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On-line preconcentration and determination of chromium in parenteral solutions by flow injection—flame atomic absorption spectrometry

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Abstract

An on-line chromium preconcentration and determination system implemented with flame atomic absorption spectrometry (FAAS) associated to flow injection (FI) was studied. For the retention of chromium, 4-(2-Thiazolylazo)-resorcinol (TAR) and Amberlite XAD-16 were used, at pH 5.0. The Cr–TAR complex was removed from the micro-column with ethanol. An enrichment 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 Cr was 20 ng l^{-1}. The precision for ten replicate determinations at the 5 μg l^{-1} Cr levels was 2.9% relative standard deviation (RSD), calculated from the peak heights obtained. The calibration graph using the preconcentration system for chromium was linear with a correlation coefficient of 0.9997 at levels near the detection limits up to at least 100 μg l^{-1}. The method was successfully applied to the determination of chromium in parenteral solution samples.

Keywords: FAAS; Flow injection; On-line preconcentration; Chromium in parenteral solutions samples; 4-(2-Thiazolylazo)-resorcinol

1. Introduction

Chromium is ubiquitous in nature, occurring under various chemically, physically and morphologically different forms [1]. It is an essential element for all vertebrates. In humans, it plays a role in the metabolism of glucose and some lipids. Chromium deficiency is associated with cardiovascular diseases and diabetes, while excessive amounts of this element, particularly in the more toxic Cr(VI) valence state, are detrimental to health as it may be involved in the pathogenesis of some diseases such as lung and gastrointestinal cancer [2,3].

Parenteral nutrition (PN) consists of administering intravenously all nutrients necessary to

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patients who cannot receive normal alimentation due to various pathologies and is, therefore, an important therapeutic requirement in many clinical situations [4,5]. Infusion solutions and solutions for PN have been identified as chromium sources [6,7]. However, the quantitative requirements or the toxicity of trace elements in parenteral solutions are difficult to assess. The importance of trace elements in the nutritional management of patients receiving total parenteral nutrition (TPN) is now widely recognised [4–7]. Long-term TPN patients can inadvertently receive significant amounts of chromium present as contaminant in TPN. Many of the solutions for parenteral nutritional support could have a chromium content which exceeds, in part considerably, the suggested threshold concentration of 5 μg l^{-1} [6,7].

In the past years, several methods have been developed for the determination of low concentrations of chromium, among them, neutron activation analysis (NAA) [8], inductively coupled plasma mass spectrometry (ICP-MS) [6,9], inductively coupled plasma optical emission spectrometry [10] (ICP-OES), electrothermal atomisation atomic absorption spectrometry (ETAAS) [11], and flame atomic absorption spectrometry (FAAS) [12–15]. The NAA method is time-consuming, and routine analysis of numerous samples is laborious. This method also requires sophisticated instrumentation that may be not available in most analytical laboratories. Within the last decade, ICP-MS has proved ideally suited as an alternative approach for the determination of chromium in various matrices, however, the cost of instrumentation may be prohibitive to many laboratories.

Although FAAS or ETAAS are the most commonly used techniques in the determination of Cr traces, the low level of Cr concentration in parenteral solutions is not compatible with the detection limit of FAAS. In order to achieve accurate, reliable and sensitive results, preconcentrations and separations are needed when the concentrations of analyte elements in the sample are too low to be determined directly by FAAS. On the other hand, FAAS is widely applied in routine laboratories due to its lower cost and greater simplicity as compared with ETAAS.

Various preconcentration techniques [8,16–18] for the determination of Cr have been proposed, including chelation and extraction, precipitation, coprecipitation, and ion-exchange resins. However, many of these methodologies are performed in batch, thus requiring great sample volumes in order to reach low detection limits. Besides, these systems pose higher contamination risks. When preconcentration techniques are applied in batch mode, the time of analysis is increased, thus turning these procedures unpractical for application in routine analysis. This situation has been significantly improved utilising flow injection (FI) associated with FAAS [19], such that general drawbacks of batch preconcentration procedures have been largely eliminated, and currently the preconcentrations can be achieved almost as efficiently as by simple FAAS determination. In order to be used as packing in preconcentration columns for FI [19], materials have to meet several requirements. Among these, they must have the appropriate kinetic and mechanical conditions so that they can easily retain and elute the analyte and they must allow the performing several retention and elution runs without exhaustion of column material. XAD resins meet many of these requirements [20] and they have been used as supports for immobilisation of chelating agents and metal complexes [21]. 4-(2-Thiazolylazo)-resorcinol (TAR) forms stable complexes with numerous metal ions [16,22], and it is, therefore, a suitable reagent for chromium preconcentration on a XAD resin [16].

Table 1
FAAS instrumental parameters employed to chromium determination

Flame type	Air–C_2H_2
Burner height	13 mm
Wavelength	357.9 nm
Slit width	0.5 nm
Lamp current	10 mA
Measurement mode	Height
Air flow rate	8.0 l min^{-1}
Acetylene flow rate	2.8 l min^{-1}
Sample introduction flow rate	3.0 ml min^{-1}

In the present work, a method for preconcentration and determination of chromium in parenteral solutions using a microcolumn filled with a macroporous resin Amberlite XAD-16 is proposed. Chromium was retained under the form of Cr–TAR complex. The determination was performed using FAAS associated with a FI methodology.

2. Experimental

2.1. Reagents

The Amberlite XAD-16 resin (Rohm & Haas, Philadelphia, PA, USA) was used. Particle size was between 20–50 mesh with a surface area of 825 m^2 g^{-1}. Before use, the surface of the resin was activated by immersion in a solution of methanol and 4 mol l^{-1} HCl (1+1). Subsequently, metal impurities were removed by further washing with 2 mol l^{-1} HCl solution.

A solution of TAR (Merck, Darmstadt, Germany) 10^{-2} mol l^{-1} was prepared by dissolution in ethanol. Lower concentrations were prepared by serial dilution with ethanol.

A stock standard solution of 1000 mg l^{-1} Cr(III) was prepared from 7.6960 g chromium nitrate ($Cr(NO_3)_3 \cdot 9H_2O$) (Merck) dissolved in ultrapure water and diluted to 1000 ml with a final HNO_3 concentration of 0.05 mol l^{-1}. Working solutions were prepared by dilution of stock solution.

A buffer solution was prepared by diluting a 2.0 mol l^{-1} acetic acid solution adjusted to pH 5.0 with sodium hydroxide.

Ultrapure water (18 MΩ cm^{-1}) was obtained from a EASYpure RF (Barnstedt, Iowa, USA).

All the reagents were of analytical-reagent grade and the presence of chromium was not detected in the working range.

2.2. Apparatus

The measurements were performed with a Shimadzu Model AA-6800 atomic absorption spectrometer (Tokyo, Japan) equipped with a chromium hollow-cathode lamp. The FAAS instrumental and operating conditions that provided the best sensitivity and no differences between the Cr(III) and Cr(VI) signal are listed in Table 1. The flow injection system used is shown in Fig. 1. A Minipuls 3 peristaltic pump (Gilson, Villiers Le-Bell, France) was used. The sample injection was achieved using a Rheodyne (Cotati, CA, USA) Model 50, four way rotary valve. A microbore glass column (50 mm length; 3 mm internal diameter) fitted with porous 25 μm glass frits was used as the resin holder. Tygon type pump tubes (Ismatec, Cole-Parmer Instrument Company, Niles, IL, USA) were employed to propel the sample, reagent and eluent.

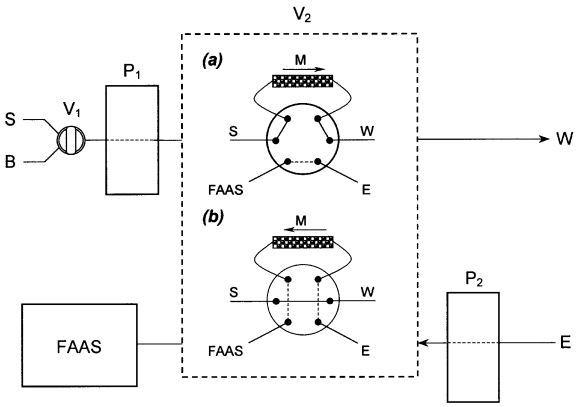

Fig. 1. Schematic diagram of the instrumental setup. S, sample; B, buffer diluted; E, eluent; W, waste; P_1 and P_2, peristaltic pump; M, microcolumn; V_1, two way valve; V_2, load-injection valve ((a) Load position; (b) Injection position).

2.3. Synthesis of the Cr–TAR complex

Previous to the preconcentration and determination of chromium, a procedure was developed in order to reach the formation of the Cr–TAR complex. 1.0 ml of 10^{-2} mol l^{-1} TAR solution and 2.0 ml of acetic/acetate buffer solution were added to a precipitation vessel containing 50 ml of sample solution. The mixture was placed in a water bath at a temperature of 90 °C for 60 min. Finally, it was allowed to cool to room temperature (25 °C).

2.4. Study of the retention of the Cr–TAR complex on the resin

In order to optimise the retention of the metal complex on the column, the following variables were assessed: pH, TAR concentration, and loading flow-rate.

Solutions were prepared with a known amount of chromium and 100-fold excess in moles of TAR to form the metal complex, and pH was varied by adding diluted HCl or NaOH solutions so that 50 ml of each solution were obtained at the corresponding pH and containing 50 μg l^{-1} of chromium. Each of the solutions was loaded on the XAD-16 resin and subsequently the retained metal complex was eluted with 10 ml of ethanol in a 10 ml volumetric flask.

The optimum concentration of TAR was determined by the same procedure, working at pH 5.0.

Assessment of retention with respect to the loading flow rate of the solutions was performed at flow rates between 2 and 15 ml min^{-1}.

The concentration of chromium was determined by FAAS. Recovery was calculated against the theoretical concentration.

2.5. Preconcentration procedure and determination

Before loading, the column was conditioned for preconcentration at the correct pH with buffer diluted (1:10) solution, valve V_1 in position B (Fig. 1). The complex was then loaded on the XAD-16 resin at a flow rate of 10 ml min^{-1}, with valve V_1 in position S and valve V_2 in load position (a) After the loading time, the sample still present in the lines and the column was removed by further washing with buffer diluted solution, with valve V_1 again in position B. Finally, valve V_2 was switched to the injection position (b) and the retained metal complex was eluted in countercurrent (i.e. reversal of the flow direction through the column during elution with respect to sample loading) with ethanol at a flow rate of 3.0 ml min^{-1}, directly into the nebulizer and subsequently the flame. Since the metal complex Cr–TAR is not totally retained on the resin (ca. 80%), the standard solution must be also passed through the micro-column. The operating conditions were established and the determination was carried out.

3. Results and discussion

3.1. Optimisation of the retention variables

The retention conditions of the metal complex were optimised and the chromium signal was monitored by measuring it with FAAS while changing the pH of the solution that passes through the sorption micro-column. Fig. 2 shows the responses at different pH values of the solution. The optimal pH values were in the 4.5–5.6 range, which was to be expected, since better complexing occurs within this range. Considering these results, the selected pH was 5.0. It must be noted that no differences were found between the behaviour of Cr(III) and Cr(VI) in any of the steps included in the here proposed methodology (complexing, micro-column retention, elution and determination). The proposed method was separately applied to the determination of Cr(III) and Cr(VI) in synthetic samples at levels of 50 μg l^{-1}, obtaining 80% recovery for both oxidation states. Therefore, the present methodology appears suitable for the determination of total chromium.

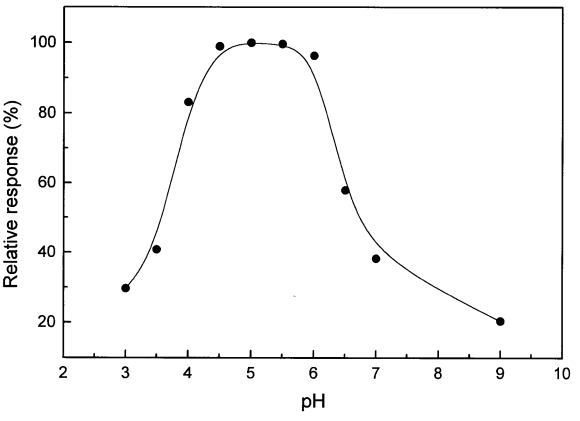

Fig. 2. Dependence of retention of Cr–TAR complex on pH of loading solutions. Preconcentration of 50 ml of Cr–TAR complex. Cr concentration was 50 μg l^{-1}; ethanol concentration 2% (v/v); TAR concentration was 2.0×10^{-4} mol l^{-1}.

In the present work, a bead size of resin of 20–50 mesh was considered adequate for the preconcentration procedure in the micro-column. Smaller resin particles could have improved retention capacity, but they would have increased back-pressure of the micro column, making it necessary to reduce the flow rate, with the subsequent increase in preconcentration time. Choice of the Amberlite XAD-16 resin was motivated by the fact that it is highly stable in both acidic and basic solution and exhibits an adequate surface area. This allows the use of the micro-column for an indefinite number of samples without degradation of performance. The dimensions of the micro-column used here were optimised in previous works [23,24].

It is well known that complex retention on XAD resins is modified by the concentration of organic solvents [20]. Furthermore, the formation of metal complexes with TAR is also affected by the solvent. Higher retention was observed for lower ethanol percentages. The value selected was 2%, since it was the lowest value compatible with the complex stability.

As regards the variation of preconcentration with TAR concentration, the signal remained constant between 10^{-4} mol l^{-1} and at least 5×10^{-3} mol l^{-1}. Chromium did not react with the complexing reagent at room temperature; however, the complex was formed on heating the reactants to 85–95 °C. A heating time of at least 60 min allowed for a constant recovery value.

The flow rate sample through the micro-column is a very important parameter, since this is one of the steps that controls the time of analysis. It could be verified that with flow rates up to 10 ml min^{-1}, there is no effect on analyte recovery, which in optimum conditions is approximately 80%. As shown in Fig. 3, at higher flow rates the retention of the Cr–TAR complex decreases.

3.2. Optimisation of the elution conditions

In order to elute Cr–TAR complex adsorbed on the resin, ethanol in countercurrent (i.e. reversal of sample loading flow direction) was used as eluent. Countercurrent elution substantially improves the elution profiles as compared with unidirectional flow. Chromium was completely eluted from the resin with 1.0 ml of ethanol. The use of ethanol as eluent and carrier solution had the advantage of increasing the sensitivity value for chromium determination by five times with respect to the aqueous medium. The effect of the eluent flow rate was studied, obtaining the best response at 3.0 ml min^{-1}. A typical elution profile for Cr obtained

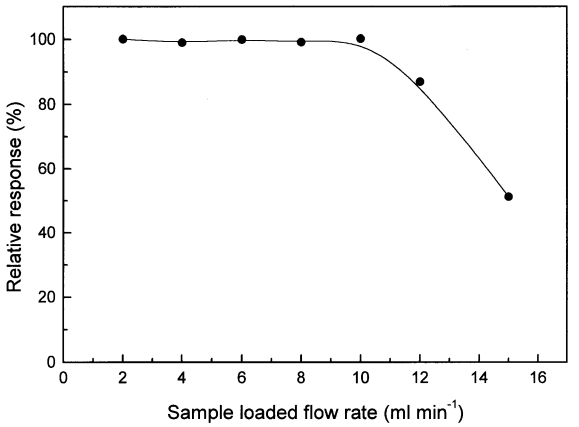

Fig. 3. Dependence of recovery of metal complex on sample flow rate. Preconcentration of 50 ml of Cr–TAR complex at pH 5.0. Cr concentration was 50 μg l^{-1}; TAR concentration was 2.0×10^{-4} mol l^{-1}.

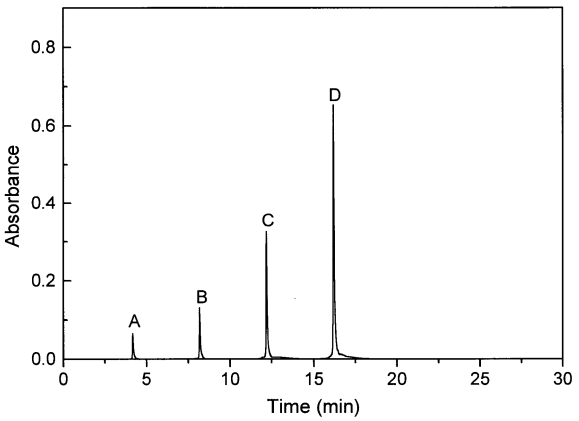

Fig. 4. Peak profiles obtained by preconcentration of 50 ml of Cr–TAR complex using the system depicted in Fig. 1. Loaded flow rate was 10 ml min^{-1}; the elution flow rate was 3.0 ml min^{-1}; TAR concentration was 2.0×10^{-4} mol l^{-1}. The Cr concentration in the standards solutions were: (A) 0.5 μg l^{-1}; (B) 1.0 μg l^{-1}; (C) 2.5 μg l^{-1}; and (D) 5.0 μg l^{-1}.

with the preconcentration system is shown in Fig. 4.

3.3. Interferences

The effects of representative potential interfering species (at the concentration levels at which they may occur in the sample studied) were tested. Cu^{2+}, Zn^{2+}, Pb^{2+}, Cd^{2+}, Ni^{2+}, Co^{2+}, Mn^{2+}, and Fe^{3+} could be tolerated up to at least 2500 μg l^{-1}. Commonly encountered matrix components such as alkali and alkaline earth elements generally do not form stable complexes and are not retained on the resin. On the other hand, anions such as CO_3^{2-}, F^-, SO_4^{2-}, Cl^-, PO_4^{3-}, malonate, and ascorbate could be tolerated up to at least 500 000 μg l^{-1}.

The recoveries were not influenced by these ions because they are not complexed with TAR reagent at the working pH value of 5.0. Thus, they are not retained on the resin prior to the elution of the retained Cr and can be easily washed out through the Amberlite XAD-16 resin allowing for highly selective determination of Cr in the presence of other ions. The value of the reagent blank signal was not modified by the presence of the potentially interfering ions assayed.

3.4. Performance of the preconcentration system

The overall time required for preconcentration of 50 ml of sample (5.0 min, at flow rate of 10 ml min^{-1}), washing (0.2 min), elution (0.4 min, at flow rate of 3.0 ml min^{-1}) and conditioning (0.2 min) was about 5.8 min; hence, throughput was approximately 10 samples h^{-1}.

A total enhancement factor of 250 was obtained with respect to the chromium determination in aqueous medium by FAAS (50 for preconcentration system and five due to the use of ethanol as solvent).

3.5. Method validation

In order to demonstrate the validity of this method, 500 ml of parenteral solution were collected and divided into ten portions of 50 ml each. The proposed method was applied to six portions and the average quantity of chromium obtained was taken as a base value. Then, increasing quantities of chromium were added to the other aliquots of sample and chromium was determined by the same method. As shown in Table 2, the recovery values are in the 95–100% range.

Additionally, the proposed method was validated by comparison with the ETAAS technique (Table 3). The results were compared with the *t*-test and no significant differences at the 95% confidence level were observed [25,26]

3.6. Determination of chromium in parenteral solutions

The reproducibility of the preconcentration method was evaluated by passing 50 ml of standard solution of chromium (5 μg l^{-1}) through the micro-column and repeating this procedure ten times (Table 4). The relative standard deviation (RSD) was 2.9%, calculated from the peak heights obtained. The calibration graph using the preconcentration system for chromium was linear with a correlation coefficient of 0.9997 at levels close to the detection limits up to at least 100 μg l^{-1}. The detection limit (DL) was calculated as the amount of chromium required to yield a net peak that was equal to three times the standard deviation of the background signal (3σ). The value of DL obtained for the preconcentration of 50 ml of aqueous solutions of Cr was 20 ng l^{-1}. Better DLs are to be expected with larger samples, but this would increase the time of analysis. Finally, the results of the method applied to chromium determination in parenteral solutions samples are shown in Table 3.

The concentrations were in the range 0.54–1.23 μg l^{-1} of chromium. The results obtained are in good agreement with those of Pluhator–Murton et al. [6]. The maximum concentration of chromium reported in parenteral solutions by these authors is 5 μg l^{-1}.

4. Conclusions

The work described in this paper has shown that adequate sensitivity and accuracy can be attained

Table 2
Recovery study

Aliquots	Base value (μg l^{-1})	Quantity of Cr added (μg l^{-1})	Quantity of Cr found (μg l^{-1})	Recovery (%)[a]
1	–	0.00	0.65±0.09	–
2	0.65	2.00	2.63	97.7
3	0.65	6.00	6.65	100.0
4	0.65	10.00	10.64	99.2
5	0.65	20.00	20.65	100.0

[a] 100 × [(found-base)/added].

Table 3
Concentrations of chromium in parenteral solutions samples (95% confidence interval; $n = 6$)

Sample	Cr concentration (μg l^{-1})	
	ETAAS	Proposed method
1[a]	1.26±0.09	1.23±0.10
2[b]	0.92±0.10	0.91±0.09
3[c]	0.64±0.08	0.65±0.09
4[d]	0.89±0.09	0.90±0.10
5[e]	0.52±0.08	0.54±0.10

[a] Ringer physiological solution.
[b] NaCl physiological solution.
[c] Isotonic dextrose 5% physiological solution.
[d] Dextrose 10% physiological solution.
[e] Sterile distilled water.

Table 4
Analytical performance of the FI preconcentration system

Linear range of response	0–100 μg l^{-1}
Calibration equation	Absorbance = 0.0013 + 0.136*C
Correlation coefficient (r)	0.9997
RSD[a]	2.9% ($n = 10$)
Limit of detection (3σ)	20 ng l^{-1}

[a] Relative standard deviation (5 μg l^{-1} Cr).

using an on-line preconcentration system with a FI-FAAS method. The coupling of an on-line preconcentration system with FI-FAAS increases the speed of the preconcentration and analysis process, and reduces sample consumption and contamination risks. The manifold presented provided a recovery of 80% of the Cr–TAR complex from the micro-column. The proposed system of preconcentration associated with FAAS allowed for chromium determination in parenteral solutions samples at concentrations as low as μg l^{-1}. The determination procedure shows good reproducibility and accuracy.

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References

[1] J.M. Christensen, K. Byrialsen, K. Vercoutere, R. Cornelis, Ph. Quevauviller, Fresenius J. Anal. Chem. 363 (1999) 28–32.
[2] J.L. Burguera, M. Burguera, C. Rondon, L. Rodríguez, P. Carrero, Y. Petit de Peña, E. Burguera, J. Anal. Atom. Spectrom. 14 (1999) 821–825.
[3] R.A. Goyer, in: C.D. Klassen, M.O. Amdur, J. Doull (Eds.), Casarett and Doull's Toxicology: the Basic Science of Poisons, Third ed., Macmillan, New York, 1986.
[4] Y. Berner, T. Shuler, F. Nielsen, C. Flombaun, S. Farkouh, M. Shike, Am. J. Clin. Nutr. 50 (1989) 1079–1083.
[5] B. Harraki, P. Guiraud, M.H. Rochat, J. Alary, A. Favier, Pharm. Acta Helv. 70 (1995) 269–278.
[6] M.M. Pluhator-Murton, R.N. Fedorak, R.J. Audette, B.J. Marriage, R.W. Yatscoff, L.M. Gramlich, JPEN 23 (1999) 222–227.
[7] F.Y. Leung, Clin. Biochem. 28 (1995) 561–566.
[8] E. Orvini, M. Gallorini, J. Radioanal. Chem. 71 (1982) 75–95.
[9] I.I. Stewart, J.W. Olesik, J. Chromatogr. A 872 (2000) 227–246.
[10] J. Posta, A. Alimonti, F. Petrucci, S. Caroli, Anal. Chim. Acta 325 (1996) 185–193.

[11] S. Nielsen, E.H. Hansen, Anal. Chim. Acta 366 (1998) 163–176.
[12] D.M. Adriá-Cerezo, M. Llobat-Estellés, A.R. Maurí-Aucejo, Talanta 51 (2000) 531–536.
[13] M.J. Marqués, A. Morales-Rubio, A. Salvador, M. de la Guardia, Talanta 53 (2001) 1229–1239.
[14] B. Demirata, Mikrochim. Acta 136 (2001) 143–146.
[15] A. Gáspár, C. Sógor, J. Posta, Fresenius J. Anal. Chem 363 (1999) 480–483.
[16] M. Soylak, U. Divrikli, M. Dogan, J. Trace Microprobe Tech. 15 (1997) 197–204.
[17] S. Tokalioglu, S. Kartal, L. Elçi, Anal. Sci. 16 (2000) 1169–1174.
[18] I. Narin, M. Soylak, L. Elçi, M. Dogan, Talanta 52 (2000) 1041–1046.
[19] Z.L. Fang, Flow Injection Separation and Preconcentration, VCH, Weinheim, 1993, pp. 85–102.
[20] D.J. Pletrzyk, C.H. Chu, Anal. Chem. 49 (1977) 757–764.
[21] N. Kabay, M. Demircioglu, H. Ekinci, M. Yüksel, M. Saglam, M. Streat, React. Func. Polym. 38 (1997) 219–226.
[22] B. Subrahmanyam, M.C. Eshwar, Mikrochim. Acta 2 (1976) 579–584.
[23] O. Vicente, A. Masi, L. Martinez, R. Olsina, E. Marchevsky, Anal. Chim. Acta 366 (1998) 201–207.
[24] O. Vicente, A. Padró, L. Martinez, R. Olsina, E. Marchevsky, Spectrochim. Acta Part B 53 (1998) 1281–1287.
[25] D.L. Massart, B.G.M. Vandeginste, S.N. Deming, Y. Michotte, L. Kaufman, Chemometrics: a Textbook, Third ed., Elsevier, New York, 1990.
[26] J.C. Miller, J.N. Miller, Estadística para Química Analítica, Second ed., Addison–Wesley Iberoamerican, New York, 1993.