



Copper concentration using micelle-mediated extraction in real samples

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Abstract

A simple and sensitive method is described for the pre-concentration by cloud point extraction followed by atomic absorption spectrometric determination of copper ion in real samples. At a solution pH of 5.5, 5, 5-diphenylimidazolidine-2, 4-dione (phenytoin) (DFID) and TritonX-114 were used as hydrophobic ligand and non-ionic surfactant. The adopted concentrations for DFID, Triton X-114 and HNO₃, bath temperature, centrifuge rate and time were optimized. Detection limits (3SDb/m) of 1.7 ng mL⁻¹ along with enrichment factors of 35 for these ions were achieved. The proposed procedure was applied to the analysis of real samples.

Keywords: Cloud point extraction, copper, triton X-114, flame atomic absorption spectrometry

1. Introduction

Copper has received considerable attention owing to its uses in metallurgy and chemical industries. Moreover, it is an essential constituent of about thirty enzymes and glycoproteins and is required for the synthesis of hemoglobin and for some biological processes. Also promotes iron absorption from the gastrointestinal system, is involved in the transport of iron from tissues into plasma, helps to maintain myelin in the nervous system, is important in the formation of bone and brain tissues and is necessary for other many important functions. When levels of Cu exceed certain values, however, defense mechanisms to protect against excess Cu are overcome and toxicity results. For this reason, several methods for the determination of copper ion are available for copper ion determination [1, 2].

The determination of trace copper in biological samples is particularly difficult because of the complex matrix and the usually low concentration of copper, which requires sensitive instrumental techniques and frequently a pre-concentration step [3-6]. Micelles and other organized amphiphilic assemble are increasingly utilized in analytical chemistry especially in separation and pre-concentration procedures. Their unique micro-heterogeneous structures capable of selective interaction with different solute molecules can strongly modify solubility, chemical equilibrium, kinetics and the spectroscopic properties of analytes and reagents [7-12].

Separation and pre-concentration based on cloud point extraction (CPE) are becoming an important and practical application of surfactants in analytical chemistry [13, 14]. CPE has been

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used to separate and pre-concentrate organic compounds as a step prior to their determination by liquid chromatography [15] and capillary electrophoresis [16]. The phase separation phenomenon has been also used for the extraction and pre-concentration of metal ions after the formation of sparingly water-soluble complexes [9, 13]. CPE as a pre-concentration step in conjunction with detection by spectrophotometry, FIA-spectrofluorimetry, FAAS, ICP-AES and HPLC for the determination of various metal ions has been widely studied [16-23]. The aim of this work was to extend the use of cloud point pre-concentration technique to the determination of Copper in different samples for the first time by using 5, 5-diphenylimidazolidine-2, 4-dione (phenytoin) (DFID) as complexing agent and Triton X-114 as surfactant. FAAS was used for the detection. Triton X-114 was chosen as nonionic surfactant because of its wide use as a cloud point surfactant as well as its low price, commercial availability and lower toxicity.

2. Experimental

2.1. Apparatus

The evaluation of copper content was carried out on a Shimadzu 680 A atomic absorption spectrometer with a hollow cathode lamp and a deuterium background corrector, at respective resonance line using an air-acetylene flame. The conditions for FAAS determination of copper is presented in Table 1. Absorbance measurements were carried out with a Perkin-Elmer UV-Vis spectrophotometer Lambda25. A Metrohm 691 pH/Ion meter with a combined glass-calomel electrode was used for adjustment of test solution pH. An MP4 centrifuge (International Equipment Company, USA) was used to accelerate the phase separation.

Table 1

Hollow cathode lamp conditions and flame conditions for FAAS in determination of copper ion

Instrument condition		Flame condition		Measurement condition	
Lamp current (mA)	3.0	Fuel (l/min)	1.8	Signal proc	direct
Slit (nm)	0.5	Oxidant (l/min)	8.0	Pre-spray (sec)	3.0
Wavelength (nm)	324	Burner (cm)	10.0	Integ time (sec)	5.0

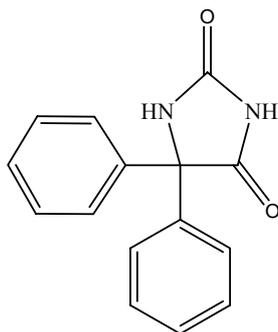
2.2. Reagents

All chemicals used in this work, were of analytical reagent grade and purchased from Merck, (Darmstadt, Germany). They were used without further purification. Deionised water was used for all dilutions. Stock solutions of copper at a concentration of 1000 $\mu\text{g mL}^{-1}$ were obtained from Spectrosol. Working standard solutions were obtained by appropriate dilution of the stock standard solutions. A 1.0 % (w/v) Triton X-114 from Merck, (Darmstadt, Germany) was prepared by dissolving 1.0 gr of Triton X-114 in distilled water in 100 mL volumetric flask with stirring. The 5, 5-diphenylimidazolidine-2, 4-dione (phenytoin) (DFID) was synthesized according to the procedure given in the literature (Scheme 1) [24].

2.3. Spectrophotometric titrations

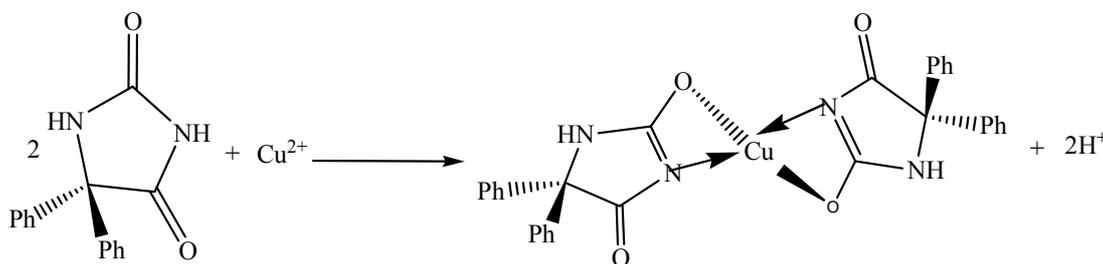
Standard stock solutions of DFID (1.0×10^{-2} M) and the copper ion (1.0×10^{-2} M) were prepared by dissolving appropriate and exactly weighed pure solid compounds in pre-calibrated 25.0 mL volumetric flasks and diluted to the mark with methanol. Working solutions were prepared by appropriate dilution of the stock solutions. According to the spectra reported in Fig.1, titration of the ligand solution with 1×10^{-5} M was carried out by the addition of amounts of a concentrated standard solution of the copper ion in methanol with 1.0×10^{-3} M using a pre-calibrated micropipette, followed by absorbance intensity reading at 25.0 °C at the related λ_{max} .

Since the volume of titrant added during titration was negligible (at the most 0.05 mL) as compared with the initial volume of the ligands (2.6 mL), no volume correction was carried out.



Scheme 1 5, 5-diphenylimidazolidine-2, 4-dione (phenytoin) (DFID)

5, 5-diphenylimidazolidine-2, 4-dione (phenytoin) (DFID) have nitrogen and oxygen as donor atoms, allowing them to act as monodentate oxygen and nitrogen donors. The existence of a donating nitrogen atom as well as $-NH$ group in DFID was expected to increase both the stability and selectivity of its copper complex. The spectrum of DFID in methanol shows absorption band at 267.3 nm. As it can be seen from Fig. 1, a decrease in absorbance is observed upon addition of increasing quantities of copper ions to L Solution, whereas the absorption intensity changes as a function of the $[Cu^{2+}]/[L]$ molar ratio (Fig. 2). These changes could be attributed to the complexation between the ligand DFID and Cu^{2+} ion. From the inflection point in the absorbance/mole ratio plot at $[Cu^{2+}]/[L]$ value 0.5, it can be inferred that 1:2 complexes is formed in methanol solution. When the ligand molecule reacts with the copper ion, it may form 1:2, $Cu(DFID)_2$, complexes (Scheme 2).



Scheme 2 The complexation between the ligand DFID and Cu^{2+} ion

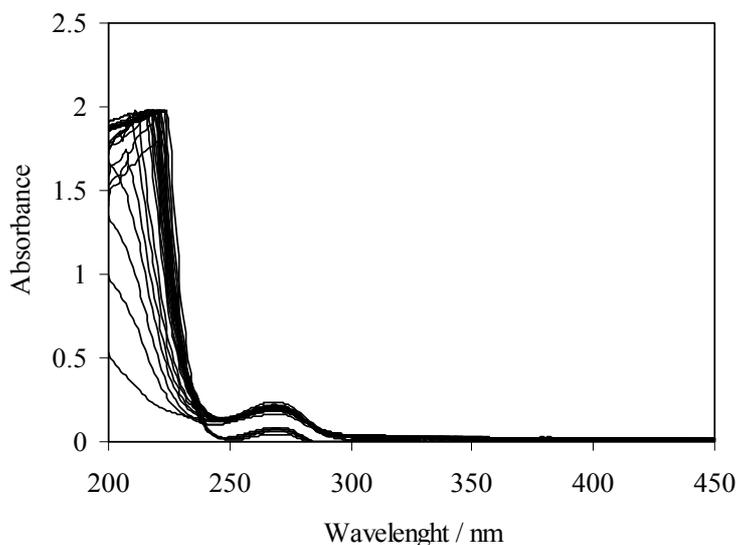


Fig. 1 UV-visible spectra for titration of DFID (1×10^{-5} M) with Cu^{2+} (1×10^{-3} M) in methanol, $\lambda_{max} = 267.3$ nm

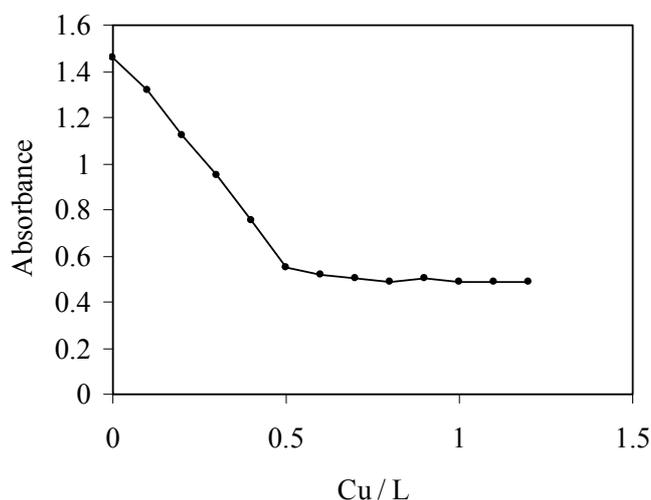


Fig. 2 Molar ratio plot for complexation between the ligand DFID and Cu^{2+} ion

2.4. Procedure for cloud point extraction

The extraction step was achieved by using Triton X-114 as the non-ionic surfactant and 5, 5-diphenylimidazolidine-2, 4-dione (phenytoin) (DFID) as the extractant. In a typical extraction experiment, aliquots of 15 mL of the sample solution containing the analyte ($0.2 \mu\text{g mL}^{-1}$ of Cu), DFID (1.5mM) and Triton X-114 (0.08% w/v) were adjusted to the appropriate pH value (pH = 5.5) with HCl. Subsequently, the sample was kept for 15 min in the thermostatic bath at 40°C . Separation of the two phases was accomplished by centrifugation for 15 min at 4000 rpm. On cooling in an ice-bath, the surfactant-rich phase become viscous and was retained at the bottom of the tubes. In a later step, in order to reduce the viscosity and facilitate sample handling, 500 μL of a solution of methanol containing 0.1mol L^{-1} HNO_3 were added to the surfactant-rich phase. The resultant solution was directly introduced into the FAAS by conventional aspiration.

2.5. Preparation of water samples

Analysis of water samples for determination of copper ions content was performed as following: 400 mL of sample was poured in a beaker and 8 mL concentrated HNO_3 and 3 mL of 30% (v/v) H_2O_2 for elimination and decomposition of organic compound were added. The samples, while stirring was heated to one tenth volume. After adjustment of samples pH to desired value the CPE were performed according to general described procedure. The soil and blood samples were digested as follow, 40 gr (soil sample) or 40 mL (blood sample) or put in a beaker and 20 mL of distilled deionized water, 24 mL HNO_3 and 6 mL of H_2O_2 (30%) added to solution. While stirring heat on a plate until decrease its volume to half, it was filtrate and completed in 200 mL volumetric flask with distilled water. The pre-concentration procedure given above was applied to these solutions [3].

3. Results and Discussion

3.1. Effect of pH

The cloud point extraction of copper ion was performed in solutions of pH ranging from 2 to 9. Extraction recovery depends on the pH at which complex formation occurs. The effects of pH on the extraction of copper complex are given in Fig. 3. In the pH range 5.5–6.0, extraction was quantitative. The decrease in recoveries at $\text{pH} > 6.0$ is probably due to the precipitation of copper ions in the form of hydroxide, and at $\text{pH} < 5.5$ may be due to competition from hydronium ion toward ions for complexation with DFID or decomposition of complex at pH values smaller than

5.5, which led to the decrease in recoveries. In subsequent experiments were performed at pH 5.5.

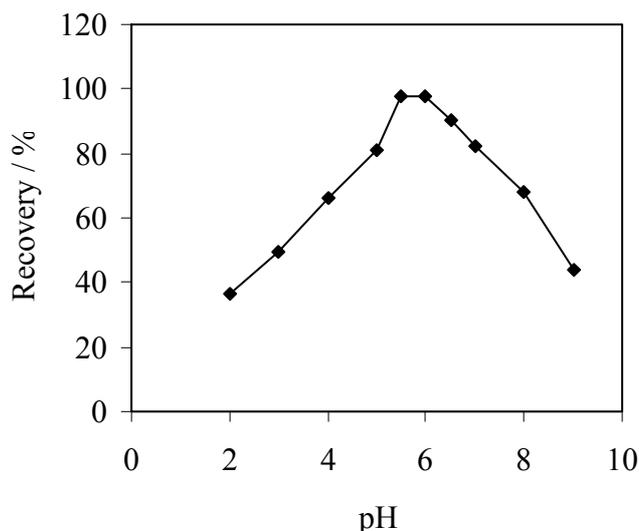


Fig. 3 Effect of pH on the cloud point extraction using DFID reagent of copper conditions: Cu^{2+} : $0.2 \mu\text{g mL}^{-1}$, Triton X-114: 0.08% (w/v), DFID: 1.5 mM

3.2. Effect of Triton X-114 concentration

The pre-concentration efficiency was evaluated using variable Triton X-114 concentrations ranging from 0.03% to 0.1% (w/v). The results are demonstrated in Fig. 4. As it is seen, the highest copper ion recovery was obtained with 0.08% (w/v) Triton X-114. By decreasing the surfactant concentration to 0.03% (w/v) the recovery is reduced. The copper ion recovery also decreased for a Triton X-114 concentration greater than 0.08% (w/v). This result might be related to the presence of the high amount of surfactant, resulting in an increase in the volume of the surfactant-rich phase. In addition, the viscosity of the surfactant-rich phase increases, leading to poor sensitivity [25, 26]. At lower Triton X-114 concentrations (below 0.03% w/v), the pre-concentration efficiency of the complex was very low, probably due to inability for quantitatively entrapment of hydrophobic complex [27].

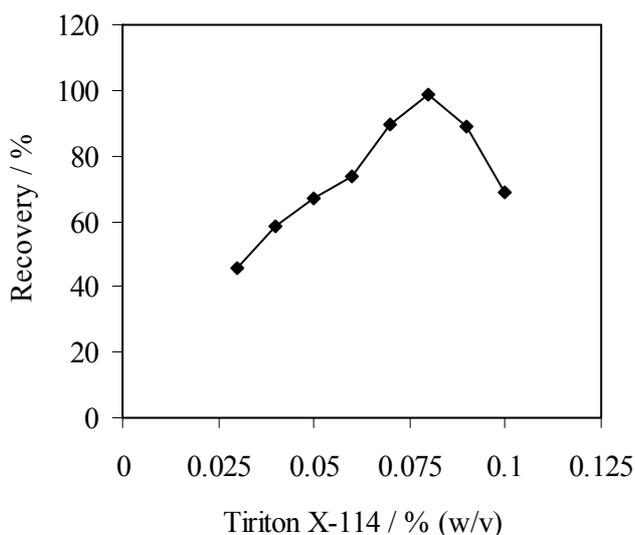


Fig. 4 Effect of Triton X-114 concentration on the cloud point extraction using DFID reagent of copper conditions: Cu^{2+} : $0.2 \mu\text{g mL}^{-1}$, pH: 5.5, DFID: 1.5 mM

The optimum surfactant concentrations are same for all analyses. An amount of 0.08% Triton X-114 was chosen in order to achieve the greatest analytical signal and thereby the highest extraction efficiency.

3.3. Effect of DFID concentration

The DFID was employed as a complexing agent for copper ion CPE. The concentration of MPKO was evaluated over the range 0.5-4.0 mM. There was no significant difference in the results between 1.5-4.0 mM considering the average copper ion recovery. The extraction recovery as a function of the DFID concentration is shown in Fig. 5. For this study, 15 ml of a solution containing $0.2 \mu\text{g ml}^{-1}$ copper ion in 0.08% (w/v) Triton X-114 with various amounts of DFID was subjected to the cloud point pre-concentration process. At this stated concentration of ion, ~100% extraction was achieved for a DFID concentration of 1.5 mM, which this concentration was chosen for subsequent experiments.

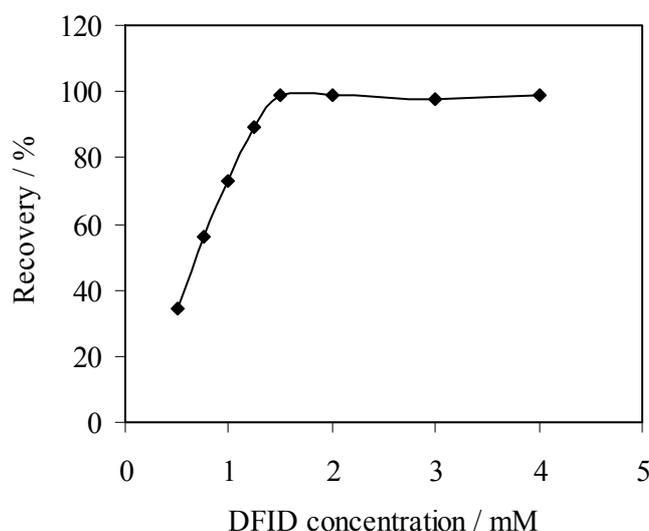


Fig. 5 Effect of DFID concentration on the cloud point extraction using DFID reagent of copper conditions: Cu^{2+} : $0.2 \mu\text{g mL}^{-1}$, pH: 5.5, Triton X-114: 0.08% (w/v)

3.4. Effect of Equilibration temperature and time

The equilibration temperature above the cloud point and equilibration time was thoroughly optimized. It was desirable to employ the shortest equilibration time and the lowest possible equilibration temperature, a compromise between completion of extraction and efficient separation of the phases. Fig. 6 clearly shows that a temperature of 40°C is adequate for the experiment. At lower temperatures separation of the two phases is not complete. The dependence of extraction efficiency on equilibration time was studied for a time interval of 5-25 min. An equilibration time of 15 min was chosen to be optimal to achieve a quantitative extraction.

3.5. Effect of centrifuge time and rates

It is required to pre-concentrate trace amount of copper ion with high efficiency in short time. Therefore, CPE on a set of experiments of 15 mL sample at pH 5.5, 1.5 mM DFID, $0.2 \mu\text{g mL}^{-1}$ copper(II) and 0.1% (w/v) NaCl by heating 40°C and centrifuging in various rate and time further cooling in various time has been carried out. The results indicate the experiment in the optimized reagent concentration after heating for 15 min in 40°C and centrifuging by 15 min in 4000 rpm and cooling in 15 min in ice-bath lead to high recovery of copper ion in short time.

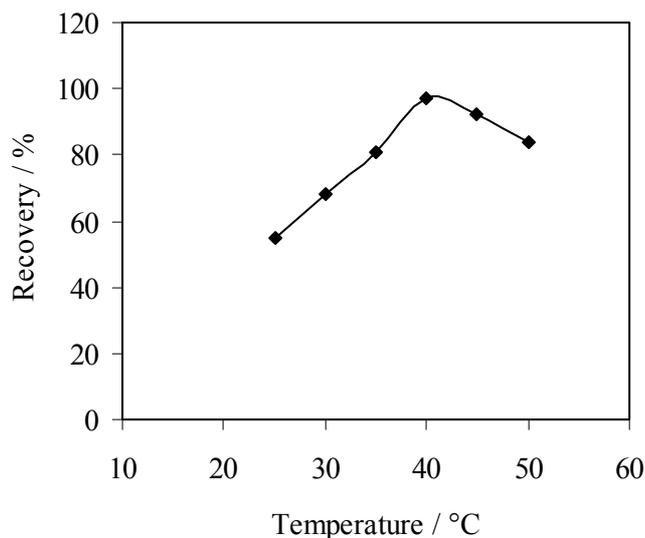


Fig. 6 Effect of equilibration temperature on the cloud point extraction using DFID reagent of copper conditions: Cu^{2+} : $0.2 \mu\text{g mL}^{-1}$, pH: 5.5, Triton X-114: 0.08 % (w/v), DFID: 1.5 mM

3.6. Effect of ionic strength

The addition of an inert salt can facilitate the phase-separation process for some nonionic surfactant systems, since it increases the density of the bulk aqueous phase [28, 29]. When the salt concentration is increased, the micelle size and the aggregation number are increased and the critical micellar concentration remains constant [30]. In addition, non-polar analytes may become less soluble in the solution at higher salt concentrations and thus contribute to higher recoveries. The results obtained indicate that the addition of salt produces an increase in the extraction of the more polar solutes while the recoveries of the less polar compounds are not affected [31-33]. Based on this discussion, NaCl was investigated as electrolyte in the concentration range from 0.1 to 1% (w/v) and the highest copper ion recovery was obtained at 0.1% (w/v) NaCl concentration. The signal decreased considerably for increasing NaCl concentrations (0.3–1% w/v). This effect might be explained by the additional surface charge when the NaCl concentration is very high, thus changing the molecular architecture of the surfactant and consequently the micelle formation process. It is necessary to emphasize that different blank solutions were also evaluated and no significant signal was obtained. In this way, 0.1% (w/v) NaCl concentration was used in all further experiments.

3.7. Effect of methanol

Since the surfactant-rich phase obtained after the cloud point pre-concentration contains a high concentration of Triton X-114 and, at the same time, the volume obtained is rather small (500 μL), methanol containing 0.1 M HNO_3 was added to the surfactant-rich phase after phase separation. Moreover, it was necessary to decrease the viscosity of the rich phase without excessive dilution of the micelle to facilitate the introduction of the sample into the atomizer of the spectrometer. There is an optimum volume (500 μL) with respect to the copper ion recovery. Smaller volumes of methanol were not tested because in this case it was not possible to quantitatively transfer the rich phase from test tubes to the graduated tubes and measuring the absorbance. Larger volumes of acidified methanol dilution are clearly predominated resulting in a gradual absorbance reduction. A 500 μL volume of methanol was therefore used throughout the remaining experiments.

3.8. Interference Studies

The effect of foreign ions on the recovery of copper was tested. Different amounts of common cations were added to the test solution containing $0.2 \mu\text{g mL}^{-1}$ of copper and the developed procedure was applied. An ion was considered as interferent, when it caused a variation in the absorbance of the sample greater than $\pm 5\%$. The tolerance limits of various foreign ions are given in Table 2. These results demonstrate that large excess amounts of common cations and anions do not interfere on the determination of trace quantities of copper.

Table 2

Effects of the matrix ions on the recoveries of the examined metal ions (n=3)

Ions ($\mu\text{g mL}^{-1}$)	Added as	Tolerance limit	Recovery (%)
Cl^- , K^+ , Na^+	KCl, NaCl	1500	99.5
Mg^{2+} , Ca^{2+}	MgCl_2 , CaCl_2	1200	102.9
Zn^{2+} , Cd^{2+} , Co^{2+}	Nitrate salt	600	101.4
Ni^{2+} , Fe^{2+}	Nitrate salt	450	102.1
HCO_3^-	NaHCO_3	550	97.2
Ag^+ , Al^{3+} , Cr^{3+}	Nitrate salt	300	97.9
SCN^-	KSCN	200	98.2
I^-	KI	150	98.1
Fe^{3+} , Hg^{2+}	Nitrate salt	100	100.3
Pb^{2+}	Nitrate Salt	50	100.0

3.9. Characteristics of the method

Calibration graphs were obtained by pre-concentrating 2.0 mL of standard solution in the presence of pH 5.5, 0.08% (w/v) Triton X-114, 1.5 mM DFID with 0.1% (w/v) NaCl, under the experimental conditions specified in the optimized procedure section. The solutions were introduced into the flame by conventional aspiration. The characteristics of the proposed method are shown in Table 3. Table 4 gives the characteristic performance of the proposed method of standard solutions subjected to the entire procedure. Limits of detection and quantification according to IUPAC are also included. The limit of detection and the linear range of the proposed method are comparable to other methods that also employed FAAS.

Table 3

Optimum conditions for the presented CPE Method

Parameter	Optimum value
pH	5.5
DFID concentration (mM)	1.5
Triton X-114 (w/v)	0.08
Eluting agent	500 μL 0.1 M HNO_3 in CH_3OH
Bath temperature ($^\circ\text{C}$)	40
Temperature time (min)	15
Centrifuge time (min)	15
Centrifuge rates (rpm)	4000

3.10. Accuracy and applications

We have explored the feasibility of the methodology using pre-concentration with DFID in surfactant media for the determination of copper ion in different matrices treated according to experimental section. The procedure was applied to the determination of copper ion in different samples, including waste water, river water, tap water, soil and blood sample by standard addition method. Reliability was checked by spiking experiments and independent analysis. The

results are presented in Table 5. The recovery of spiked samples is satisfactory reasonable and was confirmed using addition.

Table 4
Specification of method at optimum conditions for copper

Parameter	Optimum value
Linear range ($\mu\text{g mL}^{-1}$)	0.03-1.1
Detection limit (ng mL^{-1})	1.7
RSD (%)	2.3
Recovery (%)	98.5
Enrichment factor	35

Table 5
Recovery studies of copper in water samples (n=3)

Sample	Added	Found	RSD (%)	Recovery (%)
River water	0	63.5	1.4	-
	100	165.3	0.9	101.8
Tap water	0	59.8	1.6	-
	100	161.2	1.2	101.3
Waste water	0	101.3	1.6	-
	100	202.4	1.2	101.1
Soil	0	43.1	1.2	-
	100	141.0	1.4	97.9
Blood	0	57.3	1.4	-
	100	159.4	1.6	102.1

All values are for 3 measurements vs. $\mu\text{g L}^{-1}$

4. Conclusions

Cloud point extraction is an easy, safe, environmentally friendly, rapid and inexpensive methodology for the pre-concentration and separation of trace metals in aqueous solutions. Triton X-114 is of relatively low-cost and low toxicity. DFID is a very stable and fairly selective complexing reagent. The surfactant-rich phase can be directly introduced into the nebulizer of a flame atomic absorption spectrometer by dilution with acidified methanol. The proposed method can be applied to the determination of trace metals in various water samples and other environmental samples.

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