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Stir bar sorptive extraction kit for determination of pesticides in water samples with chemometric data processing

Setare Gorji a, Pourya Biparva b, *, Morteza Bahram a, Ghorbanali Nematzadeh c.

a Department of Chemistry, Faculty of Science, Urmia University, Urmia, Iran.
b Department of basic sciences, Sari agricultural sciences and natural resources university, P.O.Box 578, Sari, Iran.
c Genetic and agricultural biotechnology institute of tabarestan, Sari university of agricultural sciences and natural resources, P.O.Box 578, Sari, Iran.

Corresponding author’s name: Pourya Biparva
Corresponding author’s phone number: 98-1133687367
Corresponding author’s e-mail: p.biparva@sanru.ac.ir
Abstract

The current study describes a simple method to determine four organophosphate insecticides (i.e., ethion, phosalone, diazinon, chlorpyrifos) and an isothiazolidine acaricide (i.e., hexythiazox), as examples of pesticides in environmental water samples by stir bar sorptive extraction (SBSE). For this purpose, a self-magnetic nanocomposite monolithic (SMNM) kit coupled with high performance liquid chromatography-ultraviolet (HPLC-UV) spectroscopy was used. The size, morphology, and elemental distribution of synthesized SMNM kit were analyzed using field emission scanning electron microscopy (FESEM) coupled with energy dispersive X-ray (EDX) spectroscopy system, X-ray diffraction (XRD), and elemental mapping, respectively. An experimental design based on the central composite design (CCD) was used to optimize factors affecting SBSE. Under optimal experimental conditions, the limits of detection (LODs) were found to be in the range of 0.07 µg L\(^{-1}\) to 0.89 µg L\(^{-1}\). Linear ranges were 5-800 µg L\(^{-1}\) for ethion, hexythiazox, and chlorpyrifos, 1-1000 µg L\(^{-1}\) for phosalone, and 5-1000 µg L\(^{-1}\) for diazinon. The relative standard deviation (RSD%) did not exceed 5.48% and 6.45% for intraday and interday precisions, respectively. The enrichment factors (EFs) ranged from 15 to 39-fold (theoretical enrichment factor was 40-fold). The results showed that SMNM kit could enrich the above-mentioned pesticides effectively. The SMNMSBSE-HPLC-UV methodology was applied for the determination of pesticides in real water samples.

Keywords: Central composite design; High performance liquid chromatography-ultraviolet spectroscopy; Pesticides; Self-magnetic nanocomposite monolithic stir bar kit; Water samples
1. Introduction

Pesticides are widely used for agricultural activities because of their relatively low price and highly effective ability to control pests, weeds, and diseases [1]. The increasing production of pesticides for agricultural and non-agricultural purposes has caused the pollution of air, soil, ground, and surface water. As a result, it may seriously affect the environment and human health due to either direct exposure or through residues in food and drinking water [2].

Pesticides residues of ethion, phosalone, diazinon, chlorpyrifos, and hexythiazox are widely presented in the environment of our developing countries such as Iran. They exist in water, soils, agriculture products, and food [3]. When pesticides are absorbed by human organisms, they are very toxic due to acetyl-cholinesterase de-activation. Therefore, there is an increasing environmental concern regarding these compounds [4] and, consequently, increasing demand for developing methods for the determination of such contaminations in food and environment [5-10].

Chromatographic techniques are usually used to determine pesticides. Before determination, many preliminary steps, such as sampling, extraction, and clean-up for interference removal, are needed to be performed [11]. In the last decade, modern sample enrichment techniques run toward a great simplification, miniaturization, easy manipulation of the analytical devices, strong reduction, low-toxic reagent, and low sample volume requirements, which are in agreement with the green analytical chemistry principles [12]. Typical examples of miniaturization techniques for sample preparation include solid-phase extraction (SPE), stir bar sorptive extraction (SBSE), magnetic solid-phase extraction (MSPE), and solid-phase microextraction (SPME) have been developed for the determination of pesticides in environmental samples [13-18].

Recently, SBSE has been developed and used for the determination of volatile and semi-volatile organic compounds, such as pesticides, in various samples [19-21]. SBSE was introduced in 1999 as a solvent-free sample preparation method for the extraction and enrichment of organic compounds from aqueous matrixes. The method is based on sorptive extraction, where the solutes are extracted into a polymer coating on a magnetic stirring rod. The extraction is controlled by the partitioning coefficient of the solutes between the polymer coating and the sample matrix, and the phase ratio between the polymer coating and the sample volume [22]. After sorption, the compounds are chemically desorbed in a liquid or gas chromatography.
inlet. Polymer coatings must be physically stable and have good mechanical properties such that to ensure they do no break or degrade after their use [23]. Self-magnetic nanocomposite monolithic stir bar (SMNMSB) kit is a tool that solves the mentioned problems because of a special polymer (i.e., Polyethersulfone (PES)) used in this magnetic kit. PES shows an outstanding oxidative, thermal, and hydrolytic stability as well as a good mechanical property [24]. On the other hand, bonding of the coating to the cover of the magnetic rod, generally glass, is one of the difficulties [23]. In this regard, SMNMSB kit has not glass or magnetic rod core, which is one of their great advantages. It is directly magnetized (because of the presence of Fe₃O₄ nanoparticles (NPs) in the monolithic kit). Finally, it does not require using the magnetic rod core or glass to create the character of the magnetic coating on it to cure the polymer.

In this study, we describe the preparation of a SMNMSB kit and the optimization of SBSE procedure, followed by a magnetic nanocomposite monolithic stir bar kit in combination with chemometric data processing prior to HPLC-UV for the determination of five target pesticides (i.e., ethion, phosalone, diazinon, chlorpyrifos, and hexythiazox) in water samples.

2. Experimental

2.1. Standards and reagents

Standard pesticides (i.e., diazinon, phosalone, and chlorpyrifos) and ethion and hexthiazox were purchased from Sigma-Aldrich (St. Louis, MO, USA) and Chem Service (West, Chester, Pennsylvania, USA), respectively. Stock solutions of individual standards were prepared by dissolving each compound in methanol at a concentration of 1000 µg L⁻¹. A mixture of the standard working solutions was prepared weekly in methanol and stored in brown bottles at -18 °C. Methanol and acetonitrile were purchased from Romil Ltd. (Cambridge, UK). Dimethylsulfoxide (DMSO) (it is used as a solvent to prepare a 15% (w/w) PES solution), ethyl acetate, n-hexane, dichloromethane, iron (III)-chloride hexahydrate, and polyethylene glycol (PEG, MW =4000) were purchased from Merck (Darmstadt, Germany). Iron (II) sulfate heptahydrate was supplied from Scharlab (S.L., Spain). Ammonia solution was purchased from Chem-Lab (NV, Belgium). Polyethersulfone (PES, ultrason® E6020P with MW=58,000 g mol⁻¹) was purchased by BASF company. Multi-walled carbon nanotubes (MWCNTs >95%, OD: 5-15nm) were purchased from US Research Nanomaterials, Inc. High purity water was obtained from a Milli-Q water purification system (Millipore, Bedford, MA, USA).
2.2. Instrumentation

A KNAUER HPLC model Smartline with ultraviolet (UV) 2500 detector (Berlin, Germany) and a 20µL sample loop was used for separation and detection of pesticides. The separation was performed on a reversed-phase C18 HPLC Nucleodur column (5 μm, 250 × 4.6 mm, Macherey-Nagel Co., Düren, Germany). The isocratic elution was performed using a methanol-water solution with a ratio of 80:20 (v/v). The flow rate, injection volume, and UV wavelength were set at 0.7 mL min$^{-1}$, 20 µL, and 237 nm, respectively. The size, morphology, and elemental distribution of synthesized kit were analyzed by field emission scanning electron microscopy (FESEM) coupled with energy dispersive X-ray (EDX) system and elemental mapping using a TSCAN model MIRA3 (TESCAN, Brno, Czech Republic) operating at accelerating voltage of 20 kV.

2.3. Fe$_3$O$_4$ nanoparticles preparation

The Fe$_3$O$_4$ NPs were synthesized through the method reported by Zhu et al. [25]. A homogeneous mixture containing 90 mL 0.2 M FeCl$_3$.6H$_2$O aqueous solution and 40 mL 0.3 M FeSO$_4$.7H$_2$O aqueous solution was prepared. In the next step, 25 g PEG was added into this mixture and mixed sonically for several minutes to acquire a homogenous solution. This homogenous mixture was stirred at 70 °C and the ammonia solution was added dropwise to achieve the basic pH up to 9. Afterward, the reaction solution was stirred at the same temperature for 2 h and the Fe$_3$O$_4$ NPs were separated using an external magnet. The resultant Fe$_3$O$_4$ NPs were washed with high purity water several times and put in a vacuum oven at 40 °C for 24 h to dry.

2.4. Preparation of self-magnetic nanocomposite monolithic stir bar kit

Table S1 shows the materials required to make the kit with different weight ratios. According to this table, the fourth kit, which contains 0.100 g multi-walled carbon nanotubes (MWCNTs) and presents the highest extraction efficiency for target pesticides, was selected for further analysis. To prepare the self-magnetic nanocomposite monolithic (SMNM) kit, MWCNTs, a 15% (w/w) PES solution, and Fe$_3$O$_4$ NPs were mixed with the weight ratio of 0.10:0.80:1.00 in a dry and clean vial. To disperse MWCNTs and Fe$_3$O$_4$ NPs throughout the PES
solution and obtain a sufficiently homogeneous paste, the resultant mixture was sonicated for 30 min. Then, the prepared black paste was homogenized and loaded into an uncapped tube with 5 mm inner diameter. In the next step, the filled tube was cut into 15 mm pieces. In this way, several pastes containing cylindrical templates with the approximate size of 15 mm × 5 mm were prepared. Then, the cylindrical templates were immersed in high purity water for 48 h. Diffusion of water into the texture of the paste from the two open ends of the template helped to coagulate the black paste into a black bar and obtain the final kit. After hardening the kits, they were gently separated from the templates by clean forceps and immersed in another vial containing high purity water for 24 h.

To rigidify SMNM kits, they first were conditioned by stirring at 500 rpm for 30 min in 5 mL high purity water. Then, to remove any organic contaminants, they were stirred at 500 rpm for 30 min in 3 mL acetonitrile before using the kits for the first time. Finally, SMNM kits were regenerated by stirring at 500 rpm in 4 mL mixtures of acetonitrile-methanol (50:50) in room temperature, rinsing with high purity water, gently drying by a tissue paper, and storing at room temperature after each experimentation.

2.5. Stir bar sorptive extraction procedure

The handmade kits with a dimension of 5×15 mm were applied for simultaneous extraction and pre-concentration of target pesticides by Self-magnetic nanocomposite monolithic stir bar sorptive extraction (SMNMSBSE) process. First, a conditioned kit was placed inside a vial containing 60 mL of the water sample. The water sample was stirred using the magnetic stirrer at ambient temperature. The extraction and pre-concentration of target pesticides were performed under the following optimum conditions: extraction time: 42.00 min, stirring speed: 600.00 rpm, and 1.10% (v/v) acetonitrile as modifier. The optimum experimental conditions were achieved through central composite design (CCD) with Design-Expert® version7 software (Stat-Ease, Inc., 2005). After extracting target pesticides under optimal conditions, the kit was removed from the specimen. Then, it was washed using Milli-Q water and dried slightly using a tissue paper. For the liquid desorption, the kit was put inside a vial containing 1500 µL acetonitrile and was stirred for 10.00 min at 700.00 rpm. After desorption of analytes, the kit was removed and 100 µL of desorbed analytes was introduced to the sample loop (20 µL) for the analysis of target pesticides through HPLC-UV.
3. Results and discussion

3.1. Characterization of Fe$_3$O$_4$ NPs and SMNMSB kit

FESEM equipped with EDX spectroscopy, the X-ray diffraction (XRD), and elemental mapping were employed to determine the size, nanostructure morphology, and the elemental distribution of the produced kits, respectively. Fig. 1a displays the FESEM image of Fe$_3$O$_4$ NPs. According to this figure, the Fe$_3$O$_4$ NPs are about 5-250 nm in size. According to Figs. 1b and 1c, the FESEM image of the length and width sizes of the kit that are about 15 mm in 5 mm, respectively. Also, Fig. 1d presents the FESEM image of the kit’s nanostructures at 55.5 ×k magnification. Fe$_3$O$_4$ NPs, PES polymer, and MWCNTs are shown in Fig. 1d.

During the FESEM characterization, elemental mapping was employed to further confirm the components and element distribution. The elemental mapping from the FESEM analysis in Figs. 1e and 1f clearly confirm the presence of Fe and S in the SMNMSB kit, respectively. Also, they show a very uniform distribution of metal iron element (Fe is derived from the Fe$_3$O$_4$ NPs contained in the SMNMSB kit) and the sulfur element (S is derived from the PES polymer contained in the SMNMSB kit).

To compare the element content of nanocomposite, EDX characterization was carried out (Fig. 2). The EDX spectra of Fe$_3$O$_4$ NPs, Fe$_3$O$_4$/PES composite, and Fe$_3$O$_4$/PES/MWCNTs composite are shown in Figs. 2a to 2c, respectively. Fig. 2a illustrates the Fe and O atoms in the nanocomposite, which confirms the presence of Fe$_3$O$_4$ NPs. In Fig. 2b, in addition to Fe and O atoms, C and S atoms are also observed, which confirms the presence of polyethersulfone in Fe$_3$O$_4$/PES composite. Fig. 2c corroborates the attachment of MWCNTs onto Fe$_3$O$_4$/PES composite. Comparison between Fig. 2b and Fig. 2c shows that in Fig. 2c, the peak intensity of C and O atoms are increased, but the peak intensity of the S atom is almost constant, proving the presence of MWCNTs in Fe$_3$O$_4$/PES/MWCNTs composite. According to the FESEM images and EDX results of the Fe$_3$O$_4$ NPs and the SMNMSB kit, the observed morphology and matrix composition are in agreement with the EDX mapping results.

Fig. 3 presents the XRD pattern of the Fe$_3$O$_4$/PES/MWCNTs composite. The peaks at 2θ = 25.0° and 42.1°, which are assigned to (0 0 2) and (1 0 0) planes of the hexagonal graphite structure of MWCNTs, respectively, indicate that the presence of Fe$_3$O$_4$ NPs did not damage the
hexagonal graphite structure of the MWCNTs. Also, additional diffraction peaks at 2θ = 30.4°, 35.8°, 42.1°, 53.7°, 57.3°, and 63.0° correspond to the standard XRD data for the cubic Fe₃O₄ phase of inverse spinel crystal structure (JCPDS file No. 19-0629) [26]. No peaks corresponding to impurities appeared.

3.2. Optimization of SMNMSBSE procedure

To obtain a high extraction efficiency, the self-magnetic nanocomposite monolithic stir bar sorptive process was optimized. For this purpose, the parameters of absorption temperature, salting-out effect, extraction time, stirring speed and addition of organic modifier (acetonitrile) were evaluated. Unlike SPME, the effect of temperature on SBSE is not usually evaluated and the extractions are usually performed at room temperature. In this regard, only few authors have analyzed the effects of temperature [27, 28]. In the present study, the influence of absorption temperature on extraction efficiency of target pesticides was investigated systematically (Fig. S1). Since no significant differences in sensitivity were observed between 25 to 45 °C and, on the other hand, at temperatures above 35 °C, the Fe₃O₄ NPs existent in the kit gradually began to leach inside the sample solution, we decided to set the extraction temperature at 25 °C.

In the case of adding NaCl as a salting-out agent to hydrophobic analytes ($log K_{O/W} > 3.0$), it does not improve or even reduce the extraction efficiency, due to the increase in the viscosity of the sample, leading to slower extraction kinetics [23]. Fig. S2 presents the salting-out effect. Studied pesticides exhibited a $log K_{O/W} > 3.0$, so they are apolar and the addition of NaCl decreased their chromatographic responses. Except for hexythiazox, which has $log K_{O/W} < 3.0$ and is approximately polar, a 10% (w/v) increase in NaCl concentration increased the chromatographic response of this pesticide compared to other pesticides. In the case of pH parameter, because the organophosphorus pesticides are all non-ionizable compounds in aqueous solution, the pH of the solution would have little effect on the extraction efficiency [18]. Therefore, in this study, we decided to eliminate the factors effects of salting out and pH and carried out the extraction without adding NaCl and in the neutral solution.

3.2.1. Optimization using a central composite design

CCD was applied based on response surface methodology (RSM) and numerical optimization function of the Design Expert for the determination of the optimum conditions of
SBSE in water samples. In this method, three independent variables including extraction time (10.00-50.00 min), stirring speed (500.00-900.00 rpm), and different amounts of acetonitrile as modifier (0.00-5.00%, (v/v)) in five-level (±1.68, ±1, 0) are varied. The variables and their levels, with both natural and coded values, were investigated (Table 1). The actual experimental parameters predicted and residuals relevant to the design levels performed for the development of model are listed in Table S2. The total chromatographic peak area of five target pesticides (sum of the chromatographic peak area of each pesticide) was chosen as the experiments response (Y) and the system behavior was described by the quadratic model (Eq. 1) as follow:

\[ Y = \beta_0 + \sum_{i=1}^{k} \beta_i x_i + \sum_{i=1}^{k} \beta_{ii} x_i^2 + \sum_{i=1}^{k-1} \sum_{j=i+1}^{k} \beta_{ij} x_i x_j + \varepsilon \]  

where \( Y \) is estimated response, \( \beta_0 \) is the intercept term, \( \beta_i, \beta_{ii}, \) and \( \beta_{ij} \) are linear, quadratic, and interaction coefficients effects, respectively, \( x_i \) and \( x_j \) are independent variables, and \( \varepsilon \) is residual. The responses achieved from the set of experimental design (Table S2) were considered in multiple nonlinear regressions using the Design Expert 7 software to obtain the second order polynomial model and surface of the responses coefficients. To obtain the maximum yield, the optimal reaction parameters were generated using RSM and numerical optimization function of the software.

3.2.2. Response surface of fitting models

RSM is a set of statistical and mathematical techniques beneficial for problem analysis. In this technique, several independent variables affect a dependent variable or response and the aim is response optimization [21]. According to the data analysis, the following quadratic regression model (Eq. 2) was suggested for the relations between factors (independent variables) and response.

\[ \text{Total chromatographic peak area(response)} = +6 \cdot 24 \times 10^5 + 8 \cdot 86 \times 10^4 A + 2 \cdot 09 \times 10^4 B - 6 \cdot 58 \times 10^4 C - 2 \cdot 56 \times 10^4 AB - 3 \cdot 04 \times 10^4 A^2 + 3 \cdot 78 \times 10^4 B^2 - 1 \cdot 93 \times 10^4 C^2 \]  

In order to ensure that an adequate approximation of the true system is provided by the fitted model, and to verify that none of the assumptions of least squares regression is violated, it would be necessary to evaluate the fitted model. As can be seen in Table 2 and Table S3, the
statistical significance of the model was examined using analysis of variance (ANOVA). Probability value > F < 0.05 shows the significance of model term. The results also indicated that in the quadratic model (Eq. 2), there was no lack of fit (LOF) and the probability value > F for LOF was > 0.05. So, it is suggested that the proposed model is valid in this research. Moreover, the fitting of the model was examined using the coefficient determination ($R^2$). Model F-value of 47.48 implies the significance of the model. There is only a chance of 0.01% that such a large “Model F-Value” could happen because of noise. Probability value > F < 0.05 indicates the significance of the model term. Here, A, B, C, AB, A², B², and C² are significant model terms. Values < 0.1000 suggest that the model terms are not statistically significant. If there are many insignificant model terms (not counting those required to support hierarchy), the model reduction may improve the model.

“LOF F-value” of 3.25 for the model shows the LOF is not significant corresponding to the pure error. There is a 10.64% chance that this large LOF F-value could happen because of noise. Non-significant LOF F-value is good. The “predicted R-squared” of 0.8733 is in reasonable agreement with the “adjusted R-squared” of 0.9448. The signal to noise ratio is measured by “adequate precision”. A ratio greater than 4 is desirable. The ratio of 32.17 for model shows a sufficient signal. This model can be applied for navigation of the design space. A low coefficient of variation (CV), e.g., 4.19, indicates the desirable precision and reliability of experiments.

To compare the predicted result with the practical value, experimental rechecking was carried out using deduced optimal condition. Total chromatographic peak area (response) was achieved from actual experiments validating the RSM model. The predicted versus actual plot for the response is shown in Fig. 4. As can be seen, the actual values are distributed in the vicinity of the straight line ($y = ax+b$) with rather high $R^2$ value (0.9652).

The response surface plots obtained by plotting factors can be observed in Fig. 4. It shows the effect of extraction time and stirring speed on the response, where the value of acetonitrile addition was fixed at a center value (coded level of 0). As shown in this figure and Table S3, according to the $P$-value and $F$-ratios of the ANOVA data, the interaction between two factors of the extraction time and stirring speed was statistically significant. The response in Fig. 5 increased dramatically with the increase in extraction time at a fixed stirring speed. Thus, the maximum response was predicted when the extraction time was above 40.00 min. Similarly, the
increased stirring speed at a fixed extraction time also led to a slow increase in response content. Stirring can accelerate the molecular mass transfer rate and reduce the time to reach thermodynamic equilibrium. As a high stirring rate may cause damage to the stir bars in SBSE, a stirring rate of 600.00 rpm was employed in this work.

The effects between two factors of extraction time-acetonitrile addition (as modifier) and stirring speed-acetonitrile addition were not fit by the model in Eq. (2). Therefore, their effects were not studied.

3.2.3. Optimum conditions

Within the experimental range studied, optimum conditions for simultaneous extraction target pesticides were predicted using RSM and the optimization function of the Design Expert (Table 3). The analysis (Table 3) indicated that the maximum response was obtained at an extraction time of 42.00 min, a stirring speed of 600.00 rpm, and 1.10 % (v/v) acetonitrile addition as a modifier. Comparison of predicted and experimental values revealed a good correspondence between them, implying that empirical models derived from the RSM could be used to adequately describe the relationship between the factors and response in simultaneous extraction target pesticides.

3.3. Optimization of the desorption step

Liquid desorption (LD) was applied to desorb the target pesticides from the SMNMSB kit. Therefore, the effects of desorption solvent, desorption time, and stirring speed on desorption efficiency were investigated, systematically. For LD of the analytes, the kit was placed in a glass vial containing 1500 µL of desorption solvent and then it was stirred for certain time. The total chromatographic peak area of five target pesticides was selected as an experimental response.

3.3.1. Effect of desorption solvent

In this section, the effect of desorption solvents on desorption efficiency was studied. Methanol, acetonitrile, ethyl acetate, dichloromethane, and n-hexane were tested as the solvent for desorption of target pesticides. Table S4 shows the effect of desorption solvents on desorption efficiency, under the following experiment conditions: analyte concentration: 1.00 µg L\(^{-1}\) phosalone and 5.00 µg L\(^{-1}\) of the other pesticides, Extraction step at 25 °C, stirring rate of 700.00 rpm for 10.00 min, no acetonitrile addition as modifier, desorption step at 700.00 rpm for
10.00 min, and 1500 µL desorption solvent. It was found that dichloromethane provided a better desorption efficiency than others for target pesticides, but SMNMSB kit was dissolved in dichloromethane as solvent desorption. Therefore, dichloromethane was not selected as the desorption solvent. As can be seen in Table S4, acetonitrile was selected as a desorption solvent, because it provided higher desorption efficiency than other solvents for target analytes. Moreover, in this research, acetonitrile was one of the components of the mobile phase in subsequent HPLC separation. Thus, acetonitrile was chosen as the desorption solvent in this work.

### 3.3.2. Effect of desorption time

The effect of desorption time varying from 5.00 min to 20.00 min on desorption of target pesticides by SMNMSBSE-LD was studied with acetonitrile (1500 µL) as desorption solvent. The experiment results in Fig. S3 indicate that desorption process for ethion, diazinon, and hexythiazox approximately was established after 10.00 min while for phosalone and chlorpyrifos, the liquid desorption equilibrium was achieved after 15.00 min. Accordingly, prolonging the process from 15 to 20 min resulted in a negligible improvement in the desorption efficiency of the analytes. Generally, for total target pesticides, 15.00 min was selected as desorption time for subsequent experiments.

### 3.3.3. Effect of stirring rate in the desorption step

Stirring rate has two opposite effects on SBSE. The equilibrium state is reached faster at a higher stirring rate, while it might lead to physical damage to the SMNM kit due to the continuous friction between the surface of the kit and the bottom surface of the glass flask. Therefore, this parameter should be optimized in SBSE. The effect of stirring speed within 500.00-900.00 rpm on desorption of analytes was studied (Fig. S4). According to Fig. S4, it can be generally stated that chromatographic peak area of target pesticides was increased with the increase of stirring rate from 400.00 rpm to 700.00 rpm, and then was gradually decreased with further increase in the stirring rate to 900.00 rpm. Therefore, the moderate stirring rate of 700.00 rpm was adopted for the desorption step.

### 3.4. Reproducibility and lifetime of the prepared SMNMSB kit
The preparation reproducibility and lifetime of the SMNMSB kit are respectively outlined in Table S5 and Fig. S3. The data listed in Table S5 reveal an acceptable preparation reproducibility with the relative standard deviations (RSDs) of 3.73-7.97% (bar to bar) and 5.39-10.84% (batch to batch). The lifetime of SMNMSB kit is also presented in Fig. S5. As can be seen, the prepared SMNMSB kit could be reused in a water sample for more than 8-10 times without any significant decrease in the extraction efficiency.

3.5. Analytical performance

The analytical performance of SMNMSBSE-LD-HPLC-UV was investigated for the simultaneous determination of target pesticides under the optimum conditions (Table 4). The limits of detection (LODs), based on the signal to noise ratio (S/N) of 3, ranged from 0.07 to 0.89 µg L\(^{-1}\). The LOQs, based on the signal to noise ratio (S/N) of 10, ranged from 0.23 to 2.94 µg L\(^{-1}\). Interday and intraday precisions (n=3) were obtained by extracting analytes at a concentration level of 1.00 µg L\(^{-1}\) phosphalone and 5.00 µg L\(^{-1}\) of the other pesticides. The RSDs did not exceed 5.48% and 6.45 % for intraday and interday precisions, respectively. The obtained calibration curves illustrated satisfactory linearity in the ranges of 5 to 800 µg L\(^{-1}\) for ethion, hexythiazox, and chlorpyrifos, 1 to 1000 µg L\(^{-1}\) for phosphalone and 5 to 1000 µg L\(^{-1}\) for diazinon, respectively. Also, coefficients of determination (\(r^2\)) for all analytes were over 0.9963.

The enrichment factors, calculated by the ratio of the slope of the calibration curves obtained with and without [29] SMNMSBSE, were ranged from 15 to 39-fold. The difference in enrichment factors for five target pesticides may be related to their \(K_{O/W}\) and chemical structure and the extraction mechanism of SMNMSB kit. For polar pesticide of hexythiazox (\(K_{O/W}=2.75\)), a relatively low enrichment factor was obtained. In comparison, for apolar pesticides of diazinon (\(K_{O/W}=3.81\)), phosphalone (\(K_{O/W}=4.01\)), chlorpyrifos (\(K_{O/W}=4.76\)), and ethion (\(K_{O/W}=5.07\)), high enrichment factor was achieved because of hydrophobic interaction between these pesticides and MWCNTs existing in SMNMSB kit.

3.6. Real sample analysis

The environmental water samples were collected from our laboratory tap water (Sari-Iran), well water from Agricultural Science and Natural Resources University (Sari-Iran), and river water from Babol Roud River (Babol-Iran). In a typical assay, a SMNMSB kit was
immerses into a glass vial containing pre-filtered water sample (0.45 µm PTFE membrane). The vial was closed with a PTFE/silicone screw cap. The extraction of pesticides in water samples was performed in accordance with the procedure described in Section 2.5.

The analytical results and the recovery for the spiked samples are listed in Table 5. None of the target pesticides was found in tap water and well water. In Babol Roud river water, diazinon was detected at 1.00 µg L⁻¹. Fig. 6 presents the chromatograms obtained by SMNMSBSE-LD-HPLC-UV for Babol Roud river water before and after pesticide spiking. With respect to the chromatograms, the proposed method showed excellent clean-up for target pesticides from water samples. Also, this study led to satisfactory robustness by achieving recoveries in the range of 80.60% to 104.52%.

3.7. Comparison of SMNMSBSE-LD-HPLC-UV with other extraction methods

Extraction and determination of the target pesticides existing in water samples through the application of SMNMSBSE-LD in combination with HPLC-UV detection are compared with some other methods in Table S6. Precision relative standard deviation and recovery values of the proposed method are similar to the other methods. Apparently, the performance of SMNMSBSE-LD-HPLC-UV is superior to the other methods with respect to the limit of detection and linear range of the calibration curves. Hence, LODs and linear range for determination of pesticides outperform most of the other methods.

4. Conclusions

In the current study, a SMNMSB kit was produced and a method of SMNMSBSE-LD-HPLC-UV was presented to determine pesticides in water samples.

The extraction parameters were optimized using a five-level three-factor central composite design and desorption parameters were optimized systematically, as well. Under optimum conditions, results showed that SMNMSB kit could enrich target pesticides effectively because of its high extraction efficiency.

Three different water samples including tap water, well water, and river water were analyzed by the present methodology. The proposed method has several benefits, including simplicity of the procedure to make SMNMSB kit, low detection limits, high extraction
efficiency, excellent clean-up, and wide linear range. Ultimately, this kit can be used for commercial purposes and can easily be utilized in common microextraction procedures.

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Caption for figures:

Fig. 1. FESEM images of (a) the Fe$_3$O$_4$ NPs; (b) the length size of the SMNMSB kit; (c) the width size of the SMNMSB kit; (d) the kit’s nanostructures at 55.5 kx magnifications; (e) the distribution of elemental mapping of 1000XFeKa; (f) the distribution of elemental mapping of 1000XSKa.

Fig. 2. EDX spectra of (a) the Fe$_3$O$_4$ NPs; (b) the Fe$_3$O$_4$/PES composite; (c) the Fe$_3$O$_4$/PES/MWCTs composite.

Fig. 3. XRD pattern of the Fe$_3$O$_4$/PES/MWCTs composite.

Fig. 4. Actual versus predicted values of the responses for total chromatographic peak area of target pesticides.

Fig. 5. Estimated response surfaces obtained using CCD for total chromatographic peak area of target pesticides, by plotting extraction time vs. stirring speed.

Fig. 6. HPLC-UV chromatograms of (a) Babol Roud River water obtained by SMNMSBSE; (b) Spiked Babol Roud River water (1.00 µg L$^{-1}$ phosalone and 5.00µg L$^{-1}$ of the other pesticides) obtained by SMNMSBSE. Peaks of 1-5 represent ethion, phosalone, diazinon, hexythiazox and chlorpyrifos, respectively.

Fig. S1. Influence of the absorption temperature on the extraction efficiency of target pesticides obtained by SMNMSB kit. Extraction step conditions: extraction temperature, 25-45 °C; extraction time, 10.00 min; stirring speed, 700.00 rpm; no addition acetonitrile as modifier and desorption step conditions: stirring speed, 700.00 rpm for 10.00 min and 1500 µL acetonitrile as desorption solvent. Analyte concentration in spiked water sample: 1.00 µgL$^{-1}$ phosalone and 5.00 µgL$^{-1}$ of the other pesticides.
Fig. S2. Effect of NaCl concentration on the extraction efficiency of the analytes obtained by SMNMSB kit. Extraction step conditions: extraction temperature, 25°C; extraction time, 10.00 min; stirring speed, 700.00 rpm; no addition acetonitrile as modifier and desorption step conditions: stirring speed, 700.00 rpm for 10.00 min and 1500 µL acetonitrile as desorption solvent. Analyte concentration in spiked water sample: 1.00 µgL⁻¹ phosalone and 5.00 µgL⁻¹ of the other pesticides.

Fig. S3. Effect of desorption time (5.00-20.00 min) on the extraction of the analytes obtained by SMNMSBSE. Optimum conditions in: [Extraction step: extraction temperature, 25 °C; extraction time, 42.00 min; stirring speed, 600.00 rpm; acetonitrile addition as modifier, 1.10% (v/v)] and [desorption step: stirring speed, 700.00 rpm for 5.00-20.00 min and 1500 µL acetonitrile as desorption solvent]. Analyte concentration in spiked sample: 1.00 µg L⁻¹ phosalone and 5.00 µg L⁻¹ of the other pesticides.

Fig. S4. Effect of stirring speed (500.00-900.00 rpm) on the extraction of the analytes obtained by SMNMSBSE. Optimum conditions in: [Extraction step: extraction temperature, 25 °C; extraction time, 42.00 min; stirring speed, 600.00 rpm; acetonitrile addition as modifier, 1.10% (v/v)] and [desorption step: stirring speed, (500.00-900.00) rpm for 15.00 min and 1500 µL acetonitrile as desorption solvent]. Analytes concentration in spiked sample: 1.00 µg L⁻¹ phosalone and 5.00 µg L⁻¹ of the other pesticides.

Fig. S5. The lifetime of SMNMSB kit for extracting five target pesticides in water sample. Optimum conditions in: [Extraction step: extraction temperature, 25 °C; extraction time, 42.00 min; stirring speed, 600.00 rpm; acetonitrile addition as modifier, 1.10% (v/v)] and [desorption step: stirring speed, 700.00 rpm for 15.00 min and 1500µL acetonitrile as desorption solvent]. Analytes concentration in spiked sample: 1.00 µgL⁻¹ phosalone and 5.00 µgL⁻¹ of the other pesticides.
Table 1
Variables and their levels employed in a CCD for the evaluation of the extraction efficiency.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Coded levels</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>-α(-1.68)</td>
</tr>
<tr>
<td>Extraction time (min)</td>
<td>10.00</td>
</tr>
<tr>
<td>Stirring speed (rpm)</td>
<td>500.00</td>
</tr>
<tr>
<td>Acetonitrile addition (modifier) (%)</td>
<td>0.00</td>
</tr>
</tbody>
</table>
Table 2
ANOVA result for studied response by Design Expert.

<table>
<thead>
<tr>
<th>Response</th>
<th>Probability For model</th>
<th>R$^2_a$</th>
<th>Adj. R$^2_b$</th>
<th>Pred. R$^2_c$</th>
<th>Adeq. Precision $^d$</th>
<th>SD $^e$</th>
<th>CV $^f$</th>
<th>PERESS $^g$</th>
<th>Probability For lack of fit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total chromatographic peak area</td>
<td>&lt; 0.0001</td>
<td>0.9652</td>
<td>0.9448</td>
<td>0.8733</td>
<td>32.17</td>
<td>2.58 x 10$^4$</td>
<td>4.19</td>
<td>2.9 x 10$^{-3}$</td>
<td>0.1064</td>
</tr>
</tbody>
</table>

$^a$R$^2$: Determination coefficient.
$^b$Adj.R$^2$: Adjusted R$^2$.
$^c$Pred.R$^2$: Predicted R$^2$.
$^d$Adeq. Precision: adequate precision.
$^e$SD: Standard deviation.
$^f$CV: Coefficient of variation.
$^g$PERESS: Predicted residual error sum of squares.
Table 3
Optimum conditions, predicted and experimental response values.

<table>
<thead>
<tr>
<th>Optimum condition</th>
<th>Total chromatographic peak area of target pesticides</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Extraction time (min)</td>
</tr>
<tr>
<td></td>
<td>42.00</td>
</tr>
</tbody>
</table>

<sup>a</sup> Stirring speed in extraction step  
<sup>b</sup> Means ± relative standard deviation (n = 3).
Table 4
Analytical performance of the proposed SMNMSBSE-LD-HPLC-UV for pre-concentration and simultaneous determination of target pesticides.

<table>
<thead>
<tr>
<th>Analytes</th>
<th>Linear range (µg L(^{-1}))</th>
<th>Correlation coefficient ((r^2))</th>
<th>Limit of detection (µg L(^{-1}))</th>
<th>Limit of quantification (µg L(^{-1}))</th>
<th>Relative standard deviation (^{a}) (%, (n = 3))</th>
<th>Enrichment factor (^{b})</th>
<th>Intraday</th>
<th>Interday</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethion</td>
<td>5-800</td>
<td>0.9963</td>
<td>0.81</td>
<td>2.66</td>
<td>2.73</td>
<td>3.68</td>
<td>39</td>
<td></td>
</tr>
<tr>
<td>Phosalone</td>
<td>1-1000</td>
<td>0.9973</td>
<td>0.07</td>
<td>0.23</td>
<td>5.48</td>
<td>6.45</td>
<td>30</td>
<td></td>
</tr>
<tr>
<td>Diazinon</td>
<td>5-1000</td>
<td>0.9991</td>
<td>0.63</td>
<td>2.12</td>
<td>4.16</td>
<td>5.78</td>
<td>27</td>
<td></td>
</tr>
<tr>
<td>Hexythiazox</td>
<td>5-800</td>
<td>0.9968</td>
<td>0.89</td>
<td>2.94</td>
<td>3.23</td>
<td>4.19</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td>Chlorpyrifos</td>
<td>5-800</td>
<td>0.9980</td>
<td>0.76</td>
<td>2.53</td>
<td>5.11</td>
<td>4.84</td>
<td>38</td>
<td></td>
</tr>
</tbody>
</table>

\(^{a}\) The relative standard deviation at concentration of 1.00 µg L\(^{-1}\) phosalone and 5.00 µg L\(^{-1}\) of the other pesticides.

\(^{b}\) The enrichment factors, calculated by the ratio of the slope of the calibration curves obtained with and without SMNMSBSE.
Table 5
Analytical performance of SMNMSBSE–LD-HPLC–UV for analysis of five target pesticides in real environmental water samples.

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Tap water</th>
<th>Well water</th>
<th>River water (Babol Roud)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Added (µg L⁻¹)</td>
<td>Found (µg L⁻¹)</td>
<td>Recovery (%±RSD, n=3)</td>
</tr>
<tr>
<td>Ethion</td>
<td>0.00</td>
<td>ND a</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>5.00</td>
<td>4.87</td>
<td>97.40±2.73</td>
</tr>
<tr>
<td></td>
<td>50.00</td>
<td>46.24</td>
<td>92.48±2.80</td>
</tr>
<tr>
<td>phosalone</td>
<td>0.00</td>
<td>ND</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>1.00</td>
<td>0.91</td>
<td>91.00±6.45</td>
</tr>
<tr>
<td></td>
<td>5.00</td>
<td>4.59</td>
<td>91.80±6.21</td>
</tr>
<tr>
<td>Diazinon</td>
<td>0.00</td>
<td>ND</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>5.00</td>
<td>4.25</td>
<td>85.00±4.60</td>
</tr>
<tr>
<td></td>
<td>50.00</td>
<td>44.14</td>
<td>88.28±3.85</td>
</tr>
<tr>
<td>Hexythiazox</td>
<td>0.00</td>
<td>ND</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>5.00</td>
<td>4.33</td>
<td>87.60±4.96</td>
</tr>
<tr>
<td></td>
<td>50.00</td>
<td>50.81</td>
<td>101.62±3.62</td>
</tr>
<tr>
<td>Chlorpyrifos</td>
<td>0.00</td>
<td>ND</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>5.00</td>
<td>4.87</td>
<td>97.40±4.72</td>
</tr>
<tr>
<td></td>
<td>50.00</td>
<td>46.60</td>
<td>93.20±6.04</td>
</tr>
</tbody>
</table>

a Not detected.
Fig. 1
Fig. 2
Fig. 3

[Graph showing Fe₃O₄ NPs and MWCNTs]
Fig. 4
Fig. 5
Fig. 6
Highlights

- A simple and cheap procedure for preparation of the SMNM kit was applied.
- The SMNM material including PES solution, Fe$_3$O$_4$ NPs and MWCNTs with certain rations which was used as a sorptive kit for extraction of pesticides from water samples.
- The extraction factors were optimized using a CCD and parameters affecting desorption were optimized "one at a time".