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ORIGINAL PAPER

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# On-line complexation/cloud point preconcentration for the sensitive determination of dysprosium in urine by flow injection inductively coupled plasma–optical emission spectrometry

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**Abstract** An on-line dysprosium preconcentration and determination system based on the hyphenation of cloud point extraction (CPE) to flow injection analysis (FIA) associated with ICP–OES was studied. For the preconcentration of dysprosium, a Dy(III)–2-(5-bromo-2-pyridylazo)-5-diethylaminophenol complex was formed on-line at pH 9.22 in the presence of nonionic micelles of PONPE-7.5. The micellar system containing the complex was thermostated at 30 °C in order to promote phase separation, and the surfactant-rich phase was retained in a microcolumn 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 in the nebulizer of the plasma. An enhancement factor of 50 was obtained for the preconcentration of 50 mL of sample solution. The detection limit value for the preconcentration of 50 mL of aqueous solution of Dy was 0.03 µg $L^{-1}$. The precision for 10 replicate determinations at the 2.0 µg $L^{-1}$ Dy level was 2.2% relative standard deviation (RSD), calculated from the peak heights obtained. The calibration graph using the preconcentration system for dysprosium was linear with a correlation coefficient of 0.9994 at levels near the detection limits up to at least 100 µg $L^{-1}$. The method was successfully applied to the determination of dysprosium in urine.

**Keywords** On-line preconcentration · Dysprosium · Cloud point extraction

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## Introduction

Physical chemical and biological results support the use of chelated Dy complexes as NMR contrast agents, for example, the diethylenetriaminepentaacetic acid bis(methylamide) (DTPA-BMA) complex was used as a ligand in myocardial investigations [1] and as a potential marker of cell membrane integrity [2] and 1,4,7-tris(carboxymethyl)-10-(2′-hydroxypropyl)-1,4,7,10-tetraazacyclododecane (HP-DO3A) was a ligand in brain investigations [3]. Both dysprosium triethylenetriamine hexaaxetate (DyTTHA) and dysprosium bistriphosphate (DyPPP) were used to distinguish intra and extracellular $^{23}Na$ resonances before and after the onset of hypoxia [4].

Electrothermal atomic absorption spectrometry (ETAAS) and inductively coupled plasma–optical emission spectrometry (ICP-OES) are the most used techniques in the determination of dysprosium trace levels. Nevertheless, lower limits of detection are required if long-term toxicity studies are being performed. Consequently, a preconcentration step should be used to reach 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 the ICP–OES measurements. Furthermore, these procedures are not practical for application in routine analysis. This situation has been significantly improved by utilizing flow injection (FI) coupled to ICP–OES [5, 6], such that the general drawbacks of batch preconcentration procedures have been largely eliminated, and currently the preconcentrations can be achieved almost as efficiently as with 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.

In the last decade, increasing interest has been shown all over the world in developing surfactant-based methods

in all fields of analytical chemistry. Among other micelle-based separation methods, the cloud point extraction is an efficient extractive step for the enrichment of metal ions, allowing their quantification at ppb levels [7, 8]. Aqueous solutions of many nonionic surfactant micellar systems become turbid over a narrow temperature range, when the experimental conditions have been changed. This temperature is named the "cloud point temperature".

Above the cloud point, the solution separates into two phases: one, very small in volume—the surfactant-rich phase, and the other, the bulk aqueous solution, containing surfactant monomers. Any analyte solubilized in the hydrophobic core of the micelles will separate and become concentrated in the small volume of the surfactant-rich phase [9, 10].

The use of micellar systems as an alternative to other techniques of separation offers several advantages including low cost, safety, and high capacity to concentrate a wide variety of analytes of a widely varying nature with high recoveries and very high concentration factors [11, 12, 13]. 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 work [14, 15, 16, 17], we have used off-line CPE approaches to determine Gd and other metals coupled to ICP–OES, HPLC, and UV-Vis Spectrometry. Recently, we presented the first on-line incorporation of CPE to flow injection for the determination of Gd, after its off-line complexation [18].

In the present work, a method for preconcentration and determination of dysprosium content in urine samples using on-line CPE with FI–ICP–OES is proposed. The preconcentration step, mediated by micelles of the nonionic surfactant polyethyleneglycolmono-*p*-nonylphenylether (PONPE-7.5), is performed by means of the on-line formation of a Dy(III)–2-(5-bromo-2-pyridylazo)-5-diethylaminophenol (Dy(III)–5-Br-PADAP) complex.

## Experimental

### Apparatus

The measurements were performed with a sequential ICP spectrometer [Baird (Bedford, MA, USA) ICP 2070]. 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-Bell, France) was used. Sample injection was achieved using a Rheodyne (Cotati, CA, USA) Model 50, four-way rotatory valve. A microbore glass column (50 mm length, 3 mm i.d.) was used as the cotton holder. Tygon-type pump tubing (Ismatec, Cole–Parmer, Vernon Hills, IL, USA) was employed to propel the sample, reagent, and eluent.

The 353.170-nm spectral line was used. Table 1 shows the optimal plasma instrumental conditions. The flow injection–preconcentration system is shown in Fig. 1.

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

| | |
|---|---|
| RF generator power | 0.8 kW |
| Frequency of RF generator | 40.68 MHz |
| Plasma gas flow rate | 8.5 L $min^{-1}$ |
| Auxiliary gas flow rate | 1.0 L $min^{-1}$ |
| Carrier gas flow rate | 0.8 L $min^{-1}$ |
| Observation height (above load coil) | 15 mm |
| Analytical line (dysprosium) | 353.170 nm |

### Reagents and solutions

A standard solution of 1 mg $mL^{-1}$ Dy(III) was prepared from acidic dissolution of its oxide of analytical grade purity (Aldrich Chemical Company, Inc.). Stock solutions were standardized by a chelatometric method [19].

A $5\times10^{-2}$ mol $L^{-1}$ solution of 5-Br-PADAP (Aldrich, Milwaukee, WI, USA) was prepared by appropriate 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 surfactant polyethyleneglycolmono-*p*-nonylphenylether (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 as follows: 20 g of PONPE-7.5, 10 mL of 1 mol $L^{-1}$ $NaClO_4$ (Merck, Darmstadt, Germany), and 40 mL of distilled ethanol were mixed and made up to 100 mL with doubly distilled water.

The buffer solution ($5\times10^{-1}$ mol $L^{-1}$) was prepared by dissolving sodium tetraborate (Merck, Darmstadt, Germany) and made up to 1000 mL with ultrapure water.

Ultrapure water (18.3 MΩ $cm^{-1}$) was obtained from Barnstead EASY pure RF water system (Iowa, USA).

### Experimental procedure

#### *Determination of dysprosium content*

Urine was collected and stored in plastic containers without adding preservatives. A sample mineralization was necessary, since urine's natural occurring components normally do not allow Dy complexation with pyridylazo dyes. The samples of urine were digested as follows. A 50-mL sample of urine was accurately measured into a porcelain capsule, treated with a mixture of 2 mL of 30% (w/w) $H_2O2$ and 1 mL 65% (w/w) $HNO_3$ (conc.), and then placed in sand bath. The sample was moderately heated up to cause disappearance of the amber color. Subsequently, the sample was evaporated to incipient dryness. Then, fresh portions of 65% (w/w) $HNO_3$ were added to the dark residue and 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 0.5 mol $L^{-1}$ HCl and 0.5 mol $L^{-1}$ $HNO_3$ (3+1) and heated. This solution was diluted to approximately 40 mL with water. Blanks were prepared with the same reagents, without the samples, undergoing an identical process of mineralization.

### Preconcentration step

Before loading the column, it was conditioned for the preconcentration at the correct pH with buffer-diluted solution ($1\times10^{-2}$ mol $L^{-1}$). The solution containing dysprosium (standard solutions or samples) and a solution containing $5\times10^{-5}$ mol $L^{-1}$ 5-Br-PADAP and 1% (w/w) PONPE-7.5, buffered to pH 9.22 with sodium tetraborate, were mixed on-line at a flow rate of 8.0 and 2 mL $min^{-1}$, respectively. The solution passed trough a 100-cm mixing coil thermostated at 30 °C, and was then loaded onto the collection column, which allowed the surfactant-rich phase containing the dysprosium chelate to be collected inside the column, while the aqueous phase passed through the column. After the loading time, further washing with buffer diluted solution served to remove the sample still present in the lines and in the column. Finally, the in-

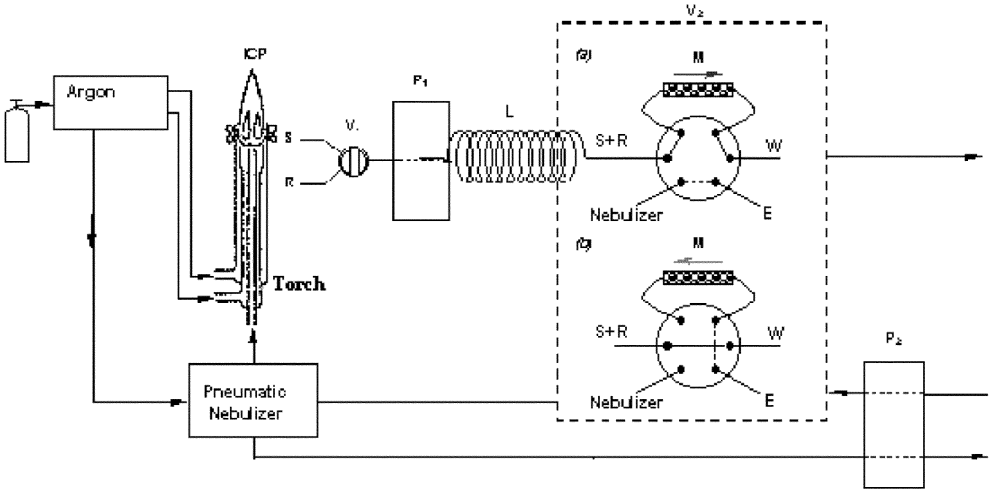


**Fig. 1** Schematic diagram of the instrumental setup. *S* sample, *R* chelating reagent + extracting solution + buffer, *E* eluent, *W* waste, $P_1$and $P_2$ peristaltic pump, L mixing coil (length 100 cm, *T* 30 °C), *M* microcolumn, $V_1$ two-way valve, $V_2$ load-injection valve (*a* load position, *b* injection position.

jection valve was switched on and the retained surfactant-rich phase was eluted with 4.0 mol $L^{-1}$ nitric acid at a flow rate of 1.5 mL $min^{-1}$ directly in the nebulizer of the plasma.

The operating conditions were established and the determination was performed. FI system measurements were expressed as peak height emission, which was corrected against the reagent blank.

## Results and discussion

The preconcentration system proposed in this paper allows the elimination of great part of the saline content in the sample, principally sodium and potassium, due to the limited tendency of these elements to form 5-Br-PADAP complexes. Besides, the on-line complexation led to a substantial improvement of the analytical performance of the method.

The effects of several experimental parameters upon the extraction parameters and sensitivity have been thoroughly evaluated and optimized. The optimization of the experimental conditions has been accomplished by the traditional method of one-at-a-time.

### Effect of pH

The retention conditions of the metal complex were optimized and the dysprosium signal was monitored by measuring it with ICP–OES while changing the pH of the sample solution that passes through the micro-column. The optimal pH values were in the 9.0–10.0 range, in accordance with the optimal complex formation pH range. Considering these results, the selected pH was 9.20 (Fig. 2a).

### Reagent excess effect

In order to determine the optimal reagent–metal ion relationship, an experiment was carried out in which the other experimental variables, except reagent concentration, remained constant. The minimum reagent to metal ion molar ratio necessary to reach the optimum response was 8.

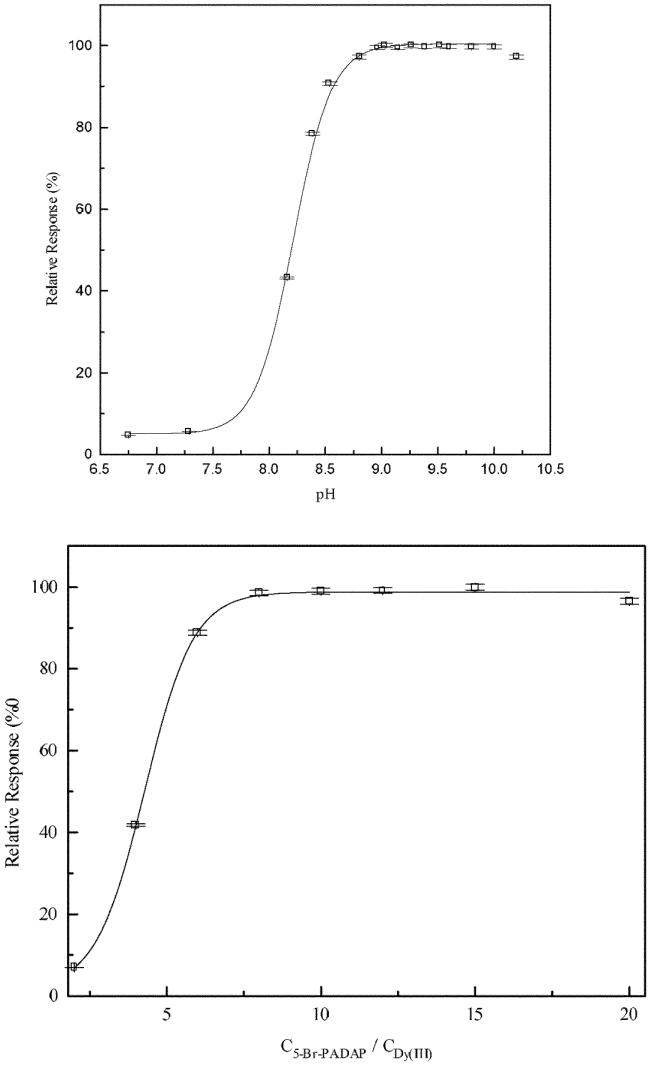


**Fig. 2** **a** Effect of pH. Conditions: $C_{PONPE-7.5}$=0.2% (w/w), $C_{ethanol}$= 0.8% (v/v), $C_{Dy(III)}$=2 µg $L^{-1}$, $C_{5\text{-Br-PADAP}}$=8×$10^{-8}$ mol $L^{-1}$, equilibration temperature 30 °C; each desired pH was obtained with additions amounts of diluted HCl or NaOH. **b** Effect of reagent excess. Conditions: $C_{PONPE-7.5}$=0.2% (w/w), $C_{sodium\ tetraborate}$=2x$10^{-4}$ mol $L^{-1}$ (pH 9.22), $C_{ethanol}$=0.8% (v/v), $C_{Dy(III)}$=2 µg $L^{-1}$, equilibration temperature 30 °C

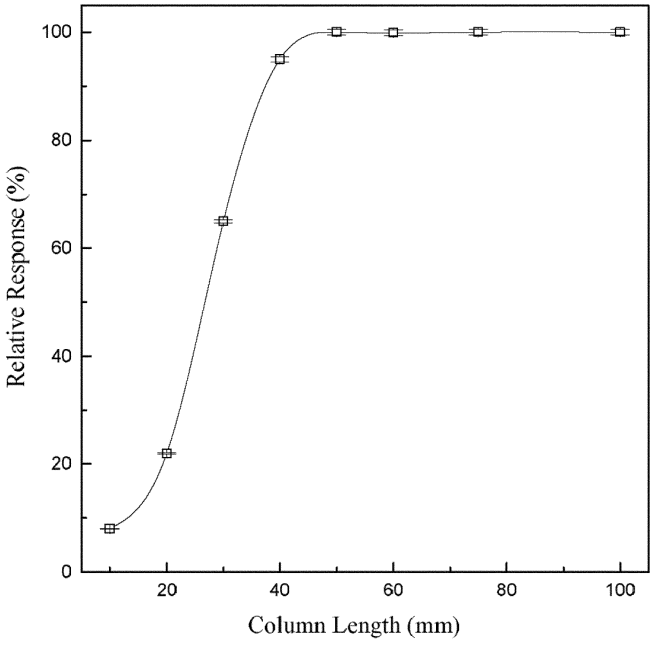


**Fig. 3** Dependence of recovery of metal complex on column length. Preconcentration of 50 mL of Dy-5-Br-PADAP-surfactant system. Conditions: $C_{PONPE\text{-}7.5}$=0.2% (w/w), $C_{sodium\ tetraborate}$=2x $10^{-4}$ mol $L^{-1}$ (pH 9.22), $C_{ethanol}$=0.8% (v/v), $C_{Dy(III)}$=2 µg $L^{-1}$, equilibration temperature 30 °C, loading flow rate 8 mL $min^{-1}$

Above this excess, no variation of the analytical response was observed. (Fig. 2b).

## Surfactant concentration

The effect of PONPE-7.5 concentration upon sensitivity and extraction parameters was studied within the surfactant concentration range 0.1–0.8%(w/w). Quantitative extraction was observed for an amphiphile concentration higher than 0.15% (w/w). In order to achieve a good preconcentration factor, 0.2%(w/w) was chosen as optimal.

## Effects of equilibration temperature

The greatest analyte preconcentration factor is reached when the CPE process is conducted with equilibration temperatures well above the cloud point temperature of the system. An equilibration temperature of 30 °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. In accordance with previous work [20], the internal diameter of the microcolumn was set at 3 mm. The column length was varied within the range 20–100 mm, allowing the optimal retention for column lengths greater than 50 mm (Fig. 3). A column length of 50 mm (approximately 50 mg of cotton) was chosen. At least 100 preconcentration/elution cycles can be performed with one cotton packing.

## Loading and elution flow rates

The flow rate sample through the microcolumn is a very important parameter, since this is one of the steps that controls the time of analysis. We could verify that with flow rates up to 8 mL $min^{-1}$ there is no effect on the analyte recovery, while 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 eluent was 1.5 mL $min^{-1}$.

## Interferences

The effects of representative 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 2,000 µg $L^{-1}$. Commonly encountered matrix components such as alkali and alkaline earth elements generally do not form stable complexes and are not CPE-extracted. 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 500.0 mg $L^{-1}$.

The value of the reagent blank signal (which was never higher than the analytical signal shown by a Dy solution containing 0.03 µg $L^{-1}$) was not modified by the presence of the potentially interfering ions assayed.

## Figures of merit and analytical performance

The relative standard deviation (RSD) obtained from the CPE–FI–ICP–OES analysis of ten replicates of 50 mL solution containing 0.4 µg $L^{-1}$ Dy was 2.2%. The calibration graph was linear with a correlation coefficient of 0.9997 at levels near the detection limits up to at least 50 µg $L^{-1}$. The detection limit (DL) was 0.03 µg $L^{-1}$; this was calculated as the amount of dysprosium required to yield a net peak and was calculated by considering three times the standard deviation of the background signal ($3\sigma$).

The overall time required for preconcentration of 50 mL of sample (6.25 min, at flow rate of 8 mL $min^{-1}$), washing (0.2 min), elution (0.25 min, at flow rate of 1.5 mL $min^{-1}$), and conditioning (0.2 min) was about 7.0 min; hence, the throughput was approximately 8 samples per hour.

A total enhancement factor of 50 was obtained with respect to dysprosium determination by ICP–OES without preconcentration.

**Table 2** Recovery study

| Aliquots | Base value ($\mu g\,L^{-1}$) | Quantity of Dy added ($\mu g\,L^{-1}$) | Quantity of Dy found ($\mu g\,L^{-1}$) | Recovery (%)[a] |
|---|---|---|---|---|
| 1[b] | – | 0.00 | 0.42±0.02 | – |
| 2 | 0.42 | 0.30 | 0.71 | 96.66 |
| 3 | 0.42 | 1.00 | 1.40 | 98.00 |
| 4 | 0.42 | 3.00 | 3.43 | 100.33 |
| 5 | 0.42 | 5.00 | 5.41 | 99.80 |

[a]100×[(found–base)/added]
[b]95% confidence interval; $n$=6

Recovery study

In order to develop recovery studies the following experiment was carriedout: 500 mL of urine sample were collected and divided into 10 portions of 50 mL each. The proposed method was applied to six portions, and the average quantity of dysprosium obtained was taken as a base value. Then, increasing quantities ofdysprosium were addedto the other aliquots of sample and dysprosium was determined by the same method. The results are given in Table 2. The recovery obtained (96.66–100.30%) was highly satisfactory and illustrated the good performance of the proposed method.

## Conclusions

The on-line CPE complexation/preconcentration of dysprosium in urine was achieved with a simple FI system, which was easily coupled to ICP–OES with pneumatic nebulization. The automated separation method offers excellent extraction efficiency and preconcentration factor. An enhancement factor of 50 with respect to dysprosium determination by ICP–OES without preconcentration was obtained. The preconcentration method allows dysprosium determinations in urine samples with good accuracy and reproducibility.

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## References

1. Nilsson S, Wikstrom G, Ericsson A, Wikstrom M, Oksendal A, Waldenstrom A (1996) Acta Radiol 37:27–35
2. Ericsson A, Bach-Gansmo T, Niklasson F, Hemmingsson A (1995) Acta Radiol 36:41–46
3. Runge VM, Wells JW (1996) Invest Radiol 31:395–400
4. Endre ZH, Allis JL, Radda GK (1989) Magn Reson Med 11:267–274
5. Posta J, Alimonti A, Petrucci SC (1996) Anal Chim Acta 325: 185–193
6. Fairman B, Sanz–Medel A (1996) Fresenius J Anal Chem 355: 757–762
7. Hinze W, Pramauro E (1993) Anal Chem 24:133–177
8. Quina FH, Hinze WL (1999) Ind Eng Chem Res 38:4150–4569
9. García Pinto C, Perez Pavón JL, Moreno Cordero B, Romero Beato E, García Sanchez S (1996) J Anal At Spectrom 11:37–41
10. Sirimanne S, Barr J, Patterson D, Ma L (1996) Anal Chem 68: 1556–1560
11. Sanz–Medel A, Fernandez de la Campana MR, Blanco Gonzales E, Fernandez–Sanchez ML (1999) Spectrochim Acta Part B 54:251–287
12. Mesquita da Silva MA, Azzolin Frescura VL, Nome Aguilera FJ, Curtius AJ (1998) J Anal At Spectrom 13:1369–1373
13. Böyükbayram AE, Volkan M (2000) Spectrochim Acta Part B 55:1073–1080
14. Silva MF, Fernández LP, Olsina RA (1998) Analyst 123: 1803–1807
15. de Wuilloud JA, Wuilloud R, Silva MF, Olsina RA, Martinez LD (2002) Spectrochim Acta Part B 57:365–374
16. Silva MF, Fernández LP, Olsina RA, Stacchiola D (1997) Anal Chim Acta 342:229–238
17. Sombra L, Luconi M, Silva MF, Olsina RA, Fernández LP (2001) Analyst 126:1172–1176
18. Ortega C, Gomez MR, Olsina RA, Silva MF, Martinez LD (2002) J Anal At Spectrom 17:530–533
19. Flaschka A (1967) EDTA titrations—An introduction to theory and practice. Pergamon Press, London
20. Wuilloud RG, Salonia JA, Gásquez JA, Olsina RA, Martinez LD (2000) Anal Chim Acta 420:73–79