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Solid Phase Extraction and Simultaneous Spectrophotometric Determination of Trace Amounts of Copper and Iron Using Mixture of Ligands

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Abstract. A novel sensitive and simple method for rapid and selective extraction, preconcentration and determination of iron (as its bathophenanthroline complex) and copper (as its neocuproine complex) using octadecyl silica cartridges and dual wavelength spectrophotometry is presented. The dual wavelength method (533 nm for the iron-bathophenanthroline and 454 nm for the copper-neocuproine as the analytical wavelength) is used to eliminate spectral interferences. Extraction efficiency and the influence of flow rates of sample solution and eluent, pH, amount of neocuproine, bathophenanthroline and hydroxylamine hydrochloride, type and least amount of eluent for elution of iron and copper complexes from cartridge, break-through volume and limit of detection are evaluated. The effects of various cationic and anionic interferences on percent recovery of iron and copper are also studied. Extraction efficiencies >95% are obtained by elution of cartridges with minimal amount of organic solvent. Iron and copper were determined in the range of 3–100 ng mL^{-1}. The limits of detection are 0.98 and 1.13 ng mL^{-1} for iron and copper, respectively. The proposed method is applied successfully to the determination of both analytes in river, tap and well water samples.

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Key words: Copper(I); iron(II); neocuproine; bathophenanthroline; octadecyl silica cartridge; solid phase extraction; dual wavelength method.

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 [1]. 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 [1]. Copper is also very important in many biological systems [2]. In medical diagnosis and biochemical research, iron and copper contents in samples such as urine, serum, liver tissue, etc., can be of considerable significance. They also play an important role in industrial and pollution studies. Iron and copper are included in the quality control of industrial and commercial products such as petroleum, alloys, foods, beverages etc [3]. Thus, the determination of trace amounts of these analytes is becoming increasingly important, especially with respect to environmental pollution. Several spectrophotometric methods have been reported for their individual determination [4, 5]. However, relatively few chromogenic reagents are suitable for the simultaneous determination of these elements. In simultaneous determinations, the sensitivities are usually different for each analyte [6, 7]. The spectrophotometric resolution of mixtures of copper and iron has been traditionally performed by solving

a set of two equations at two wavelengths [8, 9] or by sequential procedures [6, 10, 11]. This is suitable for calculating the concentration of each species in the sample. Other classic spectrophotometric approaches are based on the selective distribution of copper and iron complexes between immiscible phases and on the subsequent absorbance measurements in both phases [12]. Derivative spectrophotometry also permits the resolution and determination of binary mixtures of constituents [3].

Solid phase extraction (SPE) is an attractive technique since it reduces solvent usage and exposure, disposal costs, and extraction time for sample preparations [13]. This paper reports a method for solid phase extraction and simultaneous spectrophotometric determination of copper and iron, using a specific chromogenic reagent for each analyte (bathophenanthroline for iron and neocuproine for copper). Acetate is used as a counter ion in order to increase the sensitivity by ternary complex formation. In this application, water volumes of up to 500 mL are pulled through a cartridge, while the volume for the elution is less than 10 mL. Thus, enrichment factors of up to 50 are attained. In this method, spectral overlapping occurred. Therefore, the dual wavelength method is used to eliminate spectral interferences [14, 15].

Experimental

Chemicals and Reagents

Extra pure grade methanol, ethanol, chloroform, isopentyl alcohol, propanol, aceton, hydrochloric acid (all from Merck) were used without any further purifications. All other reagents used were of analytical grade. Doubly distilled deionized water was used throughout. The standard stock solutions of copper and iron were prepared by dissolving an appropriate amount of $CuSO_4 \cdot 5H_2O$ and $Fe_2(SO_4)_3 \cdot 5H_2O$ in deionized water. Working solutions were prepared by appropriate dilution of the stock solutions.

Apparatus

All absorbance measurements were made with a Shimadzu UV-Vis 2100 spectrophotometer. A model 642 digital Metrohm pH meter equipped with a combined glass-calomel electrode was used for pH adjustments.

Sample Extractions

Extractions were performed with Sep-Pak C18 cartridges containing 500 mg octadecyl silica (50 μm particle size, 60 Å pore size) from Waters Associates Co., MA, USA. The cartridges were used in conjunction with a vacuum apparatus. For preparing standard solutions, 40 mL doubly distilled water, 0.5 mL HCl, 1 g sodium acetate, 0.2 g hydroxylamine hydrochloride (for the reduction of Cu(II) and Fe(III) to Cu(I) and Fe(II), respectively) were added to different microliter volumes of Fe(III) and Cu(II) and the solutions were heated to boiling. The solution was cooled to room temperature, and 1 mL methanol solution containing 0.8 mg of bathophenanthroline and 1.2 mg neocuproine were added. The solution was transferred to a 50 mL volumetric flask and diluted to the mark with water. Before extraction, each cartridge was washed with 5 mL of eluting solvent by applying a slight vacuum. After the cartridge was dried completely by having air drawn through it for a few minutes, 5 mL methanol was drawn through the cartridge. A thin layer of methanol remained on the cartridge, and it was washed with 10 mL deionized water. This step protects the cartridge from water before the extraction of the complexes. It is important to note that the surface of the cartridge does not dry between the time when methanol is added and extraction is complete. Then, the sample solution containing iron–bathophenanthroline, copper–neocuproine complexes and the buffer was passed through the cartridge. After the sample had been passed through the cartridge, a few minutes of full vacuum was allowed to draw the excess water from the media. After the solid phase extraction, the complexes were stripped from the cartridge, using 8 mL of proper eluent. The concentration of iron and copper complexes are determined spectrophotometrically at 533 and 454 nm, respectively, by using the dual wavelength method.

For determination of iron and copper ions in real water samples, a 1000 mL aliquot of the water samples was first passed through a 0.45 μm pore (millipore) cellulose acetate membrane filter to remove any particles in the sample solution. After each filtration, the residue on the filter was washed with 10 mL deionized water and added to the filtered water sample. 50 mL aliquots of the water sample were then analyzed for copper and iron as described.

Results and Discussion

Some preliminary experiments were carried out in order to investigate quantitative retention of Cu(II) and Fe(III) ions by the octadecyl silica cartridges in the absence and presence of neocuproine and bathophenanthroline. It was found that while the cartridge doesn't show any tendency for retention of copper and iron ions, it is capable of retaining copper–neocuproine and iron–bathophenanthroline complexes in the same solutions quantitatively (the test solution used contained $0.3\,\mu g\,mL^{-1}$ copper, $0.6\,\mu g\,mL^{-1}$ iron, $3.84 \times 10^{-5}\,mmol\,mL^{-1}$ neocuproine and $4.8 \times 10^{-5}\,mmol\,mL^{-1}$ bathophenanthroline in 50 mL water).

Spectral Features

Cu(II) in the presence of hydroxylamine hydrochloride solution (pH 5.0–6.0) and acetate ion forms a hydrophobic complex with neocuproine that is extractable into octadecyl silica media. The complex is eluted from octadecyl silica with a proper organic solvent. The absorption spectra of the extract of the neocuproine–Cu(I)–acetate complex exhibit one band centered at 454 nm over a wavelength range of 370–560 nm (Fig. 1). Under the experimental conditions described above, Fe(II) can be extracted as bathophenanthroline–Fe(II)–acetate ion pair into octadecyl silica phase. Under these conditions the complexation and extraction

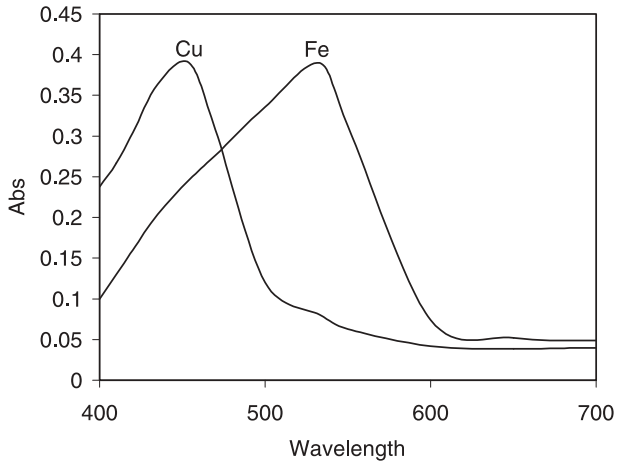

Fig. 1. Absorption spectra of neocuproine–Cu(I)–acetate ($C = 1.5\,\mu g\,mL^{-1}$, $\acute{\varepsilon}_{454} = 1.6 \times 10^4$, $\varepsilon_{533} = 3.4 \times 10^3$) and bathophenanthroline–Fe(II)–acetate ($C = 2.3\,\mu g\,mL^{-1}$, $\varepsilon_{533} = 9.4 \times 10^3$, $\acute{\varepsilon}_{454} = 5.9 \times 10^3$), complexes (eluent = methanol containing $0.163\,mmol\,mL^{-1}$ $NaClO_4$)

reactions occurred. The absorption spectra of the extract of this complex shows one band centered at 533 nm over a wavelength range of 380–620 nm (Fig. 1). Extraction of Cu(I) and Fe(II) in the presence of mixed ligands shows that the conditional formation constants of the bathophenanthroline–Fe(II)–acetate and neocuproine–Cu(II)–acetate complexes are considerably higher than those of the neocuproine–Fe(II)–acetate and the bathophenanthroline–Cu(I)–acetate complexes. When both iron and copper are present in a sample, and then acetate, neocuproine and bathophenanthroline are added to it, both analytes are quantitatively extracted in the octadecyl silica phase. Both complexes are eluted from the cartridge with a proper organic eluent. This extraction and elution process permits both analytes to be separated from the sample solution and to be preconcentrated. The absorption spectra of both analytes show that iron and copper can be determined using the dual wavelength method at 533 nm and 454 nm (i.e. $A_{533} = \varepsilon_{Fe}C_{Fe}b + \varepsilon_{Cu}C_{Cu}b$ and $A_{454} = \acute{\varepsilon}_{Fe}C_{Fe}b + \acute{\varepsilon}_{Cu}C_{cu}b$ where ε_{Fe}, ε_{Cu}, $\acute{\varepsilon}_{Fe}$ and $\acute{\varepsilon}_{Cu}$ represent molar absorptivity of bathophenanthroline–Fe(II)–acetate, neocuproine–Cu(I)–acetate at $\lambda = 533$ and bathophenanthroline–Fe(II)–acetate, neocuproine–Cu(I)–acetate at $\lambda = 454$ respectively and $b = 1$ cm is the light pass length).

Choice of Eluent

10 mL of various organic solvents (methanol, ethanol, propanol, chloroform, isopentyl alcohol, acetone, as well as methanol, ethanol, propanol, acetone containing $0.163\,mmol\,mL^{-1}$ of $NaClO_4$) were tested in order to choose a proper eluent for retained copper and iron complexes after their extraction from water. Absorbance of each solution is obtained against a blank at $\lambda = 533$ and 454 nm. Results show that a 10 mL solution of $0.163\,mmol\,mL^{-1}$ of $NaClO_4$ in methanol is a suitable eluent and that pure solvents wash only a part of the complex from the cartridge. Further experiments showed that quantitative elution of complex cannot be performed by increasing the volume of pure solvents. However, in the presence of small amounts of $NaClO_4$, quantitative elution of complexes does occur [5]. Our studies revealed that 8 mL of $0.153\,mmol\,mL^{-1}$ $NaClO_4$ in methanol are appropriate.

Effect of Amounts of Reducing Agent and Ligands

In the presence of hydroxylamine, Cu(II) and Fe(III) are reduced to Cu(I) and Fe(II). Reduced ions (Cu(I) and Fe(II)) form very stable complexes with neocuproine and bathophenanthroline, respectively. Figure 2 shows that the absorbance of copper and iron complexes increases when increasing the amount of reducing agent up to 0.2 g. Thus, 0.2 g of reducing agent was used in subsequent experiments.

In order to investigate the optimum concentrations of neocuproine and bathophenanthroline in sample solution for the quantitative solid-phase extraction of copper and iron, extraction was conducted by simultaneously increasing the concentration of ligands from 0.0 to $1.44 \times 10^{-4}\,mmol\,mL^{-1}$ ($V_{sample} = 50$ mL). The extraction of copper and iron was found to be quantitative using $>1.06 \times 10^{-4}\,mmol\,mL^{-1}$

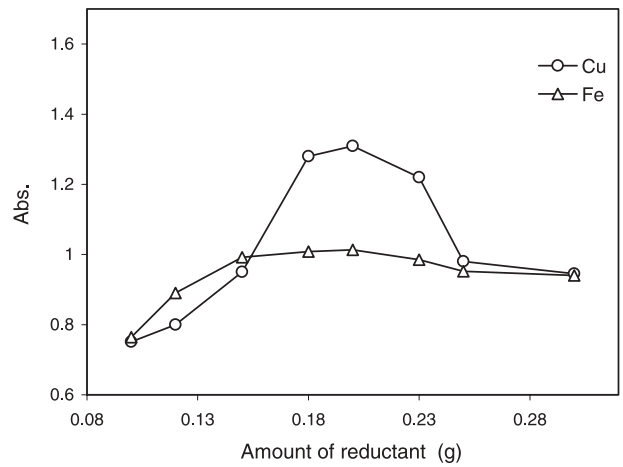

Fig. 2. Effect of the amount of hydroxylamine hydrochloride on the extraction efficiency of copper and iron complexes

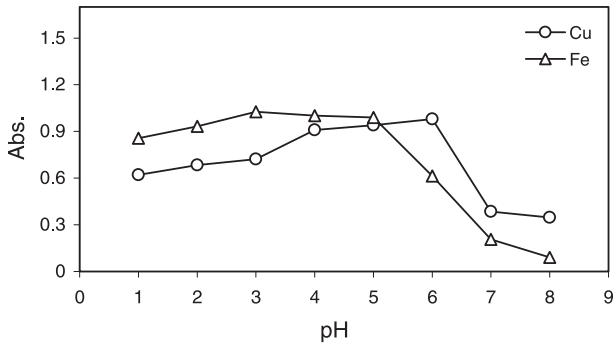

Fig. 3. Effect of pH of sample solution on extraction efficiency of copper and iron complexes

of neocuproine and $>4.21\times10^{-5}$ mmol mL^{-1} of bathophenanthroline, respectively. Hence, subsequent extraction experiments were carried out with 1.15×10^{-4} M of neocuproine and 4.82×10^{-5} M of bathophenanthroline.

Effect of pH and Flow Rates

The influence of pH on the extraction of copper and iron complexes was studied in the range of 1–8, using sodium acetate, HCl, and KOH for pH adjustment. Figure 3 shows that in the pH range of 4–6 for copper and 3–5 for iron, the percent recoveries are relatively constant. At pH values lower than 3, however, the percent recoveries decrease. This is due to the competition of H^+ ions with Cu(I) and Fe(II) ions for reaction with neocuproine and/or bathophenanthroline. At pH values higher than 5 for Fe and 6 for Cu, the percent recoveries also decrease. Higher pH values (>8) were not tested because of the hydrolysis of octadecyl silica in the cartridge. In this study, a buffer solution of pH 5 was used. The effect of sample flow rates and stripping solutions from the cartridge on the retention and recovery of copper and iron ions were investigated. It was found that, in the range of 1–20 mL min^{-1}, the retention of copper and iron ions by the cartridge is not significantly affected by the flow rates of the sample solution. However, quantitative elution of copper and iron complexes from the cartridge is achieved by flow rates in the range of 1–4 mL min^{-1}. Similar results were also reported for organic pollutants [15–17] and cations [18, 19].

Analytical Performance

Solutions of 100 mL (0.15 μg mL^{-1} Cu, 0.30 μg mL^{-1} Fe), 150 mL (0.1 μg mL^{-1} Cu, 0.20 μg mL^{-1} Fe), 250 mL (0.06 μg mL^{-1} Cu, 0.12 μg mL^{-1} Fe), 500 mL (0.03 μg mL^{-1} Cu, 0.06 μg mL^{-1} Fe), 750 mL (0.02 μg mL^{-1} Cu, 0.04 μg mL^{-1} Fe) and 1000 mL (0.015 μg mL^{-1} Cu, 0.030 μg mL^{-1} Fe) were passed through the cartridge using the recommended procedure. It was found the Cu(I) (0.03 μg mL^{-1}) and Fe(II) (0.06 μg mL^{-1}) complexes are quantitatively retained from 500 mL and smaller volumes. Thus, the breakthrough volume for the method is 500 mL. The limit of detection (LOD) of the proposed method for copper and iron determination was evaluated under optimum experimental conditions. The LOD obtained from $C_{LOD}=K_bS_bm^{-1}$ [20, 21] (where K_b is a numerical factor of 3, S_b is the standard deviation of ten replicate blank measurements and m is the slope of the calibration graph) is 1.13 and 0.98 ng mL^{-1} for copper and iron, respectively (for 500 mL of sample solution). The dynamic linear range of the method for both ions is 3–100 ng mL^{-1} (for 500 mL of sample solution). The calibration curve equations for iron and copper are $A=7\times10^{-4}+0.011\,C$ (ng mL^{-1}), ($r^2=0.999$) and $A=4.3\times10^{-4}+0.016\,C$ (ng mL^{-1}), ($r^2=0.999$), respectively. The influence of several cations and anions on the solid phase extraction and determination of copper and iron (0.3 μg mL^{-1} and 0.6 μg mL^{-1} of copper and iron respectively in 50 mL of solution) was studied. A relative error of three times the standard deviation of measurements (i.e. 5% concentration) was considered tolerable. The results are summarized in Table 1. Only some of the ions examined, such as Cd^{2+}, Hg^{2+}, Co^{2+}, Ni^{2+}, and VO_3^-, interfere with the determination of copper and iron. Using excess amounts of NaCN solution eliminates the interfering effect of Cd^{2+}, while the interfering effect of Hg^{2+} ions is eliminated by using excess amounts of KI solution. The interferences of Co^{2+}, Ni^{2+} and VO_3^- ions are eliminated by using excess amounts of neocuproine and bathophenanthroline (2:1 ligand to interfering ion). In the presence of excess interfering

Table 1. Tolerance limits of diverse ions for the recovery of 30 μg Fe and 15 μg Cu from 50 mL solutions by the cartridge

Foreign ion	Tolerated ratio of foreign ion (mg ion/mg iron)
Li^+, Na^+, K^+ Mg^{2+}, Ca^{2+}, Sr^{2+}, Ba^{2+} Pb^{2+}, Cr^{3+}, Ce^{3+}	500*
Mn^{2+}, Tl^+	300
Zn^{2+}, MoO_4^{2-}	20
Hg^{2+}, Cd^{2+}	10
VO_3^-	5
Co^{2+}, Ni^{2+}	2

* Highest ratio tested.

Table 2. Determination of iron and copper in real water samples

Sample	Add (ng mL^{-1})		Found (ng mL^{-1})		%Recovery	
	Fe	Cu	Fe	Cu	Fe	Cu
Distilled water	0.0	0.0	0.0	0.0		
	60.0	60.0	59.3	59.5	98.8 ± 1.5*	99.2 ± 1.1
Tap water	0.0	0.0	31.5	8.9		
	60.0	60.0	92.0	65.0	100.5 ± 1.8	94.4 ± 2.0
Pit water	0.0	0.0	3.2	3.6		
	60.0	60.0	65.5	61.2	103.6 ± 2.1	97.2 ± 1.6
Spring water	0.0	0.0	7.8	3.3		
	60.0	60.0	68.5	64.0	101.0 ± 1.4	101.1 ± 3.2

* Standard deviation based on four replicate analyses.

ions, a significant part of the ligand is expected to be consumed by the complexation of these ions so that it is not available for the formation of copper and iron complexes. In order to assess the applicability of this method to real samples, measures were taken to extract and separate Cu(II) and Fe(III) from 50 mL of four different water samples (i.e. distilled water, tap water, spring water and well water). Table 2 shows data from the extraction of 0.06 μg mL^{-1} of copper and 0.06 μg mL^{-1} of added iron ions from 50 mL of different water samples, demonstrating that copper and iron recoveries are almost quantitative.

Conclusions

The proposed method of solid phase extraction and simultaneous spectrophotometric determination of trace amounts of copper and iron using a mixture of ligands has the following advantages: this method is relatively rapid in comparison to the previously reported procedures for separation and determination of copper and iron [22, 23]. The total analysis time for determining copper and iron in a 500 mL water sample is at the most 15 min. It can separate copper and iron ions from many other associated metal ions, even at a high concentration of the latter. The consumption of organic solvents in the proposed method is much lower compared to that in common liquid–liquid extraction methods. In this method the spectral resolution is high, and it is not required to apply derivative spectrophotometry or multivariate calibration methods. The method has been successfully applied to the separation and determination of copper and iron in real samples. It is interesting to note that by combining the SPE procedure with sensitive determination methods such as ICP-OES, the detection limits of the method can be lowered by a factor of about 50.

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