

Short communication

Flow injection spectrophotometric determination of Al in hemodialysis solutions

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Abstract

A flow analysis (FA) system with spectrophotometric detection for Al determination in hemodialysis solutions was developed. The method was based on the reaction of Al with eriochrome cyanine R (ECR). The complex formed associated with cetyltrimethylammonium bromide (CTAB) — a cationic surfactant, which showed enough sensitivity to execute the direct analyte determination. All interferences were eliminated with the matrix matching calibration. The system presented the following analytic parameters: sensitivity (m) of $8.10 \times 10^{-4} \text{ L } \mu\text{g}^{-1}$, limit of detection (LOD) of $3.24 \mu\text{g L}^{-1}$ (3σ), linear correlation coefficient of 0.9966 and linear range response from 10.8 to $650 \mu\text{g L}^{-1}$. The accuracy of the proposed method was checked by comparison with electrothermal atomic absorption spectrometry (ET-AAS) method. There were no differences among the results obtained from both methods, at a confidence level of 95% (paired t -test). Recovery tests were also made, values obtained were from 90.4 to 109% of recovery for Al-spiked samples.

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1. Introduction

Hemodialysis and the peritoneal dialysis were developed to eliminate toxic agents, and to re-establish the electrolytes and acid–basic equilibrium in human organism [1]. Al in hemodialysis solutions can be found as impurity. Besides that, it is the most abundant metal in the earth's crust commonly used in water treatment. This element can pass through the dialyser membrane, enter the patients' blood circulation and can accumulate in the brain causing Alzheimer's [2] and/or dialysis dementia [3]. It can also accumulate in the bones leading to renal osteodystrophy [4]. Several values for Al maximum concentration in hemodialysis solutions exist in the literature: World Health Organization, $30 \mu\text{g L}^{-1}$ [4]; American Society of Parenteral and Enteral Nutrition, $25 \mu\text{g L}^{-1}$ [5]; Association for the Advancement of Medical Instrumentation, $10 \mu\text{g L}^{-1}$ [6].

Among the analytic techniques that are rendered to Al determination, the following can be mentioned: electrothermal atomic absorption spectrometry (ET-AAS) [7–9], inductively coupled plasma atomic emission spectroscopy (ICP-AES) [10], fluorimetry [11], molecular absorption spectrophotometry (vis) [12–14] and employment of flow analysis (FA) with several detectors [15–19]. The main difficulty faced on Al determination is the low concentration of the analyte ($\mu\text{g L}^{-1}$ level) and high concomitant concentrations (g L^{-1} range). Therefore, pre-treatments of sample are frequently used to eliminate interferences and/or pre-concentrate analyte [20].

It is known that the atomic absorption and atomic emission techniques are the most suitable for the determination of metals. However, the costs for the implementation and use of these techniques are not compatible with the resources of small laboratories, hospitals and hemodialysis centers. In this context, the molecular absorption spectrophotometry is more appropriate for the routine determinations due to its simplicity. Another interesting fact is the automation possibility using FA, which can improve the analytic perfor-

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mance besides reducing the previous operation, decreasing costs and reagents consumption. It is important to point out that in Brazil, there exist about 60 000 chronic renal failure patients in need of hemodialysis. Thus, the quality control of the solutions in the hemodialysis center is of great importance to public health.

The chromogenic reagents that have been used for the Al determination are chrome azurol S and cetylpyridinium chloride [21], pyrocatechol violet [22], aluminon [23], eriochrome cyanine R with (and without) cetyltrimethylammonium bromide (CTAB) and alizarin violet [13]. The association of a cationic surfactant with the chromogenic reagent promotes the micelles formation of high molar absorptivity and, therefore, high sensitivity [24]. Using the FA technique, Al was already determined in micellar medium by fluorimetry [25], in natural water samples by spectrophotometry [26] and also in hemodialysis fluid samples, however, using pre-concentration [27].

The aim of this work was to develop an FA technique for Al direct determination in hemodialysis solution using spectrophotometric detection, with the chromogenic reagent ECR associated to cationic surfactant CTAB.

2. Experimental

2.1. Reagents and solutions

All polyethylene and glass flasks were cleaned in 10% (v/v) nitric acid solution for at least 24 h, followed by washing with distilled-deionized water.

All solutions were prepared from analytical reagents, and Milli-Q water (Millipore, MA, USA) was used throughout, unless otherwise mentioned. Concentrate acids were distilled in quartz sub-boiling stills (Milestone, Italy). Al stock solution was prepared by dilution of Al nitrate (Merck) in water. Al analytical reference solutions (20–550 $\mu\text{g L}^{-1}$) were prepared by appropriate dilutions of stock solutions.

Eriochrome cyanine R (Merck), cetyltrimethylammonium bromide (Merck) and ethylenediamine (Merck) were used for chromogenic reaction. The ethylenediamine solution (3.00 mol L^{-1}) was prepared in an ice bath by mixing, carefully and slowly, ca. 80 mL of water and 51.5 mL of ethylenediamine. Then, 90 mL of hydrochloric acid was added until it reached pH 7.5. The final volume was completed with water up to 250 mL in a volumetric flask.

In order to simulate the hemodialysis solution, a synthetic sample was prepared, according to elsewhere [1]. This solution was used in the concomitant studies and in the calibration by matrix-matching technique.

2.2. Studies of concomitants

For the concomitant studies, the hemodialysis solution composition commonly used in Brazil was considered. As an example: 3220 mg L^{-1} of Na^+ ; 78.2 mg L^{-1} of K^+ ;

70.2 mg L^{-1} of Ca^{2+} ; 18.2 mg L^{-1} of Mg^{2+} ; 3798 mg L^{-1} of Cl^- ; 2360 mg L^{-1} of CH_3COO^- ; 15000 mg L^{-1} of glucose and 2135 mg L^{-1} of HCO_3^- . Most of the element concentrations are practically constant to all hemodialysis solutions; however, the glucose and bicarbonate concentrations can present wide variations, or even be absent. Nevertheless, the interference of the concomitant ones in the above-mentioned concentrations was studied. For these studies, determinations of Al solution (50 $\mu\text{g L}^{-1}$) were made, with and without concomitants.

2.3. Samples

The hemodialysis solutions samples were collected from the Hemodialysis Center of the city of Alfenas, state of Minas Gerais, Brazil, during a hemodialysis session. They were kept in polyethylene flasks, and the storage time was shorter than 1 week.

2.4. Apparatus

The equipments used in this work were an eight-channel peristaltic pump (Ismatec-Zurich, Switzerland) 7618-40 model, supplied with Tygon pump tubes for fluid propulsion; polyethylene tubes (0.8 mm i.d.) for fluid transport, loops and coils construction; a home-made proportional commutator for FA system; a UV-vis spectrophotometer (Femto), 482 model with flow cell; an atomic absorption spectrophotometer (Zeiss), AAS5 model, equipped with deuterium lamp for background correction and Al hollow cathode lamp; pH-meter (Corning) pH 10 model; Milli-Q[®] Millipore system, academic model; magnetic stirrer and heating (Fisaton), 701 model.

For data acquisition, an interface (Advantech) PCL 711S model was used. A computational program was developed in a spreadsheet of Excel[®] using statement in macros of Visual Basic[®].

2.5. The FA system

The flow analysis system is shown in Fig. 1. Through the proportional commutator, the sample is introduced on the carrier stream. In the first confluence, the sample is mixed with a solution composed of the chromogenic reagent and the cationic surfactant (R1), beginning the Al complexation. In the second confluence, the ethylenediamine buffer solution (R2) is introduced, and signal is recorded at 577 nm.

2.6. Comparison method: ET-AAS

To check the accuracy of the proposed method, some works using ET-AAS method were consulted [28–31]. After some adaptation, for our equipment and laboratory, the following experimental conditions were used: sample volume of 20 μL ; the use of chemical modifier was not necessary

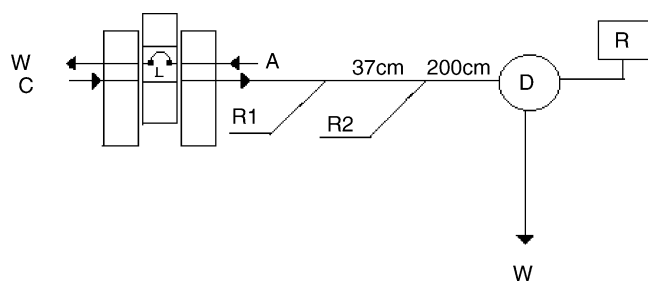


Fig. 1. Module of the FA system: A: sample; C: carrier stream (HCl, 0.012 mol L^{-1} ; flow rate = 3.96 mL min^{-1}); R1: eriochrome cyanine R, $0.0050 \text{ mol L}^{-1}$ and cetyltrimethylammonium bromide, $0.0250 \text{ mol L}^{-1}$ (flow rate = $0.572 \text{ mL min}^{-1}$); R2: ethylenediamine buffer 0.300 mol L^{-1} (flow rate = $2.903 \text{ mL min}^{-1}$); D: detector (spectrophotometer, $\lambda = 577 \text{ nm}$); W: waste; L: sample volume, 1 mL; R: register.

because the hemodialysis solutions contain high concentration of Mg, and the heating program is shown in Table 1. In these experimental conditions, the ET-AAS method presented satisfactory results for comparison with the proposed method.

3. Results and discussions

3.1. Study of elimination of the Schlieren effect

One of the problems, which occurred at the beginning of the studies, was the Schlieren effect. It provoked oscillations in the measurements, mainly in the line base, hindering the acquisition of reliable data [32–36].

The principal cause of the Schlieren effect in this work was the high concentration of the reagents that resulted in random reflections of the incident light. Thus, the best strategy to reduce Schlieren effect consisted in decreasing the ethylenediamine concentration. Low oscillation was noticed in the measurements when diluted concentrations were used and the best one was 0.30 mol L^{-1} . Therefore, all the other studies were conducted using this concentration.

3.2. Use of cationic surfactant

To improve the system's sensitivity, Al determinations with and without cationic surfactant were made. The results are presented in Table 2. When using surfactant, the limit of

Table 1
Heating program

Number	Stage	Temperature (°C)	Ramp (°C/s)	Time (s)	Inert gas
1	Drying	100	7	20	Maximum
2	Drying	150	10	20	Maximum
3	Pyrolysis	1500	300	50	Maximum
4	Atomization	2500	2500	2	Without gas
5	Cleanness	2600	2600	5	Maximum

Table 2
Comparison of the FA system with and without the use of surfactant in aqueous samples

FA system	Limit of detection ($\mu\text{g L}^{-1}$)
With cationic surfactant (BCTA)	1.85
Without surfactant	129

detection was approximately 70 times better than those found without it. The LOD obtained with surfactant was similar to the one obtained elsewhere [24].

3.3. Interference studies

In Table 3, the maximum values of interference caused by each concomitant are presented. As it can be seen, most of the tested concomitants show severe interferences; however, all the interferences were eliminated through performance of the matrix-matching technique, i.e., a synthetic hemodialysis solution prepared in our laboratory was used as carrier stream. Additionally, all standard solutions were also prepared with synthetic hemodialysis solutions.

Applying the matrix matching secured the elimination of interferences, but there was a sensitivity loss. The limits of detection obtained with and without surfactant were 0.55 and $3.24 \mu\text{g L}^{-1}$, respectively. However, the method still revealed enough sensitivity for the desired application.

The elimination of the interferences with the matrix-matching technique can be confirmed with the accuracy tests presented in the next discussion.

3.4. Figures of merit

In Fig. 2, the signals obtained from FA system are verified by the calibration curve using the matrix-matching technique, which presented the following equation: $A = 0.01042 + 0.000760C$. Recordings are also shown in triplicates of six-spiked samples (Fig. 2).

Considering the analytic parameters obtained in the FA system using a matrix-matching technique (Table 4), the LOD obtained ($3.24 \mu\text{g L}^{-1}$) is lower than the limit concentrations reported by the literature ($10\text{--}30 \mu\text{g L}^{-1}$) [4–6]. This fact yields the application of this methodology possible for the quality control of the samples.

Table 3
Maximum interference caused by each concomitant (without matrix matching)

Concomitant	Concentration (mg L^{-1})	Percentage of the interference
Sodium	3540	13%
Potassium	Up to 3500	Do not interfere
Calcium	70.2	–10%
Magnesium	Up to 3500	Do not interfere
Acetate	2596	–39%
Glucose	20000	13%
Bicarbonate	1920	–67%

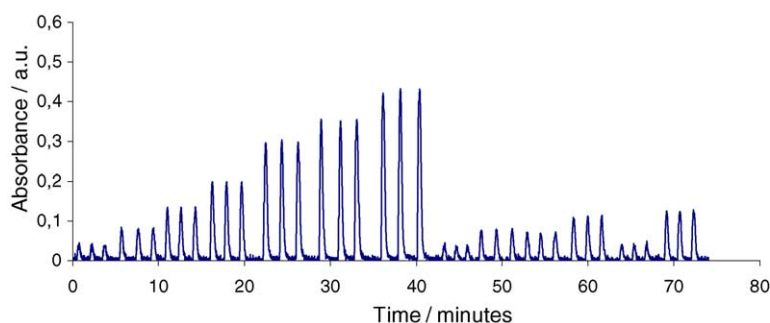


Fig. 2. Signals obtained in triplicate for concentrations of 40, 80, 150, 250, 350, 450 and 550 $\mu\text{g L}^{-1}$ of Al in hemodialysis solution and six-spiked samples.

Table 4
Analytic parameters of the FA system

Parameter	Value ^a
Sensitivity	$8.10 \times 10^{-4} \text{ L } \mu\text{g}^{-1} (\pm 2.94\%)^b$
LOD	$3.24 \mu\text{g L}^{-1} (\pm 3.5\%)$
Linear range	$10.8\text{--}650 \mu\text{g L}^{-1}$
Linear correlation coefficient	$0.9966 (\pm 0.123\%)$

^a $n = 8$.

^b Values between parentheses are the coefficients of variation.

3.5. Accuracy

In order to verify the accuracy of the proposed method, recovery tests and comparison methods were used. For the recovery tests, a sample aliquot was spiked from 20 to 300 $\mu\text{g L}^{-1}$ of Al^{3+} , and the recovery obtained ranged from 90.4 to 109%.

Before making comparison between methods, some adaptations were made in the ET-AAS, as mentioned earlier in the experimental section. After these, a typical peak shape was obtained, which is shown in Fig. 3.

There was no significant difference between FA system and ET-AAS at 95% of confidence level (paired t -test). This can be observed in Table 5.

3.6. Application

The proposed method was applied to Al determination of samples collected from the Hemodialysis Center in the city of Alfenas, state of Minas Gerais, Brazil. Al was not detected in the analyzed samples, which is in agreement with the per-

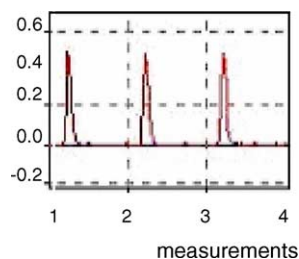


Fig. 3. Signals obtained in triplicate for a sample using the standard additions technique ($10 \mu\text{g L}^{-1}$) by ET-AAS.

Table 5
Comparison of the results

Samples	FA system ($\mu\text{g L}^{-1}$)	Atomic absorption ($\mu\text{g L}^{-1}$)
1	$43.1 (\pm 0)^a$	$37.0 (\pm 0.97\%)^a$
2	$20.7 (\pm 0)$	$19.9 (\pm 3.16\%)$
3	$37.6 (\pm 2.3\%)$	$38.3 (\pm 13.0\%)$
4	$11.7 (\pm 12.6\%)$	$10.5 (\pm 4.30\%)$
5	$17.5 (\pm 0)$	$19.4 (\pm 3.86\%)$

^a Values between parentheses are the coefficients of variation, $n = 3$.

tinent legislation (Al can not be present in concentrations superior to $10 \mu\text{g L}^{-1}$ in such solutions).

The feasibility and precision of the measurements were verified measuring the spiked samples (see registrations in Fig. 2). By these registrations, good precision of the measurements was verified with low relative standard deviation (3.18%).

4. Conclusions

The use of the cationic surfactant associated with chromogenic reagent propitiated improvement in sensitivity with a limit of detection of $3.24 \mu\text{g L}^{-1}$. This result is lower than the maximum Al concentration determined by pertinent legislation ($10 \mu\text{g L}^{-1}$), which made it possible to apply the FA system for the Al determination of hemodialysis solutions without pre-concentration.

The optimization of the reagent concentrations eliminated the Schlieren effect, and the employment of the matrix-matching technique presented satisfactory results in the elimination of interferences.

The accuracy of the method was verified using addition and recovery tests and comparison method. In the recovery tests, values between 90.4 and 109% of recovery were obtained. There was no difference, at 95% confidence level by t -test, between the proposed method and electrothermal atomic absorption spectrometry for the Al determination in hemodialysis samples.

The maximum coefficient of variation for spiked samples was 3.18%, indicating good precision of the measurements.

There was no Al detectable in the sample collected from the Hemodialysis Center in the city of Alfenas, which is in agreement with the pertinent legislation.

The proposed FA system is a simple alternative for the quality control of the hemodialysis solutions, with high analytic frequency (30 readings/h), low reagent and sample consumptions and it is of easy implementation in hemodialysis centers.

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