as to the results of the examination. Any correspondence concerning the application must include the following identification information: the U.S. APPLICATION NUMBER, FILING DATE, NAME OF APPLICANT, and TITLE OF INVENTION. Fees transmitted by check or draft are subject to collection. Please verify the accuracy of the data presented on this receipt. **If an error is noted on this Filing Receipt, please submit a written request for a Filing Receipt Correction. Please provide a copy of this Filing Receipt with the changes noted thereon. If you received a "Notice to File Missing Parts" for this application, please submit any corrections to this Filing Receipt with your reply to the Notice. When the USPTO processes the reply to the Notice, the USPTO will generate another Filing Receipt incorporating the requested corrections**

Inventor(s)

Sandra O'Connor, Hudson, NH;
Sophie Sun, Littleton, MA;
Gaauri Naik, Cambridge, MA;

Applicant(s)

Sandra O'Connor, Hudson, NH;
Sophie Sun, Littleton, MA;
Gaauri Naik, Cambridge, MA;

Assignment For Published Patent Application

Cubist Pharmaceuticals, Inc., Lexington, MA

Power of Attorney: None

Domestic Applications for which benefit is claimed - None.
A proper domestic benefit claim must be provided in an Application Data Sheet in order to constitute a claim for domestic benefit. See 37 CFR 1.76 and 1.78.

Foreign Applications for which priority is claimed (You may be eligible to benefit from the **Patent Prosecution Highway** program at the USPTO. Please see http://www.uspto.gov for more information.) - None.
Foreign application information must be provided in an Application Data Sheet in order to constitute a claim to foreign priority. See 37 CFR 1.55 and 1.76.

If Required, Foreign Filing License Granted: 12/23/2013
The country code and number of your priority application, to be used for filing abroad under the Paris Convention, is **US 14/096,346**
Projected Publication Date: To Be Determined - pending completion of Missing Parts

Non-Publication Request: No

Early Publication Request: No

Title

LIPOPEPTIDE COMPOSITIONS AND RELATED METHODS

Preliminary Class

530

Statement under 37 CFR 1.55 or 1.78 for AIA (First Inventor to File) Transition Applications: No

PROTECTING YOUR INVENTION OUTSIDE THE UNITED STATES

Since the rights granted by a U.S. patent extend only throughout the territory of the United States and have no effect in a foreign country, an inventor who wishes patent protection in another country must apply for a patent in a specific country or in regional patent offices. Applicants may wish to consider the filing of an international application under the Patent Cooperation Treaty (PCT). An international (PCT) application generally has the same effect as a regular national patent application in each PCT-member country. The PCT process **simplifies** the filing of patent applications on the same invention in member countries, but **does not result** in a grant of "an international patent" and does not eliminate the need of applicants to file additional documents and fees in countries where patent protection is desired.

Almost every country has its own patent law, and a person desiring a patent in a particular country must make an application for patent in that country in accordance with its particular laws. Since the laws of many countries differ in various respects from the patent law of the United States, applicants are advised to seek guidance from specific foreign countries to ensure that patent rights are not lost prematurely.

Applicants also are advised that in the case of inventions made in the United States, the Director of the USPTO must issue a license before applicants can apply for a patent in a foreign country. The filing of a U.S. patent application serves as a request for a foreign filing license. The application's filing receipt contains further information and guidance as to the status of applicant's license for foreign filing.

Applicants may wish to consult the USPTO booklet, "General Information Concerning Patents" (specifically, the section entitled "Treaties and Foreign Patents") for more information on timeframes and deadlines for filing foreign patent applications. The guide is available either by contacting the USPTO Contact Center at 800-786-9199, or it can be viewed on the USPTO website at http://www.uspto.gov/web/offices/pac/doc/general/index.html.

For information on preventing theft of your intellectual property (patents, trademarks and copyrights), you may wish to consult the U.S. Government website, http://www.stopfakes.gov. Part of a Department of Commerce initiative, this website includes self-help "toolkits" giving innovators guidance on how to protect intellectual property in specific countries such as China, Korea and Mexico. For questions regarding patent enforcement issues, applicants may call the U.S. Government hotline at 1-866-999-HALT (1-866-999-4258).

LICENSE FOR FOREIGN FILING UNDER

Title 35, United States Code, Section 184

Title 37, Code of Federal Regulations, 5.11 & 5.15

GRANTED

The applicant has been granted a license under 35 U.S.C. 184, if the phrase "IF REQUIRED, FOREIGN FILING LICENSE GRANTED" followed by a date appears on this form. Such licenses are issued in all applications where the conditions for issuance of a license have been met, regardless of whether or not a license may be required as set forth in 37 CFR 5.15. The scope and limitations of this license are set forth in 37 CFR 5.15(a) unless an earlier license has been issued under 37 CFR 5.15(b). The license is subject to revocation upon written notification. The date indicated is the effective date of the license, unless an earlier license of similar scope has been granted under 37 CFR 5.13 or 5.14.

This license is to be retained by the licensee and may be used at any time on or after the effective date thereof unless it is revoked. This license is automatically transferred to any related applications(s) filed under 37 CFR 1.53(d). This license is not retroactive.

The grant of a license does not in any way lessen the responsibility of a licensee for the security of the subject matter as imposed by any Government contract or the provisions of existing laws relating to espionage and the national security or the export of technical data. Licensees should apprise themselves of current regulations especially with respect to certain countries, of other agencies, particularly the Office of Defense Trade Controls, Department of State (with respect to Arms, Munitions and Implements of War (22 CFR 121-128)); the Bureau of Industry and Security, Department of Commerce (15 CFR parts 730-774); the Office of Foreign AssetsControl, Department of Treasury (31 CFR Parts 500+) and the Department of Energy.

NOT GRANTED

No license under 35 U.S.C. 184 has been granted at this time, if the phrase "IF REQUIRED, FOREIGN FILING LICENSE GRANTED" DOES NOT appear on this form. Applicant may still petition for a license under 37 CFR 5.12, if a license is desired before the expiration of 6 months from the filing date of the application. If 6 months has lapsed from the filing date of this application and the licensee has not received any indication of a secrecy order under 35 U.S.C. 181, the licensee may foreign file the application pursuant to 37 CFR 5.15(b).

PATENT APPLICATION FEE DETERMINATION RECORD

Substitute for Form PTO-875

Application or Docket Number: 14/096,346

APPLICATION AS FILED - PART I

FOR	(Column 1) NUMBER FILED	(Column 2) NUMBER EXTRA	SMALL ENTITY RATE($)	SMALL ENTITY FEE($)	OR	OTHER THAN SMALL ENTITY RATE($)	OTHER THAN SMALL ENTITY FEE($)
BASIC FEE (37 CFR 1.16(a), (b), or (c))	N/A	N/A	N/A			N/A	280
SEARCH FEE (37 CFR 1.16(k), (i), or (m))	N/A	N/A	N/A			N/A	600
EXAMINATION FEE (37 CFR 1.16(o), (p), or (q))	N/A	N/A	N/A			N/A	720
TOTAL CLAIMS (37 CFR 1.16(i))	21 minus 20 =	* 1			OR	x 80 =	80
INDEPENDENT CLAIMS (37 CFR 1.16(h))	1 minus 3 =	*				x 420 =	0.00
APPLICATION SIZE FEE (37 CFR 1.16(s))	If the specification and drawings exceed 100 sheets of paper, the application size fee due is $310 ($155 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s).						0.00
MULTIPLE DEPENDENT CLAIM PRESENT (37 CFR 1.16(j))							0.00
* If the difference in column 1 is less than zero, enter "0" in column 2.			TOTAL			TOTAL	1680

APPLICATION AS AMENDED - PART II

		(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA	SMALL ENTITY RATE($)	SMALL ENTITY ADDITIONAL FEE($)	OR	OTHER THAN SMALL ENTITY RATE($)	OTHER THAN SMALL ENTITY ADDITIONAL FEE($)
AMENDMENT A	Total (37 CFR 1.16(i))	*	Minus	**	=	x =		OR	x =	
	Independent (37 CFR 1.16(h))	*	Minus	***	=	x =		OR	x =	
	Application Size Fee (37 CFR 1.16(s))									
	FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))							OR		
						TOTAL ADD'L FEE		OR	TOTAL ADD'L FEE	

		(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA	RATE($)	ADDITIONAL FEE($)	OR	RATE($)	ADDITIONAL FEE($)
AMENDMENT B	Total (37 CFR 1.16(i))	*	Minus	**	=	x =		OR	x =	
	Independent (37 CFR 1.16(h))	*	Minus	***	=	x =		OR	x =	
	Application Size Fee (37 CFR 1.16(s))									
	FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))							OR		
						TOTAL ADD'L FEE		OR	TOTAL ADD'L FEE	

* If the entry in column 1 is less than the entry in column 2, write "0" in column 3.

** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 20, enter "20".

*** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 3, enter "3".

The "Highest Number Previously Paid For" (Total or Independent) is the highest found in the appropriate box in column 1.

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APPLICATION NUMBER	FILING OR 371(C) DATE	FIRST NAMED APPLICANT	ATTY. DOCKET NO./TITLE
14/096,346	12/04/2013	Sandra O'Connor	552815 (CPT-011USDV)

CONFIRMATION NO. 2832

113613
Cubist Pharmaceuticals, Inc.
Lathrop & Gage
65 Hayden Avenue
Lexington, MA 02421

FORMALITIES LETTER

OC000000065714835

Date Mailed: 01/02/2014

NOTICE TO FILE MISSING PARTS OF NONPROVISIONAL APPLICATION

FILED UNDER 37 CFR 1.53(b)

Filing Date Granted

Items Required To Avoid Abandonment:

An application number and filing date have been accorded to this application. The item(s) indicated below, however, are missing. Applicant is given **TWO MONTHS** from the date of this Notice within which to file all required items below to avoid abandonment. Extensions of time may be obtained by filing a petition accompanied by the extension fee under the provisions of 37 CFR 1.136(a).

- A mailing address for each inventor has not been submitted. A new application data sheet (ADS) in compliance with 37 CFR 1.76 or inventor's oath or declaration in compliance with 37 CFR 1.63 identifying the mailing address and residence (if the inventor lives at a location which is different from where the inventor customarily receives mail) is required.
  Sandra O'Connor
  Sophie Sun
  Gaauri Naik

The applicant needs to satisfy supplemental fees problems indicated below.

The required item(s) identified below must be timely submitted to avoid abandonment:

- A surcharge as set forth in 37 CFR 1.16(f) was not received.
  The surcharge is due for any one of:
  - late submission of the basic filing fee, search fee, or examination fee,
  - late submission of inventor's oath or declaration,
  - filing an application that does not contain at least one claim on filing, or
  - submission of an application filed by reference to a previously filed application.

SUMMARY OF FEES DUE:

The fee(s) required within **TWO MONTHS** from the date of this Notice to avoid abandonment is/are:
- $ **140** surcharge.
- $(**.00**) Previous Payment Amount.
- $ **140** TOTAL FEE BALANCE DUE.

Items Required To Avoid Processing Delays:

Applicant is notified that the above-identified application contains the deficiencies noted below. No period for reply is set forth in this notice for correction of these deficiencies. However, if a deficiency relates to the inventor's oath or declaration, the applicant must file an oath or declaration in compliance with 37 CFR 1.63, or a substitute statement in compliance with 37 CFR 1.64, executed by or with respect to each actual inventor no later than the expiration of the time period set in the "Notice of Allowability" to avoid abandonment. See 37 CFR 1.53(f).

- A properly executed inventor's oath or declaration has not been received for the following inventor(s):
  Sandra O'Connor
  Sophie Sun
  Gaauri Naik
- The ADS received on 12/04/2013 was not properly signed. Therefore, the Office will treat it only as a transmittal letter. See 37 CFR 1.76(e). Inventorship has not been set by this document and any foreign priority or domestic benefit claims contained therein are ineffective. See 37 CFR 1.55 or 37 CFR 1.78.

Replies must be received in the USPTO within the set time period or must include a proper Certificate of Mailing or Transmission under 37 CFR 1.8 with a mailing or transmission date within the set time period. For more information and a suggested format, see Form PTO/SB/92 and MPEP 512.

Replies should be mailed to:

Mail Stop Missing Parts
Commissioner for Patents
P.O. Box 1450
Alexandria VA 22313-1450

Registered users of EFS-Web may alternatively submit their reply to this notice via EFS-Web, including a copy of this Notice and selecting the document description "Applicant response to Pre-Exam Formalities Notice". https://sportal.uspto.gov/authenticate/AuthenticateUserLocalEPF.html

For more information about EFS-Web please call the USPTO Electronic Business Center at **1-866-217-9197** or visit our website at http://www.uspto.gov/ebc.

If you are not using EFS-Web to submit your reply, you must include a copy of this notice.

/hchin/

Office of Data Management, Application Assistance Unit (571) 272-4000, or (571) 272-4200, or 1-888-786-0101

I hereby certify that this paper (along with any paper referred to as being attached or enclosed) is being transmitted via the Office electronic filing system in accordance with § 1.6(a)(4).

Dated: December 4, 2013
Electronic Signature for Brian C. Trinque, Ph.D., Esq.: /Brian C. Trinque/

PATENT
Attorney Docket No.
552815 (CPT-011USDV)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of:
Sandra O'Connor et al.

Examiner: To Be Assigned

Application No.: To Be Assigned

Art Unit: To Be Assigned

Filed: December 4, 2013

Conf. No.: To Be Assigned

For: LIPOPEPTIDE COMPOSITIONS AND RELATED METHODS

PRELIMINARY AMENDMENT UNDER 37 C.F.R. 1.115

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

INTRODUCTORY COMMENTS

Dear Colleague:

Prior to examination, please amend the application as follows:

Amendments to the Specification begin on page 2 of this paper;

Amendments to the Claims begin on page 3 of this paper;

Remarks begin on page 6 of this paper.

AMENDENTS TO THE SPECIFICATION

Please amend the first paragraph at page 1, lines 4-5, as follows:

-- This application is a divisional of U.S. Application Ser. No. 13/511,246, filed July 10, 2012, which claims the benefit of U.S. provisional patent application 61/263,784, filed on November 23, 2009, which is incorporated herein by reference in its entirety.--

AMENDENTS TO THE CLAIMS

CLAIMS

1-21. (Canceled)

22. (New) A solid pharmaceutical daptomycin composition comprising daptomycin and at least one excipient selected from glycine and a sugar, wherein an amount of the solid pharmaceutical daptomycin composition comprising 500 mg of daptomycin dissolves in 10 mL of 0.9% aqueous sodium chloride in less than 5 minutes at about 25 degrees C.

23. (New) The solid pharmaceutical daptomycin composition of claim 22, wherein the excipient is a sugar.

24. (New) The solid pharmaceutical daptomycin composition of claim 23, wherein the sugar is a non-reducing sugar.

25. (New) The solid pharmaceutical daptomycin composition of claim 23, wherein the molar ratio of daptomycin to the sugar is about 1:1.12 to about 1:21.32.

26. (New) The solid pharmaceutical daptomycin composition of claim 23, wherein the sugar is selected from the group consisting of lactose, maltose, fructose, and dextrose.

27. (New) The solid pharmaceutical daptomycin composition of claim 23, wherein the sugar is selected from the group consisting of trehalose, sucrose, and mannitol.

28. (New) The solid pharmaceutical daptomycin composition of claim 22, wherein the solid pharmaceutical daptomycin composition comprises daptomycin and trehalose.

29. (New) The solid pharmaceutical daptomycin composition of claim 28, wherein the molar ratio of daptomycin to trehalose is about 1:2.13 to about 1:21.32.

30. (New) The solid pharmaceutical daptomycin composition of claim 22, wherein the solid pharmaceutical daptomycin composition comprises daptomycin and sucrose.

31. (New) he solid pharmaceutical daptomycin composition of claim 30, wherein the molar ratio of daptomycin to sucrose is about 1:1.12 to about 1:8.98.

32. (New) The solid pharmaceutical daptomycin composition of claim 22, wherein the solid pharmaceutical daptomycin composition comprises daptomycin and mannitol.

33. (New) The solid pharmaceutical daptomycin composition of claim 32, wherein the molar ratio of daptomycin to mannitol is about 1:2.52 to about 1:5.04.

34. (New) The solid pharmaceutical daptomycin composition of claim 22, wherein the solid pharmaceutical daptomycin composition is obtainable by:

a) forming an aqueous daptomycin solution comprising daptomycin and said excipient at a pH of about 4.5-8.0; and

b) converting the aqueous daptomycin solution to the solid pharmaceutical daptomycin composition.

35. (New) The solid pharmaceutical daptomycin composition of claim 34, wherein said Step a) comprises forming an aqueous daptomycin solution at a pH of about 4.7-7.5.

36. (New) The solid pharmaceutical daptomycin composition of claim 34, wherein said Step a) comprises forming an aqueous daptomycin solution at a pH of about 5.0-7.5.

37. (New) The solid pharmaceutical daptomycin composition of claim 34, wherein said Step a) comprises forming an aqueous daptomycin solution at a pH of about 6.5-7.5.

38. (New) The solid pharmaceutical daptomycin composition of claim 34, wherein said Step a) comprises forming an aqueous daptomycin solution at a pH of about 7.0.

39. (New) The solid pharmaceutical daptomycin composition of claim 34, wherein the aqueous daptomycin solution further comprises a buffering agent.

40. (New) The solid pharmaceutical daptomycin composition of claim 34, wherein Step b) comprises converting the aqueous daptomycin solution to a solid pharmaceutical composition by lyophilization, spray drying or fluid bed drying.

41. (New) The solid pharmaceutical daptomycin composition of claim 22, wherein an amount of the solid pharmaceutical daptomycin composition containing 500 mg of daptomycin dissolves in 10 mL of 0.9% aqueous sodium chloride in less than 2 minutes at about 25 degrees C.

42. (New) A pharmaceutical product comprising the solid pharmaceutical daptomycin composition of claim 22 and a pharmaceutically acceptable diluent.

REMARKS

Prior to examination of this application, please amend the specification as set forth above.

Prior to the entry of the instant amendments, claims 1-21 were pending in this application. *Solely for the purpose of expediting prosecution of the present application,* claims 1-21 have been canceled herein, and new claims 22-42 have been added. Accordingly, upon entry of the instant amendments, claims 22-42 will remain pending in this application.

Support for the new claims can be found throughout the application as filed and the claims as originally filed. For example:

support for new claim 22 can be found at least, for example, on page 7, lines 9-14 and page 8, lines 25-27 of the application as filed;

support for new claim 23 can be found at least, for example, on page 8, lines 25-27 of the application as filed;

support for new claim 24 can be found at least, for example, on page 8, line 32 of the application as filed;

support for new claim 25 can be found at least, for example, on page 9, lines 32-33 of the application as filed;

support for new claims 26-27 can be found at least, for example, on page 15, lines 12-13 of the application as filed;

support for new claims 28-29 can be found at least, for example, on page 10, lines 14-15 of the application as filed;

support for new claims 30-31 can be found at least, for example, on page 9, line 33 to page 10, line 2 of the application as filed;

support for new claims 32-33 can be found at least, for example, on page 10, lines 5-7 of the application as filed;

support for new claim 34 can be found at least, for example, on page 5, lines 25-28 of the application as filed;

support for new claims 35-37 can be found at least, for example, on page 5, lines 25-28 of the application as filed;

support for new claim 38 can be found at least, for example, on page 9, lines 9-12 of the application as filed;

support for new claim 39 can be found at least, for example, on page 8, lines 8-9 of the application as filed;

support for new claim 40 can be found at least, for example, on page 9, lines 23-25 of the application as filed;

support for new claim 41 can be found at least, for example, on page 14, lines 5-10 of the application as filed; and

support for new claim 42 can be found at least, for example, on page 15, lines 1-4 of the application as filed.

Accordingly, *no new matter has been added.*

The foregoing claim amendments have been made solely for the purpose of expediting prosecution of the present application. Applicants reserve the right to pursue the subject matter of the present claims prior to being amended herein in this application or in another application.

Applicants note that these amendments have been made in accordance with a Requirement for Restriction issued on January 23, 2013 for the parent application, U.S. Application Serial No. 13/511,246. The pending claims of the instant application are directed toward the invention of Group 1: a solid daptomycin preparation comprising daptomycin and a material selected from the group consisting of glycine, one or more sugars, and a combination of two or more non-reducing sugars.

CONCLUSION

Entry of the foregoing Preliminary Amendment is in order and requested. If there are any questions regarding the proposed amendment to the application, we invite the Examiner to call Applicants' representative at the telephone number below.

Dated: December 4, 2013

Respectfully submitted,

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UTILITY PATENT APPLICATION TRANSMITTAL

(Only for new nonprovisional applications under 37 CFR 1.53(b))

Attorney Docket No.	552815 (CPT-011USDV)
First Named Inventor	Sandra O'Connor
Title	LIPOPEPTIDE COMPOSITIONS AND RELATED METHODS
Express Mail Label No.	

APPLICATION ELEMENTS

See MPEP chapter 600 concerning utility patent application contents.

1. ☐ **Fee Transmittal Form** (PTO/SB/17 or equivalent)
2. ☐ **Applicant asserts small entity status.** See 37 CFR 1.27
3. ☐ **Applicant certifies micro entity status.** See 37 CFR 1.29. Applicant must attach form PTO/SB/15A or B or equivalent.
4. ☑ **Specification** [*Total Pages* 36] Both the claims and abstract must start on a new page. *(See MPEP § 608.01(a) for information on the preferred arrangement)*
5. ☑ **Drawing(s)** (35 U.S.C. 113) [*Total Sheets* 22]
6. **Inventor's Oath or Declaration** [*Total Pages* ____] *(including substitute statements under 37 CFR 1.64 and assignments serving as an oath or declaration under 37 CFR 1.63(e))*
   a. ☐ Newly executed (original or copy)
   b. ☐ A copy from a prior application (37 CFR 1.63(d))
7. ☑ **Application Data Sheet** ** See note below.* See 37 CFR 1.76 (PTO/AIA/14 or equivalent)
8. **CD-ROM or CD-R** in duplicate, large table, or Computer Program (*Appendix*)
   ☐ Landscape Table on CD
9. **Nucleotide and/or Amino Acid Sequence Submission** (*if applicable, items a. – c. are required*)
   a. ☐ Computer Readable Form (CRF)
   b. ☐ Specification Sequence Listing on:
      i. ☐ CD-ROM or CD-R (2 copies); or
      ii. ☐ Paper
   c. ☐ Statements verifying identity of above copies

ADDRESS TO: **Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450**

ACCOMPANYING APPLICATION PAPERS

10. ☐ **Assignment Papers** (cover sheet & document(s)) Name of Assignee ____
11. ☐ **37 CFR 3.73(c) Statement** (*when there is an assignee*) ☐ **Power of Attorney**
12. ☐ **English Translation Document** (*if applicable*)
13. ☐ **Information Disclosure Statement** (PTO/SB/08 or PTO-1449)
    ☐ Copies of citations attached
14. ☑ **Preliminary Amendment**
15. ☐ **Return Receipt Postcard** (*MPEP § 503*) (*Should be specifically itemized*)
16. ☐ **Certified Copy of Priority Document(s)** (*if foreign priority is claimed*)
17. ☐ **Nonpublication Request** Under 35 U.S.C. 122(b)(2)(B)(i). Applicant must attach form PTO/SB/35 or equivalent.
18. ☐ **Other:** ____

***Note**: (1) Benefit claims under 37 CFR 1.78 and foreign priority claims under 1.55 **must** be included in an Application Data Sheet (ADS).
(2) For applications filed under 35 U.S.C. 111, the application must contain an ADS specifying the applicant if the applicant is an assignee, person to whom the inventor is under an obligation to assign, or person who otherwise shows sufficient proprietary interest in the matter. See 37 CFR 1.46(b).

19. CORRESPONDENCE ADDRESS

☑ The address associated with Customer Number: 113613 **OR** ☐ Correspondence address below

Name					
Address					
City		State		Zip Code	
Country		Telephone		Email	

Signature	/Brian C. Trinque/	Date	December 4, 2013
Name (Print/Type)	Brian C. Trinque, Ph.D., Esq.	Registration No. (Attorney/Agent)	56,593

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6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).
7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (*i.e.*, GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.
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9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

Electronic Patent Application Fee Transmittal

Application Number:	
Filing Date:	
Title of Invention:	LIPOPEPTIDE COMPOSITIONS AND RELATED METHODS
First Named Inventor/Applicant Name:	Sandra O'Connor
Filer:	Brian C. Trinque
Attorney Docket Number:	552815 (CPT-011USDV)

Filed as Large Entity

Utility under 35 USC 111(a) Filing Fees

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Basic Filing:				
Utility application filing	1011	1	280	280
Utility Search Fee	1111	1	600	600
Utility Examination Fee	1311	1	720	720
Pages:				
Claims:				
Miscellaneous-Filing:				
Petition:				
Patent-Appeals-and-Interference:				

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Post-Allowance-and-Post-Issuance:				
Extension-of-Time:				
Miscellaneous:				
			Total in USD ($)	**1600**

Electronic Acknowledgement Receipt

EFS ID:	17551238
Application Number:	14096346
International Application Number:	
Confirmation Number:	2832
Title of Invention:	LIPOPEPTIDE COMPOSITIONS AND RELATED METHODS
First Named Inventor/Applicant Name:	Sandra O'Connor
Customer Number:	113613
Filer:	Brian C. Trinque/Gang Wang
Filer Authorized By:	Brian C. Trinque
Attorney Docket Number:	552815 (CPT-011USDV)
Receipt Date:	04-DEC-2013
Filing Date:	
Time Stamp:	13:53:48
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	yes
Payment Type	Deposit Account
Payment was successfully received in RAM	$1600
RAM confirmation Number	181
Deposit Account	120600
Authorized User	

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File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Specification	552815_Specification_CPT011USDV.pdf	1873839 addf5c483e3ab4932c904221bb964c664617bbae	no	36
Warnings:					
Information:					
2	Claims	552815_Claims_CPT-011USDV.pdf	172267 e7b483175f0938ffd0b7d3fc0ec166f12680ce76	no	4
Warnings:					
Information:					
3	Drawings-only black and white line drawings	552815_Drawings_CPT011USDV.pdf	631671 a43d912019e003f53ce817b04bb5c60284a33f30	no	22
Warnings:					
Information:					
4	Abstract	552815_Abstract_CPT011USDV.pdf	66431 d1e53dfdc1bb30cb0fb61623ffb201822ec24cd3	no	1
Warnings:					
Information:					
5	Application Data Sheet	552815_ADS_CPT011USDV.pdf	1279111 b3190b7ff475f68b382e22824f8d3706662bd2b0	no	7
Warnings:					
Information:					
6	Preliminary Amendment	552815_Preliminary_Amendment_CPT011USDV.pdf	265804 af9983d58f43efb89baf8445fd0b38b4d4597c4f	no	8
Warnings:					
Information:					
7	Transmittal Letter	552815_Transmittal_CPT011USDV.pdf	309711 c289d5964e07096ddb029e9c08cdb19735b9b55a	no	2
Warnings:					
Information:					
8	Fee Worksheet (SB06)	fee-info.pdf	33125 d8de6cd746430d7aa6681fea68a050033dc2cd49	no	2

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Total Files Size (in bytes):	4631959

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Lipopeptide Compositions and Related Methods

Related Applications

This application claims the benefit U.S. provisional patent application 61/263,784, filed on November 23, 2009, which is incorporated herein by reference in its entirety.

Technical Field

The present invention relates to improved lipopeptide compositions for reconstitution in a liquid diluent to form a pharmaceutical composition for parenteral administration, as well as methods of making the solid lipopeptide compositions. Preferred improved lipopeptide compositions include solid daptomycin preparations with increased rates of reconstitution in aqueous liquids and/or increased daptomycin chemical stability.

Background

Daptomycin is a cyclic lipopeptide antibiotic indicated for the treatment of complicated skin and skin structure infections and bacteremia, including bacteremia with suspected or proven infective endocarditis. Daptomycin for injection can be administered intravenously to treat indicated infections caused by susceptible strains of multiple Gram-positive microorganisms including methicillin-resistant *Staphylococcus aureus* (MRSA). Daptomycin for injection (CUBICIN®, Cubist Pharmaceuticals, Inc., Lexington, MA) is supplied as a lyophilized powder that is reconstituted and compounded as a pharmaceutical composition for parenteral administration. The reconstituted daptomycin composition can be compounded as a pharmaceutical composition for parenteral administration, for example by combination with a medically appropriate amount of pharmaceutical diluent (e.g., 0.9% aqueous sodium chloride). The diluent can be the same or different. The parenteral pharmaceutical composition including daptomycin can be administered by intravenous infusion. The lyophilized powder containing daptomycin can take 15-45 minutes to reconstitute in a pharmaceutical diluent, depending on the reconstitution procedure.

Daptomycin (Figure 1) can be derived from the fermentation product of the microorganism *Streptomyces roseosporus* with a feed of n-decanoic acid. Baltz in *Biotechnology of Antibiotics*. 2nd Ed., ed. W. R. Strohl (New York: Marcel Dekker, Inc.), 1997, pp. 415-435. Initial attempts to separate daptomycin from structurally similar components in the fermentation product lead to the identification of other structurally similar

compounds including anhydro-daptomycin (Figure 2), beta-isomer of daptomycin (Figure 3) and a lactone hydrolysis product of daptomycin (Figure 4). Anhydro-daptomycin (Figure 2) can be formed while performing techniques to separate daptomycin from structurally similar components in the fermentation product. Rehydration of the anhydro-succinimido form produces a second degradation product that contains a β-aspartyl group and is designated the β-isomer form of daptomycin (Figure 3). Kirsch et al. (Pharmaceutical Research, 6:387-393, 1989, "Kirsch") disclose anhydro-daptomycin and the beta-isomer of daptomycin produced in the purification of daptomycin. Kirsch described methods to minimize the levels of anhydro-daptomycin and the β-isomer through manipulation of pH conditions and temperature conditions. However, Kirsch was unable to stabilize daptomycin and prevent the conversion of daptomycin to anhydro-daptomycin and its subsequent isomerization to β-isomer. Kirsch was also unable to prevent the degradation of daptomycin into other degradation products unrelated to anhydro-daptomycin and β-isomer.

U.S. Patent No. 6,696,412 discloses several additional compounds present in the fermentation product from which daptomycin is derived, and provides methods for purifying daptomycin with increased purity. The additional compounds include the lactone hydrolysis product of daptomycin, having the chemical structure of Figure 4. The daptomycin purification methods can include forming daptomycin micelles, removing low molecular weight contaminants by filtration, and then converting the daptomycin-containing micelle filtrate to a non-micelle state followed by anion exchange and reverse osmosis diafiltration to obtain the high-purity daptomycin that can then be lyophilized.

One measure of the chemical stability of daptomycin in the lyophilized daptomycin powder is the amount of daptomycin (Figure 1) present in the reconstituted daptomycin composition relative to the amount of structurally similar compounds including anhydro-daptomycin (Figure 2), beta-isomer of daptomycin (Figure 3) and a lactone hydrolysis product of daptomycin (Figure 4). The amount of daptomycin relative to the amount of these structurally similar compounds can be measured by high performance liquid chromatography (HPLC) after reconstitution in an aqueous diluent. The purity of daptomycin and amounts of structurally similar compounds (e.g., Figures 2-4) can be determined from peak areas obtained from HPLC (e.g., according to Example 4 herein) to provide a measure of daptomycin chemical stability in a solid form. The daptomycin purity and chemical stability can also be measured within the liquid reconstituted daptomycin composition over time as a measure of the reconstituted daptomycin chemical stability in a liquid form.

There is a need for solid lipopeptide compositions that rapidly reconstitute (e.g., in less than about 5 minutes) in a pharmaceutical diluent to form reconstituted lipopeptide compositions that can be compounded as pharmaceutical compositions. For example, to reconstitute a 500 mg vial of lyophilized daptomycin for injection (CUBICIN®), the lyophilized powder is combined with 10 mL of 0.9% aqueous sodium chloride, allowed to stand for 10 minutes (or more) and then gently rotated or swirled "a few minutes" to form the reconstituted daptomycin composition prior to formation to prepare a parenteral daptomycin pharmaceutical composition.

There is also a need for solid daptomycin compositions with improved chemical stability in the solid and/or reconstituted form (i.e., higher total percent daptomycin purity over time), providing advantages of longer shelf life, increased tolerance for more varied storage conditions (e.g., higher temperature or humidity) and increased chemical stability after reconstitution as a liquid formulation for parenteral administration.

Summary

The present invention relates to solid lipopeptide compositions for reconstitution in aqueous diluent to form pharmaceutical compositions. The lipopeptide compositions are prepared by converting a pharmaceutically acceptable aqueous solution including the lipopeptide into the solid lipopeptide composition (e.g., by lyophilization, spray drying or the like). The solid lipopeptide composition can be subsequently reconstituted in an aqueous pharmaceutically acceptable diluent to form a pharmaceutical product for parenteral administration.

In a first embodiment, the time for reconstituting the solid lipopeptide compositions in the aqueous diluent can be unexpectedly reduced by increasing the pH of the aqueous lipopeptide solution (preferably to a pH of about 6.5-7.5, most preferably about 7.0) prior to lyophilizing the solution to form the solid lipopeptide composition. For example, solid daptomycin compositions prepared by lyophilizing liquid daptomycin solutions (without a sugar or glycine) at a pH of about 7.0 reconstituted more rapidly in 0.9% aqueous sodium chloride than otherwise comparable daptomycin formulations lyophilized at a pH of about 4.7.

The reconstitution rate of certain solid lipopeptide compositions in aqueous diluent was also accelerated by combining the lipopeptide with glycine or a sugar (preferably, a non-reducing sugar) prior to converting the solution to the solid lipopeptide. For example, 500 mg of the lyophilized pharmaceutical daptomycin compositions in Table 6 formed from

solutions including daptomycin and a non-reducing sugar or glycine at a pH of about 7.0 reconstituted in 0.9% aqueous sodium chloride in less than 2 minutes, with most compositions reconstituting in less than 1 minute.

The solid pharmaceutical lipopeptide preparations can be a product obtained by the following process: (a) forming an aqueous solution of the lipopeptide at a pH above the isoelectric point of the lipopeptide (e.g., above about 3.8 for daptomycin); (b) dissolving glycine or a sugar (preferably a non-reducing sugar) in the aqueous solution with the lipopeptide to form a liquid lipopeptide formulation; (c) adjusting the pH of the liquid lipopeptide formulation to about 6.5 to 7.5; and (d) converting the liquid lipopeptide formulation to the solid pharmaceutical lipopeptide composition (e.g., lyophilization). For example, a lyophilized daptomycin medicament preparation that reconstitutes in less than about 2 minutes in an aqueous 0.9% aqueous sodium chloride diluent can be prepared by: (a) forming an aqueous solution of daptomycin at a pH of about 4.5 – 5.0 (e.g., a pH of about 4.7); (b) adding a buffering agent including phosphate, citrate, malcate or a combination thereof to the aqueous solution of daptomycin to form a buffered daptomycin formulation; (c) dissolving one or more sugars in the buffered daptomycin formulation to form a buffered daptomycin sugar formulation containing about 2.5% w/v to about 25% w/v of the sugar(s) (e.g., about 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 21%, 22%, 23%, or 24%), the sugar(s) being selected from the group consisting of trehalose, sucrose, mannitol, and combinations thereof; (d) adjusting the pH of the buffered daptomycin sugar formulation to a pH of about 6.5 to 7.5 (e.g., 7.0); and (e) lyophilizing the buffered daptomycin sugar formulation to form the solid pharmaceutical daptomycin composition. Preferably, the sugar(s) include sucrose, sucrose and mannitol, or trehalose.

In a second embodiment, the present invention provides daptomycin compositions with improved daptomycin chemical stability, measured as higher total percent daptomycin purity over time (as determined by HPLC according to the method of Example 4). Surprisingly, the daptomycin contained in solid preparations with certain preferred compositions (e.g., daptomycin combined with sucrose or trehalose) was more chemically stable than daptomycin in daptomycin solid preparations without sugar or glycine. The chemical stability of daptomycin in a solid form was measured by comparing total daptomycin purity measurements from multiple solid daptomycin preparations each obtained according to Example 4. Higher chemical stability was measured as higher comparative

daptomycin total purity measurements between two samples according to Example 4. For example, the chemical stability of daptomycin measured from solid daptomycin compositions containing one or more non-reducing sugars such as sucrose was unexpectedly increased by between 10% and greater than 90% during a 6-month storage period prior to reconstitution in 0.9% aqueous sodium chloride (compared to daptomycin chemical stability measured from solid daptomycin compositions without any sugar).

Also surprisingly, higher daptomycin chemical stability was observed for up to 14 days in reconstituted liquid daptomycin solutions at various temperatures in daptomycin preparations containing one or more certain non-reducing sugars (e.g., sucrose) than for comparable daptomycin formulations without sugar or glycine. For example, the chemical stability of the daptomycin in the reconstituted solution over 14 days was also unexpectedly increased for compositions containing daptomycin with certain non-reducing sugars (e.g., sucrose).

Preferred examples of solid pharmaceutical daptomycin preparations include about 2.5% to 25.0% of one or more non-reducing sugars or glycine. Other preferred examples of solid pharmaceutical daptomycin preparations including about 2.5% to 25.0% of a sugar selected from the group consisting of sucrose, mannitol, and trehalose. Particularly preferred solid pharmaceutical daptomycin preparations consist essentially of daptomycin, sucrose, a sodium phosphate buffering agent (e.g., Sodium phosphate dibasic, Na_2HPO_4) and up to about 8% of other materials (e.g., as measured by HPLC peak area at 214 nm according to Example 4).

Solid pharmaceutical daptomycin preparations can be obtained by converting an aqueous solution including daptomycin and a non-reducing sugar (e.g., 15-20% sucrose w/v in the solution) at a pH above the isoelectric point of daptomycin (e.g., a pH of about 3.7 or greater). Preferably, the pH of the aqueous solution containing daptomycin and a non-reducing sugar (e.g., sucrose) is about 4.5 – 8.0 (including, e.g., pH values of 4.5-7.5, 4.7-7.5, 5.0-7.5, 5.5-7.5, 4.7-7.0, 5.0-7.0, 5.5-7.0, 6.0-7.0, and 6.5-7.0 and values therebetween) when converted to the solid pharmaceutical daptomycin preparation (e.g., a powder). Preferably, a lyophilized daptomycin medicament preparation having a reconstitution time of about 2 minutes or less in an aqueous diluent is prepared by: (a) forming an aqueous solution of daptomycin at a pH of about 4.7 – 5.0; (b) adding a buffering agent including phosphate, citrate, TRIS, maleate or a combination thereof to the aqueous solution of daptomycin; (c) dissolving a sugar (e.g., a non-reducing sugar such as sucrose) in the aqueous solution with

daptomycin to form a buffered daptomycin sugar formulation; (d) adjusting the pH of the buffered daptomycin sugar formulation to about 6.5 to 8.0 (including, e.g., pH values of 6.5-7.5, 6.5-7.0, 6.5, 7.0. 7.5, 8.0, 7.0-8.0, 7.0-7.5 and values therebetween); and (e) lyophilizing the buffered daptomycin sugar formulation to form the solid pharmaceutical daptomycin preparation.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, suitable methods and materials are described below. All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety. In case of conflict, the present specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting.

Other features and advantages of the invention will be apparent from the following detailed description, and from the claims.

Brief Description of the Drawings

Figure 1 is the chemical structure of daptomycin.

Figure 2 is the chemical structure of anhydro-daptomycin.

Figure 3 is the chemical structure of the beta-isomer of daptomycin.

Figure 4 is the chemical structure of the lactone hydrolysis product of daptomycin.

Figure 5 is Table 6 listing examples of preferred daptomycin compositions. These compositions were prepared as liquid solutions, then lyophilized to provide solid pharmaceutical daptomycin preparations that reconstitute in an aqueous pharmaceutical diluent within less than 2 minutes (including compositions that reconstitute in less than 1 minute). In Table 6, "Recon time" refers to the time required for about 500 mg the lyophilized daptomycin composition described in the "Formulation (solid state)" column to dissolve in 10 mL of 0.9% aqueous sodium chloride at room temperature (about 25 degrees C).

Figure 6 is Table 7 listing examples of other daptomycin compositions. These compositions were prepared as liquid solutions, then lyophilized to provide solid pharmaceutical lipopeptide preparations that reconstitute in an aqueous pharmaceutical diluent within 2 minutes or more. In Table 7, "Recon time" refers to the time required for

about 500 mg the lyophilized daptomycin solution to dissolve in 10 mL of 0.9% aqueous sodium chloride at room temperature (about 25 degrees C).

Figure 7 is Table 8 listing examples of daptomycin compositions containing a sugar.

Figure 8 is Table 9 showing the percent change in total daptomycin purity measured and calculated for various daptomycin formulations according to Example 4.

Detailed Description

Lipopeptide Compositions with Accelerated Reconstitution

In a first embodiment of the invention, solid pharmaceutical lipopeptide preparations are provided that have a reconstitution time less than 5 minutes in an aqueous pharmaceutical diluent. For example, 500 mg of a solid daptomycin pharmaceutical lipopeptide preparations prepared by lyophilization of a daptomycin solution including glycine or sugar(s) can be dissolved in 10 mL of 0.9% aqueous sodium chloride at room temperature (about 25 degrees C) in 4 minutes or less (including dissolution times of 4, 3, 2, 1 and less than 1 minute).

Unexpectedly, certain solid pharmaceutical lipopeptide preparations obtained from a liquid lipopeptide formulation at a pH of about 7.0 reconstituted in an aqueous pharmaceutical diluent at a faster rate than otherwise identical solid pharmaceutical lipopeptide preparations obtained from a comparable liquid lipopeptide formulation at a lower pH (e.g., 4.7). For example, two aqueous solutions of daptomycin with identical compositions (without a sugar or glycine) at pH values of 4.7 and 7.0 upon lyophilization formed powders that reconstituted in 0.9% aqueous sodium chloride diluent in 5.0 minutes (for pH 4.7) compared to 1.4 minutes (for pH 7.0) (See Table 6 and Table 7). Furthermore, adding glycine or sugars (preferably, one or more non-reducing sugars) to the daptomycin formulation also increased the rate of reconstitution of the resulting solid pharmaceutical lipopeptide preparation.

Solid pharmaceutical lipopeptide preparations having an accelerated reconstitution rate are obtainable from an aqueous solution of the lipopeptide at a suitable pH (e.g., 4.7-7.0) and temperature (e.g., 2-10 degrees C). In general, the solid pharmaceutical lipopeptide preparations can be made from an aqueous solution of the lipopeptide at a pH above the isoelectric point of the lipopeptide. Preferably, the lipopeptide includes daptomycin (Figure 1). Preferred methods for preparing solid pharmaceutical daptomycin preparations are described in Example 2a and 2b. Solid pharmaceutical daptomycin preparations can be prepared from an aqueous solution of daptomycin at a pH above the isoelectric point of

daptomycin (e.g., a pH above about 3.7 or 3.8, including pH values of 4.5, 4.7, and other higher pH values disclosed herein) and at a temperature of 2-10 degrees C. The daptomycin can be obtained in a frozen solution in sterile water for injection (sWFI) at a concentration of 125-130 mg/mL, at pH 3.0 and subsequently pH adjusted to the desired pH by adding sodium hydroxide (e.g., 3.0-10.0 N, including 3.0 N and 10.0 N) at a temperature of about 2-10 degrees C. The pH can be adjusted, for example, by adding sodium hydroxide, hydrochloric acid, phosphoric acid and/or acetic acid.

A buffering agent is optionally added to the aqueous lipopeptide solutions above a pH of about 4.7. Buffering agents can include, for example, agents including phosphate, citrate, maleate, or carbonate moieties, or a combinations thereof, and pharmaceutically appropriate counterions. The amount of the buffering agent can be selected based on the molar ratio of the buffering agent to the daptomycin (e.g., as described in Table 6). The buffering agent can be added in anhydrous or aqueous form. Specific examples of buffering agents are a sodium or potassium salt of phosphoric acid, a sodium or potassium salt of boric acid, a sodium or potassium salt of citric acid, a sodium or potassium salt of carbonic acid, sodium phosphate (e.g., Sodium phosphate dibasic), TRIS (tris(hydroxymethyl)aminomethane and salt of maleic acid. In one aspect the buffering agent is selected from sodium phosphate dibasic (Na_2HPO_4), sodium citrate, sodium bicarbonate, histidine monohydrochloride TRIS and maleate. For aqueous daptomycin solutions, the buffer preferably includes about 50 mM of a phosphate buffering agent (e.g., sodium phosphate dibasic) added to the aqueous daptomycin solution at a pH of about 4.5-6.0 (preferably at a pH of about 5.0). The pH of an acidic aqueous lipopeptide solution (e.g., pH about 3.0) can be raised prior to adding the buffering agent by adding 3N sodium hydroxide under chilled conditions (2-10° C) prior to adding the buffering agent(s).

One or more sugars (e.g., non-reducing sugars) and/or glycine can be added to the aqueous lipopeptide solution prior to converting the solution to the pharmaceutical lipopeptide preparations (e.g., by lyophilization). The amount and manner of combination of the glycine or sugar(s) with the aqueous lipopeptide solution is preferably selected to provide a liquid lipopeptide solution that can be subsequently adjusted to a pH of about 6.5 to 7.5 (e.g., by adding 3N sodium hydroxide at about 2-10 degrees C). For a liquid daptomycin formulation, the glycine and/or one or more sugars is preferably combined by stirring at a suitable temperature (e.g., 2-10 degrees C). The sugar(s) are preferably non-reducing sugars, although the aqueous daptomycin solutions can be prepared with glycine, trehalose, sucrose,

mannitol, lactose, maltose, fructose, dextrose, and combinations thereof at a pH of about 5.0 or higher. The molar ratio of the lipopeptide to the total amount of glycine and/or one or more sugars can be selected to obtain solid compositions with more rapid reconstitution rates in aqueous solvents (such as, e.g., compositions described in Table 6). For example, liquid daptomycin sugar solutions preferably include daptomycin and sucrose in a daptomycin:sucrose molar ratio of from [1.00:1.12] to about [1.00:8.98].

The pH of the lipopeptide solution can be adjusted to about 6.5 – 7.5 after combination of the lipopeptide, sugar(s) or glycine, and buffering agent(s), but prior to converting the liquid lipopeptide solution to the solid pharmaceutical preparation. Preferably, the lipopeptide includes daptomycin, and the liquid daptomycin formulation is adjusted to a pH of about 6.5 – 7.0 and most preferably to a pH of about 7.0 prior to conversion to a solid form, but after addition of the buffering agent(s) and the glycine and/or sugar(s). Figure 5 (Table 6) describes examples of preferred liquid daptomycin compositions that were lyophilized to provide solid pharmaceutical lipopeptide preparations that rapidly reconstitute (dissolve) in an aqueous diluent For each of the compositions containing glycine and a non-reducing sugar in Table 6, 500 mg of the solid daptomycin sugar composition dissolved in 0.9% aqueous sodium chloride in less than 1 minute. In contrast, many of the solid pharmaceutical preparations described in Table 7 (Figure 3) obtained from liquid daptomycin compositions at a pH of about 4.7 had longer reconstitution times than compositions in Table 6 (e.g., 500 mg of the solid pharmaceutical daptomycin compositions described in Table 7 took 2 minutes or more to reconstitute in 10 mL of 0.9% aqueous sodium chloride diluent at 25 degrees C).

The liquid lipopeptide formulation can be converted to the solid pharmaceutical lipopeptide composition by any suitable method, including lyophilization, spray-drying or fluid bed drying. Example 3 describes the lyophilization methods used to convert certain liquid daptomycin formulations in Table 6 to solid pharmaceutical daptomycin preparations prior to measuring the reconstitution times also provided in Table 6. The solid daptomycin compositions can be a lyophilized, freeze-dried, spray-dried, fluid-bed dried, spray congealed, precipitated or crystallized powder or amorphous solid. In one aspect the powder is a lyophilized or spray-dried powder. In another aspect of the invention, the powder is a lyophilized powder.

The molar ratio of daptomycin to the sugar in a solid pharmaceutical daptomycin preparation is preferably in the range of about [1:1.12] to about [1:21.32]. For example, a

solid pharmaceutical daptomycin preparation can include sucrose with a molar ratio of daptomycin to sucrose of about [1:1.12] to about [1:8.98], including daptomycin:sucrose molar ratios of [1:4.49] to [1:8.98], [1:6.73] to [1:8.98], [1:1.12], [1:1.344], [1:1.792], [1:2.24], [1:2.688], [1:3.136], [1:3.584], [1:4.032], [1:4.49], [1:4.928], [1:5.376], [1:5.824], [1:6.272], [1:6.73], [1:7.168], [1:7.616], [1:8.064], [1:8.512], or [1:8.98]. In one aspect the excipient is mannitol and the molar ratio of daptomycin to mannitol is about [1:2.52] to about [1:5.04]. In another aspect the molar ratio of daptomycin to mannitol is [1:2.52], [1:3.36], [1:4.20] or [1:5.04]. In another aspect the excipient is sucrose and the molar ratio of daptomycin to sucrose is about [1:1.12] to about [1:8.98]. In another aspect the molar ratio of daptomycin to sucrose is [1:4.49] to about [1:8.98]. In another aspect the molar ratio of daptomycin to sucrose is about [1:6.73] to about [1:8.98]. In another aspect the molar ratio of daptomycin to sucrose is [1:1.12], [1:1.344], [1:1.792], [1:2.24], [1:2.688], [1:3.136], [1:3.584], [1:4.032], [1:4.49], [1:4.928], [1:5.376], [1:5.824], [1:6.272], [1:6.73], [1:7.168], [1:7.616], [1:8.064], [1:8.512], or [1:8.98]. In another aspect the excipient is trehalose and the daptomycin to trehalose molar ratio is [1:2.13] to about [1:21.32]. In another aspect , the molar ratio of daptomycin to trehalose is [1:2.13], [1:2.556], [1:3.408]. [1:4.26], [1:5.112], [1:5.964], [1:6.816], [1:7.668], [1:8.53], [1:9.372], [1:10.224], [1:11.076], [1:11.928], [1:12.78], [1:13.632], [1:14.484], [1:14.91], [1:15.336], [1:16.188], [1:17.04], [1:17.892], [1:18.744], [1:19.592], [1:20.448], or [1:21.32].

The solid pharmaceutical lipopeptide composition can be reconstituted and combined with one or more pharmaceutically acceptable diluents to obtain a pharmaceutical composition for parenteral administration. The ratio of the daptomycin in the reconstituted liquid composition to diluent is preferably between 25 mg/mL to 200mg/mL. For example, a lyophilized composition including daptomycin can be reconstituted in a vial by adding 0.9% aqueous sodium chloride to the lyophilized composition. The reconstituted daptomycin solution can be combined with medically appropriate diluent and administered intravenously. Pharmaceutically-acceptable diluent include sterile Water for Injection (sWFI), 0.9% sterile sodium chloride injection(sSCl), bacteriostatic water for injection (bWFI), and Ringer's solution. Additional examples of suitable diluent can be found in Remington's Pharmaceutical Sciences, 17th Ed., A.R Gennaro, Ed., Mack Publishing Co., Easton, PA 1985. The diluent can be sterile Water for Injection or sterile sodium chloride injection. Preferred diluent are sWFI or lactated Ringers injection. Preferably, the diluent is not added slowly while rotating at a 45 degree angle. Also preferably, after addition of the diluent, the

vessel containing the daptomycin is not allowed to sit undisturbed for 10 minutes prior to agitation.

Optionally, the diluent further includes a pharmaceutically-acceptable preservative. In one aspect the preservative is benzyl alcohol, chlorobutanol, m-cresol, methylparaben, phenol, phenoxyethanol, propylparaben, thimerosal, phenylmercuric acetate, phenylmercuric nitrate.,benzalkonium chloride, chlorocresol, phenylmercuric salts, and methylhydroxybenzoate.

One reconstitution method includes quickly adding a diluent to a vessel containing a lyophilized daptomycin composition of Table 6, followed by swirling of the vessel if required. The diluent is preferably sWFI or sSCI. For example, the diluent can be added over a period of 1-60 seconds, more preferably 1-30 seconds and most preferably, the diluent is added in less than 20 seconds. Preferably, the weight of daptomycin in the composition to the volume of the diluent is in the range of 25mg/mL to 200 mg/mL

The parenteral pharmaceutical composition compositing daptomycin can be administered by intravenous infusion according to approved indications. For example, daptomycin for injection can be intravenously administered in 0.9% sodium chloride once every 24 hours for 7 to 14 days for the treatment of complicated skin and skin structure infections.

Compositions with Increased Daptomycin Chemical Stability

Unexpectedly, combining daptomycin with one or more non-reducing sugars (e.g., sucrose, trehalose, sucrose and mannitol) in a solid pharmaceutical preparation enhanced the chemical stability of daptomycin in both solid and reconstituted liquid phases. Daptomycin chemical stabilities were measured by comparing measurements of total daptomycin purity from multiple solid samples stored under known time periods (e.g., up to 12 months) under known conditions (e.g., constant temperatures). The daptomycin total purity for each sample was measured by high performance liquid chromatography (HPLC) (using parameters in Table 3) according to Example 4. In addition, the amount of daptomycin (Figure 1) in the reconstituted daptomycin solution was measured relative to the amount of substances selected from the group consisting of the anhydro-daptomycin (Figure 2), the beta-isomer of daptomycin (Figure 3) and the lactone hydrolysis product of daptomycin (Figure 4). Similarly, to determine daptomycin chemical stability in the reconstituted daptomycin solution, the HPLC measurement and calculation of daptomycin purity in the reconstituted

daptomycin solution was repeated according to Example 4 at various time intervals up to 14 days after preparing the reconstituted daptomycin solution.

In one aspect, a solid pharmaceutical daptomycin preparation having increased daptomycin stability can include daptomycin and a non-reducing sugar in an amount effective to increase the total daptomycin stability in the solid daptomycin preparation, as measured by total daptomycin purity according to Example 4. In another aspect, a solid pharmaceutical daptomycin preparation having increased daptomycin stability can include daptomycin and a non-reducing sugar in an amount effective to decrease the amount of substances selected from the group consisting of the anhydro-daptomycin (Figure 2), the beta-isomer of daptomycin (Figure 3) and the lactone hydrolysis product of daptomycin (Figure 4) in the daptomycin preparation (as measured by Example 4) as a solid and/or in a liquid reconstituted form compared to the stability of a daptomycin preparation without glycine or a sugar.

The solid pharmaceutical daptomycin preparation having increased daptomycin stability can include daptomycin and a sugar in an amount effective to increase the chemical stability of daptomycin as measured by changes in total purity of daptomycin in the daptomycin preparation as a solid form compared to a daptomycin preparation without glycine or a sugar, where the daptomycin purity is measured according to Example 4.

As described in Example 5, solid lipopeptide compositions with increased lipopeptide chemical stability include a non-reducing sugar (e.g., such as sucrose or trehalose) or a combination of non-reducing sugars (e.g., sucrose and trehalose). The purity of daptomycin in each solid daptomycin pharmaceutical preparation was measured after reconstitution according to Example 4 (or the reconstituted solution was frozen and the daptomycin purity according the Example 4 was later determined after thawing the reconstituted solution). The solid pharmaceutical daptomycin formulations including non-reducing sugars can have more daptomycin (Figure 1) upon reconstitution relative to substances selected from the group consisting of the anhydro-daptomycin (Figure 2), the beta-isomer of daptomycin (Figure 3) and the lactone hydrolysis product of daptomycin (Figure 4). Preferred solid pharmaceutical daptomycin preparations with a non-reducing sugar have an increased daptomycin purity (and increased shelf stability) for a period of at least up to 6 months compared to solid daptomycin preparations without a non-reducing sugar. As described in Example 5, solid daptomycin preparations were stored in vials for a various time periods (e.g., 1 month, 2 months, 3 months and 6 months) at various temperatures ranges (e.g., 2-8 degrees C, 25

degrees C and 40 degrees C), followed by reconstitution of the solid preparation followed by detection of the amount of daptomycin and substances structurally similar to daptomycin in the reconstituted liquid composition as described in Example 4.

As described in Example 6, daptomycin in reconstituted liquid pharmaceutical daptomycin preparations containing non-reducing sugar(s) unexpectedly showed improved chemical stability than reconstituted daptomycin preparations without any sugar. The increased chemical stability in reconstituted daptomycin formulations containing non-reducing sugars was measured by differences in total daptomycin purity measurements according to Example 4 for up to 14 days on samples stored at temperatures of 5 degrees C, 25 degrees C and 40 degrees C. For example, the purity of daptomycin (measured and calculated according to Example 4) in refrigerated (e.g., 2-10 degrees C) reconstituted daptomycin preparations containing about 15.0-20.0% sucrose was unexpectedly greater over a period of up to 14 days compared to reconstituted daptomycin formulations without any sugar. The reconstituted daptomycin preparations can be combined with one or more pharmaceutically acceptable diluent to obtain a pharmaceutical composition for parenteral administration (e.g., formed or stored in vessels for intravenous administration such as bags or syringes).

To assess daptomycin chemical stability in the reconstituted solution, the purity of daptomycin was measured at multiple time intervals after reconstitution (or thawing if frozen), including time periods of up to 14 days (3, 7 and 14 days). The chemical stability of daptomycin in the reconstituted liquid composition was measured after various durations as described in Example 6, by measuring daptomycin purity according to Example 4. Compositions with increased daptomycin chemical stability had higher detected amounts of daptomycin relative to detected total amounts of the substances structurally similar to daptomycin in Figures 2-4 (as measured by the method of Example 4) than compositions with lower daptomycin chemical stability.

Solid daptomycin preparations with improved chemical stability (as solids and/or in reconstituted liquids) were prepared by combining daptomycin with non-reducing sugars including sucrose and trehalose and combinations of non-reducing sugars, such as sucrose and mannitol.

In some embodiments of the solid and liquid daptomycin preparations include at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97% or at least 98% pure daptomycin as measured by Example 4. Preferably, solid pharmaceutical daptomycin

preparations are characterized in that at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97% or at least 98% of the total HPLC peak area detected at 214 nm according to Table 3 is obtained from daptomycin in a reconstituted form of the solid pharmaceutical daptomycin preparation according to the procedure of Example 4.

In some solid pharmaceutical daptomycin preparations, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97% or at least 98% by weight of the preparation consists of daptomycin, and glycine or one or more non-reducing sugars, where the pharmaceutical daptomycin preparation is characterized in that about 500 mg of the solid pharmaceutical daptomycin preparation dissolves in about 10 mL of an aqueous diluent (e.g., 0.9% aqueous sodium chloride) in less than about 2 minutes.

A preferred solid daptomycin preparation having increased reconstitution and increased daptomycin stability in powder and reconstituted forms includes a solid daptomycin preparation including daptomycin, sucrose, and a phosphate buffering agent; wherein

a. the solid daptomycin preparation includes at least 92% pure daptomycin, as calculated by the ratio of absorbance (area under curve) at 214 nm for the daptomycin divided by the total area under the curve measured by high performance liquid chromatography (HPLC) of the reconstituted daptomycin solution at 214 nm according to Table 3; and
b. the solid daptomycin preparation is obtainable by:
    i. forming an aqueous daptomycin solution including 105 mg/mL (10.5% w/v) daptomycin, a 7.1 mg/mL (50 mM) sodium phosphate dibasic buffering agent and 150 mg/mL (15% w/v) sucrose at a pH of about 7.0; and
    ii. converting the aqueous daptomycin formulation to the solid daptomycin preparation.

Preferred solid daptomycin preparations are obtained from daptomycin solutions including, about 2.5 – 25.0% w/v of one or more non-reducing sugars (e.g., sucrose, trehalose, and mannitol), and optionally further including one or more buffering agents such as sodium phosphate dibasic. Particularly preferred solid daptomycin preparations can be prepared by lyophilizing or spray drying liquid solutions containing daptomycin and sucrose (and optionally further containing about 50 mM sodium phosphate dibasic) at a pH of about 4.5 to 7.0 (including, e.g., pH values of 4.7 - 7.0).

Articles of manufacture containing the solid daptomycin preparation are also provided (e.g., enclosed sealed vials with a means for injecting the aqueous diluent into the vial, such

as a self-sealing puncturable membrane), as well as products containing a daptomycin product formulated for parenteral administration and including the solid daptomycin preparation dissolved in an aqueous diluent (e.g., a bag or syringe adapted for intravenous administration of the daptomycin product).

Preferably, 500 mg of the solid pharmaceutical daptomycin composition dissolves in 10 mL of 0.9% aqueous sodium chloride in 1 minute or less at 25 degrees C. The pH of the aqueous solution of daptomycin can be adjusted to a pH of at least 4.7 prior to dissolving the non-reducing sugar in the aqueous solution with daptomycin. Optionally, the daptomycin preparation is prepared by adding a buffering agent to the aqueous solution of daptomycin before dissolving the non-reducing sugar in the aqueous solution with daptomycin. The liquid daptomycin formulation can have a daptomycin concentration of about 105 mg/mL. The sugar in the liquid daptomycin formulation can be selected from the group consisting of trehalose, sucrose, mannitol, lactose, maltose, fructose, dextrose, and combinations thereof. In one preferred example, 500 mg of the solid pharmaceutical daptomycin composition dissolves in 10 mL of 0.9% aqueous sodium chloride in 1 minute or less at 25 degrees C, and the solid pharmaceutical daptomycin preparation is prepared by:

a. forming an aqueous solution of daptomycin at a pH of about 4.7 – 5.0;
b. adding a buffering agent comprising phosphate, citrate, maleate or a combination thereof to the aqueous solution of daptomycin;
c. dissolving a non-reducing sugar in the aqueous solution with daptomycin to form a buffered daptomycin sugar formulation;
d. adjusting the pH of the buffered daptomycin sugar formulation to about 7.0; and
e. lyophilizing the buffered daptomycin sugar formulation to form the solid pharmaceutical daptomycin composition.

Other examples of solid pharmaceutical daptomycin preparations can be prepared by:

a. forming an aqueous solution of daptomycin at a pH of about 4.7 – 5.0;
b. adding a buffering agent comprising phosphate, citrate, maleate or a combination thereof to the aqueous solution of daptomycin;
c. dissolving a sugar in the aqueous solution with daptomycin to form a daptomycin sugar formulation, the sugar selected from the group consisting of trehalose, sucrose, mannitol, lactose, maltose, fructose, dextrose, and combinations thereof;
d. adjusting the pH of the daptomycin sugar formulation to about 7.0; and

e. lyophilizing the daptomycin sugar formulation to form the solid pharmaceutical daptomycin composition.

Methods of manufacturing a lyophilized daptomycin medicament preparation having an accelerated reconstitution time in an aqueous 0.9% aqueous sodium chloride diluent can include the following steps:

a. forming an aqueous solution of daptomycin at a pH of about 4.7 – 5.0;
b. adding a buffering agent comprising phosphate, citrate, maleate or a combination thereof to the aqueous solution of daptomycin;
c. dissolving a sugar in the aqueous solution with daptomycin to form a buffered daptomycin sugar formulation containing about 2.5% to about 25% of the sugar, the sugar selected from the group consisting of trehalose, sucrose, mannitol, lactose, maltose, fructose, dextrose, and combinations thereof;
d. adjusting the pH of the buffered daptomycin sugar formulation to about 6.5 to 7.5; and
e. lyophilizing the buffered daptomycin sugar formulation to form the solid pharmaceutical daptomycin composition.

Preferably, 500 mg of the lyophilized daptomycin composition dissolves in 10 mL of 0.9% aqueous sodium chloride in 1 minute or less at 25 degrees C. The buffered daptomycin sugar formulation preferably includes a phosphate and about 2.5% to about 25% of the sugar.

Examples

The following examples are illustrative and do not limit the inventions described herein.

Improved daptomycin solid preparations were obtained by (a) forming a solid pharmaceutical preparation from a solution containing daptomycin and one or more sugars or glycine as described in Examples 2a and 2b, and (b) converting the daptomycin solution to a solid pharmaceutical preparation (e.g., by lyophilizing or spray drying), as described in Example 3. The solid pharmaceutical preparation can later be reconstituted by adding an aqueous diluent to dissolve the solid pharmaceutical preparation in about 4 minutes or less. Preferably, the solid pharmaceutical daptomycin preparations dissolve in the aqueous diluent in about 1 minute or less at 25 degrees C (optionally with gentle stirring).

According to the package insert for daptomycin for injection sold under the trademark CUBICIN® (i.e., daptomycin without glycine or a sugar):

> "The contents of a CUBICIN 500 mg vial should be reconstituted using aseptic technique as follows:

Note: To minimize foaming, AVOID vigorous agitation or shaking of the vial during or after reconstitution.
1. Remove the polypropylene flip-off cap from the CUBICIN vial to expose the central portion of the rubber stopper.
2. Slowly transfer 10 mL of 0.9% sodium chloride injection through the center of the rubber stopper into the CUBICIN vial, pointing the transfer needle toward the wall of the vial.
3. Ensure that the entire CUBICIN product is wetted by gently rotating the vial.
4. Allow the product to stand undisturbed for 10 minutes.
5. Gently rotate or swirl the vial contents for a few minutes, as needed, to obtain a completely reconstituted solution."

In contrast, the improved daptomycin solid preparations reconstitute faster in an aqueous diluent than daptomycin without sugar or glycine. Particularly preferred solid preparations can be reconstituted in an aqueous diluent in less than 2 minutes at 25 degrees C, more preferably in less than about 1 minute at 25 degrees C. Table 6 (Figure 5) and Table 5 (Figure 6) provide reconstitution times for various solid daptomycin preparations, obtained by measuring the time required to dissolve 500 mg of the solid daptomycin preparation in 10 mL of a 0.9% aqueous sodium chloride diluent at about 25 degrees C.

In addition, the Examples describe improved daptomycin solid preparations that provide greater daptomycin chemical stability in a solid form as described in Example 5 and in the reconstituted liquid form as described in Example 6. The improved daptomycin preparations can include more daptomycin relative to substances selected from the group consisting of the anhydro-daptomycin (Figure 2), the beta-isomer of daptomycin (Figure 3) and the lactone hydrolysis product of daptomycin (Figure 4), as measured by the HPLC method of Example 4. Preferably, the solid daptomycin preparation is obtained by converting a liquid daptomycin solution to a solid form, subsequently reconstituting the solid form according to Example 4, and measuring a total HPLC peak area at 214 nm according to HPLC parameters in Table 3 in the reconstituted liquid that is at least at least 92% obtained from daptomycin in the reconstituted solution. The solid daptomycin preparation can consist of daptomycin, one or more sugars selected from the group consisting of sucrose, trehalose, and mannitol, pharmaceutically appropriate salts (e.g., sodium chloride), one or more buffering agents such as sodium phosphate dibasic and materials providing up to 8% of the total HPLC peak area at 214 nm according to HPLC parameters in Table 3 in the reconstituted liquid formed according to Example 4.

Table 8 (Figure 7) describes various daptomycin pharmaceutical compositions. In Table 8,

the designation "Molar Ratio of existing components, respectively" refers to the molar ratio of daptomycin to the other components listed as [B], [C] and [D] (when present), in that order. For example, if the composition comprises daptomycin[A] and one excipient [B], the molar ratio will be expressed as [A] :[B]. If the composition comprises two excipients [B] and [C], than the molar ratio will be expressed as daptomycin[A] : excipient[B]: excipient[C] and so on. If the composition comprises daptomycin[A], and excipient[B] and a buffering agent [D], the molar ratio will be expressed as [A]:[B]:[D].

Table 6 (Figure 5) provides non-limiting examples of daptomycin compositions that reconstitute in an aqueous diluent in less than 2 minutes. Table 7 (Figure 6) provides examples of other daptomycin compositions that reconstitute in an aqueous diluent in about 2 minutes or more. Daptomycin compositions without sugar or glycine in Table 6 and Table 7 were obtained by either Method A (Example 1a) or Method B (Example 1b) followed by lyophilization according to Example 3. Daptomycin compositions with sugar or glycine in Table 6 and Table 7 were obtained by either Method A (Example 2a) or Method B (Example 2b) followed by lyophilization according to Example 3. Molar ratios in Tables 6 and 7 were calculated based on molecular weights in Table 1.

Table 1: Molecular Weights of Daptomycin and Excipients

Daptomycin	1620.67
Phosphate buffer	141.96
Sucrose	342.3
Lactose	342.3
Maltose	342.12
Trehalose	180.16
Fructose	180.16
Dextrose	180.16
Mannitol	182.17
Glycine	75.07

The present invention will be further understood by reference to the following non-limiting examples. The following examples are provided for illustrative purposes only and are not to be construed as limiting the scope of the invention in any manner.

Example 1A: *Comparative Preparation Method A (Lyophilize Daptomycin at pH 4.7 without a sugar or glycine)*

Compounding of the comparative daptomycin formulation without sugar or glycine was performed under chilled (2 - 10°C) conditions. Daptomycin Active Pharmaceutical Ingredient (API) was supplied as a frozen liquid at a concentration range of 125 – 130 mg/mL, pH 3.0. Compounding began by obtaining liquid daptomycin API (e.g., thawing of frozen daptomycin API provided at pH of about 3.0) followed by pH adjustment to the target pH of about 4.7 using 3N NaOH. The bulk solution was further diluted to the target concentration of 105 mg/mL with sWFI and mixed to ensure solution homogeneity (also at 2 - 10°C). The bulk product solution was 0.2μm filtered and filled into 10 mL vials followed by lyophilization according to the current lyophilization cycle as outlined in Example 3. The drug product formulation was stoppered under nitrogen and sealed.

Example 1B: *Comparative Preparation Method B (Lyophilize Daptomycin at pH 7.0 without a sugar or glycine))*

Compounding of the bulk formulation was performed under chilled (2 - 10°C) conditions. Daptomycin API was supplied as a frozen liquid at a concentration range of 125 – 130 mg/mL, pH 3.0. Compounding of the bulk formulation utilized thawing of the API followed by pH adjustment to the target pH of 7.0 using 3N NaOH under chilled (2 - 10°C) conditions, followed by dilution to the target concentration of 105 mg/mL with sWFI and mixing to ensure solution homogeneity. Formulated drug product was 0.2μm filtered and filled into 10 mL vials followed by lyophilization according to a modified lyophilization cycle as outlined in Example 3. The drug product formulation was stoppered under nitrogen and sealed.

Example 2A: *Preparation Method A (Lyophilize at pH 4.7)*

Compounding of improved daptomycin formulation was performed under chilled (2 - 10°C) conditions. Daptomycin Active Pharmaceutical Ingredient (API) was supplied as a

frozen liquid at a concentration range of 125 – 130 mg/mL, pH 3.0. Compounding began by obtaining liquid daptomycin API (e.g., thawing of frozen daptomycin API provided at pH of about 3.0) followed by pH adjustment to the target pH of about 4.7 using 3N NaOH, followed by addition of sugar(s) (e.g., sucrose). The bulk solution was further diluted to the target concentration of 105 mg/mL with sWFI and mixed to ensure solution homogeneity (also at 2 - 10°C). The bulk product solution was 0.2μm filtered and filled into 10 mL vials followed by lyophilization according to the current lyophilization cycle as outlined in Example 3. The drug product formulation was stoppered under nitrogen and sealed. The sugars were added as either a powder or in a suitable solution, such as sWFI.

Example 2B: *Preparation Method B (Lyophilize at pH 7.0)*

Compounding of improved daptomycin formulations was performed under chilled (2 - 10°C) conditions. Daptomycin API was supplied as a frozen liquid at a concentration range of 125 – 130 mg/mL, pH 3.0. Compounding of the bulk formulation utilized thawing of the API followed by pH adjustment to the target pH of 4.7 using 3N NaOH under chilled (2 - 10°C) conditions, followed by addition of buffering agents (phosphate, citrate, etc.) with subsequent addition of glycine or sugar(s) (sucrose, trehalose, mannitol). Once the excipients (sugars, buffering agents) were completely dissolved the solution pH of 4.7 was adjusted to 7.0 with 3N NaOH and diluted to the target concentration of 105 mg/mL with sWFI and mixed to ensure solution homogeneity. Formulated drug product was 0.2μm filtered and filled into 10 mL vials followed by lyophilization according to a modified lyophilization cycle as outlined in Example 3. The drug product formulation was stoppered under nitrogen and sealed.

Example 3: *Lyophilization of Compositions Prepared by Methods A and B*

Product vials were loaded into the lyophilizer at 5±4° C and dispersed randomly across each shelf. The composition was lyophilized to dryness, back filled with nitrogen and stoppered under vacuum. Once stoppering was complete, the lyophilization unit was bled to atmospheric pressure, using filtered nitrogen, and the product vials were removed for capping with an aluminum seal. The cycle parameters for the various formulations are summarized in Table 2.

Table 2; Summary of lyophilization cycle parameters for various compositions

Step No.	Cycle A Formulations 1 – 8, 16, 17, 18, 70 - 79	Cycle B Formulations 9 – 11, 13 - 15, 19	Cycle C Formulations 12, 20 – 27	Cycle D Formulations 35, 45, 50 - 69
1	Load product at 5 °C and hold for 60 minutes	Load product at 5° C and hold for 60 minutes	Load product at 5° C and hold for 60 minutes	Load product at 5° C and hold for 60 minutes
2	Ramp shelf to -50° C over 180 minutes and hold for 4 hours	Ramp shelf to -50° C over 180 minutes and hold for 4 hours	Ramp shelf to -50° C over 180 minutes and hold for 4 hours	Ramp shelf to -50° C over 180 minutes and hold for 4 hours
3	Apply vacuum to 90 mTorr and maintain vacuum until stoppering occurs	Apply vacuum to 90 mTorr and maintain vacuum until stoppering occurs	Apply vacuum to 90 mTorr and maintain vacuum until stoppering occurs	Apply vacuum to 90 mTorr and maintain vacuum until stoppering occurs
4	Ramp shelf to -10° C over 6 hours and hold for NLT[1] 40 hours	Ramp shelf to -17° C over 6 hours and hold for NLT 40 hours	Ramp shelf to -25° C over 6 hours and hold for NLT 40 hours	Ramp shelf to -15 °C over 6 hours and hold for NLT 40 hours
5	Ramp shelf to 40° C over 4 hours and hold for 6 hours	Ramp shelf to 40° C over 4 hours and hold for 6 hours	Ramp shelf to 40° C over 4 hours and hold for 6 hours	Ramp shelf to 40° C over 4 hours and hold for 6 hours
6	Backflush chamber with nitrogen	Backflush chamber with nitrogen	Backflush chamber with nitrogen	Backflush chamber with nitrogen
7	Stopper vials at 12.5 psia and break vacuum	Stopper vials at 12.5 psia and break vacuum	Stopper vials at 12.5 psia and break vacuum	Stopper vials at 12.5 psia and break vacuum

[1]NLT = not less than

Example 4. *Measuring the amount of daptomycin and substances structurally similar to daptomycin*

Unless otherwise indicated, the amount of daptomycin and three compounds structurally similar to daptomycin (Figures 2-4) was measured using HPLC analysis in aqueous reconstituted liquid solutions containing daptomycin, using an Agilent 1100 or 1200 high performance liquid chromatography instrument with an ultraviolet (UV) detector. Peak areas were measured using Waters Empower2 FR5 SPF build 2154 software. Unless otherwise indicated, percent purity of a solid daptomycin preparation was determined by reconstituting 500 mg of the solid daptomycin preparation in 10 mL of an aqueous diluent to form a reconstituted daptomycin solution, then measuring the absorbance of the reconstituted

sample at 214 nm by HPLC using the HPLC parameters of Table 3. The percent purity of daptomycin in the solid daptomycin preparation was calculated by the ratio of absorbance (area under curve) at 214 nm for the daptomycin divided by the total area under the curve measured by HPLC of the reconstituted daptomycin solution at 214 nm according to Table 3 and the formula below. For a 92% pure daptomycin sample, 92% of the total peak area from all peaks ≥ 0.05 area % was attributed to dapotmycin.

In addition, the amount of three substances structurally similar to daptomycin can be detected by HPLC at 214 nm according to Table 3: anhydro-daptomycin (Figure 2), the beta-isomer of daptomycin (Figure 3) and the lactone hydrolysis product of daptomycin (Figure 4). Unless otherwise indicated, the amount of these substances in solid daptomycin preparations is measured by HPLC according to Table 3 upon reconstitution of 500 mg of the solid daptomycin preparation in 10 mL of an aqueous diluent to form a reconstituted daptomycin solution, then measuring the absorbance at 214 nm of the reconstituted daptomycin by HPLC using the parameters of Table 3.

Table 3

1. Solvent Delivery System:
   - Mode: Isocratic pumping
   - Flow rate: 1.5 mL/min
   - Run time: 75 minutes
2. Solvent A: 50% acetonitrile in 0.45% $NH_4H_2PO_4$ at pH 3.25
   Solvent B: 20% acetonitrile in 0.45% $NH_4H_2PO_4$ at pH 3.25
   The target condition is approximately 45% Solvent A and 55% Solvent B to retain daptomycin at 36.0 ± 1.5 minutes; however, the solvent ratio may be adjusted to achieve the desired retention time.
3. Autosampler cooler: 5 (2 to 8) ° C
4. Injection volume: 20 µL
5. Column: IB-SIL (Phenomenex), C-8-HC, 5µ, 4.6 mm x 250 mm (or equivalent)
6. Pre-column: IB-SIL (Phenomenex), C-8, 5µ, 4.6 mm x 30 mm (or equivalent)
7. Detection wavelength: 214 nm
8. Column Temperature: 25 (22 to 28) ° C.
9. Integration: A computer system or integrator capable of measuring peak area.

The purity of daptomycin was calculated based on HPLC data, calculated as follows:

- Area % of individual substances structurally similar to daptomycin is calculated using the following equation:

Area % of daptomycin and all substances structurally similar to daptomycin as determined using absorbance at 214nm

Calculate the area of daptomycin and all other peaks ≥ 0.05 area %,

$$\% \text{ area} = (A_i/A_{tot})_x \, 100\%$$

where:

% area = Area % of an individual peak;

A_i = Peak of an individual peak; and

A_{tot} = total sample peak area including daptomycin.

- Area% of total substances structurally similar to daptomycin is calculated using the following equation:

Area% of total substances structurally similar to daptomycin equals the sum of all reported area % values from the individual substances (sum of all impurities =/> 0.05%)

- *Calculate the% purity of daptomycin in Area% using the following equation:

% Daptomycin = 100% - % total substances structurally similar to daptomycin.

Example 5. *Measuring the Chemical stability of Daptomycin in Solid Pharmaceutical Compositions*

This example shows increased daptomycin chemical stability of solid pharmaceutical daptomycin compositions in certain preferred compositions containing sucrose, mannitol, trehalose, and glycine compared to daptomycin compositions without sugar or glycine and daptomycin compositions with certain reducing sugars.

The chemical stability of various solid pharmaceutical daptomycin compositions was evaluated by placing the composition in vials at various temperatures (2-8 deg. C, 25 deg. C

and 40 deg. C). The solid pharmaceutical daptomycin compositions were obtained by lyophilizing or spray drying liquid compositions prepared according to Example 2a (Method A, at pH 4.7) or Example 2b (Method B, at pH 7.0). Lyophilization was performed according to Example 3. The amount of daptomycin and three daptomycin-related impurities was measured using the HPLC method of Example 4 in reconstituted solutions formed by dissolving about 500 mg of solid daptomycin preparations in 10 mL of 0.9% aqueous sodium chloride. The total daptomycin purity calculated according to Example 4 was plotted for measurements at 0, 1, 2, 3 and 6 months for vials of various solid pharmaceutical daptomycin compositions maintained at 40 deg. C. The slope of linear regression best fit to the plot of total daptomycin purity per month was calculated for each solid pharmaceutical daptomycin formulation (slope in % total daptomycin purity/month).

The data in Table 4 shows the ratio of the slopes for each solid daptomycin preparation normalized to the slope obtained from reconstituted solid daptomycin for injection, which does not contain sucrose. Referring to Table 4, ratios under column A were obtained from solid preparations prepared according the Method A in Example 2a (i.e., obtained from solutions containing daptomycin at a pH of 4.7), while ratios under column B were obtained from solid preparations prepared according to the Method B in Example 2b (i.e., obtained from solutions containing daptomycin at a pH of 7.0 that further contain 50 mM of a sodium phosphate buffering agent). Ratios with a "*" were from solid daptomycin preparations originally converted into solids by spray drying; all other samples were obtained from solid daptomycin preparations originally converted into solids by lyophilization (Example 3). Entries with "NT" in Table 4 were not tested. All ratios in Table 4 were obtained from linear regression of measurements of total purity of daptomycin (Figure 1) relative to substances structurally similar to daptomycin shown in Figures 2-4 at 0 (i.e., after formation of the solid material), 1 month, 2 months, 3 months and 6 months of storage at 40 deg. C, where the amount of daptomycin and substances structurally similar to daptomycin were detected and calculated according to Example 4. The ratios in Table 4 represent changes in the rate of daptomycin total purity relative to daptomycin for injection (normalized to 1.00 for Method A and Method B preparations). Ratios below 1.00 represent reduced rates in the reduction of daptomycin total purity, or increased chemical stability of the daptomycin in a formulation relative to the daptomycin chemical stability absent sucrose in the daptomycin for injection product. Accordingly, the lower the ratio in Table 4, the more

stable the daptomycin in the corresponding formulation in relation to the substances structurally similar to daptomycin in Figures 2-4.

TABLE 4: Ratio of % Change in Daptomycin Total Purity per Month Relative to Daptomycin for Injection (6 months)

Formulation (% w/v in solution prior to lyophilization or spray drying)	Synthesis Method Ex 2A	Synthesis Method Ex 2B
15.0% Sucrose	0.16	0.04
15.0% Sucrose*	NT	0.04
15.0% Sucrose	NT	0.10
5.0% Sucrose + 3.0% Mannitol	0.48	0.10
10.0% Sucrose + 3.0% Mannitol	0.22	0.13
20.0% Sucrose	0.22	0.13
10.0% Sucrose	0.21	0.15
5.0% Sucrose + 6.0% Mannitol	0.45	0.16
2.5% Sucrose + 3.0% Mannitol	0.60	0.17
2.5% Sucrose + 6.0% Mannitol	0.56	0.18
10.0% Sucrose + 6.0% Mannitol	0.24	0.20
25.0% Trehalose	0.41	0.22
10.0% Trehalose	0.47	0.26
6.0% Mannitol	0.95	0.27
5.0% Sucrose	0.35	0.27
2.5% Sucrose	0.61	0.32
5.0% Trehalose	0.67	0.35
2.5% Trehalose	NT	0.42
5% Glycine	0.97	0.74
Daptomycin (No Sugar or Glycine)	**1.00**	**1.00**
20 % Lactose	2.02	1.01
2.5% Lactose	2.85	1.19
2.5% Maltose	2.73	1.28
5% Maltose	2.29	1.37
5% Lactose	2.44	1.41
2.5% Fructose	NT	1.41
5 % Fructose	NT	1.57
5% Dextrose:Fructose	7.03	2.66
2.5% Dextrose:Fructose	8.11	2.69
5% Dextrose	8.08	3.38
2.5% Dextrose	9.90	3.51

15.0% Sucrose + 3.0% Mannitol	0.14	NT
15.0% Sucrose + 6.0% Mannitol	0.25	NT
17.5% Trehalose	0.31	NT

NT = not tested

*= prepared by spray drying, not lyophilization

The data in Table 4 show that daptomycin in a solid pharmaceutical daptomycin composition containing 15.0% sucrose showed about a 84% increase in daptomycin chemical stability compared to the daptomycin for injection in formulations prepared according to Method A (Example 2a), and a 96% increase in daptomycin chemical stability compared to the daptomycin for injection in formulations prepared according to Method B (Example 2b). Similarly, the solid pharmaceutical daptomycin containing 20.0% sucrose showed increases in daptomycin chemical stability relative to daptomycin without sucrose (i.e., daptomycin for injection) of about 78% (Method A) and 87% (Method B). Thus, combining 15-20% sucrose to a lyophilized daptomycin composition increased daptomycin chemical stability by at least 78%, and as much as 96%. In contrast, Table 4 also shows that daptomycin was about 2-9 times less stable in formulations comprising daptomycin and lactose, maltose, fructose, and/or dextrose. Table 4 therefore shows that daptomycin prepared by Methods of Example 2a and 2b (Methods A and B respectively) was stabilized when combined with non-reducing sugars or glycine (relative to daptomycin without a sugar or glycine), while daptomycin was less stable in formulations containing reducing sugars.

Figure 8 is Table 9 showing the percent change in total daptomycin purity measured and calculated for various daptomycin formulations according to Example 4. Recitation of "PO4" in Table 9 refers to formulations that contain sodium phosphate dibasic buffer agent. Recitation of a "pH" value in Table 9 refers to the pH at which the formulation was compounded (i.e., the pH of the daptomycin formulation solution that was lyophilized to form the solid daptomycin formulations that were tested to obtain the data in Table 9). NT = not tested.

To obtain the data in Table 9, each solid daptomycin formulation was maintained at 40 degrees C for various time periods (1, 2, 3, or 6 months), before reconstituting the solid daptomycin formulation and measuring the daptomycin purity according to the method of Example 4.

Table 9 shows the Daptomycin Stability Ratio, calculated as follows:

1. Prepare a control sample (daptomycin for injection commercial product, without sugar or glycine) compounded according to Example 1b and measure according to Example 4 the total percent daptomycin purity for the control sample after formulation
2. Measure the total percent daptomycin purity for a control sample according to Example 4 after storing the control sample for a given time period at 40 degrees C and subtract the total percent daptomycin purity after storage for that time period from the total daptomycin purity after formulation to provide a Total Control Percent Purity Loss;
3. Measure the total percent daptomycin purity of each formulation according to Example 4 after storing the formulation for a time period at 40 degrees C (e.g., 1 month, 2 months, etc.) and subtract the total percent purity after storage for that time period from the total daptomycin purity of the control sample after formulation to provide a Total Formulation Daptomycin Percent Purity Loss;
4. Calculate the Daptomycin Stability Ratio at 40 degrees C by dividing Total Formulation Daptomycin Percent Purity Loss obtained for each formulation after the same storage time period (from step 3) by the Total Control Percent Purity Loss (from step 2) after a given storage time period:

$$\text{Daptomycin Stability Ratio} = \frac{\text{Total Formulation Daptomycin Percent Purity Loss Measured by Step 3}}{\text{Total Control Daptomycin Percent Purity Loss Measured by Step 2}}$$

Steps 2-4 are repeated to calculate each Daptomycin Stability Ratio. The Daptomycin Stability Ratio is calculated with a separate control sample that has been stored for the same time period as the formulation. For example, Daptomycin Stability Ratio values calculated for a formulation after 1 month storage time at 40 degrees C were obtained by dividing the value from step 3 for the formulation by the value obtained from step 2 for a control that was stored for 1 month at 40 degrees C (i.e., the same storage period and storage conditions as the formulation analyzed in step 3). Similarly, Daptomycin Stability Ratio values at 2 months would be calculated with a control sample that was stored for 2 months under the same conditions as the formulation used in step 3.

Daptomycin Stability Ratio values less than 1.000 in Table 9 indicate that the corresponding formulation has a higher daptomycin chemical stability measured as a greater total daptomycin percent purity (measured by Example 4) in the sample formulation than in the control sample of daptomycin without sugar or glycine (compounded according to step 1

above) after the corresponding storage period at 40 degrees C. Preferred compositions have Daptomycin Stability Ratios of less than 0.800, more preferably less than 0.500, and most preferably Daptomycin Stability Ratios of less than 0.300.

The data in Table 9 shows that daptomycin was generally more chemically stable (as measured by daptomycin improved purity according to Example 4 upon reconstitution in aqueous diluent) for daptomycin compositions containing a non-reducing sugar compounded at pH 7.0 with a buffering agent than for daptomycin without a sugar. Notably, the formulations comprising 15% sucrose compounded according to Method A (Example 2a) or Method B (Example 2b) provided very high levels of daptomycin chemical stability among the samples tested, and significantly higher levels of daptomycin chemical stability over 12 months than observed for daptomycin of comparative formulation 0 without a sugar or glycine. The sucrose-mannitol formulations also provided improvement in daptomycin chemical stability over the daptomycin comparative formulation 0 without sugar or glycine. For example the 10% sucrose/3% mannitol, 10% sucrose/6% mannitol, and 15% sucrose/6% mannitol all compounded according to Method A (Example 2a) provided significantly improved daptomycin stability. , in contrast to the 15% sucrose/6% mannitol formulations compounded according to Method A (Example 2a). The 5% glycine formulation prepared according the Method B (Example 2b) also provided significant daptomycin stabilization, while the corresponding 5% glycine preparation from Method A (Example 2a) was less stable than daptomycin without sugar or glycine (Formulation 0). All daptomycin formulations in Table 9 containing sucrose showed increased daptomycin chemical stability compared to daptomycin without a sugar or glycine in the comparator formulation 0 (as measured by Example 4).

Example 6. *Measuring the Chemical stability of Daptomycin in Liquid Reconstituted Pharmaceutical Compositions*

This example shows increased daptomycin chemical stability in reconstituted pharmaceutical daptomycin compositions in compositions containing sucrose compared to comparable compositions without sucrose.

The chemical stability of various liquid pharmaceutical daptomycin compositions was evaluated by placing the composition in vials at various temperatures (5 deg. C, and 40 deg. C). The liquid reconstituted pharmaceutical daptomycin compositions were obtained by reconstituting about 500 mg of solid daptomycin preparations in 10 mL of sWFI. Each solid

daptomycin preparation was obtained by lyophilizing or spray drying liquid compositions prepared according to Example 1 (Method A, at pH 4.7) or Example 2 (Method B, at pH 7.0). Lyophilization was performed according to Example 3. The amount of daptomycin and daptomycin-related impurities was measured using the HPLC method of Example 4 in reconstituted solutions formed by dissolving. The % daptomycin was measured and calculated according to Example 4 for measurements at 0, 3, 7 and 14 days for vials of various solid pharmaceutical daptomycin compositions maintained at 5 deg. C or 40 deg. C.

The data in Table 5 shows the amount of % daptomycin at each measurement normalized to the % daptomycin obtained from reconstituted solid daptomycin for injection, which does not contain sucrose. Referring to Table 5, each sample was reconstituted from a solid pharmaceutical daptomycin composition prepared by Method A in Example 1 (i.e., obtained from solutions containing daptomycin at a pH of 4.7) or Method B in Example 2 (i.e., obtained from solutions containing daptomycin at a pH of 7.0 that further contain 50 mM of a sodium phosphate buffering agent), as indicated in the "Method" column. The temperature in degrees C of the reconstituted liquid is indicated under "Temp (deg C)." Numbers below 1.000 in Table 5 indicate a lower % daptomycin purity than daptomycin for injection at 0 days for a given temperature. All entries are normalized to the measurement for daptomycin for injection at the corresponding temperature (e.g., all measurements taken at 5 degrees C are normalized to the % daptomycin measured for daptomycin for injection at 5 degrees C). Accordingly, the closer the number in Table 5 is to 1.000, the more stable the daptomycin is in the reconstituted liquid form in the corresponding formulation in relation to the substances structurally similar to daptomycin in Figures 2-4.

TABLE 5: % Daptomycin Measured In Reconstituted Solution

	Method	Temp (deg C)	0	3 days	7 days	14 days
Daptomycin for Injection	A	5	1.0000	0.9957	0.9900	0.9822
15.0% Sucrose	B	5	0.9998	1.0003	0.9974	0.9977
6.0% Mannitol	B	5	1.0003	0.9998	0.9992	0.9974
Daptomycin for Injection	A	25	1.0000	0.9394	0.8618	0.7410
15.0% Sucrose	B	25	0.9998	0.9844	0.9609	0.9184
6.0% Mannitol	B	25	1.0003	0.9846	0.9609	0.9196
Daptomycin for Injection	A	40	1.0000	0.6711	0.4145	NT
15.0% Sucrose	B	40	0.9998	0.8752	0.7241	NT
6.0% Mannitol	B	40	0.9996	0.8753	0.7207	NT

NT = not tested

The data in Table 5 shows that the total % daptomycin in a liquid reconstituted pharmaceutical daptomycin composition containing 15.0% sucrose was significantly more stable than daptomycin for injection (without sucrose) at 25 degrees C after 14 days (0.9184 for the sucrose formulation compared to 0.7410 for the daptomycin for injection formulation without sucrose). This represents about a 23% increase in daptomycin chemical stability in the reconstituted solution in the presence of the reconstituted composition consisting essentially of daptomycin, about 15% sucrose, and 50 mM sodium phosphate. Accordingly, the 15.0% sucrose formulation of daptomycin demonstrated a surprisingly enhanced room temperature daptomycin chemical stability and improved shelf life after reconstitution.

Additional Exemplary Embodiments

Some specific embodiments of the invention supported by the examples include the following:

1. A solid pharmaceutical composition comprising daptomycin and glycine or a non-reducing sugar, wherein the composition has an increased rate of reconstitution, an increased rate of reconstitution being characterized by a dissolution of 500 mg of the composition in 10 mL of 0.9% aqueous sodium chloride under gentle swirling at 25 degrees C in 5 minutes or less, in particular less than 2 minutes or less than 1 minute.

2. Pharmaceutical composition of specific embodiment 1 wherein the composition has increased reconstitution chemical stability in comparison to lyophilized daptomycin, reconstitution taking place in 0.9% aqueous sodium chloride at 25 degrees C, wherein increased reconstitution chemical stability is characterized by an amount of daptomycin relative to the anhydro-daptomycin (Figure 2), the beta-isomer of daptomycin (Figure 3) and/or the lactone hydrolysis product of daptomycin (Figure 4) that is higher than the corresponding amount for lyophilized daptomycin.

3. Pharmaceutical composition according to any of specific embodiments 1 to 2 wherein the composition has increased storage chemical stability in comparison to lyophilized daptomycin, wherein the increased storage chemical stability is characterized by an amount of daptomycin relative to the anhydro-daptomycin (Figure 2), the beta-isomer of daptomycin (Figure 3) and/or the lactone hydrolysis product of daptomycin (Figure 4) which is higher

than the corresponding amount for lyophilized daptomycin when reconstituting both samples in 0.9% aqueous sodium chloride after storage of the compositions for at least 3 months at 40°C under a nitrogen atmosphere.

4. Pharmaceutical composition according to any of specific embodiments 1 to 4 wherein the composition is produced by a process comprising:
a. forming an aqueous daptomycin solution comprising daptomycin, a buffering agent, and a non-reducing sugar or mixtures thereof; or glycine and adjusting the pH to about 5 to 8, in particular 6.5 to 7.5 or about 7, and
b. converting the aqueous daptomycin solution to the solid composition, in particular by lyophilization.

5. Pharmaceutical composition according to any of specific embodiments 1 to 5 wherein the composition comprises a non-reducing sugar or mixtures thereof, in an amount effective for decreasing the rate of daptomycin degradation in comparison to a substantially identical composition lacking said non-reducing sugar, wherein the rate of degradations are defined as the respective loss of daptomycin after storage of the compositions for at least 3 months at 40°C under a nitrogen atmosphere.

6. Pharmaceutical composition according to any of specific embodiments 1 to 6 wherein the sugar is selected from non-reducing disaccharides, sugars that are substantially amorphous upon lyophilization, sucrose, dextranes, trehalose, mannitol, sorbitol or combinations thereof.

7. Pharmaceutical composition according to any of specific embodiments 1 to 7 wherein the sugar or glycine is used in amounts of about 1 to 40 wt.-%, in particular about 5-20 wt.-% or about 15 wt.-%, on basis of the weight of the total composition.

8. Liquid pharmaceutical composition comprising daptomycin and a sugar selected from sucrose, trehalose, mannitol or mixtures thereof, in an amount effective for decreasing the rate of daptomycin degradation in comparison to a solution obtained by reconstituting lyophilized daptomycin in 0.9% aqueous sodium chloride, wherein the rate of degradations are defined as the respective loss of daptomycin after storage of the liquid compositions for at least 7 days at 25 degrees C.

9. Method for preparing a composition according to any one of specific embodiments 1 to 8 comprising:

a. supplying a daptomycin preparation;

b. adding at least one excipient, optionally selected from sorbitol, mannitol, sucrose, glycine, trehalose, lactose, maltose, fructose and dextrose;

c. optionally adding a pH adjuster to obtain the desired pH;

d. optionally diluting the solution of step c with sWFI;

e. optionally filtering the solution of step d; and

f. converting the composition to a powdered form.

10. A solid pharmaceutical composition comprising daptomycin and glycine or a non-reducing sugar, wherein the composition has an increased rate of reconstitution, an increased rate of reconstitution being characterized by a dissolution of 500 mg of the composition in 10 mL of 0.9% aqueous sodium chloride under gentle swirling at 25 degrees C in 5 minutes or less, in particular less than 2 minutes or less than 1 minute; and where the solid pharmaceutical composition is further characterized in that the daptomycin preparation has a lower amount of one or more substances selected from the group consisting of anhydro-daptomycin (Figure 2), beta-isomer of daptomycin (Figure 3) and a lactone hydrolysis product of daptomycin (Figure 4) after storage for 1 month at 40 degrees C under nitrogen, compared to a solid pharmaceutical daptomycin preparation obtained by lyophilizing daptomycin and daptomycin-related compounds in 0.9% aqueous sodium chloride diluent, where the amount of the substances is detected by HPLC at 214 nm according to the method of Example 4.

Any of the specific embodiments 1-10 can pertain to a solid daptomycin preparation having a Daptomycin Stability Ratio of less than 1.000, less than 0.900, less than 0.800, less than 0.700, less than 0.600, less than 0.500, less than 0.400, less than 0.300, less than 0.200 or less than 0.100, where the Daptomycin Stability Ratio is calculated at 40 degrees C according to Example 5.

Other compositions include a powder, pharmaceutical composition comprising daptomycin and at least one excipient selected from sorbitol, mannitol, sucrose, glycine, trehalose, lactose, maltose, fructose and dextrose.

The composition of claim 1 comprising:

a. 500 mg daptomycin;
b. 714.3 mg sucrose; and
c. 35.5 mg sodium phosphate dibasic

wherein the composition is compounded at a pH of about 7.

The composition of claim 1 comprising:

a. 500 mg daptomycin;
b. 476.2 mg sucrose;
c. 142.9 mg mannitol; and
d. 35.5 mg sodium phosphate dibasic

wherein the composition is compounded at a pH of about 7.

The composition of claim 1 comprising:

a. 500 mg daptomycin;
b. 476.2 mg sucrose;
c. 285.8 mg mannitol; and
d. 35.5 mg sodium phosphate dibasic

wherein the composition is compounded at a pH of about 7.

In some solid pharmaceutical daptomycin preparations, at least at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97% or at least 98% by weight of the preparation (e.g., measured upon reconstitution as weight by volume by HPLC according to Example 4) consists of daptomycin and sucrose, where the pharmaceutical daptomycin preparation is characterized in that about 500 mg of the solid pharmaceutical daptomycin preparation dissolves in about 10 mL of an aqueous diluent (e.g., 0.9% aqueous sodium chloride) in less than about 1 minute at a pH of less than 7.0. In some solid pharmaceutical daptomycin preparations, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97% or at least 98% by weight of the preparation (e.g., measured upon reconstitution as weight by volume by HPLC according to Example 4) consists of daptomycin, sucrose and a sodium phosphate buffering agent, where the pharmaceutical daptomycin preparation is characterized in that about 500 mg of the solid pharmaceutical daptomycin preparation dissolves in about 10 mL of an aqueous diluent (e.g., 0.9% aqueous sodium chloride) in less than about 1 minute at a pH of about 7.0. In one solid pharmaceutical daptomycin preparation, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97% or at least 98% by weight of the preparation (e.g., measured upon reconstitution as weight by volume by HPLC according to Example 4) consists of daptomycin, sucrose and a buffering

agent, where the pharmaceutical daptomycin preparation is characterized in that about 500 mg of the solid pharmaceutical daptomycin preparation dissolves in about 10 mL of an aqueous diluent (e.g., 0.9% aqueous sodium chloride) in less than about 1 minute at a pH of about 7.0, and the daptomycin preparation is obtained by converting a daptomycin solution comprising 15-20% w/v sucrose to the daptomycin preparation (e.g., by lyophilization or spray drying). In one solid pharmaceutical daptomycin preparation, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97% or at least 98% by weight of the preparation (e.g., measured upon reconstitution as weight by volume by HPLC according to Example 4) consists of daptomycin, sucrose and sodium phosphate dibasic, where the pharmaceutical daptomycin preparation is characterized in that about 500 mg of the solid pharmaceutical daptomycin preparation dissolves in about 10 mL of an aqueous diluent (e.g., 0.9% aqueous sodium chloride) in less than about 1 minute at a pH of about 7.0, and the daptomycin preparation is obtained by converting a daptomycin solution comprising 15-20% w/v sucrose and 50 mM sodium phosphate dibasic to the daptomycin preparation (e.g., by lyophilization or spray drying).

In some solid pharmaceutical daptomycin preparations, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97% or at least 98% of the HPLC peak area detected at 214 nm (measured upon reconstitution as weight by volume by HPLC according to Example 4) is provided by daptomycin, and the composition consists of daptomycin, other materials detected at 214 nm by HPLC according to Example 3, glycine or one or more sugars, and a sodium phosphate buffering agent, where the pharmaceutical daptomycin preparation is characterized in that about 500 mg of the solid pharmaceutical daptomycin preparation dissolves in about 10 mL of an aqueous diluent (e.g., 0.9% aqueous sodium chloride) in less than about 1 minute at a pH of about 7.0.

In some solid pharmaceutical daptomycin preparations, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97% or at least 98% by weight of the preparation (e.g., measured upon reconstitution as weight by volume by HPLC according to Example 4) consists of daptomycin and trehalose, where the pharmaceutical daptomycin preparation is characterized in that about 500 mg of the solid pharmaceutical daptomycin preparation dissolves in about 10 mL of an aqueous diluent (e.g., 0.9% aqueous sodium chloride) in less than about 1 minute at a pH of less than 7.0. In some solid pharmaceutical daptomycin preparations, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97% or at least 98% by weight of the preparation (e.g., measured upon reconstitution

as weight by volume by HPLC according to Example 4) consists of daptomycin, trehalose and a sodium phosphate buffering agent, where the pharmaceutical daptomycin preparation is characterized in that about 500 mg of the solid pharmaceutical daptomycin preparation dissolves in about 10 mL of an aqueous diluent (e.g., 0.9% aqueous sodium chloride) in less than about 1 minute at a pH of about 7.0.

In some solid pharmaceutical daptomycin preparations, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97% or at least 98% by weight of the preparation (e.g., measured upon reconstitution as weight by volume by HPLC according to Example 4) consists of daptomycin and glycine, where the pharmaceutical daptomycin preparation is characterized in that about 500 mg of the solid pharmaceutical daptomycin preparation dissolves in about 10 mL of an aqueous diluent (e.g., 0.9% aqueous sodium chloride) in less than about 1 minute at a pH of less than 7.0.

In some solid pharmaceutical daptomycin preparations, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97% or at least 98% by weight of the preparation consists of daptomycin, mannitol, and sucrose, where the pharmaceutical daptomycin preparation is characterized in that about 500 mg of the solid pharmaceutical daptomycin preparation dissolves in about 10 mL of an aqueous diluent (e.g., 0.9% aqueous sodium chloride) in less than about 1 minute at a pH of less than 7.0. In some solid pharmaceutical daptomycin preparations, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97% or at least 98% by weight of the preparation consists of daptomycin, mannitol, sucrose and a sodium phosphate buffering agent, where the pharmaceutical daptomycin preparation is characterized in that about 500 mg of the solid pharmaceutical daptomycin preparation dissolves in about 10 mL of an aqueous diluent (e.g., 0.9% aqueous sodium chloride) in less than about 1 minute at a pH of about 7.0.

Methods of making a daptomycin pharmaceutical composition for parenteral administration are also provided. The method can include reconstituting a solid daptomycin preparation comprising a non-reducing sugar or glycine in a pharmaceutically acceptable diluent to form the composition for parenteral administration.

The compositions of the present invention can be made by a variety of methods. In one aspect, the compositions are made by:

a. supplying a daptomycin preparation

b. adding at least one excipient selected from sorbitol, mannitol, sucrose, glycine, trehalose, lactose, maltose, fructose and dextrose;

c. adding a pH adjuster to obtain the desired pH
d. diluting the solution of step c with sWFI
e. filtering the solution of step d; and
f. converting the composition to a powdered form.

In another aspect of the invention is provided a method for preparing compositions of claim 1 that are compounded with a buffer, for example at pH 7 This process comprises the steps of

a. supplying a daptomycin preparation
b. adding a pH adjuster to obtain a solution of about pH 4.7-6.0;
c. adding a buffering agent;
d. adding at least one excipient selected from sorbitol, mannitol, sucrose, glycine, trehalose, lactose, maltose, fructose and dextrose;
e. adding a pH adjuster to obtain a pH of about 7.0
f. diluting the bulk solution with sWFI
g. filtering the solution of step f; and
h. converting the composition to a powder form to obtain the solid daptomycin composition.

In another aspect of the invention is provided a method for preparing compositions of claim 1 that are compounded with a buffer, for example at pH 7. This process comprises the steps of

a. supplying a daptomycin preparation
b. adding a pH adjuster to obtain a solution of about pH 4.7-6.0;
c. adding a buffering agent;
d. adding at least one excipient selected from sorbitol, mannitol, sucrose, glycine, trehalose, lactose, maltose, fructose and dextrose;
e. adding a pH adjuster to obtain a pH of about 7.0
f. diluting the bulk solution with sWFI
g. filtering the solution of step f; and
h. converting the composition to a powder form to obtain the composition of claim 1.

A number of other embodiments of the invention have been described. Nevertheless, it will be understood that various modifications may be made without departing from the spirit and scope of the invention. Accordingly, other embodiments are within the scope of the following claims.

CLAIMS

1. A solid daptomycin preparation having improved reconstitution and increased daptomycin stability in powder and reconstituted forms compared to a daptomycin preparation compounded according to Example 1a, the solid daptomycin preparation comprising daptomycin, sucrose, and a phosphate buffering agent; wherein
   a. the solid daptomycin preparation is at least 92% pure daptomycin, as calculated by ratio of absorbance (area under curve) for the daptomycin divided by total area under a curve measured by high performance liquid chromatography (HPLC) of the reconstituted daptomycin solution at 214 nm according to Table 3 and Example 4; and
   b. the daptomycin preparation is obtainable by:
      i. forming an aqueous daptomycin solution comprising 105 mg/mL (10.5% w/v) daptomycin, a 7.1 mg/mL (50 mM) sodium phosphate dibasic buffering agent and 150 mg/mL (15% w/v) sucrose at a pH of about 7.0; and
      ii. converting the aqueous daptomycin formulation to the solid daptomycin preparation.
2. A solid daptomycin preparation comprising daptomycin and a material selected from the group consisting of glycine, one or more sugars, and a combination of two or more non-reducing sugars; the daptomycin preparation characterized in that 500 mg of the solid pharmaceutical daptomycin composition dissolves in 10 mL of 0.9% aqueous sodium chloride in 4 minutes or less at 25 degrees C.
3. The daptomycin preparation of claim 1, wherein the sugar is a non-reducing sugar and the daptomycin preparation further comprises a buffering agent.
4. The daptomycin preparation of any of claims 2-3, wherein
   a. at least 92% pure daptomycin, as calculated by a ratio of absorbance (area under curve) for the daptomycin divided by the total area under the curve measured by high performance liquid chromatography (HPLC) of the reconstituted daptomycin solution at 214 nm according to Table 3 and Example 4, and
   b. the daptomycin preparation is characterized in that 500 mg of the solid pharmaceutical daptomycin composition dissolves in 10 mL of 0.9% aqueous sodium chloride in 1 minute or less at 25 degrees C at a pH of between 4.7 and 7.0.
5. The daptomycin preparation of any of claims 2-4, wherein the sugars are selected from sucrose, trehalose, and mannitol.

6. The daptomycin preparation of any of claims 2-4, wherein the sugars comprise sucrose, and the daptomycin preparation is obtainable by:
   a. forming an aqueous daptomycin solution comprising daptomycin and about 15.0% - about 20% w/v sucrose at a pH of about 4.7-7.0, and
   b. converting the aqueous daptomycin formulation to the solid pharmaceutical daptomycin preparation.
7. The daptomycin preparation of claim 6, wherein the aqueous daptomycin solution is at a pH of about 7.0.
8. The daptomycin preparation of any of claims 6-7, wherein the daptomycin preparation is obtainable by:
   a. forming an aqueous daptomycin solution comprising daptomycin, a sodium phosphate dibasic buffering agent and about 15.0% w/v sucrose at a pH of about 7.0, and
   b. converting the aqueous daptomycin formulation to the solid pharmaceutical daptomycin preparation.
9. A solid pharmaceutical daptomycin preparation obtainable by:
   a. forming an aqueous daptomycin solution comprising daptomycin, 50 mM of a phosphate containing buffering agent, and about 15.0% sucrose at a pH of about 7.0, and
   b. converting the aqueous daptomycin formulation to the solid pharmaceutical daptomycin preparation,

   wherein the solid pharmaceutical daptomycin preparation is characterized in that 500 mg of the solid pharmaceutical daptomycin composition dissolves in 10 mL of 0.9% aqueous sodium chloride in about 2 minutes or less at 25 degrees C.
10. The solid pharmaceutical daptomycin preparation of claim 9, wherein at least 92% pure daptomycin, as calculated by ratio of absorbance (area under curve) for the daptomycin divided by total area under the curve measured by high performance liquid chromatography (HPLC) of the reconstituted daptomycin solution at 214 nm according to Table 3 and Example 4.
11. The solid pharmaceutical daptomycin preparation of any of claims 1-10, further characterized in that the daptomycin preparation has a lower amount of one or more substances selected from the group consisting of anhydro-daptomycin (Figure 2), beta-isomer of daptomycin (Figure 3) and a lactone hydrolysis product of daptomycin (Figure 4) after storage for 1 month at 40 degrees C under nitrogen, compared to a solid

pharmaceutical daptomycin preparation obtained by lyophilizing daptomycin and daptomycin-related compounds in 0.9% aqueous sodium chloride diluent, wherein the amount of the substances is detected by HPLC at 214 nm according to the method of Example 4.

12. A liquid pharmaceutical daptomycin preparation having increased daptomycin chemical stability and being obtainable by:
    a. forming an aqueous daptomycin solution comprising daptomycin, 50 mM of a phosphate containing buffering agent, and about 15.0% sucrose at a pH of about 7.0,
    b. converting the aqueous daptomycin formulation to the solid pharmaceutical daptomycin preparation, and then
    c. reconstituting the solid pharmaceutical daptomycin preparation an aqueous diluent to form liquid pharmaceutical daptomycin preparation.

13. The liquid pharmaceutical daptomycin preparation of claim 12, wherein the liquid pharmaceutical daptomycin preparation is formulated for intravenous administration.

14. An article of manufacture comprising the pharmaceutical daptomycin preparations of any of claims 1-13.

15. A method of manufacturing a solid pharmaceutical daptomycin preparation having increased daptomycin chemical stability measured by a decrease in the rate of formation of substances structurally similar to daptomycin in the solid daptomycin preparation for 3-14 days after reconstitution in an aqueous diluent, wherein the substances structurally similar to daptomycin are selected from the group consisting of the anhydro-daptomycin (Figure 2), the beta-isomer of daptomycin (Figure 3) and the lactone hydrolysis product of daptomycin (Figure 4); the rate of formation of substances structurally similar to daptomycin is measured according to Example 4; and the method comprises:
    a. forming an aqueous daptomycin solution comprising daptomycin and about 5.0 – 20.0% w/v sucrose at a pH of about 4.5 to 7.5, and
    b. converting the aqueous daptomycin formulation to the solid pharmaceutical daptomycin preparation.

16. The method of claim 15, wherein the aqueous daptomycin solution has a pH of about 7.0 and comprises daptomycin, 15% w/v sucrose and 50 nM sodium phosphate buffer; and the aqueous daptomycin solution is converted to the solid pharmaceutical daptomycin preparation by lyophilization.

17. The method of any of claims 15-16, characterized in that the % purity of daptomycin in the solid pharmaceutical daptomycin preparation as measured by the method of Example 4 is increased by at least 10%, relative to the % purity of daptomycin in a solid pharmaceutical daptomycin preparation without sucrose measured by the method of Example 4 after 6 months at 40 degrees C.
18. The method of claim 17, wherein a Daptomycin Stability Ratio at 40 degrees C as calculated by Example 5 is less than 1.000.
19. The solid pharmaceutical daptomycin preparation of any one of claims 1-11, wherein a Daptomycin Stability Ratio at 40 degrees C as calculated by Example 5 is less than 1.000.
20. The solid pharmaceutical daptomycin preparation of any one of claims 1-11, wherein a Daptomycin Stability Ratio at 40 degrees C as calculated by Example 5 is less than 0.500.
21. The solid pharmaceutical daptomycin preparation of any one of claims 1-11, wherein a Daptomycin Stability Ratio at 40 degrees C as calculated by Example 5 is less than 0.300.

Daptomycin

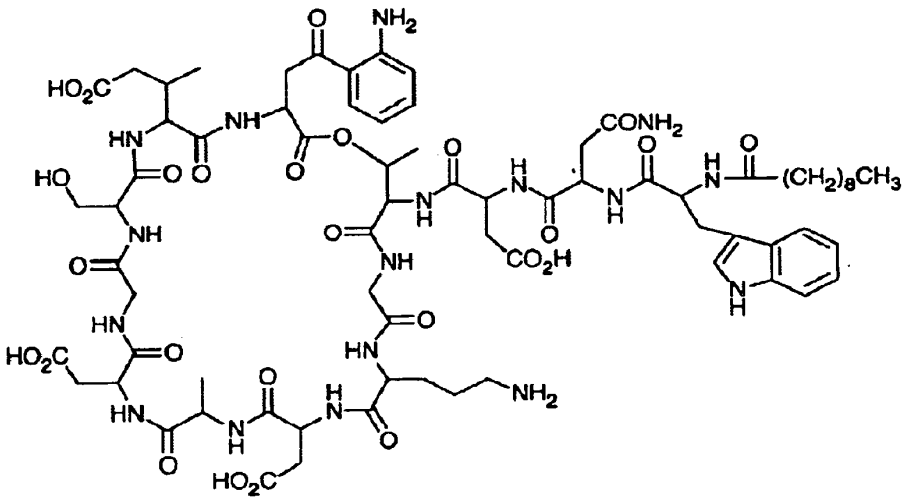


Fig. 1

"anhydro-daptomycin"

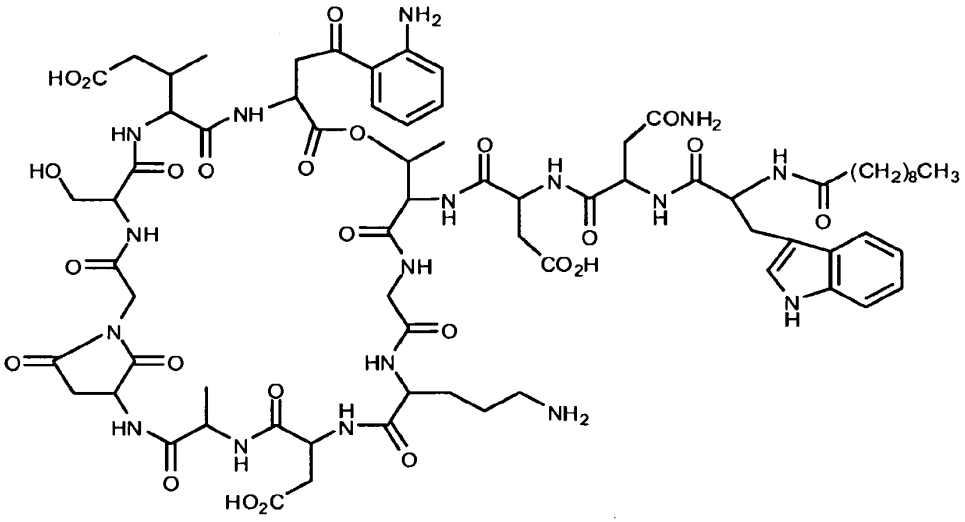


Figure 2

"β-isomer" or "β-isomer of daptomycin"

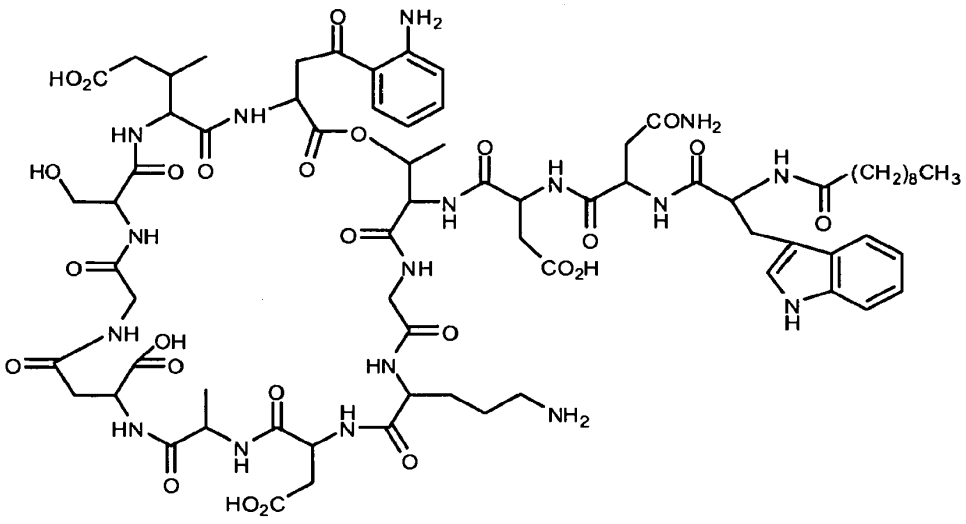
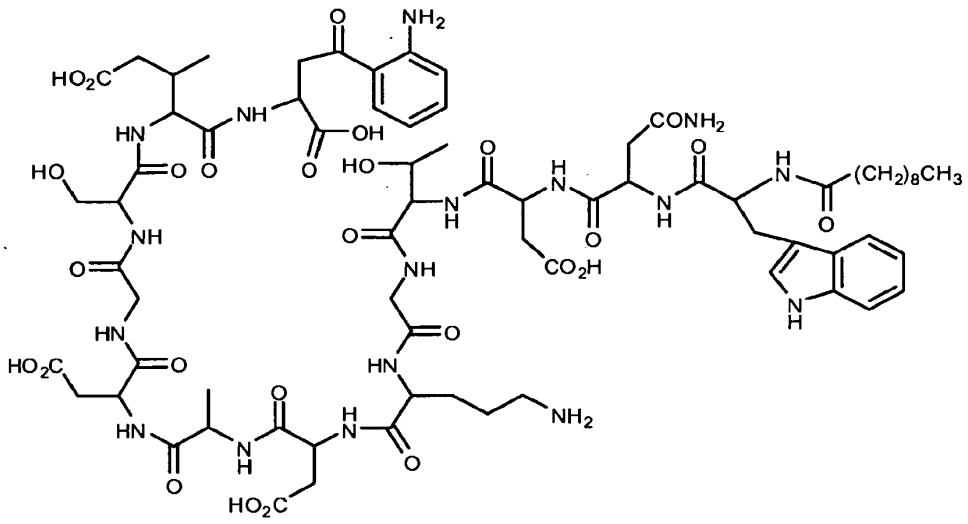


Figure 3



lactone hydrolysis product

FIG. 4

Figure 5

Table 6

No.	Liquid Formulation Components	Recon Time (min)	Formulation (%w/v in solution)	Formulation (solid state) 500 mg/vial	Ratios Dap : sugar Dap : PO4 Dap : Mannitol	Molar Ratio Dap : Sugar(s)
0	Daptomycin, 50 mm PO4, pH 7.0	1.4 min		500mg Dap		
1	2.5% Trehalose, 50mM PO4, pH 7.0	< 1	10.5% Dap 2.5% Trehalose 0.71% PO4	500mg Dap 119mg Tre 35.5mg PO4	 1 : 0.24 1 : 0.071	 1 : 2.13 1 : 0.81
2	5 % Trehalose, 50mM PO4, pH 7.0	< 1	10.5% Dap 5% Trehalose 0.71% PO4	500mg Dap 238mg Tre 35.5mg PO4	 1 : 0.48 1 : 0.071	 1 : 4.26 1 : 0.81
3	10 % Trehalose, 50mM PO4, pH 7.0	< 1	10.5% Dap 10% Trehalose 0.71% PO4	500mg Dap 476.2mg Tre 35.5mg PO4	 1 : 0.95 1 : 0.071	 1 : 8.52 1 : 0.81
4	2.5% Sucrose, 50mM PO4, pH 7.0	< 1	10.5% Dap 2.5% Sucrose 0.71% PO4	500mg Dap 119mg Sucrose 35.5mg PO4	 1 : 0.24 1 : 0.071	 1 : 1.12 1 : 0.81
5	5 % Sucrose, 50mM PO4, pH 7.0	< 1	10.5% Dap 5% Sucrose 0.71% PO4	500mg Dap 238mg Sucrose 35.5mg PO4	 1 : 0.48 1 : 0.071	 1 : 2.24 1 : 0.81
6	10 % Sucrose, 50mM PO4, pH 7.0	< 1	10.5% Dap 10% Sucrose 0.71% PO4	500mg Dap 476.2mg Suc 35.5mg PO4	 1 : 0.95 1 : 0.071	 1 : 4.48 1 : 0.81
7	2.5% Sucrose, 3% Mannitol, 50mM PO4, pH 7.0	< 1	10.5% Dap 2.5% Sucrose 3% Mannitol 0.71% PO4	500mg Dap 119mg Sucrose 142.9mg Man 35.5mg PO4	 1 : 0.24 1 : 0.29 1 : 0.071	 1 : 1.12 1 : 2.52 1 : 0.81
8	5 % Sucrose, 3% Mannitol, 50mM PO4, pH 7.0	< 1	10.5% Dap	500mg Dap		

No.	Liquid Formulation Components	Recon Time (min)	Formulation (%w/v in solution)	Formulation (solid state) 500 mg/vial	Ratios Dap : sugar Dap : PO4 Dap : Mannitol	Molar Ratio Dap : Sugar(s)
			5% Sucrose 3% Mannitol 0.71% PO4	238mg Sucrose 142.9mg Man 35.5mg PO4	1 : 0.48 1 : 0.29 1 : 0.071	1 : 2.24 1 : 2.52 1 : 0.81
9	10 % Sucrose, 3% Mannitol, 50mM PO4, pH 7.0	< 1	10.5% Dap 10% Sucrose 3% Mannitol 0.71% PO4	500mg Dap 476.2mg Suc 142.9mg Man 35.5mg PO4	1 : 0.95 1 : 0.29 1 : 0.071	1 : 4.48 1 : 2.52 1 : 0.81
10	2.5% Sucrose, 6% Mannitol, 50mM PO4, pH 7.0	<1	10.5% Dap 2.5% Sucrose 6% Mannitol 0.71% PO4	500mg Dap 119mg Sucrose 285.8 Man 35.5mg PO4	1 : 0.24 1 : 0.57 1 : 0.071	1 : 1.12 1 : 5.04 1 : 0.81
11	5% Sucrose, 6% Mannitol, 50mM PO4, pH 7.0	< 1	10.5% Dap 5% Sucrose 6% Mannitol 0.71% PO4	500mg Dap 238mg Sucrose 285.8mg Man 35.5mg PO4	1 : 0.48 1 : 0.57 1 : 0.071	1 : 2.24 1 : 5.04 1 : 0.81
12	10% Sucrose, 6% Mannitol, 50mM PO4, pH 7.0	< 1	10.5% Dap 10% Sucrose 6% Mannitol 0.71% PO4	500mg Dap 476.2mg Suc 285.8mg Man 35.5mg PO4	1 : 0.95 1 : 0.57 1 : 0.071	1 : 4.48 1 : 5.04 1 : 0.81
13	20 % Sucrose, 50mM PO4, pH 7.0	< 1	10.5% Dap 20% Sucrose 0.71% PO4	500mg Dap 952.4mg Suc 35.5mg PO4	1 : 1.90 1 : 0.071	1 : 8.96 1 : 0.81
14	25% Trehalose, 50mM PO4, pH 7.0	< 1	10.5% Dap 25% Tre 0.71% PO4	500mg Dap 1190.5mg Tre 35.5mg PO4	1 : 2.38 1 : 0.071	1 : 21.32 1 : 0.81
15	25% Trehalose, pH 4.7	< 1	10.5% Dap 25% Tre	500mg Dap 1190.5mg Tre	1 : 2.38	1 : 21.32
19	20% Sucrose, pH 4.7	< 1	10.5% Dap	500mg Dap		

No.	Liquid Formulation Components	Recon Time (min)	Formulation (%w/v in solution)	Formulation (solid state) 500 mg/vial	Ratios Dap : sugar Dap : PO4 Dap : Mannitol	Molar Ratio Dap : Sugar(s)
			20% Sucrose	952.4mg Suc	1 : 1.90	1 : 8.96
23	15 % Sucrose, 3% Mannitol, pH 4.7	0.3 – 1.5	10.5% Dap 15% Sucrose 3% Mannitol	500mg Dap 750mg Sucrose 142.9mg Man	1 : 1.5 1 : 0.29	1 : 6.73 1 : 2.52
35	20% Lactose, 50mM PO4, pH 7.0	< 1	10.5% Dap 20% Lactose 0.71% PO4	500mg Dap 952.4mg Lact 35.5mg PO4	1 : 1.90 1 : 0.071	1 : 8.80 1 : 0.81
50	2.5% Lactose, 50 mM PO4, pH 7.0	< 1	10.5% Dap 2.5% Lactose 0.71% PO4	500mg Dap 119mg Lac 35.5mg PO4	1 : 0.24 1 : 0.071	1 : 1.10 1 : 0.81
51	2.5% Maltose, 50 mM PO4, pH 7.0	0.5 – 1.2	10.5% Dap 2.5% Maltose 0.71% PO4	500mg Dap 119mg Malt 35.5mg PO4	1 : 0.24 1 : 0.071	1 : 1.12 1 : 0.81
52	2.5% Fructose, 50 mM PO4, pH 7.0	< 1	10.5% Dap 2.5% Fructose 0.71% PO4	500mg Dap 119mg Fruc 35.5mg PO4	1 : 0.24 1 : 0.071	1 : 2.13 1 : 0.81
53	2.5% Dextrose, 50 mM PO4, pH 7,0	0.6 – 1.1	10.5% Dap 2.5% Dextrose 0.71% PO4	500mg Dap 119mg Dex 35.5mg PO4	1 : 0.24 1 : 0.071	1 : 2.13 1 : 0.81
54	2.5%Dextrose/Fructose (1:1), 50mM PO4, pH 7.0	0.5 – 1.2	10.5% Dap 2.5% Dex/Fruc 0.71% PO4	500mg Dap 119mg D/F 35.5mg PO4	1 : 0.24 1 : 0.071	1 : 1.07 : 1.07 1 : 0.81
55	5% Lactose, 50mM PO4, pH 7.0	< 1	10.5% Dap 5% Lactose 0.71% PO4	500mg Dap 238mg Lact 35.5mg PO4	1 : 0.48 1 : 0.071	1 : 2.20 1 : 0.81
56	5% Maltose, 50mM PO4, pH 7.0	< 1	10.5% Dap	500mg Dap		

No.	Liquid Formulation Components	Recon Time (min)	Formulation (%w/v in solution)	Formulation (solid state) 500 mg/vial	Ratios Dap : sugar Dap : PO4 Dap : Mannitol	Molar Ratio Dap : Sugar(s)
			5% Maltose 0.71% PO4	238mg Malt 35.5mg PO4	1 : 0.48 1 : 0.071	1 : 2.24 1 : 0.81
57	5% Fructose, 50mM PO4, pH 7.0	< 1	10.5% Dap 5% Fructose 0.71% PO4	500mg Dap 238mg Fruc 35.5mg PO4	1 : 0.48 1 : 0.071	1 : 4.26
58	5% Dextrose. 50 mM PO4, pH 7.0	< 1	10.5% Dap 5% Dextrose 0.71% PO4	500mg Dap 238mg Dex 35.5mg PO4	1 : 0.48 1 : 0.071	1 : 4.26 1 : 0.81
59	5%Dextrose/Fructose (1:1), 50mM PO4, pH 7.0	< 1	10.5% Dap 5% Dex/Fruc 0.71% PO4	500mg Dap 238mg D/F 35.5mg PO4	1 : 0.48 1 : 0.071	1 : 2.13 : 2.13 1 : 0.81
60	2.5% Lactose, pH 4.7	1.1	10.5% Dap 2.5% Lactose	500mg Dap 119mg Lac	1 : 0.24	1 : 1.10
61	2.5% Maltose, pH 4.7	1.1	10.5% Dap 2.5% Maltose	500mg Dap 119mg Malt	1 : 0.24	1 : 1.12
62	2.5% Fructose, pH 4. 7	1.2	10.5% Dap 2.5% Fructose	500mg Dap 119mg Fruc	1 : 0.24	1 : 2.13
64	2.5%Dextrose/Fructose (1:1), pH 4.7	1.7	10.5% Dap 2.5% Dex/Fruc	500mg Dap 119mg D/F	1 : 0.24	1 : 1.07 : 1.07
65	5% Lactose, pH 4.7	1.6	10.5% Dap 5% Lactose	500mg Dap 238mg Lact	1 : 0.48	1 : 2.24
71	6% Mannitol, 50 mM PO4, pH 7.0	< 1	10.5% Dap 6% Mannitol 0.71% PO4	500mg Dap 285.8mg Man 35.5mg PO4	1 : 0.57 1 : 0.071	1 : 5.04 1 : 0.81

No.	Liquid Formulation Components	Recon Time (min)	Formulation (%w/v in solution)	Formulation (solid state) 500 mg/vial	Ratios Dap : sugar Dap : PO4 Dap : Mannitol	Molar Ratio Dap : Sugar(s)
73	5% Glycine, 50 mM PO4, pH 7.0	< 1	10.5% Dap 5% Glycine 0.71% PO4	500mg Dap 238mg Glycine 35.5mg PO4	1 : 0.48 1 : 0.071	1 : 10.31 1 : 0.81
75	15% Sucrose, 50mM PO4, pH 7.0	< 1	10.5% Dap 15% Sucrose 0.71% PO4	500mg Dap 714.3mg Sucrose 35.5mg PO4	1 : 1.5 1 : 0.071	1 : 6.73 1 : 0.81
76	15% Sucrose, 50mM PO4, pH 7.0	< 1	10.5% Dap 15% Sucrose 0.71% PO4	500mg Dap 714.3mg Sucrose 35.5mg PO4	1 : 1.5 1 : 0.071	1 : 6.73 1 : 0.81

Figure 6

Table 7

	Formulation ID	Recon Time (min)	Formulation (% w/v in solution)	Formulation (solid state) 500 mg/vial	Ratios Dap : sugar Dap : PO4 Dap : Mannitol	Molar Ratio Dap : Excipients
00	Daptomycin, pH 4.7	5 min		500mg Dap		
16	2.5% Sucrose, pH 4.7	2 - 4	10.5% Dap 2.5% Sucrose	500mg Dap 119mg Sucrose	1 : 0.24	1 : 1.12
17	5% Sucrose, pH 4.7	0.7 – 2	10.5% Dap 5% Sucrose	500mg Dap 238mg Sucrose	1 : 0.48	1 : 2.24
18	10 % Sucrose, pH 4.7	0.3 – 3	10.5% Dap 10% Sucrose	500mg Dap 476.2mg Suc	1 : 0.95	1 : 4.48
20	2.5% Sucrose, 3% Mannitol, pH 4.7	2 - 8	10.5% Dap 2.5% Sucrose 3% Mannitol	500mg Dap 119mg Sucrose 142.9mg Man	1 : 0.24 1 : 0.29	1 : 1.12 1 : 2.52
21	5% Sucrose, 3% Mannitol, pH 4.7	2 – 6	10.5% Dap 5% Sucrose 3% Mannitol	500mg Dap 238mg Sucrose 142.9mg Man	1 : 0.48 1 : 0.29	1 : 2.24 1 : 2.52
22	10 % Sucrose, 3% Mannitol, pH 4.7	0.5 – 2	10.5% Dap 10% Sucrose 3% Mannitol	500mg Dap 476.2mg Suc 142.9mg Man	1 : 0.95 1 : 0.29	1 : 4.48 1 : 2.52
63	2.5% Dextrose, pH 4.7	2	10.5% Dap	500mg Dap		

	Formulation ID	Recon Time (min)	Formulation (% w/v in solution)	Formulation (solid state) 500 mg/vial	Ratios Dap : sugar Dap : PO4 Dap : Mannitol	Molar Ratio Dap : Excipients
			2.5% Dextrose	119mg Dex	1 : 0.24	1 : 2.13
66	5% Maltose, pH 4.7	2.4	10.5% Dap 5% Maltose	500mg Dap 238mg Malt	1 : 0.48	1 : 2.20
67	5% Fructose, pH 4.7	2.5	10.5% Dap 5% Fructose	500mg Dap 238mg Fruc	1 : 0.48	1 : 4.26
68	5% Dextrose, pH 4.7	2.4	10.5% Dap 5% Dextrose	500mg Dap 238mg Dex	1 : 0.48	1 : 4.26
69	5%Dextrose/Fructose (1:1), pH 4.7	2.0	10.5% Dap 5% Dex/Fruc	500mg Dap 238mg D/F	1 : 0.48	1 : 2.13 : 2.13
77	5 % Trehalose, pH 4.7	3 – 4	10.5% Dap 5% Trehalose	500mg Dap 238mg Tre	1 : 0.48	1 : 4.26
	2.5% Trehalose, pH 4.7	3 – 5	10.5% Dap 2.5% Trehalose	500mg Dap 119mg Tre	1 : 0.24	1 : 2.13

Figure 7

Table 8

ID No.	Lipopeptide [A]	Compound [B]	Compound [C]	Buffering Agent [D]	Compounding pH	Molar Ratio of existing components, respectively	Formulation in Solution upon addition of diluent (weight/volume)
1	daptomycin	Trehalose		Sodium phosphate dibasic	about 7.0	1 : 2.13 : 0.77	10.5% Dap 2.5% Trehalose 0.71% Na_2HPO_4
2	daptomycin	Trehalose		Sodium phosphate dibasic	about 7.0	1 : 4.26 : 0.77	10.5% Dap 5% Trehalose 0.71% Na_2HPO_4
3	daptomycin	Trehalose		Sodium phosphate dibasic	about 7.0	1 : 8.53 : 0.77	10.5% Dap 10% Trehalose 0.71% Na_2HPO_4
4	daptomycin	Sucrose		Sodium phosphate dibasic	about 7.0	1 : 1.12 : 0.77	10.5% Dap 2.5% Sucrose 0.71% Na_2HPO_4
5	daptomycin	Sucrose		Sodium phosphate dibasic	about 7.0	1 : 2.24 : 0.77	10.5% Dap 5% Sucrose 0.71% Na_2HPO_4
6	daptomycin	Sucrose		Sodium phosphate dibasic	about 7.0	1 : 4.49 : 0.77	10.5% Dap 10% Sucrose 0.71% Na_2HPO_4
7	daptomycin	Sucrose	Mannitol	Sodium phosphate dibasic	about 7.0	1 : 1.12 : 2.52 : 0.77	10.5% Dap 2.5% Sucrose 3% Mannitol 0.71% Na_2HPO_4
8	daptomycin	Sucrose	Mannitol	Sodium phosphate dibasic	about 7.0	1 : 2.24 : 2.52 : 0.77	10.5% Dap 5% Sucrose 3% Mannitol 0.71% Na_2HPO_4

ID No.	Lipopeptide [A]	Compound [B]	Compound [C]	Buffering Agent [D]	Compounding pH	Molar Ratio of existing components, respectively	Formulation in Solution upon addition of diluent (weight/volume)
9	daptomycin	Sucrose	Mannitol	Sodium phosphate dibasic	about 7.0	1 : 4.49 : 2.52 : 0.77	10.5% Dap 10% Sucrose 3% Mannitol 0.71% Na_2HPO_4
10	daptomycin	Sucrose	Mannitol	Sodium phosphate dibasic	about 7.0	1 : 1.12 : 5.04 : 0.77	10.5% Dap 2.5% Sucrose 6% Mannitol 0.71% Na_2HPO_4
11	daptomycin	Sucrose	Mannitol	Sodium phosphate dibasic	about 7.0	1 : 2.24 : 5.04 : 0.77	10.5% Dap 5% Sucrose 6% Mannitol 0.71% Na_2HPO_4
12	daptomycin	Sucrose	Mannitol	Sodium phosphate dibasic	about 7.0	1 : 4.49 : 5.04 : 0.77	10.5% Dap 10% Sucrose 6% Mannitol 0.71% Na_2HPO_4
13	daptomycin	Sucrose		Sodium phosphate dibasic	about 7.0	1 : 8.98 : 0.77	10.5% Dap 20% Sucrose 0.71% Na_2HPO_4
14	daptomycin	Trehalose		Sodium phosphate dibasic	about 7.0	1 : 21.32 : 0.77	10.5% Dap 25% Trehalose 0.71% Na_2HPO_4
15	daptomycin	Trehalose			about 4.7	1 : 21.32	10.5% Dap 25% Trehalose
16	daptomycin	Sucrose			about 4.7	1 : 1.12	10.5% Dap 2.5% Sucrose
17	daptomycin	Sucrose			about 4.7	1 : 2.24	10.5% Dap 5% Sucrose
18	daptomycin	Sucrose			about 4.7	1 : 4.49	10.5% Dap 10% Sucrose
19	daptomycin	Sucrose			about 4.7	1 : 8.98	10.5% Dap 20% Sucrose

ID No.	Lipopeptide [A]	Compound [B]	Compound [C]	Buffering Agent [D]	Compounding pH	Molar Ratio of existing components, respectively	Formulation in Solution upon addition of diluent (weight/volume)
20	daptomycin	Sucrose	Mannitol		about 4.7	1 : 1.12 : 2.52	10.5% Dap 2.5% Sucrose 3% Mannitol
21	daptomycin	Sucrose	Mannitol		about 4.7	1 : 2.24 : 2.52	10.5% Dap 5% Sucrose 3% Mannitol
22	daptomycin	Sucrose	Mannitol		about 4.7	1 : 4.49 : 2.52	10.5% Dap 10% Sucrose 3% Mannitol
23	daptomycin	Sucrose	Mannitol		about 4.7	1 : 6.73 : 2.52	10.5% Dap 15% Sucrose 3% Mannitol
24	daptomycin	Sucrose	Mannitol		about 4.7	1 : 1.12: 5.04	10.5% Dap 2.5% Sucrose 6% Mannitol
25	daptomycin	Sucrose	Mannitol		about 4.7	1 : 2.24 : 5.04	10.5% Dap 5% Sucrose 6% Mannitol
26	daptomycin	Sucrose	Mannitol		about 4.7	1 : 4.49 : 5.04	10.5% Dap 10% Sucrose 6% Mannitol
27	daptomycin	Sucrose	Mannitol		about 4.7	1 : 6.73 : 5.04	10.5% Dap 15% Sucrose 6% Mannitol
28	daptomycin	Sucrose	Mannitol	Sodium phosphate dibasic	about 7.0	1 : 6.73 : 2.24 : 0.77	10.5% Dap 15% Sucrose 3% Mannitol 0.71% Na_2HPO_4
29	daptomycin	Sucrose	Mannitol	Sodium phosphate dibasic	about 7.0	1 : 6.73 : 5.04 : 0.77	10.5% Dap 15% Sucrose 6% Mannitol 0.71% Na_2HPO_4

ID No.	Lipopeptide [A]	Compound [B]	Compound [C]	Buffering Agent [D]	Compounding pH	Molar Ratio of existing components, respectively	Formulation in Solution upon addition of diluent (weight/volume)
30	daptomycin	Lactose		Sodium phosphate dibasic	about 7.0	1 : 4.49 : 0.77	10.5% Dap 10% Lactose 0.71% Na_2HPO_4
31	daptomycin	Maltose		Sodium phosphate dibasic	about 7.0	1 : 4.49 : 0.77	10.5% Dap 10% Maltose 0.71% Na_2HPO_4
32	daptomycin	Fructose		Sodium phosphate dibasic	about 7.0	1 : 8.52 : 0.77	10.5% Dap 10% Fructose 0.71% Na_2HPO_4
33	daptomycin	Dextrose		Sodium phosphate dibasic	about 7.0	1 : 8.52 : 0.77	10.5% Dap 10% Dextrose 0.71% Na_2HPO_4
34	daptomycin	Dextrose	Fructose	Sodium phosphate dibasic	about 7.0	1 : 4.26 : 4.26 : 0.77	10.5% Dap 5% Dextrose 5% Fructose 0.71% Na_2HPO_4
35	daptomycin	Lactose		Sodium phosphate dibasic	about 7.0	1 : 8.98 : 0.77	10.5% Dap 20% Lactose 0.71% Na_2HPO_4
36	daptomycin	Maltose		Sodium phosphate dibasic	about 7.0	1 : 8.98 : 0.77	10.5% Dap 20% Maltose 0.71% Na_2HPO_4
37	daptomycin	Fructose		Sodium phosphate dibasic	about 7.0	1 : 17.05 : 0.77	10.5% Dap 20% Fructose 0.71% Na_2HPO_4
38	daptomycin	Dextrose		Sodium phosphate dibasic	about 7.0	1 : 17.05 :0.77	10.5% Dap 20% Dextrose 0.71% Na_2HPO_4
39	daptomycin	Dextrose	Fructose	Sodium phosphate dibasic	about 7.0	1: 8.52 : 8.52 : 0.77	10.5% Dap 10% Dextrose 10% Fructose 0.71% Na_2HPO_4

ID No.	Lipopeptide [A]	Compound [B]	Compound [C]	Buffering Agent [D]	Compounding pH	Molar Ratio of existing components, respectively	Formulation in Solution upon addition of diluent (weight/volume)
40	daptomycin	Lactose			about 4.7	1 : 4.49	10.5% Dap 10% Lactose
41	daptomycin	Maltose			about 4.7	1 : 4.49	10.5% Dap 10% Maltose
42	daptomycin	Fructose			about 4.7	1 : 8.52	10.5% Dap 10% Fructose
43	daptomycin	Dextrose			about 4.7	1 : 8.52	10.5% Dap 10% Dextrose
44	daptomycin	Dextrose	Fructose		about 4.7	1 : 4.26 : 4.26	10.5% Dap 5% Dextrose 5% Fructose
45	daptomycin	Lactose			about 4.7	1 : 8.98	10.5% Dap 20% Lactose
46	daptomycin	Maltose			about 4.7	1 : 8.98	10.5% Dap 20% Maltose
47	daptomycin	Fructose			about 4.7	1 : 17.05	10.5% Dap 20% Fructose
48	daptomycin	Dextrose			about 4.7	1 : 17.05	10.5% Dap 20% Dextrose
49	daptomycin	Dextrose	Fructose		about 4.7	1 : 8.52 : 8.52	10.5% Dap 10% Dextrose 10% Fructose
50	daptomycin	Lactose		Sodium phosphate dibasic	about 7.0	1 : 1.12 : 0.77	10.5% Dap 2.5% Lactose 0.71% Na_2HPO_4
51	daptomycin	Maltose		Sodium phosphate dibasic	about 7.0	1 : 1.12 : 0.77	10.5% Dap 2.5% Maltose 0.71% Na_2HPO_4
52	daptomycin	Fructose		Sodium phosphate dibasic	about 7.0	1 : 2.13 : 0.77	10.5% Dap 2.5% Fructose 0.71% Na_2HPO_4

ID No.	Lipopeptide [A]	Compound [B]	Compound [C]	Buffering Agent [D]	Compounding pH	Molar Ratio of existing components, respectively	Formulation in Solution upon addition of diluent (weight/volume)
53	daptomycin	Dextrose		Sodium phosphate dibasic	about 7.0	1 : 2.13 : 0.77	10.5% Dap 2.5% Dextrose 0.71% Na_2HPO_4
54	daptomycin	Dextrose	Fructose	Sodium phosphate dibasic	about 7.0	1 : 1.07 : 1.07 : 0.77	10.5% Dap 2.5% 1.25% Dextrose 1.25% Fructose 0.71% Na_2HPO_4
55	daptomycin	Lactose		Sodium phosphate dibasic	about 7.0	1 : 2.24 : 0.77	10.5% Dap 5% Lactose 0.71% Na_2HPO_4
56	daptomycin	Maltose		Sodium phosphate dibasic	about 7.0	1 : 2.24 : 0.77	10.5% Dap 5% Maltose 0.71% Na_2HPO_4
57	daptomycin	Fructose		Sodium phosphate dibasic	about 7.0	1 : 4.26 : 0.77	10.5% Dap 5% Fructose 0.71% Na_2HPO_4
58	daptomycin	Dextrose		Sodium phosphate dibasic	about 7.0	1 : 4.26 : 0.77	10.5% Dap 5% Dextrose 0.71% Na_2HPO_4
59	daptomycin	Dextrose	Fructose	Sodium phosphate dibasic	about 7.0	1 : 2.13 : 2.13 : 0.77	10.5% Dap 2.5% Dextrose 2.5% Fructose 0.71% Na_2HPO_4
60	daptomycin	Lactose			about 4.7	1 : 1.12	10.5% Dap 2.5% Lactose
61	daptomycin	Maltose			about 4.7	1 : 1.12	10.5% Dap 2.5% Maltose
62	daptomycin	Fructose			about 4.7	1 : 2.13	10.5% Dap 2.5% Fructose
63	daptomycin	Dextrose			about 4.7	1 : 2.13	10.5% Dap 2.5% Dextrose

ID No.	Lipopeptide [A]	Compound [B]	Compound [C]	Buffering Agent [D]	Compounding pH	Molar Ratio of existing components, respectively	Formulation in Solution upon addition of diluent (weight/volume)
64	daptomycin	Dextrose	Fructose		about 4.7	1 : 1.07 : 1.07 :	10.5% Dap 1.25% Dextrose 1.25% Fructose
65	daptomycin	Lactose			about 4.7	1 : 2.24	10.5% Dap 5% Lactose
66	daptomycin	Maltose			about 4.7	1 : 2.24	10.5% Dap 5% Maltose
67	daptomycin	Fructose			about 4.7	1 : 4.26	10.5% Dap 5% Fructose
68	daptomycin	Dextrose			about 4.7	1 : 4.26	10.5% Dap 5% Dextrose
69	daptomycin	Dextrose	Fructose		about 4.7	1 : 2.13 : 2.13	10.5% Dap 2.5% Dextrose 2.5% Fructose
70	daptomycin	Mannitol			about 4.7	1 : 5.04	10.5% Dap 6% Mannitol
71	daptomycin	Mannitol		Sodium phosphate dibasic	about 7.0	1 : 5.04 : 0.77	10.5% Dap 6% Mannitol 0.71% Na_2HPO_4
72	daptomycin	Glycine			about 4.7	1 : 10.23	10.5% Dap 5% Glycine
73	daptomycin	Glycine		Sodium phosphate dibasic	about 7.0	1 : 10.23 : 0.77	10.5% Dap 5% Glycine 0.71% Na_2HPO_4
74	daptomycin	Sucrose			about 4.7	1 : 6.73	10.5% Dap 15% Sucrose

ID No.	Lipopeptide [A]	Compound [B]	Compound [C]	Buffering Agent [D]	Compounding pH	Molar Ratio of existing components, respectively	Formulation in Solution upon addition of diluent (weight/volume)
75	daptomycin	Sucrose		Sodium phosphate dibasic	about 7.0	1 : 6.73 : 0.77	10.5% Dap 15% Sucrose 0.71% Na_2HPO_4
76	daptomycin	Sucrose		Sodium phosphate dibasic	about 7.0	1 : 6.73 : 0.77	10.5% Dap 15% Sucrose 0.71% Na_2HPO_4
77	daptomycin	Trehalose			about 4.7	1 : 4.26	10.5% Dap 5% Trehalose
78	daptomycin	Trehalose			about 4.7	1 : 8.53	10.5% Dap 10% Trehalose
79	daptomycin	Trehalose			about 4.7	1 : 14.92	10.5% Dap 17.5% Trehalose

Figure 8

Table 9

Formulation ID	Formulation Description	Daptomycin Stability Ratio at 40 Degrees C				
		T0	1 month	2 months	3 months	6 months
0	Daptomycin Control with 50mM Phosphate buffer at pH 7.0 (without sugar or glycine)	0.000	1.000	1.000	1.000	1.000
1	2.5% Trehalose, 50mM PO4, pH 7.0	0.000	0.667	0.800	0.667	1.000
2	5 % Trehalose, 50mM PO4, pH 7.0	0.000	0.867	0.867	0.714	0.871
3	10 % Trehalose, 50mM PO4, pH 7.0	0.000	0.400	0.400	0.381	0.613
4	2.5% Sucrose, 50mM PO4, pH 7.0	0.000	0.533	0.467	0.524	0.742
5	5 % Sucrose, 50mM PO4, pH 7.0	0.000	0.467	0.533	0.476	0.645
6	10 % Sucrose, 50mM PO4, pH 7.0	0.000	0.267	0.133	0.238	0.355
7	2.5% Sucrose, 3% Mannitol, 50mM PO4, pH 7.0	0.000	0.267	0.133	0.238	0.387
8	5 % Sucrose, 3% Mannitol, 50mM PO4, pH 7.0	0.000	0.267	0.133	0.190	0.258
9	10 % Sucrose, 3% Mannitol, 50mM PO4, pH 7.0	0.000	-0.200	0.267	0.190	0.226
10	2.5% Sucrose, 6% Mannitol, 50mM PO4, pH 7.0	0.000	-0.067	0.333	0.238	0.355
11	5% Sucrose, 6% Mannitol, 50mM PO4, pH 7.0	0.000	-0.200	0.133	0.238	0.290
12	10% Sucrose, 6% Mannitol, 50mM PO4, pH 7.0	0.000	0.000	0.067	0.190	0.419
13	20 % Sucrose, 50mM PO4, pH 7.0	0.000	-0.267	0.133	0.143	0.226
14	25% Trehalose, 50mM PO4, pH 7.0	0.000	0.133	0.533	0.381	0.484
15	25% Trehalose, pH 4.7	0.000	-0.067	NT	0.286	0.323
16	2.5% Sucrose, PO4, pH 4.7	0.000	0.333	0.600	0.429	0.581
17	5% Sucrose, PO4, pH 4.7	0.000	0.133	0.267	0.190	0.323
18	10 % Sucrose, PO4, pH 4.7	0.000	0.067	0.133	0.095	0.194
19	20% Sucrose, PO4, pH 4.7	0.000	-0.467	-0.067	0.000	0.097
20	2.5% Sucrose, 3% Mannitol, pH 4.7	0.000	0.000	0.200	0.429	0.484
21	5% Sucrose, 3% Mannitol, pH 4.7	0.000	0.000	0.133	0.333	0.387

Formulation ID	Formulation Description	Daptomycin Stability Ratio at 40 Degrees C				
		T0	1 month	2 months	3 months	6 months
22	10 % Sucrose, 3% Mannitol, pH 4.7	0.000	0.333	0.200	0.381	0.226
23	15 % Sucrose, 3% Mannitol, pH 4.7	0.000	0.133	0.000	0.190	0.129
24	2.5% Sucrose, 6% Mannitol, pH 4.7	0.000	0.400	0.400	0.571	0.516
25	5% Sucrose, 6% Mannitol, pH 4.7	0.000	0.333	0.333	0.476	0.419
26	10% Sucrose, 6% Mannitol, pH 4.7	0.000	0.200	0.067	0.238	0.226
27	15% Sucrose, 6% Mannitol, pH 4.7	0.000	0.200	0.067	0.286	0.226
35	20%Lactose with 50mM Phosphate buffer at pH 7.0	0.000	2.600	0.800	0.524	0.484
45	20% Lactose at pH 4.7	0.000	2.267	2.867	1.571	2.161
50	2.5%Lactose with 50mM Phosphate buffer at pH 7.0	0.000	2.667	4.733	3.286	2.935
51	2.5%Maltose with 50mM Phosphate buffer at pH 7.0	0.000	2.933	4.467	3.476	3.129
52	2.5% Fructose with 50mM Phosphate buffer at pH 7.0	0.000	3.133	4.800	3.905	4.032
53	2.5%Dextrose with 50mM Phosphate buffer at pH 7.0	0.000	7.467	12.400	9.333	8.516
54	2.5% Dextrose/Fructose (1:1) with 50mM Phosphate buffer at pH 7.0	0.000	5.400	8.267	6.857	6.419
55	5.0% Lactose with 50mM Phosphate buffer at pH 7.0	0.000	3.067	4.800	3.810	3.419
56	5.0% Maltose with 50mM Phosphate buffer at pH 7.0	0.000	3.400	4.800	4.048	3.355
57	5.0%Fructosewith 50mM Phosphate buffer at pH 7.0	0.000	2.533	4.133	3.190	3.355
58	5.0%Dextrosewith 50mM Phosphate buffer at pH 7.0	0.000	7.667	11.133	8.905	8.258
59	5.0% Dextrose/Fructose(1:1)with 50mM Phosphate buffer at pH 7.0	0.000	4.267	7.600	6.524	6.161
60	2.5% Lactose pH 4.7	0.000	2.267	3.533	2.905	2.774
61	2.5% Maltose pH 4.7	0.000	2.133	3.600	2.905	2.645
62	2.5% Fructose pH 4.7	0.000	3.133	4.933	3.905	3.968

Formulation ID	Formulation Description	Daptomycin Stability Ratio at 40 Degrees C				
		T0	1 month	2 months	3 months	6 months
63	2.5%Dextrose pH 4.7	0.000	9.267	14.400	10.952	9.903
64	2.5%Dextrose/Fructose(1:1) pH 4.7	0.000	5.000	9.267	7.571	7.645
65	5.0%Lactose pH 4.7	0.000	2.333	3.333	2.571	2.452
66	5.0%MaltosepH 4.7	0.000	2.133	3.600	2.905	2.645
67	5.0%Fructose pH 4.7	0.000	2.200	4.467	3.810	3.581
68	5.0%Dextrose pH 4.7	0.000	4.200	8.867	7.000	7.516
69	5.0%Dextrose/Fructose(1:1) pH 4.7	0.000	3.333	7.200	6.048	6.452
70	6% Mannitol, pH 4.7	0.000	0.533	0.867	0.667	0.903
71	6% Mannitol, 50 mM PO4, pH 7.0	0.000	0.533	0.600	0.524	0.645
72	5% Glycine, pH 4.7	0.000	0.600	1.000	0.667	0.935
73	5% Glycine, 50 mM PO4, pH 7.0	0.000	1.267	1.867	1.524	1.742
74	15 % Sucrose, PO4, pH 4.7	0.000	0.000	0.200	-0.095	0.161
75	15% Sucrose, 50mM PO4, pH 7.0	0.000	0.000	0.200	0.286	0.065
76	15% Sucrose, 50mM PO4, pH 7.0	0.000	0.067	0.267	0.048	0.226
77	5 % Trehalose, pH 4.7	0.000	0.487	NT	0.595	0.639
78	10 % Trehalose, pH 4.7	0.000	0.420	NT	0.490	0.458
79	17.5% Trehalose, pH 4.7	0.000	0.293	NT	0.257	0.313

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(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PE, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

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— *as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii))*

Published:

— *with international search report (Art. 21(3))*

— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*

(88) Date of publication of the international search report:
13 October 2011

(54) Title: LIPOPEPTIDE COMPOSITIONS AND RELATED METHODS

Daptomycin

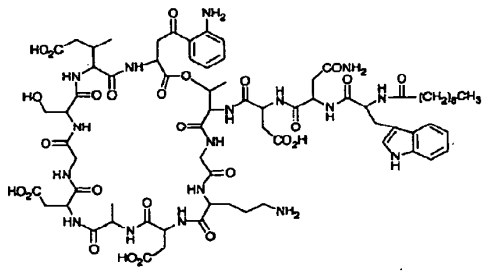

Fig. 1

(57) Abstract: The present disclosure provides novel powder daptomycin formulations which have improved chemical stability and faster reconstitution times when in the solid state. Some examples of the compositions comprise daptomycin and sucrose.

WO 2011/063419 A3

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Application Data Sheet 37 CFR 1.76	Attorney Docket Number	552815 (CPT-011USDV)
	Application Number	
Title of Invention	LIPOPEPTIDE COMPOSITIONS AND RELATED METHODS	

The application data sheet is part of the provisional or nonprovisional application for which it is being submitted. The following form contains the bibliographic data arranged in a format specified by the United States Patent and Trademark Office as outlined in 37 CFR 1.76.
This document may be completed electronically and submitted to the Office in electronic format using the Electronic Filing System (EFS) or the document may be printed and included in a paper filed application.

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☐ Portions or all of the application associated with this Application Data Sheet may fall under a Secrecy Order pursuant to 37 CFR 5.2 (Paper filers only. Applications that fall under Secrecy Order may not be filed electronically.)

Inventor Information:

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Legal Name

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	Sandra		O'Connor	

Residence Information (Select One) ◉ US Residency ○ Non US Residency ○ Active US Military Service

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Inventor 2 Remove

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	Application Number	
Title of Invention	LIPOPEPTIDE COMPOSITIONS AND RELATED METHODS	

City	Cambridge	**State/Province**	MA	**Country of Residence** i	US

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City	Cambridge	**State/Province**	MA
Postal Code	02138	**Country** i	US

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Customer Number	113613		
Email Address	bostonpatent@lathropgage.com	Add Email	Remove Email

Application Information:

Title of the Invention	LIPOPEPTIDE COMPOSITIONS AND RELATED METHODS		
Attorney Docket Number	552815 (CPT-011USDV)	**Small Entity Status Claimed**	☐
Application Type	Nonprovisional		
Subject Matter	Utility		
Total Number of Drawing Sheets (if any)	22	**Suggested Figure for Publication (if any)**	

Publication Information:

☐ Request Early Publication (Fee required at time of Request 37 CFR 1.219)

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Application Data Sheet 37 CFR 1.76	Attorney Docket Number	552815 (CPT-011USDV)
	Application Number	
Title of Invention	LIPOPEPTIDE COMPOSITIONS AND RELATED METHODS	

Customer Number	113613

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When referring to the current application, please leave the application number blank.

Prior Application Status	Pending		Remove
Application Number	Continuity Type	Prior Application Number	Filing Date (YYYY-MM-DD)
	Division of	13511246	2012-07-10
Prior Application Status			Remove
Application Number	Continuity Type	Prior Application Number	Filing Date (YYYY-MM-DD)
13511246	a 371 of international	PCT/US2010/057910	2010-11-23
Prior Application Status			Remove
Application Number	Continuity Type	Prior Application Number	Filing Date (YYYY-MM-DD)
PCT/US2010/057910	Claims benefit of provisional	61263784	2009-11-23
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Foreign Priority Information:

This section allows for the applicant to claim priority to a foreign application. Providing this information in the application data sheet constitutes the claim for priority as required by 35 U.S.C. 119(b) and 37 CFR 1.55(d). When priority is claimed to a foreign application that is eligible for retrieval under the priority document exchange program (PDX) [i] the information will be used by the Office to automatically attempt retrieval pursuant to 37 CFR 1.55(h)(1) and (2). Under the PDX program, applicant bears the ultimate responsibility for ensuring that a copy of the foreign application is received by the Office from the participating foreign intellectual property office, or a certified copy of the foreign priority application is filed, within the time period specified in 37 CFR 1.55(g)(1).

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Application Number	Country [i]	Filing Date (YYYY-MM-DD)	Access Code [i] (if applicable)
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Application Data Sheet 37 CFR 1.76	Attorney Docket Number	552815 (CPT-011USDV)
	Application Number	
Title of Invention	LIPOPEPTIDE COMPOSITIONS AND RELATED METHODS	

Statement under 37 CFR 1.55 or 1.78 for AIA (First Inventor to File) Transition Applications

☐ This application (1) claims priority to or the benefit of an application filed before March 16, 2013 and (2) also contains, or contained at any time, a claim to a claimed invention that has an effective filing date on or after March 16, 2013.
NOTE: By providing this statement under 37 CFR 1.55 or 1.78, this application, with a filing date on or after March 16, 2013, will be examined under the first inventor to file provisions of the AIA.

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In accordance with 37 CFR 1.14(c), access may be provided to information concerning the date of filing this Authorization.

Applicant Information:

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Application Data Sheet 37 CFR 1.76	Attorney Docket Number	552815 (CPT-011USDV)
	Application Number	
Title of Invention	LIPOPEPTIDE COMPOSITIONS AND RELATED METHODS	

Applicant 1 Remove

If the applicant is the inventor (or the remaining joint inventor or inventors under 37 CFR 1.45), this section should not be completed. The information to be provided in this section is the name and address of the legal representative who is the applicant under 37 CFR 1.43; or the name and address of the assignee, person to whom the inventor is under an obligation to assign the invention, or person who otherwise shows sufficient proprietary interest in the matter who is the applicant under 37 CFR 1.46. If the applicant is an applicant under 37 CFR 1.46 (assignee, person to whom the inventor is obligated to assign, or person who otherwise shows sufficient proprietary interest) together with one or more joint inventors, then the joint inventor or inventors who are also the applicant should be identified in this section.

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Name of the Deceased or Legally Incapacitated Inventor :

If the Applicant is an Organization check here. ☒

Organization Name	Cubist Pharmaceuticals, Inc.		
Mailing Address Information:			
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Address 2			
City	Lexington	**State/Province**	MA
Country [i]	US	Postal Code	02421
Phone Number		Fax Number	
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	Application Number	
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Mailing Address Information For Assignee including Non-Applicant Assignee:

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Signature	/Brian C. Trinque, Ph.D., Esq.			Date (YYYY-MM-DD)	2013-12-04
First Name	Brian C.	Last Name	Trinque	Registration Number	56593

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