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Extractive analysis of aluminum traces in dialysis solutions

M.A. Raggi [a,*], C. Sabbioni [a], G. Cantelli Forti [b]

[a] *Department of Pharmaceutical Sciences, Via Belmeloro 6, 40126 Bologna, Italy*
[b] *Department of Pharmacology, Via Irnerio 48, 40126 Bologna, Italy*

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Abstract

A very sensitive procedure for the fluorimetric determination of aluminum traces in dialysis solutions by means of Mordant Red 19 dyestuff is described with the extraction of the Al complex in isobutylmethylketone. The experimental conditions were studied, in order to obtain the best extraction yield. The emission intensity of the metal chelate, extracted in the organic layer, was measured at 549 nm, exciting at 485 nm. Linearity between emission intensity and Al concentration was found in the 1–30 ng/ml range. The limit of detection was 0.25 ng/ml. The method resulted to be suitable for the determination of Al traces in commercial dialysis solutions for toxicological purposes.

Keywords: Aluminum; Mordant Red 19 reagent; Aluminum microdosage; Extractive fluorimetric determination; Dialysis solutions

1. Introduction

Today, the severe toxic effects of Al on patients with renal failure subjected to dialysis, such as bone disease, anemia, encephalopathy and 'dialysis dementia', are well known [1–4].

In the last few years, the Official Pharmacopeias (Ph. Eur., F.U.I. [5,6]) require an accurate check of the levels of Al traces in dialysis commercial solutions, which must fall within 10 ng/ml, for hemofiltration solutions and peritoneal dialysis solutions.

A recent paper [7] reports that, despite extensive measures to control aluminum exposure, chronic and acute episodes of Al intoxication still occur, particularly in developing countries. From this, the need for accurate and sensitive analytical procedures suitable for this control is apparent, in order to have available reliable analytical methods for the Al microquantities determination.

Numerous papers for the analysis of Al traces in dialysis solutions have been reported in literature, and they include emission spectrometry [8], electrochemical procedures [9,10] and above all atomic absorption spectrometry [11–14]. A few papers report spectrofluorimetric methods [15,16] for aluminum microdosage.

Some years ago, two fluorimetric procedures have been developed in our group, one based on the use of Morin and Pontachrome blue-black

* Corresponding author. Tel.: +39-051-209-9700; fax: +39-051-209-9734.
E-mail address: raggima@kaiser.alma.unibo.it (M.A. Raggi)

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reagents [17] and the other on the extraction of the Al-Morin complex [18].

Recently we proposed the use of the dyestuff Mordant Red 19 (MR19) for the determination of Al in mineral waters [19] and in dialysis solutions [20]. Now we propose the same reagent for a modified fluorimetric procedure with an extraction step in isobutylmethylketone (IBMK), which seems to improve the sensitivity and selectivity of the method.

2. Experimental

2.1. Apparatus and chemicals

The pH values of all solutions were measured with a Crison (Barcelona, Spain) Model 501 pH meter (pH $\pm$ 0.01). Fluorescence intensities were measured with a Perkin–Elmer (Beaconsfield, Buckinghamshire, England) LS-5 spectrofluorimeter. Absorbance spectra were recorded using a Jasco (Tokyo, Japan) UVIDEC-610 double-beam spectrophotometer.

All reagents were of analytical grade. Aluminum nitrate nonahydrate (pure for atomic absorption spectroscopy) stock solution, glacial acetic acid, sodium hydroxide, *n*-propylic alcohol, isobutylmethylketone (IBMK) were purchased from Merck (Darmstadt, Germany); 5-chloro-3-[(4,5-dihydro-3-methyl-5-hydroxy-1-phenyl-1H-pyrazol-4-yl)azo]-2-hydroxy-benzensulfonic acid monosodic salt or Mordant Red 19 (MR19) was purchased from Sigma Chemicals (St. Louis, MO, USA). All solutions were prepared using ultrapure water from a Millipore (Bedford, MA, USA) MilliQ apparatus.

The samples of hemofiltration solutions (SIF-BH 504/B, SIF-BH 499) and of peritoneal dialysis solutions (SIF-BP 466/A) were from Sifra (Isola della Scala, Verona, Italy).

2.2. Standard solutions

All solutions, except the Mordant Red 19 stock solution, were prepared in aluminum free polyethylene (PMP) volumetric flasks.

Mordant Red 19 stock solution (3.9×10^{-5} M): 5.12 mg of MR19 were dissolved in 200 ml of *n*-propylic alcohol, or of ultrapure water. The commercial product contains about 65% of pure dye, the rest being mostly NaCl. The solution was used after being stored for at least 24 h.

pH 5.15 buffer solution: 100 ml of a 1 N NaOH solution and 138 ml of glacial acetic acid were brought up to 1 l with ultrapure water. The resulting buffer solution had pH 5.15 and ionic strength 0.1.

Aluminum stock solution (1 g/l): a vial containing the standard aluminum for atomic absorption was diluted with ultrapure water to 1 l. Aluminum standard solution (10 μg/ml): an aliquot of 1 ml of aluminum stock solution was diluted to 100 ml with pH 5.15 buffer solution.

2.3. Extraction step

In a PMP flask, 0–2.5 ml of *n*-propylic alcohol, 2.5 ml of MR19 stock solution in *n*-propylic alcohol or in water, were brought up to 12.5 ml with pH 5.15 buffer solution. 100 μl of standard aluminum solution (100 μg/ml) were added. The resulting solution was thermostatted at 70°C for 60 min, then 0.5–2.0 ml of IBMK were added and the mixture was shaken for 2 min. The organic layer was subjected to spectrofluorimetric analysis at wavelength (λ) = 549 nm, while exciting at λ = 485 nm.

2.4. Procedures

Calibration curve: the solution were prepared and analyzed as in Section 2.3 above, except: 0 ml of *n*-propylic alcohol, 2 ml of IBMK and several aliquots (from 0 to 100 μl) of standard aluminum solution were added.

Sample analysis: flasks were prepared as above, except that 2 ml of buffer were substituted with the hemodialysis solution sample, and were subjected to the same treatment. Emission intensities were plotted against added Al concentration. The aluminum concentrations in the sample were determined by the standard addition method.

Recovery analyses were performed by spiking hemodialysis solution samples with known

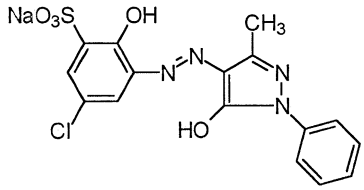

Fig. 1. Chemical structure of Mordant Red 19.

amounts of standard aluminum solution, and then analyzing by the usual analytical procedure.

3. Results and discussion

3.1. Complex formation

The Mordant Red 19 dyestuff (Fig. 1) reacts with Al ions to give a very stable Al-MR19 complex, which produces an intense fluorescence emission. The reaction reaches completeness under the following conditions [20]: pH 5.15; dye concentration 7.8×10^{-6} M in *n*-propylic alcohol.

In order to improve the sensitivity and the selectivity of the method, an extraction step of the Al-MR19 complex in IBMK was introduced. The optimal conditions to obtain the highest fluorescence emission using the smallest volume of organic layer were studied.

3.2. Extraction studies

Preliminary experiments were performed starting from the experimental conditions of the direct method [20] (7.5 ml of pH 5.15 buffer, 2.5 ml of MR19 solution in *n*-propylic acid, 2.5 ml of *n*-propylic alcohol) and adding very small volumes of IBMK (< 1.5 ml). The results were not satisfactory because the organic layer showed opalescence. In fact, the presence of alcohol favored the IBMK solubilization [21]. Numerous trials were then performed (Table 1).

The next step was the study of the complexation between Al and MR19 without *n*-propylic alcohol in the reaction medium (using an aqueous solution of MR 19). Under these conditions (Table 1, assay 1), the subsequent extraction with IBMK posed no problems for extracted volumes and opalescence, but gave rise to a very low fluorescence. Therefore, the presence of *n*-propylic alcohol was necessary to get a good fluorescence, so different ratios between aqueous medium, IBMK and *n*-propylic alcohol were studied. The amount of *n*-propylic alcohol (not containing MR19) was varied, to keep the complexing agent concentration constant. The performed assays are reported in Table 1. As can be seen, the total volume of the complexation step does not change (12.5 ml). In fact, every decrease in the *n*-propylic alcohol volume corresponds to an identical increase in the aqueous medium volume. IBMK volumes reported relate to the minimum volume necessary to obtain a good separation (no opalescence) between aqueous and organic phases.

The conditions reported in the seventh assay (no *n*-propylic alcohol addition, 0.5 ml of IBMK) gave good phase separation, but the extraction yield was low. In order to obtain a high extraction, the minimum necessary IBMK volume is 2

Table 1
Extraction studies

Steps	Experimental conditions	Analysis[a]							
		1	2	3	4	5	6	7	8
Complexation	*n*-propylic alcohol (ml)	0	2.5	2.0	1.5	1.0	0.5	0	0
	MR19 in *n*-propylic alcohol (ml)	0	2.5	2.5	2.5	2.5	2.5	2.5	2.5
	Buffer (ml)	12.5	7.5	8.0	8.5	9.0	9.5	10.0	10.0
Extraction	IBMK (ml)	2.0	2.0	1.5	1.0	0.8	0.5	0.5	2.0
	Extracted organic layer (ml)	2.0	6.0	4.0	3.0	1.5	1.0	0.7	3.0

[a] Aluminum concentration: 10 ng/ml.

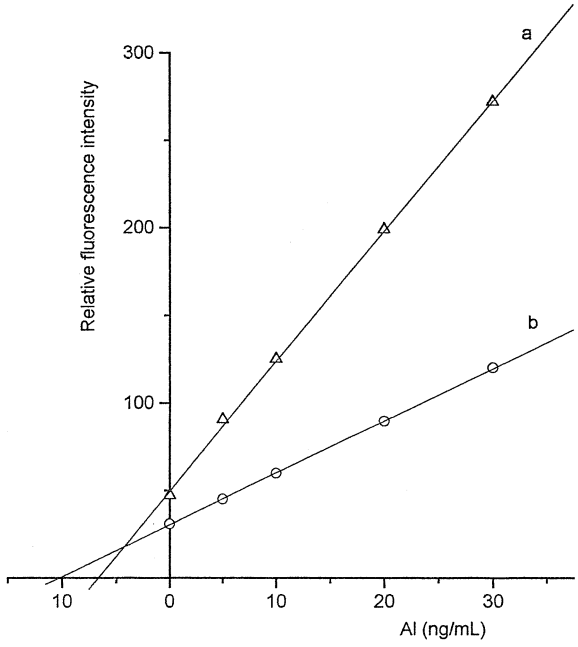

Fig. 2. (a) Calibration curve. (b) Determination of Al in a diluted hemofiltration solution by the extrapolation method.

ml, while the final extracted volume (a mixture of *n*-propylic alcohol and IBMK) is 3 ml, as reported in the eighth assay. Under these experimental conditions, the extraction efficiency is very good. In fact, absolute recovery with only one extraction step is almost complete, because the remaining aqueous solution shows no fluorescence emission.

The morphology of the Al-MR19 complex, extracted with IBMK, does not change appreciably, if compared to that obtained with the direct procedure. The emission band maximum was at 549 nm (instead of 555 nm), when exciting at 485 nm (instead of 478 nm). On the contrary, a considerable hypercromic effect was observed, which led to a higher sensitivity in the Al analysis.

3.3. Method validation

A calibration curve was obtained by plotting relative emission intensity values against Al concentrations (ng/ml). Linearity was found in the 1–30 ng/ml Al concentration range (Fig. 2a), that is, a 1.5–45 ng/ml Al range, if one considers the dilution coefficient. The regression equation was $y = 7.43x + 49.22$ ($Rc = 0.998$), where y represents the fluorescence emission intensity values (expressed as arbitrary units), while x represents the Al concentration values (expressed as ng/ml). The quantitation limit (LOQ) resulted to be 0.4 ng/ml, while the detection limit (LOD) was 0.25 ng/ml. The repeatability (or intraday precision) was good; the percent relative standard deviation (RSD%) values of fluorescence intensity were 2.1 and 1.6 for Al concentrations of 10 and 20 ng/ml ($n = 6$), respectively.

3.4. Application to diluted dialysis solutions

Having validated the method, it was applied to the analysis of Al traces in some commercially available diluted dialysis solutions.

The 10 ml volume of pH 5.15 buffer used for the calibration curve was substituted with 2 ml of dialysis solution and 8 ml of the same buffer, and subjected to the described procedure.

The Al level was determined by applying the standard addition method. Various amounts of standard Al solution (10 μg/ml), corresponding to 0, 5, 10, 20 and 30 ng/ml, were added to 2 ml aliquots of dialysis solution sample and subjected to complexation and extraction procedure. The emission values found were plotted against the concentration of added Al and the Al level in the dialysis solution was determined by extrapolating the least-square fitting line to zero emission.

As an example, Fig. 2 shows the results obtained by analyzing a SIF-BH 504/B dialysis solution sample (b) and the corresponding calibration curve (a).

The difference between the intercepts on the x axis of lines 2b and 2a is 3.2 ng/ml, which, multiplied by 1.5 (dilution coefficient) gives a final Al value equal to 4.8 ng/ml. This value is in a good agreement with the value obtained using the direct procedure previously developed by us [20]. The results obtained analyzing some commercial dialysis solutions are reported in Table 2. The accuracy of the procedure was verified by means of recovery studies, spiking the sample with a known amount of Al standard solution. A recovery value of 96% was found after a 10 ng/ml Al addition.

Table 2
Al analysis in commercial dialysis solutions

Sample	Name and kind of solution	Composition	Al (ng/ml)[a]
1	SIF-BH 504 B, diluted hemodialysis solution	NaCl 5.73 g KCl 0.11 g $CaCl_2$ $2H_2O$ 0.22 g $MgCl_2$ $6H_2O$ 0.10 g Na lactate 4.70 g Glucose, monohydrate 2.20 g Water for injectables to 1000 ml	4.8 ± 0.6
2	SIF-BH 499, diluted hemodialysis solution	NaCl 6.28 g KCl 0.15 g $CaCl_2$ $2H_2O$ 0.26 g $MgCl_2$ $6H_2O$ 0.10 g Na lactate 4.76 g Water for injectables to 1000 ml	6.1 ± 0.8
3	SIF- BP 466 A, peritoneal dialysis solution	NaCl 5.67 g $CaCl_2$ $2H_2O$ 0.26 g $MgCl_2$ $6H_2O$ 0.07 g Na lactate 3.92 g Glucose, monohydrate 16.50 g Water for injectables to 1000 ml	8.9 ± 1.2
4	Solution reconstructed by us following the declared SIF-BH 504 B composition	As SIF-BH 504 B	4.0 ± 0.7

[a] Each value is the mean of three independent assays $\pm$ S.D.

4. Conclusion

These results suggest that the fluorimetric extractive procedure, based on the use of MR19 and the subsequent extraction of the Al complex in IBMK, is suitable for the toxicological check of Al traces in dialysis solutions, having a good precision and satisfactory accuracy. The proposed method is surely better, with respect to sensitivity and selectivity, than the direct procedure which uses the same reagent. For this reason, it seems highly promising with regard to the analysis of Al in concentrated dialysis solutions and in biological fluids, for whose samples the direct method with MR19 was not suitable because of matrix interference. An investigation in this direction is under way.

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