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Combination of Directly Suspended Droplet Microextraction and Flame Atomic Absorption Spectrometry for Determination of Trace Amounts of Iron and Copper

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Um procedimento de extração em fase líquida miniaturizado, baseado na microextração direta em gota suspensa combinada com espectrometria de absorção atômica com chama foi desenvolvido para a determinação de traços de ferro e cobre, sem a necessidade da etapa de centrifugação para a separação de fases. O método consiste na extração de complexos de ferro e cobre com 2-mercaptopiridina-*N*-óxido em uma microgota de metil-isobutil-cetona (MIBK) como solvente extrator. Os fatores que afetam a extração foram otimizados. Nas condições otimizadas um fator de enriquecimento de ~25 foi obtido para ambos, ferro e cobre, a partir de 6,5 mL de fase aquosa. As curvas analíticas mostraram-se lineares no intervalo de 40 a 800 µg L^{-1} e de 25 a 1200 µg L^{-1} para ferro e cobre, respectivamente. Os limites de detecção foram 3,76 e 1,84 µg L^{-1} para ferro e cobre, respectivamente. O método proposto foi aplicado para a determinação de íons ferro e cobre em águas, frutas, vegetais e também em materiais de referência certificados, com resultados bastante eficientes.

A miniaturized liquid-phase extraction procedure based on directly suspended droplet microextraction combined with flame atomic absorption spectrometry was developed for determination of trace amounts of iron and copper, without any need for the centrifugation step for phase separation. The method was based on the extraction of the iron and copper complexes with 2-mercaptopyridine-*N*-oxide onto a microdrop of methyl isobutyl ketone (MIBK) as extractant solvent. The factors influencing the extraction were optimized. Under optimum conditions, an enrichment factor of ~25 was obtained for both iron and copper from only 6.5 mL of aqueous phase. The analytical curves were linear between 40-800 µg L^{-1} and 25-1200 µg L^{-1} for iron and copper, respectively. The limits of detection were 3.76 µg L^{-1} and 1.84 µg L^{-1} for iron and copper, respectively. The developed method has been successfully applied for the determination of iron and copper ions in environmental waters, fruits, vegetables and also certified reference materials, with high efficiency.

Keywords: directly suspended droplet, microextraction, iron, copper

Introduction

Recently, pollution of water and food samples by appreciable amount of heavy metals has been considered as a result of human activities. Heavy metal composition of foods is of interest because of their essential or toxic nature. For example, iron, zinc, copper, chromium, cobalt and manganese are essential, while lead, cadmium, nickel and mercury are toxic at certain levels. Toxic elements can be very harmful even at low concentrations when ingested over a long time. Other elements (iron, zinc, copper, etc.), are essential for human life at low concentrations, however,

they can also be toxic at high concentrations.[1] Iron is an essential nutritional element for all life forms, i.e., it is a cofactor for a number of enzymes and is essential for oxygen transport and electron transfer. Although daily requirements for iron are 8 and 18 mg for men and women, respectively, it is potentially toxic in excess concentrations because of its prooxidant activity. Hence, its concentrations in body samples should be frequently controlled.[2] On the other hand, copper is an important trace bioelement for mammals and plants and plays a significant role as a cofactor in at least 30 important enzymes. Study of this metal in real samples is of a great interest because of its critical biological effects as an essential element. But in higher levels it is toxic and can cause nausea, vomiting, irritation of nose and throat, liver trouble or even death.

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Because of this, there is an increasing need to control the trace copper content in water and food samples on a daily basis.[3]

However, due to matrix effect and low concentration of metal ions, efficient separation and preconcentration steps are essential prior to analytical measurements. Several preconcentration methods such as co-precipitation,[4] liquid-liquid extraction (LLE)[5] and solid phase extraction (SPE)[6,7] have been developed for the separation and preconcentration of iron from environmental matrices. Among the methods of copper preconcentration reported are liquid-liquid extraction,[8,9] coprecipitation[10] and also several systems based on solid phase extraction.[11-14] However, the uses of classical extraction methods for these purposes are usually time-consuming, labor-intensive and require large amounts of high purity solvents for extraction.

In recent years, there has been a growing interest in the development of miniaturized preconcentration methods based on liquid-liquid or solid-phase extraction.[15-18] Liquid-phase microextraction (LPME) technique, which emerged in the 1990s,[19-22] is a miniaturized format of LLE and overcomes many of disadvantages of solid phase microextraction (SPME), e.g., non-dependence on a commercial supplier. It is generally simple to use, a quick procedure, which is characterized by affordability and reliance on widely available materials. Research on this technique began by using small droplets of organic solvents suspended from the tip of a microsyringe needle. However, new approaches have been developed to analyze compounds of a different nature and to obtain large enrichment factors using relatively short extraction times.[23] LPME based on static microdroplets does have some drawbacks. First of all, the microdroplet can be lost from its support due to gravity, shear force and flow-field turbulence. Also, constrained stirring decreases the extraction efficiency or enrichment factor. The volume of the microdroplet is limited to 5 μL, which results in poor compatibility with some instruments that require larger injection volumes (e.g., high performance liquid chromatography (HPLC)). In recent years, Yangcheng and co-workers[24] developed directly suspended droplet microextraction (DSDME) as a new sampling method. In this method, a stirring bar is placed at the bottom of a vial containing an aqueous sample and rotated at a speed required to cause a gentle vortex. If a small volume of an immiscible organic solvent is added to the surface of the aqueous solution, the vortex results in the formation of a single droplet at or near the center of rotation. The droplet itself may also rotate on the surface of the aqueous phase, increasing mass transfer. In comparison with the other LPME techniques based on droplet systems (e.g., SDME), it provides more flexibility in the choice of the operational parameters, especially the amount of the solvent and the stirring frequency. The possibility of applying larger volumes of organic solvents in this method also makes it a useful technique to match with HPLC in addition to gas chromatography (GC).[25]

The aim of this study was the combination of green and simple directly suspended droplet microextraction (DSDME) with flame atomic absorption spectrophotometry (FAAS) for determination of Cu(II) and Fe(III) in different samples for the first time without need of any special glass tube for extraction solvent collection.

Experimental

Instrumentation

Determination of iron and copper was performed on a Shimadzu AA-670 atomic absorption spectrometer (Kyoto, Japan) under the recommended condition for each metal ion. The instrumental parameters were as follows: wavelengths 248.3 and 324.8 nm for iron and copper, respectively. Bandwidths were 0.2 and 0.5 nm for iron and copper, respectively. All pH measurements were made using a Metrohm E-691 digital pH meter with a combined glass electrode. A 100 μL Hamilton syringe (Hamilton Bonaduz AG, Bonaduz, Switzerland), was used for methyl isobutyl ketone (MIBK) injection and withdrawal of the enriched drop at the end of the DSDME process. Stirring of the solution was carried out by a magnetic stirrer (Rodwell Monostir, England) and a stir bar (8 mm × 3 mm). A home-made microsample introduction system was constructed from a 25 mL pipette valve and was coupled to the nebulizer needle by a small length of the PTFE capillary tube.[26,27]

Reagents and materials

Nitric acid, hydrogen peroxide, formic acid, perchloric acid, hydrofluoric acid and MIBK from Merck (Darmstadt, Germany) were of analytical grade. The stock solution of 1000 mg L^{-1} of iron and copper were prepared by dissolving Fe(NO$_3$)$_3$.9H$_2$O and Cu(NO$_3$)$_2$.3H$_2$O from Merck (Darmstadt, Germany) in 1% HNO$_3$.[1] The calibration standards for the analytes were prepared using the atomic absorption standard solutions (Fe(NO$_3$)$_3$ 1000 mg L^{-1} Fe, and Cu(NO$_3$)$_2$ 1000 mg L^{-1} Cu from Merck (Darmstadt, Germany)).

A 1.0 g L^{-1} solution of 2-mercaptopyridine-N-oxide sodium salt (Alfa Aesar, Germany) in water was prepared. Other metal salts were analytical grade and purchased from Merck. Working solutions were prepared daily by appropriate dilution of the stock solutions. All experiments were done three times and mean values were evaluated.

General procedure

A 5 mL water sample solution (0.5 μg mL^{-1} Fe^{3+} and Cu^{2+}) or real samples, a stir bar (8 mm × 3 mm), 1.0 mL of 0.1 mol L^{-1} formate buffer (concentration in solution is 0.0154 mol L^{-1}), and 0.5 mg of 2-mercaptopyridine-*N*-oxide (equal to 0.5 mL of 1.0 g L^{-1} solution in water) were placed in a 10 mL glass tube. The magnetic stirrer was subsequently turned on and the stirring rate fixed at 700 rpm in order to form a steady vortex. Then, 210 μL of MIBK was injected onto the surface of the aqueous solution with a microsyringe. The solvent immediately formed a drop-shape upon contact with aqueous solution. After 27 min, the remaining microdrop (50 ± 3 μL) was taken back into the microsyringe and diluted with ethanol to 250 μL. Then 100 μL of the final solution was aspirated into the flame atomic absorption spectrometer for each metal using a home-made microsample introduction system.[26,27] The described method was successfully applied for the determination of Fe(III) and Cu(II) in water, vegetable, fruit and certified reference material samples (JA-1a, JB-3, SRM 1643e and 1640a). The samples were prepared as follows.

Preparation of natural water and water certified reference materials

River water from Sepid Rood (Astaneh Ashrafieh, Iran), mineral water (Hayat, Iran) and drinking water (Sanandaj, Iran) were acidified to pH < 2.0 with concentrated HNO$_3$, immediately filtered (for river water) and stored in precleaned polyethylene bottles. In order to determine the total iron and copper, a 50.0 mL aliquot of each sample was oxidized by addition of 5.0 mL concentrated HNO$_3$ and 1.0 mL concentrated H$_2$O$_2$ (30% m/m). The beaker was covered with watch glass and heated at 100 °C for 30 min to complete the oxidation.[5,28] Then, the sample was transferred into the 50 mL flask and diluted to mark with deionized water. Further, 5 mL of this solution was tested for determination of iron and copper under the general procedure. Preparation of natural water certified reference materials (SRM 1643e and SRM 1640a) followed the same method.

Preparation of vegetable and fruit samples

One gram of sample was placed in a 100 mL beaker, and 10 mL of concentrated HNO$_3$ (65% m/m) was added to the beaker. The mixture was evaporated near to dryness on a hot plate at about 130 ˚C for 4 h. After cooling to room temperature, 3 mL of concentrated hydrogen peroxide (30% m/m) was added. The mixture was again evaporated near to dryness. The resulting solution was diluted to 25 mL

with distilled water. The filtration procedure was made for some vegetable samples which were not completely dissolved.[1] For the analysis of concentrated samples another dilution was done. After adjusting the pH, analysis was done as previously mentioned.

Preparation of rock certified reference materials

The rock sample was analyzed according to the literature[29-31] with a little modification: 0.1 g of powdered rock was weighed in a 50 mL Teflon beaker and 4 mL HNO$_3$ (65% m/m), 3 mL HClO$_4$ (70% m/m) and 5 mL HF (40% m/m) were added. These were mixed well and this mixture was kept for more than 30 min at room temperature, then the beaker was covered and heated at ca. 160 °C for one day. Then the mixture was recovered and evaporated to dryness at ca. 140 °C for 2-3 days. The residue was dissolved with 10 mL (1+1) HCl by heating and dilution to 50 mL for analysis. After another dilution (1000 and 500 times for Fe and Cu, respectively) and adjusting the pH, analysis was done as previously mentioned.

Results and Discussion

In order to obtain high enrichment factor and absorbance, the effect of different parameters such as MIBK volume, pH, amount of 2-mercaptopyridine-*N*-oxide, extraction time, stirring rate and salt addition was optimized. Then, the effect of coexisting ions was investigated and different real samples such as natural water, vegetable and fruit were subjected to the proposed method to evaluate the concentration of iron and copper. Finally, method validation was made using rock certified reference material.

For DSDME, as a special type of LLE, the equilibrium organic phase concentration is given by

$$C_o^{eq} = \kappa\, C_{aq}^{eq} = \kappa\, C_{aq}^0 / (1 + \kappa\, (V_o / V_{aq})) \tag{1}$$

where C_o^{eq} and C_{aq}^{eq} are the equilibrium concentration in the organic phase and aqueous phase, respectively, C_{aq}^0 is the initial concentration in the aqueous phase, κ is the distribution coefficient, V_{aq} is the sample volume, and V_o is the organic solvent volume.

The C_o/C_{aq} at time t is defined as κ_t, and the enrichment factor E is expressed by[24]

$$E = C_o / C_{aq} = \kappa_t / (1 + \kappa_t\, (V_o / V_{aq})) \tag{2}$$

The organic solvent volume has great impact on the extraction of target analytes, since the kinetics of extraction depend upon the interfacial area (A) and the organic solvent volume (V_o) in accordance with the following equation:

$$k = (A / V_o) \, \beta_o \, (1 + \kappa \, (V_o/V_{aq})) \qquad (3)$$

where k is the observed rate constant, V_{aq} is the sample volume, V_o is the extractant organic phase and β_o is the overall mass transfer coefficient with respect to the extractant phase.[32]

The recovery of extraction (ER) was calculated according to:

$$ER = (C_o \, V_o) / (C_{aq}^0 \, V_{aq}) \times 100 \qquad (4)$$

where C_o is the concentration in the organic phase.[33]

Selection of organic solvent and effect of organic solvent volume

Selection of organic solvent is very important for achieving efficient analyte preconcentration. The analyte in the sample solution (donor phase) should have high partition coefficient into the organic solvent. The appropriate organic solvent in this work should also have low solubility in water to minimize dissolution in the aqueous phase. It should also have high viscosity to hold the microdroplet and a lower density than water to float over the aqueous sample solution.[34]

Three low-density solvents (toluene, n-hexane, MIBK) differing in polarity and water solubility were tested for this purpose. In the same experimental condition, the absorbance results for MIBK were better than other solvents. Therefore, methyl isobutyl ketone (MIBK) (density: 0.80 g mL^{-1}; solubility in water: 1.91 g per 100 mL (20 °C)) was selected as extraction solvent for subsequent experiments. In order to evaluate the effect of the extraction solvent volume, different volumes of MIBK were added to 6.0 mL aqueous phase (5.0 mL of sample solution containing 500 µg L^{-1} of Fe^{3+} and 500 µg L^{-1} Cu^{2+} ions and 1.0 mL ligand 1.0 g L^{-1}) in the range of 150-300 µL and the remained organic phase diluted to 250 µL with ethanol and the determination of iron and copper was done according to the recommended procedure. The results are shown in Figure 1. As can be seen, the absorbance increases by increasing the MIBK volume in the range of 150-200 µL. These results are consistent with equation 3, since a larger interfacial area-to-drop volume ratio is provided by smaller drops.[26] Furthermore, the use of MIBK volumes higher than 250 µL gradually decreases the absorbance signal which is due to increasing the volume of the organic phase. Therefore, in the subsequent studies, 210 µL of MIBK was selected as the optimum volume of the extraction solvent.
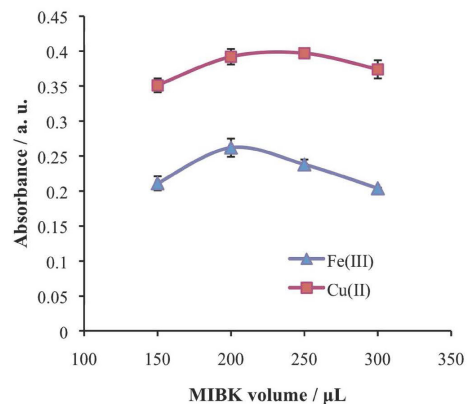

Figure 1. Effect of volume of extraction solvent on the absorbance of iron and copper. Conditions: water sample volume, 5.0 mL; 1.0 mL 2-mercaptopyridine-N-oxide 1.0 g L^{-1}; extraction solvent, MIBK; concentration of Fe(III) and Cu(II), 0.5 µg mL^{-1}; extraction time, 20 min; stirring rate, 600 rpm; n = 3.

Effect of pH

One of the most important parameters affecting the preconcentration procedure is the pH of the solution, because the formation of soluble metal complexes and their stabilities in aqueous solutions are strongly related to the pH of the medium.[35] The effect of pH on the complex formation and extraction of Fe^{3+} and Cu^{2+} from 6.0 mL of aqueous phase into organic phase (210 µL MIBK) was studied in the range of 1.0-9.0. The pH values were adjusted by either nitric acid or sodium hydroxide solution. The experimental results are illustrated in Figure 2, showing that the maximum absorbance is obtained at pH 3.0 and pH 3.0-4.0 for iron and copper, respectively. The decrease in extraction of iron and copper ions at higher pH is due to the competition of hydroxyl ions with pyrithion for reaction with analytes. Therefore, pH 3.0 was chosen for subsequent experiments and the pH adjustment was carried out by formic acid/sodium formate buffer solution.
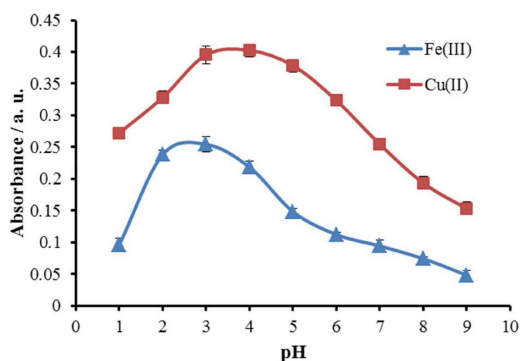

Figure 2. Effect of pH on the absorbance of iron and copper. Conditions: water sample volume, 5.0 mL; 1.0 mL 2-mercaptopyridine-N-oxide 1.0 g L^{-1}; extraction solvent, MIBK 210 µL; concentration of Fe(III) and Cu(II), 0.5 µg mL^{-1}; extraction time, 20 min; stirring rate, 600 rpm; n = 3.

Effect of amount of 2-mercaptopyridine-*N*-oxide

In order to study the influence of the 2-mercaptopyridine-*N*-oxide amount on the extraction and analytical response for iron and copper, 6.0 mL of aqueous phase (5.0 mL of sample solution containing 0.5 µg mL^{-1} of Fe^{3+} and 0.5 µg mL^{-1} of Cu^{2+} ions and 1.0 mL of 0.1 mol L^{-1} formate buffer) were extracted into organic phase (210 µL of MIBK) using various amounts of ligand (1.0 g L^{-1}) ranging from 0.0-1.0 mg. As can be seen from Figure 3, the absorbance increased rapidly as the amount of ligand increased from 0.2 to 0.4 mg, and then slowly decreased upon further increasing in the ligand amount. Therefore, a 2-mercaptopyridine-*N*-oxide amount of 0.5 mg (equal to 0.5 mL of 1.0 g L^{-1} ligand solution) was chosen for subsequent experiments.
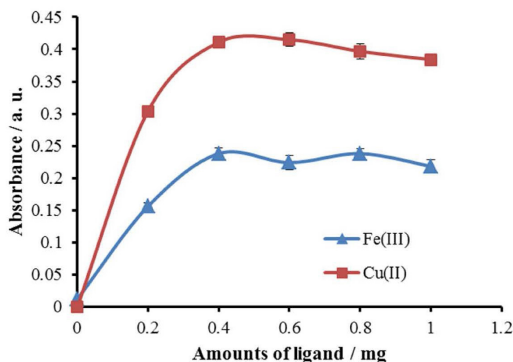

Figure 3. Effect of amount of 2-mercaptopyridine-*N*-oxide on the absorbance of iron and copper. Conditions: water sample volume, 5.0 mL; 1.0 mL formate buffer 0.1 mol L^{-1}; extraction solvent, MIBK 210 µL; concentration of Fe(III) and Cu(II), 0.5 µg mL^{-1}; extraction time, 20 min; stirring rate, 600 rpm; n = 3.

Effect of extraction time

Extraction is an equilibrium process, and the maximum extraction efficiency is obtained when the system is at equilibrium. Therefore, optimum time is required to reach equilibrium.[36] Thus, the effect of time on extraction efficiency of iron and copper from 6.5 mL of aqueous phase (5.0 mL of sample solution containing 0.5 µg mL^{-1} of Fe^{3+} and 0.5 µg mL^{-1} of Cu^{2+} ions, 1.0 mL 0.1 mol L^{-1} formate buffer and 0.5 mL 2-mercaptopyridine-*N*-oxide solution) into organic phase (210 µL of MIBK) was examined in the range of 5-35 min. The results showed that absorbance increased by the increase in time, up to 25 min and then remained constant upon further increasing in extraction time. Thus, the extraction time of 27 min was selected for further experiments.

Effect of stirring rate

Stirring speed is one of the major factors that affect the extraction efficiency. Agitation of the sample is routinely applied to the mass transfer coefficient in aqueous solution and accelerates the extraction kinetics. Increasing the stirring rate can decrease the thickness of the diffusion film in the aqueous phase and improve the repeatability the extraction method.[37] The effect of the stirring rate was studied in the range of 200-1000 rpm. The results showed that the analytical signal increased with increasing stirring rate from 0 to 600 rpm, and then it remained nearly constant upon further increase in the stirring rate up to 800 rpm. Thus, 700 rpm was chosen as the optimum stirring rate.

Effect of salt

The effect of salt on extraction efficiency was studied by varying the concentration of NaCl within the range of 0-10% m/v. Based on the obtained results (Figure 4), addition of salt did not improve the extraction efficiency and the extraction efficiency was higher without addition of sodium chloride. In fact, apart from the salting out effect, salt addition causes a second effect and changes the physical properties of the Nernst diffusion film and reduces the rate of diffusion of the target analyte into the droplet.[38] Hence, the extraction experiments were carried out without additional salt.
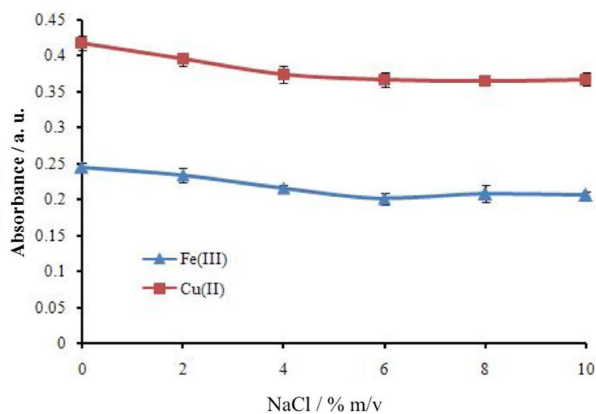

Figure 4. Effect of salt on the absorbance of iron and copper. Conditions: water sample volume, 5.0 mL; 1.0 mL formate buffer 0.1 mol L^{-1}; 0.5 mL 2-mercaptopyridine-*N*-oxide 1.0 g L^{-1}; extraction solvent, MIBK 210 µL; concentration of Fe(III) and Cu(II), 0.5 µg mL^{-1}; extraction time, 27 min; stirring rate, 700 rpm; n = 3.

Effect of coexisting ions

The effects of common coexisting ions on the extraction of iron and copper were also studied. In these experiments, 5.0 mL of solutions containing 0.5 µg mL^{-1} of metal ions and various amounts of interfering ions were treated according to the recommended procedure. A species was considered to interfere if it resulted in a ± 5% variation of the absorbance signal. The results are given in Table 1. As

can be seen from Table 1, the majority of the investigated ions have no significant influence on the extraction of Fe(III) and Cu(II) under the selected conditions. This may be due to formation of more stable complexes of Fe(III) and Cu(II) with pyrithione than the other metal ions studied. Lofts showed that the order of stability constants and, hence, the reactivity of the ligand toward metal ions followed the trend of Fe(III) > Cu(II) > Pb(II) > Zn(II) > Ni(II) > Co(II) > Cd(II) > Mn(II) > Ca(II).[39] Thus, it is not surprising that other cations cannot significantly interfere in the extraction of Fe^{3+} and Cu^{2+} from aqueous solution. In the case of Hg^{2+} ions the data given in Table 1 shows that the interference effect of this ion is higher than the other metal ions studied. This is probably because Hg^{2+} ion as a soft acid has a high affinity for the sulfur atom of the pyrithion as a soft base, which results in strong interactions that increase the stability of Hg^{2+}-pyrithion complex and therefore, an increase in interference of this ion.

Table 1. Effect of coexisting ions on the extraction of 0.5 µg mL^{-1} Fe(III) and 0.5 µg mL^{-1} Cu(II)

Coexisting ions	Maximum tolerable limit / (mg L^{-1})
Na$^+$, K$^+$	5000
Cl$^-$	3500
Mg^{2+}, Ba^{2+}, ClO$_4^-$, Br$^-$, Ca^{2+}, oxalate	1500
Urea	1000
Sr^{2+}, SO$_4^{2-}$, I$^-$, Cd^{2+}, Ni^{2+}, NH$_4^+$, citrate, thiourea	500
Co^{2+}, Zn^{2+}, Pb^{2+}, PO$_4^{3-}$, SCN$^-$	250
Sn^{2+}, Al^{3+}	150
Hg^{2+}	50

Analytical figures of merit

The analytical characteristics of the proposed method, including linear range, limit of detection, relative standard deviation (RSD), correlation coefficient (R^2), and enrichment factor, were obtained. Under the optimum experimental conditions, analytical curves were achieved by analyzing 5.0 mL of standard solution containing a known amount of target ions in the range of 10-1400 µg L^{-1}. The organic phase (50 ± 3 µL) was diluted to 250 µL with ethanol, and 100 µL of this solution was aspirated into the flame atomic absorption spectrometer for determination of each metal. The linear dynamic range was 40-800 µg L^{-1} and 25- 1200 µg L^{-1} for iron and copper respectively. The limit of detection (LOD), calculated as the concentration of the absolute amount of analyte yielding a signal equivalent to three times the standard deviation of the blank

(LOD = 3 σ_{blank} / m, n = 10), where m is the slope of the analytical curve in accordance to IUPAC recommendation, were 3.76 and 1.84 µg L^{-1} for Fe^{3+} and Cu^{2+}, respectively. The limit of quantification (LOQ = 10 σ_{blank} / m, n = 10), were 12.53 and 6.13 µg L^{-1} for Fe^{3+} and Cu^{2+}, respectively.

The results are summarized in Table 2.

Table 2. Analytical figures of merit

Analytical parameters	Fe^{3+}	Cu^{2+}
Linear dynamic range / (µg L^{-1})	40-800	25-1200
Slope / (abs mg^{-1} L)	0.253	0.816
Intercept	0.001	0.007
Correlation coefficient	0.999	0.998
RSD / %[a]	1.7	2.1
Limit of detection / (µg L^{-1})	3.76	1.84
Limit of quantification / (µg L^{-1})	12.53	6.13
Recovery of extraction / %	98	96
Enrichment factor[b]	25.7	25.4
Enrichment factor[c]	20.0	20.0

[a]Relative standard deviation (n = 10, 0.5 µg mL^{-1}); [b]the enrichment factor is the ratio of iron and copper concentration in ethanol (250 µL) to that of the bulk phase initially; [c]the enrichment factor is the ratio of sample volume/final volume (5 mL/0.25 mL).

Applications

The proposed method was successfully used for the determination of total iron and copper in several water, fruit and vegetable samples. The results, along with the recovery for the spiked samples, were given in Tables 3 and 4. As can be seen, added iron and copper are quantitatively recovered from all samples. The accuracy of the proposed method was evaluated by means of recovery experiments and analysis of certified reference materials JA-1a and JB-3 (andesite and basalt, Geological Survey of Japan), SRM 1643e and SRM 1640a (trace elements in natural water from NIST). The results are shown in Table 5. These results indicate the validity of the proposed methodology for analysis of iron and copper in real samples.

Conclusions

In the present study, an efficient and straightforward mode of microextraction technique (DSDME) has been developed for trace analysis of metallic cations such as iron and copper. This technique provides multi-element enrichment capability, simplicity, low consumption of organic solvent, such as MIBK, which is less toxic than other chlorinated extracting solvents, and doesn't need any centrifugation step for phase separation. The

Table 3. Application of proposed method for analysis of natural waters (n = 3)

Sample	Added / (μg L^{-1})	Found[a] / (μg L^{-1})		Recovery / %	
		Fe	Cu	Fe	Cu
	0	113 ± 3	BDL[b]	–	–
Tap water (Sanandaj, Iran)	50	161 ± 3	48 ± 2	96	96
	100	208 ± 5	96 ± 2	95	96
	0	BDL	BDL	–	–
Mineral water (Hayat, Iran)	50	49 ± 2	49 ± 3	98	98
	100	97 ± 3	96 ± 3	97	96
	0	214 ± 5	32 ± 2	–	–
River water (Sepid Rood, Iran)	50	261 ± 5	79 ± 3	94	94
	100	311 ± 6	127 ± 3	97	95

[a]Mean ± standard deviation; [b]BDL: below detection limit.

Table 4. Application of the proposed method for analysis of vegetable and fruit samples (n = 3 and three samples for each type of fruit and vegetable were analyzed)

Sample	Added / (μg g^{-1})	Found[a] / (μg g^{-1})		Recovery / %	
		Fe	Cu	Fe	Cu
	0	59.2 ± 1.1	1.8 ± 0.09	–	–
Parsley (Sanandaj, Iran)	10	69.7 ± 1.5	11.6 ± 0.6	105	98
	50	110.3 ± 1.3	51.2 ± 0.5	102	99
	0	110.1 ± 2.3	4.6 ± 0.4	–	–
Mint (Sanandaj, Iran)	10	120.8 ± 1.6	14.9 ± 0.5	107	103
	50	162.2 ± 2	54.3 ± 0.5	104	99
	0	39.7 ± 1	1.3 ± 0.08	–	–
Carrot (Sanandaj, Iran)	10	50.1 ± 1.3	11.4 ± 0.4	104	101
	50	91.1 ± 2	52 ± 0.4	103	101
	0	37.3 ± 1	2.4 ± 0.07	–	–
Apple (Sanandaj, Iran)	10	47.9 ± 1.1	12.1 ± 0.4	106	97
	50	89.4 ± 1.3	53.4 ± 0.9	104	102

[a]Mean ± standard deviation.

Table 5. Analytical results for certified reference materials (n = 3)

Sample	JA-1a		JB-3		SRM 1643e		SRM 1640a	
	Fe / %	Cu / ppm	Fe / %	Cu / ppm	Fe / ppb	Cu / ppb	Fe / ppb	Cu / ppb
Certified value	5.08	41.7	8.27	194	95.7 ± 1.4[a]	BLDR[b]	BLDR	85.75 ± 0.51
Found	5.14 ± 0.5	41.1 ± 1.9	8.35 ± 0.2	188 ± 2.3	96.5 ± 2.1	–	–	81.70 ± 2.5
Recovery / %	101	98	101	97	98	–	–	95

[a]Mean ± standard deviation; [b]BLDR: below linear dynamic range.

developed DSDME method possesses a high potential for the separation of iron and copper ions from a host of coexisting alkali, alkaline earth, transition and heavy metal ions. Determination of iron and copper can be easily achieved by flame atomic absorption spectrometry with an inexpensive homemade micro sample introduction system. DSDME-FAAS was compared with other reported methods for determination of iron and copper (Table 6). As can be seen, the proposed procedure shows a good limit of detection and precision, wide linear dynamic range and more drastic preconcentration factor, which are better in most cases and are comparable with reported methods in

Table 6. Comparison of the proposed method with other reported methods for determination and preconcentration of iron and copper

Analytical technique	Analyte	LOD / ($\mu g\ L^{-1}$)	LDR / ($\mu g\ L^{-1}$)	Ef[a]	Sample volume / mL	RSD[b] / %	Ref.
CPE[c]-FAAS	Fe, Cu	2.2, 1.4	15-200, 10-250	30	15	0.6- 4.1	40
DLLME[d]-UV/Vis	Fe	7.5	25-1000	5	5	1.2	41
SPE[e]-FAAS	Fe, Cu	0.82, 0.64	250-5000	62.5	40	3.7, 2.6	42
LLE[f]-UV/Vis	Cu	27	200-400	10	200	–	43
SPE-FAAS	Cu	3	–	20	250	< 12	44
USAEME[g]-FAAS	Fe	7.4	40-800	14.2	5	2.5	45
USAE-SFODME-FAAS[h]	Fe, Cu	8.6, 4.1	40-800, 20-1200	13	5	2.9,1.2	46
DSDME- FAAS	Fe, Cu	3.76, 1.84	40-800, 25-1200	25	5	1.7, 2.1	this work

[a]Ef: enrichment factor; [b]RSD: relative standard deviation; [c]CPE: cloud point extraction; [d]DLLME: dispersive liquid-liquid microextraction; [e]SPE: solid phase extraction; [f]LLE: liquid-liquid extraction; [g]USAEME: ultrasound-assisted emulsification microextraction; [h]USAE-SFODME: ultrasound-assisted emulsification-solidified floating organic drop microextraction.

other cases. The application of MIBK as an extracting solvent in comparison with dodecanol and chlorinated solvents have advantages like adaptability with flame AAS and no need for dilution or solvent evaporation.[45,46] The method was successfully applied to the separation and determination of iron and copper in different real samples.

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