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Assessment of trace aluminium content in parenteral solutions by combined cloud point preconcentration—flow injection inductively coupled plasma optical emission spectrometry

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Abstract

A micelle-mediated phase separation without added chelating agents to preconcentrate trace levels of aluminium in parenteral solutions as a prior step to its determination by flow injection inductively coupled plasma optical emission spectrometry has been developed. The enrichment step is based on the cloud point extraction of aluminium with the non-ionic surfactant polyethyleneglycolmono-*p*-nonylphenylether (PONPE 7.5). The chemical variables affecting the sensitivity of the extractive-spectrometric procedure were studied in detail. After optimization, a preconcentration factor of 200 and a %E higher than 99.9 were achieved. The detection limit (DL) value of aluminium for the preconcentration of 50 ml of parenteral solution was 0.25 μg l^{-1}. The calibration graph using the preconcentration system for aluminium was linear with a correlation coefficient of 0.9997 at levels near the DLs up to at least 200 μg l^{-1}. The developed hyphenated assay, which thoroughly satisfies the typical requirements for pharmaceutical control processes, is appropriate to monitor the aluminium concentration in parenteral nutrition.

Keywords: Parenteral solutions; Aluminium; Cloud point extraction; Inductively coupled plasma atomic emission spectrometry

1. Introduction

Aluminium (Al) is a nonessential, toxic metal to which humans are frequently exposed. It is present in food, water and pharmaceutical compounds. This element has been involved as a causative factor in several clinical and neuropathological diseases, particularly in-patients with chronic renal failure. Some of the reported implications of aluminium exposure on human pathologies include microcytic anemia, osteomalacia, encephalopathy/dialysis dementia [1], Parkinson's disease [2] of Guam, amyotrophic lateral sclerosis and, Alz-

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heimer's disease [3]. Parenteral exposure to aluminium can occur via contaminated total parenteral nutrition (TPN), intravenous solutions, or contaminated dialysates. This fluid contains sodium, potassium, calcium, magnesium, and chloride ions at concentrations designed to control the loss of the major electrolytes from blood. In addition, the fluids also contain glucose and amino acids at varying concentrations.

In view of more recent publications [4–7], the aluminium contamination may be a potential hazard to patients with prolonged parenteral nutrition. Patients can inadvertently receive significant amounts of aluminium present as contaminant in TPN. The great variability between the solutions of different manufacturers and of different batches suggests that the contamination takes place during manufacture. Thus, the production of this medicines in pharmaceutical laboratories calls for very strict quality control, because they are injected directly in the blood stream at high volumes.

The suggested threshold concentration of aluminium in solutions for parenteral nutritional support recommended by the American Society for Clinical Nutrition and the American Society for Parenteral and Enteral Nutrition is 25 $\mu g\,l^{-1}$. The sensitivity (2 $mg\,l^{-1}$) of the standard fluorimetric method for aluminium determination recommended by the British Pharmacopoeia [8] is not suitable for determining aluminium at the levels normally found in TPN solutions.

Although, electrothermal atomic absorption spectrometry and inductively coupled plasma-optical emission spectrometry (ICP-OES) are the most used techniques in the determination of trace level metals, the aluminium concentration in parenteral solutions is not compatible with the limit of detection of such techniques. In order to achieve accurate, reliable and sensitive results, a preconcentration step is essential.

In the last decade, an increasing interest is shown all over the world in developing surfactant-based methods in all fields of analytical chemistry. Aqueous solutions of many non-ionic surfactant micellar systems become turbid over a narrow temperature range, when the experimental conditions have been changed. This temperature is named 'cloud point temperature'. Above the cloud point, the solution separates into two phases: one, very small in volume, the surfactant-rich phase; and the other, the bulk aqueous solution, containing surfactant monomers. The use of micellar systems as an alternative to other techniques of separation offers several advantages including low cost, safety and high capacity to concentrate a wide variety of analytes of widely varying nature with high recoveries and very high concentration factors [9–12]. From an analytical point of view, the surfactant-rich phase can be used to separate and/or preconcentrate different analytes before their injection into any hydrodynamic analytical system. There is a useful overlapping of ordered media and atomic spectrometry techniques. The peculiar properties of ordered media can also be exploited in atomic spectrometry basically in two different directions: by changing the physical properties (density, viscosity, surface tension) of the liquid sample solution to be fed into the atomizer and by altering chemical properties of such solutions in order to enhance the sensitivity of atomic detectors and improve nebulization efficiency [13].

Among other reported applications, the cloud point extraction (CPE) has been used to preconcentrate metals [14–18] based on the formation of chelates in the surfactant aggregate. Indeed, the coupling of CPE with atomic methodologies [19,20] has recently been reported. Nevertheless, in the present paper we have demonstrated quantitative extraction of aluminium in the absence of chelating reagent. This new-type of micelle-mediated extraction has never been used as a prior step to ICP-OES.

In the present work we have developed and optimized a powerful CPE-ICP-OES combined methodology for Al (III) determination, which shows excellent preconcentration parameters. The enrichment step is based on the CPE of aluminium with PONPE 7.5 in the absence of chelating agent. By introducing the surfactant-rich phase diluted with hydrochloric acid by means of the flow injection (FI) system, an extensive use of the spectrometer without cleaning and re-optimization is allowed.

2. Experimental

2.1. Reagents

Working standard solutions were prepared by stepwise dilution from 1.00 mg ml^{-1} Al stock standard solution (Merck, Darmstadt, Germany) immediately before use. A 2×10^{-2} mol l^{-1} Acetate buffer solution was prepared, the desired pH being obtained by the addition of dilute $HClO_4$ or NaOH solution (Merck).

All solutions were prepared with ultra-high-quality water obtained from a Barnstead Easy pure RF compact ultrapure water system.

All the other reagents were of analytical-reagent grade. Tokio Kasei Industries (Tokio, Japan) supplied the non-ionic surfactant polyethyleneglycolmono-*p*-nonylphenylether (PONPE 7.5).

Solution A was prepared following the procedure described by Silva et al.[14] by mixing 10 ml of PONPE 7.5, 10 ml of $NaClO_4$ (Merck) (1 mol l^{-1}) and 40 ml of distilled ethanol and diluting to 100 ml with doubly distilled water.

2.2. Apparatus

The measurements were performed with a sequential inductively coupled plasma spectrometer (Baird (Bedford, MA) ICP 2070). The 1 m Czerny–Turner monochromator had a holographic grating with 1800 grooves mm^{-1}. The ICP-OES operating conditions are listed in Table 1. The 309.278 nm spectral line was used and measurements of FI system were expressed as peak-height emission, which was corrected against the reagent blank.

A Minipuls 3 peristaltic pump (Gilson (Villiers Le-Bell, France)) was used. Sample injection was achieved using a Rheodyne (Cotati, CA) Model 50, four way rotary valve. Tygon type pump tubes (ismatec, Cole-Parmer Instrument Company, Illinois, USA) were employed to propel the sample.

pH values were measured with an Orion 940 pH meter equipped with glass combined electrode. A centrifuge was used to accelerate the phase separation process.

2.3. Recommended procedure. CPE and ICP-OES determination

Fifty millilitre parenteral solution sample, 0.5 ml acetate buffer solution 2×10^{-2} mol l^{-1} (pH 6.3) and 0.5 ml of solution A, were placed in a graduated centrifuge tube. This solution was kept at 90 °C for 10 min for equilibration and then centrifuged for 5 min at 3500 rpm ($1852.2 \times g$). After being cooled at −18 °C for 5 min the surfactant phase which had separated became a viscous gel and the aqueous phase could be poured off. The surfactant-rich phase in the tube was then made up to 1 ml by adding HCl_{conc} (Merck). In order to diminish the viscosity of the sample. One hundred microlitre of the enriched phase were injected into the ICP with a FI system using pneumatic nebulization. Table 1 shows the optimal experimental conditions for the preconcentration-determination of Al (III) in parenteral solutions.

Table 1
Experimental conditions for the CPE-ICP determination of Al (III) in parenteral solutions and ICP-OES instrumental parameters

Equilibration temperature	90 °C
Equilibration time	10 min
Centrifugation time	5 min
Cooling time	5 min
CPE extractant solution	1 ml sol A in 50 ml sample
Working pH	6.0
Buffer solution	Acetate buffer 4×10^{-3} mol l^{-1}
Surfactant-rich phase diluting agent	HCl_{conc}
RF generator power	0.8 kW
Frequency of RF generator	40.68 MHz
Plasma gas flow rate	8.5 l min^{-1}
Auxiliary gas flow rate	1 l min^{-1}
Carrier gas flow rate	0.8 l min^{-1}
Observation height (above load coil)	15 mm
Analytical line (Aluminium)	309.278 nm

A concentric glass nebulizer was used.

3. Results and discussion

3.1. Selection of surfactant. Extraction process

Several non-ionic surfactant were tested: TX-100 (Merck), TX-405 (Fluka, Sweden), Igepal CO 720 (Aldrich Chemical Company, Inc, Milwaukee, USA) and Tween 80 (Sigma Chemical Co., San Louis, USA). The obtained results never showed quantitative extraction. PONPE 7.5 has been successfully used as extracting surfactant of metals chelates [14,21,22]. The cloud point of the studied system with PONPE 7.5 is near room temperature, offering advantages in terms of the experimental procedure.

Besides, although regular solution theory predicts that partition constants of the metal chelates will be almost independent of the metal ion nature, they vary with the kind of extracted metal in the case of CPE with PONPE 7.5 [9,15]. The mechanism in the variation of the partition constants could be explained in terms of the presence of microscopically ordered structures in the surfactant phase, such as those in liquid crystals, which can distinguish slight differences in molecular size, shape and structural factors [11].

From our previous work [21,23], and considering the nature of the extracting species and Al distribution equilibria [24], and we concluded that Al forms a complex with PONPE 7.5 through their polyoxyethylene groups and is consequently located in the micelle surface.

3.2. Effect of experimental variables on CPE parameters and optimization of system

3.2.1. Effect of ethanol

The presence of ethanol prior to extraction step produces an adequate increase on the cloud point temperature of the system. Besides, the preconcentration factor ($f = v_w/v_s$, where v_w represents the volume of aqueous phase and v_s the volume of surfactant-rich phase) is influenced by the ethanol concentration prior to CPE step. The optimal preconcentration factor was achieved with ethanol concentration above 4% (v/v).

3.2.2. Effect of buffer concentration and ionic strength

Several buffer agents were tested but the optimal results were obtained with acetate buffer solution. The influence of buffer concentration prior to CPE was investigated. The results are shown in Fig. 1. Acetate buffer 4×10^{-3} mol l^{-1} was chosen as optimal.

The ionic strength has no considerable effect upon the magnitude of extraction and sensitivity within the interval $\mu = 0.005$–1 mol l^{-1}. Thus, the ionic strength was kept constant at 0.01 mol l^{-1} with sodium perchlorate.

3.2.3. Effect of pH

Experiments were made in order to locate the optimal pH range for the quantitative aluminium extraction. Each desired pH value was obtained by the addition of $HClO_4$ (Merck) (d) and/or NaOH (d). The results obtained are shown in Fig. 2. As can be seen, the extraction begins at pH 4.6 and starts to decrease at pH 7.2, offering a relatively wide range for quantitative extraction. A pH of 6 was chosen.

3.2.4. Effect of surfactant concentration

The variation on extraction efficiency was studied within the surfactant concentration range

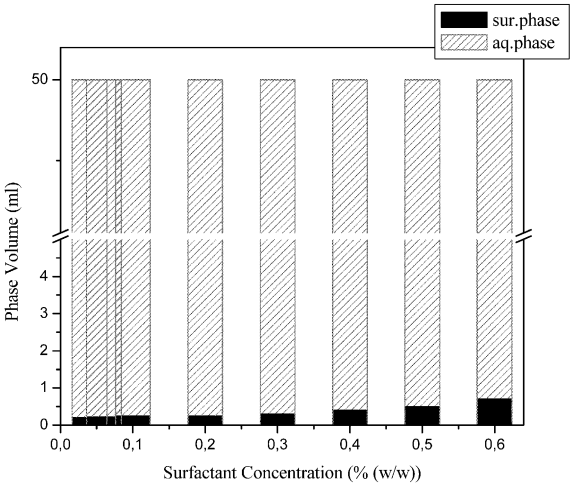

Fig. 1. Effect of buffer concentration. $C_{PONPE\ 7.5} = 0.2\%$ (w/w); $C_{Al\ (III)} = 10$ μg l^{-1}; equilibration temperature = 90 °C; equilibration time = 10 min; and ionic strength = 0.01.

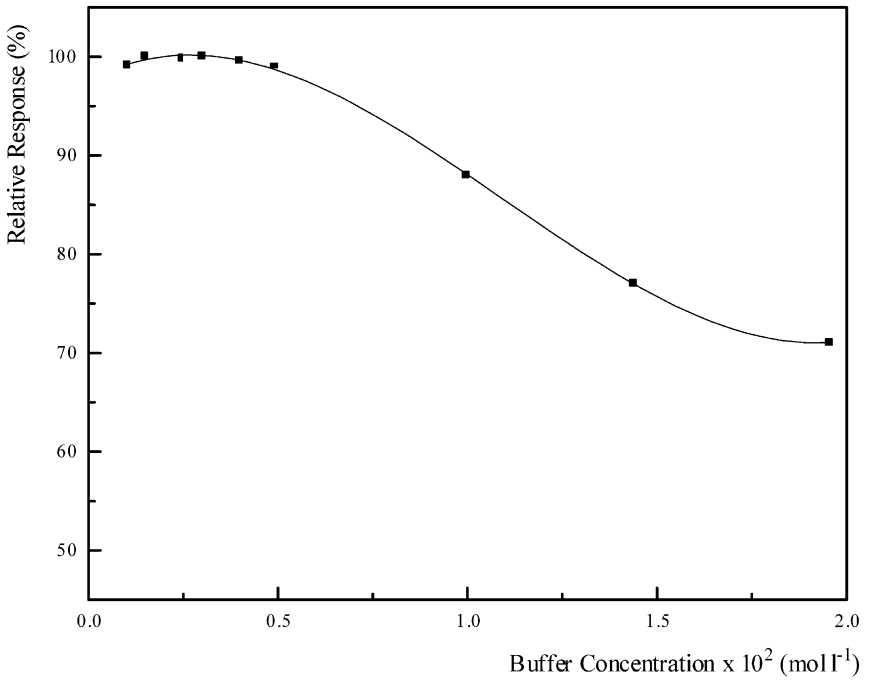

Fig. 2. Effect of pH. $C_{PONPE\ 7.5} = 0.2\%$ (w/w); $C_{Al\ (III)} = 10\ \mu g\ l^{-1}$; equilibration temperature = 90 °C; and equilibration time = 10 min. Each desired pH was obtained with additions of suitable amount of diluted HCl and NaOH.

of 0.05–0.6% (w/w). Quantitative extraction was observed for the whole concentration range. 0.2% (w/w) was chosen in order to achieve a good preconcentration factor. The results are shown in Fig. 3.

3.2.5. Effects of equilibration temperature and time

The greatest analyte preconcentration factor is reached when the CPE process is conducted with equilibration temperatures above the cloud point temperature of the system. It was observed that the volume of the surfactant-rich phase of PONPE 7.5 decreased by a factor of approximately 5 when the temperature was increased from 25 to 90 °C working at a surfactant concentration of 0.2% (w/w).

The dependence of extraction efficiency upon equilibration time was studied within a range of 2–40 min. An equilibration time of 10 min was chosen as the best solution to achieve quantitative extraction and experimental convenience.

3.2.6. Effect of centrifugation time

A centrifuge time of 5 min was selected as optimum since complete separation occurred at this time and no appreciable improvements were observed for longer times.

3.2.7. Selection of the dilution agent for the surfactant-rich phase

Different solvents for the surfactant-rich phase were tried so as to select the one producing the optimal results regarding sensitivity. The very high viscosity of the surfactant-rich phase (≈ 20 cP) is markedly decreased with a small amount of an appropriate diluting agent. Nevertheless, the selection of eluent was critical for this particular case.

Most of the reported CPE literature uses ethanol to dilute the surfactant-rich phase prior to detection step. This was not possible in this case since organic solvents generate instability in the ICP, which can eventually lead to its extinction. From other eluents tested, hydrochloric acid turned out to be a good one for surfactant-rich

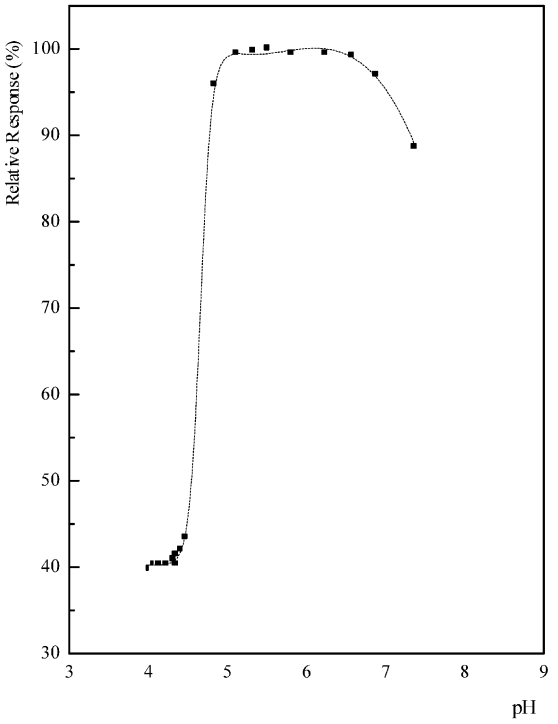

Fig. 3. Effect of surfactant-concentration volumes calculated by the height measurement of each phase after 48 h equilibration time; $n = 6$. $C_{PONPE\ 7.5} = 0.2\%$ (w/w); $C_{Al\ (III)} = 10\ \mu g\ l^{-1}$; $C_{Na_2B_4O_7} = 1 \times 10^{-3}$ mol l^{-1}; equilibration temperature = 90 °C; working pH 6.0; and ionic strength = 0.01.

phase. The effect of the eluent concentration was studied and the best ICP-OES signal was achieved for HCl_{conc}. In this situation, the CPE fractions may be appropriately manipulated and injected into the ICP.

3.3. Method validation

In order to demonstrate the validity of our method, 1.0 l of sample water was collected and divided in 10 portions of 100 ml each. The proposed method was applied to six portions and the average quantity of aluminium obtained was taken as a base value. Then increasing quantities of aluminium were added to the other aliquots of sample and aluminium was determined by the same method (Table 2).

3.4. Interferences

The effects of representative potential interfering species (at the concentration levels at which they may occur in the samples studied) were tested. Thus, Cu^{+2}, Zn^{+2}, Cd^{+2}, Ni^{+2}, Co^{+2}, Mn^{+2} and Fe^{+3} could be tolerated up to at least 2500 $\mu g\ l^{-1}$. Commonly encountered matrix components such as alkali and alkaline earth elements generally are not extracted into the surfactant-rich phase. The value of the reagent blank signal was not modified by the presence of the potentially interfering ions assayed. Even though iron is normally present in TPN solutions at trace levels, Fe (III) could be tolerated up to at least 1×10^{-4} mol l^{-1}.

3.5. Analytical performance. Determination of aluminium in parenteral solutions samples

After optimization, a preconcentration factor (f) of 200 and a %E (extraction percentage) higher than 99.9 were achieved. The relative standard deviation for 10 replicates containing 8.0 $\mu g\ l^{-1}$ of Al (III) was 3.1%. The calibration graph using the preconcentration system for aluminium was linear with a correlation coefficient of 0.9997 at levels near the DLs up to at least 200 $\mu g\ l^{-1}$. The DL value for the preconcentration of 50 ml of parenteral solution calculated as the amount of Al (III) required to yield a net peak that was equal to three times the standard deviation of the background signal (3σ) was 0.25 $\mu g\ l^{-1}$. The overall time required for loop loading, injection, and FI signal development was about 30 s. Thus, the number of determinations per hour was approximately 120.

Finally, the results of the method applied to Al (III) determination in different parenteral solutions samples are shown in Table 3.

4. Conclusions

The results demonstrate the usefulness of this new-type of micelle-mediated extraction to quantitatively extract and preconcentrate aluminium in parenteral solutions in the absence of chelating

Table 2
Method validation

Aliquot No.	Base value (μg l^{-1})	Al (III) added (μg l^{-1})	Al (III) found (μg l^{-1})[a]	S[b]	Recovery (%)[c]
I	8.2		8.21	0.031	
II	8.2	2.8	10.99	0.116	99.64
III	8.2	5.3	13.46	0.087	99.14
IV	8.2	18.0	26.09	0.175	99.38

[a] $n = 6$.
[b] Standard deviation.
[c] 100 (found-base value)/added.

Table 3
Concentrations of aluminium in commercial parenteral solutions (95% confidence interval; $n = 6$)

Sample	Al concentration (μg l^{-1})
I[a]	8.2 ± 0.2
II[a]	11.3 ± 0.2
III[b]	4.8 ± 0.3
IV[b]	5.2 ± 0.2
V[c]	30.1 ± 0.2
VI[c]	27.8 ± 0.2

[a] Ringer physiological solution: Na^+ 145.5 meq l^{-1}; K^+ 1.3 meq l^{-1}; Ca^{2+} 2.7 meq l^{-1}; Cl^- 149.5 meq l^{-1}.
[b] NaCl physiological solution: Na^+ 145 meq l^{-1}; K^+ 145 meq l^{-1}.
[c] Balanced electrolytic solution: Na^+ 140.5 meq l^{-1}; K^+ 10 meq l^{-1}; Ca^{2+} 5.0 meq l^{-1}; Mg^{2+} 3 meq l^{-1}; Cl^- 103.5 meq l^{-1}; acetate and citrate 55 meq l^{-1}.

agent. The proposed system of preconcentration associated with ICP-OES allowed aluminium determination in parenteral solutions at ppb levels. The determination procedure shows quantitative extraction, good reproducibility and accuracy.

The in situ CPE procedure represents a promising approach in the area of pharmaceutical monitoring due to its low cost, simplicity, safety, efficiency and versatility. Due to the characteristics of the method, parenteral solutions can be analyzed without previous treatment.

It has to be pointed out that CPE without chelating agents has never been used for the enrichment of metals prior to their determination by ICP-OES.

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