

Development of a cloud point extraction-spectrofluorimetric method for trace copper(II) determination in water samples and parenteral solutions

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Abstract

A simple and efficient cloud point extraction-spectrofluorimetric method for the determination of copper(II) in different samples has been proposed. The procedure is based on the oxidation of thiamine with copper(II) to form highly fluorescent thiochrome, its extraction to Triton X-114 micelles and spectrofluorimetric determination. The variables affecting the analytical performance were studied and optimized. The calibration graphs using the preconcentration system for copper were linear over the range 1.0–250 ng ml⁻¹ with limit of detection of 0.29 ng ml⁻¹. Relative standard deviation for five replicate determinations of copper at 100 ng ml⁻¹ concentration level was 2.12%. Average recoveries between 94 and 105% were obtained for spiked samples. The method has been applied to water samples and parenteral solutions and the amounts of copper found are very similar to those obtained by a standard method.

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1. Introduction

Copper is an essential element not only for life in mammals but also for plants, and plays an important role in carbohydrate and lipid metabolism. It has many biological effects as an essential element as well as a toxic one [1]. In general, a daily copper intake of 1.5–2 mg is essential and copper at nearly 40 ng ml⁻¹ is required for normal metabolism of many living organisms [1,2]. But, in higher levels is toxic and severe oral intoxication will affect mainly the blood and kidneys. At these levels, copper can bind to the cell membrane and hinder the transport process through the cell wall [1,2]. Because of these and other copper effects on the taste of waters and corrosion, the trace copper content in water and food must be controlled on a daily basis and the European Commission has fixed the limit of 2 µg ml⁻¹ for Cu in drinking water and the allowed limit of copper is set to 1.3 µg ml⁻¹ in the USA similar to that in Canada (1.0 µg ml⁻¹) [3,4].

The determination of copper is usually carried out by flame [5–7] and graphite [8,9] atomic absorption spectrometry (AAS),

as well as spectrophotometry [3,10], chemiluminescence [11] and electrochemical methods [4,12]. However, due to presence of copper in low levels in environmental samples and the matrix effects, different separation and preconcentration techniques such as liquid–liquid [13] or solid phase [1,5,7,14–16], precipitation [17], ion-exchange [18] or flotation [19] are necessary. Disadvantages, such as significant chemical additives, solvent losses, complex equipments, large secondary wastes, prefiltration problems and time consuming, limit the application of these techniques [6].

Other methodologies have been developed with a view to elimination or minimizing the use of organic solvents and simplifying the operating procedures. The use of extraction and preconcentration steps based on phase separation by the cloud point extraction (CPE) methodology offers a convenient alternative to more conventional extraction systems. The principle, advantages and applications of CPE have been well established and identified in recent years and comprehensive reviews of the theory and applications of surfactant-mediated separations in analytical chemistry are available [20–23]. The use of preconcentration steps based on CPE offers a conventional alternative to more traditional extraction systems and permits the design of extraction schemes that are simple, cheap, of high efficiency and of lower toxicity than extractions that use organic solvents. To

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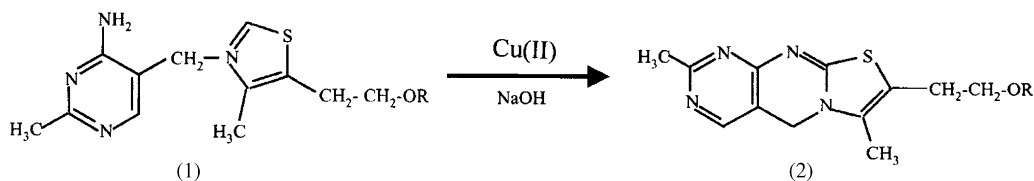


Fig. 1. Scheme of oxidation of thiamine with copper. (1) Thiamine and (2) thiochrome derivative.

date, CPE has been used for the extraction and preconcentration of metal ions after the formation of sparingly water-soluble complexes, and then the complex in the surfactant-rich (SR) phase is determined by different spectrometric methods [24–33].

In the present work the results obtained from applying the CPE-spectrofluorimetric method for the determination of copper is reported. The method is based on chemical oxidation of thiamine to thiochrome by using copper(II), extraction of thiochrome to non-ionic micelles and its determination by spectrofluorimetry. Potential factors affecting the CPE preconcentration and analytical performance are investigated in detail. The proposed method permits analysis and quantification of the copper in different samples with simple spectrofluorimetric method. Thus, time and cost of the analysis can significantly decrease in addition to other well-known advantages of CPE methodologies.

2. Experimental

2.1. Apparatus

All fluorescence measurements were performed using a Shimadzu RF-5301 PC spectrofluorophotometer equipped with a 150 W Xenon lamp and using 1.00 cm quartz cells. Instrument excitation and emission slits both were adjusted to 5 nm. A thermostated bath (636 Friedberg/Hessen, Germany), was used for CPE experiments. A Hettich centrifuge (EBA 20) was used to accelerate the phase separation process. A Corning M120 pH-meter and 25 ml calibrated centrifuge tubes were also used.

2.2. Reagents

The non-ionic surfactant Triton X-114 (Sigma, St. Louis, MO, USA) was used without further purification. Stock standard solution of copper(II) at a concentration of 1000 $\mu\text{g ml}^{-1}$ was prepared from copper(II) sulphate pentahydrate (E. Merck) and working standard solutions were obtained by appropriate dilution of this solution. A solution of thiamine at a concentration of 500 $\mu\text{g ml}^{-1}$ was prepared from thiamine hydrochloride (E. Merck) and was kept in refrigerator (4 °C) for 1 week. Also, a 1.0 mol l⁻¹ NaOH solution was prepared.

All other reagents were of analytical-reagent grade (E. Merck) and all solutions were prepared in double distilled water.

2.3. Recommended procedure for calibration

Aliquots of 25 ml solution containing copper(II) in the range of 1.0–250 ng ml^{-1} , thiamine (15.0 $\mu\text{g ml}^{-1}$), NaOH

(0.1 mol l⁻¹) and Triton X-114 (0.1%, v/v) were kept in a controlled temperature bath for 10 min at 40 °C. Separation of the two phases was achieved by centrifuging for 5 min at 3800 rpm. After cooling in an ice bath for 5 min, the SR phase became viscous, and the supernatant aqueous phase was easily decanted. The SR phase was diluted to 2.5 ml with ethanol–water (1:1) and the fluorescence intensity was measured at 438 nm with the excitation wavelength set at 368 nm.

2.4. Procedure for real samples

Bottled mineral water samples and parenteral solutions were obtained from local sources. Aliquots of 10.0 ml of each sample were subjected to the CPE and spectrofluorimetric determination as described in the previous section.

3. Results and discussions

Copper(II) reacts with thiamine in alkaline medium and forms thiochrome (Fig. 1), which is subsequently trapped in the surfactant micelles and separated from the aqueous phase. The excitation and emission spectra of thiamine as thiochrome in micellar medium are given in Fig. 2, with maximums at 368 ± 3 and 438 ± 3 nm, respectively. CPE conditions were studied and suitably adjusted as follows.

3.1. The concentration of thiamine

The effect of thiamine concentration on the fluorescence intensities was optimized as shown in the Fig. 3. It was found

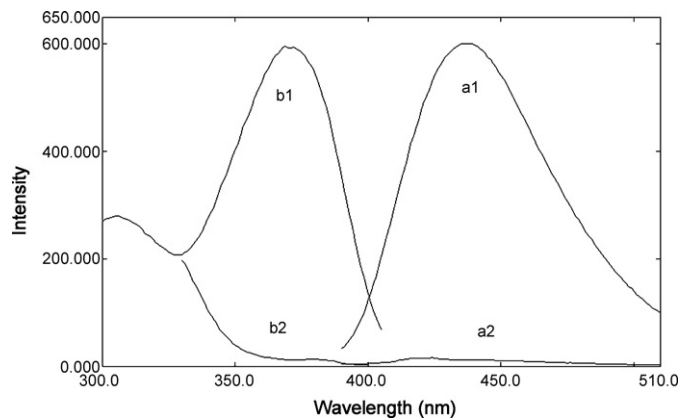


Fig. 2. Emission and excitation spectra—a1 and b1: emission and excitation of thiamine as thiochrome; a2 and b2: emission and excitation of reagents blank; 15.0 $\mu\text{g ml}^{-1}$ thiamine; 200 ng ml^{-1} copper(II); 0.1 mol l⁻¹ OH⁻; 0.1% (v/v) Triton X-114.

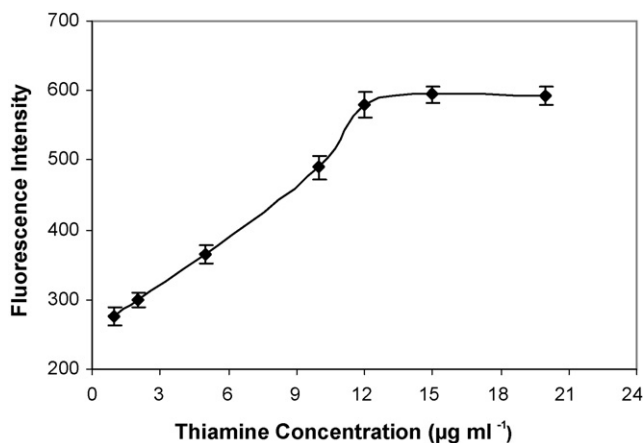


Fig. 3. Effect of thiamine concentration on the spectrofluorimetric responses: 200 ng ml⁻¹ copper(II); 0.1 mol l⁻¹ OH⁻; 0.1% (v/v) Triton X-114.

that concentrations over 12.0 µg ml⁻¹ led to the constant and maximum signals. Hence, a concentration of 15.0 µg ml⁻¹ from thiamine was used for other experiments. At concentrations lower than this range the fluorescence intensity decreases due to insufficient concentration of thiamine for complete oxidation by copper.

3.2. The concentration of NaOH

The Oxidation yield of thiamine depends on the pH and the time taken to reach equilibrium decreases with increasing OH⁻ concentration. The influence of OH⁻ concentration on the fluorescence intensity was studied. As shown in Fig. 4 the fluorescence rapidly increased up to 0.1 mol l⁻¹ from NaOH and then remained constant. Hence, this concentration was chosen for other experiments. A comparison between OH⁻ and different buffer systems on analytical signals was performed. For this purpose, pH of a series of solutions containing known amount of copper and other reagents was adjusted in 12.0 by using OH⁻ or different buffer systems (such as ammonia, phosphate or borate) and solutions were subjected to the CPE and spectrofluorimetric determination as described above. The results showed that higher

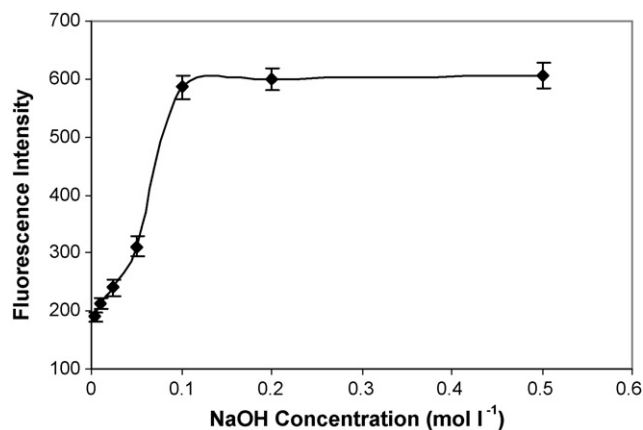


Fig. 4. Effect of OH⁻ concentration on the spectrofluorimetric responses: 200 ng ml⁻¹ copper(II); 15.0 µg ml⁻¹ thiamine; 0.1% (v/v) Triton X-114.

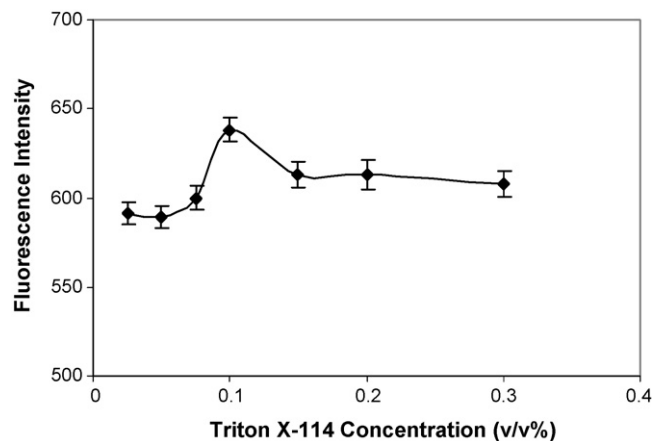


Fig. 5. Effect of Triton X-114 concentration on the spectrofluorimetric responses: 15.0 µg ml⁻¹ thiamine; 200 ng ml⁻¹ copper(II); 0.1 mol l⁻¹ OH⁻.

analytical signals were obtained by using OH⁻. Therefore, the pH adjustment was not performed by using buffer systems and addition of proper amount of OH⁻ (2.5 ml from 1.0 mol l⁻¹ NaOH) is sufficient for adjustment of pH and achievement of higher sensitivity.

3.3. The concentration of Triton X-114

Fig. 5 shows the effect of concentration of Triton X-114 on the analytical signals. As can be seen in this figure, the change of Triton X-114 concentration in the studied range (i.e. 0.025–0.3%) has not significant effect on the fluorescence intensities. Hence, a 0.1% (v/v) surfactant concentration corresponding to the maximum intensity was chosen as the optimum for other experiments.

3.4. Effects of other experimental factors

Optimal incubation time and equilibration temperature are necessary to complete extraction and to achieve easy phase separation. The temperature at which the sample solution is heated was examined in the range 30–60 °C and an optimum of 40 °C was selected. The dependence of extraction efficiency upon equilibration time was studied within a range of 10–60 min. An equilibration time of 10 min was chosen as the best. The effect of the centrifugation time on extraction efficiency was the other parameter that was studied within a range of 5–25 min. A centrifuge time of 5 min at 3800 rpm was selected for the entire procedure. Also, the effect of ethanol–water ratio as diluting agent was studied and the results showed that the ratios above 1:2 ratio (ethanol:H₂O) resulted to higher and constant analytical signals. A 1:1 ratio was used in the rest of work.

3.5. Analytical performance

Calibration graphs were obtained by CPE of 25 ml of standard solutions containing known amount of the copper in the presence of 0.1% (v/v) Triton X-114 and under the experimental conditions specified in the procedure. The SR phase (≈0.2 ml) was diluted to 2.5 ml with ethanol–water (1:1) and the fluo-

Table 1
Tolerance limits of interfering species in the determination of 100 ng ml⁻¹ of copper(II)

Interferent-to-anlyte ratio	Interferent species
1000:1	As ³⁺ , Ni ²⁺ , Sn ²⁺ , Bi ³⁺ , Li ⁺ , Na ⁺ , K ⁺ , Ca ²⁺ , Mg ²⁺ , ClO ₄ ⁻ , CO ₃ ²⁻ , SO ₄ ²⁻ , NO ₃ ⁻ , tartrate and urea
500:1	Zn ²⁺ , Cd ²⁺ , Pb ²⁺ , Mn ²⁺ , Co ²⁺ , Cr ³⁺ , Cl ⁻ , F ⁻ , PO ₄ ³⁻ , glycine and oxalate
100:1	I ⁻ and thiourea
50:1	Citrate
5:1	Fe ³⁺ and Hg ²⁺
1:1	EDTA

Table 2
Determination of copper in some spiked samples

Analyte	Sample	Spiked (ng ml ⁻¹)	Found ^a (ng ml ⁻¹)	Recovery (%)
Cu	Bottled mineral water	20	18.8 ± 0.43	94
		40	39.2 ± 0.85	98
	NaCl physiological solution	20	20.6 ± 0.45	103
		40	42.0 ± 0.91	105

^a Average of three determinations ± standard deviation.

cence was measured. Thus, the preconcentration factor of 10 was achieved. In all cases, linear relationships between the fluorescence intensity and the concentration of the copper in the solution were obtained. The linear concentration range was from 1.0 to 250 ng ml⁻¹ of copper with linear regression equation as follows:

$$I_F = 12.577 C + 9.475, \quad r = 0.9989,$$

where I_F is the fluorescence intensity, C is the concentration of copper as ng ml⁻¹ and r is the regression coefficient.

The relative standard deviation (R.S.D.) obtained for five replicates determination of 100 ng ml⁻¹ of copper was 2.12% and limit of detection (LOD) calculated, as three times the standard deviation of the blank signals was 0.29 ng ml⁻¹. The obtained LOD was sufficiently low as to be valuable for detecting copper in different samples and by considering the preconcentration factor achieved in this work (≈ 10), is comparable with those obtained in other CPE methods used for extraction and preconcentration of copper [27,31–33]. It is obvious that this LOD can be improved by using a larger volume of initial solution or diluting of the SR phase by small volumes of diluting agent.

3.6. Interferences

To investigate the interference effect of different species, aliquots of 25 ml solution containing 100 ng ml⁻¹ of copper and interfering species in different interferent-to-analyte ratios were subjected to the complete procedure. Copper recoveries were almost quantitative in the presence of other species with tolerance limits (error <5%) indicated in Table 1. As can be seen in this table, some metal ions including Fe³⁺ and Hg²⁺ can interfere with the determination of copper. The interference effect of Fe³⁺ can be eliminated by using F⁻ (2×10^{-3} mol l⁻¹) as masking agent. The presence of Hg²⁺ in this concentration levels is not probable in studied samples.

Table 3
Determination of copper in different samples

Sample	Copper ^a (ng ml ⁻¹)	
	Proposed method	Standard method
Tap water	25.3 ± 0.58	24.5 ± 0.43
Mineral water 1	11.2 ± 0.23	11.5 ± 0.18
Mineral water 2	15.2 ± 0.32	15.7 ± 0.26
Mineral water 3	13.5 ± 0.29	13.1 ± 0.21
NaCl physiological solution	8.9 ± 0.18	8.7 ± 0.14
Ringer physiological solution	11.8 ± 0.24	11.4 ± 0.19

^a Average of three determinations ± standard deviation.

3.7. The validation and application of the method

Proposed method was applied to the determination of copper(II) in different samples. Accuracy of the proposed method was proved by spike-recovery test. Aliquots of 10.0 ml of different samples were spiked with two different concentrations of copper at 20 and 40 ng ml⁻¹ and recovery experiments were conducted as well for these samples. The results are summarized in Table 2 and calculated amounts of recoveries varied between 94 and 105%.

For the practical application of this method, a 10.0 ml portion of each water sample or parenteral solution was analyzed for copper with proposed method and standard method [34] and the results are shown in Table 3. A comparison using t -test at 95% confidence interval demonstrates that there is not significant difference among the achieved results using the proposed spectrofluorimetric method and standard method.

4. Conclusions

The proposed method permits analysis and quantification of copper(II) at ng ml⁻¹ levels in different samples with simple and low-cost spectrofluorimetric method. This methodology offers a simple, rapid, sensitive and inexpensive alternative to

other separation/preconcentration techniques with the concomitant benefits from the use of CPE (safety, cost, high separation yield, versatility and non-polluting respect). The proposed CPE method gives low LOD, good R.S.D. and solvent-free extraction of the copper from its initial matrix without previous treatment. The method was verified with real samples and applied satisfactory to the determination of copper in different samples.

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