

Application of Ultrasound-assisted Emulsification Solidification of Floating Organic Drop Microextraction for Preconcentration and Trace Detection of Iron (III) in Water and Human Urine Samples

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Received April 08, 2017; Revised June 29, 2017; Accepted September 23, 2017

This paper is available on-line at <http://ijoh.tums.ac.ir>

ABSTRACT

At present, metals are used in many industries and working places so the consequence use and exposure to toxic metals is some human health risks. In order to protect the exposed people to these metals, identification of toxic metals in biological samples can be an effective strategy in the controlling of people's health. In the present research, ultrasound-assisted emulsification with solidification of floating organic droplet microextraction procedure (USAE-SFODME) combined with atomic absorption spectroscopy was used for pre-concentration and determination trace amount of iron (III) from water and urine matrices. This research consisted of preparation of samples containing iron (III), iron extraction and analysis with flame atomic absorption spectroscopy equipped with iron hollow cathode lamp with analysis wavelength of 248.33 nanometers. Optimization of the extraction conditions was carried out for ligand volume, solvent volume, temperature, sonication time and pH. Detection of limit (LOD) and limit of quantification (LOQ) were respectively 0.378 and 1.147 $\mu\text{g mL}^{-1}$ in this research. The proposed method in pre-concentration and analysis trace amount of iron in aquatic samples can be established as a successful method. One of the important advantages of this approach reduces the operator exposure to toxic substances.

KEYWORDS: *USAE-SFODME, Urine sample, Iron, Flame atomic absorption spectroscopy*

INTRODUCTION

Widespread use of metals in industrial activities can lead to increasing occupational exposure to these toxic substances [1-2]. Exposure

to some metals, for example, zinc, aluminum, iron, and copper can cause toxic effects on human [3- 4].

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Several studies have been investigated about toxic effects of metals from micron to nano size on human, however, increasing the problem of bronchial obstruction is due to exposure to iron oxides in the casting workers being exposed to Fe²O³ nanoparticles [5]. Due to the toxicity of various metals, investigation about biological monitoring of these substances is important.

Common laboratory methods have been defined for analyzing metals in biological samples for example iron was analyzed in bone, liver, hair, blood, and urine with atomic absorption spectrometry [6]. In biological monitoring researches, various extraction techniques are applied for analyzing toxic metals in different matrices so some of them are single drop microextraction (SDME) [7-8], continuous-flow microextraction, hollow fiber liquid-phase microextraction (HF-LPM) [9], dispersive liquid-liquid microextraction (DLLME), and recently, solidified floating organic drop microextraction (SFODME) [10-11]. The iron cations in the body so a research was carried out for spectrophotometric determination of iron in water samples using dispersive liquid-liquid microextraction based on solidification of floating organic drop (DLLME-SFO) technique, this technique is simple and sensitive with low cost [12].

There are advantages and disadvantages in all methods of preparation and extraction of biological samples. Generally, solvent exposure is high in classical methods and these methods are expensive with toxicity and fire hazards. Extraction and analysis of chemicals from some samples require the removal of other chemical elements so other chemicals required for purification [13].

In analysis of different samples, simple and safe methods are needed and SFODME is part of this group of methods. In some papers SFODME technique was used to analysis iron ($r=0.992$, $RSD=3.2$), copper ($r=0.9989$, $RSD=3.83\%$) and lead ($r=0.999$, $RSD=5.4\%$) cations in water samples so this method was done with precision and acceptable accuracy [14-17].

This method in one research was applied for speciation of ultra-trace quantities of gold in real samples, RSD and percentage R were respectively 1.7% and 97.88 [18].

Because of little information about application of SFODME in biological monitoring, in the present research, this technique was used to detect trace amount of iron (III) in water and urine samples.

MATERIALS AND METHODS

Standard solution and reagents: Iron (Fe³⁺) standard solution with concentration of 1000 $\mu\text{g mL}^{-1}$ (Merck Co./Germany), 1-(2-Pyridylazo)-2-naphthol (PAN) (Merck Co./Germany) PAN as a tridentate chelator, nitric acid (1 molar), sodium hydroxide (1molar), 1-dodecanol and ethanol (Merck Co. /Germany).

Instrument: Atomic absorption spectroscopy (Analytik Jena AG - AAS vario 6/Germany) equipped with iron hollow cathode lamp, pH meter (Model: 3510, Jenway/England), laboratory centrifugal device (Model: Rotofix 32A, Hettich/Germany), ultrasound apparatus (Bandelin Co./U.S.A).

Sample preparation: 24-h urine sample was drawn from a volunteer. Urine samples were collected in a clean polyethylene container then transported to the laboratory and kept at a temperature of 4 °C. All samples were passed through Whatman filter paper.

Extraction procedure: Urine sample containing Fe³⁺ cation was prepared with the concentration of 0.8 $\mu\text{g mL}^{-1}$ in a 20 mL centrifugal tube. Then, it was mixed with 2mL of PAN (0.0001 mole L⁻¹) as ligand. PH value was adjusted ~ 5.5 and 90 μL of 1-dodecanol was added to the samples by using Hamilton microsyringe. The solution was placed in the ultrasound apparatus for 20 min under 35 °C, then it was placed for 5 min in the centrifugal device with 2000 rpm. Extraction time and temperature was 20 min and 35 °C, respectively. After the centrifugal stage, the test tube was immediately put in an ice bath for 5 min and the extraction solvent was frozen in the ice bath. Then, the extracted concentrate was easily taken by a small spatula and placed in another vial to melt rapidly, finally, it was immediately diluted by injecting 500 μL of ethanol to it and injected manually to flame the atomic absorption apparatus. Atomic absorption Analysis was done in 248.33 nanometers.

RESULTS

Optimization of USAE-SFODME: Optimal conditions include some parameters such as ligand volume, volume of solvent extraction, temperature, extraction time and pH. Recovery was calculated using the following formula.

$$\% \text{Recovery} = (C_{\text{spiked sample}} - C_{\text{unspiked sample}}) / C_{\text{added}}$$

Influence volume of PAN: The effect of PAN as ligand with concentration of 0.0001 mole L⁻¹ in different volume (0.5-2.5mL) was

investigated on this extraction. The best extraction occurred at 2 ml of PAN (Fig. 1).

Effect of pH: The pH in the range of 2 to 10 was studied and for 5.5 the best effect on iron extraction was obtained. In lower amounts and above of this value, the extraction efficiency was decreased (Fig. 2).

Effect of temperature: The increase in temperature causes the ligand to be analyzed and resulting in reduced extraction efficiency (Fig.3). Many metal complexes in expose to high temperatures are separated and therefore extraction efficiency is reduced. In this study, temperature in the range of 15-45 °C was evaluated and maximum efficiency was obtained at 35 °C, therefore, this value was determined as optimal temperature.

The influence of ultrasonic time: Mass transfer of analyses from aqueous solution to solvent extraction can be done quickly and ultrasonic process plays an important role in separation metal cations with the proposed microextraction method. In this research, ultrasonic time was evaluated in the range of 10 to 40 min (Fig. 4).

Effect of solvent extraction volume: The density of 1-dodecanol is greater than water and this solvent is insoluble in water, so it can collect easily. The effect of 1-dodecanol volume was investigated in the range of 30-60-90 and 120 μ l. The volume of 90 μ l was selected as the optimal volume so higher volume can lead to reduction of iron metal complexes with ligands.

Effect of solvent extraction volume on extraction of Fe^{3+} by USAE-SFODME method, extraction condition: 2mL PAN, pH=5.5, temperature=35°C, sonication time = 20 min is seen in Fig. 5.

Method validation: Deionized water sample (Merck Co.) and urine samples were used to determine the accuracy and precision" of USAE-SFODME technique. Thus, by spiking the appropriate amount of Fe^{3+} to definite volumes of deionized water (Merck Co.) and urine samples, three standard concentrations of Fe^{3+} with the concentrations of 0.3, 0.6 and 0.8 μ g mL^{-1} were prepared, respectively. The spiked samples in optimum conditions were evaluated and after extraction, relative standard deviation (RSD) was used to determine the accuracy (Table 1) and the proposed has appropriate accuracy.

Calibration curve: For calibration and determining the linear equation and correlation coefficient, iron standards with concentrations of

0.05, 0.1, 0.5, 1.0, 2 μ g mL^{-1} were prepared, then, they were evaluated in the obtained optimum conditions. The equation of calibration curve was obtained as $y = 0.0601x - 0.0034$, such that "y" and "x" are equal to the rate of absorption and concentration of Fe^{3+} , respectively. Calibration equation indicates appropriate linearity with the correlation coefficient of $R^2 = 0.9819$ according to the considered technique (Fig. 6). The detection of limit defined as $\text{LOD} = 3S_{a/b}$ (18) and limit of quantification were respectively 0.378(μ g mL^{-1}) and 1.147 μ g mL^{-1} (Table 2).

Comparison of USAE-SFODME with other methods: Efficiency of USAE-SFODME method with other techniques shows that there is good agreement between this method with the references (Table3).

DISCUSSION

Identifying trace amounts of Fe^{3+} cations in urine samples could be done in the present study with appropriate accuracy by USAE-SFODME technique [19]. Filtration is a suitable solution to eliminate water and urine interveners before performing this method [20]. Extracting iron (III) by USAE-SFODME technique in water samples was done and has successful efficiency for these types of samples[21-22]. A strong chelating factor in extraction and pre-concentration of metal cations is used in the new method of microextraction [23].

In this research, different volumes of PAN as ligand were selected (0.5-1-1.5-2-2.5mL) and optimum volume was 2mL because in this volume highest absorption rate of Fe^{3+} cations was observed. The other important factor in extraction efficiency is the type of the extraction solvent so 1-dodecanol has low solubility in water, low volatility, and melting point near ambient temperature (14) and it is selected as the extraction solvent in this study. An appropriate amount of the solvent in the present study was 90 μ L and by increasing its volume, reductions were observed in the absorbing rate of Fe^{3+} cations. Increasing the volume of extraction solvent over 90 μ L leads to increases the rate of analyte absorption but after reaching maximum absorption, increasing in this volume will have no effects on the absorption rate and remains fixed. Using ultrasound waves with centrifugal process will help to the establishment of smaller drops of 1-dodecanol organic solvent in the water phase in shorter time.

In this research, ultrasound in the range of 10,20,30,40 min was examined and in 20 min, the best results have been obtained. The ultrasound apparatus creates organic drops and increases the contact surface between extraction solvent and analyte by intensive movements and vibrations. Temperature is effective on solvency of organic

solvents in water and on the contact surface between the sample solution and extraction solvent so it can affect on the trend of mass transfer and extraction efficiency. Temperature was examined in different ranges (15-25-35-45 °C) and results show that sonication in 20 min under 35 °C has the highest effect on absorbing of Fe³⁺. Increasing temperature and sonication time can lead to reduction the rate of Fe³⁺ absorbing; hence, the extraction efficiency will be decreased. Another important factor is pH examined in four ranges (2-5.5-7-10) and the most appropriate pH in the present study was 5.5, and reductions in the extraction efficiency were observed by increasing or decreasing this rate. In high amounts of pH, the

extraction efficiency is reduced due to the reaction between the hydroxide anion and the metal cations. In low amount of pH, the efficiency of extraction is reduced due to the high concentration of proton and its competition with metal cations for the formation of the complex. Therefore, pH value of 5.5 was selected as the optimum amount.

Finally, this method is suitable for the extraction of iron from urine samples and some parameters such as sonication time, extraction temperature, the solvent type, ligand type and pH are some important factors affecting pre-concentration and extraction of Fe³⁺ cations.

Table 1. Recovery and %RSD in deionized water and urine samples (n=3)

| Sample | Added (µg L ⁻¹) | Found (µg L ⁻¹) | Recovery (%) | RSD (%) |
|------------------------------------|-----------------------------|-----------------------------|--------------|---------|
| Deionized water sample (Merck Co.) | – | ND* | 98.6±0.005 | 1.4 |
| | 0.8 | 0.788 (±.002) ^b | 99.2±0.004 | 0.8 |
| | 0.6 | 0.59(±.005) | 99.3±0.005 | 0.7 |
| | 0.3 | 0.298(±.001) | | |
| urine samples | – | ND | 97.4±0.005 | 2.6 |
| | 0.8 | 0.78 (±.01) | 95.5±0.004 | 4.5 |
| | 0.6 | 0.57(±.01) | 95.5±0.002 | 4.5 |
| | 0.3 | 0.286(±.005) | | |

Table 2. Parameters obtained using the proposed method

| | |
|-----------------------------|---------------------------|
| Recovery (%) | 97.4±0.005 |
| Quantification of limit | 1.147 µg mL ⁻¹ |
| Detection of limit | 0.378 µg mL ⁻¹ |
| R.S.D. (%) (n = 3) | 2.6 |
| Slope | 9.8 × 10 ⁻¹ |
| Correlation coefficient (r) | 0.99 |
| R ² | 0.98 |

*Not Detected

Table 3. Comparison of the proposed method with other methods for extraction iron (LOD=0.378 µg L⁻¹ and LOQ=1.147 µg mL⁻¹)

| instrument type | Extraction method | r | Concentration range | RSD (%) | Recovery (%) | EF ^a | LOD ^b | Reference |
|-------------------|-------------------|--------|-------------------------------|---------|--------------|-----------------|--------------------------|-----------|
| FAAS | USAE-SFODME | 0.9900 | .05-2 µgrL ⁻¹ | 2.6 | 97.4 | - | 0.378 µg L ⁻¹ | This work |
| Spectrophotometry | DLLME | 0.9980 | 0.025–1.0 g m L ⁻¹ | 1.2 | - | - | 7.5 g L ⁻¹ | [24] |
| FAAS | SPE | 0.9970 | 0.2–10 g m L ⁻¹ | 1.4 | - | - | 19 g L ⁻¹ | [25] |
| FAAS | LLE | 0.9988 | 25–150 g L ⁻¹ | 7 | - | - | 9 g L ⁻¹ | [26] |
| FAAS | LLE | 0.9990 | up to 5 g m L ⁻¹ | 2.1 | 93–107 | - | 0.24 g L ⁻¹ | [27] |
| UV–Vis | IL-USA-DLLME | - | 5.0–140.0 µg L ⁻¹ | 1.5 | 103.1 | - | 0.2 µg/L | [28] |

^a Enhancement factor.

^bLimit of detection.

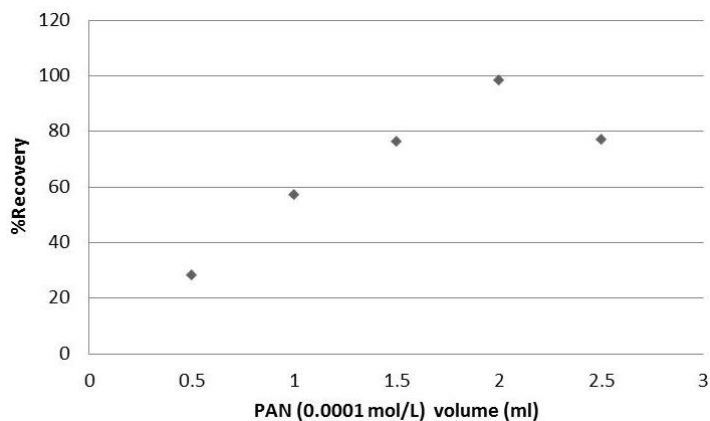


Fig. 1. Effect PAN volume on extraction of Fe^{3+} by USAE-SFODME method. Extraction conditions: 90 μl -dodecanol, pH=5.5, temperature=35 °C, sonication time = 20 min

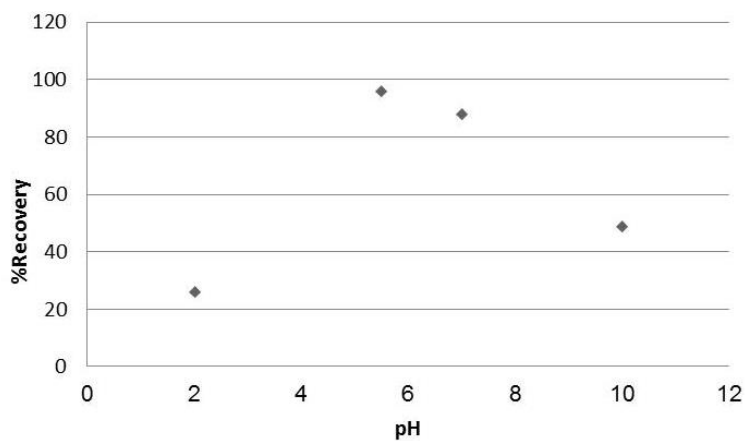


Fig. 2. Effect of the pH on extraction of Fe^{3+} by USAE-SFODME method.. Extraction conditions: 90 μl -dodecanol. 2 mL PAN, temperature=35 °C, sonication time = 20 min

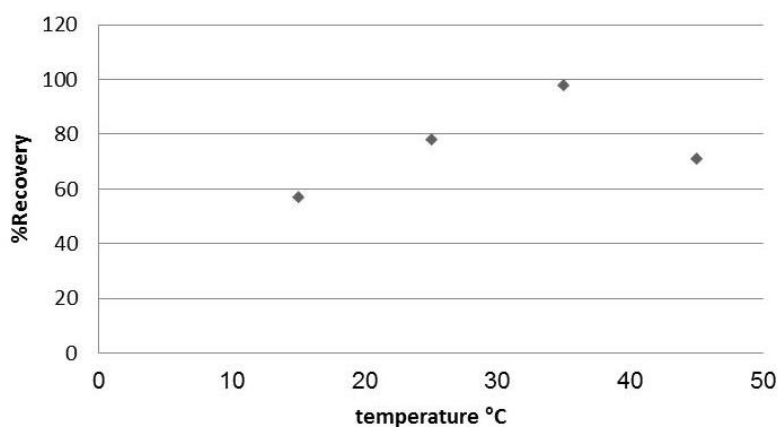


Fig. 3. Temperature effect on extraction of Fe^{3+} by USAE-SFODME method. Extraction condition: 90 μl -dodecanol. 2mL PAN, pH=5.5, sonication time = 20 min

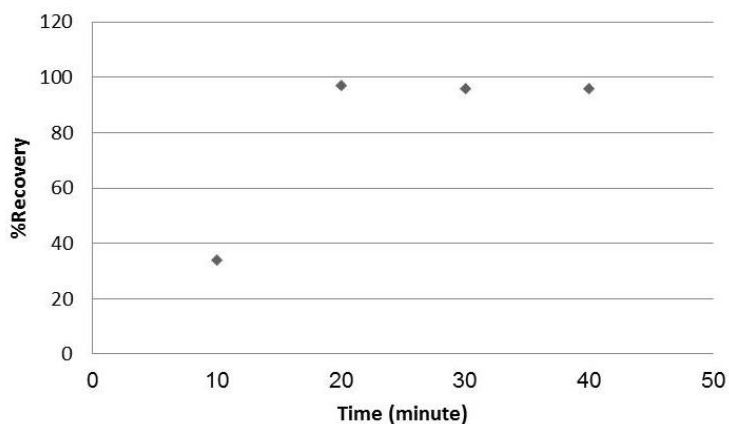


Fig. 4. Ultrasonic time effect on extraction of Fe^{3+} by USAE-SFODME method, extraction condition: 90 μl -dodecanol. 2mL PAN, pH=5.5, temperature=35 °C

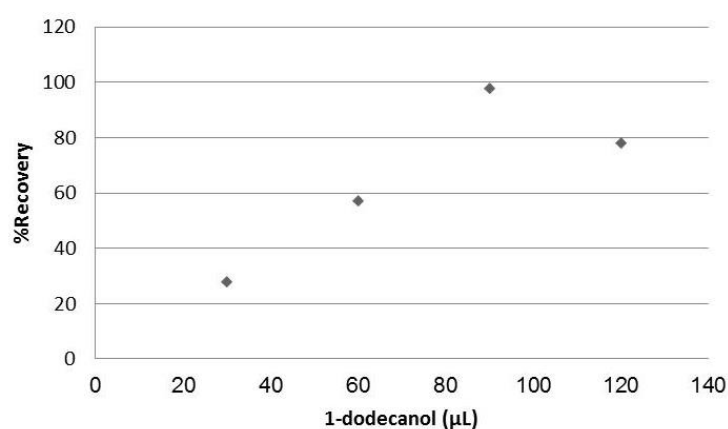


Fig. 5. Effect of solvent extraction volume on extraction of Fe^{3+} by USAE-SFODME method, extraction condition: 2mL PAN, pH=5.5, temperature=35 °C, sonication time = 20 min

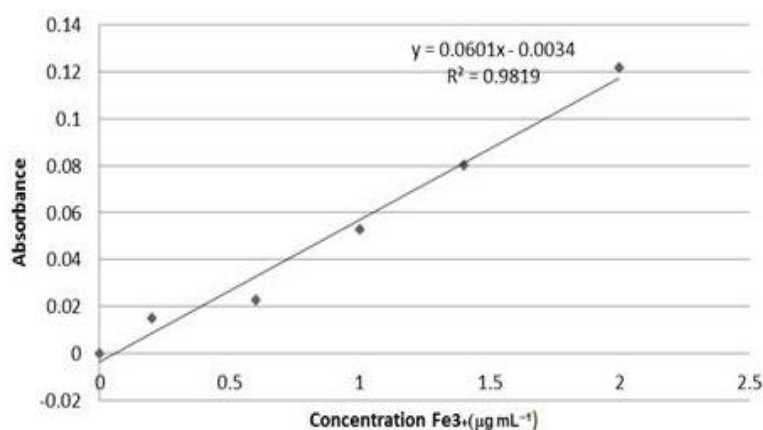


Fig. 6. Calibration curve in extraction of Fe^{3+}

CONCLUSION

Solidified floating organic drop microextraction has some benefits including, need small sample volume, reduction in the analysis time, using green solvents, inexpensive analysis costs, and proper accuracy. The proposed technique could probably create suitable results in extraction of other metals in biologic samples also it was

successful in identifying and evaluating toxic metals in water samples. This method could be used for biological monitoring in the exposed people to toxic metals.

To achieve more results, this technique used in order to extraction of trace amount of other toxic metals in biological samples.

ACKNOWLEDGMENTS

This manuscript is the thesis results in the master's course with code 394939 and the authors thank all the people involved in this research. The authors declare that there is no conflict of interest.

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