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Cloud Point Extraction of Copper, Zinc, Iron and Nickel in Biological and Environmental Samples by Flame Atomic Absorption Spectrometry

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Abstract: Cloud point methodology was successfully employed for preconcentration of trace copper, zinc, iron, and nickel prior to their determination by flame atomic absorption spectrometry (FAAS). The metals react with 2-phenyl-1H-benzo[d] imidazole (PHBI) in a surfactant Triton X-114 medium. The effects of analytical parameters including pH, amounts of reagents etc. were investigated on the recoveries of analytes. No influences were observed from the matrix ions. The detection limits ($3SD_b/m$) of 1.8, 2.8, 1.4, and 2.1 ng mL^{-1} for Cu^{2+}, Fe^{3+}, Zn^{2+}, and Ni^{2+}, respectively. The method was applied to metal determination in various real samples. The validation of the procedure was carried out by analysis of a certified reference biological material, BCR 185 R liver samples.

Keywords: 2-phenyl-1H-benzo[d]imidazole, cloud point extraction, preconcentration, triton X-114

INTRODUCTION

Monitoring the presence of toxic trace elements in biological fluids is an extremely important task to evaluate occupational and environmental exposure (1,2). Trace element determinations are misconsidered a difficult analytical task, mostly due to the complexity of the matrix and the low

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concentration of these elements, which requires sensitive instrumental techniques and often a preconcentration step (1–3). The traditional extraction and other conventional separation methods are time-consuming and labor-intensive approaches, besides requiring relatively large amounts of high-purity and frequently toxic solvents, which have to be disposed off properly (4,5). Compared with other extraction methods such as conventional liquid-liquid extraction, cloud point extraction (CPE) exhibits much more environmentally friendly properties, and it is safer because small volumes of noxious surfactants are used instead of toxic organic solvents. Separation and preconcentration based on cloud point extraction (CPE) is becoming an important and practical application of surfactants in analytical chemistry (6,7). Micelles and other organized amphiphilic assemble are increasingly utilized in separation and preconcentration procedures (8–10). 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 (8–13). The small volume of the surfactant-rich phase obtained with this methodology permits the design of extraction schemes that are simple, cheap, highly efficient, speedy, and of lower toxicity to the environment than those extractions that use organic solvents (14–21).

The efficiency of the CPE depends on the hydrophobicity of the ligand and of the complex formed, on the apparent equilibrium constants in the micellar medium and on the formation kinetics of the complex and on the transference between the phases (22). Triton X-114 was chosen as the non-ionic surfactant for the presented work because of its low cloud-point temperature and high density of the surfactant rich phase as well as its low cost, commercially available and lower toxicity.

This work presents an alternative application of cloud point extraction for the precise and accurate determination of trace metals at the low concentration level ($\mu g\ L^{-1}$ or $\mu g\ kg^{-1}$) in environmental samples by FAAS after the pre-concentration step with 2-phenyl-1H-benzo[d]imidazole (PHBI)/Triton X-114 system. This combination is first used in the cloud point extraction studies.

In the present work, a cloud point extraction procedure for the preconcentration-separation of copper, zinc, iron, and nickel ions in environmental samples has been established.

EXPERIMENTAL

Instrumentation

A Shimadzu AA-680 atomic absorption spectrometer equipped with a deuterium background correction and respective hollow-cathode lamp

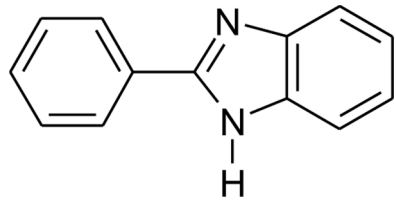
as the radiation source was used for absorbance measurements at resonance wavelength according to instrument instruction. The instrumental parameters were adjusted according to the manufacturer's recommendations. A 30 E 148 Sheme fan or Hettich centrifuge was used to accelerate the phase separation process. A Metrohm 692 pH meter furnished with a combined glass-saturated calomel electrode was used for pH measurements.

Reagents and Materials

All chemicals used in this work were of analytical reagent grade and purchased from Merck. They were used without further purification. De-ionized water was used for all dilutions. A standard solution was prepared by dissolving an appropriate amount of nitrate salt of these ions in deionized water. A 1.0% (w/v) Triton X-114 from E. Merck, Darmstadt, Germany was prepared by dissolving 1.0 mL of Triton X-114 in distilled water in 100 mL volumetric flask with stirring. Methanol solvents were purchased from Merck Company. Cloud points of Triton X-114 in aqueous solution are 24°C (23). The ligand PHBI was synthesized according to literature (Scheme 1) (24).

Procedure

A typical cloud point experiment required the following steps: an aliquot of 15 mL of a solution containing 0.26 $\mu g\,mL^{-1}$ of Cu^{2+}, Ni^{2+}, Fe^{3+} and 10.13 $\mu g\,mL^{-1}$ of $2n^{2+}$ ions, 0.13% Triton X-114 and 0.7 mM of PHBI was adjusted to pH 8.5 with addition of KOH. The mixture was shaken for 1 min and left to stand in a thermo-stated bath at 50°C, for 20 min. Separation of the phases was achieved by centrifugation at 3500 rpm, for 15 min. The whole system was cooled in an ice-bath so for 15 min the surfactant rich phase would regain its viscosity. In this way, the bulk aqueous



Scheme 1. Structure of ligand.

phase was easily decanted. The remaining micellar phase was dissolved in 0.5 mL of 2.0 M HNO_3 in methanol and then the analyte contents were readily evaluated by FAAS.

Application of Real Samples

The real samples were treated according to a previous publication (23,25–28). Triplicate samples from each certified real sample were weighed in glass flasks and 5–10 mL of HNO_3 was added. The flasks were capped and then digested at 60–70°C for 1–2 h until semidried. The digests were treated with 5 mL nitric acid and a few drops of H_2O_2. Then they were heated on a hot plate at approx. 80°C until the color of the digestion solution became bright yellow. The digests were cooled and diluted to 25 mL in volumetric flasks with water. Then the procedure given in the section titled "procedure" was performed.

RESULTS AND DISCUSSION

Effect of pH

Cloud point extraction yield depends on the pH at which complex formation occurs (29–35). pH plays a unique role on metal-chelate formation and subsequent extraction. CPE of copper, nickel, zinc and iron

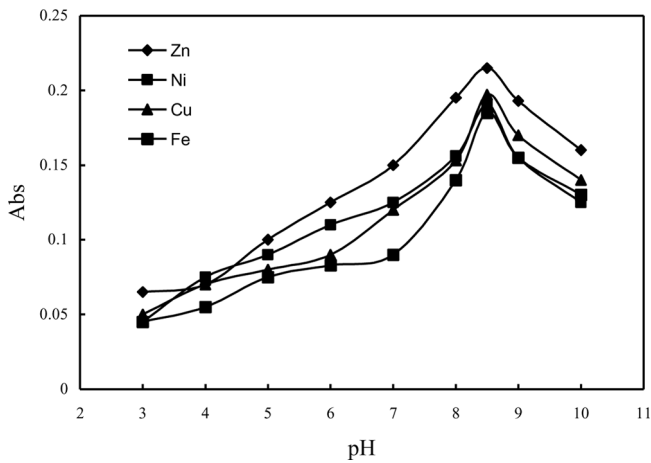


Figure 1. Effect of pH on cloud point extraction of analyte ions (N = 3).

ions was performed in solutions of pH ranging from 3.0 to 10.0. Figure 1 shows the effect of pH on the extraction of the complexes of these ions. It was found that in the alkaline pH range, pH of 8.5, extraction efficiency was quantitative. In subsequent experiments a pH of 8.5 was selected.

Effect of PHBI Concentration

2-phenyl-1H-benzo[d]imidazole (PHBI) as ligand is selected for this work. It was used at first for the cloud point extraction of traces metal ions. The experiments were first performed without 2-phenyl-1H-benzo-[d]imidazole under optimal conditions. The recoveries of the analytes were not quantitative. A set of similar experiment with different concentration of ligands in the range of 0.3–2.7 mM was conducted in order to investigate the effect of the ligand on ions recovery. The extraction recovery as a function of the PHBI concentration is shown in Fig. 2. At 0.7 mM of PHBI concentration, the recovery values for copper, nickel, zinc, and iron ions were found to be quantitative, therefore 0.7 mM of a PHBI concentration was chosen for subsequent experiments. The composition of the complex produced is critical in attaining highest extraction percentages. It has been reported that charged complexes are formed at high concentrations of chelating agents, instead of uncharged complexes, thus decreasing extraction since the latter are preferentially extracted into the hydrophobic core of the micelles (29).

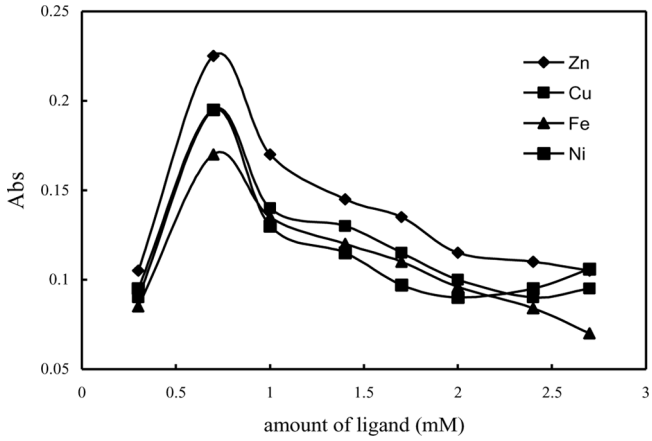


Figure 2. Effect of ligand concentration on the recoveries of analytes (pH = 8.5, N = 3).

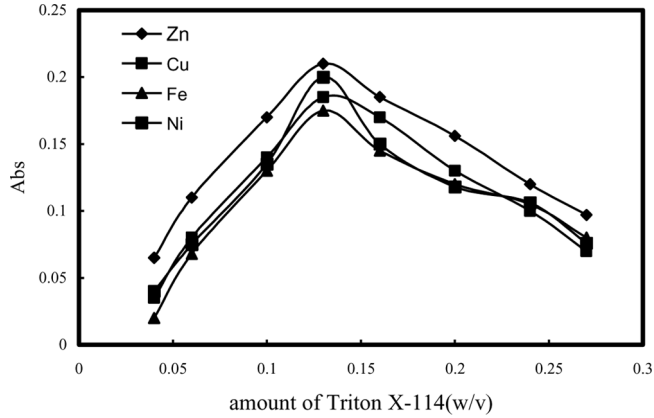


Figure 3. Effect of concentration of Triton X-114 on cloud point extraction of analytes (pH = 8.5, N = 3).

Effect of Triton X-114 Concentration

The non-ionic surfactant Triton X-114 was chosen because of its commercial availability in a high purified homogeneous form, low toxicological properties, and cost. Additionally the cloud point (23–26°C) of Triton X-114 permits its use in the extraction and/or pre-concentration of a large number of molecules and chelate (30,31). The preconcentration efficiency was evaluated using Triton X-114 concentrations ranging from 0.03% to 0.27% (w/v) (Fig. 3). Quantitative recoveries for analytes were obtained with 0.13% (w/v) Triton X-114. By decreasing the surfactant concentration to 0.03% (w/v) the recovery was reduced. At lower Triton X-114 concentrations (below 0.03% w/v), the preconcentration efficiency of the complex was very low, probably due to assemblies that were inadequate to quantitatively entrap the hydrophobic complex (31). The recoveries of copper, nickel, zinc, and iron ions were decreased for a higher Triton X-114 concentration. It may be related to reducing interaction between ligand and metal ions at higher Triton X-114 concentration. Since, 0.13% (w/v) of Triton X-114 showed the highest copper, nickel, zinc, and iron ions recovery; a surfactant concentration of 0.13% (w/v) was selected as a compromise between the results obtained (in terms of sensitivity) and the surfactant concentration.

Effect of NaCl Concentration

The concentration of NaCl as electrolyte was investigated in the range from 0.0 to 0.25 M (Fig. 4). The signal of the analytes were increased

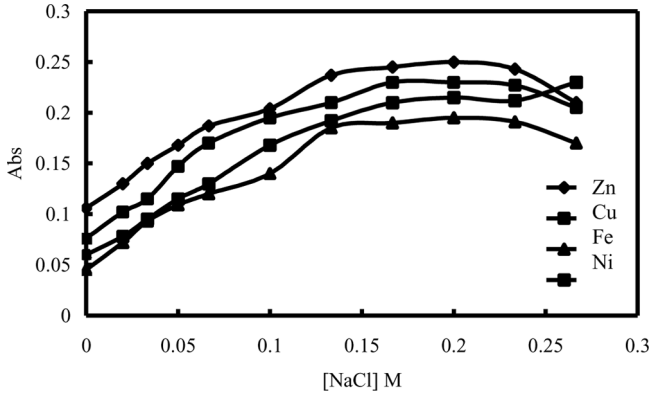


Figure 4. Effect of concentration of NaCl on the recoveries of analyte ions (pH = 8.5, N = 3).

considerably for increasing NaCl concentrations from 0.0–0.25 M. 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, 0.2 M NaCl concentrations were used in all further experiments.

Effect of Methanol Volume

Since the surfactant-rich phase obtained after application of presented procedure, it contains a high concentration of Triton X-114. At the same time, the volume obtained is rather small. Due to these points, 0.5 mL various concentration of HNO_3 in methanol was added to the surfactant-rich phase after phase separation. There is an optimum volume of 0.5 mL of 2.0 M HNO_3 in methanol. 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 for ions. For larger volumes of acidified methanol, dilution was clearly predominated, resulting in a gradual absorbance reduction. A 0.5 mL of 2.0 M HNO_3 in methanol was therefore used throughout the remaining experiments.

Effect of Temperature

The cloud point temperature of Triton X-114 is 23–25 °C which is almost room temperature is the preferred temperature for cloud point extraction of various analytes. It was desirable to employ the shortest incubation time and the lowest possible equilibration temperature, which compromise completion of the reaction and efficient separation of phases. It was observed that temperatures in the range of 45–50 °C are adequate for analyte ions. Higher temperatures lead to the decomposition of PHBI-ions complexes and the reduction of the analytical signal. At lower temperatures the separation of the two phases is not complete.

Effect of Centrifuge Time and Rate

It is required to preconcentrate the trace amount of copper, nickel, zinc, and iron ions with high efficiency in a short time. Therefore, CPE on a set of experiments of 15 mL sample at pH 8.5, 0.7 mmol PHBI, 0.26 $\mu g\,mL^{-1}$ of Cu^{2+}, Ni^{2+}, Fe^{3+} and 10.13 $\mu g\,mL^{-1}$ of $2n^{2+}$ ions, and iron ions and 0.2 M NaCl by heating in 50 °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 20 min in 50 °C and centrifuging by 15 min in 3500 rpm and cooling in 15 min in ice-bath lead to high recovery of copper, nickel, zinc, and iron ions in short time.

Effect of Foreign Ions

The effect of foreign ions on the determination of ions by the proposed method was investigated. The results are presented in Table 1. An ion

Table 1. Effects of the interferences ions on the recoveries of the examined metal ions

Ion	Interference/analyte amount
Ba^{2+}, Mg^{2+}, Ca^{2+}, Ti^{3+}, Na^{+}, K^{+}, Li^{+}, Al^{3+}	1000
Pb^{2+}, Cd^{2+}, Co^{2+}, Hg^{2+}, Ni^{2+}	250
CH_3COO^{-}	900
Ag^{+}	150
Mn^{2+}, $SO_4{}^{2-}$	600
Cl^{-}	400

was considered as an interferent, when it caused a variation in the absorbance of the sample greater than ±5%. The results show that a high limit of various alkaline, alkaline earth, and transition metal ions could be tolerated by the proposed method.

Analytical Features

In order to calculate the experimental preconcentration factors (28,29) which were obtained as the ratio of the slope of the calibration graph with and without preconcentration, the calibration graphs were prepared for iron, nickel, copper, and zinc in the interval of 0.02–0.26 mg L^{-1}. Experimental preconcentration factors were 58, 45, 64, and 38 for Cu^{2+}, Ni^{2+}, Zn^{2+}, and Fe^{3+}, respectively were obtained. The preconcentration factor for all ions was 30.

The limit of detection (LOD) of a method is the lowest analyte concentration that produces a response detectable above the noise level of the system, typically, three times the standard deviation (s) of the blank (n = 10), were found to be detection limits (3SDb/m, SDb: standard deviation of blanks, m: slope of the calibration curve) of 1.8, 2.8, 1.4, and 2.1 ng mL^{-1} for Cu^{2+}, Fe^{3+}, Zn^{2+}, and Ni^{2+}. The limit of quantification (LOQ) is the lowest level of analyte that can be accurately and precisely measured. The limits of quantification, defined as 10 times the standard deviation (s) of the blank (n = 10), were found to be 15 μg L^{-1} for Ni^{2+}(II), 5 μg L^{-1} for Cu^{2+} and Zn^{2+}, and 15 μg L^{-1} for Fe^{3+}. The precision of the proposed method was evaluated by ten successive CPE and elution with 0.26 μg mL^{-1} 1 of Cu^{2+}, Ni^{2+}, Fe^{3+} and 10.13 μg mL^{-1} of $2n^{2+}$ ions in 15 mL of sample solutions. The relative standard deviations (RSD) were 3.6%, 2.0%, 2.7%, 2.1% for iron, copper, nickel, and zinc, respectively.

Accuracy of the Method

In order to evaluate the accuracy of the proposed procedure, bovine liver (BCR 185 R) certified reference material was analyzed. The obtained results are given in Table 2. In Table 2 the values given represent the average and standard deviation of the three determinations of each sample. There is no significant difference between the achieved results by the proposed method and certified values. The recoveries of ions were in the acceptable range.

The reliability of the presented method was checked by spiking experiments and independent analysis. The results for this study are presented in Tables 3–5 for apple fruit, lotus tree, blood, brewed fennel milk, and orange juice samples. The recovery of spiked samples is

Table 2. Analysis result of bovine liver (BCR 185 R) certified reference material by the presented method

Ion	Certified value, mg/kg	Our value, mg/kg	RSD %	Recovery %
Cu	277	283.4	0.9	102.3
Zn	138.6	143.6	1.0	103.6

Table 3. Levels of Analyte ions in blood and lotus samples (N = 3)

		Blood			Lotus (tree)		
Ion	Added ($\mu g\ g^{-1}$)	Found ($\mu g\ g^{-1}$)	RSD %	Recovery %	Found ($\mu g\ g^{-1}$)	RSD %	Recovery %
Fe	0.0	0.490	1.5	—	0.261	1.4	—
	0.2	0.693	1.2	101.5	0.468	1.0	103.5
Cu	0.0	0.162	1.3	—	0.260	1.4	—
	0.2	0.368	1.1	103.0	0.467	1.2	103.5
Zn	0.0	0.149	1.3	—	0.145	1.4	—
	0.2	0.356	1.0	103.5	0.351	1.1	103.0
Ni	0.0	0.097	1.4	—	0.128	1.3	—
	0.2	0.305	1.1	104.0	0.334	1.2	103.0

Table 4. Recovery of trace elements from milk and orange juice samples after application of presented procedure

		Milk sample			Orange juice		
Ion	Added ($\mu g\ mL^{-1}$)	Found ($\mu g\ mL^{-1}$)	RSD %	Recovery %	Found ($\mu g\ mL^{-1}$)	RSD %	Recovery %
Fe	0.0	0.428	1.3	—	0.692	0.9	—
	0.2	0.634	1.0	103.0	0.895	0.8	101.5
Cu	0.0	0.254	1.2	—	0.162	1.1	—
	0.2	0.461	1.0	103.5	0.370	0.9	104.0
Zn	0.0	0.163	1.3	—	0.302	1.1	—
	0.2	0.368	1.1	102.5	0.508	1.0	103.0
Ni	0.0	0.089	1.5	—	0.032	1.4	—
	0.2	0.295	1.0	103.0	0.240	1.0	104.0

Table 5. Recovery of trace elements from Apple fruit and Brewed fennel samples after application of presented procedure

		Apple fruit			Brewed fennel		
Ion	Added ($\mu g\ mL^{-1}$)	Found ($\mu g\ g^{-1}$)	RSD %	Recovery %	Found ($\mu g\ g^{-1}$)	RSD %	Recovery %
Fe	0	87.9	1.1	—	8.8	1.4	—
	20.0	108.9	1.1	105.0	29.2	1.1	102.0
Cu	0	10.8	1.4	—	5.1	1.6	—
	20.0	31.4	1.1	103.0	25.8	1.2	103.5
Ni	0	1.6	1.4	—	1.6	1.6	—
	20.0	22.0	1.1	102.0	21.9	1.3	101.5
Zn	0	16.3	1.7	—	9.4	1.7	—
	20.0	37.0	1.4	103.5	29.9	1.3	102.5

Added value for apple are $\mu g\ mL^{-1}$ and for fennel brewed is ($mg\ g^{-1}$).

satisfactorily reasonable and was confirmed using addition method, which indicates the capability of the system in the determination of ions. A good agreement was obtained between the added and measured analyte amounts. The recovery values calculated for the added standards were always higher than 95%, thus confirming the accuracy of the procedure and its independence from the matrix effects.

CONCLUSIONS

In this work, the use of micellar systems as a separation and preconcentration for copper, nickel, zinc, and iron offers several advantages including low cost, safety, preconcentration copper, nickel, zinc, and iron with high recoveries and very good extraction efficiency. The method presented could be compared with the other preconcentration and separation methods including cloud point extraction and solid phase extraction for these properties (36–40). The surfactant-rich phase can be easily introduced into the flame after dilution with 2.0 M HNO_3 and directly determined by AAS. The proposed method can be applied to the determination of trace amounts of copper, nickel, zinc, and iron in various samples.

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