Preconcentration and determination of iron and copper in spice samples by cloud point extraction and flow injection flame atomic absorption spectrometry

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ABSTRACT

A flow injection (FI) cloud point extraction (CPE) method for the determination of iron and copper by flame atomic absorption spectrometer (FAAS) has been improved. The analytes were complexed with 3-amino-7-dimethylamino-2-methylphenazine (Neutral Red, NR) and octylphenoxypolyethoxyethanol (Triton X-114) was added as a surfactant. The micellar solution was heated above 50 °C and loaded through a column packed with cotton for phase separation. Then the surfactant-rich phase was eluted using $0.05\,\mathrm{mol\,L^{-1}}$ H_2SO_4 and the analytes were determined by FAAS. Chemical and flow variables influencing the instrumental and extraction conditions were optimized. Under optimized conditions for 25 mL of preconcentrated solution, the enrichment factors were 98 and 69, the limits of detection ($3s$) were 0.7 and $0.3\,\mathrm{ng\,mL^{-1}}$, the limits of quantification ($10s$) were 2.2 and $1.0\,\mathrm{ng\,mL^{-1}}$ for iron and copper, respectively. The relative standard deviation (RSD) for ten replicate measurements of $10\,\mathrm{ng\,mL^{-1}}$ iron and copper were 2.1% and 1.8%, respectively. The proposed method was successfully applied to determination of iron and copper in spice samples.

1. Introduction

Trace metals play an important role in human metabolism and either excess or deficiency of them in the living organism can lead to biological disorder [1]. Iron is vital for almost all living organisms due to the fact that it occurs in a wide variety of metabolic process, including oxygen transport, DNA synthesis, and electron transport. However, iron concentrations in body tissue must be carefully regulated, because excessive iron leads to tissue damage as a result of formation of free radicals [2]. Copper is an essential micronutrient required for good health. However, high levels of this element can be harmful. Ingesting high levels of copper can cause vomiting, nausea, diarrhea, liver or kidney damage or even death [3,4].

Due to these reasons, the accurate and precise determinations of trace metals are important for analytical chemists [5–8]. Because of its experimental rapidity, simplicity, and wide application, flame atomic absorption spectrometry is the one of the most widely applied methods those are based on spectrometry. But the direct determination of metal ions at trace levels FAAS is limited not only due to insufficient sensitivity, but also by matrix interference. Despite the selectivity and sensitivity of analytical techniques, there is a pivotal need for the preconcentration and/or separation of trace elements before their analysis. A powerful technique to effectively enhance atomic absorption spectrometry's sensitivity and selectivity is CPE. The method is based on the property of most non-ionic surfactants in aqueous solutions to form micelles and become turbid when heated to a temperature known as the cloud point temperature. Above this temperature, the micellar solution separates in a surfactant-rich phase of a small volume and in a dilute aqueous phase [9]. Any species present in the solution that interact with the micellar aggregates are thus extracted and may be preconcentrated in the small volume of the surfactant-rich phase [10–12]. Off-line CPE has been used for the extraction and preconcentration of organic compounds [13,14] and metal ions in various samples [15–18]. It involves a series of bulky steps including incubation, centrifugation, and separation of the surfactant-rich phase from aqueous phase and dilution of surfactant-rich phase. All these steps can cause poor reproducibility, low preconcentration factor and time consuming procedure [19]. The combination of on-line systems with CPE puts together the vantage of both systems: high velocity and analytical reproducibility, reduced sample and reagent consumption, minimized risk of analyte loses and matrix contamination [20,21]. Nevertheless, these advantages have not been benefited yet; there is only a few amount of published work involving coupling [22].

Fang et al. [21] proposed, for the first time, the on-line incorporation of CPE and FIA. Ortega et al. [23–25] were the first in coupling CPE on-line with ICP-OES for determination of rare earth elements.

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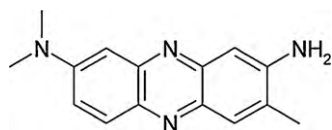*E-mail address:* carpa@hacettepe.edu.tr (Ç.A. Şahin).

Fig. 1. Molecular structure of NR.

Nan et al. [26] developed an FI-CPE procedure for ETAAS determination of trace Pb in biological samples. The potential of hypenating CPE to FAAS was illustrated by Curtius et al. [27] in 1998 for the preconcentration and determination of Ag and Au in geological samples. Recently, Silva and Roldan [28] and Lemos and David [29] have reported methods for trace heavy metals enrichment prior to FAAS measurements.

Molecular structure of NR is given in Fig. 1. NR is an azine dye, which is similar to the other planar dyes in the chemical structure belonging to the acridine, thiazine and xanthene groups having nitrogen as the heteroatom instead of divalent oxygen or sulphur [30]. Due to these reactive groups NR can be used as a complexing agent for CPE. The complex formation reactions between NR and iron and copper are very fast and the selectivity of NR is enhanced by suitable selection of pH. Even so, according our knowledge, the use of NR for preconcentration has not been reported before.

In the present work, the incorporation of CPE into the FI-AAS determination of iron and copper in spice samples using NR as chelating agent and optimization of chemical and flow conditions was introduced.

2. Experimental

2.1. Instrumentation

A PerkinElmer Model AAnalyst 800 Atomic Absorption Spectrophotometer equipped with deuterium back ground correction and air–acetylene burner was used for Fe and Cu measurements in standard and sample solutions. Iron and copper hollow cathode lamps were used as radiation source. The most sensitive wavelengths (nm) and lamp currents (mA) used for the determination of the analytes were as follows: Fe 248.3 and 30, and Cu 324.8 and 15, respectively. Slit widths were 0.2 nm. All of the absorbance measurements were carried out in using an air/acetylene flame at flow rates of 18 and 2.3 L min^{-1} for Fe and 17 and 2.1 L min^{-1} for Cu. 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 0.05 mol L^{-1} sulphuric acid. A Fisher Scientific Accumet Model 15 pH meter was used to measure pH values. A Clifton Model NE1-22 thermostatic bath, maintained at the desired temperature, was used for cloud point temperature experiments. A PerkinElmer model flow injection system, FIAS 400, was used for sample loading, separation and elution. The FIAS 400 system comprises two peristaltic pumps, a two-positional and six-port valve and a handmade mini-column. A mini-column manufactured from glass (30 mm length and 2.5 mm i.d.) packed with 30 mg of cotton was used. To control the complex formation UV–vis spectrometer was used (Shimadzu, Japan).

2.2. Reagents and solutions

All chemicals used were of analytical reagent grade. All solutions were prepared with deionized water (18.1 MΩ cm) obtained from a Barnstead, Nanopure Diamond purification system. Stock solutions were prepared from appropriate amounts of the respective nitrates (Merck, Darmstadt, Germany) at a concentration 1000 mg L^{-1} in deionized water, and diluted daily for obtaining working solutions prior to use. The standard solutions used for the calibration procedures were prepared before use by dilution of the stock solution with deionized water. The non-ionic surfactant Triton X-114 (Sigma–Aldrich, Milwaukee, USA) was used without further purification. A 0.1% (w/v) NR solution was prepared dissolving an appropriate amount of NR obtained from Sigma–Aldrich in deionized water. This solution was stable for at least 1 month. Buffer solution of pH 6 and pH 7 was prepared by using sodium acetate (Merck) and acetic acid (Merck) at appropriate concentrations. 0.05 mol L^{-1} H$_2$SO$_4$ was prepared by dilution of 0.5 mol L^{-1} H$_2$SO$_4$ (Merck) and used as eluent. Laboratory glassware was kept overnight in 10% nitric acid solution. Before use, the glassware was rinsed with deionized water and dried in dust free environment. The following certified reference materials were used for method validation: the standard bush branches and leaves sample (NCS DC 73349) purchased from China National Analysis Center for Iron and Steel (Beijing, China) and fortified water sample (TM 23.2) purchased from LGC (Teddington, UK).

2.3. Procedure

For CPE preconcentration, aliquots of 25 mL solution containing sample or standard solution of iron and copper were prepared and placed in graduated tube. After pH adjustment with 2 mL acetate buffer (pH 6.0 for Fe and pH 7.0 for Cu) the amount of 500 μL chelating agent NR (0.1%, w/v) was added and metal-chelate was formed. Then, 250 μL Triton X-114 (10%, v/v) was added and mixed well in vortex and kept in a thermostated bath at 50 °C for 30 min. At that point, the initial solution separates into two phases—one bulk aqueous phase containing the surfactant monomers and the other, a surfactant-rich phase of small volume containing the trapped metal by micelles. After this step, the obtained solution was passed through the mini-column with the help of the FI 400 system in fill position of valve [Fig. 2(a)]. So the surfactant-rich phase trapped inside of the mini-column while the aqueous phase passes through the mini-column. When the sample loading was finished, to elude the retained analytes, the valve switched to the inject position and the eluent solution sent in reverse flash direction [Fig. 2(b)]. The carrier solution was directly introduced into the FAAS nebulizer.
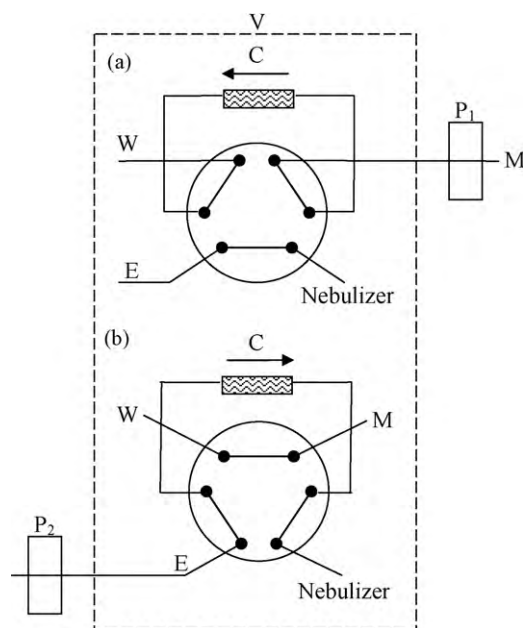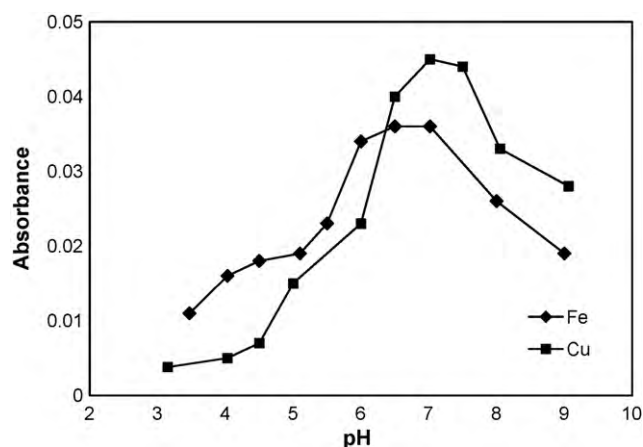

Fig. 2. Schematic diagram of the FI-CPE system. M, micellar solution [sample + NR + Triton X-114 (50 °C)]; E, eluent; W, waste; P$_1$ and P$_2$, peristaltic pumps; C, column; V, six-port 2-position injection valve. (a) Fill position and (b) inject position.

Fig. 3. Influence of the pH on CPE of iron and copper. Sample, 25 mL, 10 ng mL^{-1}; surfactant, 0.05% Triton X-114; ligand, 0.1% NR; eluent, 0.05 mol L^{-1} H$_2$SO$_4$; equilibration temperature, 50 °C; equilibration time, 30 min.

Fig. 4. Influence of NR concentration on CPE of iron and copper. Sample, 25 mL, 10 ng mL^{-1}; surfactant, 0.05% Triton X-114; pH, 6 (for Fe) and 7 (for Cu); eluent, 0.05 mol L^{-1} H$_2$SO$_4$; equilibration temperature, 50 °C; equilibration time, 30 min.

2.4. Samples and sample pretreatment

Red pepper, black pepper, cumin, cinnamon, mint and thyme samples were purchased from local supermarkets at Ankara in Turkey, grounded and dried. Masses of 1000 mg of spice samples were transferred into separate 250 mL beakers and 5 mL of 0.5 mol L^{-1} nitric acid was added to moisten the samples thoroughly. This was followed by adding 10 mL of concentrated nitric acid and heating on a hot plate (130 °C) for 3 h. After cooling to room temperature, 5 mL of concentrated perchloric acid was added drop wise. The beaker was heated gently until completion of sample decomposition resulting in a clear solution. This was left to cool down and then was transferred into a 250 mL volumetric flask by rinsing the interior of the beaker with small portions of 0.1 mol L^{-1} nitric acid and the solution was filled to the mark with the same acid [31].

3. Results and discussion

3.1. Optimization of chemical variables

3.1.1. Effect of pH

The pH plays a unique role on metal-chelate formation and subsequent extraction [5–8]. Separation of metal ions by cloud point method involves the prior formation of a complex with sufficient hydrophobicity to be extracted into the small volume of surfactant-rich phase. Extraction recovery depends on the pH at which complex formation occurs. In order to find optimum pH, the effect of pH in the range 3–9 on the complex formation reactions was investigated. Fig. 3 shows the effect of pH on the extraction of the Fe and Cu complexes. As can be seen in Fig. 3, a pH value of around 6 and around 7 found to be the optimum for the quantitative extraction of the NR–iron complex and the NR–copper complex, respectively.

3.1.2. Effect of NR concentration

NR is an azine dye which acts as a ligand. It complexes the metal ions through the azine-nitrogen atom and amino-nitrogen atom. In order to study the influence of NR concentration on the analytical response for iron and copper, different concentrations of the NR in the range of 0.01–1.00% (w/v) were used, and the general procedure was applied. The absorbances as a function of the concentration of NR are shown in Fig. 4. As can be seen CPE efficiency increased rapidly as the concentration of NR increased from 0.01 to 0.05%, then kept almost constant upon further increase
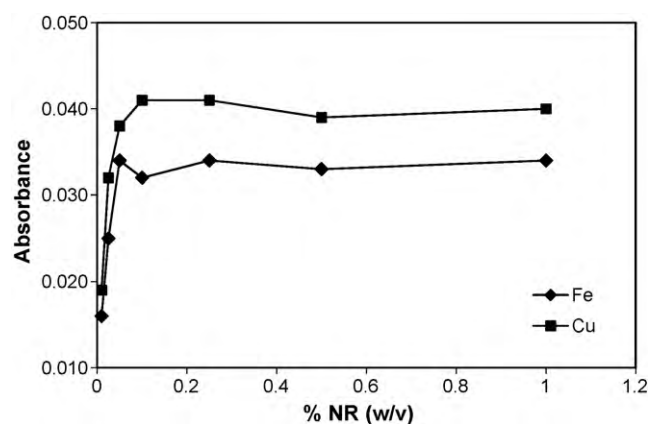
in the NR concentration up to 1.00%. Therefore, NR concentration of 0.1% was chosen for subsequent experiments. Complex formation was controlled with UV–vis spectrometer and stable at least 24 h.

3.1.3. Effect of Triton X-114 concentration

The amount of Triton X-114 not only affected the extraction efficiency, but also the volume of surfactant-rich phase. A successful CPE should maximize the extraction efficiency by minimizing the phase volume ratio ($V_{org}/V_{aqueous}$), thus improving its concentration factor [32]. There is a narrow range within which easy separation, maximum extraction efficiency and analytical signal are accomplished. The variation of the analytical signal of Fe and Cu within the Triton X-114 concentration range of 0.01–0.2% (v/v) was examined (Fig. 5). Quantitative extraction was observed when the Triton X-114 concentration was higher than 0.05% (v/v). At lower concentrations, the extraction efficiency of complex is low probably because of the inadequacy of the assemblies to entrap the hydrophobic complex quantitatively. At higher concentrations of the surfactant, the volume of the surfactant-rich phase is increasing and the column is saturating with the surfactant and could not retain the surfactant-rich phase completely. For this reason the signal of the analyte is decreasing. Since, a concentration of 0.05% (v/v) was chosen as optimum concentration for the determination of iron and copper.
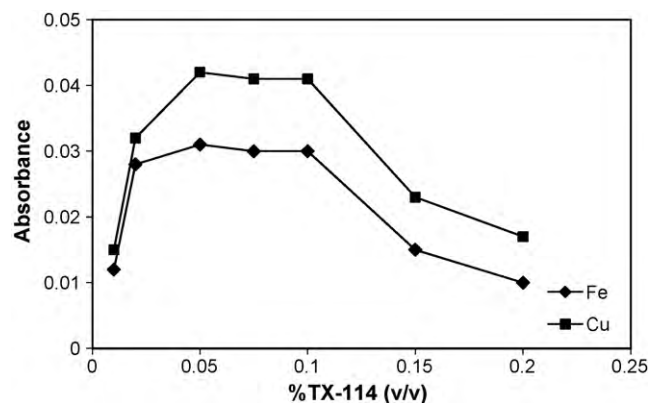
Fig. 5. Influence of Triton X-114 concentration on CPE of iron and copper. Sample, 25 mL, 10 ng mL^{-1}; ligand, 0.1% NR; pH, 6 (for Fe) and 7 (for Cu); eluent, 0.05 mol L^{-1} H$_2$SO$_4$; equilibration temperature, 50 °C; equilibration time, 30 min.

3.1.4. Effect of equilibration temperature and time

When temperature increases, the system is further away from the cloud point, causing the non-ionic surfactant to be less water soluble. To achieve easy phase separation, optimal equilibration temperature and incubation time are necessary to complete extraction. The effect of the equilibration temperature and time was studied with a range of 20–80 °C and 5–60 min, respectively. It was found that an equilibration temperature of 50 °C and a time of 30 min were adequate to achieve quantitative extraction.

On the other hand, it appears that the phase volume ratio of all non-ionic surfactants decrease as the equilibration temperature increase. The greatest analyte preconcentration factors are thus expected under conditions where the CPE is conducted using equilibration temperatures that are well above the cloud point temperature of the surfactant. It was found that a temperature of 50 °C is adequate for iron and copper.

3.1.5. Effect of ionic strength

For investigating the influence of ionic strength on performance of CPE, various experiments were performed by adding different amount of NaCl (0.005–0.05 mol L^{-1}). Other experimental conditions were kept constant. The results showed that ionic strength has no significant effect on the enrichment factor. Thus ionic strength was kept constant at 0.01 mol L^{-1} with NaCl.

3.1.6. Selection of the eluent and eluent concentration

After the preconcentration step, an appropriate solvent should be used to remove the metal ion-containing surfactant aggregated within the mini-column. This eluent is also employed to transport the metal ions to FAAS hence the selection of proper eluent is an essential factor determining the analytical signal of iron and copper [33]. Desorption of the retained metal ions from the cotton mini-column has been investigated. In different concentrations of some eluents such as water, ethanol, methanol, acetone, nitric acid, hydrochloric acid, sulphuric acid, and ethanolic and methanolic solutions of these acids were tested as eluent to achieve reasonable desorption efficiency and at the same time a satisfied analytical signal for iron and copper. Mixtures containing ethanol or methanol were found to be efficient for the elution but these eluents were not able to provide a good analytical signal. The use of ethanol, methanol, acetone or water alone resulted in poor elution and poor analytical signal. Therefore, mixtures containing ethanol were not used as eluent in subsequent experiments. Acid solutions presented the best results in terms of elution and signal. Sulphuric acid has been chosen for subsequent experiments because this eluent provided more precise results. Different H$_2$SO$_4$ concentrations in the range of 5.0×10^{-3} to 1.0×10^{-1} mol L^{-1} were tested for the quantitative elution of iron and copper ions from the mini-column. The results indicated that the signals for iron and copper have highest values when the concentration of sulphuric acid is up to 5.0×10^{-2} mol L^{-1}. A 5.0×10^{-2} mol L^{-1} sulphuric acid aqueous solution was used as eluent in all subsequent experiments.

3.2. Optimization of FI flow conditions

3.2.1. Column dimension and effect of packing material

The home-made glass mini-column was packed with different filtering materials was employed to carry out phase separation. Glass wool, cotton and silica gel were tested as packing material and it was found that cotton packed mini-column acts well for the entrapment of the analyte-containing organized surfactant assemblies. The length of the column is important parameter [34,35]. The shorter columns were inadequate for retaining the surfactant-rich phase, while the longer column decreased the signal due to insufficient elution of the micellar aggregates. The internal diameter of the mini-column was chosen as 2.5 mm and the column length was var-

Table 1
Optimum conditions for the CPE of iron and copper ions.

Optimum conditions for FI-CPE of Fe and Cu	Value
Concentration of chelating agent	0.1% (w/v)
Concentration of surfactant	0.05% (v/v)
pH	~6.0 for Fe, ~7.0 for Cu
Equilibrium temperature (°C)	50
Equilibrium time (min)	30
Column dimension (mm)	30 × 2.5 i.d
Amount of packing material (mg)	30
Eluent	0.05 mol L^{-1} H$_2$SO$_4$
Sample flow rate (mL min^{-1})	1.5
Eluent flow rate (mL min^{-1})	3.0

ied within the range 10–50 mm. The experimental results showed that the optimal retention was achieved with mini-column length greater than 25 mm. Therefore, a mini-column with an effective length of 30 mm and inner diameter of 2.5 mm was used to obtain suitable sensitivity and precision. The amount of cotton wool in the mini-column is also important for collecting the surfactant-rich phase; hence it was optimized for the selected column by testing different amounts of dry cotton wool. The best results were obtained when 30 mg cotton was used. Larger amounts of cotton led to the deterioration of the signal due to blockage of column and insufficient elution of the retained complex; when a smaller amount was used, collection was not efficient due to reduced filtering capacity. Further experimental studies revealed that at least 50 preconcentration/elution cycles can be performed with the same column without a significant change in the performance of system.

3.2.2. Sample loading rate

The sample flow rate through the mini-column is a very important parameter, since this is one of the steps that control the analysis time [36]. The influence of sample flow rate on the preconcentration efficiency was tested at flow rates of 0.40–3.00 mL min^{-1}. It was found that analytical signal decreased slightly by increasing the sample flow rate above 1.5 mL min^{-1} because of incomplete retention of the surfactant-rich phase in the mini-column. Therefore, a sample flow rate of 1.5 mL min^{-1} was selected for further studies.

3.2.3. Eluent flow rate

The flow of eluent is a parameter that affects the rate of elution of iron and copper from the mini-column. The effect of elution rate on the preconcentration efficiency was studied at flow rates of 0.40–3.00 mL min^{-1}. It was observed that the analytical signal increased by increasing the eluent flow rate. At flow rates above 1.5 mL min^{-1} sharper peaks were obtained. Below 1.5 mL min^{-1} broader peaks were observed due to the incompatibility between elution and nebulization flow rate. Therefore, the highest elution flow rate of 3.0 mL min^{-1} was selected for further studies to compensate with the rate of nebulizer.

Obtained optimum condition values for CPE and FI-AAS flame atomic absorption spectrometry system for preconcentration and determination of iron and copper ions are presented in Table 1.

3.3. Interferences

The effect of foreign ions on the determination of Fe and Cu by the proposed method was investigated by measuring the absorbance of the solutions containing 10 ng mL^{-1} of each metal ion in the presence of various amounts of other ions. The tolerance limit was defined as the concentration of added ion that caused less than ±5% relative error in the determination of Fe and Cu. The maximum tolerances of the investigated cations and anions are given

Table 2
Tolerance limits of interfering ions in the determination of $10\,ng\,mL^{-1}$ Fe and $10\,ng\,mL^{-1}$ Cu.

Ions	Metal to interferent ratio (w/w)	
	Fe	Cu
Al^{3+}	1/500	1/1000
Fe^{3+}	–	1/1000
Cr^{3+}	1/2000	1/1000
Sr^{3+}	1/1000	1/500
As^{3+}	1/1000	1/1000
Zn^{2+}	1/500	1/1000
Ca^{2+}	1/1000	1/2000
Cd^{2+}	1/500	1/500
Cu^{2+}	1/3000	–
Pb^{2+}	1/2000	1/1000
Mn^{2+}	1/500	1/1000
Ni^{2+}	1/100	1/2000
Mg^{2+}	1/1000	1/1000
Hg^{2+}	1/1000	1/500
Na^{+}	1/5000	1/3000
CO_3^{2-}	1/3000	1/2000
SO_4^{2-}	1/1000	1/1000
SCN^{-}	1/1000	1/1000
NO_3^{-}	1/3000	1/3000
Cl^{-}	1/5000	1/3000
PO_4^{3-}	1/3000	1/3000

Table 3
Analytical characteristics of the method.

Parameter	Analytical feature	
	Fe	Cu
Enrichment factor	98	69
Sample volume, mL	25	25
Limit of detection, $ng\,mL^{-1}$ (3s)	0.7	0.3
Limit of quantification, $ng\,mL^{-1}$ (10s)	2.2	1.0
Precision ($10\,ng\,mL^{-1}$, $n=10$) RSD (%)	2.1	1.8
Linear range, $ng\,mL^{-1}$	2.50–200	1.00–200

in Table 2. Fe and Cu recoveries were nearly quantitative in the presence of other ions.

3.4. Analytical features

The calibration graphs were linear in the range of 2.50–200 $ng\,mL^{-1}$ iron and 1.00–200 $ng\,mL^{-1}$ copper under the optimum conditions of the general procedure. The regression equations for iron and copper determination were $A = 4.46 \times 10^{-3}C + 2.76 \times 10^{-3}$ and $A = 3.39 \times 10^{-3}C + 3.86 \times 10^{-3}$, respectively, where A is the absorbance and C is the metal concentration in solution ($ng\,mL^{-1}$). The correlation coefficient of the calibration curve equations was higher than 0.990 for all elements, which indicates that a good linear regression was established between the absorbances and the concentrations. The equation obtained by direct aspiration in FAAS without the preconcentration procedure, the linear equation for iron (500–4000 $ng\,mL^{-1}$) was $A = 3.51 \times 10^{-5}C + 6.96 \times 10^{-4}$ ($R^2 = 1.00$) and for copper (500–4000 $ng\,mL^{-1}$) was $A = 4.88 \times 10^{-5}C + 1.05 \times 10^{-2}$ ($R^2 = 1.00$). The enhancement factor for Fe and Cu calculated as the ratio of slope of calibration curve of the analytes after preconcentration to

that prior preconcentration and found as 98 and 69 for Fe and Cu, respectively.

The precision of the method, calculated as the relative standard deviation of ten independent measurements carried out 10 $ng\,mL^{-1}$ iron and copper. The limit of detection (LOD) is defined as the concentration equivalent to three times the standard deviation (3s) of ten measurements of the blank [33] and is the lowest analyte concentration that produces a response detectable above the noise level of the system. The limit of quantification (LOQ) is the lowest level of analyte that can be accurately and precisely measured. LOQ, defined as ten times the standard deviation (10s) of ten measurements. Table 3 gives the analytical features of the method.

Table 5
Determination of iron and copper ions in some spice samples using proposed methodology ($n=3$).

Sample	Ion	Added ($\mu g\,g^{-1}$)	Found ($\mu g\,g^{-1}$)	Recovery %
Red pepper	Fe	0	366.3 ± 1.4	–
		20	385.7 ± 1.3	99.8
		50	411.1 ± 1.3	98.7
	Cu	0	3.6 ± 0.6	–
		20	23.9 ± 1.1	101.3
		50	52.6 ± 0.7	98.1
Black pepper	Fe	0	298.1 ± 1.1	–
		20	325.9 ± 1.0	102.4
		50	343.3 ± 0.9	98.6
	Cu	0	7.3 ± 0.4	–
		20	27.5 ± 0.9	100.6
		50	56.9 ± 0.9	99.2
Cumin	Fe	0	218.0 ± 1.4	–
		20	229.3 ± 1.5	100.6
		50	258.4 ± 1.7	96.4
	Cu	0	22.5 ± 1.1	–
		20	42.2 ± 1.3	99.3
		50	73.0 ± 1.2	100.7
Cinnamon	Fe	0	584.3 ± 1.6	–
		20	615.5 ± 1.6	101.8
		50	624.9 ± 1.7	98.5
	Cu	0	21.3 ± 1.0	–
		20	43.4 ± 1.1	105.1
		50	70.8 ± 1.4	99.3
Mint	Fe	0	680.8 ± 1.5	–
		20	697.1 ± 1.1	99.5
		50	720 ± 1.6	98.5
	Cu	0	1.2 ± 0.4	–
		20	21.8 ± 0.6	102.8
		50	51.1 ± 1.0	99.8
Thyme	Fe	0	358.2 ± 1.1	–
		20	380.2 ± 1.4	100.5
		50	397.9 ± 1.5	97.4
	Cu	0	6.2 ± 0.7	–
		20	26.4 ± 1.0	100.8
		50	56.1 ± 0.8	99.8

Table 4
Determination of iron and copper ions in certified reference materials using proposed methodology ($n=3$).

Sample	Ion	Certified value	Found value	R.S.D. %	Recovery %
NCS DC 73349	Fe	1070 $\mu g\,kg^{-1}$	1056.2 $\mu g\,kg^{-1}$	1.2	98.7
	Cu	6.6 $\mu g\,kg^{-1}$	6.4 $\mu g\,kg^{-1}$	0.8	97.0
TM 23.2	Fe	12.7 $\mu g\,L^{-1}$	12.4 $\mu g\,L^{-1}$	0.9	97.6
	Cu	9.7 $\mu g\,L^{-1}$	9.2 $\mu g\,L^{-1}$	1.3	94.8

Table 6
Comparison of proposed method with the other CPE coupled with FI-AAS methods.

Analyte	Complexing agent	SPR[a] diluting agent	Sample matrix	Linear range (ng mL^{-1})	LOD (ng mL^{-1})	Sample volume (mL)	RSD %	EF[b]	Ref.
Fe	Ferron	Ethanol	Water, milk	10–250	1.7	20	2.1	75	[38]
Cu	TAN[c]	0.1 mol L^{-1} HNO$_3$	Water	0.27–100	0.27	50	1.6	64.3	[39]
Cu	APDC[d]	1 mol L^{-1} HNO$_3$ in methanol	Water	0.5–30	NA[e]	250	NA	NA	[40]
Fe				10–100	NA	250	NA	NA	
Fe	APDC	Methanolic solution of HNO$_3$	Wine	<350	20	10	2.4	NA	[41]
Cu	APDC-CTAB[f]	0.1 mol L^{-1} HNO$_3$ in methanol	Water	4–40	0.9	10	NA	100	[42]
Cu	DDTP[g]	0.1 mol L^{-1} HNO$_3$ in methanol	Water, serum, hair	5–200	0.94	10	1.5	33	[43]
Cu	Capric acid octyl amine	Distilled water	Water	0–2.0	0.01	100	2.0	NA	[44]
Fe	APDC	Methanolic solution	Water	<100	3.5	10	NA	50	[45]
Fe	NR	0.05 mol L^{-1} H$_2$SO$_4$	Spice	2.50–200	0.7	25	2.1	98	This work
Cu				1.00–200	0.3	25	1.8	69	

[a] Surfactant-rich phase.
[b] Enhancement factor.
[c] 1-(2-Thiazolylazo)-2-naphthol.
[d] Ammonium pyrrolidinedithiocarbamate.
[e] Non-available data.
[f] Cetyl trimethylammonium bromide.
[g] Diethyldithiophosphate.

3.5. Accuracy of the method

In order to validate the proposed method, recovery experiments were carried out by certified reference materials, NCS DC 73349 (the standard bush branches and leaves sample) and TM 23.2 (fortified water) were used for method validation. Results are the average of three replicates. As Table 4 indicates there is a good agreement between the obtained results and the known values. The recoveries are close to 100% and indicate that the proposed system was helpful for the determination of iron and copper in the real samples.

3.6. Determination of iron and copper in real samples

The proposed method was applied to the determination of iron and copper in spice samples (red pepper, black pepper, cumin, cinnamon, mint and thyme). These samples were subjected to preconcentration and metal ions determination using the proposed procedure. The results are given in Table 5. The percentage recovery (R) was calculated by using the equation: $R = \{100(C_m - C_0)/m\}$, where C_m is a value of metal in a spiked sample, C_0 is a value of metal in a sample and m is the amount of metal spiked [37]. The obtained recoveries were reasonable for trace iron and copper analysis in spice matrices, in a range of 96–105%.

3.7. Comparison of proposed method with other CPE coupled with FI-AAS methods

A comparison of the represented method with the other reported FI-CPE methods for the iron and copper extraction and FAAS determination is given in Table 6. Apparently, presented method has low LODs, high-enrichment factors and these characteristics are comparable or even better than most of the other methods in Table 6.

4. Conclusion

In this work, a FI-CPE system coupled to FAAS was developed for the extraction, preconcentration and determination of iron and copper in real samples. Actually, formation of metal–NR complexes, addition of surfactant and heating to cloud point processes were carried out in batch mode. But the cloudy mixture obtained after heating was transferred to cotton packed mini-column for separation of surfactant-rich phase by means of FI system. Some of

the operations in batch procedures were not used in this new system, such as cooling, removal of supernatant and dilution. Removal of these steps decreased the analysis time. In addition, this study offers a simple, inexpensive, and nonpolluting technique for the preconcentration and determination of trace metals. Triton X-114 is of relatively low-cost and toxicity. NR is a very stable and fairly selective complexing reagent. The proposed preconcentration method exhibits good precision, accuracy and sensitivity and allows iron and copper determination in spice samples at μg kg^{-1} levels.

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