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On-line cloud point preconcentration and determination of gadolinium in urine using flow injection inductively coupled plasma optical emission spectrometry

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The on-line incorporation of cloud point extraction (CPE) into flow injection analysis (FIA) associated with ICP-OES for determining metal ions is presented and evaluated. The methodology is based on the complexation of Gd(III) with 2-(5-bromo-2-pyridylazo)-5-diethylaminophenol in the presence of non-ionic micelles of PONPE-7.5. The micellar system containing the complex was thermostated at 25 °C and loaded into the FIA manifold at a flow rate of 5 ml min^{-1}, and the surfactant rich-phase was retained in a micro-column packed with cotton, at pH 9.2. The surfactant-rich phase was eluted with 4 mol l^{-1} nitric acid at a flow rate of 1.5 ml min^{-1} directly into the nebulizer of the plasma. An enhancement factor of 20 was obtained for the preconcentration of 10 ml of sample solution. The detection limit for the preconcentration of 10 ml of aqueous solution of Gd was 40 ng l^{-1}. The precision (RSD) for 10 replicate determinations at the 2.0 μg l^{-1} Gd level was 1.9%, calculated from the peak heights obtained. The calibration graph using the preconcentration system for gadolinium was linear, with a correlation coefficient of 0.9997 at levels near the detection limit up to at least 50 μg l^{-1}. The method was successfully applied to the determination of gadolinium in urine.

Introduction

Chelated Gd complexes have been found to be useful as NMR contrast agents for obtaining physical, chemical and biological results. Due to the tendency of the paramagnetic lanthanide cations to complex with naturally occurring agents, Gd-DTPA and Gd-DOTA chelates have been introduced as contrast agents in magnetic resonance imaging (MRI) and computer tomography scanning.[1–3] A rapid and complete excretion of the contrast agent is desired when a diagnostic examination of a patient is being carried out.

$[Gd(DPTA)]^{2-}$ and $[Gd(DOTA)]^{2-}$ are rapidly excreted mainly into urine, while the elimination of free Gd(III) is slow, with the liver being the main repository.[4] Immediate tolerance to free Gd(III) ions is very poor and their long-term effect is unknown. It is therefore very important to determine the Gd content in urine.[5]

Although ETAAS and ICP-OES are the most used techniques in the determination of trace levels of gadolinium, the concentration in urine samples after using MRI is not compatible with the limit of detection of such techniques. Consequently, a preconcentration step is essential in order to achieve an adequate performance.

When preconcentration techniques are applied in a batch mode, the time of analysis increases and the operations are usually too tedious to be compatible with ICP-OES measurements. Furthermore, these procedures are not practical for routine analysis. This situation has been significantly improved by utilizing flow injection (FI) associated with ICP-OES,[6,7] such that the general drawbacks of batch preconcentration procedures have been largely eliminated and currently preconcentration can be achieved almost as efficiently as a simple ICP-OES determination. Reagent consumption is usually reduced to a small percentage of that in batch procedures. Sample contamination is also reduced, which becomes important when trace concentrations are determined. In fact, to date the most dramatic improvements achieved in FI-ICP-OES have been in the field of on-line preconcentration.

The use of micellar solutions in different areas of analytical chemistry has attracted much attention in recent years. Among other micelle-based separation methods, cloud point extraction is an efficient extraction step for the enrichment of metal ions, allowing their quantification at ppb levels.[8,9] Aqueous solutions of many non-ionic surfactant micellar systems become turbid over a narrow temperature range, when the experimental conditions are changed. This temperature is called the "cloud point temperature". Above the cloud point, the solution separates into two phases: the surfactant-rich phase, which is very small in volume; and the bulk aqueous solution, containing surfactant monomers. Any analyte that is dissolved in the hydrophobic core of the micelles will separate and become concentrated in the small volume of the surfactant-rich phase.[10,11]

The use of micellar systems as an alternative to other techniques of separation offers several advantages including low cost, safety, and a high capacity to concentrate a wide variety of analytes of widely varying nature with high recoveries and very high concentration factors.[12–14] 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.

In previous works,[15–18] we have used off-line cloud point extraction (CPE) approaches in order to determine Gd and other metals coupled with ICP-OES, HPLC and UV–Vis spectrometry. Recently, Fang *et al.*[19] presented for the first time the on-line incorporation of CPE into flow injection for the analysis of coproporphyrin. Nevertheless, this type of coupled approach has never been used for determining metal ions.

The purpose of the present paper is to demonstrate the

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feasibility of on-line CPE with FI-ICP-OES for the preconcentration and determination of total gadolinium content in urine samples. The preconcentration step, mediated by micelles of the non-ionic surfactant poly(ethylene glycol) mono-*p*-nonylphenyl ether (PONPE 7.5), is performed by means of the formation of a Gd(III)-2-(5-bromo-2-pyridylazo)-5-diethylaminophenol [Gd(III)-5-Br-PADAP] complex.

Experimental

Apparatus

The measurements were performed with a sequential ICP spectrometer (ICP 2070, Baird, Bedford, MA, USA). The 1 m Czerny–Turner monochromator is based on a holographic grating with 1800 grooves mm^{-1}. A Minipuls 3 peristaltic pump (Gilson, Villiers-le-Bel, France) was used. Sample injection was achieved using a Rheodyne (Cotati, CA, USA) Model 50, four-way rotatory valve. A microbore glass column (30 mm length; 3 mm id) was used as the cotton holder. Tygon-type pump tubing (Ismatec, Cole-Parmer, Vernon Hills, IL, USA) was employed to carry the sample, reagent and eluent.

The 335.047 nm spectral line was used. Table 1 shows the optimal plasma instrumental conditions.

The flow injection-preconcentration system is shown in Fig. 1.

Reagents and solutions

A 1 mg ml^{-1} standard solution of Gd(III) was prepared from acid dissolution of its oxide of analytical grade purity (Aldrich Chemical Company, Inc.). Stock solutions were standardized by a chelatometric method.[20]

A 1×10^{-3} mol l^{-1} solution of 5-Br-PADAP (Aldrich, Milwaukee, WI, USA) was prepared by dissolution in ethanol (Merck, Darmstadt, Germany). Lower concentrations were prepared by serial dilution with ethanol.

As it is not possible to obtain a real aqueous solution of the poly(ethylene glycol) mono-*p*-nonylphenyl ether surfactant (PONPE-7.5, Tokyo Kasei Industries, Chuo-Ku, Tokyo, Japan) (cloud point below room temperature), it was experimentally convenient to prepare a stock surfactant solution. 20 g of PONPE-7.5, 10 ml of $NaClO_4$ (1 mol l^{-1}, Merck, Darmstadt, Germany) and 40 ml of distilled ethanol were mixed and made up to 100 ml with doubly distilled water.

The buffer solution (5×10^{-2} mol l^{-1}) was prepared by dissolving sodium tetraborate (Merck, Darmstadt, Germany) and making up to 1000 ml with ultrapure water.

Ultrapure water (18.3 MΩ cm) was obtained from a Barnstead EASY pure RF water system (IA, USA).

Procedures

Determination of gadolinium content. Urine was collected and stored in plastic containers without any preservatives. Acid mineralization of the sample was necessary due to the fact that naturally occurring components of urine do not normally allow Gd complexation with pyridylazo dyes. The samples of urine were digested as follows. 10 ml of urine sample were accurately measured into a porcelain capsule, and treated with a mixture of 2 ml of H_2O_2 and 1 ml of concentrated HNO_3. The capsule was placed in a sand bath. The sample was moderately heated until the amber color disappeared. Subsequently, the sample was evaporated to incipient dryness. Fresh portions of concentrated HNO_3 were added to the dark residue, which was then heated to dryness. This procedure was repeated until a white ash was obtained. The residue was taken up with 1 ml of a mixture of HCl and HNO_3 (3 + 1) and heated. This solution was diluted up to approximately 8 ml with water.

Blanks were prepared using the same reagents, without the samples, undergoing an identical process of mineralization.

Table 1 Plasma instrumental conditions with the use of a concentric glass nebulizer

Rf generator power	0.8 kW
Frequuency 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 (gadolinium)	335.047 nm

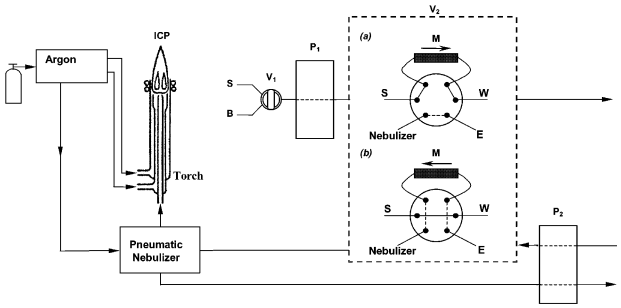

Fig. 1 Schematic diagram of the instrumental setup: S, sample + extracting solution (25 °C); B, buffer + surfactant; E, eluent; W, waste; P_1 and P_2, peristaltic pump; M, micro-column; V_1, two way valve; V_2, load-injection valve [(a) load position; (b) injection position].

Preconcentration step. The on-line incorporation of CPE with FIA was performed as follows. In the first stage, the sample solution containing gadolinium, 1 ml of stock surfactant solution, 50 µl chelating agent solution and 0.8 ml of buffer agent were placed in a plastic tube, and the resultant solution was equilibrated in a thermostatic bath at 25 °C. Before loading, the column was conditioned for preconcentration at the correct pH with a buffer-diluted solution containing 0.1% (w/w) PONPE 7.5.

The sample solution was then loaded into the FIA manifold at a flow rate of 5 ml min^{-1} by using a volume-based sampling mode. The solution passed through the collection column, which allowed for the surfactant-rich phase containing the gadolinium chelate to be collected inside the column, while the aqueous phase passed through the column. After loading, further washing with buffer-diluted solution served to remove any sample still present in the lines and in the column. Finally, the injection valve was switched on and the retained surfactant-rich phase was eluted with 4 mol l^{-1} nitric acid at a flow rate of 1.5 ml min^{-1} directly into the nebulizer of the plasma.

Since the metal chelate is not totally retained on the resin (90%) the standard solution must also be passed through the micro-column. The operating conditions were established and the determination was performed.

Recovery study. In order to develop recovery studies, the following sequence was carried out. 100 ml of urine sample were collected and divided into 10 portions of 10 ml each. The proposed method was applied to six portions and the average quantity of gadolinium obtained was taken as a base value. Then, increasing quantities of gadolinium were added to the other aliquots of the sample and gadolinium was determined by the same method (Table 2).

Results and discussion

The excretion half-life of Gd complexes is enormously different from that of free Gd: 5 min *versus* 7 d.[4,5] Gd(III) is retained in the liver and then very slowly eliminated *via* the urinary tract. Consequently, and due to the elevated toxicity of Gd(III) and

Table 2 Recovery study (95% confidence interval; $n = 6$)

Aliquots	Base value/ μg l^{-1}	Quantity of Gd added/μg l^{-1}	Quantity of Gd found/μg l^{-1}	Recovery (%)[a]
1	—	0.00	0.52 ± 0.02[b]	—
2	0.52	0.50	1.01 ± 0.03	98.0
3	0.52	1.00	1.50 ± 0.04	98.0
4	0.52	2.00	2.52 ± 0.03	100.0
5	0.52	4.00	4.51 ± 0.05	99.7

[a]100 × [(found-base)/added]. [b]Standard deviation.

the extensive use of gadolinium-based pharmaceuticals, it is clear that the determination of gadolinium in urine is of outstanding importance.

Preconcentration of gadolinium in the urine samples was necessary because its concentration in healthy subjects can be too low to be compatible with ICP-OES detection limits: for example, Allain *et al.*[21] reported gadolinium quantities in urine to be as low as 0.3 μg l^{-1}.

In addition, the preconcentration system proposed in this paper allows the elimination of a large part of the saline content in the sample, principally sodium and potassium, which have a limited tendency to form 5-Br-PADAP complexes.

Effect of pH

The retention conditions of the metal complex were optimized and the gadolinium signal was monitored using ICP-AES while changing the pH of the sample solution that passed through the micro-column. The optimal pH values were in the 8.0–10.5 range, in accordance with the optimal complex formation pH range. Considering these results, a pH of 9.20 was selected.

Surfactant and chelating agent concentrations

The effect of PONPE 7.5 concentration upon sensitivity and extraction was studied within the surfactant concentration range of 0.1–2.0% (w/w). Quantitative extraction was observed for an amphiphile concentration higher than 0.6% (w/w). In order to achieve a good preconcentration factor, 1% (w/w) was chosen as optimal. The minimum reagent to metal ion molar ratio necessary to reach the optimum response was 10. Above this ratio, no variation in the analytical response was observed.

Equilibration temperature

The greatest analyte preconcentration factor is reached when the CPE process is conducted at equilibration temperatures well above the cloud point temperature of the system. In order to induce phase separation, the sample temperature was maintained at 25 °C and the phase behavior of the system was studied. Fig. 2 shows the phase diagram of the studied system. An equilibration temperature of 25 °C was chosen as optimal.

Collection column

A home-made column packed with suitable filtering material was employed to carry out phase separation. Commercial cotton proved to be highly efficient at retaining the surfactant-rich phase; 90% retention of the analyte was achieved. In accordance with previous work,[22–24] the internal diameter of the micro-column was set at 3 mm. The column length was varied within the range 10–50 mm, and optimal retention was achieved for column lengths greater than 20 mm. A column length of 30 mm was chosen.

Loading and elution flow rates

The sample flow rate through the micro-column is a very important parameter, since this is one of the steps that controls

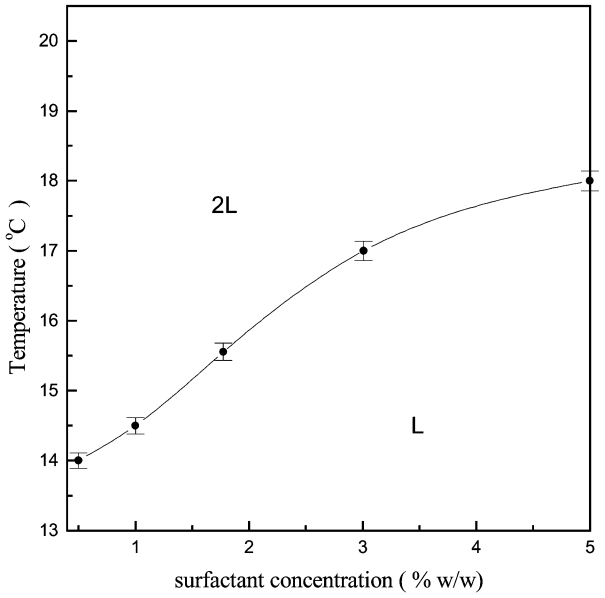

Fig. 2 Variation of the cloud point temperature as a function of the surfactant concentration. Conditions: $C_{\text{PONPE 7.5}} = 0.1$–5% (w/w); $C_{\text{sodium tetraborate}} = 4 \times 10^{-3}$ mol l^{-1} (pH = 9.20); $C_{\text{ethanol}} = 4\%$ (v/v); $C_{\text{Gd(III)}} = 5$ μg l^{-1}; $C_{\text{5-Br-PADAP}} = 4 \times 10^{-7}$ mol l^{-1}; $\mu = 0.01$. L, Single isotropic amphiphilic solution phase region, and 2 L, presence of two coexisting isotropic phases.

the time of analysis. For flow-rates up to 5 ml min^{-1} there was no effect on the analyte recovery, which under optimum conditions is 90%. Fig. 3 shows that, at higher loading flow-rates, the recovery decreases.

In order to elute the surfactant-rich phase retained on the column, 4 mol l^{-1} nitric acid was used as the eluent. The analyte was completely eluted from the cotton in 15 s. The optimum flow rate of the eluent was 1.5 ml min^{-1}.

Interferences

The effects of some potential interfering species 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 2000 μg l^{-1}. Commonly encountered matrix components, such as alkali and alkaline earth elements, generally do not form stable complexes and are not suitable for CPE. On the other hand, anions such as CO_3^{2-}, F^-, SO_4^{2-}, Cl^- and PO_4^{3-} could be tolerated up to at least 500 000 μg l^{-1}.

The value of the reagent blank signal (which was never higher than the analytical signal for a Gd solution containing 40 ng l^{-1}) was not modified by the presence of the potentially interfering ions studied.

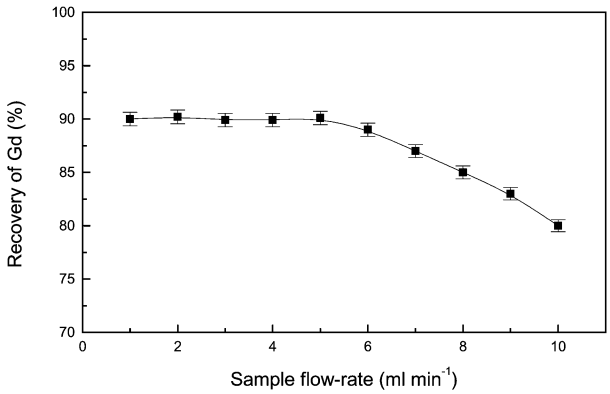

Fig. 3 Dependence of recovery of metal complex on loading sample flow rate. Preconcentration of 10 ml of Gd-5-Br-PADAP-surfactant system at pH 9.2 and $C_{\text{Gd(III)}} = 5$ μg l^{-1}.

Table 3 Concentrations of gadolinium in urine samples (95% confidence interval; $n = 6$)

Sample	Gd concentration/μg l^{-1}
1	0.25 ± 0.03
2	0.40 ± 0.03
3	0.52 ± 0.02
4	0.47 ± 0.02
5	0.50 ± 0.02
6	0.37 ± 0.03

Figures of merit and analytical performance

The relative standard deviation (RSD) resulting from the CPE-FI-ICP-OES analysis of 10 replicates of 10 ml of a solution containing 2.0 μg l^{-1} Gd was 1.9%. The calibration graph was linear with a correlation coefficient of 0.9997 at levels near the detection limits and up to at least 50 μg l^{-1}. The detection limit (DL) was 40 ng l^{-1}, calculated as the amount of gadolinium required to yield a net peak, considering three times the standard deviation of the background signal (3*s*).

The overall time required for preconcentration of 10 ml of sample (2.0 min, at a flow rate of 5 ml min^{-1}), washing (0.2 min), elution (0.25 min, at a flow rate of 1.5 ml min^{-1}) and conditioning (0.2 min) was about 2.65 min; hence, the throughput was approximately 22 samples per hour.

A total enhancement factor of 20 was obtained with respect to gadolinium determination by ICP-OES without preconcentration.

Determination of gadolinium in urine

The results of the gadolinium determination in urine are shown in Table 3. The concentrations were in the range 0.25–0.52 μg l^{-1} of gadolinium. The results obtained were in good agreement with those of Allain *et al.*[21]

Conclusions

In this work, the on-line incorporation of cloud point extraction (CPE) with flow injection analysis (FIA) associated with ICP-OES is presented and evaluated for the first time. The possibility of performing CPE on-line opens up an attractive alternative in the area of automated separation methods, particularly in view of the excellent extraction efficiencies and preconcentration factors associated with the technique. The CPE preconcentration of gadolinium in urine was achieved with a simple FI system, which was easily coupled to ICP-OES with pneumatic nebulization. The results demonstrate the possibility of using the 5-Br-PADAP-PONPE-7.5 system for the preconcentration of gadolinium. A 20-fold enhancement factor with respect to gadolinium determination by ICP-OES without preconcentration was obtained. The preconcentration method allowed gadolinium to be determined in urine samples with good accuracy and reproducibility.

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