ISSN-1996-918X

Pak. J. Anal. Environ. Chem. Vol. 14, No. 2 (2013) 16 – 27

Speciative Determination of Dissolved Inorganic Fe(II), Fe(III) and Total Fe in Natural Waters by Coupling Cloud Point Extraction with FAAS

Ramazan GÜRKAN* and Nail ALTUNAY

University of Cumhuriyet, Faculty of Sciences, Department of Chemistry, TR-58140 Sivas, TURKEY

Received 03 May 2013, Revised 28 December 2013, Accepted 31 December 2013

Abstract

A new cloud point extraction (CPE) method for the preconcentration of trace iron speciation in natural waters prior to determination by flame atomic absorption spectrometry (FAAS) was developed in the present study. In this method, Fe(II) sensitively and selectively reacts with Calcon carboxylic acid (CCA) in presence of cetylpyridinium chloride (CPC) yielding a hydrophobic complex at pH 10.5, which is then entrapped in surfactant-rich phase. Total Fe was accurately and reliably determined after the reduction of Fe(III) to Fe(II) with sulfite. The amount of Fe(III) in samples was determined from the difference between total Fe and Fe(II). CPC was used not only as an auxiliary ligand in CPE, but also as sensitivity enhancement agent in FAAS. The nonionic surfactant, polyethylene glycol tert-octylphenyl ether (Triton X-114) was used as an extracting agent. The analytical variables affecting CPE efficiency were investigated in detail. The preconcentration/enhancement factors of 50 and 82 respectively, were obtained for the preconcentration of Fe(II) with 50 mL solution. Under the optimized conditions, the detection limit of Fe(II) in linear range of 0.2-60 µg L^{-1} was 0.06 µg L^{-1}. The relative standard deviation was 2.7 % (20 µg L^{-1}, N: 5), recoveries for Fe(II) were in range of 99.0-102.0% for all water samples including certified reference materials (CRMs). In order to verify its accuracy, two CRMs were analyzed and the results obtained were statistically in good agreement with the certified values.

***Keywords*:** Inorganic iron speciation; micellar effect; FAAS; CCA; CPE.

Introduction

Iron plays an important role in chemical reactions, such as in geological processes, environmental and atmospheric chemistry, and in biochemistry [1]. The environmental and biological impact of iron depends, in large, on its chemical properties, such as valence, solubility and the degree of complex formation. Iron plays an important role in plant metabolism where it is essential for photosynthetic and respiratory electron transport, nitrate reduction, chlorophyll synthesis and detoxification of reactive oxygen species [2-5]. Due to the presence of iron in environmental and biological materials, and the lack of sufficient understanding of the role of its oxidation state, accurate and reliable determination of both Fe(II) and Fe(III) is of great importance [6-8].

Many techniques such as spectrophotometry [9–12], atomic absorption spectrometry (AAS) [13-17], inductively coupled plasma-optical emission spectrometry (ICP-OES) [18, 19], inductively coupled plasma-mass spectrometry (ICP-MS) [4, 20-21], cathodic or anodic stripping voltammetry (CSV or ASV) [22, 23], chromatography [24, 25] and spectroscopic sensors [26], have recently been reported for the determination of inorganic dissolved Fe species.

*Corresponding Author Email: rgurkan@cumhuriyet.edu.tr

Among these analytical techniques, atomic absorption spectrometry as an element-selective detection tool is generally preferred in the determination of traces metal ions in environmental samples due to its simplicity and its lower cost than other instrumental techniques like ICP-MS and ICP-OES, which are sensitive, expensive and requiring expert users. However, lower analyte levels than its detection limit and high salt contents of the real samples are two main limitations in the determination of metal ions by atomic absorption spectrometry. The researchers have been focused to solve these limitations. Separation–preconcentration procedures including liquid–liquid extraction [27-28], cloud point extraction (CPE) [29-30], coprecipitation [31], ion-exchange [32] and electro-analytical methods [33-34] has been widely used for that purpose.

Among them, CPE is considered as the most versatile and simple approach for separation and preconcentration of trace metal ions from aqueous matrices, because this approach provides some advantages such as safety, low cost, high extraction efficiency, easy disposal of the surfactants, and low toxicity of the utilized reagents compared with classical organic solvents [35].

In CPE, nonionic surfactants such as Triton X-100, Triton X-114, Ponpe 7.5 and Tween 80 tend to form micelles in aqueous solutions and become turbid when they are heated to the cloud point temperature. Above the cloud point, the micellar solution separates into a surfactant rich phase, known as the coacervate phase with a small volume, and into a diluted aqueous phase, with a large volume. When the analyte ions, which are primarily present in the aqueous solution and bound to the micelles, form hydrophobic compounds with a chelating agent, they are extracted to the surfactant-rich phase, and hence they can easily be separated and preconcentrated by this way [36, 37].

The present study presents a CPE procedure for the separation and preconcentration of Fe(II) and Fe(III) ions based on the formation of a hydrophobic complex with Calcon carboxylic acid (CCA) in presence of cetylpyridinium chloride (CPC) as an auxiliary ligand at pH 10.5 borate buffer. Triton X-114 was used as phase separating nonionic surfactant, and the hydrophobic complex was extracted into the surfactant-rich phase. Then, surfactant-rich phase was diluted with acetonitrile, and its dissolved inorganic iron content was directly determined by means of FAAS.

Experimental

Instrumentation

An atomic absorption spectrometer (Shimadzu AAS-6300), equipped with an iron hollow cathode lamp and an air-acetylene flame atomizer, was used for in all determinations. The wavelength, lamp current, slit width and burner height used, was 248.3 nm, 12 mA, 0.2 nm, 7.0 mm, respectively. The absorbance measurements were carried out using an air/acetylene flame at flow rates of 18 and 2.2 L min^{-1}. The nebulizer flow rate and the burner height were adjusted in order to obtain the maximum absorbance signal by aspirating a solution containing the analyte in acetonitrile. The pH measurements were carried out with a pH meter (Sartorious Docu-pH-meter). A centrifuge was used to accelerate the phase separation process (Mistral 2000). A thermostatic water-bath maintained at the desired temperature, was used in the CPE experiments.

Reagents and standard solutions

Ultra-pure water with a resistivity of 18.2 MΩ cm^{-1} was prepared during trace analysis by Milli-Q water purification system. Before use for trace analysis, all containers (glassware, polyethylene bottles with low density and high density, LDPE and HDPE) were treated for one week at least, first with 1:4 (v/(v) HNO_3 then with 1:4 (v/v) HCl, finally they were abundantly rinsed with water prior to use when not used the vessels were kept in 1:4 (v/v) HCl. All chemicals used were of analytical reagent grade. The stock solution of 1000 mg L^{-1}Fe(II) was prepared by dissolving 0.102 g of $FeSO_4 \times 2H_2O$ supplied from Merck (Darmstadt, Germany) with water. The stock solution of 1000 mg L^{-1}Fe(III) was prepared by dissolving 0.477 g of $Fe_2(SO_4)_3 \times 7H_2O$ with water. Working solutions of Fe(II) and Fe(III) were prepared by a stepwise dilution of the stock solutions at suitable ratios. The nonionic surfactant

Triton X-114 supplied from Sigma (St. Loius, MO, USA) was used without further purification. A 5.0% (v/v) Triton X-114 was prepared by dissolving 5.0 mL of Triton X-114 in distilled water in 100 mL volumetric flask with stirring. The chelating ligand solution (3.0×10^{-3} mol L^{-1} CCA) was prepared by dissolving an appropriate amount of CCA supplied from Sigma in water. To create a mixture of 100 mL, borate buffers at pH 10.5 was prepared by mixing a suitable volume of 0.05 mol L^{-1} sodium tetraborate solution with a certain volume of diluted NaOH or HCl and adjusting the pH to 10.5. The cationic surfactant solution (3.0×10^{-3} mol L^{-1}) was prepared by dissolving an appropriate amount of CPC supplied from Sigma in water. The NaCl solution of 20% (w/v) was prepared by dissolving 20 g of solid NaCl supplied from Merck in water.

The general CPE procedure

An aliquot of the sample or pretreated-sample containing Fe(II) in the range of 0.2–60 µg L^{-1}, was transferred into a centrifuge tubes with glass stopper (50 mL in capacity). Added 0.6 mL of 3.0×10^{-3} mol L^{-1} CCA, 0.2 mL of 20% (w/v) NaCl solution, 1.0 mL of 3.0×10^{-3} mol L^{-1} CPC, 0.1 mL of 5.0% (v/v) Triton X-114, then the pH was adjusted approximately to 10.5 using borate buffer. Then solutions were mixed and kept in a thermostatic water bath for 10 min at 50 ^{o}C. The separation into two phases was accelerated by centrifuging at 3000 rpm for 5 min. The mixture was then cooled in an ice-bath for 5 min in order to increase the viscosity of the surfactant-rich phase and facilitate the removal of the aqueous phase. Then, the aqueous phase was easily separated from surfactant-rich phase by inverting the tube. 1.0 mL of acetonitrile solution was added to the surfactant-rich phase to reduce its viscosity prior to determination of iron by FAAS at 248.3 nm. Finally, the iron concentrations were determined by using the calibration curve and standard addition curve approaches where necessary.

Sampling and sample pretreatment

Water samples were collected from the city of Sivas, in Turkey. The only pretreatment was acidification to pH 2.0-2.5 with HCl, which was performed immediately after collection, in order to prevent adsorption of the metal ions on the flask walls. Samples were filtered in the laboratory using a 0.45 µm pore size membrane filter to remove suspended solids before analysis. 20 mL of acidic mine water samples were directly analyzed without any other pretreatment procedure. 25 mL of tap water samples were independently analyzed by means of the present CPE approach with and without using 1.5 mL of 10 mg L^{-1} NH_4F solution in order to suppress the matrix effect. At least one blank solution was run for each sample in order to evaluate possible metal contamination by the reagents used. The lake water samples were collected from the three different sampling point of Hafik Lake, which is a shallow, partly eutrophic lake (Hafik, Sivas, Turkey). Surface-water samples were collected directly into 250-mL HDPE bottles. The samples were immediately cooled in an ice cooler, brought back to the laboratory, and filtered through a 0.2-µm-pore size polycarbonate membrane filter (Nuclepore, Whatman, Brentford, UK). The filtrates were stored frozen (-20 °C) in HDPE bottles until speciation analysis. Separate samples were stored at 4 °C in Teflon vials after acidification to pH 2.0-2.5 with HCl for the determination of total Fe. The HDPE bottles were cleaned by soaking into 3 mol L^{-1} of HCl for 3 days and then rinsing with MQ. For speciation analysis, the preconcentration of Fe(II) with CPE due to be 13.5-fold more sensitive was firstly preferred. Due to be slow of complexation rate of Fe(III) with ligands in low concentrations at pH 10.5, it was not directly considered as an analyte in the method development step, and its preconcentration with CPE wasn't used as a quantitative tool in analysis of samples. Total Fe was accurately and reliably determined after the reduction of Fe(III) to Fe(II) by using sulfite as reducing agent at 40 ^{o}C and pH 4.5. The amount of Fe(III) in samples was indirectly calculated from the difference between total Fe and Fe(II) amounts.

Results and Discussion

Optimization of experimental variables

In order to achieve the best analytical performance of CPE procedure for determination of iron (II) at level of 20 µg L^{-1}, the effects of analytical variables such as pH, concentrations of ligand, auxiliary ligand and nonionic surfactant, centrifugation rate and time, equilibration

temperature and time on the analytical signal were independently studied and optimized. The pH is a critical factor affecting complex formation reaction between metal ions and ligand molecules, and the extractability of metallic complex into the surfactant-rich phase. In this context, CCA has four acidic groups. The sulfonic acid group completely dissociates in aqueous solutions, but the dissociation of other groups depends on the solution's pH [38, 39]. Due to the presence of these groups and competition between proton, H^+ and Fe^{2+}ions for the binding sites in the ligand molecule, the pH is a critical parameter. Because of not being obtained a metal-ligand complex at low pHs, the effect of pH on the CPE was studied in the range of 8.0-11.0. For this purpose, NH_3/NH_4Cl, $H_2PO_4^-/HPO_4^{2-}$ and borate buffer systems at equimolar concentrations were initially chosen and used. But, the best analytical signal was obtained with borate buffer system at pH 10.5. The reason of this high sensitivity may be the esterification reaction of the boric acid to CCA containing carboxyl and phenol groups at orto-position in terms of protection and stabilization of ligand or metal-complex against environmental factors.

As can be seen from (Fig. 1(a)), the maximum absorbance was obtained at pH 10.5. The effect of borate buffer concentration on the analytical signal was also studied in the range of 0-4.0×10^{-3} mol L^{-1} (in 50.0 mL final volume), and as can be seen from (Fig. 1(b)), the best analytical signal was obtained with buffer concentration of 1.0×10^{-3} mol L^{-1}whereas it decreased at lower and higher buffer concentrations. Therefore, the buffer concentration 1.0×10^{-3} mol $L^{-1}$was considered as optimal value for further studies.

The variation of analytical signal as a function of concentration of CCA, which was chosen as chelating ligand, is presented in (Fig. 2). The dependence of CPE to ligand concentration was examined in the range of (0.012-0.120)×10^{-3} mol L^{-1}. As can be seen from Figure 2, the extraction of Fe(II) increases up to a ligand concentration of 3.6×10^{-5} mol L^{-1} and then decreases with increasing slope and keeps constant in range of (0.36-1.20)×10^{-4} mol L^{-1}. This signal decreasing effect in this range may be due to either aggregation of ligand with dimerization at higher concentrations or a new weak complex formation of ligand with metal ions at pH 10.5. In this ligand concentration, the extraction at levels of maximum 100 µg L^{-1} Fe(II) can be considered to be complete. Therefore, a ligand concentration of 3.6×10^{-5} mol L^{-1} was considered as the optimal value for the further studies.

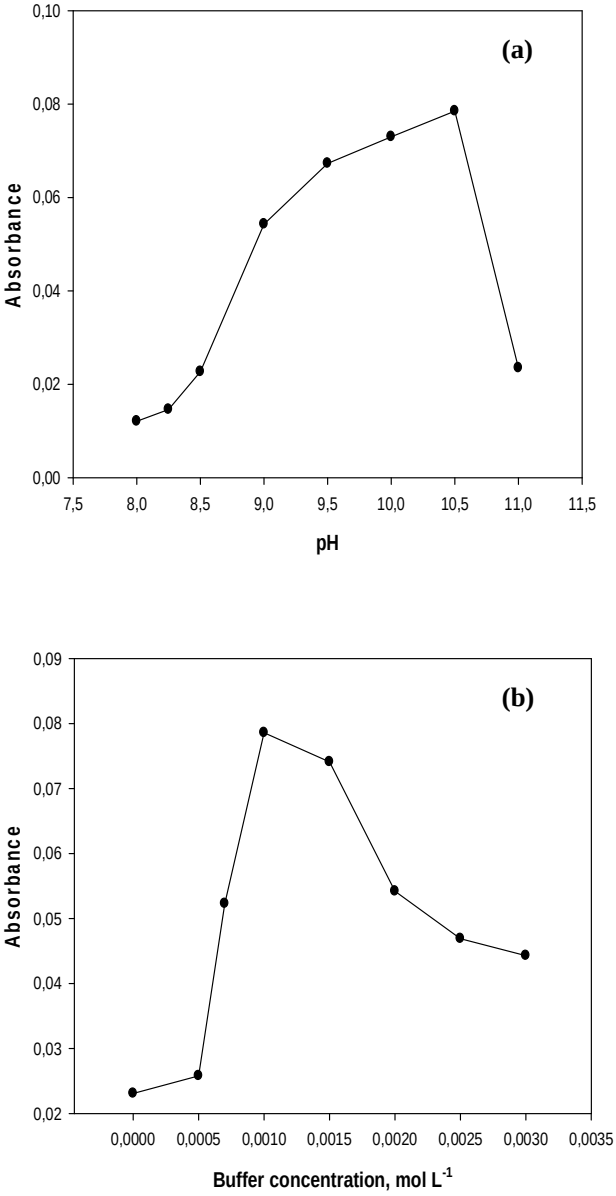

Figure 1. **(a) The effect of pH on analytical signal (b) The effect of buffer concentration on analytical signal**

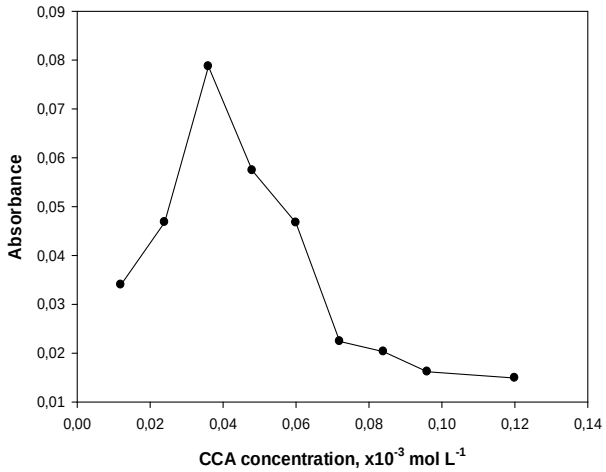

Figure 2. **The effect of CCA concentration on analytical signal**

The variation of analytical signal as a function of concentration of cationic surfactant, CPC, which was chosen as auxiliary ligand, is presented in (Fig. 3(a)). The dependence of CPE efficiency to concentration of auxiliary ligand as an ionic surfactant was studied in the range of $(0.012\text{-}0.132)\times10^{-3}$ mol L^{-1}. As can be seen from Figure 3(a), the extraction of Fe(II) sharply increases up to an auxiliary ligand concentration of 6.0×10^{-5} mol L^{-1} (in a premicellar region with a CMC of 0.12 mmol L^{-1}) and gradually decreases due to decrease in the rate and equilibrium constant of complexation reaction in higher concentrations. Therefore, an auxiliary ligand concentration of 6.0×10^{-5}mol L^{-1} was considered as the optimal value for the subsequent studies.

Triton X-114 was chosen as non-ionic surfactant in the CPE procedure due to its commercial availability, low toxicity, high density, and low cloud point temperature. The effect of the Triton X-114 concentration on CPE of Fe(II) was evaluated by varying the nonionic surfactant concentration in the range of 0.0025-0.2% (v/v). As can be seen from (Fig. 3(b)), iron absorbance sharply increases as surfactant concentration is sharply increased up to a concentration of 0.01% (v/v). Above this concentration, the analytical signal sharply is decreased in range of 0.01-0.2% (v/v) and remained constant at approximately in range of 0.05-0.15% (v/v). This decrease in analytical signal may be due to the increase of the surfactant concentration, deteriorating the FAAS signal. At lower concentrations than 0.01% (v/v), the decrease in extraction efficiency of complex may probably be due to the inadequacy of the surfactant assemblies to entrap the hydrophobic ternary complex quantitatively. Therefore, an extractant concentration of 0.01% (v/v) was considered as optimal value in all further studies.

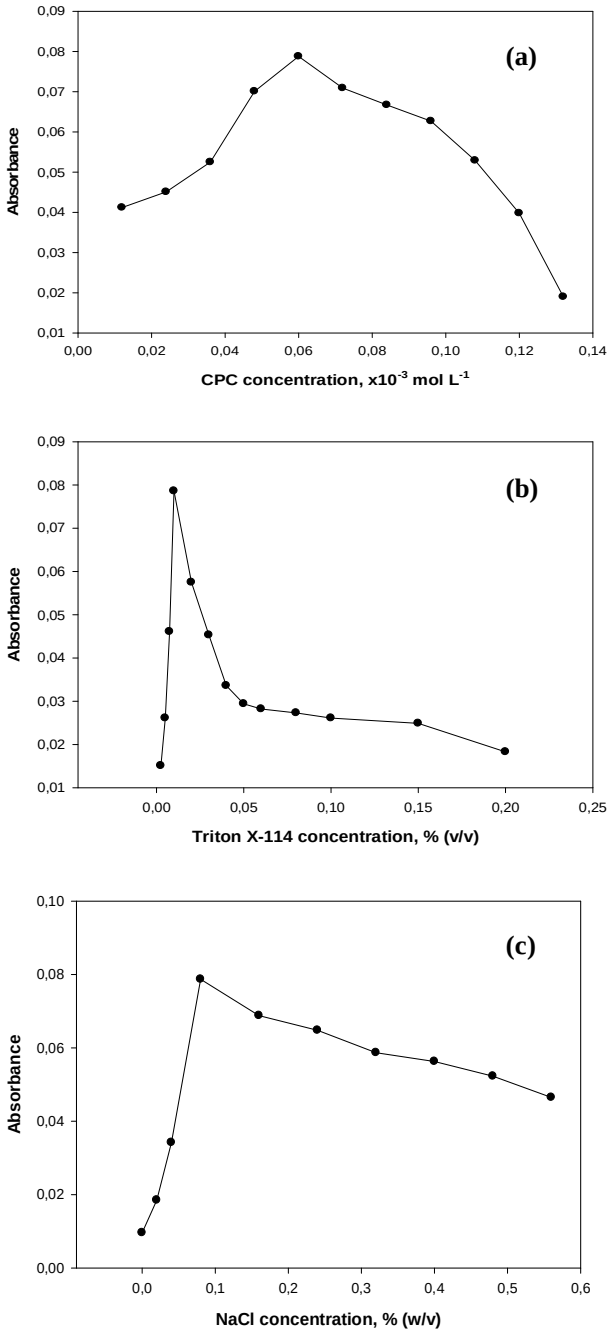

Figure 3. **(a) The effect of CPC concentration on analytical signal (b) The effect of Triton X-114 concentration on analytical signal (c) The effect of ionic strength on analytical signal**

Ionic strength of the aqueous phase can affect the efficiency of extraction for metal analysis based on the formation of hydrophobic metal complex by salting out effect. So, the effect of ionic strength on CPE was initially studied at fixed concentrations by using aqueous solutions of ionic salts such as NaCl, KCl, NaF and thiourea. From the results, the best absorbance change as a function of sensitivity or CPE efficiency was observed for NaCl in order of NaCl>KCl>NaF> Thiourea. So, it was decided to the use of NaCl as signal enhancement salt for further studies. The effect of NaCl concentration on CPE was studied in the range of 0-0.56% (w/v). The absorbance increased with an increasing salt concentration up to 0.08% (w/v), and then gradually decreased at higher volumes than 0.08% (w/v). In range of 0.08-0.56 % (w/v), the decrease in absorbance may be due to the dissociation the hydrophobic ternary complex with increasing salt effect (Fig. 3(c)). For this reasons, a concentration of 0.08 % (w/v) was considered as the optimal value for further studies.

It is desirable a CPE procedure, which employs the shortest incubation time and the lowest incubation temperature possible, considering the compromise between the completion of the reaction and efficiency of the extraction and phase separation. The effect of the incubation temperature on the CPE of iron was studied within the range of 25-80 ºC. From the results, the maximum absorbance for Fe(II) was obtained around 50 ºC. A further increase in temperature leads to a significant decrease in the absorbance. This may probably be due to the thermal instability of the hydrophobic ternary complex of Fe(II) ion formed with CCA in presence of CPC at pH 10.5. So, an incubation temperature of 50 ºC was adopted as optimal value for further experiments.

The effect of the incubation time on the absorbance was studied in the range of 5-30 min. The maximum extraction efficiency was obtained at 10 min. At shorter and longer incubation times the extraction efficiency was significantly decreased. So, an incubation time of 10 min was decided to be enough in terms of completeness of extraction.

The effect of centrifugation time on analytical signal at 3000 rpm was also studied in the range of 0-20 min. From the results, it was found that a centrifugation time of 10 min provided a quantitative phase separation. At lower and higher centrifugation times analytical signal was significantly decreased. Therefore, a centrifugation time of 10 min was decided to be enough in terms of efficient phase separation.

The surfactant rich phase acquired after enrichment with CPE was obtained with a very small volume of 250 µL for detection of analyte with FAAS. The various solvents such as 1 mL of ethanol, 1 mL of THF, 1.0 mL of acetonitrile, 1.0 mL of methanol containing 1.0 mol L^{-1} HNO_3 and 1.0 mL of 2.5 mol L^{-1} H_2SO_4 were added to surfactant-rich phase after phase separation. The absorbance signals as a function of each solvent added to the surfactant-rich phase were measured, and the maximum absorbance was obtained in the presence of 1.0 mL acetonitrile. So, it was decided to use 1.0 mL acetonitrile for further studies.

Optimization studies for speciation analysis

It is well known that Fe(III) prevails in contact with atmospheric oxygen and Fe(II) might be present in reducing environments, and different factors such as dissolved oxygen, pH and presence of natural organic ligands affect the distribution of iron between these two oxidation states. It was established that Fe(III) could be reduced to Fe(II) by using reducing agents such as ascorbic acid, sulfite, hydrazine and hydroxylamine. It is expressed that Fe(II) is also stable in the presence of oxygen at low pHs (<2.0), this oxidation state changes above this pH, especially pH>4.0. So, to be controlled of the valence of iron in aqueous solutions is of great importance [40, 41].

In order to reduce Fe(III) to Fe(II) in standard Fe(III) solutions ranging from 5 to 50 µg L^{-1}, various reducing agents such as ascorbic acid, sulfite, hydroxylamine and hydrazine at equimolar concentrations were initially studied in acidic media. Under the optimized conditions, reducing agent giving the best calibration

sensitivity and reproducible signals to provide quantitatively a complete reduction was considered for speciation analysis of iron. It has been found that other reducing agents except for sulfite (0.291) are not suitable due to give lower signals in order of ascorbic acid (0.286)> hydrazine (0.240)> hydroxylamine (0.234) by using 1.0 mL of 0.01 mol L^{-1} reducing agents in final volume of 50 mL. Moreover, hydrazine and hydroxylamine are not toxic reducing agents not to be eco-friendly due to give interfering oxidation products such as NO_2^-, N_2O and NO. Therefore, sulfite was decided to be used for further studies. With this aim, prior optimization studies were conducted for binary mixtures.

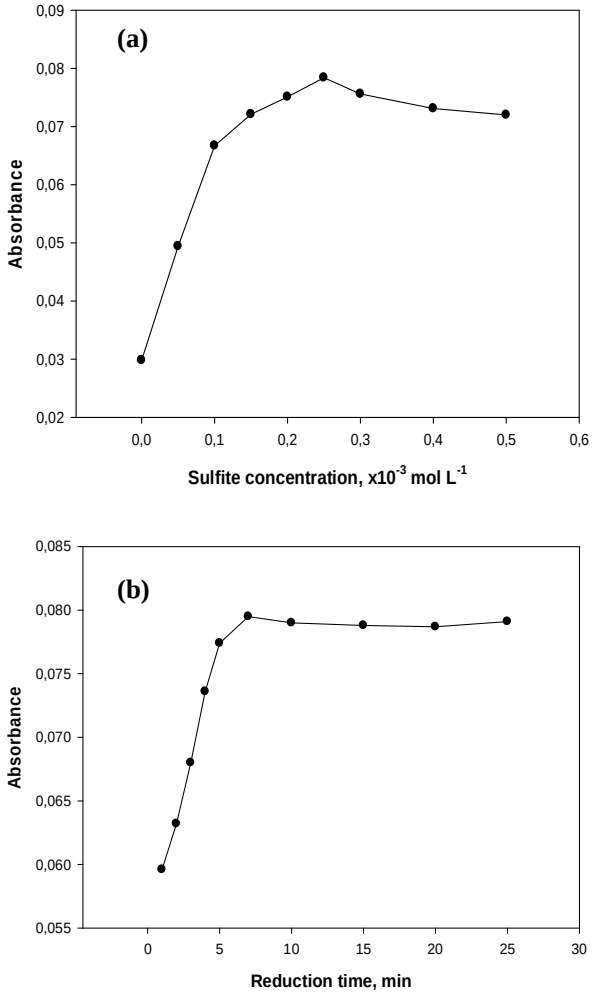

Figure 4. **(a) The effect of sulfite concentration on analytical signal for reduction of 20 µg L^{-1}Fe(III) to Fe(II) at 40 ºC under the optimized conditions (b) The effect of reduction time on analytical signal for reduction of 20 µg L^{-1}Fe(III) to Fe(II) at 40 ºC under the optimized conditions**

Under the optimized conditions, firstly by keeping constant the other variables the effect of pH on reduction of Fe(III) to Fe(II) was studied by using formate buffer in pH range of 2.0-6.0 at reducing character, and a maximum sensitivity was obtained at pH 4.5. Secondly, the effect of sulfite concentration on the sensitivity as a measure of completeness of reducing procedure was studied in the range of (0-0.5)×10^{-3} mol L^{-1}, and a concentration of 0.25×10^{-3} mol L^{-1} was found to be sufficient for further studies (Fig. 4(a)). Also, the effect of reaction time and temperature was studied in time period of 1.0-25 min and in the range of 20-60 ^oC, respectively and a reducing duration of 7 min and a temperature of 40 ^oC were selected as optimum values (Fig. 4 (b)).

Analytical performance features

The calibration curves were obtained by collecting the analytical signals of standard iron solutions submitted to the CPE procedure under the optimized conditions. After preconcentration of 50 mL of sample, the calibration curve obtained for Fe(II) was linear from 0.2 to 60 µg L^{-1} with a regression equation, A= 2.70×10^{-3}[Fe(II), µg L^{-1}] +0.0225 (r^2: 0.9988) while those of Fe(III) was linear from 6.0 to 1000 µg L^{-1} with a regression equation, A= 2.00×10^{-4}[Fe(III), µg L^{-1}] + 0.0035 (r^2: 0.9995). A series standard Fe(III) solutions changing in range of 0.2-4.0 µg mL^{-1} in 0.15 mol L^{-1} HNO_3 were also analyzed by direct aspiration without preconcentration for comparison with CPE-FAAS. From absorbance readings, a calibration curve was plotted, and the linear regression equation, A= 3.32×10^{-5} [Fe(III), mg L^{-1}] - 8.63×10^{-3}(r^2: 0.9974) with the detection and quantification limits of 0.02 and 0.06 mg L^{-1} was obtained. The precision was 2.2% (N: 5, 2.0 mg L^{-1}). The combination of CPE caused to a pronounced increase of sensitivity for iron measurements by FAAS and makes feasible the determination of trace amounts of iron in water samples. Considering the slopes of the calibration curves, it can be seen that CPE-FAAS presents a higher sensitivity of 13.5-fold especially for Fe(II) when compared to conventional FAAS based on the direct

aspiration. From ten replicate analyte blank measurements the detection and quantification limits of the method have been calculated to be 0.06 and 0.2 μg L^{-1}Fe(II) according to $3\sigma_{blank}/m$ method (σ_{blank} is the standard deviation of ten blank replicate determinations, m is the slope of calibration curve) while they are 1.5 and 5.0 μg L^{-1} for Fe(III) respectively. The relative standard deviations (RSDs) of 25 and 100 μg L^{-1} of Fe(II) and Fe(III) for five replicate measurements were 2.7 and 2.4%, respectively. The preconcentration factor, which is defined as the ratio of the analyte concentrations in linear range after and before CPE, was 50 for Fe(II). An enhancement factor of 82 was obtained as the ratio between the slopes of the calibration curves for the preconcentrated samples (50 mL) and the ones not submitted to preconcentration [42].

Interference studies

The effect of different interfering species on the determination of 20 μg L^{-1} of iron (II) ions by the proposed method was evaluated. An anionic or cationic ion was considered as an interfering ion when it caused an error greater than ±5.0% in the determination of Fe(II). The results are given in (Table 1). At the given mole ratio no interference was observed and the recovery of Fe(II) was quantitative in the presence of all foreign ions studied. For speciative determination of Fe(II) in presence of Fe(III) ions in tap water, ammonium fluoride at 1.5 mL of 10 mg L^{-1} was preferably used as masking agent when necessary. In the case of calcium and magnesium-rich waters such as lake water as well as Al^{3+}, Fe^{3+}, Ni^{2+} and Co^{2+}ions, a mixture of NH_4F (1.5 mL of 10 mg L^{-1}) and $(NH_4)_2C_2O_4$ (2.5 mL of 10 mg L^{-1}) in presence of formate buffer at pH 4.5 also was used to eliminate the possible matrix effect. These results indicate the efficiency of the proposed CPE approach and its analytical potential for real sample applications.

Table 1. **The effect of interfering species on the determination of 20 μg L^{-1} of Fe(II) ions.**

Interfering ions	Tolerance ratio	Recovery%
	Anions	
NO_2^-, SO_3^{2-}, $HCOO^-$, CH_3COO^-, Cl^-, NO_3^- and I^-	550-1500	97.5-100.0
Br^-, $C_2O_4^{2-}$, F^- and PO_4^{3-}	300-500	97.5-101.0
SO_4^{2-}	250	98.5
HCO_3^-	150	98.5
SCN^- and CN^-	25, 50	97.0-99.0
	Cations	
Na^+, NH_4^+, K^+, $Ca^{2+}$and Mg^{2+}	>1000	97.5– 102.0
Pb^{2+}, Cd^{2+}, Sr^{2+} and Ba^{2+}	500- 750	98.5-100
Cu^{2+}, Bi^{3+}, Al^{3+} and Cr^{6+}	350-450	98.0-100.0
Ag^+, Hg_2^{2+} and Sn^{4+}	225-300	97.5-100.5
Hg^{2+}, Zn^{2+}, Mn^{2+} andV^{4+}	100-200	98.5-101.5
Sn^{2+}, Mo^{5+}andV^{5+}	50-75	98.0-101.0
Fe^{3+}, Cr^{3+}	12.5, 25	98.5-101.0
Co^{2+} and Ni^{2+}	10	97.0-98.5

The recovery studies for speciation of Fe(II) and Fe(III) in binary mixtures

In order to study the effect of Fe(II)/Fe(III) ratio, the binary mixtures prepared at Fe(II)/Fe(III) concentration ratios ranging from 0.25 to 5 after reducing with sulfite at pH 4.5 formate buffer were analyzed by the CPE/FAAS procedure, and the results obtained are given in (Table 2). As could be seen from the results, Fe(II) and Fe(III) are completely separated for further studies, and quantitatively recovered with a RSD ranging from 1.4 to 3.3% in range of 97.5-99.5 % for Fe(II) and in range of 97.0-99.0 % for total Fe.

Table 2. **The speciation analysis results obtained by the proposed CPE/FAAS method.**

Fe(II)/Fe(III) ratio	Fe(II)				**Fe(III)				
	Added, µg L^{-1}	*Found, µg L^{-1}	RSD %	Recovery %	Added, µg L^{-1}	Total Fe	RSD %	*Found, µg L^{-1}	Recovery%
0.25	10	9.8 ±0.3	3.1	98.0	40	59.7±0.9	1.5	39.7	99.0
0.50	15	14.8±0.4	2.7	99.0	30	44.6±0.7	1.6	29.6	99.0
0.50	20	19.8 ±0.5	2.5	99.0	40	59.8±0.9	1.5	39.8	99.5
2.00	20	19.7±0.5	2.5	99.0	10	29.8±0.6	2.0	9.8	98.0
2.00	30	29.9±0.5	1.7	100.0	15	44.7±0.7	1.6	14.7	98.0
2.00	40	39.8±0.6	1.5	99.0	20	59.8±0.8	1.3	19.8	99.0
3.00	30	29.8±0.6	2.0	99.0	10	39.7±0.7	1.8	9.7	97.0
4.00	40	39.5±0.6	1.5	99.0	10	49.7±0.7	1.4	9.8	98.0
5.00	50	49.7±0.7	1.4	99.0	10	59.9±0.9	1.5	9.9	99.0

*The mean value and its standard deviation of three replicate measurements at 95% confidence level.
**The results determined by calculating the difference between the total Fe and Fe(II) amounts before and after reduction with 1.25 mL of 0.01 mol L^{-1} sulfite at 40 ºC and 5 min under the optimized conditions.

Analytical applications

The applicability of the method was investigated for determination of iron in different matrices. It was applied to tap water, acidic mine water, river water and lake water samples. The lake and river water samples were collected from three various sampling points of lake (Hafik, Sivas, Turkey) and Kızılırmak river (Sivas, Turkey), respectively. The acidic mine water samples, which are used for healthy purposes and marketed commercially, were supplied by a firm. Its accuracy was checked by recovery studies based on analysis of water samples spiked at levels of 5, 10 and 15 µg L^{-1}. The results were extensively represented in (Table 3(a)). From the results, it is clear that the recoveries are highly quantitative in range of 100.0-101.0 % for Fe(II) and in range of 99.0-102.0 % for Fe(III). The results show that the proposed CPE/FAAS method is very suitable for the studied sample types in view of analytical point. In order to control its validation in terms of accuracy and precision, the method was also applied to two certified water samples, ERM-CA011a and NIST-1643e. It was observed that the obtained values (20.7 ± 0.2 and 19.7 ± 0.2 µg L^{-1}) by calibration curve after their dilutions at 1:10 and 1:5 ratios, respectively were highly compatible with certified values of 20.7±0.6 and 19.6±0.6 µg L^{-1} for five replicate analyses. As can be seen from (Table 3(b)), there was statistically no significant difference between the certified values and the found values at 95% confidence level. Also, the recovery studies were conducted by analysis of the spiked standard Fe(II) and Fe(III) solutions before and after reduction with sulfite, and it was observed that the recoveries are highly quantitative. Thus, it can be said that the CPE/FAAS method proposed is highly accurate and reliable for monitoring of dissolved Fe(II) and Fe(III) present especially at low concentrations in a wide range of samples.

When compared with several CPE techniques previously published in literature, the proposed method has advantages such as a low detection limit of 0.06 µg L^{-1}, preconcentration and enhancement factors of 50 and 82 respectively in a linear range of 300-fold. As a result, a sensitivity improvement has been achieved by the proposed method when compared to previously reported works using CPE and spectrophotometry, FAAS, FI-FAAS, GF-AAS [13, 30, 43-47] except for ET-AAS [48]. Especially, ET-AAS, ICP-OES and ICP-MS are highly sensitive, but cost-effective detection techniques as well as being a tedious and

time-consuming, and need an experienced user according to FAAS. Moreover, FAAS is economical and a versatile element selective detection tool, which may be available in almost every laboratory. Validation of the present method was verified by determining inorganic dissolved iron contents of the certified water samples by using CPE/FAAS approach, and the results obtained by using the present method, were highly good in view of accuracy and precision. The method can be very useful tool in a local laboratory for the monitoring of inorganic dissolved iron species in environmental water samples.

Table 3. **(a) Speciative determination of total Fe and dissolved inorganic iron species in environmental water samples.**

Samples		Added (μg L^{-1})		Found by CPE/FAAS (μg L^{-1}) *			Recovery %	
		Fe(II)	*Fe(III)*	*Fe(II)*	*Total Fe*	*Fe(III)***	*Fe(II)*	*Fe(III)*
Acid mine water$_1$		-	-	21.6 ±0.2	32.4±0.2	10.8	-	-
		5	10	26.6±0.2	47.4±0.3	20.8	101.0	100.0
		10	5	31.7±0.3	47.4±0.3	15.8	101.0	100.0
Acid mine water$_2$		-	-	20.1 ±0.2	41.5±0.2	21.4	-	-
		5	10	25.1±0.2	56.5±0.4	31.4	101.0	100.0
		10	5	30.2±0.3	56.5±0.3	26.4	101.0	99.0
Acid mine water$_3$		-	-	13.8±0.2	18.6±0.2	4.8	-	-
		5	15	18.9±0.2	38.6±0.3	19.7	103.0	99.0
		15	5	28.7±0.2	38.6±0.3	9.9	100.0	102.0
Tap water in absence of NH_4F		-	-	14.7±0.2	25.0±0.2	10.4	-	-
		5	15	19.7±0.2	45.1±0.2	25.4	101.0	100.0
		15	5	29.7±0.2	45.1±0.2	15.6	100.0	100.0
Tap water in presence of 1.5 mL of 10 mg L^{-1} NH_4F		-	-	14.5±0.2	18.7±0.2	4.2	-	-
		5	15	19.6±0.2	38.7±0.3	19.2	101.0	100.0
		15	5	29.6±0.2	38.7±0.3	9.1	101.0	99.0
River water		-	-	12.2±0.2	32.5±0.2	20.3	-	-
		5	10	17.2±0.2	47.5±0.3	30.3	101.0	100.0
		10	5	22.2±0.2	47.5±0.3	25.3	100.0	100.0
***Lake water	**Entry**	-	-	7.9±0.2	15.2±0.2	7.2	-	-
		10	15	18.1±0.2	40.2±0.3	22.2	101.0	100.0
		-	-	7.5±0.2	14.7±0.2	7.2	-	-
		10	15	17.6±0.2	39.8±0.3	22.2	101.0	100.0
	Middle	-	-	5.1±0.2	7.7±0.2	2.5	-	-
		10	15	15.2±0.2	32.7±0.2	17.5	101.0	100.0
		-	-	5.0±0.2	11.7±0.2	6.7	-	-
		10	15	15.1±0.2	36.7±0.3	21.6	101.0	100.0
	Coastal	-	-	7.4±0.2	12.2±0.2	4.7	-	-
		10	15	17.5±0.2	37.2±0.3	19.7	101.0	100.0
		-	-	6.7±0.2	12.0±0.2	5.3	-	-
		10	15	16.7±0.2	37.1±0.3	20.4	101.0	102.0

*The mean value and its standard deviation of five replicate measurements at 95% confidence level.
**The results found by subtracting the amount of Fe(II) from those of total Fe after reducing with sodium sulfite at pH 4.5 formate buffer media.
***The chemical properties of lake water samples (Hafik, Sivas, Turkey). The mean analysis values obtained by means of thirty replicate measurements: pH: 7.45, total hardness (FS^o) 17.66, total alkalinity 134.67 mg L^{-1}, Ca 58.40 mg L^{-1}, Mg 6.66 mg L^{-1}, Cl^- 34.10 mg L^{-1}, HCO_3^- 134.55 mg L^{-1}

Table 3. **(b) The analysis results of certified water samples**

Certified environmental water sample	Dilution ratio	Certified value, µg L^{-1}		Added, µg L^{-1}		*Found value, µg L^{-1}			Recovery%		***The statistical t- and F-values
		Fe(II)	Fe(III)	Fe(II)	Fe(III)	Fe(II)	Total Fe	**Fe(III)	Fe(II)	Fe(III)	
ERM-CA011a	1:10	20.7±0.6	-	-	-	20.7±0.2	-	-	-	-	1.59
Hard drinking				5	15	25.2±0.2	40.2±0.4	15.0	101.0	100.0	(0.56)
water–Metals				15	5	35.5±0.3	40.2±0.3	4.9	102.0	97.0	
				10	10	30.4±0.3	40.2±0.4	9.9	102.0	99.0	
NIST-1643e	1:5	19.62±0.6	-	-	-	19.7±0.2	-	-	-	-	0.28
Simulated				5	15	24.7±0.3	39.8±0.3	15.1	99.0	101.0	(0.85)
fresh water-				15	5	34.7±0.3	39.7±0.4	5.0	101.0	101.0	
Trace elements				10	10	29.8±0.3	39.8±0.4	10.0	101.0	100.0	

Conclusions

In this work, a new CPE approach coupled to FAAS was developed for the extraction, preconcentration and determination of inorganic dissolved iron species (Fe(II), Fe(III) and total Fe) in environmental waters. Actually, formation of iron–CCA complexes, addition of nonionic surfactant in presence of CPC and heating to cloud point processes were carried out by operations such as cooling, removal of supernatant and dilution. This CPE approach offers a simple, inexpensive, and eco-friendly technique for the preconcentration and speciative determination of trace iron species before and after reduction of Fe(III) to Fe(II) with sulfite under a short preheating of 5 min at 40 ^{o}C and pH 4.5. Triton X-114 is of relatively low-cost and toxicity. CCA for Fe(II) is especially a very stable and fairly selective complexing agent at pH 10.5 borate buffer. The proposed preconcentration method exhibits good precision, accuracy and sensitivity as well as relatively selectivity, and allows speciative determination of iron species in water samples at µg L^{-1} levels. In comparison, the proposed method provides good results in terms of detection limits, preconcentration and enhancement factors. The method has been successfully applied to the preconcentration and determination of Fe(II), Fe(III) and total Fe in both different water samples and two CRMs, and statistically verified and validated by comparing the results obtained with certified values as well as recoveries old spiked samples.

Acknowledgements

Authors wish to thank Professor Mehmet Akçay due to critical contributions in the preparation of this manuscript. The present study was also presented as a poster in the 5th National Analytical Chemistry Congress, June 21th-25th 2010, held in Faculty of Pharmacy, Erzurum, Turkey.

References

1. R. Cornelis, J. Caruso, H. Crews, K. Hramann, *Handbook of Elemental Speciation II, Species in the Environment, Food, Medicine and Occupational Health* (John Wiley & Sons) 2005.
2. J. H. Martin and S. E. Fitzwater, *Nature*, 331 (1988) 341.
3. J. H. Martin, R. M. Gordon, S. E. Broenkow, and W. W. Fitzwater, *Deep. Sea. Res. Part A*, 36 (1989) 649.
4. X. Pu, B. Hu, Z. Jiang and C. Huang, *Analyst*, 130 (2005) 1175.
5. E. P. Achterberg, T. W. Holland, A. E. Bowie, R. Fauzi, C. Mantroura and P. J. Worsfold, *Anal. Chim. Acta*, 442 (2001) 1.
6. M. A. Aki, *Microchem. J*, 75 (2003) 199.
7. D. Kara and M. Alkan, *Talanta*, 55 (2001) 415.

8. M. Yaman and G. Kaya. *Anal. Chim. Acta,* 540 (2005) 77.
9. P. K. Tarafder and R. Thakur, *Microchem. J,* 80 (2005) 39.
10. Y. Huang, D. Yuan, J. Ma, M. Zhang and G. Chen, *Microchim. Acta,* 166 (2009) 221.
11. A. S. Amin and A. A. Gouda, *Talanta,* 76 (2008) 1241.
12. O. Inan and Y. Özdemir, *J. Food Sci. Tech.,* 46 (2009) 320.
13. F. Shakerian, S. Dadfarnia and A. M. Haji Shabani, *J. Iran. Chem. Soc.,* 6 (2009) 594.
14. M. Noroozifar, M. Khorasani-Motlagh and R. Akbari, *Anal. Sci.,* 22 (2006) 141.
15. E. Pehlivan and D. Kara, *Microchim. Acta,* 158 (2007) 137.
16. S. Saçmacı and S. Kartal, *Anal. Chim. Acta,* 623 (2008) 46.
17. S. L. C. Ferreira, H. S. Ferreira, R. M. de Jesus, J. V. S. Santos, G. C. Brandao and A. S. Souza, *Anal. Chim. Acta,* 602 (2007) 89.
18. C. Xiong, Z. Jiang and B. Hu, *Anal. Chim. Acta,* 559 (2006) 113.
19. L. Xia, Y. Wu, Z. Jiang, S. Li and B. Hu, *Int. J. Environ. Anal. Chem.,* 83 (2003) 953.
20. B. H. Li and X. P. Yan, *J. Sep. Sci.,* 30 (2007) 916.
21. M. Grotti, F. Soggia, F. Ardini and R. Frache, *J. Anal. Atom. Spectrom.,* 24 (2009) 522.
22. O. Mikkelsen, C. M. G. van den Berg and K. H. Schroder, *Electroanal.,* 18 (2006) 35.
23. P. L. Croot and M. Johansson, *Electroanal.,* 12 (2000) 565.
24. S. Roncevic and I. Steffan, *Atom. Spectrosc.,* 25 (2004) 125.
25. S. Ichinoki, S. Fujita and Y. Fujii, *J. Liq. Chromatogr. R. T.,* 32 (2009) 281.
26. A. Abbaspour, M. A. Mehrgardi, A. Noori, M. A. Kamyabi, A. Khalafi-Nezhad and M. N. S. Rad, *Sensor. Actuator. B.,* 113 (2006) 857.
27. E. R. Pereira, B. M. Soares, J. V. Maciel, S. S. Caldas, C. F. F. Andrade, E. G. Primel and F. A. Duarte, *Anal. Methods*, 5 (2013) 2273.
28. F. S. Rojas, C. B. Ojeda and J. M. C. Pavón, *Am. J. Chem.,*2 (2012) 28.
29. M. Ghaedi, A. Shokrollahi, R Mehrnoosh, O. Hossaini and M. Soylak, *Cent. Eur. J. Chem.,* 6 (2008) 488.
30. D. L. Giokas, E. K. Paleologos and M. I. Karayannis, *Anal. Bioanal. Chem.,* 373 (2002) 237.
31. L. Elci, M. Soylak and B. Ozcan, *Anal. Lett.,* 36 (2003) 987.
32. H. Z. Şenyuva, D. Yurtsever Sarıca and T. Özden, *Turk. J.Chem.,* 26 (2002) 425.
33. N. Yu. Stozhko, O. V. Inzhevatova and L. I. Kolyadina, *J. Anal.Chem.,*60 (2005) 668.
34. M. Lu, N. V. Rees, A. S. Kabakaev and R. G. Compton, *Electroanal.,*24 (2012) 1693.
35. Z. Shi, X. Zhu and H. Zhang, *J. Pharmaceut. Biomed.,*44 *(*2007) 867.
36. G. L. Donati, C. C. Nascentes, A. R. A. Nogueira, M. A. Z. Arruda and J. A. N´obrega, *Microchem. J.* 82 (2006) 189.
37. Y. Yamini, M. Faraji, S. Shariati, R. Hassani and M. Ghambarian, *Anal. Chim. Acta,* 612 (2008) 144.
38. M. K. Amini and M. Kabiri, *J. Iranian Chem. Soc.,* 2 (2005) 32.
39. S. Bahrami, S. Abbasi, Y. A. Ghorbani and A.A. Miran-Beigi, *Russian J. Electrochem.,*45 (2009) 208.
40. P. Pohl and B. Prusisz, *TrAC, Trends Anal. Chem.,* 25 (2006) 909.
41. C. J. Borman, B. P. Sullivan, C. M. Eggleston and P. J. S. Colberg, *Sensors*,9 (2009) 4390.
42. W. Wei, X-B. Yin and X-W. He, *J. Chromatogr. A,* 1202 (2008) 212.
43. C. Duran, D. Özdeş¸ E. Çelenk Kaya¸ H. Kantekin, V.N. Bulut and M. Tüfekçi¸ *Turk. J. Chem.,* 36 (2012) 445.
44. X-L. Song and S-K. Liang, *Metallurgical Analysis*, 30 (2010) 70.
45. P. Liang, H. Sang and Z. Sun, *J. Colloid Interface Sci.*, 304 (2006) 486.
46. D. L. Giokas, E. K. Paleologos, S. M. Tzouwara-Karayanni and M. I. Karayannis, *J. Anal. At. Spectrom.,*16 (2001) 521.
47. A. Ohashi, H. Ito, C. Kanai, H. Imura and K. Ohashi, *Talanta,* 65 (2005) 525.
48. N. N. Meeravali, M. A. Reddy and S. J. Kumar, *Anal. Sci.,* 23(2007) 351.