

Application of Response Surface Methodology and Genetic Algorithm for Optimization and Determination of Iron in Food Samples by Dispersive Liquid–Liquid Microextraction Coupled UV–Visible Spectrophotometry

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Received: 13 March 2017 / Accepted: 4 October 2017 / Published online: 24 October 2017
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Abstract A simple and facile method was developed for the determination of trace amount of iron. The method is based on the complex formation between Fe (III) and picrate anion in the presence of piroxicam, as a complexing agent. Dispersive liquid–liquid microextraction (DLLME) was applied to extract the formed ion associate, Fe (III)-piroxicam. The absorbance of the extracted iron in the sedimented phase was measured by UV–Vis spectrophotometry. Two statistical methods of response surface methodology and genetic algorithm (GA) based on artificial neural network (ANN) were employed for prediction and optimization of a four-constituent DLLME. Plackett–Burman design was used for screening the influential parameters including pH, the volume of picrate anion, disperser, and extraction solvents. Central composite design (CCD) was used to obtain the optimum levels in the proposed method. The experimentally obtained data were used to train the GA model. CCD and GA models were compared for their predictive abilities. The result showed that both models have the ability to predict the proposed process, but ANN model is more reliable than CCD. The absorbance of the extracted iron obeys Beer's law in the range of 0.03–0.96 $\mu\text{g mL}^{-1}$ ($R^2 = 0.998$), and the limit of detection of 0.008 $\mu\text{g mL}^{-1}$ and enhancement factor of 88.84 were achieved for the process. The developed

procedure was successfully applied to the determination of iron in water samples and two types of common vegetation sample, i.e., tea and mint.

Keywords Artificial neural network · Genetic algorithm · Central composite design · Iron (III) · Dispersive liquid–liquid microextraction · UV–Vis spectrophotometry · Food samples

1 Introduction

One of the most significant and essential elements in environmental and biological systems is iron. It plays a leading role in hemoglobin, myoglobin, catalase, and other proteins responsible for oxygen and electron transfer. Its absence in any living organism causes anemia, a disease which is treated with iron salts. Interestingly enough, the excess of the element during treatment may lead to serious poisoning [1]. Chemical properties such as valence, solubility, and the degree of chelating are the most important factors in the environmental effectiveness of iron, which exists in many oxidation states (–II to +VI). The most prevalent forms of dissolved Fe in natural water are Fe (II) and Fe (III). In this regard, Fe (II) oxidizes in oxygen-rich environments and Fe (III) makes stronger complexes. The World Health Organization (WHO) states that the permissible value of iron in drinking water ranges from 0.3 to 3.0 mg L^{-1} [2]. For instance, tea as one of the most popular beverages and mint as one of the most useful medicinal plants are found in many households. Based on the medical research, excessive drinking of tea exactly after eating could cause anemia. Consequently, determination of iron species in environmental and biological systems and vegetation is definitely essential due to its direct effect on health. According to the literature sur-

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vey, different techniques such as spectrophotometry [3–5], inductively coupled plasma optical emission spectrometry [6,7], capillary electrophoresis [8], voltammetry [9], and flame atomic absorption spectrometry [10] have been applied for the determination of iron. Generally, a preconcentration step is required for the determination of the low concentration of iron. Different methods such as solid-phase extraction [11], co-precipitation [12], and cloud point extraction [13] have been used for this purpose. Recently, researchers have focused on the development of techniques using efficient and low-cost organic solvents as well as the ease of operation and low consumption of organic solvents [14]. Consequently, various microextraction systems such as dispersive liquid–liquid microextraction (DLLME) have been developed for preconcentration and determination of both organic and inorganic samples [15,16]. DLLME is a miniaturized sort of liquid–liquid extraction (LLE) in which microliter volumes of extraction solvent are used. An appropriate mixture of the extraction solvent and the dispersive solvent is injected into the aqueous sample. As a result of this injection, a cloudy solution is formed. This solution is then exposed to centrifugation, and the fine droplets are settled at the bottom of the conical test tube. The interest analyte is extracted from the initial solution and concentrated to a small volume of the settled phase [17–19]. However, due to the difficulties in measuring the small volume of the settled phase, the number of studies in which DLLME is combined with UV–Vis spectrophotometry is limited. In recent years, the problem dealing with measuring the absorbance of relatively small volumes in UV–Vis spectrophotometry has often been tackled with the evaporation or dilution of sedimented phase as well as using special instruments such as fiber optic-linear array detection spectrophotometry (FO-LADS) or digital colorimetry (DC) [20]. Dealing with this practical problem, spectrophotometry as a simple, low-cost, rapid, and sensitive technique has been applied for DLLME determination of Rhodamine 6G [21], cadmium and copper [22], iron [2], and cobalt [23].

In order to optimize the effective parameters in DLLME and other techniques in analytical chemistry, mostly one-factor-at-a-time approach has been used in which the interaction between the effective parameters of the process is neglected. Besides, a larger amount of time and more reagents are required. In recent years, the use of chemometric methods such as response surface methodology (RSM) based on the experimental designs has resolved such major problems [24]. The use of a reliable model is very important to optimize the process. RSM consists of a group of mathematical procedures to study the relationship between a number of variables and one or more responses. Central composite design is an effective optimization process. A full factorial CCD was applied to optimize the effective parameters on microextraction. In 2017, Habila and coworkers presented a new method for determination of lead. In this method, DLLME was used for

preconcentration of lead in aqua solutions using 2,20 dithio-bis (benzothiazole) as complexing agent. Plackett–Burman was utilized to study the impact of analytical parameters on DLLME recovery. The limit of detection was found to be 4.3 mg L^{-1} [25]. In another study, a novel vortex-assisted dispersive liquid–liquid microextraction method for the preconcentration and separation of copper(II) using 1-(2-pyridylazo)-2-naphthol using as chelating agent has been reported [26]. These studies show the high potential of DLLME in separation and preconcentration of trace amount of heavy metals.

Lutfullah Sharma presented a method for determination of Fe (III) in which piroxicam was able to form a colored complex with Fe (III) with K_f equal to 6.12×10^{10} [27]. Also, azmi reported another study for determination of piroxicam by using Fe (III) [28]. Based on the Lutfullah and azmis studies, piroxicam was chosen as a proper complexing agent to determine the concentration of Fe (III). Accordingly, ion association can increase the sensitivity of the method. Thus in this work, a DLLME coupled with UV–Vis spectrophotometry was used for preconcentration and determination of the trace amount of iron (III) which is based on the formation of ion associate between Fe (III)-piroxicam and picrate anion.

To promote the previous studies on iron, spectrophotometry as a simple technique was applied to determine trace amount of iron. The impact of various parameters on DLLME was studied by Plackett–Burman design. Then, central composite design (CCD) was used to optimize the significant factors including sample pH, the volume of the disperser, extraction solvents, and picrate anion.

2 Experimental

2.1 Reagents and Solutions

A stock standard solution of Fe (III) at the concentration of $0.1 \text{ } \mu\text{g L}^{-1}$ was prepared by dissolving analytical grade of $\text{Fe}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}$ (Merck) in doubly distilled water. The standard solutions were prepared daily by the stepwise dilution of stock solution. All chemicals such as chloroform, carbon tetrachloride, acetonitrile, dichloromethane, methanol, ethanol, and hydrochloric acid, with the purity of higher than 99%, were purchased from Merck. Hydrochloric acid (1.0 mol L^{-1}), acetate, and phosphate buffer were used to adjust the pH of the solutions.

2.2 Apparatus

The absorbance measurement and spectra recording were carried out on a UV–Vis Ultrospec 3100 pro with a 1.00-cm quartz microcell and the personal computer with swift II soft-

ware. Inductively coupled plasma (Perkin-Elmer, OPTIMA 7300-DV) was used to check the reliability of the method by determination of the concentration of iron ions in real samples. The pH values were controlled by a Jenway 3300 pH meter with a glass combined electrode. Also, in order to accelerate the separation process, a Sigma 3K30 model centrifuge with 15-mL centrifuge tubes was used.

2.3 Experimental Designs Strategy

In the present study, two statistical methods of response surface methodology (RSM) and genetic algorithm (GA) based on artificial neural network (ANN) were employed for prediction and optimization of a four-constituent DLLME. The experimental designs plan was used in two stages: (1) considering the most important factors affecting the extraction efficiency using Plackett–Burman design and (2) optimizing the significant factors applying a CCD. This method can reduce the number of the experiments to study the effect of parameters and their interaction. Then, the results were analyzed using MINITAB 16 software.

2.4 Procedure

Eight milliliters of Fe (III) standard solution ($0.1 \mu\text{g mL}^{-1}$) was transferred to a 15-mL test tube with conical bottom, and pH was adjusted to 4.2. One milliliter of piroxicam solution (0.001 mol L^{-1}) and $71 \mu\text{L}$ picrate anion were added into this sample solution to form ion association. A mixture of $136 \mu\text{L}$ of chloroform (as extracting solvent) and $531 \mu\text{L}$ of ethanol (as dispersive solvent) was rapidly injected into the sample tube using a 2.0-mL Hamilton syringe. Immediately, fine droplets of extraction solvent were dispersed in the aqueous phase as a result of which a cloudy solution was produced. This mixture was centrifuged for 2 min at 4000 rpm so that the analytes are concentrated to a small volume of the settled phase [23,28,29]. About $40 \mu\text{L}$ of the sedimented phase was withdrawn using a Hamilton syringe. At the end, it was inserted into the quartz microcell to measure the absorbance.

2.5 Preparation of Water Sample

Tap water was prepared from Shahrekord University, and well water was obtained by a well located in a personal farm in Najafabad, Isfahan. Two drops of nitric acid were added to the water samples. The samples were filtered through a cellulose membrane filter of pore size $0.45 \mu\text{m}$ and adjusted to pH 4.2. A defined volume treated according to the procedure mentioned in Sect. 2.3.

2.6 Determination of Iron in Vegetation

Tea was purchased from a local supermarket in Shahrekord. Mint was also purchased from a local greengrocer's. To prepare the samples, 1.0 g of each individual powdered sample weighed and was mixed with 20 mL of nitric acid solution into a clean glass beaker. The acid digestion was carried out by heating the mixture till nearly dryness. After cooling, deionized water was added to the residue; then, it was neutralized to pH 7 by sodium hydroxide solution. The solutions were separately filtered and diluted to 50 mL with distilled deionized water in a calibrated flask. After adjusting the pH to 4.2, the total iron content was determined by the proposed DLLME method.

3 Results and Discussion

3.1 Reaction Mechanism

Previous studies have shown that the use of picrate as the counter ion increased the sensitivity and selectivity of the spectrophotometric determination. The introduction of a third component into a binary complex leads to formation ion association system. So, in this study picrate anion was selected to form ion association. The Fe (III)–piroxicam–picrate complex would more comfortably transfer into organic solvent. This is probably due to more solubility of iron (III)–piroxicam–picrate complex in organic solvents. Therefore, the analytical signal increased in the presence of picrate anion. Therefore, ion associate recombination seems to be the easiest way to extract and determine Fe (III). Since there are lots of factors affecting the extraction, the experimental design was applied to consider the important factors affecting the process.

3.2 Selection of the Extraction and Disperser Solvents

In DLLME, the extraction solvent should be denser than water in order to settle at the end of the tube easily. Moreover, it should have enough potential to extract compounds of interest, and the least solubility in water to minimize dissolution in the aqueous phase [15,18]. Thereby, chloroform, carbon tetrachloride, and dichloromethane were investigated.

The disperser solvent has to be miscible with both the extraction solvent and aqueous phase [17,29]. As a result, all combinations of extracting solvents and disperser solvents such as ethanol, methanol, and acetonitrile were tested. It should be noted that in the case of dichloromethane, no sedimented phase was observed. Both chloroform and carbon tetrachloride had enough sedimented phases, but the intensity of analytical signals increased in the former. This may be explained by the high extractability of the iron ion asso-



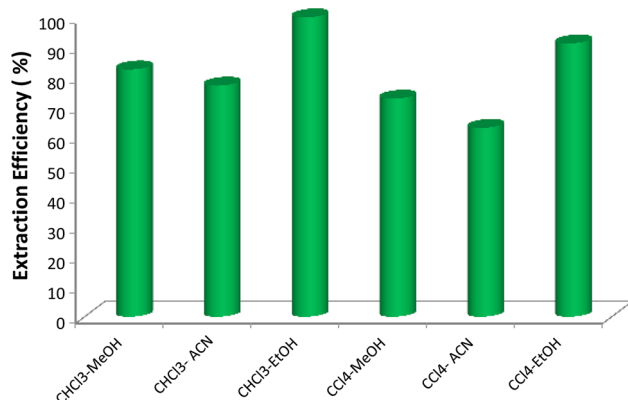


Fig. 1 The effect of the type of extraction–disperser solvent pair on absorbance, conditions: 8 ml of Fe (III) standard solution ($0.1 \mu\text{g L}^{-1}$), 1 ml of piroxicam solution (0.001 mol L^{-1}), $71 \mu\text{L}$ of picrate anion, $136 \mu\text{L}$ of chloroform (as extracting solvent) and $531 \mu\text{L}$ of ethanol (as dispersive solvent), pH:4.2

ciate in this solvent. Therefore, chloroform was chosen as the extraction solvent. Since acetonitrile was used as disperser solvent, the cloudy solution was not stable. Ethanol formed more stable two-phase systems, and it compares favorably with the intensity of the analytical signals. Thus, ethanol was chosen as dispersive solvent for the next experiments. The results indicated that the maximum absorbance was achieved by applying ethanol–chloroform as disperser–extraction solvents pair. The results are represented in Fig. 1.

3.3 Considering Significant Factors Using Plackett–Burman Design

First, experimental Plackett–Burman design was utilized for screening and considering the main parameters affecting the extraction. Since the interaction is ignored in this design, one can calculate the main effects with fewer experiments. In the present study, the effects of the following eight factors were studied: pH of the sample, the concentration of the chelating agent, extraction solvent volume, disperser solvent

volume, salt effect, extraction time, centrifugation speed, and the amount of picrate. Table 1 shows the levels of factors selected according to the outcomes of the preliminary experiments. Following this, twelve trials were done in duplicate and the results were listed.

The analysis of variance (ANOVA) method was utilized for the determination of main effects [30,31]. The data of Plackett–Burman design are indicated in Table 2. As it is represented, at 95% confidence level, centrifugation speed, the concentrations of piroxicam and salt as well as extraction time have no significant influence on the response (p -values ≥ 0.05). The results prove that DLLME method is time-independent technique. This independence is due to the large surface area between the extraction solvent and the aqueous phase after the formation of cloudy solution. Moreover, the greatest advantage of this technique is the high speed of the DLLME process. Other factors, such as pH, the volume of disperser, picrate, and extraction solution whose p values are less than 0.05, have noticeable impacts on the extraction efficiency. Hence, the aforementioned factors were considered in the next step of the design.

3.4 The Optimization of the Main Microextraction Parameters Using CCD

In the next step, CCD was conducted in order to determine the optimal conditions for the four screened significant factors. This design is one of the factorial designs for statistical analysis, and it has been previously reported for optimization of microextraction parameters [31]. The experimental design was performed by MINITAB software version 16.0. Table 3 reveals different variables, symbols, and levels. In the present CCD, thirty experiments were predicted. Four independent variables, i.e., the volume of dispersive solvent (L), extracting solvent (P), pH (E), and picrate volume (F), were studied at five levels at the central point. For each of the four variables, high and low points were selected to construction a full factorial design was used to study the effect of each

Table 1 The experimental variable and levels of the Plackett–Burman design

Variable	Symbol	Variable levels	
		Low	High
pH	E	3	6
Concentration of chelating agent (mol L^{-1})	G	10^{-4}	10^{-3}
Volume of picrate (μL)	F	10	100
Salt effect	M	0	10
Volume of dispersive solvent (μL)	L	100	800
Volume of extraction solvent (μL)	P	90	150
Time (s)	T	30	300
Centrifugation speed (r min^{-1})	S	2000	10,000

Table 2 Regression coefficients and their significances for the response of Plackett–Burman design

Term	Effect	Coefficient	Standard error	T	<i>p</i> -value ^a
Constant	–	0.3770	0.0067	56.22	0.000
E	–0.1875	–0.0937	0.0067	–13.98	0.000
G	–0.0225	–0.0112	0.0067	–1.68	0.114
F	–0.1858	–0.0929	0.0067	–13.85	0.000
M	–0.0175	0.0087	0.0067	–1.30	0.212
L	0.0475	0.0237	0.0067	3.54	0.003
P	0.1725	0.0862	0.0067	12.86	0.000
T	0.0191	–0.0095	0.0067	–1.43	0.164
S	0.0258	–0.01292	0.0067	1.93	0.073

^a Probability of seeing the observed “*F* value” if the null hypothesis is true

Table 3 Factors, their symbols and levels for CCD

Variable	Symbol	Level				
		– <i>a</i>	–1	0	+1	+ <i>a</i>
pH	E	1.8	3.3	4.8	6.3	7.8
Volume of picrate (μL)	F	0	40	80	120	160
Volume of dispersive (μL)	L	200	400	600	800	1000
Volume of extraction (μL)	P	60	90	120	150	180

parameter and their interaction. In the present CCD, thirty experiments were predicted.

Table 4 depicts the matrix and the corresponding responses. ANOVA was considered for evaluating the model and the significance of effects (Table 5). The results of the statistical analysis including the estimated regression coefficients and *p* values are calculated. The fact that all variables with the $0.05 \geq p$ values are significant reveals that the applied model has successfully made an ideal prediction for the process with a linear relationship between the variables. All variables with the *p* values of ≥ 0.05 should be removed from the proposed equation. The *F*-value of 30.66 implies that the model is significant [30,31]. There is no chance that this large model *F*-value could occur due to the noise. Moreover, the results indicated that *p* value for lack of fit (0.667) was not significant. The coefficient of determination (*R*² and adjusted-*R*²) was used for expressing the quality of the polynomial model. *R*² is an indicative factor for the amount of variations around the mean explained by the model which was equal to 96.95. The adjusted-*R*² is adjusted for the number of terms in the model, and it was equal to 93.69. The large adjusted *R*² value depicted a good relationship between the experimental data and the fitted model. The suggested model is given by Eq. (1). In this equation, *b*₀ is the intercept and the *b* terms represent those parameters of the model which are optimized iteratively to fit.

$$Y = b_0 + b_1E + b_2L + b_3P + b_4E^2 + b_5F^2 + b_6L^2 + b_7P^2 + b_8EF + b_9EL \tag{1}$$

In which:

$$b_0 = -1.57836 \quad b_1 = 0.239611 \quad b_2 = 0.935417$$

$$b_3 = 0.0187667 \quad b_4 = -0.0336111 \quad b_5 = -9.765 \times 10^{-6}$$

$$b_6 = -1.39062 \quad b_7 = -7.29 \times 10^{-5} \quad b_8 = 0.0003125$$

$$b_9 = 0.0625$$

The most relevant fitted response surfaces for the design are shown in Fig. 2. In each figure, two factors are constant at the central point and other ones are allowed to vary. The point is that the curvatures of these plots indicate the interaction between the factors. As it can be seen, by increasing the amount of chloroform from 110 to 140 μL, and ethanol from 400 to 600 μL, in turn, the volume of the sedimented phase, as well as absorbance, will increase (Fig. 2d). Further increase in chloroform and ethanol volumes will decrease the analytical signals as a consequence of decreasing the enrichment factor. In addition, decreasing the absorbance may be interpreted by increasing the solubility of the complex in aqueous phase when more ethanol is used. In the case of lower volumes, the interface decreases so that the ion association cannot completely transfer into organic phase (Fig. 2d). As it is shown in Fig. 2a, c, and f, the extraction of the ion association in acidic media is more probable (pH = 3–5.5), and in more acidic media (*pH* < 2.5), the certainty of extraction is decreased. Finally, using optimization option of the software, the optimal conditions were obtained as the following: *pH* = 4.2, the volume of picrate, disperser, and extraction solvents 71, 531, and 136 μL, respectively.

Table 4 Design and response for CCD and ANN

Run	Block	<i>E</i>	<i>F</i>	<i>L</i>	<i>P</i>	ABS (Actual)	ABS (Neural)	ABS (CCD)
1	2	4.8	80	0.6	120	0.43	0.3500007	0.29751
2	2	4.8	80	0.6	60	0.02	0.0199807	0.06565
3	2	4.8	80	1	120	0.11	0.0897562	0.18809
4	2	7.8	80	0.6	120	0.05	0.0866138	0.18919
5	2	4.8	160	0.6	120	0.37	0.3698907	0.58268
6	2	4.8	80	0.6	120	0.39	0.3500007	0.54601
7	2	4.8	0	0.6	120	0.34	0.3400724	0.39554
8	2	4.8	80	0.2	120	0.28	0.2806187	0.46233
9	2	4.8	80	0.6	180	0.29	0.2904503	0.47701
10	2	1.8	80	0.6	120	0.18	0.1794744	0.31063
11	1	6.3	120	0.4	90	0.01	0.0102799	0.37183
12	1	6.3	120	0.8	150	0.19	0.1904439	0.44039
13	1	6.3	120	0.4	150	0.22	0.2202288	0.53071
14	1	4.8	80	0.6	120	0.37	0.3500007	0.54601
15	1	3.3	120	0.4	150	0.26	0.4485494	0.52663
16	1	4.8	80	0.6	120	0.39	0.3500007	0.54601
17	1	6.3	40	0.4	90	0.05	0.0518516	0.21347
18	1	3.3	120	0.8	90	0.02	0.0202039	0.18383
19	1	6.3	40	0.4	150	0.16	0.1602044	0.37235
20	1	3.3	120	0.8	150	0.22	0.1856894	0.43631
21	1	3.3	40	0.4	90	0.21	0.2104735	0.33899
22	1	6.3	120	0.8	90	0.02	0.0200539	0.18791
23	1	3.3	40	0.8	150	0.27	0.1519902	0.40755
24	1	4.8	80	0.6	120	0.33	0.3500007	0.54601
25	1	3.3	40	0.8	90	0.05	0.0498799	0.15507
26	1	6.3	40	0.8	90	0.01	0.0096522	0.02955
27	1	6.3	40	0.8	150	0.13	0.1301216	0.28203
28	1	3.3	120	0.4	90	0.15	0.149994	0.36775
29	1	4.8	80	0.6	120	0.35	0.3500007	0.54601
30	1	3.3	40	0.4	150	0.33	0.4159634	0.49787

3.5 Genetic Algorithm

In this research study, a feed-forward neural network (FF ANN) was utilized to consider the relation between the input parameters and the output. First, the number of neurons in the hidden layer was determined. According to the available methods, the number of neurons was selected using trial and error [32–35]. Then, the learning algorithm was chosen to achieve the best weights for the neural network. Since the best training of network would be achieved by minimizing the error function, the choice of a suitable learning method is a crucial part of modeling by ANN. The back-propagation algorithm was used to train the understudy feed-forward ANN, and the Levenberg–Marquardt optimization algorithm was employed for its training. Then, the validation of the prediction model on the basis of error function was considered. The mean square error (MSE) and the correlation coefficient

(*r*) were exploited to show the error function and the predictive ability of the network, respectively. MSE can be defined as follows:

$$MSE = \frac{1}{N} \sum_{i=1}^N (y_{i,\text{exp}} - y_{i,\text{pred}})^2$$

where

MSE is the mean squared error

N is the number of experimental data points

$y_{i,\text{exp}}$ is the experimental value of training sample *i*

$y_{i,\text{pred}}$ is the predicted value from the neural network for training sample *i*

In order to assess the fitting and prediction accuracy of models made, the root-mean-squared error (RMSE) is other parameter which is stated as follows:

Table 5 Results of ANOVA for CCD

Source	df ^a	Seq SS	Adj SS	Adj MS	F-value ^b	p value
Block	1	0.018727	0.018727	0.018727	16.05	0.001
Regression	14	0.500808	0.500808	0.035772	30.66	0.000
Linear	4	0.203183	0.203183	0.050796	43.54	0.000
E	1	0.040017	0.040017	0.040017	34.30	0.000
F	1	0.000150	0.000150	0.000150	0.13	0.725
L	1	0.028017	0.028017	0.028017	24.02	0.000
P	1	0.135000	0.135000	0.135000	115.73	0.000
Square	4	0.283150	0.283150	0.070787	60.68	0.000
E ²	1	0.105125	0.156868	0.156868	134.47	0.000
F ²	1	0.000006	0.006696	0.006696	5.74	0.031
L ²	1	0.059894	0.084868	0.084868	72.75	0.000
P ²	1	0.118125	0.118125	0.118125	101.26	0.000
Interaction	6	0.014475	0.014475	0.002412	2.07	0.123
EF	1	0.005625	0.005625	0.005625	4.82	0.45
EL	1	0.005625	0.005625	0.005625	4.82	0.45
EP	1	0.000100	0.000100	0.000100	0.09	0.774
FL	1	0.000625	0.000625	0.000625	0.54	0.476
FP	1	0.000900	0.000900	0.000900	0.77	0.395
LP	1	0.001600	0.001600	0.001600	1.37	0.261
Residual error	14	0.016332	0.016332	0.001167		
Lack of fit	10	0.010732	0.010732	0.001073	0.14	0.667
Pure error	4	0.005600	0.005600	0.001400		
Total ^c	29	0.535867				

^a Degrees of freedom

^b Test for comparing model variance with residual (error) variance

^c Total of all information corrected for the mean

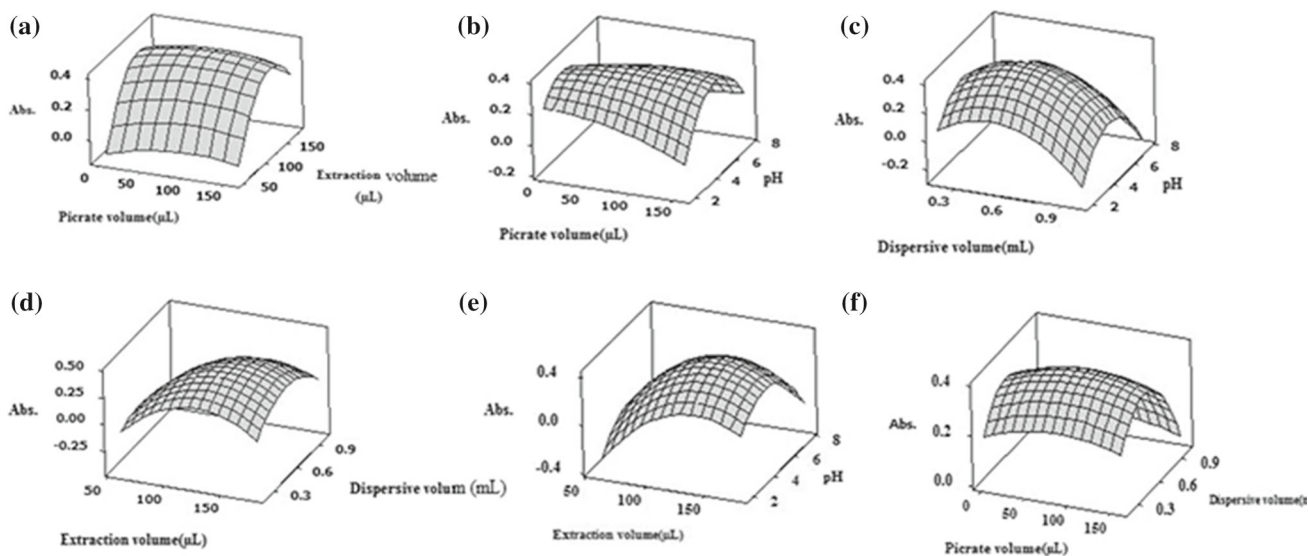


Fig. 2 The response surface plots of the effect of variables and their interactions on the absorbance, conditions: 8 ml of Fe (III) standard solution ($0.1 \mu\text{g L}^{-1}$), 1 ml of piroxicam solution (0.001 mol L^{-1}), 71 μL of

picrate anion, 136 μL of chloroform (as extracting solvent) and 531 μL of ethanol (as dispersive solvent), pH: 4.2

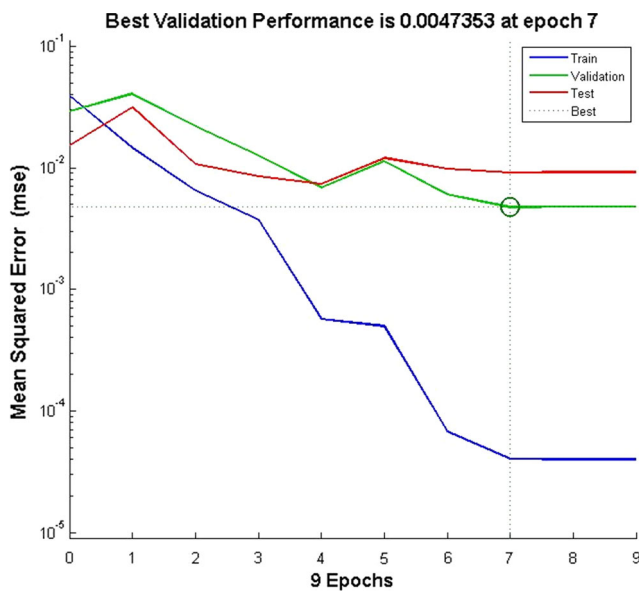


Fig. 3 Evolution of network performance (MSE) during training phase using Levenberg–Marquardt

Table 6 Optimal values of the first layer of the network weights

	HN* 1	HN 2	HN 3	HN 4	HN 5
Input 1	0.3610	3.2714	-0.7245	1.1729	1.2339
Input 2	0.6659	-0.3602	-0.0200	-1.3750	-0.1513
Input 3	0.4662	-1.0087	2.1874	-0.0189	-0.2914
Input 4	1.6073	-1.0415	1.2711	-2.0386	3.0048
Bias	2.1681	-1.1595	-1.2234	1.7887	1.1895

$$\text{RMSE} = \sqrt{\text{MSE}}$$

Normalized root-mean-squared error (NRMSE) is determined as follows:

$$\text{NRMSE} = \frac{\text{RMSE}}{Y_{\max} - Y_{\min}}$$

in which Y_{\max} and Y_{\min} are the maximum and minimum response obtained by each of the models.

Table 4 shows the experimental data which were used for training the artificial neural network. The data were randomly dispersed into four groups, 70% in the training set, 15% in the test set, and 15% in the validation set. After repetition of the trails, a network with 5 hidden neurons showed the best performance. The MSE value was obtained to be 0.002. The results are illustrated in Fig. 3. Table 6 shows the weights which are obtained from the final trained network. The ANN prediction results are indicated in Table 7.

Table 7 Optimal values of the output layer of the network weights

	Output 1
HN 1	-0.8445
HN 2	-0.8149
HN 3	-0.7905
HN 4	-0.1435
HN 5	0.8428
Bias	-0.4559

Table 8 Comparison of the precision and accuracy of RSM and GA-ANN

Method	MSE	RMSE	NRMSE
RSM-CCD	0.034	0.184	0.333
GA-ANN	0.002	0.044	0.100

3.6 Optimization by Genetic Algorithm

In this work, the genetic algorithm was applied to obtain the optimal conditions. GA-ANN was successfully employed to find the best conditions. The properties which were used for determining the controllers' parameters were reported as followed.

Chromosome population = 40
 The number of generation = 100
 Crossover fraction = 0.8
 Elite count = 5%
 Migration fraction = 0.2
 Migration interval = 20

The optimum formulation using the genetic algorithm displayed. Validation was performed in triplicate, and the optimum conditions for pH, volume of picrate, the volume of dispersive, and the volume of extraction were 3.4, 160, 200, and 170, respectively.

3.7 Comparison CCD and ANN

The predicted data obtained by central composite design and ANN were compared with each other. In order to evaluate the precision and accuracy of both applied models, MSE, RMSE, and NRMSE for both models were calculated and are summarized in Table 8.

Moreover, a regression analysis for predicted data by ANN and experimental data was done. As shown in Fig. 4, the correlation coefficient (r) was 0.81 and 0.93 for RSM and ANN, respectively. According to the obtained results, ANN had higher predictive accuracy than CCD even with a limited number of experiments.

Fig. 4 Network model with training, validation, test, and all prediction

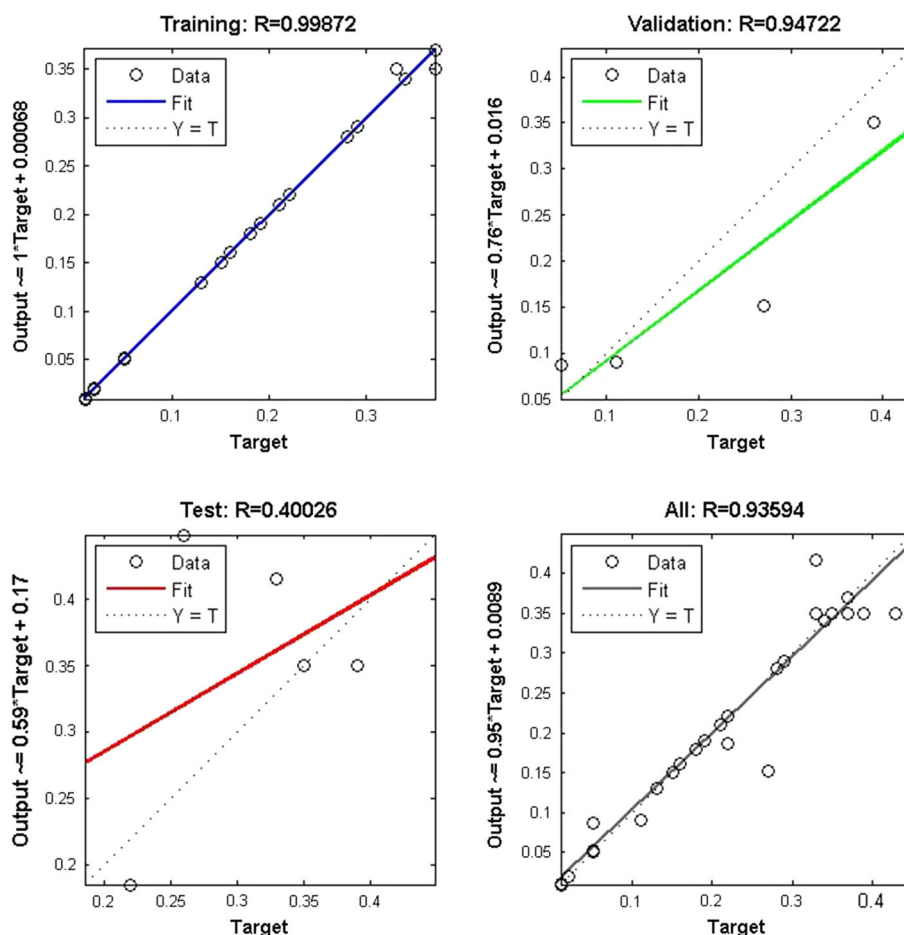


Table 9 Comparison of optimization powers of GA-ANN and RSM

Method	Component	Parameter	Abs.
Optimization by GA-ANN	pH	3.4	0.48
	Volume of picrate (μL)	160	
	Volume of dispersive solvent (μL)	200	
	Volume of extraction solvent (μL)	170	
Optimization by RSM	pH	4.2	0.42
	Volume of picrate (μL)	71	
	Volume of dispersive solvent (μL)	531	
	Volume of extraction solvent (μL)	136	

3.8 Comparison of Optimization by RSM and GA-ANN

RSM and GA-ANN were employed to predict the optimize parameters. Table 9 shows the comparison of the results obtained by two methods for multi-objective. As this is identified, both RSM and CCD are suitable for this study.

3.9 Analytical Performance

The calibration graph was obtained by DLLME of 8.0 mL standard solutions containing the known amount of Fe

(III) under optimal conditions. The linear range of 0.03 – 0.960 mg L⁻¹ and the calibration equation of $A = 0.165C + 0.081 (R^2 = 0.998)$ were achieved. The limit of detection (LOD), which is calculated as three times the standard deviation of the measurement of blanks divided by the slope of the calibration graph, was found to be

0.008 mg L⁻¹. The enhancement factor (EF), defined as the ratio of the slope of the calibration curve after and before preconcentration, was found to be 110. The relative standard deviation (RSD) of 3.9 % for the solution containing 0.10 mg L⁻¹ of Fe (III) in optimal conditions for eight replicated measurements ($n = 8$) was acquired.

3.10 Effect of Coexisting Ions

The effect of the common coexisting ions on the analytical signal of analyte was investigated. Eight mL of the solution containing 0.10 mg L^{-1} of Fe (III) with various amount of interfering ions was treated by the recommended procedure. The tolerable amount of each ion was taken as a Fe (III): interference ratio that caused an error in the absorbance value of no longer than $\pm 5\%$. As the results indicated in Table 10, the presence of the common cations and anions in natural water has no significant influence on the determination of iron (III) under the optimum conditions. The tolerance of the method to metal ions indicated that these cations can react with piroxicam and picrate to form pink complexes. Among the organic species, only EDTA and SCN, which form relatively strong

Table 10 Tolerance limits of interfering species in the determination of 0.1 mg L^{-1} of Fe (III)

Interferent-to-analyte ratio	Interference species
1000:1	Na^+ , Mg^{2+} , Ca^{2+} , CH_3COO^- , NO_3^- , SO_4^{2-} , Br^- , I^- , tartrate, citrate
800:1	Pb^{2+} , Mn^{2+} , Urea, Ni^{2+}
500:1	Co^{2+} , oxalate, Zn^{2+} , Cr^{3+}
100:1	Cd^{2+} , PO_4^{3-}
50:1	Hg^{2+} , Cu^{2+}
5:1	EDTA, SCN

Table 11 Analytical results (mean \pm s, $n = 3$) for determination of Fe in food samples

Sample	Spiked ($\mu\text{g L}^{-1}$)	Found ($\mu\text{g L}^{-1}$)	ICP ($\mu\text{g L}^{-1}$)	Recovery (%)
Tap water	–	62.1 ± 2.3	63.3 ± 0.2	
	100	155.1 ± 3.2	–	91.7
	200	270.6 ± 1.2	–	103.6
Well water	–	274.9 ± 3.1	275.2 ± 0.3	
	100	373.1 ± 2.2	–	97.7
	200	476.2 ± 4.3	–	100.4
Mint	–	232.7 ± 4.5	–	
Tea	–	102.5 ± 3.3	–	

Table 12 Analytical characteristics of the different extraction methods

System	Detection	R	LDR*	LOD**	RSD%	Refs.
DLLME-SFO	Spectrophotometry	0.998	31–350	8	4.2	[17]
DLLME	Spectrophotometry	0.998	25–1000	7.5	1.2	[2]
CPE	FASS	0.999	10–250	1.7	2.1	[36]
LLE	FAAS	0.999	Up to 5000	0.24	2.1	[37]
LLE	FAAS	0.998	25–150	9	1.2	[38]
DLLME	Spectrophotometry	0.998	30–960	8	3.9	This work

* Linear dynamic range ($\mu\text{g L}^{-1}$)

** Limit of detection ($\mu\text{g L}^{-1}$)

complex with iron (III), make interference. It should be mentioned that in the sample type studied, the concentration of such organic species is not significant.

4 Analysis of Real Sample

To evaluate the efficiency of the developed method, two types of water samples (tap water and well water) and two types of vegetation samples (tea and mint) were studied. Standard addition technique was used in DLLME method. Reliability and accuracy of the method were considered either by spiking the sample or by comparing the results with data obtained by ICP-OES. As the results shown in Table 11, the recovery of spiked sample is good, and there is satisfactory agreement between the results and data obtained by ICP-OES (at 95% confidence limit), suggesting the proposed procedure is reliable for the sample type examined.

4.1 Comparison of the Proposed Method with the Other Studies

The determination of iron (III) in the water samples by aforementioned method was compared with some extractive methods, the outcome of which is summarized in Table 12. Clearly, the proposed method is comparable in LOD and LDR to the previous studies.

In fact, this study is a comparison between using two models of statistical methods including GA and CCD for optimiza-

tion of the significant factors for determination of iron. Comparing the results of two methods showed that ANN is more successful for modeling and optimizing the proposed process. While most of the previous studies have been applied for determination of iron in water sample, the proposed method was applied in real sample such as tea and mint. And it can be mentioned that, in none of the previous studies done to measure iron microextraction, the combination of chemometrics and UV–visible spectrophotometry has not been reported.

5 Conclusions

The proposed method of (DLLME) was successfully applied for separation, preconcentration, and final determination of iron (III) in food samples by UV–visible spectrophotometry with perfect accuracy and precision. The efficient combination DLLME with UV–visible spectrophotometry presented high extraction efficiencies for separation and preconcentration of iron (III). Furthermore, the powerful statistical method of response surface methodology was efficiently employed for prediction and optimization of the influential parameters in the proposed process. Comparing the result of two methods including GA and CCD showed that ANN is more successful for modeling and optimization this process. Using experimental design and computer software not only make to find a model for optimization the response, but also the number of the experiments reduced. The prominent advantages of the present study are minimum organic solvent consumption, reducing the number of the experiment, low cost, low detection limit, easy and rapid operation, and short analysis time. Also, the detection limit and the calibration range of this method are comparable to the previous works.

Acknowledgements The financial support of this work provided by University of Shahrekord is highly appreciated. The authors are also partially supported by the Center of Excellence for Mathematics, Shahrekord University. They are also deeply indebted to Dr. H. Hadadzadeh and Dr. F. Shemirani without whose valuable contribution we could never finish the work.

Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflict of interest.

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