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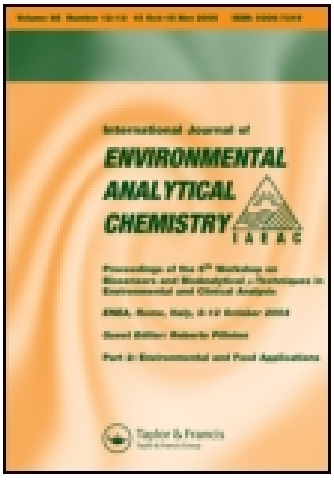

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Speciation and determination of iron using dispersive liquid-liquid microextraction based on solidification of organic drop followed by flame atomic absorption spectrometry

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A simple and efficient method for iron speciation (Fe^{2+}, Fe^{3+}) using dispersive liquid–liquid microextraction based on solidification of organic drop (DLLME-SFO) technique followed by flame atomic absorption spectrometry was developed. In this method, 1 mL of ethanol (as the disperser solvent) containing 50 μL of 1-undecanol (as the extracting solvent) was injected rapidly into the sample solution containing iron (II), iron (III) species and 1.1×10^{-3} mol L^{-1} oxine (as the complexing agent). The procedure is based on the complexation of Fe^{3+} with 8-hydroxyquinoline (oxine) at pH 3, and extraction of the resulting complex using DLLME-SFO. The total Fe was determined after the oxidation of Fe^{2+} to Fe^{3+} with concentrated nitric acid. The concentration of Fe^{2+} was determined from the difference between the concentration of total Fe and the Fe^{3+}. Some parameters such as pH, oxine concentration, ratio of disperser and extractant solvent were investigated. Under the optimum conditions, the calibration graph was linear in the range of 25–250 μg L^{-1} for Fe^{3+} with a limit of detection (LOD) of 4.8 μg L^{-1}. The relative standard deviation (RSD, %) for 10 replicate determinations of Fe^{3+} at 50 μg L^{-1} levels was 3.2%. To validate the developed method, a certified reference material (BRGM R52) was analysed and the determined value was in very good agreement with the certified value. The proposed method was successfully employed for determination of iron species in tap, well and spring water samples.

Keywords: dispersive liquid–liquid microextraction; flame atomic absorption spectrometry; iron speciation; solidification of organic drop

1. Introduction

Iron is an important element in biological systems, environment, industries, and medicines. It is a necessary trace element found in nearly all living organisms and plays an important role in biology, forming complexes with molecular oxygen in haemoglobin and myoglobin; these two compounds are common oxygen transport proteins in vertebrates [1]. The oxidation state of iron in an environment can indicate its electrical potential and microbial activity [2].

Several techniques such as atomic absorption spectrometry (AAS) [3], inductively coupled plasma-optical emission spectrometry (ICP-OES) [4], and high-performance liquid chromatography [5] have been reported for determination of Fe or its species. Flame atomic absorption spectrometry (FAAS) is one of the most widely applied methods for heavy metal analysis because of its experimental rapidity, simplicity, and wide application. The direct determination of trace elements by spectroscopic methods, such as FAAS is often difficult because of

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insufficient sensitivity and selectivity of the used methods. For this reason, preconcentration is often required. Several procedures such as liquid–liquid microextraction (LLME) [6–9], solid phase extraction (SPE) [10], cloud point extraction (CPE) [11] and co-precipitation [12] have been used for preconcentration of different metal ions from different matrices. Dispersive liquid–liquid microextraction (DLLME) [13–15] is a new approach in liquid–liquid microextraction procedures which has advantages of high rapidity, simplicity, and consumption of small amounts of extractant solvent.

One of the main drawbacks of DLLME is the need to use extractant solvents with higher density than water. With this strategy, after centrifuging and discarding the aqueous solution, the enriched phase at the bottom of the centrifuge tube is determined by an appropriate analytical instrument. Different methods have been developed to use extractant solvents with lower densities than water, but some of them are complicated and need modification of the microextraction procedure [16,17]. However, Zanjani *et al*. [18] used solidification of organic drop microextraction (SFOME) as a new and simple liquid phase microextraction method. Recently, dispersive liquid–liquid microextraction based on solidification of floating organic droplet (DLLME-SFO) [19] has been developed as a novel microextraction procedure, which follows the same principle of the DLLME technique and made it possible to use solvents with lower densities than water.

Atomic absorption spectrometry could not directly used for speciation of elements [20]. Thus, speciation of elements by AAS methods requires a separation and preconcentration step [21]. Here, we used DLLME-SFO followed by flame atomic absorption spectrometry for speciation and determination of iron species (Fe^{2+}, Fe^{3+}). This method is based on the complexation of Fe^{3+} with 8-hydroxy quinolone (oxine) at the optimum pH, and employing DLLME-SFO technique to extract the complex followed by FAAS for determination of iron. This method has the advantages of simplicity, rapidity, and low cost.

2. Experimental

2.1 *Instrumentation*

A shimadzu AA-670 (Shimadzu, Japan) flame atomic absorption spectrometer equipped with a 100 mm burner head, deuterium background correction and an air–acetylene flame was utilised. The pH values were measured with a pH-meter (Metrohm 632, Switzerland) supplied with a glass-combination electrode. Phase separation was assisted using a Centurion Scientific Centrifuge (Model Andreas Hettich D72, Tuttlingen, Germany).

2.2 *Reagents*

All chemicals used were of analytical reagent grade. All solutions were prepared with deionised water (18.1 MΩcm) obtained from a Barnstead, Nanopure Diamond purification system. A stock solutions of 1000 mg L^{-1} Fe^{3+} and Fe^{2+} ions were prepared by dissolving the appropriate amounts of $Fe(NH_4)(SO_4)_2.12H_2O$ (Merck, Germany) and $Fe(NH_4)_2(SO_4)_2.6H_2O$ (Merck) in 1% (v/v) HCl (Merck, Darmstadt, Germany), respectively. Working standard solutions were prepared freshly at various concentrations by diluting the stock standard solution with water. Suprapur HNO_3 (65%), H_2SO_4 (98%), and HF (38–40%) were purchased from Merck (Germany) and used for sample digestion. The chelating agent, 8-hydroxyquinoline (oxine), was purchased from Merck. A solution of 2×10^{-2} mol L^{-1} oxine was prepared in 0.1 mol L^{-1} acetic acid and diluting to 50 mL with water. 1-Undecanol (for synthesis) as extraction solvent, methanol (for spectroscopy), ethanol (for spectroscopy), acetonitrile (HPLC grade) and acetone (suprasolv) were purchased from Merck (Darmstadt, Germany). Other chemicals used:

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potassium cyanide, thiourea, potassium chloride, and other metal ion salts (to study the effect of interfering ions) were obtained from Merck (Darmstadt, Germany). To keep the pH at a constant value, 1 mol L^{-1} sodium acetate/ acetic acid buffer solution was used.

2.3 *Microextraction procedure*

(1) Determination of Fe^{3+}/Fe^{2+}: 10 mL of sample solution containing Fe^{3+}/ Fe^{2+}, 1 mL of 1 mol L^{-1} sodium acetate/acetic acid buffer solution at pH 3, and 1.1×10^{-3} mol L^{-1} oxine were transferred into a conical centrifuge tube. 1 mL of ethanol as disperser phase containing 50 μL extraction solvent (1-undecanol) was rapidly injected into the sample solution using a 2 mL syringe. A cloudy solution (water, ethanol and 1-undecanol) was formed in the centrifuge tube and the complex was extracted into the fine droplets of extraction solvent. The mixture was centrifuged at 4000 rpm for 4 min. The dispersed fine droplets of the extraction phase were collected on the top of the centrifuge tube. Then, the centrifuge tube was transferred to a beaker containing ice and the extraction organic solvent (50 ± 3 μL) was solidified after 10 min. The solidified organic solvent was transferred into a conical vial, in which it melted immediately. After dilution to 500 μL with ethanol, the resulting solution was aspirated into the flame atomic absorption spectrometer for quantification.

(2) Speciation of Fe^{2+} and Fe^{3+}: To measure the amounts of each ion in the mixture, Fe^{3+} was analysed according to the microextraction procedure. For determination of total Fe, the mixture was acidified with concentrated HNO_3 to convert Fe^{2+} to Fe^{3+} [22] and the same procedure was conducted for determination of total Fe^{3+} and then the concentration of Fe^{2+} was determined from the subtraction of these results.

2.4 *Preparation of real samples*

Water samples, including tap, well (Ghasem Abad, Mashhad, Iran) and spring waters (Now Chah, Mashhad, Iran) were collected from their local sources. After sampling, spring and well waters were filtered through 0.45 μm filter paper to remove suspended particulate matters and analysed according to the following procedure:

- After addition of 1.1×10^{-3} mol L^{-1} oxine and 1 mL buffer solution to 5 mL of different water samples and diluting to 10 mL with water, these samples were analysed for their Fe^{3+} contents according to the microextraction procedure. For determination of Fe^{2+} in real samples, concentrated HNO_3 was used for converting Fe^{2+} to Fe^{3+}. The accuracy of the method was verified by the analysis of samples spiked with known amounts of Fe^{3+} and Fe^{2+} and the mixtures of Fe^{2+}/Fe^{3+} at different levels.
- A 0.1000 g of certified reference material, BRGM R52, was dissolved in 20 mL of the mixture of HF, H_2SO_4, and HNO_3 (5:3:1). The mixture was heated on heating block at low temperature to near dryness and diluted to 25 mL with deionised water. After diluting the stock solution to achieve the desired concentration, the iron content was determined according to the microextraction procedure.

3. Results and discussions

Effects of different parameters that influence the microextraction procedure were completely investigated and optimised conditions were selected.

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3.1 *Effect of pH*

pH plays an important and critical influence on the speciation and efficiency of the method. The effect of pH was studied in the range of 3–8. The results in Figure. 1 show that at pH 3, Fe^{3+} formed a hydrophobic complex with oxine (Fe^{3+}-oxine) which can be extracted into an organic extraction solvent but Fe^{2+} remains in an aqueous solution. This difference makes it possible to separate Fe^{3+} from the sample solution with DLLME-SFO. For a pH higher than 3, both Fe^{2+} and Fe^{3+} were extracted. Therefore pH 3 was selected as the optimum pH value.

3.2 *Effect of ligand concentration*

To study the effect of ligand concentration on the sensitivity of the method, different concentrations of oxine were used in the range of 3.6×10^{-5}–1.1×10^{-3} mol L^{-1}. The results show that (data not shown), the absorbance increased up to 7.2×10^{-4} mol L^{-1} oxine concentration and remained constant afterwards. To ensure adequate concentration of oxine, 1.1×10^{-3} mol L^{-1} was selected as the optimum ligand concentration for subsequent analysis.

3.3 *Effect of the disperser solvent type*

In this study 1-undecanol was used as the extraction solvent. The selection of a dispersive solvent is limited to solvents such as methanol, acetonitrile, ethanol and acetone, that are miscible with both water and extraction solvents. In all cases, 50 µL of extraction solvent was diluted to 1 mL with different disperser solvents and injected into the sample solution. The results show that (data not shown), acetonitrile and ethanol have higher sensitivities compared with methanol and acetone. Because of the safety, cheapness and availability of ethanol, this solvent was used as disperser solvent for subsequent analysis.

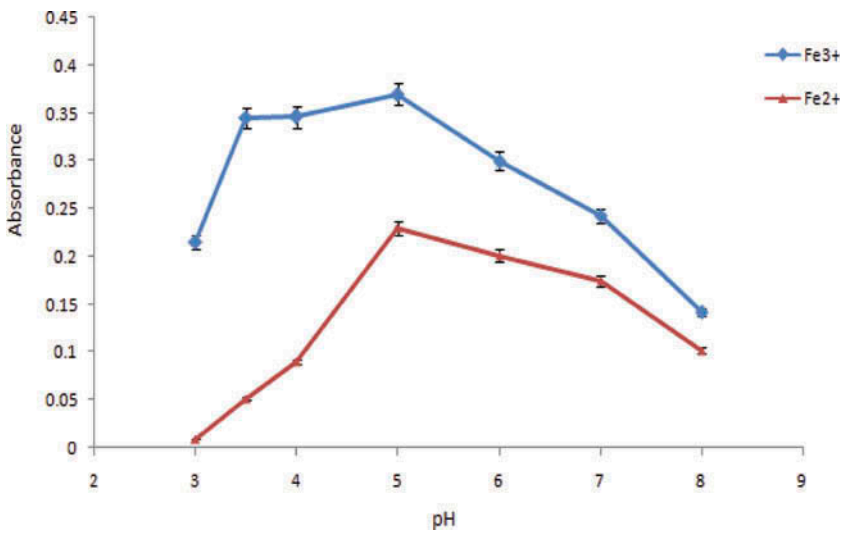

Figure 1. Effect of pH on the absorbance of Fe^{2+} and Fe^{3+} solutions. Conditions: 200 µg L^{-1} Fe^{2+}/Fe^{3+}, 1.1×10^{-3} mol L^{-1} oxine, 1 mL of ethanol containing 50 µL 1-Undecanol.

3.4 *Effect of the extraction and disperser solvent volume*

The extracting solvent must have low volatility, low water solubility, high solubility in dispersive solvent, be capable of formation of cloudy solution in water in the presence of dispersive solvent and a melting point near to room temperature (in the range of 10–30°C). 1-undecanol has the advantages mentioned above and can be used as extraction solvent for SFO [9,23]. To examine the effect of extraction solvent volume on the extraction efficiency, solutions containing different volumes of 1-undecanol with a fixed volume of disperser solvent were used with the same DLLME-SFO procedure. The experimental conditions were fixed to include the use of 1.0 mL ethanol containing different volumes of 1-undecanol. According to the obtained results (data not shown), the absorbance increases with increasing the 1-undecanol concentration up to 50 μL and it decreases gradually with increasing the extraction solvent volume. The decrease in absorbance could be related to the increasing viscosity of the final solution aspirated to FAAS [24]. Therefore, 50 μL of 1-undecanol was selected as the optimum value. The effect of disperser solvent volume was also investigated in the range of 0.5–2 mL containing 50 μL of 1-undecanol. To achieve more stable cloudy solution, 1.0 mL of ethanol was selected as the optimum volume. Therefore, 1 mL of ethanol containing 50 μL of 1-undecanol was used as the injecting solution.

3.5 *Centrifugation time*

The effect of centrifugation time on analytical signal was studied in the range of 1–10 min. A very short centrifugation time may not insure satisfactory phase separation. Therefore, 4 min was chosen as the optimum centrifuge time.

3.6 *Effect of the ionic strength*

To investigate the influence of the ionic strength on the DLLME-SFO performance, several experiments were performed with different KCl concentrations (1–8% g mL^{-1}) while keeping other experimental parameters constant. The results show that (data not shown), with increasing the ionic strength, the signals were constant up to 1% g mL^{-1} KCl but decreased gradually by increasing the salt concentration. This effect may be attributed to the decrease of 1-undecanol (extraction solvent) solubility in aqueous phase at higher ionic strength [23,24]. Therefore, further experiments were performed without addition of KCl.

3.7 *Interferences study*

The effect of other ions on the extraction of iron was studied under the optimised conditions. Solutions containing 100 μg L^{-1} Fe^{3+} and different concentration of other ions were prepared and followed according to the proposed procedure. The results are shown in Table 1. An ion was considered to interfere when its presence produced a variation of more than 5% in the absorbance of the sample. Some species, such as Cu^{2+} and Co^{2+} interfered with the determination of Fe^{3+}. These interferences were eliminated by using 0.05 mol L^{-1} KCN, and 0.1 mol L^{-1} thiourea as masking agents for Co^{2+} and Cu^{2+}, respectively.

3.8 *Analytical figures of merit*

Under the optimum conditions, the calibration graph was linear in the range of 25–250 μg L^{-1} of Fe^{3+} with a correlation coefficient (R^2) of 0.9993. The regression equation was A =

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Table 1. Effect of foreign ions on the recovery of 100 μg L^{-1} Fe^{3+}.

Ions	Ion/Fe mole ratio	Recovery
K^+	500	100
Na^+	500	98
Mg^{2+}	500	99
Cd^{2+}	200	100
Pb^{2+}	200	99
Ca^{2+}	200	100
Mn^{2+}	100	96.5
Ni^{2+}	100	97.5
Cu^{2+}	25	96.8
Co^{2+}	25	95.5
NO_3^-	500	100
Cl^-	500	99
Br^-	200	96
SO_4^{2-}	200	99

0.001C + 0.008, where A is the absorbance and C is the concentration of Fe^{3+} in μg L^{-1}. The relative standard deviation (RSD) for the analysis of ten replicates of 50 μg L^{-1} Fe^{3+} solution was 3.2%. The detection limit (calculated as the concentration equivalent to three times of the standard deviation of the blank divided by the slope of the calibration curve after preconcentration) was 4.8 μg L^{-1}. The preconcentration factor calculated as the ratio between the volume of the aqueous phase (10 mL) and the final volumes of the extraction phase (500 μL), was 20. The sensitivity of the proposed method for determination of Fe^{3+} based on 0.0044/m (where m is the slope of calibration curve) was 3.6 μg L^{-1}. The enhancement factor was calculated as the ratio of the slope of a calibration curve prepared from aqueous solutions submitted to the recommended extraction procedure, and that obtained without the preconcentration and found to be 17.2.

3.9 *Application of the proposed method*

The proposed method was applied for the determination of iron species in different water samples. To show the validity of this method, recovery experiments were also performed by spiking the samples with different amount of Fe^{2+} or Fe^{3+}. The obtained results are given in Table 2. Good agreement was obtained between the added and found analyte content using the microextraction procedure. The accuracy of the proposed method was successfully verified with the analysis of a certified reference material (CRM), BRGM R52, with certified Fe content of 2.203% (w/w). Using the method developed in this study, the Fe content found in the CRM was 2.190 ± 0.05 (w/w) (mean ± standard deviation based on three replicate analyses). This result shows that the analytical result was in very good agreement with the certified value.

3.10 *Comparison to other methods*

The figures of merit of the determination of iron species using solidified floating organic drop microextration with some of the previously reported methods are given in Table 3. In comparison with other reported methods, the proposed method has low LOD, short analysis time and wide dynamic range. This methodology is a reproducible, simple, and low-cost technique and does not require further instrumentation and it can be used with regular FAAS equipment.

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Table 2. Determination of iron species in the real and spiked samples.

Sample	Measured (μg L^{-1})		Added (μg L^{-1})		Found (μg L^{-1})		Recovery (%)	
	Fe^{2+}	Fe^{3+}	Fe^{2+}	Fe^{3+}	Fe^{2+}	Fe^{3+}	Fe^{2+}	Fe^{3+}
Tap water[a]	ND[b]	ND	–	–	–	–	–	–
			50	–	52.8	–	105.6	–
			–	100	–	102.3 ± 0.9	–	102.3
			50	100	52.1 ± 0.6	104.8 ± 1.1	104.2	104.8
Spring water[c]	ND	27	–	–	–	–	–	–
			50	–	49.8	–	99.6	–
			–	100	–	124.5 ± 1.3	–	98.03
			50	100	54.1 ± 0.3	132.5 ± 1.8	108.2	104.3
Well water[d]	ND	43	–	–	–	–	–	–
			50	–	51.8 ± 0.4	–	103.6	–
			–	100	–	150.1 ± 1.9	–	104.9
			50	100	49.5 ± 0.3	146.1 ± 1.8	99.0	102.1

Notes: [a]From Mashhad, Iran.
[b]Not detected.
[c]From Now Chah, Mashhad, Iran.
[d]From Ghasem Abad, Mashhad, Iran.

Table 3. Comparison of DLLME-SFO method with other methods for preconcentration and determination of iron.

Extraction method	Linear range (μg L−1)	LOD (μg L−1)	RSD (%)	Extraction time (min)	Reference
DLLME	25–1000	7.5	1.2	<1	[6]
LLE	50–20,000	3.2	3.9–4.5	<30	[25]
LLE	25–150	9	7	15	[26]
DLLME-SFO	31–350	8	3.9	<1	[27]
USAEME[a]	40–800	7.4	2.5	20	[28]
USAEME	1–1000	0.27	1.93	9	[29]
DLLME-SFO	25–250	4.8	3.2	<1	This work

Note: [a]Ultrasound assisted emulsification microextraction.

4. Conclusions

Dispersive liquid–liquid microextraction based on solidification of organic drop (DLLME-SFO) was used for speciation and determination of iron followed by flame atomic absorption spectrometry. The preconcentration method was successfully applied to iron speciation in different water samples, with good accuracy and good reproducibility.

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