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Nutrition

Influence of the glass packing on the contamination of pharmaceutical products by aluminium. Part III: Interaction container-chemicals during the heating for sterilisation

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Abstract

The interaction of chemicals with the container materials during heating for sterilisation was investigated, storing the components of parenteral nutrition solutions individually in sealed glass ampoules and in contact with a rubber stopper, and heating the system at 121 °C for 30 min. Subsequently, the aluminium content of the solutions was measured by atomic absorption spectrometry (AAS). The assay was also carried out with acids, alkalis and some complexing agents for Al. The containers were decomposed and also assayed for aluminium. 30 different commercial solutions for parenteral nutrition, stored either in glass or in plastic containers, were assayed measuring the aluminium present in the solutions and in the container materials. The results of all investigated container materials revealed an aluminium content of 1.57% Al in glass, 0.05% in plastic and 4.54% in rubber. The sterilisation procedure showed that even pure water was able to extract Al from glass and rubber, 22.5 ± 13.3 µg/L and 79.4 ± 22.7 µg/L respectively, while from plastic the aluminium leached was insignificant. The Al released from glass ampoules laid between 20 µg/L for leucine, ornithine and lysine solutions and 1500 µg/L for solutions of basic phosphates and bicarbonate; from rubber stoppers it reached levels over 500 µg/L for cysteine, aspartic acid, glutamic acid and cystine solutions. Ion-exchange properties and influence of pH can explain the interaction of glass with some chemicals (salts, acids and alkalis), but only an affinity for aluminium could explain the action of some amino acids and other chemicals, as albumin and heparin, on glass and rubber, considering the aluminium release. Experiments with complexing agents for Al allowed to conclude that the higher the stability constant of the complex, the higher the Al release from the container material.

Key words: aluminium, contamination, glass, rubber, sterilisation, parenteral nutrition

Introduction

Parenteral nutrition (PN) is the administration of nutrients intravenously to patients that cannot be fed via gastrointestinal tract. Products for parenteral nutrition are commercialised in form of sterile solutions, and include electrolytes (Na, K, Ca, Mg, chloride, bicarbonate, acetate and phosphate), amino acids, carbohydrates (glucose and polyols), oligoelements (Cu, Zn, Cr, Mn), albumin, vitamins and lipids. Although the presence of Al in pharmaceutical products used parenterally is known at least for 18 years (1, 2), this is still an unsolved problem today.

Al has a toxic effect to children and adults with chronic renal failure and to patients on long-term parenteral

Dedicated to Professor Dr. Georg Schwedt on the occasion of his 60th birthday.

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nutrition (3–7). Moreover, Al can also be associated with impaired neurologic development in preterm infants, which receive prolonged intravenous feeding (8–11).

The American Societies for Clinical Nutrition (ASCN), for Parenteral and Enteral Nutrition (ASPEN) (12) and for Pediatric Gastroenterology and Nutrition (ASPGN) (13) proposed to restrict the Al contamination of large volume parenterals to a maximum of 25 µg/L, and although for small volume parenterals no maximum level was proposed, the label should inform how many µg Al/L the product contains. Studies have been showing, however, that Al found in these formulations is almost always over the proposed limit (14–16).

Several investigations (17–20) dealt with the presence of Al in these formulations but none of them offered explanations for the origin of the Al contamination. The results found by all authors revealed, however, that Al in these products can reach levels over 5000 µg/L, and that even being produced by different manufacturers and in different countries, the distribution of the contamination in these products is the same: calcium gluconate, phosphate salts and oligoelements are the most contaminated, followed by vitamins and albumin, while dextrose and amino acids are among the least contaminated.

Two possible sources can be suspected of contributing to the presence of Al in these products: raw material and containers, not considering any step of the industrial processing of these formulations. In spite of the high probability of the contamination by these sources, we found only two papers which related the contamination of albumin (21) and gluconate (22) solutions to their storage in glass containers.

In previous works we investigated the influence of the storage time of the solutions in contact with the glass and plastic surface of the containers considering the shelf-live of the products (23, 24), and the contribution of the Al present as impurity in the chemicals to the contamination of the solutions for PN (25).

In the present work we investigated glass, plastic and rubber as container materials considering their interaction with the chemicals and possible Al releasing during the heating for the sterilisation procedure.

Experimental

Apparatus

A Varian SpectrAA-200 atomic absorption spectrometer equipped with a GTA-100 graphite furnace and an autosampler (Melbourne, Australia), a Trox class 100 clean bench (Curitiba, Brasil), a Phoenix AV 50 N 6281 autoclave (São Paulo, Brasil), a Berghof BSB 939-IR sub-boiling distillation apparatus (Eningen, Germany) and a Digimed pHmeter D-20 (São Paulo, Brasil) were used.

Reagents

The water used throughout was distilled, deionised and further purified by a Milli-Q high purity water device (Millipore, Bedford, USA). An Al standard solution containing 1000 mg/L Al (Merck, Germany) was used to prepare the working standard solutions. HNO_3 (65%, 1.40 g/mL) from Merck was further purified by sub-boiling distillation.

Contamination control

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

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

Procedures

The assayed chemicals included besides salts, amino acids, glucose, albumin, heparin and some polyols used in parenteral nutrition: acetic acid, hydrochloric acid, sodium hydroxide, sodium nitrate, sulfate and gluconate, calcium chloride, magnesium chloride, zinc chloride, manganese chloride, copper chloride, chromium chloride, and the complexing agents for Al EDTA, NTA, oxalic acid and citric acid. Although heparin is not used for parenteral nutrition, it was included in the experiment. The salts above cited that are not used in parenteral nutrition were included in the experiment in order to compare the influence of different cations and anions on the interaction with the container material. The substances were of "for analysis" quality and from different manufactures (Merck, Sigma, Aldrich, Riedel-de Haën or Ajinomoto). For the analysis, solutions containing 0.1, 1.0 or 10.0% (m/v) of the substance were prepared in polyethylene volumetric flasks, and the Al content measured by flame or electrothermal atomic absorption spectrometry (FAAS or ETAAS, respectively), following the conditions described in Table 1. The chloride containing solutions were analysed directly by FAAS or by ETAAS, after a treatment to separate the saline matrix according to a method earlier described (26), because chloride ions cause interferences on graphite furnace measurements.

Solutions of amino acids were also prepared 0.1, 1.0 or 10.0% (m/v) according to their solubility or Al content. For the amino acids which solubility did not allow complete dissolution, HNO_3 was added to promote the solubilisation.

Heparin was dissolved to give a solution with the same concentration (5000 UI/mL) as the commercial product.

All samples were prepared in triplicates and the measurement was carried out just after the preparation.

Analysis of the containers

Polyethylene ampoules (10 and 20 mL), glass ampoules (10 and 20 mL), bottles for amino acid solutions (100, 250 and 500 mL), and grey and red elastomeric closures for amino acid bottles, all from commercial products, were assayed for their Al content.

The glass containers were crushed into fragments about 1 mm in size and well mixed. One hundred milligrams of the glass fragments was placed in a PTFE vessel with 5 mL of 48% (m/m) hydrofluoric acid 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 Al measured by FAAS.

The plastic ampoules were cut into small pieces and a portion of 1 g was mixed with approximately 2 g of KNO_3 and heated in a platinum crucible at 250 °C for 3 hours. The ash was dissolved in 4 mL of 96% (m/m) H_2SO_4 and diluted to 50 mL with water. Al was measured by FAAS.

The decomposition of the rubber stoppers was carried out by heating 0.2 g of the rubber in a platinum crucible at 600 °C for 2 hours. The ash was dissolved by addition of 6 mL hydrofluoric acid 1+1 with water, and gently heated. The final volume was made up to 100 mL with water. The Al measurement was carried out by FAAS.

A blank experiment was carried out using only the reagents and including all steps of the three different decomposition procedures.

Preparation of the samples for moist heat sterilisation

As the analysis revealed a very low level of Al in plastic containers, the heating procedure was carried out only with glass containers; moreover, the usual procedure for sterilising parenterals in plastic containers is not through heat but by using ethylene dioxide or radiation (27).

10 mL new glass ampoules for injectables (Schott do Brasil) were filled with a 0.5% (m/v) solution of each substance selected for the assay. The ampoules were sealed and heated at 121 °C in an autoclave for 30 min. After cooling down they were opened and the Al content of the solution measured by FAAS or ETAAS. The Al already present in the substances was discounted from the Al measured. The experiment was carried out in triplicates. Twelve ampoules filled only with pure water were also submitted to the sterilisation procedure. Three new ampoules were assayed individually for their Al content as described in the previous section.

Table 1. Atomic absorption spectrometer operating conditions.

Instrument			
Wavelength (nm)	309.3		
Lamp current (mA)	10		
Spectral slit width (nm)	0.5		
Background correction	Deuterium lamp		
Flame	Acetylene/nitrous oxide		
Graphite furnace	Pyrolytic coated furnace with L'vov platform and argon as purge gas		
	Temperature programme		
Step	Temperature (°C)	Time (s)	Gas flow (L/min)
1	85	5	3.0
2	95	35	3.0
3	120	10	3.0
4	1100	20	3.0
5	1100	10	3.0
6	1100	2	0.0
7*	2500	0.7	0.0
8*	2500	1	0.0
9	2600	1	3.0

*read

Closure experiments

Among the investigated commercial products for parenteral nutrition only the amino acids and albumin solutions were stored in bottles with elastomeric closures. Therefore the assay was carried out with amino acid solutions, and also with the complexing agents EDTA, NTA, citric acid and oxalic acid, and with hydrochloric acid, acetic acid and sodium hydroxide. A rubber stopper (grey) was stored individually in 200 mL of each solution in a polypropylene flask and submitted to the sterilisation procedure as described above. The assay was also carried out with the closure in contact with pure water. The experiments were carried out in triplicates. Three pieces of these closures were also assayed to determine their Al content.

Analysis of commercial products

Commercial solutions of salts, glucose, heparin and albumin and formulations containing amino acids were analysed. With the exception of 10% and 20% NaCl, 10% and 19.1% KCl and ampoules of oligoelements (Zn, Cu, Cr and Mn salts), the Al was measured directly by FAAS or ETAAS (some samples had to be diluted to have their Al concentration in the range of the calibration curves). The Al present in these salt solutions was determined according to the method mentioned above, after matrix separation (26). For all products, at least 3 samples of the same batch were analysed, and the reported results correspond to the mean value calculated from these replicates.

Results

Containers and closures

The analysis of containers and closures used for storing of commercial parenteral solutions showed that Al was present in all three types of materials: plastic, glass and rubber (Table 2). However, whereas in plastic containers only a small amount was found, probably from catalysts used for plastic polymerisation, Al is present in high levels in glass and rubber. Aluminium oxide is added to glass to improve its chemical resistance, so that glass for parenterals contains normally from 2.6 to 6.6% Al_2O_3 (28). Rubber can contain Al as well. Hydrate or calcined aluminium silicate (clay) is used as filler in elastomeric materials to improve hardness, abrasion resistance and density (29). In spite of the fact that other materials can be used as filler, Al was found in all rubber analysed in this work.

Sterilisation

The ampoules used for this experiment presented an Al level of 2.14 ± 0.47%.

Table 2. Aluminium present as contaminant in commercial parenteral solutions and in container materials. All solutions were within their guaranteed period of shelf-life.

Product	Al in solution ±SD (µg/L)	Container	Al in container (%)
NaCl 20%	149 ± 10	glass ampoule	1.43
	13 ± 4	polyethylene	0.04
KCl 10%	68 ± 6	glass ampoule	1.25
	23 ± 5	polyethylene	0.06
Magnesium sulfate 50%	560 ± 85	glass ampoule	1.25
	380 ± 288	glass ampoule	1.43
Sodium acetate 2 meq/mL	45 ± 7	glass ampoule	2.14
	17 ± 8	polyethylene	0.05
Potassium phosphate 2 meq/mL	988 ± 76	glass ampoule	1.98
	1325 ± 142	glass ampoule	2.45
Sodium phosphate 0.5 mol/L	933 ± 88	glass ampoule	1.65
	879 ± 203	glass ampoule	2.05
Calcium gluconate 10%	5621 ± 1165	glass ampoule	1.51
	5960 ± 62	glass ampoule	2.21
Sodium bicarbonate 8.4%	833 ± 141	glass bottle	0.99
	922 ± 102	glass bottle	1.03
Oligoelements [a]	1129 ± 33	glass ampoule	2.14
Oligoelements [b]	1854 ± 744	glass ampoule	2.21
Amino acids 10%	164 ± 6	glass bottle	0.82
		rubber closure	3.91
Amino acids 10%	116 ± 30	glass bottle	0.76
		rubber closure	4.23
Amino acids 10%	93 ± 23	glass bottle	0.84
		rubber closure	5.34
Amino acids 10%	65 ± 13	glass bottle	0.89
		rubber closure	5.70
Amino acids 10%	23 ± 8	plastic bag	0.01
Glucose 50%	13 ± 1	polyethylene	0.04
	293 ± 14	glass ampoule	1.15
Glucose 25%	9 ± 3	polyethylene	0.08
	370 ± 23	glass ampoule	1.87
Albumin 20%	644 ± 58	glass flask	0.67
		rubber closure	4.06
	149 ± 23	glass flask	0.66
		rubber closure	3.99
Heparin 5000 UI/mL	732 ± 23	glass ampoule	2.88
	738 ± 54	glass ampoule	3.03

[a] Composition: 22.0 mg $ZnSO_4$, 6.3 mg $CuSO_4$, 2.46 mg $MnSO_4$, 102.5 µg $CrCl_3$ per ampoule

[b] Composition: 8.8 mg $ZnSO_4$, 1.60 mg $CuSO_4$, 123.04 µg $MnSO_4$, 20.50 µg $CrCl_3$ per ampoule

Suppliers: Abbott, Ariston, Aster, Baxter, Behring, B. Braun, Darrow, Fresenius, Fujisawa, Gayer, Halex Istar, Hypofarma, Santisa, Roche, Zenalb

The first step of the investigation on Al release from glass during the sterilisation procedure was carried out with pure water. The results of ampoules filled only with water showed that even pure water is capable to extract Al from the glass surface (Fig. 1). A mean value of 22.5 ± 13.3 µg/L Al was measured in the water samples after heating for sterilisation.

Fig. 2 shows the Al extracted from glass ampoules filled with solutions of the different investigated substances during the moist heat procedure. Among the salt solutions, those having a basic character showed the highest interaction. Whereas chlorides and sulfates extracted a mean of 200 µg/L Al, basic phosphates and bicarbonate extracted more than 900 µg/L Al (see also Fig. 3a). Al released by the NaOH solution (4500 µg/L) confirms the stronger interaction of the alkaline solutions with glass. Among the amino acids, there were some that did not extract Al from the glass ampoule, and others that were able to extract a large amount of Al. Solutions of cystine, cysteine, aspartic acid, glutamic acid and tyrosine extracted more than 200 µg/L respectively, whereas solutions of lysine, ornithine and leucine extracted as much Al as pure water. Fig. 2 also shows Al extracted by action of complexing agents and the polyols mannitol, sorbitol and xylitol.

Fig. 3 shows Al extracted by action of salts, grouped according to their constituents: different anions having the same cation in Fig. 3a, and different cations bound to the same anion in Fig. 3b. For this comparison, salts not used in parenterals were included; the aim was to evaluate the action of cations and anions respectively. Con-

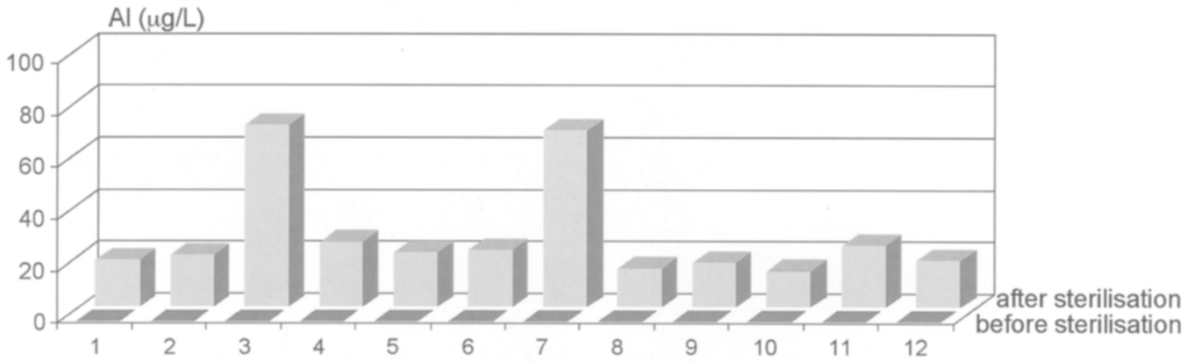

Fig. 1. Aluminium extracted from glass ampoules during sterilisation at 121 °C for 30 min by action of pure water.

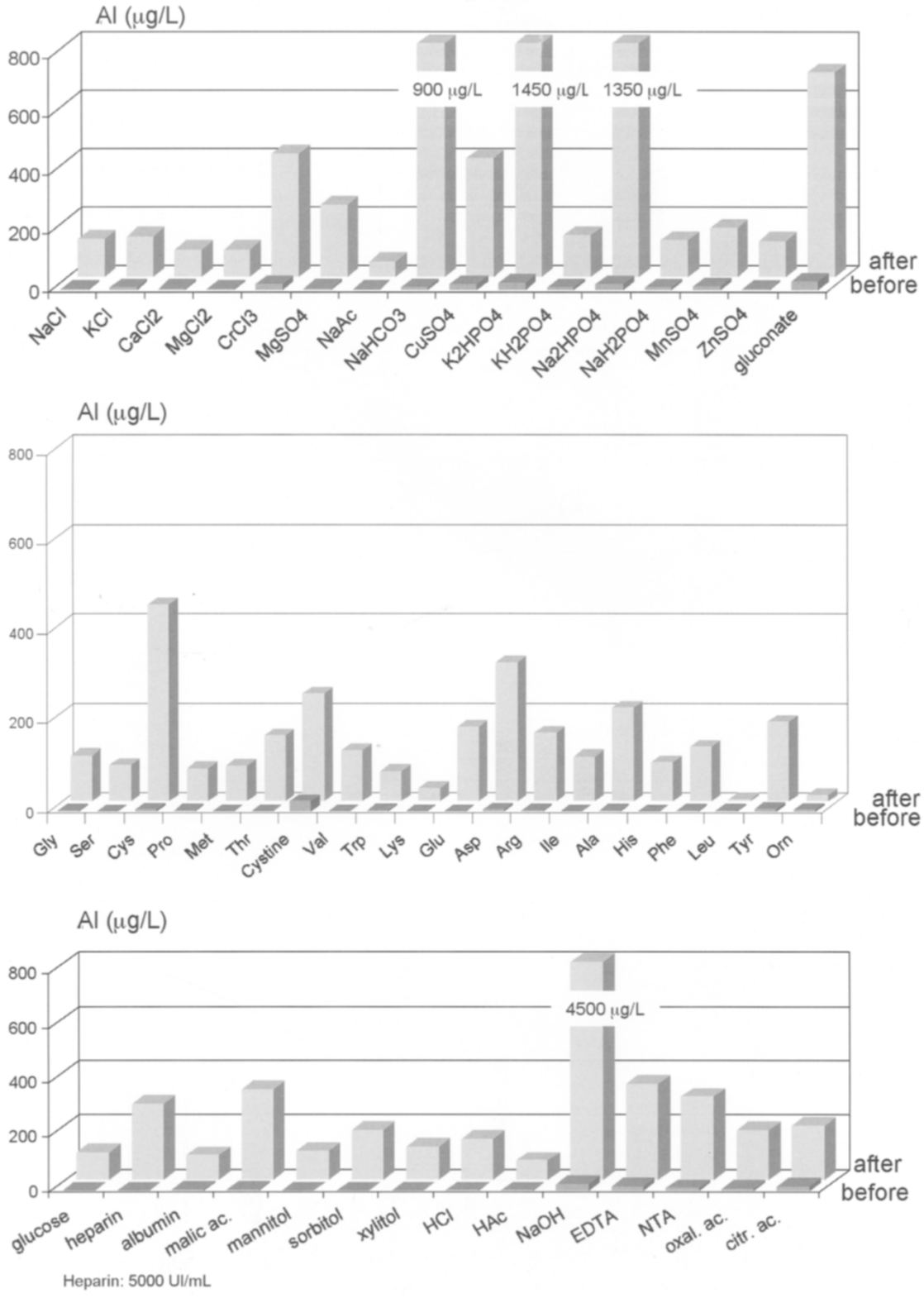

Fig. 2. Aluminium extracted from glass ampoules during sterilisation at 121 °C for 30 min by action of solutions of different substances at a concentration of 0.5% (m/v). Results are a mean of three replicates. After = after sterilisation, before = before sterilisation.

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