Talanta 133 (2015) 120–126

Contents lists available at ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

High-throughput automatic flow method for determination of trace concentrations of aluminum in dialysis concentrate solutions using salicylaldehyde picolinoylhydrazone as a turn-on fluorescent probe

Arnau Garau [a], Miquel Oliver [a], María Rosende [a], Manuel P. Mánuel-Vez [b], Manuel Miró [a,*]

[a] FI-TRACE Group, Department of Chemistry, University of the Balearic Islands, Carretera de Valldemossa, km 7.5, E-07122 Palma de Mallorca, Spain
[b] Department of Analytical Chemistry, Faculty of Marine and Environmental Sciences, University of Cádiz, Puerto Real, E-11510 Cádiz, Spain

ARTICLE INFO

Article history:
Received 30 July 2013
Received in revised form
2 April 2014
Accepted 21 April 2014
Available online 18 July 2014

Keywords:
Flow analysis
Salicylaldehyde picolinoylhydrazone
Aluminum
Dialysis concentrate solutions
Turn-on probe
Fluorescence

ABSTRACT

A simple and expedient flow-based assembly capitalizing on programmable flow is herein proposed for reliable determination of trace level concentrations of aluminum as a potential contaminant in dialysis concentrate solutions without any prior sample clean-up/preconcentration procedure. Using salicylaldehyde picolinoylhydrazone in weakly acidic media as a turn-on fluorescent probe, the manifold is devised to handle three samples concurrently in stopped-flow reaction mode for simultaneous improvement of the analytical sensitivity and sample throughput.

Analytical parameters influencing the sensitivity and repeatability of the assays, namely, probe concentration, reaction temperature and reaction time were investigated using a multivariate optimization protocol composed of a full factorial screening design followed by a Doehlert matrix model. The analysis of the Pareto chart and surface response revealed that the reaction time and amount of fluorescent probe were critical parameters for reliable assays of aluminum at the low ng mL^{-1} level.

Under the optimized chemical and physical variables, a detection limit of 1.1 ng mL^{-1} Al(III) at the $3s_{blank}$ level, relative standard deviations better than 1.0%, a dynamic linear range of 5.0–80 ng mL^{-1} and a sample throughput up to 25 h^{-1} were obtained with no need for either sample preconcentration or the use of organized supramolecular systems. Demonstrated with the analysis of hemodialysis and peritoneal concentrate solutions, and dialysis waters, the flow-through method copes with the requirements of regulatory bodies (e.g., European Pharmacopeia) for quality control of aluminum in high salinity containing dialysis concentrates.

1. Introduction

The quality and the purity of dialysis fluids prepared in-situ by dilution of dialysis concentrates are of major concern in renal replacement therapies as large volumes of dialysis fluids come into contact with the patient's bloodstream during each treatment [1]. Dialysis fluids are regarded as the main routes of inadvertent exposure of renal failure patients to unwanted metal species. Quality control programs of dialysis fluids are usually targeting aluminum. Free and labile aluminum species would in turn diffuse through the perm-selective dialysis membranes mimicking the renal function and penetrate into the blood stream of the patient whereupon they might induce clinical disorders, such as demineralizing osteodystrophy, dialysis encephalopathy and anemia, and might trigger Alzheimer's disease [2–4].

Aiming at protecting the health of patients with chronic renal failure several pharmacopoeias legislated the maximum allowable aluminum concentration in commercially available hemodialysis concentrates, dialysis water for dilution and peritoneal dialysis solutions, which are typically set to 10 μg L^{-1}, 100 μg L^{-1} and 15 μg L^{-1}, respectively [5]. Containing elevated electrolyte concentrations, routine quality assessment of dialysis solutions is not straightforward and imposes severe constraints to the instrumental methods available for determination of trace level concentrations of aluminum.

Electrothermal atomic absorption spectroscopy (ETAAS) on account of its unrivaled sensitivity might be deemed the method of choice for monitoring the concentration level of aluminum in dialysis concentrates and water for dilution. Direct sample analysis is however hindered because of large background signals and non-spectroscopic matrix interferences [6–8], unless samples are

* Corresponding author. Tel.: +34 971172746; fax: +34 971173426.
E-mail address: manuel.miro@uib.es (M. Miró).

http://dx.doi.org/10.1016/j.talanta.2014.04.094
0039-9140/

diluted several fold. Sample dilution is oftentimes making the analysis unfeasible, with aluminum concentrations falling below the limit of quantification of ETAAS. Likewise, analysis of hemodialysis and peritoneal dialysis concentrate solutions by ICP-MS is troublesome as a result of salt deposition onto the MS sampling cones as well as the generation of isobaric and polyatomic spectroscopic interferences that are proven to jeopardize accurate measurements [9]. A variety of sample clean-up and preconcentration procedures for aluminum encompassing (micro) solid-phase extraction, (micro) liquid-phase extraction, co-precipitation and cloud-point extraction have been employed for minimization of matrix interfering effects in atomic spectrometric detection [6,8,10] (also to improve sensitivity in molecular absorption procedures [11–13]), though they usually account for more than 70% of labor time of the analytical process.

Turn-on fluorometric systems are alternate sensitive methods, yet at expense of employing organized supramolecular media to ameliorate quantum yields [14] or mild to elevated temperatures in which case nuisance gas bubble formation in flow-based procedures might occur [15]. Conventional fluorescence ligands for aluminum chemosensing including morin [16], 8-hydroxyquinoline [17] and derivates thereof [14] however lack selectivity and react among other free metal species with Ca(II), and Mg(II), which are frequently found in dialysis concentrates in concentrations above 1000 mg L^{-1}. Masking agents or chelating resins to eliminate interferences or hyphenation with chromatographic separations [18,19] are thus imperative for reliable determination of aluminum in dialysis fluids and concentrates [20–23]. Despite the improvement of analytical properties (namely, sensitivity and accuracy) by preliminary sample processing methods, a Round–Robin interlaboratory comparison program with fifty-eight participants for assays of aluminum in dialysis solutions rendered unsatisfactory results because of the ubiquitous nature of the target species [24]. Thus, there is a quest of novel analytical procedures for expedient and reliable assays of low abundance aluminum as unwanted matrix ingredient in dialysis concentrates and dialysis fluids.

In this work, a simple and affordable multicommutated flow analyzer for in-line fluorometric detection of trace level concentrations of aluminum is developed and optimized for expeditious quality control of hemodialysis and peritoneal dialysis concentrates, and dialysis waters with no need of either micellar media/high reaction temperatures or prior sample clean-up/extraction procedures. The proposed method involves the utilization of a dedicated fluorescence probe, that is, salicylaldehyde picolinoylhydrazone (SAPH) bearing arylhydrazone moieties that give rise to a characteristic blue-green fluorescent chelate with aluminum at a 1:3 metal-to-ligand stoichiometric ratio $Al(SAPH)_3$ [25]. To the best of our knowledge, the arylhydrazone probe is herein exploited for the first time for reliable analysis of troublesome dialysis concentrate solutions.

2. Experimental

2.1. Reagents and solutions

All chemicals were of analytical reagent grade. Ultra-pure water (specific resistivity of 18.2 MΩ cm) obtained from a Milli-Q system (Millipore, Bedford, USA) was employed to prepare all solutions and standards. Plastic containers for standard preparation were soaked in 10% (v/v) nitric acid for 24 h and rinsed with Milli-Q water prior to use.

A stock standard solution of aluminum (1000 μg mL^{-1}) was prepared by dissolving 1.0 g of aluminum foil (Merck, Darmastadt, Germany) in a minimum volume of 2 mol L^{-1} HCl (Scharlab, Barcelona, Spain) as per APHA-AWWA-WPCF recommendations [26] prior to making up to 1 L. Working standard solutions were prepared by stepwise dilution of the stock.

A stock buffer solution of 2 mol L^{-1} acetic acid/acetate was prepared by adding 3.3 mL of glacial acetic acid (Sigma Aldrich, St. Louis, MO, USA) to 90 mL Milli-Q water to which concentrated ammonia was added dropwise until pH 5.4 followed by making up to 100 mL with Milli-Q water.

A solution consisting of 50% ethanol (Scharlab)/water was used as a carrier and rinsing solution as well to minimize carryover effects in the flow network. The buffer and carrier were both filtered across a cationic exchange membrane (EMPORE 3M, St. Paul, Minnesota, USA) so as to eliminate traces of aluminum as contaminant.

Salicylaldehyde picolinoylhydrazone (SAPH), used as turn-on fluorescent probe, was synthesized by condensation of equimolar amounts of salicylaldehyde (SA, CAS no. 90-02-8) and picolinoylhydrazide (PH) in line with a previous synthesis [25] but with slight modifications. SA is commercially available (Merck); however, PH was in-house synthesized as follows: 10 mL of picolinic acid ethyl ester (Sigma Aldrich, CAS no. 2524-52-9) and 5.2 mL of hydrazine (Sigma Aldrich, CAS no. 10217-52-4) were added to 5 mL of ethanol. The resulting solution was gently stirred and heated at 85 °C under reflux for 30 min followed by cooling down at 4 °C for 6 h to complete the precipitation of the product. The yellow PH precipitate was filtered off through a nylon membrane and recrystallized from benzene. Then, 1.0 g of the synthesized PH was suspended in 1 mL of SA with gentle stirring and heating at 40 °C to trigger the condensation reaction. The white SAPH precipitate was isolated by filtration and dried overnight in a drying oven at 50 °C. A working solution of 7×10^{-4} mol L^{-1} SAPH in 24% (v/v) ethanol was used throughout as recommended elsewhere [27].

The dialysis solutions (viz., hemodialysis concentrate, peritoneal dialysis solution and dialysis water) were kindly supplied by the University Hospital of Puerto Real, Cádiz, Spain. The concentrated acidic hemodialysis solution of elevated ionic strength contained the following concentrations of electrolytes and organic species: 210.70 g L^{-1} NaCl; 5.22 g L^{-1} KCl; 3.56 g L^{-1} $MgCl_2 \cdot 6H_2O$, 7.72 g L^{-1} $CaCl_2 \cdot 2H_2O$; 6.31 g L^{-1} acetic acid and 35 g L^{-1} glucose. The peritoneal dialysis solution was composed of 5.4 g L^{-1} NaCl; 0.26 g L^{-1} $CaCl_2 \cdot 2H_2O$; 0.05 g L^{-1} $MgCl_2 \cdot 6H_2O$; 75 g L^{-1} icodextrin and 4.5 g L^{-1} sodium lactate as main ingredients.

Since the formation of the Al-SAPH chelate is strongly influenced by the acidity of the medium [25,27,28], samples were diluted batchwise at distinct ratios (viz., ratios of 1:1 and 1:3 for dialysis water and dialysis concentrates, respectively) and buffered to a final pH of 5.4 in a 0.2 mol L^{-1} acetic acid/acetate medium. Samples were analyzed by the flow setup (re. below) without any other previous treatment.

2.2. Instrumentation and flow analyzer

The hybrid flow set-up for fluorometric determination of aluminum in dialysis concentrates and diluents is illustrated in Fig. 1. It is composed of a dual-syringe pump (SP1 and SP2) with programmable speed as a liquid driver (MicroBU 2030, Crison, Alella, Barcelona, Spain) furnished with two syringes of 5 mL (syringes of 2.5 and 1 mL were also used in the multivariate optimization procedure), two solenoid valves at their heads (SV1 and SV4) along with four ancillary solenoid valves (SV5, SV6, SV7 and SV8 all from *N*-Research, Caldwell, NJ, USA) with dead volumes of 58 μL each assisting in sample loading and delivery of well-defined sample aliquots to the several mixing coils for reaction development.

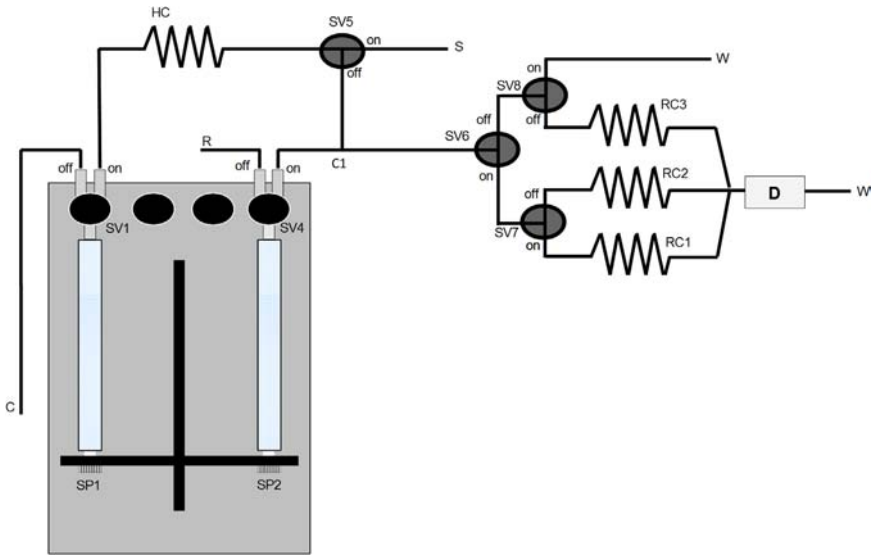

Fig. 1. Diagrammatic description of the fluorometric flow set-up for determination of low abundance aluminum in dialysis solutions. SP, syringe pump; SV, solenoid valve; RC, reaction coil; HC, holding coil; C, carrier solution; R, reagent solution; S, sample; D, fluorometric detector; and W, waste.

An 84 cm-long holding coil (HC) with an inner volume of ca. 4.1 mL was made of 1.5 mm ID polytetrafluoroethylene (PTFE) tubing. All of the other tubing lines were made of 0.8 mm ID PTFE tube. Three reaction coils (RC1, RC2 and RC3) with a capacity of 1.3 mL each were constructed by interlacing PTFE tubing of 0.8 mm ID in knots of ca. 1.5 cm diameter. The sample zone and fluorescent probe merged in a three-way (C1) poly(methylmethacrylate) (PMMA) confluence. A four-way PMMA juncture was used for connecting the three RC with the flow-through detector consisting of an SLM-AMINCO AB2 fluorometer equipped with a Xe emission light powered at 150 W and a quartz flow-through cell of 1 cm pathlength and 20 μL internal volume. The excitation and emission wavelengths were fixed to 384 and 468 nm, respectively, using slit widths for the excitation and emission monochromators of 4 and 8 nm, respectively. Peak height was used as analytical signal. Instrumental control of the flow analyzer was carried out with a software package (Autoanalysis 5.0, Sciware) based on dynamic link libraries.

2.3. Analytical procedure

Automatic fluorometric assays of low abundance aluminum in dialysis concentrate solutions and diluents using the turn-on SAPH organic probe were performed according to the operational sequence given in Table 1.

Initially, the syringes SP1 and SP2 are filled with carrier (50% v/v EtOH) and the complex forming organic probe, respectively, and the HC and three reaction coils are cleansed with 1.5 mL of carrier solution so as to circumvent Al-probe carryover effects and the potential sorption of organic matrix components onto the PTFE tubing. On changing the sample, SP1 is set to draw up a well-defined volume of sample or standard (namely, 600 μL) past SV5 to rinse the sampling line, whereupon the flow was reversed and the surplus of sample is directed to waste through SV8.

The hybrid flow systems is next programmed to aspirate 600 μL of sample in HC followed by the concurrent activation of SP1 and SP2 for on-line mixing of the sample segment with an equal volume of the picolinoylhydrazone-based fluorescent probe at a total flow rate of 2.0 mL min^{-1}. The overall composite sample/probe zone is then delivered to RC1 whereupon the flow is halted. Taking into account the slow kinetics of the complex forming reaction, the flow analyzer is devised so as to handle simultaneously three replicates or three distinct samples in on-line parallel operational mode to ameliorate the sample throughput in quality control procedures. Hereto, the flow system is programmed to analyze the next replicate/sample using a virtually identical analytical procedure while the first sample is kept for 150 s in RC1 to react with the probe. The third replicate/sample is analogously handled in RC3 while the former is being processed in RC2. After completion of the stopped-flow approach for 150 s the Al-SAPH chelate of each sample is detected in-line sequentially by activation of SP1 as a result of which the reaction product is delivered to the flow-through fluorometer at a considerably high flow rate (viz., 7.0 mL min^{-1}) to minimize dispersion of the fluorescent Al-probe chelate toward the flow cell.

2.4. Multivariate analysis

A multivariate optimization procedure was undertaken in this work to ascertain the effects of temperature, reaction time and volume of the SAPH probe upon the normalized fluorescence response so as to obtain the optimum operating conditions for the hybrid flow system in terms of sensitivity.

A two-level full factorial design was employed to elucidate those main factors that significantly influence the fluorescence response and discard those with negligible effects [29,30]. The experimental design was built in a dimensionless coordinate system using factor coding wherein the highest and the lowest levels are given as +1 and −1, respectively, as illustrated in Table 2. Distinct factors may affect the system response interactively, i.e., the effect of one factor may depend on the levels of others. Any interactions must also be distinguished from random measurement errors. Therefore, along with the main effects associated with individual factors, the relevance of the interaction between two factors was also evaluated to get knowledge about the potential degree of twisting of the first-order planar model [31]. In addition, three replicates of the center point were also included in the design to ensure that the variability found is on account of the factor effect in lieu of the random error. Investigation of the significance of the factors' influence upon the fluorescence signal was undertaken by ANOVA [29].

A Doehlert matrix-based multivariate design [32,33] was selected in this work because factors can be explored at different levels as per their dependence upon the analytical response.

The statistical computer package StatGraphics Centurion XV (Stat Point Inc., Herndon, VA, USA, 2005) was used to build the

Table 1
Operational sequence for fluorometric determination of aluminum in dialysis concentrate solutions and waters using a multicommutated hybrid flow system[a].

Step	Operation	Solenoid valve position						Description
		1	4	5	6	7	8	
1	Dispense 1.5 mL at 10.0 mL min^{-1}	ON	OFF	OFF	ON	ON	OFF	Washing of HC and reaction coils
2	Dispense 1.5 mL at 10.0 mL min^{-1}	ON	OFF	OFF	ON	OFF	OFF	
3	Dispense 1.5 mL at 10.0 mL min^{-1}	ON	OFF	OFF	OFF	OFF	OFF	
4	Aspirate 600 μL at 10.0 mL min^{-1}	ON	OFF	ON	OFF	OFF	OFF	Washing of sampling line
5	Dispense 2.0 mL at 10.0 mL min^{-1}	ON	OFF	OFF	OFF	OFF	ON	
6	Aspirate 600 μL at 3.0 mL min^{-1}	ON	OFF	ON	OFF	OFF	OFF	Draw up sample into the HC
7	Dispense 70 μL at 1.0 mL min^{-1}	ON	OFF	OFF	ON	ON	OFF	Bring the heading sample plug to C1
8	Dispense 600 μL sample (at 1.0 mL min^{-1}) +600 μL probe (at 1.0 mL min^{-1})	ON	ON	OFF	ON	ON	OFF	On-line mixing of sample with fluorescent probe (SAPH) in RC1
9	Dispense 270 μL at 1.0 mL min−1	ON	OFF	OFF	ON	ON	OFF	Bring the overall composite sample/SAPH zone into RC1
10	Aspirate 600 μL at 3.0 mL min^{-1}	ON	OFF	ON	OFF	OFF	OFF	Aspirate the second replicate/sample into the HC
11	Dispense 70 μL at 1.0 mL min^{-1}	ON	OFF	OFF	ON	OFF	OFF	Bring the heading sample plug to C1
12	Dispense 600 μL sample (at 1.0 mL min^{-1}) +600 μL probe (at 1.0 mL min^{-1})	ON	ON	OFF	ON	OFF	OFF	On-line mixing of sample with fluorescent probe (SAPH) in RC2
13	Dispense 270 μL at 1.0 mL min^{-1}	ON	OFF	OFF	ON	OFF	OFF	Bring the overall composite sample/SAPH zone into RC2
14	Aspirate 600 μL at 3.0 mL min^{-1}	ON	OFF	ON	OFF	OFF	OFF	Aspirate the third replicate/sample into the HC
15	Dispense 70 μL at 1.0 mL min^{-1}	ON	OFF	OFF	OFF	OFF	OFF	Bring the heading sample plug to C1
16	Dispense 600 μL sample (at 1.0 mL min^{-1}) +600 μL probe (at 1.0 mL min^{-1})	ON	ON	OFF	OFF	OFF	OFF	On-line mixing of sample with fluorescent probe (SAPH) in RC3
17	Dispense 270 μL at 1.0 mL min^{-1}	ON	OFF	OFF	ON	OFF	OFF	Bring the overall composite sample/SAPH zone into RC3
18	Wait 13 s							Additional step for a total halting time of sample+probe in RC1 for 150 s
19	Start reading							
20	Dispense 3.5 mL at 7.0 mL min^{-1}	ON	OFF	OFF	ON	ON	OFF	In-line detection of the fluorescent Al-SAPH chelate of sample 1
21	Wait 39 s							Additional step for a total halting time of sample+probe in RC2 for 150 s
22	Dispense 3.5 mL at 7.0 mL min^{-1}	ON	OFF	OFF	ON	OFF	OFF	In-line detection of the fluorescent Al-SAPH chelate of sample 2
23	Wait 39 s							Additional step for a total halting time of sample+probe in RC3 for 150 s
24	Dispense 3.5 mL at 7.0 mL min^{-1}	ON	OFF	OFF	OFF	OFF	OFF	In-line detection of the fluorescent Al-SAPH chelate of sample 3
25	Stop reading							

[a] For the sake of simplicity operational steps for syringe refilling are not indicated.

Table 2
Screening design for multivariate analysis of potentially significant variables in in-line fluorometric assays of trace concentrations of Al(III) using the SAPH probe.

Run	Temperature (°C)		Reaction time (stopped-flow) (s)		Probe volume (μL)		Normalized fluorescence (response)
	Uncoded	Coded	Uncoded	Coded	Uncoded	Coded	
1	50	+1	200	+1	300	+1	16.4
2	50	+1	200	+1	120	−1	16.1
3	50	+1	0	−1	300	+1	18.5
4	50	+1	0	−1	120	−1	16.4
5	25	−1	200	+1	300	+1	24.4
6	25	−1	200	+1	120	−1	18.5
7	25	−1	0	−1	300	+1	15.2
8	25	−1	0	−1	120	−1	9.2
9	37.5	0	100	0	210	0	19.0
10	37.5	0	100	0	210	0	18.8
11	37.5	0	100	0	210	0	18.9

two-level factorial design with 11 runs including center points (2^3+3) and the Doehlert matrix-based surface response model.

3. Results and discussion

3.1. Multivariate optimization of the hybrid flow system

The optimization by one variable at a time does not guarantee that the real conditions for maximum sensitivity in the in-line fluorometric determination of aluminum will be hit. This approach would be valid only if the variables to be optimized would be totally independent from each other. On the contrary, experimental design approach considers some related variables at the same time, taking into account variable interactions. Hence, the optimization process was performed in two consecutive steps. Firstly, a two-level full factorial design with three replicates of the center point was carried out using 600 μL of 25 μg L^{-1} Al standard at pH 5.4, and a probe concentration of 0.7 mmol L^{-1} to ascertain what factors and second-order interactions thereof have a significant influence on the response of the hybrid flow system. Temperature, reaction time (stopped-flow mode) and probe volume within the domains of 25–50 °C, 0–200 s and 120–300 μL, respectively, were selected as main factors in the factorial design. The normalized fluorescence intensity was taken as analytical response. The normalization of the analytical readout is imperative to offset the dilution effect as a result of the variation in the probe volume in the course of the factorial design assays. The normalized fluorescence intensity was calculated as follows:

$$I_f = (I_s - I_b)[(V_s + V_p)/V_s)]$$

where I_f stands for the normalized fluorescence intensity; I_s for the analytical signal of the sample; I_b for the blank signal; V_s for the sample volume and V_p for the probe volume.

The standardized factor effects can be readily visualized using Pareto histograms (see Fig. 2) where the standardized effects of main factors (namely, temperature (A), reaction time (B) and probe volume (C) and interactions as well) are displayed in descending order and each bar length equates the value of a calculated Student's *t*. A given factor effect is deemed statistically significant whenever its *t*-value is equal or larger than the *t*-critical value at a 0.05 significance level (represented by the vertical line on the chart), corresponding in our case to 2.78 for four degrees of freedom. The positive (white) or negative (blue) bars denote those scenarios where the normalized fluorescence intensity increases or diminishes, respectively when increasing a given factor from the lowest to the highest coded level. According to ANOVA results

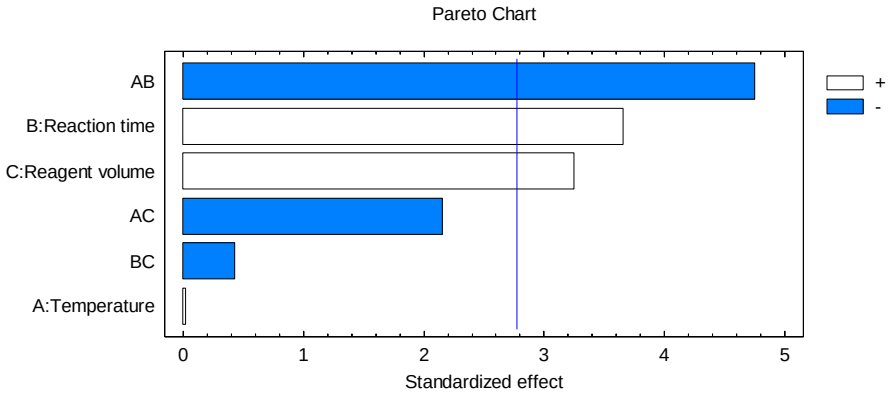

Fig. 2. (Colour online) Pareto chart of standardized effects (α=0.05) for two-level screening of the influence of main factors and two-term interactions upon the analytical response. (A) Temperature; (B) reaction time; and (C) probe volume. Experimental conditions: 600 μL Al at the 25 μg L^{-1} level, pH 5.4; 0.7 mmol L^{-1} SAPH. Regression equation: normalized signal = 17.41 + 0.015*A* + 2.01*B* + 1.78*C* − 2.60*AB* − 1.18*AC* − 0.23*BC*.

the reaction time and probe volume were statistically significant within the experimental domain at the 0.05 significance level, while the reaction temperature proved to be not significant. The greater the amount of SAPH injected in the flow system and the longer the reaction time the higher were the analytical readouts. The Pareto chart revealed that the interaction between the reaction temperature and reaction time is the factor having a more significant influence upon method sensitivity. This is most likely due to the fast decomposition of the fluorescent Al-probe chelate at increasing temperatures. One-at-a-time univariate methods would hence have lead to biased results. The lack of fit test [33] (p=0.0027≪0.05) demonstrated however that the first-order model is inappropriate to describe the analytical system. A response surface based on the second-order Doehlert matrix design was therefore selected for further multivariate optimization taking the most significant variables from the factorial design as experimental factors. In a two-variable Doehlert design one variable is explored at five levels while the other at solely three levels. The probe volume was explored in a wider and expanded domain at five increasing levels starting next to the center point in the first order design (namely, 200, 300, 400, 500 and 600 μL) because the factorial design indicated that the greater the SAPH amount the better was the analytical response. The influence of this parameter was precisely evaluated so as to reduce the expenses of the turn-on fluorescent probe to the extent possible without jeopardizing the sensitivity of the analytical procedure. The reaction time was explored at three levels within the range spanning from 150 to 400 s. Longer reaction times were deemed not necessary inasmuch as steady-state reaction conditions were expected to be reached within the new experimental domain.

The response surface model was proven to fit to a first-order planar model within the experimental domain. The higher the SAPH volume the better was the reaction efficiency while the reaction time did not significantly influence the analytical response. A probe volume of 600 μL (to merge on-line with the sample plug at a 1:1 volume ratio) along with a reaction time of 150 s, that is, the lowest uncoded value in the Doehlert design, for improved sample throughput, were chosen as optimum values for the remainder of the work.

3.2. Analytical performance and analysis of dialysis concentrate solutions and diluents

The analytical performance of the hybrid multicommutated flow setup for fluorometric determination of trace level concentrations of Al(III) is summarized in Table 3 including the linear dynamic range, calibration curve, limits of detection and quantification, repeatability, sensitivity and sampling frequency.

An eight-level external calibration plot with standards ranging from 5 to 80 μg L^{-1} was used for Al(III) quantification. The plot of fluorescence intensity (I_f) against analyte concentration (Al in μg L^{-1}) rendered a linear regression equation, viz., I_f=1.33 [Al (III), μg L^{-1}] − 1.6. The limits of detection (LOD) and quantification (LOQ) were calculated as per the $3s_{blank}$ and $10s_{blank}$ criteria, respectively. On the basis of the LOQ and the bottom end concentration of the dynamic range the automated flow system is proven well suited for sensitive assays of traces of aluminum in hemodialysis concentrates, peritoneal dialysis solutions and waters used for dialysis fluid preparation, taking into account that the maximum allowed concentrations (MAC) endorsed by the European Pharmacopoeia are ≤10 μg L^{-1}, ≤100 μg L^{-1} and ≤15 μg L^{-1} for dialysis waters, dialysis concentrates and peritoneal dialysis solutions, respectively [5].

Though the stopped-flow approach is used to ameliorate the reaction yield between Al(III) and SAPH the on-line execution of three reactions concurrently fosters the sample throughput to be increased from 12 injection h^{-1} – whenever employing a single reaction coil – up to 25 injections h^{-1} as demanded in quality control procedures for screening of potential contamination of dialysis concentrates by hazardous trace elements. Similar flow arrangements have been reported in the literature for expedient and high-throughput in-line derivatization reactions and liquid and solid-phase microextraction procedures [34,35].

Potential interfering effects of concomitant metal species in the determination of Al(III) using the picolinoylhydrazone-based probe were investigated in detail. A given metal was considered as interference when its concentration affected the fluorescence intensity by ±10%. Earlier reports indicated that Cu(II), Fe(III) and Zn(II) are the main potential interfering species in the SAPH-based fluorometric detection of Al(III) as a result of competitive reactions [25,27,28]. Concentrations up to 500 μg L^{-1} Zn (II) and 1000 μg L^{-1} Cu(II) for a concentration of Al(III) of 30 μg L^{-1} were however tolerated by using 5 and 20 mg L^{-1} thioglycolic acid, respectively, as a masking agent. By using 20 mg L^{-1} $Fe(CN)_6^{4-}$ the tolerated concentration of Fe(III) increased up to 1000 μg L^{-1} as a consequence of the precipitation of the Prussian Blue dye, in line with earlier observations [36]. It should be borne in mind that concentrations of Cu(II), Zn(II) and Fe(III) above 250 μg L^{-1} are not likely to occur in dialysis concentrate solutions and diluents.

The reliability of the hybrid flow system as a potential quality control tool for determination of low abundance Al(III) in dialysis solutions was ascertained by the analysis of real-life dialysis samples.

They were processed with minimum handling so as to minimize sample contamination. Due to the lack of certified reference materials with matrix composition resembling dialysis fluids, the as-received samples from the University Hospital of Puerto Real (Spain) were doped at Al(III) concentration levels below MAC specified by the current European Pharmacopoeia [5] and analyzed on-line without addition of neither masking agents nor surfactants for evaluation of method trueness (see Table 4). It should be stressed that the analytical validation of several methods reported earlier in the literature for the analysis of dialysis solutions [10,11] were conducted using moderately or highly contaminated dialysis concentrates exceeding the MAC.

Excellent agreement was found between spiked and measured concentrations of Al(III) at $\leq 10\ \mu g\ L^{-1}$ in dialysis waters with recoveries $\geq 96\%$ using external calibration (see Table 4), thereby demonstrating the applicability of the proposed optimized multicommuted method for fast screening of the analytical quality of waters employed as diluents in hospitals in terms of potential aluminum contamination. Satisfactory relative recoveries of Al(III) were also encountered in doped hemodialysis acid concentrates at concentration levels $\leq 32.0\ \mu g\ L^{-1}$ using external calibration with deviations $\leq 10\%$. Relative recoveries within the range of 86–106% have been reported when combining batchwise microcolumn chelating preconcentration with ETAAS detection for analysis of dialysis concentrates [6]. More importantly, sorptive sample treatment procedures are in several instances rather cumbersome with the sample loading step plus column rejuvenation lasting about 60 min [6].

Method trueness was evaluated using a *t* test [37] for comparison of the doped against measured concentrations in individual spikes of the hemodialysis concentrate solution and the water for dilution. As the overall calculated values of *t* were below the critical *t* value (that is, 4.30 for two degrees of freedom), no matrix interfering effects were detected for analysis of either hemodialysis concentrates or water for dialysis.

Determination of trace metal concentrations in peritoneal dialysis solutions is deemed challenging inasmuch as they contain large quantities of icodextrin, that is, a water-soluble polysaccharide with molecular weights ranging from 13 to 19 kDa used for the absorption of waste products from the blood. The application of the standard additions method at 3 levels was proven necessary in this sample to offset multiplicative matrix effects. As can be seen in Table 4, no significant differences at the 0.05 significance level were found between the spiked and the measured concentrations of Al(III) even though the analyzed peritoneal solution contains as much as $75\ g\ L^{-1}$ of icodextrin.

The analytical figures of merit of the proposed multicommutated fluorometric method are compared in Table 5 against several spectrophotometric, fluorometric and atomic absorption spectrometric methods reported in the literature for determination of trace levels aluminum in dialysis concentrates. All of the previous methods (see Table 5) involved a prior sample clean-up step for isolation of Al(III) from those matrixes containing high concentrations of electrolytes and mono/polysaccharides with subsequent preconcentration of the target species by sorptive or liquid-phase

Table 3
Analytical performance of the hybrid flow analyzer for fluorometric determination of Al(III).

Analytical parameter	Value
Linear dynamic range ($\mu g\ L^{-1}$)	5–80
Limit of detection ($\mu g\ L^{-1}$)	1.1
Limit of quantification ($\mu g\ L^{-1}$)	3.7
Repeatability (%) ($n=12$) at the 40 $\mu g\ L^{-1}$ level	0.84
Correlation coefficient (r)	0.9989
Sensitivity ($L\ \mu g^{-1}$)	1.33
Sampling frequency (injections h^{-1})	25

Table 4
Analysis of dialysis concentrate solutions and water for dialysis by on-line fluorometry using a turn-on fluorescent probe.

Sample	Concentration added ($\mu g\ L^{-1}$)	Concentration found ($\mu g\ L^{-1}$)[a]	Recovery (%)	t_{exp}[b]
Water for preparation of dialysis fluids	0.0	<1.1	–	–
	6.0	5.77 ± 0.04	96	3.30
	8.0	7.83 ± 0.04	98	2.47
	10.0	9.60 ± 0.05	96	4.03
Hemodialysis acid concentrate	0.0	<1.1	–	–
	16.0	17.6 ± 0.4	110	2.40
	24.0	26.3 ± 0.8	109	1.69
	32.0	34.8 ± 1.0	109	1.56
Peritoneal dialysis solution	0.0	<1.1	–	–
	12.0	12.6 ± 0.3	105	1.07
	14.0	13.8 ± 0.3	98	0.36
	15.0	16.0 ± 0.1	106	4.09

[a] Results are expressed as the mean of three assays ± standard deviation.
[b] $t_{crit}=4.30$.

Table 5
Analytical performance of analytical methods reported in the literature for determination of Al(III) in hemodialysis concentrates.

Detection	Reagent	LOD ($\mu g\ L^{-1}$)	Precision (%)	Linear range ($\mu g\ L^{-1}$)	Clean-up/preconcentration system	Ref.
Spectrophotometric	Chrome azuroS	4.25	9.9	5–80	Solid phase extraction using polyethylene powder	[11]
	3,5-Ditertbutylsalicylfluorone	0.06	1.2	0.06–12	Liquid/liquid extraction using 1-butyl-3-trimethylsilylimidazolium hexafluorophosphate	[12]
	Eriochrome cyanine-R	0.2	<6	NR	Coprecipitation using 8-hydroxyquinoline/Co^{2+}	[13]
FAAS/ICP-MS	–	10/0.1	10.7/7.0	10–500	Solid phase extraction using chromotrope 2B immobilized on AG1-X8 ion exchange resin	[10]
ETAAS	–	0.5	9	NR	Solid phase extraction using chelex 100	[6]
	–	0.3	3	0.3–21	Continuous liquid/liquid extraction using acetylacetone/8-hidroxyquinoline/methylisobutylketone	[8]
Fluorometric	8-Hydroxyquinoline	0.384	<6	10–50	Solid phase extraction and cloud point extraction	[20]
	8-Hydroxyquinoline	0.7–2	8.7	2–1000	Liquid–liquid extraction	[21]
	5,7-Dibromo-8-quinolinol	0.3	3	1–50	Solid phase extraction using chromotrope 2B and liquid–liquid extraction	[23]
	Salicylaldehyde picolinoylhydrazone (SAPH)	1.1	0.84	5–80	None	This work

FAAS: flame atomic absorption spectrometry; ICP-MS: inductively coupled plasma mass spectrometry; ETAAS: electrothermal atomic absorption spectrometry; Ref.: reference; NR: not reported.

extraction. On the contrary, no preliminary sample clean-up/preconcentration or luminescence enhancer is needed in our method as a consequence of the unique sensitivity and selectivity of the fluorescent probe. The LOD falls inside the range of LODs of the methods mentioned above (0.06–10 μg L^{-1}) but without preconcentration and copes with the requirements of the European Pharmacopeia for Al(III) assays in dialysis concentrates. In addition, the proposed flow method features one order of magnitude better precision than that reported in previous procedures (see Table 5) because of elimination of sources affecting measurement uncertainty.

4. Conclusion

An automatic flow-based analyzer has been presented in this work for reliable determination of traces of aluminum occurring as unwanted matrix component in high salinity-laden dialysis solutions. Employing a turn-on fluorescent picolinoylhydrazone-based probe, the flow method is endowed with unusual selectivity for Al(III). Optimized via a multivariate Doehlert matrix approach, the fluorometric method showcases superior performance over previous atomic spectrometric or colorimetric/fluorometric procedures in terms of shortening of analysis times up to 2.4 min/sample as three samples are processed in parallel. The LOQ of the proposed method is below the MAC specified by regulatory authorities with no need of any prior sample clean-up/preconcentration protocol and/or the use of supramolecular organized entities or masking reagents. Besides simplicity minimum risk of sample contamination is thus ensured. No bias was detected in the analysis of complex hemodialysis concentrates and peritoneal dialysis solutions with recoveries over the range of 96–110%.

Current work is underway to tailor the optimized flow system for in-situ unattended monitoring of potentially occurring aluminum in dialysis solutions just prior loading in hemodialysis machine equipments by unskilled personnel in hospital lab facilities within quality control programmes that oversee all processes related to dialysis treatment.

Acknowledgment

The authors acknowledge financial support from the Spanish Ministry of Economy and Competitiveness through Project CTM2010-17214 and from the Czech Ministry of Education, Youth and Sports through Project no. CZ.1.07/2.3.00/20.0235.

References

[1] ANSI/AAMI RD52:2004, Dialysate for Hemodialysis, American National Standards Institute, 2004.
[2] H.H. Malluche, M.C. Faugere, Am. J. Kidney Dis. 6 (1985) 336–341.
[3] E. Reusche, B. Lindner, H. Arnholdt, Virchows Arch. 424 (1994) 105–112.
[4] G. Berthon, Coord. Chem. Rev. 149 (1996) 241–280.
[5] European Pharmacopoeia, 5th Ed. Monograph 01/2005: 1167 Water for diluting concentrated hemodialysis solutions, pp. 1699; Monograph 01/2005:0128 solutions for hemodialysis, pp. 1700; Monograph 01/2005:0862 solutions for peritoneal dialysis, pp. 2212; Council of Europe, Strasbourg, France, 2004.
[6] S. Knežević, R. Milačič, M. Veber, Fresenius J. Anal. Chem. 362 (1998) 162–166.
[7] D. Bohrer, P. Cícero do Nascimento, M. Guterres, M. Trevisan, E. Seibert, Analyst 124 (1999) 1345–1350.
[8] J. Komarek, R. Cervenka, T. Ruzicka, V. Kuban, J. Pharm. Biomed. Anal. 45 (2007) 504–509.
[9] A. Berkkan, N. Erta, Talanta 64 (2004) 423–427.
[10] A. Martín-Esteban, P. Fernández, C. Pérez-Conde, A. Gutiérrez, C. Cámara, Anal. Chim. Acta 304 (1995) 121–126.
[11] D. Bohrer, A. Gioda, R. Binotto, P. Cícero do Nascimento, Anal. Chim. Acta 362 (1998) 163–169.
[12] L. Zaijun, L. Naping, Z. Xia, S. Qijun, J. Pharm. Biomed. Anal. 43 (2007) 1609–1614.
[13] V.N. Bulut, D. Arslan, D. Ozdes, M. Soylak, M. Tefekci, J. Hazard. Mater. 182 (2010) 331–336.
[14] G. de Armas, M. Miró, A. Cladera, J.M. Estela, V. Cerdà, Anal. Chim. Acta 455 (2002) 149–157.
[15] D. Kara, A. Fisher, S.J. Hill, Anal. Chim. Acta 611 (2008) 62–67.
[16] H.Z. Lian, Y.F. Kang, S.P. Bi, A. Yasin, D.L. Shao, Y.J. Chen, L.M. Dai, L.C. Tian, Anal. Bioanal. Chem. 376 (2003) 542–548.
[17] A. Alonso, M.J. Almendral, M.J. Porras, Y. Curto, C. García de María, Anal. Chim. Acta 447 (2001) 211–217.
[18] H.-Z. Lian, Y.-F. Kang, A. Yasin, S.-P. Bi, D.-L. Shao, Y.-J. Chen, L.-M. Dai, L.-C. Tian, J. Chromatogr. A 993 (2003) 179–185.
[19] J. Wu, C.Y. Zhou, H. Chi, M.K. Wong, H.K. Lee, H.Y. Ong, C.N. Ong, J. Chromatogr. B 663 (1995) 247–253.
[20] T.G. Kazi, S. Khan, J.A. Baig, N.F. Kolochi, H.I. Afridi, A. Shah, Anal. Methods 2 (2010) 558–563.
[21] M. Buratti, C. Valla, O. Pellegrino, F.M. Rubino, A. Colombi, Anal. Biochem. 353 (2006) 63–68.
[22] M.R. Pereiro-García, M.E. Díaz-García, A. Sanz-Medel, Analyst 115 (1990) 575–579.
[23] P. Fernández, C. Pérez-Conde, A. Gutiérrez, C. Cámara, Talanta 38 (1991) 1387–1392.
[24] A. Taylor, R.J. Briggs, C. Çevik, Clin. Chem. 40 (1994) 1517–1521.
[25] M.P. Mánuel-Vez, M. García-Vargas, Talanta 41 (1994) 1553–1559.
[26] American Public Health Association (APHA), American Water Works Association (AWWA), Water Pollution Control Federation (WPCF), Standard Methods for the Examination of Water and Wastewaters, 21st ed., Washington DC, 2005.
[27] G. Albendín, M.P. Mánuel-Vez, C. Moreno, M. García-Vargas, Talanta 60 (2003) 425–431.
[28] M.P. Mánuel-Vez, C. Moreno, D.J. González, M. García-Vargas, Anal. Chim. Acta 355 (1997) 157–161.
[29] B. Dejaegher, Y. Van der Heyden, LC–GC Eur. 21 (2008) 96–102.
[30] R. Leardi, Anal. Chim. Acta 652 (2009) 161–172.
[31] D.C. Montgomery, Design and Analysis of Experiments, 7th ed., John Wiley & Sons, New York, 2009.
[32] S.L.C. Ferreira, W.N.L. dos Santos, C.M. Quintella, B.B. Neto, J.M. Bosque-Sendra, Talanta 63 (2004) 1061–1067.
[33] L.A. Sarabia, M.C. Ortiz, Surface response methodology, in: S. Brown, R. Tauler, R. Walczak (Eds.), Comprehensive Chemometrics, vol. 1, Elsevier, Oxford, 2009, pp. 345–390.
[34] M. Miró, A. Cladera, J.M. Estela, V. Cerda, Anal. Chim. Acta 438 (2001) 103–116.
[35] M. Manera, M. Miró, J.M. Estela, V. Cerda, Anal. Chim. Acta 582 (2007) 41–49.
[36] M.P. Manuél-Vez, Spectrofluorometric assays of Al(III) and Ti(IV) and simultaneous spectrophotometric assays of Fe(III), Co(II) and Ni(II) using picolinoylhydrazone derivatives for characterization of agriculture soils (in Spanish) (Ph. D. dissertation), University of Cádiz, 1990.
[37] J.N. Miller, J.C. Miller, Statistics and Chemometrics for Analytical Chemistry, 5th ed., Pearson Education Ltd., Essex, UK, 2005.