Original Paper

Aluminium release of pharmaceutical glass containers: Determination by GFAAS in the extract solutions and study of the inner surfaces by XPS and SIMS

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It is well-known that aluminium accumulation in the body is responsible for severe neurological diseases to which patients receiving haemodialysis are particularly exposed. This problem, which could arise for any kind of solutions for hypodermoclysis, seems to be overcome if the aluminium content of the injectable preparations is less than 10 µg/l.

In the present paper the aluminium release has been determined for different glass containers for pharmaceutical use after autoclave treatment with distilled water for 60 min at 121 °C. The determinations have been carried out by Graphite Furnace Atomic Absorption Spectrometry in the extracts: The best result was obtained for the glass of type II whose release was lower than 2 µg/l for any capacity. An explanation of the aluminium release mechanism from these glasses is attempted on the basis of XPS and SIMS data obtained for the inner surface of the containers.

The sulphur treatment depletes the surface almost completely of calcium and sodium ions, thus forming a highly durable SiO_2-rich layer. After autoclaving the pH value of the solution remains in the range of neutral values and the dissolution process is limited to about 5 nm. No preferential extraction of aluminium has been detected in the bloom.

Herauslösen von Aluminium aus pharmazeutischen Glasbehältern: Bestimmung in den Extraktionslösungen durch GFAAS und Untersuchung der inneren Oberflächen durch XPS und SIMS

Es ist bekannt, daß Aluminiumanreicherungen im Körper verantwortlich sind für schwere neurologische Krankheiten, denen besonders Blutdialysepatienten ausgesetzt sind. Dieses Problem, das bei jeder Art von Lösungen für eine subkutane Infusion auftreten könnte, scheint bewältigt werden zu können, wenn der Aluminiumgehalt der Infusionslösungen unter 10 µg/l liegt.

In der vorliegenden Arbeit wurde das Herauslösen von Aluminium für verschiedene Glasbehälter, die in der Pharmazie verwendet werden, nach einer 60 min langen Autoklavenbehandlung mit destilliertem Wasser bei 121 °C ermittelt. Die Aluminiumbestimmungen wurden an den Extraktionslösungen der Gläser nach der AAS-Methode mit einer Graphitrohrküvette durchgeführt. Die besten Ergebnisse wurden für den Glastyp II erhalten, dessen Abgabefähigkeit für jede Behältergröße niedriger als 2 µg/l war. Eine Erklärung für den Aluminiumfreisetzungsmechanismus aus diesen Gläsern wurde auf der Basis von XPS- und SIMS-Daten versucht, die für die innere Oberfläche der Behälter ermittelt wurden.

Die Schwefelbehandlung läßt die Oberfläche nahezu vollständig an Calcium- und Natriumionen verarmen, wodurch eine sehr widerstandsfähige SiO_2-reiche Schicht ausgebildet wird. Nach einer Autoklavenbehandlung blieb der pH-Wert der Lösung im neutralen Bereich, und der Auflösungsprozeß wurde auf 5 nm beschränkt. Es konnte keine bevorzugte Aluminiumextraktion in der Ausblühung festgestellt werden.

1. Introduction

It is well known that the accumulation of aluminium in the human body is responsible for severe neurological diseases which can evolve in encephalopathy, osteopathy, Alzheimer's syndrome [1 to 3]. Patients receiving peritoneal dialysis or haemodialysis are particularly exposed to such a risk due to aluminium contamination of the dialysis fluids which cannot be totally overcome even by accurate monitoring [4]. The aluminium level in the blood is normally so low that a concentration of 60 µg/l of this element in the blood is already a signal for an accumulation process.

In order to reduce this risk the Commission Européenne de Pharmacopée [5] has recently stated that the aluminium concentration must not exceed 10 µg/l either in the dialysis fluids or in the Aqua Purificata to be used as a diluting agent.

Glass containers are not normally used for dialysis fluids but they are largely employed for infusion purposes. The release of glass components other than aluminium to pharmaceutical preparations during an autoclaving process or long-term storage has been extensively investigated [6 and 7] and the behaviour of the glass surfaces is well-recognised [8]. On the other hand, few data are available as far as the aluminium release is concerned.

The object of this paper is twofold:

a) to measure the aluminium release from the glass surfaces during the sterilisation process (autoclaving for 1 h at 121 °C) and to state which additional contribution to the total amount of the aluminium present in a solution is due to the glass itself;

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Table 1. Chemical composition (in wt%) of different glass container types

	type I	types II and III
SiO_2	67.8	72.0
Al_2O_3	6.10	1.80
Na_2O	10.0	13.0
K_2O	1.40	1.00
CaO	0.60	10.0
MgO	–	2.20
BaO	4.00	–
B_2O_3	10.0	–

Explanations: The chemical durability of the glasses of types I and II is high, that of the glass of type III is moderate. The glasses of type II were obtained by surface dealkalisation of glass containers of type III with ammonium sulphate and formation of a SiO_2-rich layer with high chemical durability.

b) to study with SIMS (Secondary Ion Mass Spectrometry) and XPS (X-ray Photoelectron Spectrometry) techniques how the inner surfaces of the containers are modified by the sterilisation process, in order to identify the release mechanism of aluminium.

2. Experimental and results

Containers of glass types I, II and III with a capacity of 100 and 500 ml (for smaller capacities the aluminium accumulation is not a serious problem at all) have been examined as intended to come into contact with injectable preparations according to the recommendations of the European Pharmacopoeia [9]. For such containers the following definitions will apply:

a) glass container of type I: made of borosilicate glass having a high hydrolytic resistance due to the chemical composition of the glass as a material;

b) glass container of type II: having a high hydrolytic surface resistance resulting from an appropriate surface treatment, normally of soda-lime-silica glass;

c) glass container of type III: made of soda-lime-silica glass having a moderate chemical resistance due to the composition of the glass as a material.

The compositions of the glasses used in this study are reported in table 1.

Three glass containers of each type and capacity (100 and 500 ml) were carefully washed and filled with "grade 1 water" (complying with ISO 3696 specifications [10]) and autoclaved for 1 h at 121 °C according to the prescriptions of the European Pharmacopoeia [9]. The use of water as a filling agent allows severe matrix effects due to the salts normally present in the pharmaceutical preparations to be avoided and to consider the contribution of the blank (aluminium signal of the filling liquid before autoclaving) near to zero [11].

For the analysis of the solutions a Varian AA 1475 (Varian PTY LTD, Victoria, Australia) atomic absorption spectrometer was used in conjunction with a GTA 95 graphite tube atomizer. The extract solutions were acidified with concentrated HNO_3 (Suprapur, Merck, Darmstadt (FRG)) to give the final concentration of 1 % and aluminium was determined by Graphite Furnace Atomic Absorption Spectrometry (GFAAS). 10 µl were spiked manually in the graphite tube and the firing repeated 5 times for each sample. The operating parameters are shown in table 2.

A BDH (British Drug House Ltd, Poole (UK)) aluminium saturated solution (1 g/l) as aluminium nitrate was used for the preparation of the calibration solution; tips and glassware were decontaminated by soaking overnight in 5 % HNO_3. The results are reported in table 3.

To investigate the aluminium release mechanism, the final pH value and the release of Na_2O, CaO and SiO_2 were determined in the extract solutions from 500 ml containers of each type of glass. Sodium and calcium were analysed by flame AAS and SiO_2 by the "molybdenum blue" spectrophotometric method [12]. The compositions of these extract solutions are summarised in table 4.

In order to ascertain the possible influence of the sulphur treatment on glass of type II as far as the aluminium release is concerned, the bloom formed on the glass surface of 500 ml containers after the dealkalisation process was removed by washing with grade 1 water and analysed. The concentration of Al_2O_3 in the bloom was about 2 µg per bottle and that of SiO_2 of the order of 100 µg per bottle.

To analyse the inner surfaces of the 500 ml containers of the three types of glasses before and after autoclaving, fragments of (5 × 5) mm^2 were cut

Table 2. Operating parameters of Varian AA 1475 atomic absorption spectrometer and GTA 95 graphite tube atomizer

spectrophotometer		furnace programme						
HCL lamp:	10 mA	step:	1	2	3	4	5	6
slit:	0.2 nm	temperature						
wavelength:	309.3 nm	in °C:	50	90	120	1200	2500	2700
background corrector	off	time in s:	5	30	20	15	0	2
gas:	argon	isotherm:	–	–	10	15	2[1])	–
integration:	peak height							

[1]) gas stop.

from the walls with a diamond wheel, rinsed with distilled water, dried and stored in a desiccator until needed. Freshly fractured surfaces were used as bulk composition references. XPS was the preferred analytical method for quantitative compositional analysis of the surfaces, but SIMS was also used as a complementary technique.

The argon ion-beam sputtering has been used in conjunction with XPS to provide an in-depth analysis: Nevertheless, it should be taken into account that this approach can yield erroneous results which are more acute when analyses of multicomponent glasses are performed [13 to 15].

SIMS profiles for sodium and aluminium were obtained with a Cameca IMS 4f (Cameca, Paris (F)). The profiles of sodium for glass of type I and those of aluminium for each glass type do not show any significant difference before and after autoclaving and are not reported here; sodium profiles of glasses of types II and III before and after the sterilisation process are reported in figure 1. It is evident from the figure that the bulk composition for sodium in glass containers of type II is not yet reached at 140 nm in both cases, while in glass containers of type III sodium profiles before and after autoclaving reach the bulk level at about 20 and 40 nm, respectively. The sodium SIMS profiles were used to normalize the quantitative XPS data and to provide a better understanding as regards reaching the bulk composition.

XPS quantitative analysis was performed by means of a VG ESCA3 MK1 spectrometer (VG Scientific Limited, East Grinstead, West Sussex (UK)) with a hemispherical electrostatic analyser. The pressure in the analytical chamber was of the order of $1 \cdot 10^{-7}$ Pa. The Al-K_α radiation was used with a power of 12 kV · 10 mA and the calibration of the spectrometer was done with the signal of the Au 4f7/2 term at 84.0 eV binding energy (b.e.). To avoid charging effects on the sample surface all spectra were calibrated with the C 1s signal, due to the contamination at 285.0 eV (b.e.). The sputtering was done in the preparation chamber with Ar^+ ions at 2 kV and 10 μA/cm^2. In this condition the sputtering rate was of the order of 2 nm/min.

The compositions of the surfaces before and after autoclaving of the three types of glass containers are reported in table 5 together with the in-depth composition (10; 40; 100 nm from the inner surfaces) of the samples after autoclaving.

3. Discussion

As might be expected the reported results emphasize a deeply different behaviour of the three types of glass containers as far as the aluminium release is concerned. Nevertheless, it is rather astonishing to note that the aluminium release of the glass of type II

Table 3. Determination of aluminium release by GFAAS

glass type	capacity in ml	aluminium in μg/l	CV in %
extract solutions:			
I	100	68 ± 6	8.8
		68 ± 6	8.8
		67 ± 6	8.8
mean value:		68 ± 6	
I	500	66 ± 2	3
		102 ± 6	5.9
		84 ± 6	7.1
mean value:		84 ± 6	
II	100/500	<2	
III[2])	500	339 ± 10	3.1
		328 ± 34	10.3
		342 ± 35	10.2
mean value:		336 ± 29	

testing of analytic accuracy:

sample of extract solution	aluminium added in μg/l	aluminium found in μg/l	aluminium found in %
A	–	34	–
A with aluminium added	20	53	95
B	–	11	–
B with aluminium added	10	20	90

[2]) For glasses of type III only 500 ml containers were analysed.

Table 4. Chemical composition (in mg) and final pH value of the 500 ml glass containers extract solution

glass type	Na_2O	CaO	SiO_2	pH value
I	0.19	<0.05	0.45	6.8 to 7.2
II	0.038	<0.05	0.38	6.4 to 7.0
III	1.90	0.74	7.5	9.5

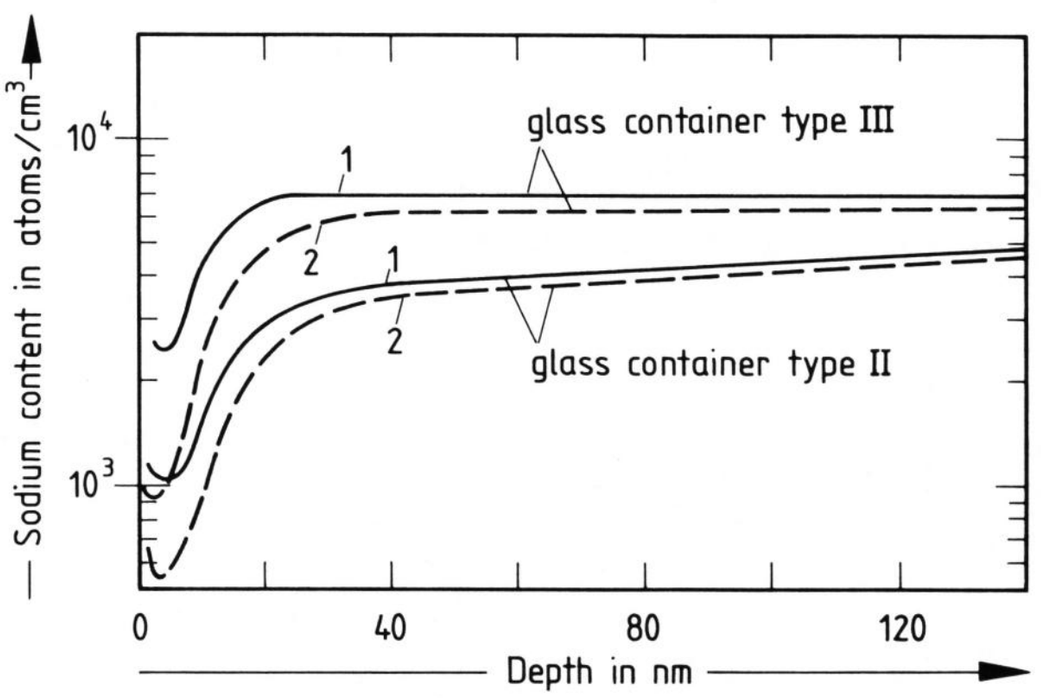
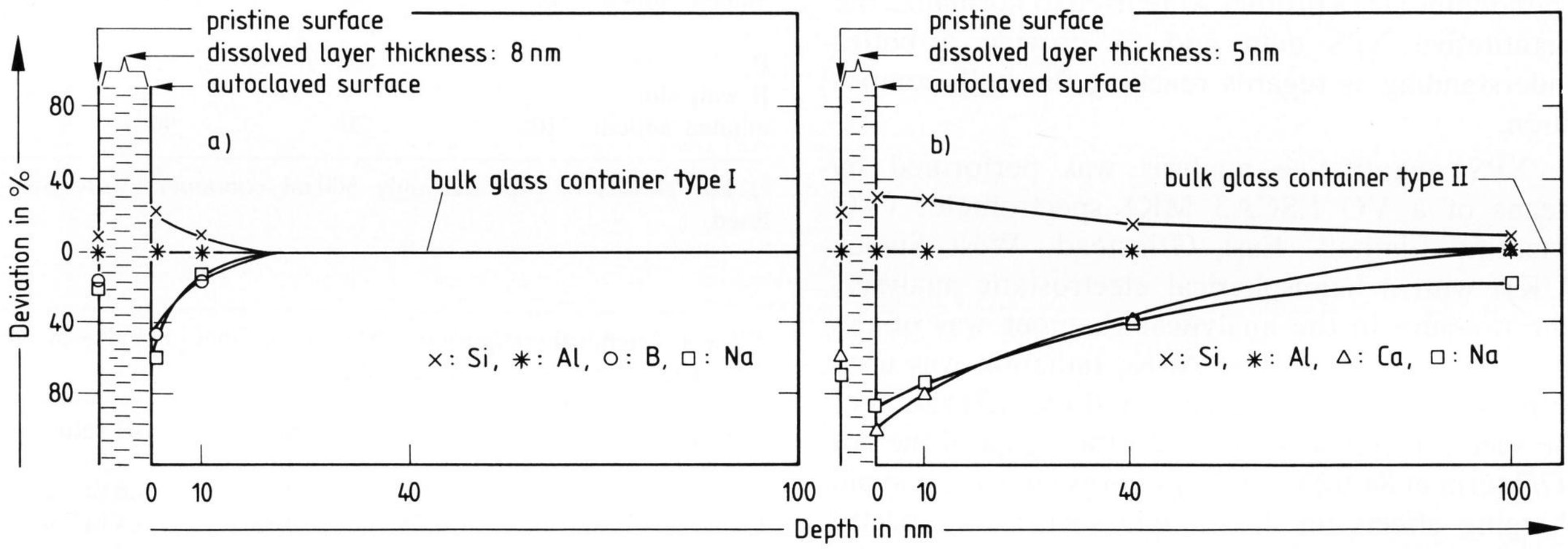


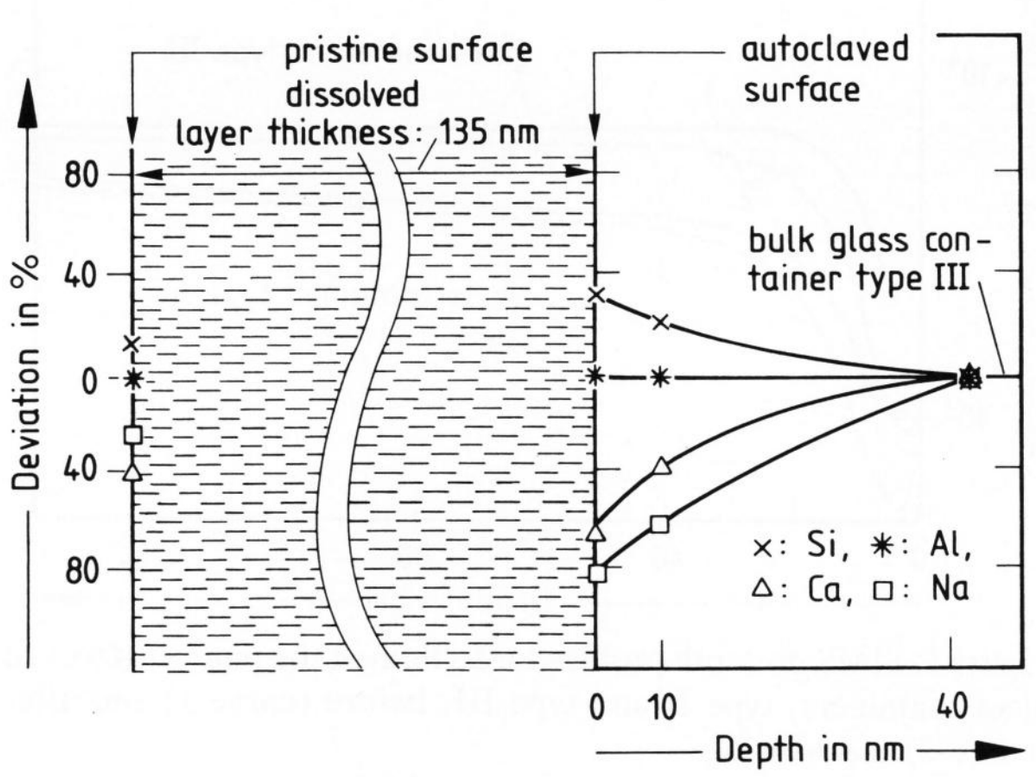
Figure 1. SIMS in-depth profiles for sodium of the inner surfaces of glass containers, type II and type III, before (curve 1) and after (curve 2) autoclaving.

Table 5. XPS quantitative determination (in wt%) of the surface composition of the three types of glass containers before (pristine) and after autoclaving (autoclaved). The in-depth compositions at 10, 40, 100 nm from the autoclaved surfaces are also reported

	bulk composition before autoclaving	composition of the pristine surface	composition of the autoclaved surface	composition of the glasses with autoclaved surfaces at a sample depth (in nm) of		
				10	40	100
type I						
SiO_2	67.8	74.0	83.0	70.0	67.8	67.8
Al_2O_3	6.1	6.1	6.1	6.1	6.1	6.1
Na_2O	10.0	8.0	4.0	9.0	10.0	10.0
B_2O_3	10.0	8.5	5.0	8.5	10.0	10.0
BaO	4.0	2.5	1.5	4.0	4.0	4.0
type II						
SiO_2	72.0	88.0	95.0	93.0	83.0	77.0
Al_2O_3	1.8	1.8	1.8	1.8	1.8	1.8
Na_2O	13.0	5.0	1.5	3.0	8.0	10.0
MgO	2.2	1.5	1.0	1.5	2.2	2.2
CaO	10.0	3.0	1.0	2.0	6.0	10.0
type III						
SiO_2	72.0	81.0	91.0	86.0	72.0	72.0
Al_2O_3	1.8	1.8	1.8	1.8	1.8	1.8
Na_2O	13.0	10.0	2.0	4.0	13.0	13.0
MgO	2.2	2.2	1.0	2.2	2.2	2.2
CaO	10.0	6.0	3.0	6.0	10.0	10.0



Figures 2a and b. Surfaces composition (main oxides) before and after autoclaving and the in-depth composition of the samples after autoclaving (XPS profiles) given as per cent deviation from the bulk glass composition; a) container glass of type I; b) container glass of type II.



is below the detection limit of the analytical method (2 µg/l). Therefore, from this point of view it behaves even better than glass of type I that is in principle the most durable one.

On the whole, the results clearly show that the mechanism of the aluminium release in all three glasses can be explained by a dissolution process [16], which implies that the species forming the glass are dissolving into solution in the same ratios as they

Figure 3. Container glass of type III; surface composition (main oxides) before and after autoclaving and the in-depth composition of the sample after autoclaving (XPS profiles) given as per cent deviation from the bulk glass composition.

occur in the bulk glass. Indeed the Si/Al ratios in the extract solutions are nearly stoichiometric as compared to the chemical composition of glasses. On the other hand, the analysis of the bloom of glass of type II demonstrates that during the sulphurisation process no preferential extraction of aluminium occurred: In fact Al_2O_3 is present in the bloom in a very low amount and in the same ratio to SiO_2 as in the original glass.

This mechanism of aluminium release is confirmed also by surface analysis: The Al_2O_3 concentration on the surfaces of the three glasses before and after autoclaving does not differ significantly from the bulk composition. These results demonstrate also that during the sterilisation process no aluminium reprecipitation occurs as was observed by other authors under more stressed conditions [17]. On the basis of the total migration (table 3), the inner surface of the containers (315 cm^2), the volume of the containers (500 ml) and the glass density (2.5 g/cm^3), the thickness of the dissolved layer during the sterilisation process could be calculated and resulted to be about 8 nm for glass of type I, 5 nm for glass of type II and 136 nm for glass of type III.

The mentioned dissolution process is summarised in figures 2a and b and 3 where:

a) the thickness of the dissolved glass layer;

b) the surface compositions (as per cent deviation from the bulk composition) before and after autoclaving;

c) the thickness of the modified layer and the profiles of the main oxides in the autoclaved samples

are reported. As regards glass of type I (figure 2a), the thickness of the dissolved layer is about 8 nm; as a consequence of autoclaving the leaching process resulted in a surface which was depleted of sodium and boron but enriched in silicon. No significant aluminium variation could be detected.

The XPS profiles after autoclaving indicate that for sodium and boron a diffusion process is taking place over a depth of about 20 nm. Such a diffusion process was not detected before autoclaving where the bulk composition was already reached after about 5 nm.

In glass of type II (figure 2b) the thickness of the dissolved layer is of about 5 nm; unlike glass of type I the compositions of the "pristine" surface and the "autoclaved" surface are quite similar to each other as reported by other authors [8 and 18]. Only a slight decrease of sodium and calcium concentrations and a moderate silicon increase is evident. These moderate differences are consistent with the sulphurisation process which promotes the formation of a highly durable SiO_2-rich layer and a modification of the glass composition to a depth of hundreds of nanometres. This situation is not influenced by autoclaving; particularly the Al_2O_3 content remains constant at the bulk value also for glass surfaces of type II.

The dissolved layer of glass of type III (135 nm) is much more important compared with the other two glasses (figure 3). This result is in agreement both with the moderate chemical durability of this glass and with the final pH value of the extract solution.

In comparison with the "pristine" one, the "autoclaved" surface is deeply depleted of sodium and calcium, whose profiles indicate leaching to a depth of about 40 nm. The leaching of these ions is a diffusion process and the difference in the extent between sodium and calcium is probably proportional to their individual diffusivity coefficient [19]. On the contrary, the surface concentration of aluminium remains constant during the whole process and no diffusion profiles were obtained.

4. Conclusions

The release of aluminium from pharmaceutical glass containers during autoclaving for 1 h at 121 °C was investigated through analysis of the extract solutions and study of the inner container surfaces. The results show some differences in the behaviour of the three types of glass under investigation, but a common releasing mechanism due to a dissolution process. The better performance of glass of type II (aluminium release less than 2 µg/l is probably due to two main reasons:

a) The sulphur treatment depletes the surface almost completely of CaO and Na_2O, thus resulting in a SiO_2-rich surface layer with an extremely high resistance to leaching during autoclaving; the pH value of the solution remains therefore in the range of neutral values.

b) Although the thickness of the dissolved layer is of the same order of magnitude as that of glass of type I, the aluminium content of the bulk glass is three times lower.

The intrinsic features of glasses of type I and II ensure an effective protection against a hypothetical aluminium accumulation even in case of prolonged treatments. Glass of type II particularly complies with the European Pharmacopoeia recommendations for dialysis solutions.

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