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Arsenic release from glass containers by action of intravenous nutrition formulation constituents

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Abstract

Pharmacopoeias prescribe tests to determine the levels of arsenic in raw materials and glass containers. In this study, glass ampoules for injectables containing individually the main components of intravenous nutrition formulations were submitted to the hydrolytic resistance test by heating at 121 °C for 30 min. As(V) and As(III) levels in these solutions after heating were determined by hydride generation atomic absorption spectrometry. The arsenic content of substances used in these formulations was previously determined, as well as the arsenic content of the glass containers. The results showed that raw substances as well as glass containers contain arsenic. Moreover, arsenic is released during the heating (hydrolytic resistance test). However, the amount released and the arsenic species present in solution depend on the solution composition. While As(V) was the predominant specie in glass, solutions containing reducing substances such as glucose and vitamins had As(III) in higher concentration. Therefore, arsenic is released from glass containers during the heating for sterilization, and reacts with formulation constituents depending on their reducing properties.
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Keywords: Arsenic species; Intravenous nutrition; Glass; Contaminants

1. Introduction

Glass containers for parenterals must meet specifications concerning stability (USP 26, BP, IP). One such specification is related to the extractability of arsenic during the heating cycle for product sterilization. The specification, however, deals neither with arsenic speciation nor considers the action of formulation constituents on the glass surface since the test addresses water attack only. Glass can contain arsenic as a constituent because arsenic oxide(III) may be added to glass melt as a fining agent to improve its transparency (Scholze, 1988), a feature specially important for solutions for intravenous administration that must be subjected to visual inspection of the content before use (Bacon, 1986).

Extraction of arsenic from glass containers could be increased or reduced by action of formulation constituents. Moreover, depending on the nature of the substance, it could increase the extraction rate of arsenic from glass and also change the arsenic species in solution by converting As(III) into As(V) or vice-versa. In a previous work we developed a procedure to investigate the presence of As(III) and As(V) in commercial solutions for parenteral nutrition (Bohrer et al., 2005). While in water for injection and in solutions of salts, As(V) predominated over As(III), in solutions of some amino acids, glucose and vitamins, As(III) was the most abundant species. As the later substances possess reducing properties, the presence of As(III) in higher concentration was attributed to the reduction of As(V) in solution by these substances.

Parenteral nutrition (PN) is the administration of nutrients intravenously to patients that cannot be fed via the gastrointestinal tract. Products for PN are commercialized in form of sterile solutions, and include electrolytes, amino acids, carbohydrates, albumin, vitamin and lipids emulsions. Previous studies have shown that PN solutions can be contaminated by toxic metals (Buchman et al., 2001; Leung, 1995). Such contamination can lead to organ deposition of the metals with subsequent deleterious effects. The presence of arsenic in pharmaceuticals is a matter of great consideration due to its high toxicity. Stud-

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ies have found that variations in the level of contamination depend on formulation and brand of constituents (Leung, 1995; Pluhator-Murton et al., 1999). The main problem associated with contamination in solutions for PN lies in their intravenous route of administration and the large volume necessary for feeding patients regularly.

In this work arsenic release from glass containers by action of formulation constituents was evaluated. This included an investigation of the possibility of As(V) from glass to react with formulation constituents to form the more toxic As(III) species.

2. Material and methods

2.1. Instrumentation

A SpectrAA 200 spectrometer (Varian, Australia), equipped with a VGA 77 system for hydride generation and a GTA 100 for electrothermal atomization were used. The operating conditions of the spectrometer for the determination of arsenic species are shown in Table 1. A Phoenix AV 50 N 6281 autoclave (São Paulo, Brazil), a Berghof BSB 939-IR sub-boiling distillation apparatus (Eningen, Germany) and a Digimed pH meter D-20 (São Paulo, Brazil) were used.

2.2. Reagents

The water used throughout was distilled, deionized and further purified by a Milli-Q high purity water device (Millipore, Bedford, USA). Arsenic(V) and As(III) standard solutions containing 1000 mg/L As were prepared by dissolving $Na_2HAsO_4{\cdot}7H_2O$ and As_2O_3 (Merck, Darmstadt, Germany) in water and in 1.0 mol/L NaOH, respectively. Working standard solutions were prepared daily diluting the standards in 6 mol/L HCl.

Freshly prepared solution of sodium tetrahydroborate(III) ($NaBH_4$) (Merck) was used as a 1% (w/v) solution in 0.1 mol/L NaOH. Citrate buffers were prepared with 0.1 and 0.5 mol/L citric acid and had the pH adjusted to 2.0 or 4.5 with 1.0 mol/L NaOH.

All other reagents were of analytical-reagent grade. HCl (36%, 1.19 g/mL) (Merck) was further purified by sub-boiling distillation.

Table 1
Operating conditions of the atomic absorption spectrometer

Spectrometer conditions	
Wavelength	193.7 nm
Band width	0.5 nm
Lamp current	10 mA
Hydride generation	
Quartz cell	15 cm path length × 8 mm i.d.
Heating	Electrothermal
Temperature	925 °C
Nitrogen flow rate	1.5 L/min
Reductant concentration	1% (m/v) in 0.1 mol/L NaOH
Reductant flow rate	1.0 mL/min
Sample flow rate	8 mL/min
As(III) reduction milieu	
Sample pH ≥ 7	0.5 mol/L citric acid pH 2.0
Sample pH < 7	0.5 mol/L citric acid pH 4.5
Flow rate	1.0 mL/min
Total As(III + V) reduction milieu	6.0 mol/L HCl
Flow rate	1 mL/min
Electrothermal atomization	Pyrolytic coated partitioned graphite tube
Gas flow	3.0 L/min

Temperature program

Drying		Pyrolysis		Atomization	
Temperature (°C)	Time (s)	Temperature (°C)	Time (s)	Temperature (°C)	Time (s)
95/120	40/10	1400	8	2600	2.6

2.3. Contamination control

To avoid contamination, only plastic materials were used. All laboratory ware (pipette tips, volumetric flasks, etc.) was immersed for at least 24 h in a 10% (v/v) HNO_3/ethanol solution and shortly before the use, washed with Milli-Q purified water.

2.4. Arsenic species measurement

The determination of As(V) and As(III) in solutions of amino acids, salts, vitamins, glucose and heparin was carried out following the conditions set in an earlier work (Bohrer et al., 2005). Arsenic(III) was measured carrying out the reduction in citrate buffer pH 2.0 for samples which pH was ≥7 or in citrate buffer pH 4.5 for those which pH was <7. Arsenic(V) was evaluated measuring the total As in solution by changing the citrate buffer to 6.0 mol/L HCl solution, and discounting As(III) from the total found. Sodium tetrahydroborate(III) in a concentration of 1% (m/v) was used as reducing agent for both species determination. Analytical curves were obtained with standards containing from 5 to 40 μg/L As of each species.

2.5. Analysis of substances

The assayed chemicals included most substances used in parenteral nutrition. Although heparin is not used for parenteral nutrition, it was included in the experiment. At least three samples of different manufacturers for each substance (Merck, Aldrich, Sigma, Ridel-de Haën and Ajinomoto) were assayed. For the analysis, solutions containing 0.1 or 0.5% (m/v) of the substance were prepared in polyethylene volumetric flasks, and the As content measured by ET AAS following the conditions described in Table 1. Heparin was dissolved to give a solution with the same UI/mL (5000) as the commercial product. All samples were prepared in triplicate and the measurement was done just after the preparation.

2.6. Analysis of glass containers

Clear and amber glass ampoules (5, 10 and 20 mL) and bottles for amino acids solution (250, 500 and 1000 mL) were assayed

for their As(V) and As(III) content according to a method elsewhere described (Nascimento et al., 2005).

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 vial with 5 mL of HF (48%, m/m) and 5 mL of water and let to stand for 48 h. After the dissolution, the sample was filtered (because in bottle samples a small residue was left), the volume was adjusted to 100 mL with water and the As species measured by HG AAS. The residual mass was subtracted from the initial glass mass. A blank experiment was carried out using the reagents and including all steps of the decomposition procedure.

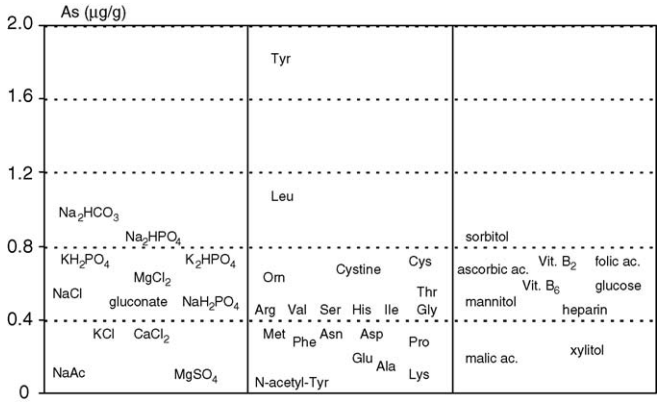

Fig. 1. Arsenic level in substances used in formulations for parenteral nutrition.

2.7. Preparation of samples for hydrolytic resistance test

Ten-milliliter new glass ampoules for injectables (Schott, Brazil) were rinse three times with water and filled with 0.1 or 0.5% (m/v) solution of each substance selected for the assays. The ampoules were sealed by fusion of the glass and heated at 121 °C in autoclave for 30 min. After cooling down they were opened and the As species in solution measured by HG-AAS. The As already present in the substances was discounted from the total arsenic measured. The experiment was carried out in triplicate. Ten ampoules filled with pure water only were also submitted to the glass hydrolytic resistance test according to the British Pharmacopoeia.

2.8. Analysis of commercial products

Commercial solutions of salts, glucose, heparin, vitamins, lipid emulsions and formulations containing amino acids were

Table 2
Arsenic species and total arsenic found in containers used for storing intravenous formulations

Container	Glass type	Total As (mg/g)	As(III) (mg/g) ± R.S.D.[a]	As(V) (mg/g) ± R.S.D.[a]
Ampoule 5 mL	Clear	1.85	0.43 ± 3.1	1.42 ± 1.1
Ampoule 5 mL	Amber	0.60	0.30 ± 3.1	0.30 ± 4.2
Ampoule 10 mL	Clear	1.98	0.49 ± 0.9	1.49 ± 0.5
Ampoule 10 mL	Amber	0.34	0.12 ± 1.2	0.22 ± 1.9
Ampoule 20 mL	Clear	2.93	0.42 ± 2.1	2.51 ± 0.6
Ampoule 20 mL	Amber	0.19	0.09 ± 1.1	0.10 ± 1.5
Bottle for amino acids (250 mL)	Clear	0.056	0.008 ± 0.5	0.048 ± 1.4
Bottle for amino acids (500 mL)	Clear	0.054	0.019 ± 0.6	0.035 ± 0.8
Bottle for amino acids (1000 mL)	Clear	0.099	0.027 ± 2.2	0.072 ± 2.3

[a] $n = 3$.

Table 3
Arsenic species present as contaminant in commercial parenteral solutions

Product	Glass container		Solution[a]	
	As(V) (mg/g) (%)	As(III) (mg/g) (%)	As(V) (μg/L) (%)	As(III) (μg/L) (%)
Water for injection	2.0 (80)	0.5 (20)	36 (92)	3 (8)
KCl 19.1%	3.4 (85)	0.6 (15)	41 (100)	n.d. (0)
NaCl 20%	2.9 (84)	0.6 (16)	41 (100)	n.d. (0)
Sodium acetate 2 meq/mL	2.4 (83)	0.5 (17)	42 (91)	4 (9)
Sodium phosphate 0.5 M	2.5 (85)	0.4 (15)	37 (100)	n.d. (0)
Calcium gluconate 10%	2.6 (82)	0.6 (18)	73 (78)	20 (21)
Magnesium sulfate 50%	2.5 (85)	0.4 (15)	40 (74)	14 (26)
Glucose 25%	2.8 (85)	0.5 (25)	17 (76)	5 (24)
Vitamins (Tiaminose)	2.4 (83)	0.5 (17)	86 (83)	18 (17)
Vitamins (Dextrovitase)	2.8 (83)	0.6 (17)	10 (71)	4 (29)
Vitamins (Frutovena)	2.6 (83)	0.5 (17)	22 (35)	40 (65)
Amino acids 8%	0.7 (73)	0.03 (27)	17 (77)	5 (33)
Lipid emulsion	0.4 (65)	0.02 (35)	17 (100)	n.d. (0)
Heparin 5000 UI/mL	1.4 (77)	0.4 (23)	17 (30)	40 (70)

Brands: B. Braum, Fresenius, Baxter, Halex Istar, JP, Merck, Aché, Farmalab, Abbott, Elkins Sinn. n.d. = not detected.
[a] $n = 3$; 0.1 < R.S.D. < 5.7.

analyzed for their As(III) and As(V) content by HG AAS. The glass containers in which they were stored were also assayed for both arsenic species as previously described. For all products, at least three samples of the same batch were analyzed, and the reported results correspond to the mean value calculated from these replicates.

3. Results and discussion

Arsenic is present as a trace impurity in practically all substances used in PN. Fig. 1 shows the arsenic level found in the assayed substances. However, most have arsenic levels below 0.6 μg/g, which is much lower than the concentration allowed by pharmacopoeias (when prescribed). Arsenic above this level was found in phosphates and bicarbonate and in some amino acids. However, even these higher levels are still lower than the allowed ones. For example, the limit for arsenic in sodium hydrogencarbonate stated by the International Pharmacopoeia (vol. 5, 2003) is 3 μg/g.

The analysis of containers for parenterals (bottles and ampoules) revealed that all glasses contain both arsenic species (Table 2). However, while the total arsenic content in bottles is not greater than 0.1 mg/g, it reaches almost 3 mg/g in clear ampoules. There is also a difference in the arsenic content of clear and amber ampoules; while the arsenic level in the amber ones ranged between 0.2 and 0.6 mg/g, it was not lower than 2 mg/g in the clear ampoules (see also Table 3). The speciation analysis revealed that As(V) is the most abundant species in all samples, however, the ratio As(V)/As(III) is different for clear and amber containers. While this ratio is around 50:50 in amber glass, only up to 25% of the arsenic present in the clear glasses is As(III). These results can be explained by the reactions experienced by arsenic oxides in the glass melt. Arsenic oxide is added to the glass melt as fining agent (to improve glass transparency). The As_2O_3 is supposed to react with potassium nitrate to release nitrogen oxides and oxygen, and is converted into As_2O_5 by this reaction. These gases form large bubbles that rapidly rise to the surface stirring the bath and sweeping small bubbles, formed by decomposition of batch materials. Bubbles in glass for pharmaceuticals are undesirable because they compromise glass transparency, affecting the visual inspection of the contents. The As_2O_5 produced in this first step can still be decomposed to As_2O_3 because trioxide is more stable at high temperatures (Shelby, 1997). Therefore, As(V) is expected to be the main species, but As(III) may also be found in glasses. Because ampoules should be as transparent as possible, their arsenic content is higher than the bottles.

3.1. Hydrolytic resistance test and arsenic release

Before starting the tests, the glass ampoules (clear) used for storing the solutions were assayed for arsenic speciation and revealed an arsenic content of 1.5 ± 0.6 mg/g As(V) and 0.4 ± 0.2 mg/g As(III). The first experiment was to carry out the hydrolytic resistance test with water only. The results showed that even pure water is able to extract arsenic species from glass surface. In average, 31 μg/L As(V) and 2 μg/L As(III) were extracted from 10 assayed ampoules, corresponding to a proportion of 94% As(V) and 6% As(III). Because all solutions for PN are aqueous solutions, the arsenic extracted by pure water was taken as standard for comparison for action of the substances.

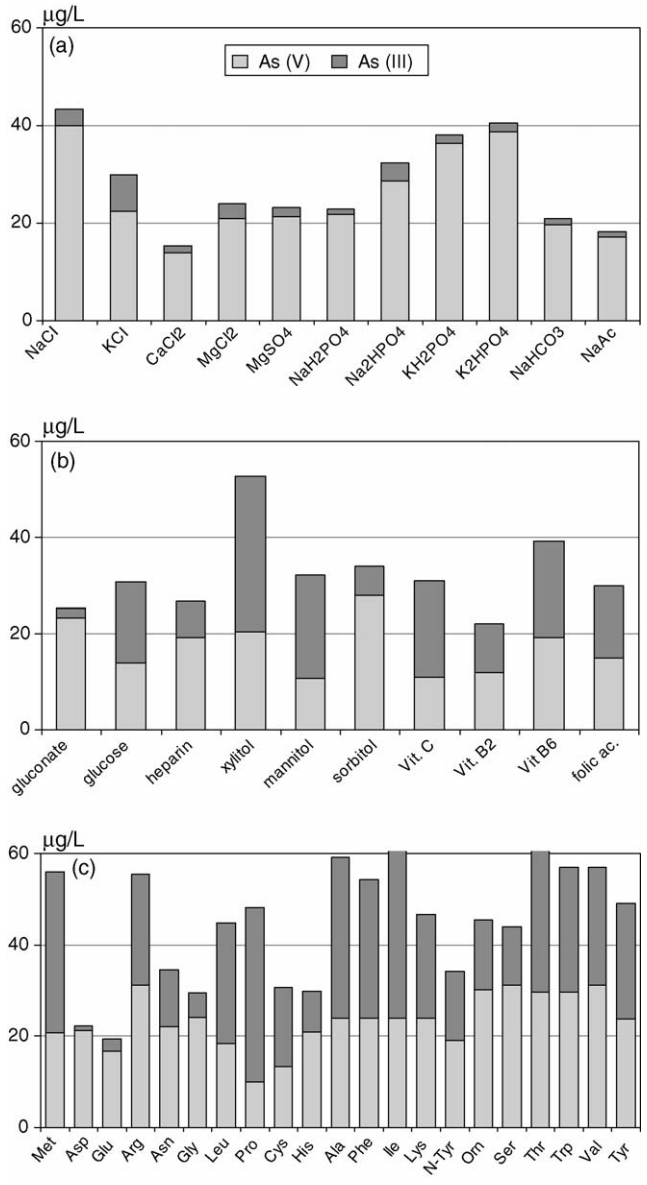

Fig. 2. As(V) and As(III) extracted from glass ampoules during the test for surface hydrolytic resistance for parenteral preparations.

Fig. 2a shows As(V) and As(III) found in solutions of salts after the heating procedure. The results are similar to those obtained with pure water. Much more As(V) than As(III) was found in solution and the extraction yields lay around 30 μg/L for As(V) and 2 μg/L for As(III). Different values were found in solutions of organic substances, however. While the total amount of arsenic is not so different (30–40 μg/L), the ratio As(V) to As(III) reached 40:60 in some solutions (Fig. 2b and c). Because the ratio As(V)/As(III) in the glass ampoules used for the test is 80:20 and the selective extraction of As(III) from glass is unlikely, As(III) might be formed in solution by reduction of As(V).

Table 4
Arsenic species and total arsenic found in commercial parenteral solutions of different ages

Product	Product age (months)	Total As (μg/L)	As(V) (μg/L ± R.S.D.)[a]	As(III) (μg/L ± R.S.D.)[a]
Water for injection	4	39.3	36.4 ± 3.7	2.9 ± 0.2
Water for injection	11	30.9	27.5 ± 1.2	3.4 ± 0.4
KCl 10%	6	23.6	23.6 ± 0.8	n.d.
KCl 19.1%	24	41.3	41.3 ± 1.6	n.d.
NaCl 20%	5	15.9	15.9 ± 3.2	n.d.
NaCl 20%	20	43.8	40.9 ± 5.7	2.9 ± 0.2
Sodium bicarbonate 8.4%	15	198.3	147.2 ± 1.2	51.1 ± 0.8
Sodium bicarbonate 8.4%	23	248.6	227.8 ± 0.5	20.8 ± 2.0
Calcium gluconate 10%	6	73.1	46.8 ± 4.1	26.3 ± 2.3
Calcium gluconate 10%	13	92.7	72.8 ± 0.5	19.9 ± 1.9
Calcium gluconate 10%	24	239.6	176 ± 7.2	63.6 ± 2.0
Magnesium sulfate 50%	9	15.7	15.7 ± 0.2	5.7 ± 0.2
Magnesium sulfate 50%	24	33.5	12.2 ± 1.2	21.3 ± 1.8
Magnesium sulfate 50%	24	53.8	39.9 ± 5.6	13.9 ± 3.0

[a] $n = 3$.

In order to confirm this supposition, tests were carried out with solutions of ascorbic acid, glucose, leucine, xylitol, and NaCl individually sealed in ampoules spiked with 40 μg/L As(V). The results showed that, with exception of the NaCl solution, As(V) was reduced to As(III) during the heating cycle. Fig. 3 depicts the percentage of As(V) and As(III) in these solutions after the heating cycle.

3.2. Arsenic speciation in commercial formulations

Both the commercial formulations and their glass containers were assayed for arsenic speciation (Table 3). As already presented, arsenic levels in glass containers correspond to approximately 80% As(V) and 20% As(III). This proportion, however, was not found in solutions. Since the arsenic contribution of the raw material is negligible, it must have migrated from the glass container into the solution. In spite of As(V) being the predominant species in the glass containers, it was not the predominant one in some formulation contents. While in solutions of KCl, NaCl, sodium acetate and sodium phosphate, As(III) contributed less than 20% of the total arsenic, in formulations containing glucose, vitamins, heparin, calcium gluconate, magnesium sulfate and sodium bicarbonate, As(III) level raised up to 60%. Considering that these substances can react as reducing agents, As(V) may be extracted from glass and reduced to As(III) in solution.

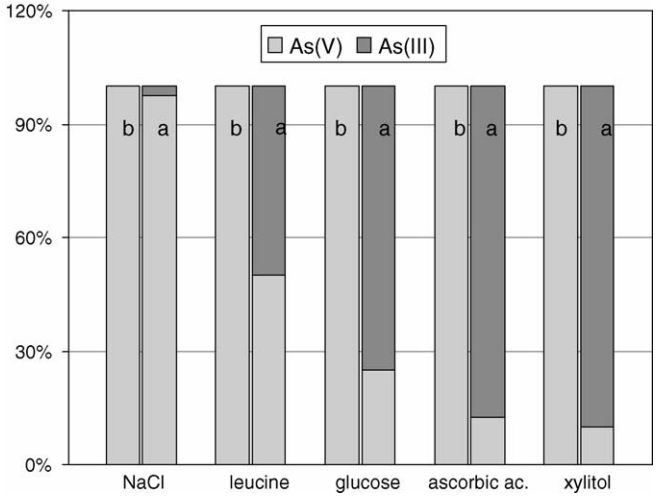

Fig. 3. As(V) and As(III) measured before (b) and after (a) heating for 30 min solutions spiked with 40 μg/L As(V).

Because the total amount of arsenic found in some commercial products was considerable higher than in the hydrolytic resistance test with the substances, we compared the age of these samples at the time they were analyzed with their arsenic content. It seems that arsenic migration may be an ongoing process (as already observed for aluminium; Bohrer et al., 2001) because the older the solution the higher its arsenic content (Table 4). However, differently from aluminium, that is selectively extracted by action of substances that present affinity for this element, the presence of higher concentrations of arsenic in older samples seems to be related to the dissolution of the glass network itself. Mainly in alkaline solutions (able to dissolve the network) the arsenic content increased with the age of the product.

4. Conclusions

Arsenic was found as impurity in almost all substances used in PN. Since the levels are not high, they do not contribute significantly for arsenic contamination in PN products.

Ampoules and bottles for parenteral formulations contain arsenic in amounts varying from 0.4 to 3.4 mg/g As(V) and from 0.01 to 0.6 mg/g As(III), corresponding to a ratio of about 80:20, respectively. Arsenic present in glass may migrate from the container surface into the solution. This process can be accelerated by heating for sterilization of the final product (sealed ampoules or closed bottles) and, mainly in those with pH over 7, it can last until the use. Consequently, because the shelf-life of these prod-

ucts is normally 2 years, controlling the quality just after production would not be enough to guarantee that arsenic level would be below that allowed at the time of the use. Arsenic speciation carried out after the heating cycle for each formulation constituent individually, revealed that the ratio 80:20 (As(V)/As(III)) found in glasses was not kept after extraction. Even the total amount of arsenic extracted from glass being approximately constant (30–40 μg/L) the ratio As(V)/As(III) changed to 40:60, depending on the substance. Therefore, considering that As(III) is more toxic than As(V) and that by reaction with As(V) the formulation constituents oxidize themselves, it would be advisable not to prescribe total arsenic quantification but arsenic speciation instead.

The total arsenic found in the samples after the processing carried out in this study (fill, seal and sterilization) has not exceeded the usual limit of 0.1 mg/L for arsenic in infusion solutions prescribed by pharmacopoeias. The arsenic levels in some commercial products, mainly sodium bicarbonate, however, were much higher than this limit. For these products it would be advisable either to shorten the shelf-life or to avoid packaging in glass containers.

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