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Original Paper

Influence of the glass packing on the contamination of pharmaceutical products by aluminium. Part II: Amino acids for parenteral nutrition

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Abstract

The presence of aluminium in amino acids parenteral nutrition solutions can be related to the affinity of the amino acids for aluminium present in glass containers used for storage. For this study solutions of 19 amino acids used in parenteral nutrition were stored individually in glass flasks and the aluminium measured at determined time intervals. Solutions of complexing agents for aluminium, as ethylene-diaminetetraacetic acid, nitrilotriacetic acid, citrate, oxalate and fluoride ions were also stored in the same flasks and the aluminium measured during the same time interval. The measurements were made by electrothermal atomic absorption spectrometry. The aluminium content of the glass containers was also measured. The results showed that the glasses have from 0.6% to 0.8% Al. Only solutions of cysteine, cystine, aspartic acid and glutamic acid became contaminated by aluminium. As the same occurred with the complexing agents, aluminum can be released from glass due to an affinity of the substances for aluminium. Comparing the action of complexing agents and amino acids for which the stability constants of aluminium complex are known, it is possible to relate the magnitude of the stability constant with the aluminium leached from glass, the higher the stability constant, the higher the aluminium released. The analysis of commercial formulations with and without cysteine, cystine, glutamic acid or aspartic acid stored in glass containers confirms that the presence of these amino acids combined with the age of the solution are, at least partially, responsible for the aluminium contamination. The results demonstrated that the contamination is an ongoing process due to the presence of aluminium in glass combined with the affinity of some amino acids for this element.

Key words: aluminium contamination, glass containers, amino acids, parenteral nutrition

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Introduction

The presence of aluminium as contaminant in parenteral nutrition (PN) solutions is well-known and has been very discussed in the literature in least years (1–5). Many studies showed that PN solutions are contaminated with aluminium even when they have different compositions and are the same products but from different brands (6, 7).

Although aluminium contamination of PN solutions is suspected to cause impaired bone growth or neurological development in preterm infants since at least 15 years (8–14), the contamination of commercial intravenous-feeding solutions is still today a trouble (15–18).

Parenteral preparations should be stored in containers that do not interact physically or chemically with the preparations, and are made of a transparent material that do not permit diffusion into or across the walls of the container. Glass complies these general requirements, and pharmacopoeias (19, 20) prescribe the type of glass preferable for each parenteral preparation in the individu-

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al monographs. Containers composed of glass should meet the requirements for chemical stability (19, 20), but this chemical stability is expressed only by the hydrolytic resistance, that is evaluated by tritating the alkalinity released into water under prescribed conditions of contact. According to their hydrolytic resistance glass containers are classified in Type I, II, III or IV, being Type I or II, due to their high hydrolytic resistance, suitable for aqueous preparations for parenteral use. This high chemical resistance is however obtained by addition of oxides to glass, mainly boric and aluminium oxides (21); and consequently turns glass a source of aluminium, and increases the possibility of contamination.

The presence of aluminium in PN solutions could be related to an interaction of these solutions with the aluminium present in the glass container. The release of aluminium from glass into acidic or alkaline solutions can be explained by the action of the medium on the glass surface; low pH favours exchange of metal ions from glass, whereas high pH solutions promotes the dissolution of the glass surface itself (21). Solutions of amino acid do not show pH at extreme values and therefore their action could not be related to processes similar to those of acids and alkalis. Considering that they form complexes with some metals and therefore act as ligands for metal ions (22), this complex formation could be responsible for the extraction of aluminium from glass by some amino acids.

In this work the action of amino acids used for PN on the aluminium present in glass containers was studied and compared with the action of substances that complex aluminium. The aim is to show that the affinity of some amino acids for aluminium is the main factor in their leaching action on glass, and therefore that the glass container can be source of contamination of amino acids PN solutions by this metal.

Material and Methods

Apparatus

A Varian Spectra AA200 atomic absorption spectrometer equipped with a GTA-100 graphite furnace and an autosampler (Melbourne, Australia), a Trox class 100 clean bench (Curitiba, Brazil), Digimed pHmeter D-20 (São Paulo, Brazil) and a Philco microwave oven (São Paulo, Brazil) were used.

Reagents

All chemicals were of analytical-reagent grade. All aqueous solutions were prepared with distilled and deionized water, that was further purified by a Milli-Q high purity water device (Millipore, Bedford, USA). An aluminium stock standard solution containing 1000 mg/l Al was prepared from aluminium nitrate nonahydrate (Merck, Darmstadt, Germany) and working standard solutions by suitable dilutions of the stock solution. The amino acids were from different suppliers and the solutions were prepared by dissolution of the amino acid in Milli-Q purified water at the concentration showed in Table 1.

Contamination control

To avoid contamination, only plastic materials were used. All laboratory ware (pipette tips, volumetric flasks, etc.) was immersed for at least 48 h in a 10% HNO_3 in ethanol (v/v) mixture and shortly before the use washed with Milli-Q purified water.

To avoid contamination from the air, all steps in the sample and reagents preparation were carried out in a class 100 clean bench.

Glass analysis

All glasses used throughout this work were analysed to determine their hydrolytic resistance (19) and aluminium content. The glass containers were crushed into fragments about 1 mm in size, and 0.1 g of these was placed in a PTFE vessel with 5 ml of HF (48% m/m) (Merck, Darmstadt, Germany) and 5 ml of water, and heated in a domestic microwave oven at a minimal power (174 W) for 10 min, the procedure was repeated twice. After the total dissolution of the sample the volume was completed to 200 ml with water and the aluminium measured by flame atomic absorption spectrometry (FAAS).

First experiment

Solutions of 19 amino acids were stored separately in 10 ml type II glass flasks. The amino acids concentrations (Table 1) were the same of Soramin 10% (Darrow Laboratórios S.A., São Paulo, Brazil) or Aminon 20 (J.P. Indús-

Table 1. Aluminium content of amino acids

Amino acid	Brand	Aa conc. (g/L)	Al solution[1] (µg/L)	Al substance (µg Al/g Aa)
Alanine	Riedel de Haën	15.0	8.9 ± 0.3	0.59
Arginine	Merck	12.0	3.6 ± 0.4	0.30
Aspartic acid	Labsynth	2.7	2.6 ± 0.4	0.96
Cysteinium chloride	Merck	0.72	4.0 ± 0.03	5.55
Cystine	Merck	0.3	9.8 ± 0.2	32.67
Glutamic acid	Ecibra	4.6	10.1 ± 0.2	2.20
Glycine	Merck	8.0	3.8 ± 0.02	0.48
Histidine	Verp	5.0	14.8 ± 1.3	2.96
Isoleucine	Sigma	5.1	< 1	< 0.2
Leucine	Merck	9.8	2.8 ± 0.3	0.29
Lysine chlorhydrate	Riedel de Haën	6.6	16.2 ± 1.9	2.45
Methionine	Merck	5.3	< 1	< 0.2
Phenylalanine	Merck	5.4	10.3 ± 0.7	1.91
Proline	Merck	15.0	1.8 ± 0.2	0.12
Serine	Sigma	2.5	< 1	< 0.4
Threonine	Sigma	4.9	4.5 ± 0.6	0.92
Tryptophan	Vetec	2.0	2.5 ± 0.3	3.65
Tyrosine	Merck	1.6	< 1	< 0.6
Valine	Merck	6.2	< 1	< 0.2

[1] n = 3

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tria Farmacêutica S.A., São Paulo, Brazil) parenteral solutions. The flasks were closed and left to shake at room temperature during all the time of the experiment. The aluminium content of these solutions was measured by electrothermal atomic absorption spectrometry (ETAAS) 15, 60 and 90 days after they have been stored. The blank was the aluminium measured in these solutions just before their storage. The solutions were prepared in polyethylene volumetric flasks previously decontaminated as above described. The samples were assayed in triplicate.

All glass flasks for storage of amino acid solutions were placed before the use in a muffle oven at 560 °C for 12 h to make their surface free of contamination from adsorbed substances.

Second experiment

The second experiment was carried out with the amino acids that extracted aluminium from the glass containers in the first experiment. 500 ml of solution 0.017 mol/l of cystine, cysteine, aspartic acid and glutamic acid were placed separately in PN glass bottles (500 ml), that were previously heated at 560 °C as above described. The flasks were shaken during all the time of the experiment. The aluminium content of these solutions was measured 15, 30 and 60 days after the storage at room temperature by ETAAS. The pH of the solutions was measured at the beginning and at the end of the experiment. The same procedure was carried out with solutions of citric acid, sodium fluoride, oxalic acid, ethylene-diaminetetraacetic acid (EDTA) and nitrilotriacetic acid (NTA), all from Merck (Darmstadt, Germany), at the same concentration of 0.017 mol/l. All samples were assayed in duplicate. As the aluminium concentration in these solutions after storing was too high, the analysis was done either by FAAS or ETAAS.

Extraction rates

Solution 0.028 mol/l of cystine, cysteine, aspartic acid, glutamic acid, arginine, lysine glycine and alanine were stored in glass bottles for PN solutions (500 ml) at room temperature and left to shake. The aluminium in these solutions was measured by ETAAS at intervals of 10 days for 60 days and after that at intervals of 30 days for a year. The samples were assayed in duplicate.

Analysis of commercial amino acid parenteral solutions

Seven different formulations of amino acids for parenteral nutrition were analysed. At least 3 bottles of each batch were analysed. The formulations showed different compositions, but only one of them had no cysteine, cystine, aspartic or glutamic acid; from this one three sets of 3 bottles with different storage times were analysed. The other six formulations had different storage times. From one of these formulations ten bottles of the same batch were analysed.

The aluminium determination was carried out by ETAAS; when necessary the samples were diluted to have their aluminium concentration within the range of the analytical curve.

To check the accuracy of the aluminium measurement in these amino acid formulations recovery experiments were carried out. Three different formulations were spiked with 25 µg Al/l and the aluminium measured by ETAAS as above.

Table 2. Aluminium leached from glass container by different amino acid solutions as a function of time of storage at room temperature. The concentration of amino acids is described in Table 1

Amino acid	pH initial	Al leached (µg Al/g Aa/L) ± RSD			pH final
		15 days	60 days	90 days	
Alanine	5.5	*	*	< 1	6.0
Arginine	8.5	3 ± 0.5	4 ± 1	4 ± 1	8.5
Aspartic acid	1.7	35 ± 4	75 ± 5	106 ± 9	2.5
Cysteine	1.3	230 ± 22	725 ± 67	1056 ± 99	1.0
Cystine	6.0	458 ± 24	1661 ± 75	3026 ± 246	6.0
Glutamic acid	2.8	27 ± 2	68 ± 8	87 ± 10	3.0
Glycine	5.9	*	*	*	5.9
Histidine	8.1	< 1	1 ± 0.1	3 ± 0.3	8.1
Isoleucine	6.1	*	*	*	6.2
Leucine	5.9	*	*	*	6.0
Lysine	7.0	2 ± 0.2	4 ± 0.2	6 ± 0.4	7.0
Methionine	5.6	*	*	*	5.6
Phenylalanine	5.5	*	*	*	5.5
Proline	6.2	*	*	*	6.3
Serine	7.0	*	*	*	7.0
Threonine	6.8	*	*	*	6.8
Tryptophan	5.8	*	*	*	5.8
Tyrosine	7.0	*	*	*	6.9
Valine	5.8	< 1	< 1	1 ± 0.2	5.9

* No significant difference (n=3) between the mean value and the blank. For blank values see Table 1

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Results and discussion

The glass flasks used to store the individual amino acid solutions had an aluminium content of 0.6% and the glass bottles of commercial amino acid parenteral solutions about 0.8% Al.

In Table 1 it is shown the aluminium already present as impurity in the assayed amino acids. Whereas isoleucine, methionine, serine, tyrosine and valine showed no contamination, cysteine and cystine were the highest contaminated: ca 6 and 33 µg Al/g amino acid respectively. The analysis of other cystine samples of different batches and from different brands showed also high aluminium content: two samples from Merck had 29 and 63 µg Al/g, and one sample from Sigma 45 µg Al/g.

The results in Table 2 show that solutions of cystine, cysteine, aspartic acid, glutamic acid, lysine and arginine extracted aluminium from glass, whereas other amino acids did not.

The higher releasing occurred in solutions of cystine, cysteine, aspartic acid and glutamic acid. The action of these amino acids is still stronger than salts (showed in Part I of this work). As the pH of the solutions of these amino acids is between 1 and 6, and they are no ionic substances, their action cannot be related to pH, as occurred with HCl and NaOH, nor to an ion exchange process as with salts.

In Figure 1 it can be seen that all investigated complexing agents extract an elevate amount of aluminium from glass and the action of amino acids can be compared with the action of these substances. As the molar concentration of the substances, nature of the glass, temperature and time of contact are the same, it could be possible to relate the leaching action of the substance to its affinity for aluminium.

Amino acids complex metals (22); the aluminium complexes of glutamic and aspartic acids have known stability constants, and although the stability constant for cys-Al is not found in the literature, cysteine must interact with Al as it interacts with other metals; this could explain its great leaching action on the aluminium present in glass. Whether the stability constants (23–26) are placed in decreased order, the aluminium in the solutions of these substances arising from glass is in decreased order too (Figure 1). Cysteine is a ligand for aluminium as stronger as citrate or oxalate.

The pH of the solution could play a small role in the action of arginine, lysine and histidine. They do not form any known complex with aluminium but due to their alkaline character they act on the glass surface and the aluminium is released into the solution.

Figure 2 shows that an equilibrium between the aluminium in solution and on the glass surface is established, but it depends on the nature of the substance and the time of contact. Whereas for arginine, lysine, aspartic and glutamic acid this equilibrium is reached in nearby 90

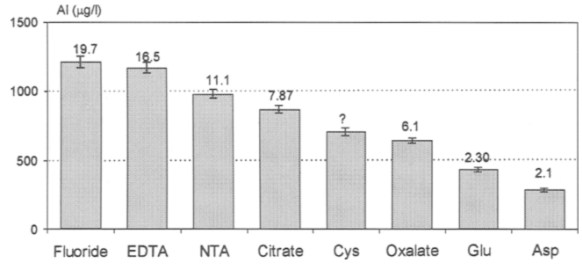

Figure 1. Aluminium released from PN glass bottles by action of 0.017 mol/l solution of amino acids and complexing agents after 60 days storage at room temperature. The numbers above the bars are the stability constants (log *K*) of the aluminium complexes (24, 25).

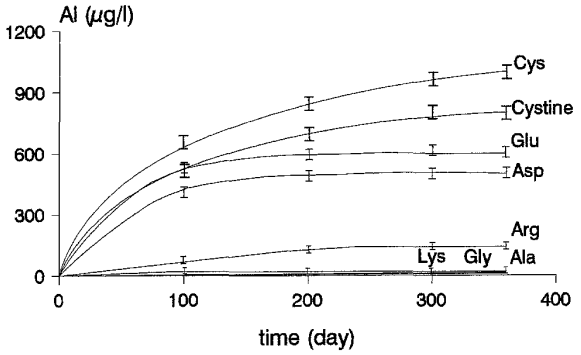

Figure 2. Aluminium leached from glass containers by amino acids as function of time at room temperature. Amino acids concentration: 0.028 mol/L.

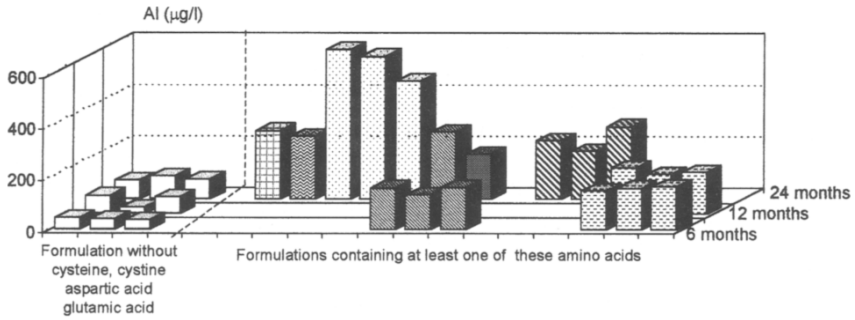

Figure 3. Aluminium measured in different commercial amino acid parenteral formulations. The samples were shared according to the age, 6, 12 or 24 months old. Each bar corresponds to a mean value of three samples. Each bar type corresponds to a different formulation.

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days, for cysteine and cystine after 120 days the aluminium in solution still increases.

When polyethylene containers were used in place of glass, no increase in the aluminium concentration was observed over the same time period.

Commercial amino acid parenteral solutions

Seven types of commercial amino acid parenteral solutions were analysed. Only one of these formulations contained no cysteine, cystine, aspartic acid or glutamic acid.

As the samples were supplied by the university hospital, they had different storage times, and to simplify the presentation of the data, they were shared in three groups according to their age at the time of the analysis: 6, 12 or 24 months. From some formulations we had samples of three different batches (and different storage times) but from others samples only one batch (but three bottles) was obtained. The results of this analysis are in Figure 3. It can be seen that formulations with cysteine, cystine, aspartic acid or glutamic acid showed more aluminium than the formulation without these amino acids. The results also shown that the storage time could be responsible for higher contamination. Whereas in the formulation without cysteine, cystine aspartic acid or glutamic acid the aluminium accumulation was not higher than 80 µg/l, even after two years storage, all the others showed accumulations above this value even after 6 months storage.

In Figure 4 are the results of ten samples of the same batch. The aluminium in these samples varied from 101 to 193 µg/l with a mean deviation of ±18 µg/l. It can be seen that the aluminium present in these samples is not random, within the same lot of samples the aluminium levels were reproducible, and the contamination always occurred.

To assess the accuracy of measuring aluminium in commercial amino acid formulations, three different formulations were spiked with 25 µg Al/l and the aluminium content measured by ETAAS. Recoveries between 88 and 105% showed that the results of the aluminium present in these solutions are reliable.

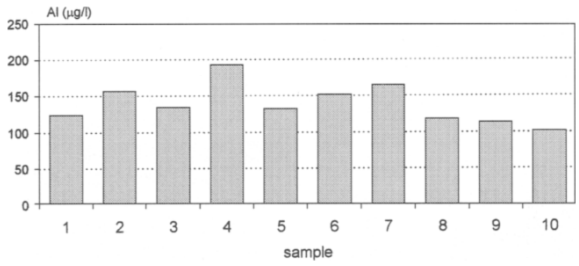

Figure 4. Aluminium measured in ten samples of one batch of the same formulation. Pediamino PLM 10% (Lab. B. Braun S.A.), containing cysteine and glutamic acid. Measurement done after 6 months storage.

Conclusion

Cysteine, cystine, aspartic acid and glutamic acid are able to release aluminium from glass whereas the other amino acids are not.

This action can be related to their affinity for aluminium like some complexing agents, EDTA, oxalic acid, citric acid and fluoride ions, considering that they extracted aluminium from glass in the same extension of these agents. A relationship between the stability constant of the aluminium complexes and the aluminium released from glass was observed; the higher the stability the higher the aluminium released.

All analysed commercial formulations of amino acids showed aluminium, and like occurred with prepared individual solutions, the composition and age of the solution are important factors for the aluminium contamination. Formulations containing cysteine, cystine, glutamic acid and aspartic acid that have been stored for several months are the most contaminated.

The difference of the aluminium extracted by different formulations can be due to the composition and interactions between amino acids; with regard to the individual solutions of cystine, cysteine, aspartic acid and glutamic acid, the difference could be attributed, besides the presence of several amino acids in the formulations, to the maintenance of the pH at a value near the neutral in the commercial formulations.

Although aluminium in amino acid parenteral solutions is not very high, the contamination of these solutions occurs and could be, at least partially, attributed to their storage in glass containers. This contribution is the most troubling because it is ongoing until the product is used; over many months of storage, the aluminium contribution of the glass container predominates.

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