Trace determination of safranin O dye using ultrasound assisted dispersive solid-phase micro extraction: Artificial neural network-genetic algorithm and response surface methodology

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Abstract

In this study, ultrasound assisted dispersive solid-phase micro extraction combined with spectrophotometry (USA-DSPME-UV) method based on activated carbon modified with Fe₂O₃ nanoparticles (Fe₂O₃-NPs-AC) was developed for pre-concentration and determination of safranin O (SO). It is known that the efficiency of USA-DSPME-UV method may be affected by pH, amount of adsorbent, ultrasound time and eluent volume and the extent and magnitude of their contribution on response (in term of main and interaction part) was studied by using central composite design (CCD) and artificial neural network-genetic algorithms (ANN-GA). Accordingly by adjustment of experimental conditions suggested by ANN-GA at pH 6.5, 1.1 mg of adsorbent, 10 min ultrasound and 150 μ L of eluent volume led to achievement of best operation performance like low LOD (6.3 ng mL⁻¹) and LOQ (17.5 ng mL⁻¹) in the range of 25-3500 ng mL⁻¹. In following stage, the SO content in real water and wastewater samples with recoveries between 93.27–99.41% with RSD lower than 3% was successfully determined.

Keywords: Artificial Neural Network; Central composite design, Genetic algorithms; Safranin O; Ultrasound assisted dispersive solid-phase micro extraction.

1. Introduction

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Huge content of contaminant in different industrial waste water via their following entrance to aqueous media led to serious the damaging their quality of in subsequently destroy health of living things [1-3]. Some of these contaminants due to their natural toxicity, carcinogenic or mutagenic property and also their high bioaccumulation efficiency received special attention even at very low concentrations [4-6]. Recently, much attention was devoted to quality of environmental, which encourage the researchers to design appropriate control procedure to reduce health hazards [7-9].

Safranin O (SO) used as food additive for generation of colour candies and cookies, textile industries, leather, paper as well as in researches related to histology, textile, cytology and bacteriology [10]. While this material is not toxic with hazardous nature in comparison to other respective ones, its permanent exposure and consumption led to creation of such as cornea and conjunctiva in human and rabbit eyes and also cause skin and respiratory tract irritation [11].

Therefore, there is a need for an accurate and precise method for determining these organic pollutant in various matrices such as organism, atmosphere, water, and soil to grasp the dynamics of toxic compounds in various environments.

Application of ultrasound irradiation accelerate chemical process via production of acoustic cavitation that achieved by the propagation of pressure wave through a liquid [12]. Ultrasound energy improve the mass transfer through convection pathway and probable activation of reactive surface that is emerged from physical phenomena such as micro-streaming and turbulence, acoustic (or shock) waves and microjets [13]. Shock waves have the potential of creating microscopic turbulence within interfacial films surrounding neighbor solid particles. Ultrasonic radiation is powerful tools to intensify the mass transfer (adsorption and desorption) process by various known and probable mechanism [14-17].

Ultrasound assisted dispersive solid-phase microextraction (USA-DSPME-UV) is a sample handling technique characterized as being a real non-solvent technique [18]. The choice of extracting phase is essential in USA-DSPME-UV to achieve selective and efficient extraction of the analytes to be determined [19]. USA-SPME-UV can be considered as a very green sample preparation technique, because of its solvent-free characteristics [3, 17]. USA-DSPME-UV allows pre-concentrating the analytes in an easy way without the need for solvent evaporation after the extraction process, as typically occurs with conventional extraction techniques [20, 21]. The total removal of organic solvents for sample preparation avoids or minimizes the waste generation derived from the sample preparation step.

Moreover, it can be used for the extraction and preconcentration of target analytes present in very small samples because of the convenient dimensions of the USA-DSPME-UV system [22, 23].

In application of extraction process, it is crucial to improve process efficiency, reduce operational cost and time to minimum and take into account the most important factors, what can be achieved by applying the optimization techniques such as response surface methodology (RSM) and artificial neural network-genetic algorithm (ANN-GA) [24, 25].

Response surface methodology (RSM) is the collection of experimental design techniques that uses statistical and mathematical methods for designing the experiments, evaluating the effects of several factors, and achieving the optimum conditions for desirable responses with a limited number of planned experiments. RSM consists of an adequate functional relationship between a response of interest and a number of associated control variables. The purpose of RSM method is to find the optimum response and realize how the response changes in a given direction by modulating the design variables [2, 26, 27].

The most popular artificial neural network (ANN) architecture is associated with the common multi-layered perceptron comprised of input, hidden and output layers, and different numbers of neurons in each layer. As ANN models are exclusively data-based, it is not possible to guarantee their smoothness in the optimization phase. Hence, the conventional gradient-based optimization methods, which require a continuous, differentiable and, more importantly, smooth objective function, in general may not be used efficiently to optimize the function learned by ANN. The optimization of ANN parameters can be improved using GA, a stochastic global search algorithm, achieving better performances. GA simulate the survival-of-the-fittest principle of nature while searching for an objective function that helps in its natural optimization. GA use string coding of variables for dividing the search space into discrete ones, which further helps in searching for global optimum in the entire solution space. GA are used for optimization based on weights and biases obtained ANN. The genetic algorithm is applicable to solve a variety of optimization problems specifically the discontinuous, no differentiable, stochastic or highly nonlinear objective function [28-31].

The present study describes the efficiency of Fe_2O_3 -NPs-AC as solid phase (adsorbent) for extraction of SO from wastewater samples. The effects of various parameters including pH, amount of adsorbent, ultrasound time and eluent volume were investigated RSM and ANN-GA. The aims of this study are: (i) to obtain a predictive model based on RSM and ANN-GA techniques for prediction of the extraction percentage of SO dye, (ii) to maximize the

extraction percentage of SO dye using RSM and ANN-GA models, (iii) to develop a simple and fast method for extraction of SO dye.

2. Experimental

2.1. Chemicals and instruments

Ultrapure water (obtained by Milli-Q system, Bedford, MA, USA) was used in all experiments. Stock standard solution of safranin O (SO) dye with a concentration of 100 mg L⁻¹ were obtained by dissolving appropriate amounts of SO dye (Merck, Darmstadt, Germany). Working standard solutions were obtained by appropriate dilution of the stock standard solutions. THF, methanol, ethanol, acetone, acetonitrile, and DMF, which were used as eluent solvents, were from Merck (Merck, Darmstadt, Germany) were used. X-ray diffraction (XRD, Philips PW 1800) was performed to characterize the phase and structure of the prepared Fe₂O₃-NPs-AC using Cu_{Kα} radiation (40 KV and 40 mA) at angles ranging from 10 to 80°. The morphology of the Fe₂O₃-NPs-AC were observed by scanning electron microscopy (SEM: Hitachi S-4160) under an acceleration voltage of 26 KV. The absorbance was measured by a UV-vis spectrophotometer (model V-530, Jasco, Japan) at 520 nm using quartz micro cells. A Hermle Labortechnik GmbH centrifuge model Z206A (Germany) was used to accelerate the phase separation. A Metrohm digital pH-meter model 686 (Switzerland, Swiss) with a combined Ag/AgCl glass electrode was used for pH adjustments. An ultrasonic bath with heating system (Tecno-GAZ SPA Ultrasonic System) at 40 kHz of frequency and 130 W of power was used for the ultrasound assisted adsorption procedure. The STATISTICA and Design-Expert software (Stat Soft Inc., Tulsa, USA) was used for experimental design analysis and their subsequent regression analysis.

2.2. Ultrasound assisted dispersive solid phase microextraction method

10 mL solutions consisting of 500 ng mL⁻¹ concentration of SO dye at pH 6.5 was mixed with 1.1 mg adsorbent in test tube by 15 mL and the test tube was entered in ultrasound system for 8 min. Subsequently, the enriched SO dye was eluted by 150 µL of DMF as eluent solvent. In this step, SO were extracted into fine droplets of DMF. After 8 min sonication, the test tube was placed on the vertex for 4 min and then the mixture solution was centrifuged for 5 min at 3500 rpm. The eluent solvent was separated using a syringe. Then 50 µL of eluent solvent consisting SO entered in quartz microcells and determination of extraction percentage by spectrophotometric method. To develop

the USA-DSPME-UV procedure for enrichment of SO, some parameters controlling extraction (pH, amount of adsorbent, ultrasound time and eluent volume) were investigated by RSM and ANN-GA. Additionally, under optimum condition obtained from ANN-GA calculated quantitative parameters. Extraction recovery (ER %) and enrichment factor (EF) of SO were calculated as follows [22, 23]:

$$ER\% = \frac{C_{org} V_{org}}{C_{initial} V_{aq}} \times 100 \quad (1)$$

$$EF = \frac{C_{sed}}{C_0} \quad (2)$$

where C and V are the concentration and volume, and the suffixes org and initial indicate eluent and initial solution, respectively. The relative recoveries (RR %) of the analytes were used as the parameters to evaluate the method efficiency. The RR% was calculated by eq. (3).

$$RR\% = \left(\frac{C_{found} - C_{real}}{C_{added}} \right) \times 100 \quad (3)$$

The C_{found} , C_{real} , and C_{added} are defined as the concentration of analyte after addition of a known amount of standard in the real sample, the concentration of analyte in the real sample and the concentration of known amount of standard which was spiked to the real sample, respectively [32].

2.3. Preparation Fe_2O_3 -NPs-AC

The Fe_2O_3 -NPs-AC were synthesized via an in situ crystallization technique. Briefly, 1.5 g active carbon (AC) were added to 20 mL of distilled water and then transferred to an ultrasonic bath to obtain a uniform suspension for 20 min. Then 1.52 g $Fe(NO_3)_3 \cdot 6H_2O$ was added and sonication was contention used for 10 min. After that, 1 g NaOH was directly added to the $Fe(OH)_3$ and AC suspension under fierce stirring for 30 min. Then, the resultant system

was transferred into a stainless steel autoclave and held at 160 °C for 22 h. After cooling to room temperature naturally, the as-prepared products were filtered, washed with distilled water, and dried at 80 °C for 12 h.

2.4. Experimental design and statistical analysis

Response Surface Methodology (RSM) is a technique for optimization and analyzing the relationship between independent variables and response (ER %), both as linear or nonlinear forms to determine an optimum response. Central Composite Design (CCD) in RSM is the most popular class of design used for fitting a second order model, especially in the extraction process [33]. In this study, four important factors were considered in experiments with five levels. Table 1 shows the ranges and levels of the four independent variables with actual and coded values of each parameter. The independent variables are coded to two levels namely: low (-1) and high (+1), whereas the axial points are coded as -2 and +2. A five-level-four-factor central composite design was employed to fit a second-order response surface model which required 31 experiments, including 16 factorial, 8 axial points and 7 replicates at the center points, which are used to determine the experimental error (pure error) and the reproducibility of the data. 31 experiments were conducted in random order and the extraction percentage was calculated. The complete CCD design matrix in terms of real independent variable is presented and the corresponding results are given in Table 2. The experiments were run in replicates to minimize errors. The linear and quadratic model with interactions can be seen in the following equation [10]:

$$y = \beta_0 + \sum_{i=1}^k \beta_i x_i + \sum_{i=1}^k \beta_{ii} x_i^2 + \sum_{i=1}^k \sum_{j=1}^k \beta_{ij} x_i x_j + \varepsilon \quad (4)$$

where y expresses response (extraction percentage), x_i and x_j represent independent variables, β_0 indicates the intercept constant, β_i show the coefficients of linear terms, β_{ii} represent the coefficients of quadratic terms, β_{ij} show the coefficients of interaction terms, ε indicates experimental error and k represents the number of independent variables. The data were analyzed using STATISTICA and Design-Expert software and the coefficients were interpreted using F-test. Analysis of variance (ANOVA), regression analysis and plotting of 3D plot were used to establish the optimum conditions for the extraction percentage. The accuracy and general ability of the polynomial model could be evaluated by the coefficient of determination R^2 . The experiment was carried out in triplicates [34].

2.5. Artificial neural network (ANN)

An artificial neural network (ANN) is a simplified computational and mathematical model that is inspired by the structure of biological neural networks. In this approach weighted sum of inputs arriving at each neuron is passed through an activation function (generally nonlinear) to generate an output signal [35]. Artificial neural network has been used successfully for modelling of dye extraction. MATLAB (The Math works, Inc., Ver. 7.0.1) was chosen to develop the ANN model using neural network toolbox from the data. In this present study, a three-layer feed forward back propagation neural network with a linear transfer function was developed for modeling of SO extraction by Fe_2O_3 -NPs-AC. ANN has three layers: an input layer represents as independent variables; the output layer gives the dependent variables and one or more intermediate nodes act as a collection of feature detectors. As we have chosen pH, amount of adsorbent, ultrasound time and eluent volume as independent variables, and extraction percentage of SO as dependent variables, network model with four neurons in the input layer and one in the output layer was the obvious choice. The number of hidden nodes determines the model complexity of a neural network. However, too many neurons in the hidden layer may also result in the over fitting of the model data. After a number of training trials, the best neural network model was generated. The maximum number of epochs (training cycles) was chosen by a trial and error approach [36].

2.6. Genetic Algorithm (GA)

GA is an adaptive search procedure based on the mechanics of natural genetics and natural selection and has been used for a variety of search problems. GA starts by generating a random population. Each individual of the population represents a possible solution and is coded by binary string (called 'chromosome c') [37]. The bit value "1" represents a select variable; the bit value "0" represents an unselect variable. The "fitness", which is measure of adaptation to environment, is calculated for each individual. After the evaluation of all the individuals, the next population is obtained using genetic operations. As the algorithm proceeds, the individual's of the population are gradually improved [38]. In the proposed method, after developing the central composite design and trained ANN, the genetic algorithm can be used to optimize the input variables, with an objective to maximize the percentage of SO dye extraction. GA based optimization processes were executed by using central composite design and trained ANN models as the fitness functions to give the global optimized solutions.

3. Results and discussion

3.1. Characterization of adsorbent

The microstructure and morphology of the Fe₂O₃-NPs and Fe₂O₃-NPs -AC (Fig. 1a, b) shows the Fe₂O₃ nanoparticles were well decorated on the activated carbon. These results clearly indicate the presence of strong interaction between AC and Fe₂O₃ nanoparticles. The highly dispersed metal oxide nanoparticles on high surface area of AC may create a better communication between the adsorbent and analyte.

Transmission electron microscopy (TEM) was employed to analyze detailed the crystal structure of Fe₂O₃-NPs (Fig. 2a) present the high magnification TEM images of Fe₂O₃-NPs, where the uniform distribution of particles is observed with the particle size of 10-25 nm.

The crystalline nature of the Fe₂O₃-NPs-AC was investigated by powder XRD (Fig. 2b) shows the presence of well-defined peaks that are centered at ca. 23.9° (012), 33° (104), 35.4° (110), 40.7° (113), 49.3° (024), 53.9° (116), 57.4° (018), 62.3° (214), 63.9° (300), 69.5° (208), 72.7° (119) and 76.3° (220), where all the diffraction peaks were well indexed to the rhombohedra crystal structure of Fe₂O₃ (JCPDS No. 33-0664). All the peaks observed in the composites are related to the Fe₂O₃ nanoparticles. The crystallite size was estimated from the width of (110) plane according to the Scherer's formula $D = k\lambda/\beta \cos\theta$, where constant k is 0.9-1, β is the full width half maxima and θ is the Bragg angle. The size was found to be 17.5 nm.

3.2. Optimization of eluent solvent

Preparatory experiments were done to choose the best eluent solvent. Therefore, USA-NSPME method was applied for extraction of 500 ng mL⁻¹ of SO from 10.0 mL aqueous solution. In this stage, desorption of SO dye from Fe₂O₃-NPs-AC was studied by means of 200.0 μ L of different organic solvents (acetonitrile, acetone, methanol, THF, ethanol and DMF). After desorption process, 100.0 μ L of organic phase transmission to micro cell for subsequent analysis. It was found that among the five used solvents, the maximum extraction percentage was obtained using DMF as desorption solvents for eluting adsorb SO dye from Fe₂O₃-NPs-AC. Therefore, DMF was chosen for subsequent experiments and the effect of type of eluent on the extraction percentage of SO dye were shown in Fig. 3. The effect of the volume of desorption solution on desorption efficiency of the SO dye was also investigated by subsequent experimental design.

3.3. Central composite design (CCD)

In the first step of the study, the effects of operating variables such as initial pH, amount of adsorbent, sonication time and eluent volume on extraction of SO were investigated using RSM according to CCD. In the second step, the main objective was to achieve optimal condition for determination of SO dye in wastewater. The batch runs were conducted in CCD designed experiments to visualize the effects of independent factors on extraction of SO and the results along with the experimental conditions. The experimental results were evaluated and the approximating function of extraction of SO was obtained in eq. (5) by utilizing STATISTICA 10. The least squares method was used to estimate the parameters in the approximating polynomial, and then the response surface analysis was done in terms of the fitted surface. The analysis of the fitted surface is approximately equivalent to analysis of the actual system if the fitted surface is an adequate approximation of the true response function. In most cases, the second-order model is adequate. Eq. (5) could be used in predicting the response for the given values of initial pH, amount of adsorbent, ultrasound time and eluent volume.

$$\text{ER\% SO} = -61.80 + 43.57A - 5.93B + 5.02C + 0.02D + 1.46AB + 0.70AC + 0.10AD - 0.60BC + 0.06BD - 0.02CD - 5.10A^2 - 5.10B^2 - 0.24C^2 - 0.02D^2 \quad (5)$$

In eq. (5), y is the extraction recovery of SO dye. A, B, C and D are corresponding to independent variables of initial pH, amount of adsorbent, ultrasound time and eluent volume, respectively. The negative coefficients for the model components B, BC, CD, A^2 , B^2 , C^2 and D^2 indicate the unfavorable effects on the extraction recovery of SO dye. While, the positive coefficients for A, C, D, AC and BD indicate favorable effects on the extraction recovery of SO dye. The significance of each model parameter will be determined by the statistical analysis.

ANOVA results of this quadratic model presented in Table 3 indicating that it could be used to navigate the design space. The appropriate procedure for testing the equality of several means is the analysis of variance (ANOVA) which has a much wider application. The goodness of fit of regression equation developed could be measured by adjusted determination coefficient. The R^2 value of 0.9929 (Fig. 4a) and adjusted R^2 of 0.9867 shows that the model could be significant predicting the response and explaining 95% of the variability in the extraction of SO dye. The Predicted R^2 of 0.9642 is in reasonable agreement with the “Adjusted R^2 of 0.9867. The statistical significance of the equation was evaluated by F-test and ANOVA (analysis of variance) which showed that the model was statistically significant at 95% confidence level ($p < 0.0001$). ANOVA reported the model F-value of 160.4 implying that the model is significant. P-value denotes the importance of each coefficient, helping in understanding

the interactions among the variables. The model also depicted the statistically non significant lack of fit ($p > 0.05$), indicating that the responses are adequate for employing in this model [39].

Fig. 4b shows the residual plot versus run orders. The random pattern of residuals would indicate the model adequacy. Additionally, assuming that the data have a normal distribution is necessary to integral analysis of the experimental data [40]. Therefore, the normal probability plot of residual values is shown in Fig. 4c. Evidently, the figure demonstrates that the experimental data fall close enough to the straight line suggesting normal distribution of the data [41]. To test the normality assumption, Box-Cox normality plot was assessed and the lowest point on the plot represented the best value of lambda (1.01) that suggested no need for transformation of extraction yield response (Fig. 4d). Adequate precision measures the signal to noise ratio and a ratio greater than 4 is desirable. Therefore, in the quadratic model of extraction of SO, the ratio of 54.01 indicate an adequate signal for the model to be used to navigate the design space [42].

3.4. Response surface plot

Response surface plot was used to determine extraction of SO dye over interactive variables adsorbent and ultrasound time, and pH and eluent volume (Fig. 5). Fig. 5a shows the effect of adsorbent dosage and ultrasound time on the extraction of SO. Adsorbent dosage and ultrasound time are the most important parameters influencing on the extraction of SO. As can be seen from Fig. 5a, the maximum extraction percentage of SO dye was obtained at about 1.5 mg of adsorbent and ultrasound time 8 min. The extraction percentage of SO dye increases with decreasing adsorbent up to 1.5 mg and afterward shows a slight decrease. The effect of pH and eluent volume on extraction of SO is shown in Fig. 5b as a 3D response surface plot. The extraction of SO increased with the decrease in eluent volume. Also the extraction of SO dye increased up to pH 7.0 and above this value decreased with the increase pH.

3.5. Optimization process

The profile for predicted values and desirability option in the STATISTICA and Design-Expert software is used for the optimization process. The scale in the range of 0.0 (undesirable) to 1.0 (very desirable) must be maximized following best and efficient selection of variables. The CCD design matrix results (Table 2) show the maximum (95.95%) and minimum (59.61%) extraction of SO dye. According to these values, DF settings for each dependent

variable of extraction percentage are depicted on the right hand side of Fig. 6: desirability of 1.0 was assigned for maximum extraction (95.95%), 0.0 for minimum (59.61%) and 0.5 for middle (77.78%). The left hand side of Fig. 6 (bottom) shows the application of individual desirability scores calculation of the extraction percentage. Since desirability 1.0 was selected as the target value, the overall response obtained from these plots with the current level of each variable in the model is depicted at the top (left) of Fig. 6. The CCD optimization design matrix (Fig. 6) show that maximum extraction percentage of SO (96.12% and desirability of 1.0) was achieved at following conditions: 1.5 mg of adsorbent, ultrasound time 8 min, eluent volume 180 μ L and pH 7.0.

3.6. Modeling and optimization by ANN-GA

ANN has been successfully utilized for prediction extraction processes of different analyte from aqueous solution. In the present work, a feed-forward back propagation network with tangent sigmoid transfer function (tansig) at hidden layer and a linear transfer function (purelin) at output layer was successfully applied. During the net training process, the mean square error (MSE) that was chosen as the function of error performance has minimum value at 9 neurons among examined neurons from 1 to 15. Network training performed in 9 epochs [43].

The relationship between the input and output data was achieved by ANN to approximate any function with finite number of discontinuities by learning their relationships. The criterion for selection of the optimum ANN structure is the MSE of the test data as well as the correlation coefficient (R^2). The smallest MSE was considered for train function, while the result of Table 4 shows the relation between the number of neurons, R^2 and MSE for presented ANN. It was found that using 9 hidden neurons, the R^2 and MSE reached values of 0.9993 and 0.09964, respectively. Also ANN offered the weights that are coefficients between the artificial neurons and are considered analogous to synapse strengths between the axons and dendrites in real biological neurons. Table 5 shows the provided weights by ANN in the present study. The intensity of influence of each input variable on the output variable was calculated using the neural weight data.

Fig. 7a shows the MSE versus the number of epochs for optimal ANN models show that the training was stopped after 9. The good simulation and correlation of experimental and predicted results (test data) for the optimum number of hidden layer neurons, which confirm accuracy of the neural network for modeling and prediction of the outlet composition of extraction percentage, while, Fig. 7b Show the plot of Error Histogram for extraction

percentage of SO dye by Fe₂O₃-NPs-AC and also this plot indicates that the errors in this extraction process is very low.

In recent years, combination ANN-GA has been successfully applied for the optimization of various systems. In this study, GA used by equations that achieved from ANN models. The method starts with a population of random regimes using pH, adsorbent mass (mg), ultrasound time (min) and eluent volume (μL) as the optimization parameters. The objective function was that equation achieved from ANN model that correlates the inputs with output and can be defined as follows [37]:

$$y = -\text{purelin}\left(w_2 * \text{tansig}\left(w_1 * [x(1); x(2); x(3); x(4)] + b_1\right) + b_2\right) \quad (6)$$

where, x(1), x(2), x(3) and x(4) represent the inputs, w₁ and b₁ are the weight and bias of hidden layer and w₂ and b₂ are the weight and bias of output layers. Table 5 shows the weight and bias values of each layer, which achieved from the best ANN model. The Matlab R2013a genetic algorithm toolbox has been applied for GA processes with 51 generation (Fig. 8a), population size of 20 with scattered crossover function of 0.8, Rank scaling function and Stochastic uniform option. For extraction percentage of SO dye the ANN-GA method predicts maximum extraction percentage (99.92%) at pH 6.5, 1.1 mg of adsorbent, ultrasound time 10 min and 150 μL of eluent volume (Fig. 8b).

3.7. Comparison of CCD and ANN-GA

The CCD and ANN-GA are techniques capable to solve linear and non-linear multivariate problems. Both models were used for optimization of extraction of SO dye by Fe₂O₃-NPs-AC. In addition, both models were well fitted to experimental response. The CCD approach has a regression equation for forecasting and achieving optimum conditions of variables unlike ANN-GA. However, the major limitation of CCD is the capability to solve only quadratic non-linear correlation. Besides, the ANN-GA model is able to solve any form of non-linearity. Therefore, the advantage of GA can overcome that of CCD. The predicted optimum conditions resulted from CCD and ANN-GA presented in Table 6. The experimental results showed an average SO dye extraction efficiency of 99.74 ± 1.81% (n=5). The existence of the high degree of agreement between the experimental results and predicted optimum results indicating that the ANN-GA could be used effectively for the evaluation and optimization of the effects of the extraction independent variables on the extraction efficiency of SO dye from wastewater.

3.8. Method validation

After optimization, the results showed that the USA-DSPME-UV has the higher extraction efficiency (in terms of enrichment factor) for the SO dye in the samples. Under the optimum conditions obtained from ANN-GA, limits of detection (LOD), limits of quantification (LOQ), linear dynamic ranges (LDR), and relative standard deviations (in terms of repeatability) of USA-DSPME-UV method were calculated (Table 7). Based on the calibration curves, sensitivity of the method was evaluated in terms of LOD and LOQ, which were statistically calculated as 3 and 10 times of the standard deviations of five replicate extractions of SO dye minimum detectable concentrations divided on the calibration slope. Repeatability (intra-day and inter-day precisions) were evaluated by analyzing five replicates of the model sample in the same day and five different days. The enrichment factors was calculated based on eq. (2). Five replicate extractions were performed in all calculations.

3.9. Study of selectivity

To evaluate the selectivity of the developed system, the effect of typically potential interfering ions and dyes were investigated and the results are shown in Table 8. According to the data, some major matrix ions present in natural water samples have no obvious interferences, while some other heavy metal ions have less tolerable limits due to the competition and co-extraction effect. These ions had no interference with the USA-DSPME-UV method under the optimum conditions due to probably low stabilities of their SO dye. These metal ions and dyes did not show any interfering effect at their studied levels for the extraction of SO dye in some various aqueous samples.

3.10. Analysis of real samples and validity of the method

Water is one of the important pathways of pollutants entering human bodies. Considering the biological and environmental significance of SO dyes, several real water and wastewater samples and certified reference water and industry wastewater samples were analyzed to validate the accuracy and applicability of the established method. The analytical results were summarized in Table 9. The results of certified reference samples were in good agreement with the certified values. For real water and wastewater samples, the extraction percentage for the spiked samples were in the acceptable range of 93.27–99.41%.

4. Conclusion

In this study, The Fe₂O₃-NPs-AC was successfully synthesized and it was characterized by XRD, FE-SEM and TEM methods, then used to extraction of SO dye in USA-DSPME-UV method. Afterwards, the significant variables were optimized using RSM and ANN-GA. Under the optimum conditions, the enrichment factors was 124.59. The linear range was 25-3500 ng mL⁻¹ and LODs was 6.3 ng mL⁻¹. The relative standard deviations (RSDs) were in the range of 2.8%. The relative recoveries of SO dye from different water and wastewater samples were 93.27- 99.41%. The resulting Optimized procedure allowed for the quantification of ultra-trace levels of SO dye in water and wastewater samples using USA-DSPME-UV coupled to spectrophotometry method. The main benefits of the proposed methodology for extraction and determination of SO dye were low sample consumption, minimum use of toxic organic solvent, rejection of matrix constituent, simplicity and high enrichment factor.

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Table 1. Independent variables and levels used for response surface design.

Factors	Symbol (unit)	Levels				
		Lowest ($-\alpha$)	Low (-1)	Central (0)	High ($+1$)	Highest ($+\alpha$)
pH	A (-)	4.0	5.0	6.0	7.0	8.0
Adsorbent mass (mg)	B (mg)	0.5	1.0	1.5	2.0	2.5
Ultrasound time (min)	C (min)	2	4	6	8	10
Eluent volume	D (μ L)	120	150	180	210	240

Table 2. CCD matrix of independent variables and their corresponding experimental and predicted extraction percent of safranin O.

Experiment	Actual values of variables				Response (ER% SO)		
	A	B	C	D	Experimental	Predicted	Residual
1	7.0	1.0	4	150	87.674	86.728	0.946
2	6.0	1.5	6	180	92.536	92.425	0.111
3	6.0	1.5	6	120	91.618	91.202	0.416
4	7.0	1.0	8	210	92.994	93.306	-0.312
5	6.0	1.5	6	180	92.719	92.425	0.294
6	5.0	1.0	4	150	82.813	83.805	-0.992
7	5.0	2.0	4	150	77.402	77.193	0.209
8	6.0	2.5	6	180	82.63	82.924	-0.294
9	7.0	1.0	4	210	88.959	88.523	0.436
10	5.0	1.0	4	210	74.65	74.200	0.450
11	6.0	1.5	6	180	92.352	92.425	-0.073
12	7.0	1.0	8	150	95.948	97.459	-1.511
13	5.0	1.0	6	210	73.916	74.771	-0.855
14	6.0	1.5	6	240	81.097	81.312	-0.215
15	5.0	2.0	8	150	79.42	79.955	-0.535
16	6.0	1.5	6	180	91.343	92.425	-1.082
17	6.0	1.5	6	180	92.077	92.425	-0.348
18	7.0	2.0	4	210	87.95	88.698	-0.748
19	4.0	1.5	6	180	59.608	59.351	0.257
20	7.0	2.0	8	210	91.985	91.092	0.893
21	6.0	1.5	2	180	83.757	84.773	-1.016
22	5.0	2.0	8	210	67.221	68.270	-1.049
23	7.0	2.0	4	150	83.364	83.035	0.329
24	7.0	2.0	8	150	91.252	91.377	-0.125
25	5.0	2.0	4	210	72.868	71.456	1.412
26	6.0	1.5	6	180	93.728	92.425	1.303
27	6.0	0.5	6	180	92.246	91.750	0.496
28	5.0	1.0	8	150	89.601	88.956	0.645
29	6.0	1.5	6	180	92.436	92.425	0.011
30	6.0	1.5	10	180	93.322	92.319	1.003
31	8.0	1.5	6	180	85.041	85.096	-0.055

Table 3. Analysis of variance (ANOVA) for the fitted quadratic polynomial model for optimization of SO extraction.

Source	Sums of squares	Degree of freedom	Mean squares	F-value	p-value (Prob > F)	
Model	2310.1	14	165.01	160.40	< 0.0001	*
A	978.55	1	978.55	951.25	< 0.0001	*
B	115.01	1	115.01	111.80	< 0.0001	*
C	78.874	1	78.874	76.673	< 0.0001	*
D	144.41	1	144.41	140.39	< 0.0001	*
AB	8.3222	1	8.3222	8.0900	0.01172	*
AC	27.694	1	27.694	26.922	< 0.0001	*
AD	126.91	1	126.91	123.37	< 0.0001	*
BC	5.0769	1	5.0769	4.9353	0.04109	*
BD	14.609	1	14.609	14.202	0.00168	*
CD	31.468	1	31.468	30.590	< 0.0001	*
A ²	726.90	1	726.90	706.62	< 0.0001	*
B ²	46.111	1	46.111	44.825	< 0.0001	*
C ²	26.818	1	26.818	26.070	0.00011	*
D ²	67.775	1	67.775	65.884	< 0.0001	*
Residual	16.459	16	1.0287			
Lack of Fit	13.372	10	1.3372	2.5989	0.12732	**
Pure Error	3.0872	6	0.51453			
Cor Total	2326.5	30				
R ²	Adj-R ²	Pred-R ²		Adequate Precision		
0.9929	0.9867	0.9642		54.014		

* Significant.

** Not significant.

Table. 4. Comparison of 1-15 neurons in the hidden layer for extraction efficiency by ANN model development with the Levenberg–Marquardt algorithm for preconcentration and determination of SO.

Neurons	R^2_{train}	R^2_{test}	$R^2_{\text{validation}}$	R^2	$\text{MSE}_{\text{train}}$	MSE_{test}	$\text{MSE}_{\text{validation}}$	MSE
1	0.933018	0.918241	0.913803	0.923737	10.0779	12.27129	14.72252	11.07052
2	0.975097	0.858537	0.983397	0.974884	3.910377	2.424262	4.535992	3.785466
3	0.983102	0.975507	0.979073	0.979936	2.66566	4.385099	4.264996	3.147418
4	0.95098	0.752418	0.948011	0.939401	7.957128	11.97373	14.08372	9.429527
5	0.999389	0.990067	0.99126	0.995911	0.099671	1.854985	2.12348	0.648253
6	0.99943	0.998408	0.998739	0.998886	0.098101	0.603087	0.115799	0.173974
7	0.999233	0.969813	0.998071	0.993437	0.10912	6.205884	0.448885	1.043455
8	0.996283	0.996887	0.990607	0.994829	1.205446	0.549324	1.617628	1.170035
9	0.999452	0.99915	0.999337	0.999336	0.089323	0.138593	0.108604	0.099643
10	0.998699	0.986223	0.994588	0.98998	0.135729	8.842882	8.869638	2.667496
11	0.998098	0.996741	0.993644	0.996524	0.336667	0.228297	1.97769	0.559149
12	0.99849	0.9327	0.923563	0.980338	0.299694	8.600074	11.91352	3.190465
13	0.999236	0.906145	0.878618	0.965487	0.107634	17.67611	17.6893	5.210074
14	0.999211	0.926304	0.922563	0.941593	0.095877	22.98083	40.3452	9.260531
15	0.928914	0.934976	0.968736	0.932931	13.07545	11.26508	6.094459	11.79929

Table 5. The weight and bias of trained ANN for predicting the extraction of SO dye.

w1				w2	b1	b2
1.2485490	-3.9314979	-3.817844	1.5531901	0.00116399	-0.155	0.0872
3.92957743	4.7907632	1.1705057	1.6146074	-0.05529279	-6.3302	
3.01075718	-4.0399144	1.83234730	2.9846349	-0.10375259	-2.9514	
1.31982239	3.00215787	-3.3184370	-2.6252863	-0.12428062	0.7856	
-3.2083859	-3.7331529	-4.5483728	1.9366376	-0.19341471	1.8433	
-1.3496414	0.3548539	-3.8888162	-4.2049562	-0.13064912	2.8859	
1.4050600	-0.5649643	-5.7394318	-0.4829760	-0.05423904	3.2829	
0.0056189	-2.0426111	-2.4841625	-6.3500128	0.062040953	2.3076	
-5.310292	0.5615058	1.786162	2.1490475	-0.47518742	-0.8281	

Table 6. The comparison between optimum conditions of CCD and ANN-GA models.

Approach	Variables				ER%	
	pH	Amount of adsorbent (mg)	Ultrasound time (min)	Eluent volume (μL)	Experimental (RSD, N=5)	Predicted
CCD	7.0	1.5	8	180	96.12 (2.4)	95.47
ANN-GA	6.5	1.1	10	150	99.92 (1.8)	99.74

Table 7. Analytical characteristics of the proposed method.

Quantitative analysis	
Sample volume (mL)	10
Volume of eluent (μL)	150
Regression equation before preconcentration	$y = 0.0626x + 0.0014$; $R^2 = 0.9999$
Regression equation after preconcentration	$y = 7.7996x + 0.0747$; $R^2 = 0.9972$
Linear range (ng mL^{-1})	25-3500
Limit of detection (ng mL^{-1})	6.3
Repeatability (RSD, %) ($n=5$, $0.5 \mu\text{g mL}^{-1}$)	2.8
Limit of quantification (LOQ) (ng mL^{-1})	17.5
Average extraction recovery	95.60%
Preconcentration factor	66.67
Enrichment factor	124.59

Table 8. Tolerance limits of interfering species in the determination of 500 ng mL⁻¹ SO (N = 3).

Interference	Added as	Tolerance ratio (mg L ⁻¹)	ER% (RSD)
Na ⁺	NaNO ₃	500	96.32 (1.7)
Ca ²⁺	Ca(NO ₃) ₂	500	97.22 (0.9)
SO ₄ ²⁻	Ca(NO ₃) ₂	500	96.32 (1.6)
Mg ²⁺	Mg(NO ₃) ₂	500	95.45 (1.3)
I ⁻	KI	500	96.11 (2.0)
Br ⁻	KBr	500	97.22 (1.4)
NH ₄ ⁺	NH ₄ NO ₃	500	97.95 (1.5)
Cl ⁻	KCl	500	95.76 (1.0)
NO ₃ ⁻	KNO ₃	500	97.25 (0.8)
K ⁺	KNO ₃	500	97.12 (1.3)
Ba ²⁺	Ba(NO ₃) ₂	500	96.35 (1.2)
Ag ⁺	AgNO ₃	200	95.04 (1.5)
Fe ³⁺	FeCl ₃	200	94.76 (0.7)
Co ²⁺	Co(NO ₃) ₂	200	95.23 (1.8)
Ni ²⁺	Ni(NO ₃) ₂	150	96.54 (2.3)
Cr ²⁺	Cr(NO ₃) ₂	150	95.01 (2.1)
Pb ²⁺	Pb(NO ₃) ₂	150	96.78 (1.4)
Cu ²⁺	Cu(NO ₃) ₂	150	97.76 (2.1)
Ponceau 4R	-	100	96.75 (1.4)
Carmoisine	-	100	97.21 (1.6)
Sunset Yellow FCF	-	100	98.00 (1.7)
Methylene blue	-	100	95.45 (2.3)
Tartrazine	-	100	94.90 (2.8)
Allura Red AC	-	100	95.21 (1.4)

Table 9. Extraction recoveries and RSD in water and industry wastewater samples at spiked level by USA-DSPME-UV method (N=5).

Samples	SO added (ng mL ⁻¹)	SO Found (µg mL ⁻¹)	ER% (RSD)
Wastewater ^a	500	488.20	97.64 (1.6)
Tap water ^a	500	497.05	99.41 (0.9)
Wastewater ^b	500	474.00	94.80 (2.7)
Wastewater ^c	500	466.35	93.27 (1.8)
Wastewater ^d	500	471.80	94.36 (2.4)

^a Refinery in Gachsaran city

^b Sugar factory in Yasouj city

^c Hadaf Textile Industries Company Esfahan city

^d Kimia Polyester Qom Company

Fig. 1. (a) SEM images of Fe_2O_3 -NPs and (b) Fe_2O_3 -NPs-AC.

Fig. 2. a) TEM image of Fe_2O_3 -NPs and b) XRD pattern of the Fe_2O_3 -NPs-AC.

Fig. 3. The effect of the type of extraction solvent on the extraction recovery of SO using the proposed USA-NSPME method. Extraction conditions: volume of extraction solution: 200 μ L; pH of extraction solution = 6.0; ultrasound time: 6 min; volume of sample: 10.0 mL and 1.5 mg adsorbent mass.

Fig. 4. a) Normal % probability plot of the residuals, b) Plot of residuals versus run number, c) plot showing model predicted value versus actual value and d) The Box-Cox plot for extraction yield; pink color lines represent the upper (2.06) and the lower (0.015) limits of the 95% confidence interval calculated for the Box-Cox lambda value, the blue and green lines represent the current (1) and the best (1.01) lambda values, respectively. A horizontal line is drawn for the Ln (Residual SS) value corresponding to confidence limits for SO extract yields.

Fig. 5. Response surface plots showing effects of variables (A: pH, B: adsorbent mass (mg), C: ultrasound time (min) and D: eluent volume (μL)) on the extraction yield of melatonin.

Fig. 6. Profiles for predicted values and desirability function for ER% of SO. Dashed line indicated current values after optimization.

Fig. 7. MSE versus the number of epochs (a) and plot of Error Histogram (b) for removal percentage of SO dye by Fe_2O_3 -NPs-AC.

Fig. 8. Plots of best fitness and best individual for extraction of SO dye by ANN-GA method.

Graphical abstract