



# Spectrophotometric determination of iron species using a combination of artificial neural networks and dispersive liquid–liquid microextraction based on solidification of floating organic drop

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## ABSTRACT

A dispersive liquid–liquid microextraction based on solidification of floating organic drop (DLLME-SFO) and artificial neural networks method was developed for the simultaneous separation/preconcentration and speciation of iron in water samples. In this method, an appropriate mixture of ethanol (as the disperser solvent) and 1-undecanol (as the extracting solvent) containing appropriate amount of 2-thenoyltrifluoroacetone (TTA) (as the complexing agent) was injected rapidly into the water sample containing iron (II) and iron (III) species. At this step, the iron species interacted with the TTA and extracted into the 1-undecanol. After the phase separation, the absorbance of the extracted irons was measured in the wavelength region of 450–600 nm. The artificial neural networks were then applied for simultaneous determination of individual iron species. Under optimum conditions, the calibration graphs were linear in the range of 95–1070  $\mu\text{g L}^{-1}$  and 31–350  $\mu\text{g L}^{-1}$  with detection limits of 25 and 8  $\mu\text{g L}^{-1}$  for iron (II) and iron (III), respectively. The relative standard deviations (R.S.D.,  $n = 6$ ) were lower than 4.2%. The enhancement factor of 162 and 125 were obtained for  $\text{Fe}^{3+}$  and  $\text{Fe}^{2+}$  ions, respectively. The procedure was applied to power plant drum water and several potable water samples; and accuracy was assessed through the recovery experiments and independent analysis by graphite furnace atomic absorption spectrometry.

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## 1. Introduction

Speciation of iron in water samples is very important from the environmental point of view. The oxidation state of iron in an environment can indicate its electrical potential and microbial activity [1]. Thus, the demand of the method development for determination of  $\text{Fe}^{2+}$  and  $\text{Fe}^{3+}$  as the most important iron species in water samples has been increased [2]. According to the literature survey, different techniques such as spectrophotometry [3–5], capillary electrophoresis [6], inductively coupled plasma optical emission spectrometry [7,8], inductively coupled plasma mass spectrometry [9], voltametry [10], flame atomic absorption spectrometry (FAAS) [11], polarography [12] and chemiluminescence [13] have been used for the speciation of iron. However, for the determination of extremely low concentration of iron species, a separation and preconcentration step is usually required. Various methods such as stripping voltametry [14] liquid–liquid extraction [15,16,7], solid phase extraction [11,17–19], co-precipitation [20] and cloud point extraction [3,21] have been used for this purpose. In recent years, the liquid phase microextraction techniques have received a

growing attention due to their simplicity, low consumption of organic solvents, low cost, ease of the operation and possibility of obtaining high enrichment factors [22].

In 2006 and 2007 two new liquid–liquid microextraction techniques, namely dispersive liquid–liquid microextraction (DLLME) [23] and solidified floating organic drop microextraction (SFODME) [24] were introduced for the purpose of separation and preconcentration of various analytes. DLLME is based on a ternary solvent system in which a mixture of extracting and disperser solvents is rapidly injected into an aqueous sample containing the analytes of interest, causing the formation of a cloudy solution. The advantages of this method are simplicity, rapidity, low cost, low organic solvent volume, high recovery and high enrichment factor. However, one of its drawbacks is the limitation of the choice of the extraction solvents as it must be a high-density water immiscible solvent. In the SFODME method, a droplet of an immiscible solvent with a melting point of 10–30 °C is floated on the surface of an aqueous sample containing the analytes. The mixture is agitated in order to maximize the contact area between the two solutions. The sample vial is then placed in an ice bath until the organic drop is solidified. The droplet is then easily removed and the amount of analytes in the melted drop is determined. This method had formerly been used for the extraction of metal ions [25–27] and the organic compounds [24] from water samples. In 2008, Leong and

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Huang [28] reported a novel variation of SFODME called DLLME-SFO; this method is based on the principle of DLLME and SFODME, i.e. instead of maintaining one droplet of the extraction solvent on the surface of the sample, a mixed solution of the extracting solvent with a melting point near to the room temperature and the dispersive solvent is rapidly injected into the sample producing fine dispersed droplets. This brings about a vast contact area between the extraction solvent and the sample which caused faster mass transfer and shorter extraction time. Like SFODME, the DLLME-SFO has the advantages of speed, simplicity, high efficiency, low cost, simple extraction apparatus and consumption of very small amounts of low-toxic organic solvents. In addition, the extraction time of DLLME-SFO is even shorter than the SFODME. The DLLME-SFO has been used for the determination of various organic [29–33] and inorganic [34–36] analytes.

Although the spectrophotometric techniques are known as simple, low cost, rapid and sensitive methods of analysis, they often lack the required selectivity for simultaneous determination of analytes whose absorption spectra overlap. During the past two decades, it had been attempted to solve this problem through the combination of chemometric methods with spectrophotometric determination. It is to be noted that multivariate calibration chemometric methods such as partial least squares (PLS) and principal component regression (PCR) can only be applied to linear systems, whereas artificial neural networks (ANNs) are more convenient for resolving the problem in nonlinear systems. The theoretical aspects of the artificial neural networks are described by Zupan and Gasteiger [37]. The most popular method for data compression in chemometrics is the principal component analysis (PCA). In practice, for the multicomponent analysis, principle components (PCs) are often successfully used as the inputs of ANNs. PCs can reduce the number of inputs to a network which results in the reduction of the training time and the repetition of the input data. Among the chemometric methods, the principle component artificial neural network (PC-ANN) is one of the most powerful methods in this field [38,39].

In this study, the possibility of the combination of DLLME-SFO and PC-ANNs for the separation/preconcentration and the spectrophotometric determination of iron species was considered and a rapid and selective method using 2-thenoyltrifluoroacetone (TTA) as the complexing agent and the chromogenic reagent was developed.

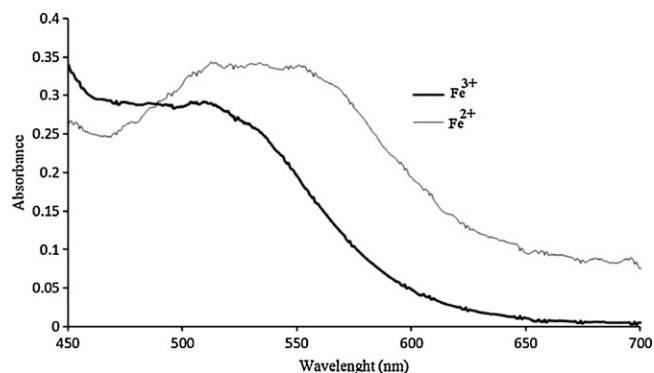
## 2. Experimental

### 2.1. Reagents and chemicals

All of the chemicals used were of analytical reagent grade obtained from Merck (Darmstadt, Germany). All the solutions were prepared with doubly distilled water.  $1000 \text{ mg L}^{-1} \text{ Fe}^{2+}$  was prepared by dissolving 0.7021 g of  $\text{Fe}(\text{NH}_4)_2(\text{SO}_4)_2 \cdot 6\text{H}_2\text{O}$  in  $0.1 \text{ mol L}^{-1} \text{ H}_2\text{SO}_4$  in a 100 mL volumetric flask.  $\text{Fe}^{3+}$  was prepared by dissolving 0.4333 g of  $\text{Fe}(\text{NO}_3)_3$  in 2%  $\text{HNO}_3$  in a 100 mL volumetric flask. Diluted working solutions were prepared daily from the stock solutions. A stock solution of acetate buffer ( $0.1 \text{ mol L}^{-1}$ ) was prepared by dissolving appropriate amounts of sodium acetate and acetic acid solutions in distilled water and adjusting the pH to 4.8. The stock thenoyltrifluoroacetone (TTA) solution ( $0.16 \text{ mol L}^{-1}$ ) was prepared by dissolving an appropriate amount of TTA in 5 mL of 1-undecanol.

### 2.2. Apparatus and software

An Avantes photodiode array spectrophotometer model, AvaSpec-2048, equipped with a source model of



**Fig. 1.** Absorption spectra of  $400 \mu\text{g L}^{-1}$  of  $\text{Fe}^{2+}$  and  $150 \mu\text{g L}^{-1}$  of  $\text{Fe}^{3+}$  at optimum conditions: sample volume, 60.0 mL; disperser solvent, 800  $\mu\text{L}$ ; extraction solvent, 90  $\mu\text{L}$ ; pH, 4.8; [TTA], 0.16  $\text{mol L}^{-1}$ .

AvaLight-DH-S-BAL and a 10-mm micro flow cell, an Ismatic peristaltic pump model, MS-REGLO/8-100 (Switzerland), and a rotary injection valve (Rheodyne, CA, USA) with 100  $\mu\text{L}$  loop were used. A Pentium 4 personal computer was applied for controlling the spectrophotometer and collecting data. All the spectra measurements were performed against a reagent blank solution. All the pH measurements were done with a Metrohm 691 pH meter using a combined glass calomel electrode. The centrifuge (Hitachi, Universal 320, Tuttlingen, Germany) was used for the phase separation. The PCA program for the determination of the necessary principle components was written in Matlab according to the algorithm described by Martens and Naes [40]. The ANNs calculations were performed using Net Toolbox in Matlab 7.0.

### 2.3. Procedures

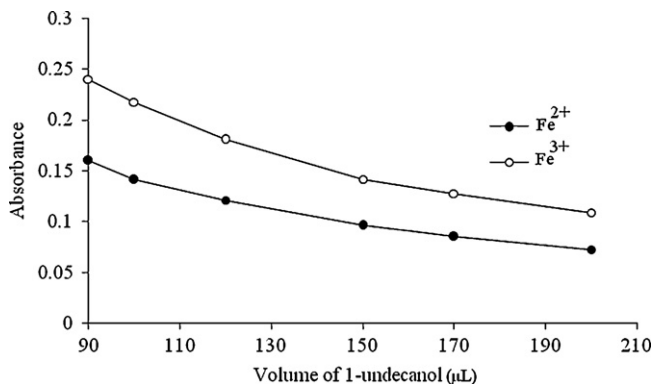
The pH of 60 mL of the sample solution was adjusted to 4.8 using acetate buffer and then it was transferred into a 100 mL vial. A mixture of 90  $\mu\text{L}$  1-undecanol (extraction solvent) containing  $0.16 \text{ mol L}^{-1}$  of TTA as the complexing agent and 800  $\mu\text{L}$  ethanol (dispersive solvent) was rapidly injected into the aqueous sample containing  $\text{Fe}^{2+}$  and  $\text{Fe}^{3+}$ . At this stage, a cloudy solution containing many dispersed fine droplets of TTA in 1-undecanol was formed; iron ions interacted with TTA and extracted into 1-undecanol in a few seconds. The cloudy solution was then centrifuged for 3 min at 2500 rpm; the organic solvent droplets floated on the surface of the aqueous solution due to their low density. The vial was transferred into an ice bath and the organic solvent was solidified after 5 min. Then the solidified solvent was transferred into a conical vial where it melted immediately. In order to decrease the viscosity of organic phase, 40  $\mu\text{L}$  ethanol was added. Finally, the loop of a flow injection system was filled with 100  $\mu\text{L}$  of target phase and was transferred into the flow cell of spectrophotometer equipped with a CCD detector and its absorbance was measured in the wavelength region of 450–600 nm against the reagent blank. The spectra were analyzed by the PC-ANNs method for simultaneous determination of  $\text{Fe}^{2+}$  and  $\text{Fe}^{3+}$ .

## 3. Results and discussion

In this study, a combination of PC-ANNs and DLLME-SFO was developed for the simultaneous preconcentration and spectrophotometric determination of iron species. The preliminary experiments indicated that  $\text{Fe}^{2+}$  and  $\text{Fe}^{3+}$  form colored complexes with TTA that quickly extract into the fine droplets of the 1-undecanol. However, the spectra of these complexes against the reagent blank (Fig. 1) showed strong overlap in the region of 450–600 nm so that direct spectrophotometric determination of

**Table 1**  
Properties of common extraction solvents for the DLLME-SFO method.

Extraction solvent	Density (g mL <sup>-1</sup> )	Boiling point (°C)	Melting point (°C)
1-Undecanol	0.83	243	13–15
n-Hexadecane	0.77	287	18
1,10-Dichlorodecane	0.99	140–142	14–16



**Fig. 2.** Effect of the volume of extraction solvent on the extraction of Fe<sup>2+</sup>-TTA and Fe<sup>3+</sup>-TTA complexes. Conditions: sample volume, 60.0 mL; disperser solvent, 800 µL; pH, 5.5; [Fe<sup>2+</sup>], 750 µg L<sup>-1</sup>; [Fe<sup>3+</sup>], 290 µg L<sup>-1</sup>; [TTA], 0.05 mol L<sup>-1</sup>.

the individual compound is not possible. Nevertheless, this problem may be solved by combination of chemometric analysis with the spectrophotometric determination. Thus, in order to obtain a high enrichment factor, the parameters affecting the extraction of each iron species were optimized initially. Then at the optimum conditions the PC-ANNs as a suitable chemometrics method was applied for the analysis of the overlapping spectra for simultaneous spectrophotometric determination of Fe<sup>2+</sup> and Fe<sup>3+</sup> in the mixtures.

### 3.1. Optimization of extraction conditions

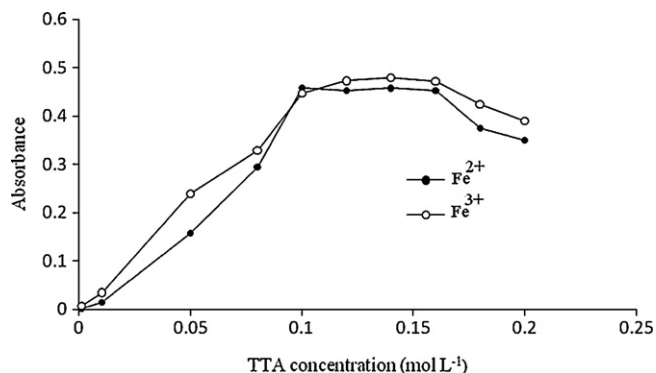
#### 3.1.1. Effects of the nature and volume of the extraction solvent

One of the most important factors affecting the extraction efficiency and enrichment factor of the metal complexes in DLLME-SFO is the nature of organic solvent and its volume. Several extracting solvents including 1-undecanol, n-hexadecane and 1,10-dichlorodecane were investigated. Table 1 illustrates the properties of the solvents. The experiments were performed by using 100 µL of each extracting solvent and 800 µL of ethanol (as the disperser solvent). The signals obtained with 1-undecanol for both iron species were higher than the other extracting solvents. Therefore, 1-undecanol was chosen for further experiments.

In order to select the optimum volume of extraction solvent, several experiments were performed using 800 µL of ethanol and different volumes of 1-undecanol. It was observed that by increasing the volume of 1-undecanol from 90 to 200 µL, the volume of the sediment phase increases from 67 to 155 µL while the absorbance was decreases accordingly (Fig. 2). A volume of less than 90 µL of 1-undecanol resulted in a sediment volume less 60 µL which was insufficient for determination by the designed flow injection system. Thus, in order to have a high enhancement factor and good repeatability, 90 µL of 1-undecanol was selected as the optimum volume of the extracting solvent.

#### 3.1.2. Effects of the nature and volume of the disperser solvent

The main criterion for disperser solvent in DLLME-SFO is its miscibility with both water and the extraction solvent. In this study acetone, methanol and ethanol were evaluated as disperser solvents. With 90 µL of 1-undecanol and 800 µL of each disperser



**Fig. 3.** Effect of TTA concentration on the extraction of Fe<sup>2+</sup>-TTA and Fe<sup>3+</sup>-TTA complexes. Conditions: sample volume, 60.0 mL; disperser solvent, 800 µL; extraction solvent, 90 µL; pH, 5.5; [Fe<sup>2+</sup>], 750 µg L<sup>-1</sup>; [Fe<sup>3+</sup>], 290 µg L<sup>-1</sup>.

solvent, the analytical signals for both iron species with ethanol were comparable with that obtained with methanol and were about 10% higher than that obtained with acetone. So, because of its lower toxicity, ethanol was selected as the disperser solvent.

The effect of the volume of disperser solvent on the extraction recovery was also considered. Variation in the ethanol volume changes the final volume of the extraction solvent. Thus, the effects of various volumes of ethanol were investigated at the fixed volume of 1-undecanol (90 µL). It was observed that by using 750–850 µL of ethanol as a disperser solvent the volume of the solidified phase remains constant (67 ± 2 µL), while when the volume of ethanol is less than 400 µL, the stable cloudy solution is not formed and with the volume greater than 850 µL, the extraction efficiency is decreased due to the increase in solubility of the complex in aqueous phase. Therefore, in order to achieve a stable cloudy solution and high recovery, 800 µL of ethanol was selected as optimum volume of disperser solvent.

#### 3.1.3. Effect of the TTA concentration

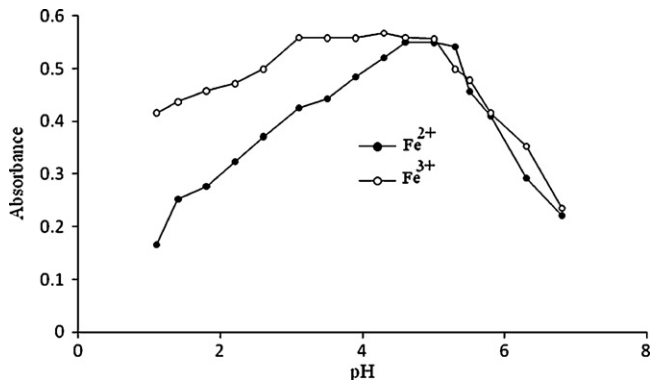
The extraction efficiency for iron species was dependent on the TTA concentration as shown in Fig. 3. The analytical signal was increased by increasing the TTA concentration up to 0.10 and 0.14 mol L<sup>-1</sup> for Fe<sup>2+</sup> and Fe<sup>3+</sup>, respectively and remained constant up to 0.16 mol L<sup>-1</sup> of TTA. Further increase in TTA concentration, however, resulted in a slight decrease of the analytical signals. This might be due to the saturation of organic phase with TTA which resulted in the back extraction of the complexes into the aqueous phase. So, in order to minimize the effect of the interferences, 0.16 mol L<sup>-1</sup> of TTA was selected as the optimum concentration for further studies.

#### 3.1.4. Effect of the sample pH

The pH of the aqueous phase has a unique role in the extraction efficiency as it affects the metal-chelate formation and its subsequent extraction. The effect of the pH on the extraction of iron complexes was investigated in the pH range of 1.0–7.0. Fig. 4 shows that the absorbance of Fe<sup>2+</sup> and Fe<sup>3+</sup> complexes with TTA reaches a maximum in the pH range of 4.6–5.3 and 3.1–5.0, respectively. The decrease in signals at pH greater than 5 can be related to the hydrolysis of iron species, whereas the decreases at low pH may be related to the competition of proton with iron species for complexation with TTA. Thus, a pH of 4.8 was chosen as the optimum pH for the simultaneous extraction of both iron species.

#### 3.1.5. Extraction time

Extraction time is another factor affecting the extraction efficiency and speed of the analysis. The extraction time was considered as the time interval between the injection of the



**Fig. 4.** Effect of pH on the extraction of  $\text{Fe}^{2+}$ -TTA and  $\text{Fe}^{3+}$ -TTA complexes. Conditions: sample volume, 60.0 mL; disperser solvent, 800  $\mu\text{L}$ ; extraction solvent, 90  $\mu\text{L}$ ; [TTA], 0.16 mol  $\text{L}^{-1}$ ; [ $\text{Fe}^{2+}$ ], 750  $\mu\text{g L}^{-1}$ ; [ $\text{Fe}^{3+}$ ], 290  $\mu\text{g L}^{-1}$ .

ethanol/1-undecanol mixture and the beginning of centrifugation process. The effect of the extraction time on the extraction efficiency was examined by changing the extraction time from 0.5 to 5 min at constant experimental conditions. According to our observation, formation of adducts and their extraction into the organic phase were fast. Thus, time has no significant effect on the extraction efficiency.

### 3.1.6. Analytical performance

Calibration curves were constructed for  $\text{Fe}^{2+}$  and  $\text{Fe}^{3+}$  at the wavelength of 516 nm and 450 nm, respectively. The calibration

**Table 2**

Optimized parameters used for construction of PC-FFANNs in simultaneous determination of  $\text{Fe}^{2+}$  and  $\text{Fe}^{3+}$ .

Parameter	$\text{Fe}^{2+}$	$\text{Fe}^{3+}$
Number of layers	2	2
Input nodes (number of PCs)	3	3
Hidden nodes	3	4
Output nodes	1	1
Update weight function algorithm	Levenberg Marquardt	Levenberg Marquardt
Hidden layer transfer function	Radbas	Logsig
Output layer transfer function	Purelin	Purelin
Number of epochs	34	48
Performance ratio	0.0015	0.00004

graph for  $\text{Fe}^{2+}$  was linear in the range of 95–1070  $\mu\text{g L}^{-1}$  and the equation was  $A = 0.0006C + 0.1036$  ( $R^2 = 0.9988$ ), whereas for  $\text{Fe}^{3+}$  the calibration graph was linear in the range of 31–350  $\mu\text{g L}^{-1}$  and the equation was  $A = 0.0017C + 0.0735$  ( $R^2 = 0.9987$ ), where  $A$  is the absorbance and  $C$  is the concentration value ( $\mu\text{g L}^{-1}$  level in aqueous phase). The limits of detection (LOD) defined as the ratios of the three times of the standard deviations of the blank signals over the slope of the calibration curves were found to be 25 and 8  $\mu\text{g L}^{-1}$  for  $\text{Fe}^{2+}$  and  $\text{Fe}^{3+}$ , respectively. The relative standard deviations ( $n=6$ ) at 540  $\mu\text{g L}^{-1}$  of  $\text{Fe}^{2+}$  and 290  $\mu\text{g L}^{-1}$  of  $\text{Fe}^{3+}$  were found to be 4.2% and 3.9%, respectively. The enhancement factors defined as the ratio of the slope of the calibration curve with and without preconcentration were found to be 125 and 162 for  $\text{Fe}^{2+}$  and  $\text{Fe}^{3+}$ , respectively.

**Table 3**

Actual, prediction and RMSEP values for training, validation and test data sets.

Training set				Validation set				Test set			
$\text{Fe}^{2+}$ ( $\mu\text{g L}^{-1}$ )		$\text{Fe}^{3+}$ ( $\mu\text{g L}^{-1}$ )		$\text{Fe}^{2+}$ ( $\mu\text{g L}^{-1}$ )		$\text{Fe}^{3+}$ ( $\mu\text{g L}^{-1}$ )		$\text{Fe}^{2+}$ ( $\mu\text{g L}^{-1}$ )		$\text{Fe}^{3+}$ ( $\mu\text{g L}^{-1}$ )	
Actual	Predict	Actual	Predict	Actual	Predict	Actual	Predict	Actual	Predict	Actual	Predict
1070	1075.9	70	67.185	95	92.357	350	348.88	310	316.68	115	121.4
95	91.69	31	30.698	160	165.29	70	66.823	540	529.3	70	72.015
540	540.93	180	176.95	540	521.28	31	35.09	310	294.81	290	304.87
160	162.78	31	29.617	160	166.66	250	248.07	95	100.15	115	114.36
1070	1068.3	31	33.013	1070	1091	250	242.68	540	522.78	350	358.03
160	155.71	290	292.09	760	760.5	250	247.81	1070	1095.5	350	352.35
1070	1050.9	290	290.69	95	94.756	70	67.919				
95	90.414	180	182.98								
160	168.22	115	117.65								
310	298.03	31	31.953								
760	766.87	70	65.604								
540	542.28	115	117.82								
310	315.91	250	249.74								
95	98.684	250	245.33								
760	755.43	31	31.871								
760	764.88	180	180.43								
1070	1071.4	180	181.67								
310	307.7	350	350.77								
95	88.039	290	291.02								
1070	1079.9	115	112.1								
160	169.26	350	350.02								
310	308.58	70	67.488								
540	531.19	290	289.04								
310	301.59	180	182.01								
760	761.05	350	348.46								
540	538.38	250	249.5								
160	164.26	180	176.91								
760	758.15	115	119.39								

RMSEP		Validation set		Test set	
$\text{Fe}^{2+}$	$\text{Fe}^{3+}$	$\text{Fe}^{2+}$	$\text{Fe}^{3+}$	$\text{Fe}^{2+}$	$\text{Fe}^{3+}$
6.6	2.3	11.2	3.7	15.1	7.5

### 3.2. Multivariate calibration

After optimizing the extraction conditions, multivariate calibration techniques were used to analyze the spectra of Fe<sup>2+</sup> and Fe<sup>3+</sup> species for their simultaneous determination. In order to make a suitable model, 41 calibration samples of binary mixtures were designed. The concentration levels for the analytes were as follows: Fe<sup>2+</sup> (95, 160, 310, 540, 760 and 1070 µg L<sup>-1</sup>); and Fe<sup>3+</sup> (31, 70, 115, 180, 250, 290 and 350 µg L<sup>-1</sup>). Then different multivariate calibration techniques including PLS1, PLS2, PCR and PC-ANNs were tested for the construction of calibration model in the spectral region between 450 and 600 nm. Initial calculations indicated that with a linear technique (PLS1, PLS2 or PCR), the construction of suitable model was not possible; whereas the nonlinear model of PC-ANNs gave acceptable responses. So, PC-ANNs were selected for further studies. To do the PC-ANNs analysis, the 41 calibration samples were randomly divided into three sets; training set (containing 28 trials), validation set (containing 7 trials) and test set (containing 6 trials), and then the parameters affecting the PC-ANNs were optimized.

#### 3.2.1. Optimizing the network variables in PC-FFANN

Determination of the optimum values for parameters affecting ANN is very important. The ANN with a layered structure is a mathematical system that stimulates the biological neural network, containing neurons (computing units) and synapses (connections between neurons). An ANN consists of several neuron layers including input, output, and at least one hidden layer. Synapses connect input neurons to the hidden ones and hidden neurons to the output ones. The strength of the synapse from one neuron to the next is determined by means of the weight. Each neuron from the hidden and output layers is associated with a real value named the neuron's bias and a nonlinear function named the transfer or activation function. Feed forward artificial neural networks (FFANNs) as one of the most popular types of ANNs were selected for this study. In FFANNs the information moves only in the forward direction without any cycles or loops, that is, from the input nodes through the hidden one to the output nodes. In this work, a three-layer network was designed for each FFANN. Furthermore, in order to decrease the number of the inputs, a principle component analysis (PCA) technique was applied on the spectrum data and the proper number of PCs was selected. Then before training the networks, the input and output values were normalized between -1 and +1 and the Levenberg Marquardt was selected as the update weight function algorithm [41]. The network was then trained using the training set by the back propagation strategy for optimization of the weights and the bias values. The important parameters such as the type of the transfer function and the number of the inputs were optimized according to the root mean square error (RMSE) values for each item. The results of the optimization of PC-FFANNs parameters are summarized in Table 2.

The root mean square errors of prediction (RMSEP) for each set were calculated for the calibration test set by the following equation:

$$\text{RMSEP} = \sqrt{\frac{\sum_{i=1}^n (\hat{y}_i - y_i)^2}{n}}$$

where  $\hat{y}_i$  and  $y_i$  are the desired output and the actual output sets respectively, and  $n$  is the number of the samples in each set. The actual, prediction and RMSEP values of binary mixtures for training, validation and test data sets are summarized in Table 3.

**Table 4**

Effect of diverse interfering species on the recovery of iron: at concentrations of 540 and 290 µg L<sup>-1</sup> of Fe<sup>2+</sup> and Fe<sup>3+</sup>, respectively.

Interfering species	Molar ratio (ion/Fe <sup>3+</sup> )	Recovery (%) <sup>a</sup>	
		Fe <sup>2+</sup>	Fe <sup>3+</sup>
K <sup>+</sup>	1000	95.2 ± 4.4	97.1 ± 3.4
Na <sup>+</sup>	1000	97.7 ± 5.3	98.0 ± 5.5
Ba <sup>2+</sup>	1000	96.8 ± 4.1	99.5 ± 2.1
Mg <sup>2+</sup>	1000	98.2 ± 3.3	102.3 ± 3.5
Ca <sup>2+</sup>	1000	104.5 ± 3.6	104.5 ± 5.4
Al <sup>3+</sup>	1000	97.1 ± 6.1	95.9 ± 5.8
Cu <sup>2+</sup>	800	96.0 ± 5.3	96.7 ± 4.6
Pb <sup>2+</sup>	1000	93.3 ± 3.2	95.6 ± 6.2
Zn <sup>2+</sup>	700	106.6 ± 3.9	97.8 ± 4.8
Cr <sup>3+</sup>	600	95.4 ± 3.9	96.1 ± 4.4
Cd <sup>2+</sup>	1000	103.6 ± 4.5	105.3 ± 5.5
SO <sub>4</sub> <sup>2-</sup>	1000	104.1 ± 3.2	96.0 ± 5.2
ClO <sub>4</sub> <sup>-</sup>	1000	104.4 ± 5.7	105.3 ± 5.2
CO <sub>3</sub> <sup>2-</sup>	1000	98.8 ± 4.1	99.9 ± 4.0
CH <sub>3</sub> COO <sup>-</sup>	1000	98.9 ± 4.3	102.0 ± 4.5
NO <sub>3</sub> <sup>-</sup>	1000	95.3 ± 5.5	103.3 ± 6.4
PO <sub>4</sub> <sup>3-</sup>	600	98.5 ± 5.9	104.4 ± 3.1
Cl <sup>-</sup>	800	96.9 ± 2.3	97.9 ± 4.3
Br <sup>-</sup>	1000	95.1 ± 3.3	95.8 ± 4.8
Tartrate	500	97.0 ± 4.3	96.2 ± 4.1
Urea	500	98.2 ± 3.6	95.1 ± 3.6
Citrate	30	98.9 ± 3.3	94.7 ± 4.6
Oxalate	10	99.0 ± 4.4	95.1 ± 4.5
EDTA	3	95.1 ± 5.3	94.8 ± 3.6

<sup>a</sup> Results are mean and standard deviation of three independent measurements.

### 3.3. Interference study

The selectivity of the method was evaluated for the simultaneous determination of Fe<sup>2+</sup> and Fe<sup>3+</sup> in the presence of common foreign ions in water metrics. The effect of different inorganic ions and organic species was studied on simultaneous determination of 540 and 290 µg L<sup>-1</sup> of Fe<sup>2+</sup> and Fe<sup>3+</sup>, respectively. A relative error of less than ±5% was considered to be within the range of the experimental error. The results of this investigation have been summarized in Table 4. It can be seen that the presence of the inorganic cations and anions at the given mole ratio has no significant influence on the determination of iron species under the optimum conditions, thus, the method can tolerate high concentration of inorganic species. However, the tolerance of the method to organic species, such as EDTA, which form relatively strong complex with iron is lower. It should be noted that in the sample type studied the concentration of such organic species is not significant.

### 3.4. Analysis of real and synthetic samples

The proposed method was applied for the determination of Fe<sup>2+</sup> and Fe<sup>3+</sup> in tap water, well water, mineral water and drum water of Yazd power plant. The samples were first filtered through a Millipore 0.45 µm pore-size membrane into cleaned polyethylene bottles and were treated according to the given procedure. The accuracy of the method was verified by the analysis of the samples spiked with the known amount of Fe<sup>2+</sup> and Fe<sup>3+</sup>, and comparing the results with the data obtained by graphite furnace atomic absorption spectrometry (GFAAS). As indicated in Table 5, the recoveries of added iron were satisfactory (92.5–108.7%), and at 95% confidence limit there was no significant difference between the results of the developed method and GFAAS. Thus, the method is reliable for the determination of the iron species in the examined sample type.

**Table 5**  
Analytical results for determination of Fe<sup>2+</sup> and Fe<sup>3+</sup> in water samples.

Sample	Spiked ( $\mu\text{g L}^{-1}$ )		Found <sup>a</sup> ( $\mu\text{g L}^{-1}$ )		Recovery (%)		GFAAS <sup>a</sup> ( $\mu\text{g L}^{-1}$ ) Total Fe
	Fe <sup>2+</sup>	Fe <sup>3+</sup>	Fe <sup>2+</sup>	Fe <sup>3+</sup>	Fe <sup>2+</sup>	Fe <sup>3+</sup>	
Tap water	–	–	N.D. <sup>b</sup>	35 ± 3	–	–	36.2 ± 2.1
	600	200	618 ± 21	241 ± 11	103.0	103.0	
	750	100	739 ± 35	140 ± 7	98.5	105.0	
	350	200	366 ± 14	245 ± 12	104.6	105.0	
Well water	–	–	184 ± 11	41 ± 7	–	–	221.4 ± 8.6
	150	70	328 ± 15	109 ± 9	96.0	97.1	
	360	250	534 ± 34	297 ± 12	97.2	102.4	
	70	150	250 ± 18	204 ± 13	94.3	108.7	
Mineral water	–	–	N.D.	N.D.	–	–	3.1 ± 0.2
	350	40	330 ± 5	37 ± 2	94.3	92.5	
	200	100	197 ± 8	105 ± 4	98.5	105.0	
	250	200	249 ± 8	204 ± 7	99.6	102.0	
Power plant drum water	–	–	181 ± 5	65 ± 3	–	–	241.7 ± 4.3
	85	190	265 ± 5	254 ± 9	98.8	99.5	
	380	250	558 ± 10	325 ± 11	99.2	104.0	
	600	60	801 ± 23	127 ± 6	103.3	103.3	

<sup>a</sup> The results are mean of three measurements ± standard deviation.

<sup>b</sup> Not detected.

**Table 6**  
The analytical characteristics of some extractive methods for iron speciation.

Method	Complexing agent/species	EF <sup>a</sup>		Dynamic range ( $\mu\text{g L}^{-1}$ )		LOD <sup>b</sup> ( $\mu\text{g L}^{-1}$ )		%RSD		Extraction time (min)	Detector	Ref.
		Fe <sup>2+</sup>	Fe <sup>3+</sup>	Fe <sup>2+</sup>	Fe <sup>3+</sup>	Fe <sup>2+</sup>	Fe <sup>3+</sup>	Fe <sup>2+</sup>	Fe <sup>3+</sup>			
LLE	AMPC <sup>c</sup> /Fe <sup>3+</sup>	20	20	Up to 5000	Up to 5000	0.24	0.24	2.1	2.1	>5	FAAS	[42]
LLE	PAN <sup>d</sup> /Fe <sup>2+</sup>	12.5	–	25–150	250–3000	9	–	7	–	20	FAAS	[15]
DLLME	O-Phen <sup>e</sup> /Fe <sup>2+</sup>	10	10	25–1000	25–1000	7.5	7.5	1.2	1.2	<5	UV-Vis	[16]
CPE <sup>c</sup>	APDC <sup>f</sup> /Fe <sup>3+</sup>	50	50	Up to 100	Up to 100	3.5	3.5	1.8	1.8	10	FAAS	[3]
DLLME-SFO	TTA/Fe <sup>2+</sup> & Fe <sup>3+</sup>	125	162	95–1070	31–350	25	8	4.2	3.9	<1	UV-Vis	[This work]

<sup>a</sup> Enhancement factor.

<sup>b</sup> Limit of detection.

<sup>c</sup> 4-Acetyl-5-methyl-1-phenyl-1H-pyrazole-3-carboxylic acid.

<sup>d</sup> 1-(2-Pyridylazo)-2-naphthol.

<sup>e</sup> O-phenanthroline.

<sup>f</sup> Ammonium pyrrolidinedicarbodithioate.

### 3.5. Comparison with other methods

Determination of iron species in the water samples by the developed DLLME-SFO was compared with some extractive methods used for the determination of iron species and the outcome of which has been summarized in Table 6. As it is shown, the enhancement factor of proposed DLLME-SFO is higher and the extraction time is shorter than the other reported methods. Furthermore, most of these methods required an oxidizing or reducing agents for speciation of iron, whereas in the proposed method, Fe<sup>2+</sup> and Fe<sup>3+</sup> are extracted simultaneously and the speciation is affected by the chemometric method.

## 4. Conclusion

A selective, sensitive, simple, environmentally friendly and low cost DLLME-SFO method followed by determination with a fiber optic-linear photodiode array spectrometer was developed for iron speciation in water samples. PC-ANNs analysis was successfully applied for the analysis of the overlapping spectra and the determination of iron species. The method does not need the time consuming and labor-intensive steps of oxidation or reduction of iron species. The main benefits of the proposed method are simplicity, use of a common spectrophotometer instrument, enhancement of sensitivity, minimum organic solvent consumption, low cost as well as a high enrichment factor.

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