Talanta 99 (2012) 13–21

Contents lists available at SciVerse ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

Multi-response optimization of magnetic solid phase extraction based on carbon coated Fe_3O_4 nanoparticles using desirability function approach for the determination of the organophosphorus pesticides in aquatic samples by HPLC–UV

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ARTICLE INFO

Article history:
Received 10 February 2012
Received in revised form
28 March 2012
Accepted 18 April 2012
Available online 26 April 2012

Keywords:
Carbon
Magnetic nanoparticles
Magnetic solid phase extraction
Organophosphorus pesticides
Desirability function
Water analysis

ABSTRACT

The present study investigates the application of carbon coated Fe_3O_4 (Fe_3O_4/C) magnetic nanoparticles as an adsorbent for magnetic solid phase extraction (MSPE) of trace amounts of organophosphorus pesticides (OPPs) from environmental water samples and their determination using high performance liquid chromatography with ultraviolet detection (HPLC–UV). The Fe_3O_4/C magnetic nanoparticles were synthesized by a simple hydrothermal reaction and the resultant material was characterized by X-ray powder diffraction, field emission scanning electron microscopy and Fourier transform infrared. Central composite design combined with desirability function (DF) was applied to find the experimental conditions providing the highest global extraction efficiency. These conditions were found in correspondence with a solution pH of 9.16, 97.4 mg Fe_3O_4/C and 10 mmol L^{-1} NaCl added to samples. Under the optimal conditions, the proposed method was evaluated, and applied to the analysis of OPPs in water samples. The results demonstrated that our proposed method had wide dynamic linear range (0.05–400 ng mL^{-1}) with a good linearity ($R^2 > 0.9949$) and low detection limits (4.3–47.4 pg mL^{-1}). High enrichment factors were achieved ranging from 330–1200. The established MSPE–HPLC–UV method has been successfully applied for the determination of the OPPs in spiked water samples (well, tap, river and mineral). Satisfactory recovery results showed that the matrices under consideration do not significantly affect the extraction process.

1. Introduction

Sample preparation methods generally involve a solvent- or solid-phase-based extraction technique [1,2]. The objective of sample preparation is to transfer the analyte from the sample matrix into a form that is pre-purified, concentrated and compatible with the analytical system. The disadvantages such as intensive labor, time consuming, unsatisfactory enrichment factor and large quantity of toxic solvent, limit application of classical sample preparation methods such as liquid–liquid extraction (LLE). In order to avoid some of these drawbacks, solid-phase extraction (SPE) is increasingly used [3]. SPE needs smaller volumes of reagents than LLE, thus generating less toxic waste. Moreover, better enrichment factors are obtained by SPE [4]. In some cases, however, due to the limited rate of diffusion and mass transfer, extraction time of ordinary SPE processes is usually long [5], it is particularly evident when extracting very low amount of the target analytes from large volumes of samples.

Interest in studying nanostructured materials has grown significantly in recent years due to unique size and physical properties. These materials have many important applications in the fields of biotechnology, biomedicine, engineering, material science and environmental areas [6–8]. Magnetic nanoparticles (MNPs) have attracted much research interest due to many potential technological applications in different areas, e.g., in catalysis as support [9], in targeted drug delivery [10], adsorption processes [11] and environmental remediation [12]. Also, MNPs can be used as a novel and excellent adsorbents due to their unique advantages over traditional micro-sized adsorbents [13]. Therefore, in recent years, a new procedure for SPE, based on the use of magnetic or magnetically modified adsorbents called magnetic solid-phase extraction (MSPE) [14], has been developed. It adopts magnetic particles as adsorbents, which endow some unique features in extraction and solve some problems associated with ordinary SPE. A distinct advantage of this technology is that magnetic materials can be readily isolated from sample solutions by the application of an external magnetic field. This advantage solved some inherent limitations associated with the use of nanomaterials in SPE column. For example, when column dynamic extraction mode is used, the nanosized particles packed SPE column exhibits high backpressure, making it very difficult to adopt

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http://dx.doi.org/10.1016/j.talanta.2012.04.023

high flow rates; when the static batch mode is used, the nanosized SPE adsorbents often lead to a very low filtration rate [15].

Among the most widely used magnetic particles, bare Fe_3O_4 play a major role in many areas. However, bare Fe_3O_4 magnetic nanoparticles can easily form large aggregates, which may alter their magnetic properties. Moreover, these nm-sized metal oxides are not target selective and are unsuitable for samples with complicated matrices. Magnetic particles can be coated with a protective layer of different materials to improve their stability and to introduce new surface properties and functionalities. Carbon is a versatile coating material due to its chemical stability, biocompatibility, possibility of surface modification and pore creation.

Organophosphates are the basis of many pesticides and chemical warfare agents. Organophosphorus pesticides (OPPs) are a class of chemicals that generally act as cholinesterase inhibitors and have been widely used in agriculture due to their high efficiency as insecticides [16]. Slow degradation of pesticides in the environment and extensive or inappropriate use by farmers can lead to environmental contamination [17]. OPPs are known to be highly neurotoxic and they disrupt the cholinesterase that regulates acetylcholine, a neurotransmitter needed for proper nervous system function [18].

Contamination of surface water and groundwater with hazardous compounds has attracted increasing attention in recent decades all over the world. Therefore, for the sake of human health and environmental pollution control, the determination of trace OPPs in environmental samples is of tremendous importance. According to the European Union (EU) Directive on water quality (98/83/CE) [19], the maximum admissible concentration (MAC) for pesticides is $0.1\,\mu g\,L^{-1}$ for each individual substance and $0.5\,\mu g\,L^{-1}$ is the maximum allowed for the total concentration of all organophosphorus. Development of an efficient analytical method to detect such contaminants is an important topic for environment protection.

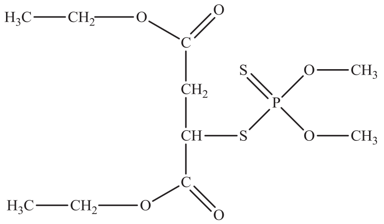
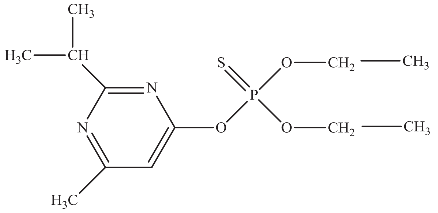
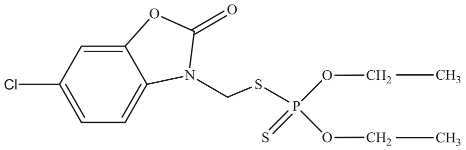
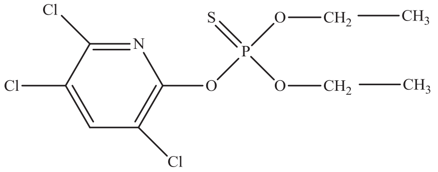
At present, a number of methods have been used for the preconcentration and determination of OPPs in aquatic samples like ionic liquid-based dispersive liquid–liquid microextraction (IL-DLLME) [20], SPE [21], solid phase microextraction (SPME) [22], single-drop microextraction (SDME) [23] and cloud point extraction (CPE) [24]. However, most of these methodologies are laborious and time consuming and therewith cannot achieve low detection limits. In this study MNPs were synthesized by chemical coprecipitation technique and was modified with carbon by a simple hydrothermal reaction. Scanning electron microscopy, X-ray powder diffraction and Fourier transform infrared spectroscopy were applied to characterization of Fe_3O_4 and carbon coated Fe_3O_4 (Fe_3O_4/C) MNPs. The prepared MNPs were employed for preconcentration of OPPs (see Table1) from aquatic samples prior to be determined by HPLC–UV. Central composite design (CCD) combined with desirability function (DF) was applied for optimization purposes. The optimization procedure of MSPE (CCD combined with DF) can be used as a new approach to obtain the optimized values of variables in experimental design. To the best of our knowledge, this methodology has not been employed previously in the extraction and determination of trace OPPs from aquatic samples.

2. Experimental

2.1. Chemicals and water samples

Pesticide analytical standards include malathion (Mala), diazinon (Diaz), phosalone (Phos) and chlorpyrifos (Chlor) were provided by Fluka (Germany) (Table 1 shows their structures and IUPAC names). All pesticide standards were of 97.7–99.5% purity. Standard solutions of each compound at a concentration of $100\,mg\,L^{-1}$ were prepared in methanol and stored at 4 °C. Methanol LC-grade from Merck

Table 1
Name, abbreviation and chemical structures of the target analytes.

Name	Abbreviation	Chemical structure
Malathion Diethyl 2-[(dimethoxyphosphorothioyl)sulfanyl]butanedioate	Mala	
Diazinon O,O-Diethyl O-[4-methyl-6-(propan-2-yl)pyrimidin-2-yl] phosphorothioate	Diaz	
Phosalone 6-chloro-3-(diethoxyphosphinothioylsulfanylmethyl)-1,3-benzoxazol-2-one	Phos	
Chlorpyrifos O,O-Diethyl O-3,5,6-trichloropyridin-2-yl phosphorothioate	Chlor	

(Darmstadt, Germany) was used for standard preparation and chromatographic analysis. $FeCl_2 \cdot 4H_2O$, $FeCl_3 \cdot 6H_2O$, sodium hydroxide and hydrochloric acid used for pH adjustment and MNPs synthesis and sodium chloride used for ionic strength studies were also purchased from Merck (Darmstadt, Germany). All other chemicals were obtained from Merck (Darmstadt, Germany).

The recovery studies were carried out using tap water, well water, river water and mineral water samples. Tap water sample was collected freshly from our laboratory (Azarbaijan University of Tarbiat Moallem, Tabriz, Iran) and well water came from deepground water in Tabriz (Iran). River water (Aji chai River, Tabriz, Iran) was picked up a few days before analysis. Mineral water sample was collected from kandowan (East Azarbaijan Province, Iran). All samples were collected in brown bottles, stored in the dark place at 4 °C until analysis. Initial analysis confirmed that they were free of target analytes.

2.2. Synthesis of magnetite nanoparticles

The coprecipitation technique is probably the simplest and most efficient chemical pathway to obtain magnetic particles [25]. Therefore, in this work Fe_3O_4 MNPs were prepared by chemical coprecipitation technique [26]. Firstly, 5.2 g of $FeCl_3.6H_2O$, 2.0 g of $FeCl_2.4H_2O$, were dissolved into 25 mL deoxygenated deionized water followed by adding 0.85 mL of 12 mol L^{-1} HCl under N_2 protection. Then, the resulting solution was added dropwise into 250 mL of 1.5 mol L^{-1} NaOH solution under vigorous stirring N_2 protection at 80 °C. The produced MNPs were rinsed with deionized water (5 × 300 mL) and then were dispersed into appropriate amount of deionized water to get 5 mg mL^{-1} suspension of Fe_3O_4 MNPs.

The Fe_3O_4/C MNPs were synthesized based on an established procedure [26], with slight modification. Briefly, for the preparation of Fe_3O_4/C MNPs, 4 g of glucose was dissolved in 40 mL of 5 mg mL^{-1} Fe_3O_4 MNPs aqueous solution. And then, the reactant mixture was placed in a 50 mL Teflon-sealed autoclave and heated at 170 °C for 4 h. Finally, the autoclave was cooled naturally in air, and the products were isolated with the help of a magnet and washed with deionized water and ethanol for ten times, respectively. Fig. 1 shows the synthesis procedure for Fe_3O_4/C MNPs.

2.3. Instrumentation

A Jasco HPLC system, consisted of a PU-1580 isocratic pump, a Rheodyne 7725i injector with a 20–µL loop (Rheodyne, Cotati, CA, USA) and a UV-1575 spectrophotometric detector was used in the experiment. The chromatographic system was controlled by HSS-2000 provided by Jasco using the LC-Net II/ADC interface. The data were processed using BORWIN software (version 1.50). An analytical 250 mm × 4.6 mm ID, 5-µm particle, Perfectsil Target ODS–3 column (MZ–Analysentechnik, Germany) with a ODS–3 precolumn (10 × 4.0 mm I.D., 5 µm), which was maintained at ambient temperature, was employed for separation.

X-ray powder diffraction (XRD) pattern of the sample were recorded on Brucker AXF (D8 Advance) X-ray power diffractometer with a CuKα radiation source (λ=0.154056 nm) generated at 40 kV and 35 mA. The samples were scanned in 2θ range 10°–80° at a scan rate of 5° min^{-1}. Fourier transform infrared (FTIR) spectra were recorded using a Bruker model Vector 22 FTIR Spectrometer (Ettlingen, Germany) on KBr pellets. Scanning electron microscopy images were obtained using an S–4800 field emission scanning electron microscope (FESEM) (Hitachi, Tokyo, Japan).

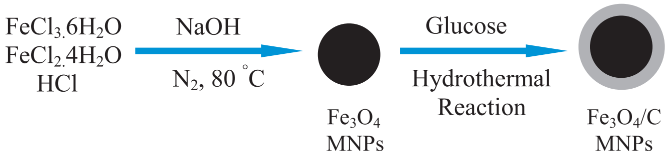


Fig. 1. Schematic illustration of the preparation strategy for Fe_3O_4/C MNPs.

2.4. MSPE procedure

MSPE of all the samples involved in this study was carried out as follows. An aliquot of 800 mL of filtered water samples was transferred to 1000 mL glassware beakers and 10 mmol L^{-1} NaCl was added into the sample, the pH was adjusted to 9.16 with 1 mol L^{-1} NaOH. Then, 4.87 mL of Fe_3O_4/C MNPs suspension (20 mg mL^{-1}) were added into the sample followed by sonication for 10 min at 25 ± 3 °C. Then an Nd–Fe–B strong magnet (100 × 50 × 40 mm) was positioned at the bottom of the beaker, and the Fe_3O_4/C MNPs were isolated from the suspension. The preconcentrated target analytes adsorbed on Fe_3O_4/C MNPs were desorbed with 8 mL acetonitrile with 5 min sonication at 25 ± 3 °C. The acetonitrile was transferred into another beaker and was evaporated to near dryness under a nitrogen stream at 25 °C and volume of acetonitrile was adjusted to 0.5 mL. A 20 µL of this solution was injected into the HPLC system for analysis.

2.5. Chromatographic conditions

The isocratic mobile phase consisted of methanol–phosphate buffer (25 mM) (pH=5) in the ratio of 80:20 v/v, flowing through the column at a constant flow rate of 1 mL min^{-1} [27]. The eluent was monitored using UV detection at a wavelength of 225 nm. The mobile phase was filtered through a 0.22 µm membrane–type GV filter (Millipore). A 40 kHz and 138 W ultrasonic water bath with temperature control (sonic bath model LBS2–FALC instruments S.r.l TREVIGLIO, Italy) was applied to degassing the mobile phase.

2.6. Desirability approach

When several responses were evaluated in an experimental design, the optimum points reached individually for each factor did not coincide in all cases. There are many statistical methods for solving multiple response problems such as overlaying the contours plot for each response, constrained optimization problems and the desirability approach [28].

The Derringer function or DF [29] is the most important and most currently used multi-criteria methodology in the optimization of analytical procedures. DF involves the transformation of each predicted response to a dimensionless partial DF d_i. The scale of the DF ranges between $d=0$, for a completely undesirable response, to $d=1$ for a fully desired response above which further improvements would have no importance. With the individual desirabilities, it is then possible to obtain the overall desirability (D). The D is defined as the weighted geometric average of the individual desirability (d_i).

$$D=\left(\prod_{i=1}^{n} d_i\right)^{1/n}$$

where d_i indicates the desirability of the response and n is the number of responses in the measure.

3. Results and discussion

3.1. Characterization of Fe_3O_4 and Fe_3O_4/C MNPs

The surface chemistry of Fe_3O_4 and Fe_3O_4/C MNPs was studied using FTIR spectrum. The typical FTIR spectra of MNPs with and without modification were shown in Fig. 2A. As can be seen, an absorption band appeared at 585 cm^{-1} corresponding to the Fe–O bond in the Fe_3O_4 particles. Compared with the spectrum of bare Fe_3O_4, the presence of carbon in Fe_3O_4/C could be proven by the peaks at 1699.2 and 1616.7 cm^{-1} corresponding to stretching frequencies of C=O and C=C, respectively, and by the peaks at 1289.0 and 1381.1 cm^{-1} corresponding to the C–O stretching and O–H bending vibrations [30]. These functional groups on the surface of modified Fe_3O_4 MNPs suggesting the presence of large amount of hydrophilic groups. These results confirm the generation of the Fe_3O_4/C MNPs.

XRD is a powerful tool for crystal structure characterization at the present. Therefore, the crystalline structure of the synthesized MNPs was characterized by XRD. The XRD spectra of the Fe_3O_4 and the Fe_3O_4/C MNPs were shown in Fig. 2B. The presence of the peaks corresponding to the planes (220), (311), (222), (400), (511), and (440) in the pattern of the sample confirm the formation of spinel structure. Also, Fe_3O_4 and Fe_3O_4/C MNPs had similar diffraction peaks, this result indicated that the crystal structure of Fe_3O_4 MNPs was not changed after modification with carbon.

Fig. 3 shows FESEMs of typical surface regions of the Fe_3O_4 (A) and Fe_3O_4/C MNPs (B), obtained with 1,00,000 magnifications. It can be seen that they have nearly uniform distribution of particle size. The particle sizes of both Fe_3O_4 and Fe_3O_4/C MNPs were measured in SEM micrographs. The diameter of Fe_3O_4 MNPs is in the range of 35–45 nm and that of Fe_3O_4/C MNPs is a little larger.

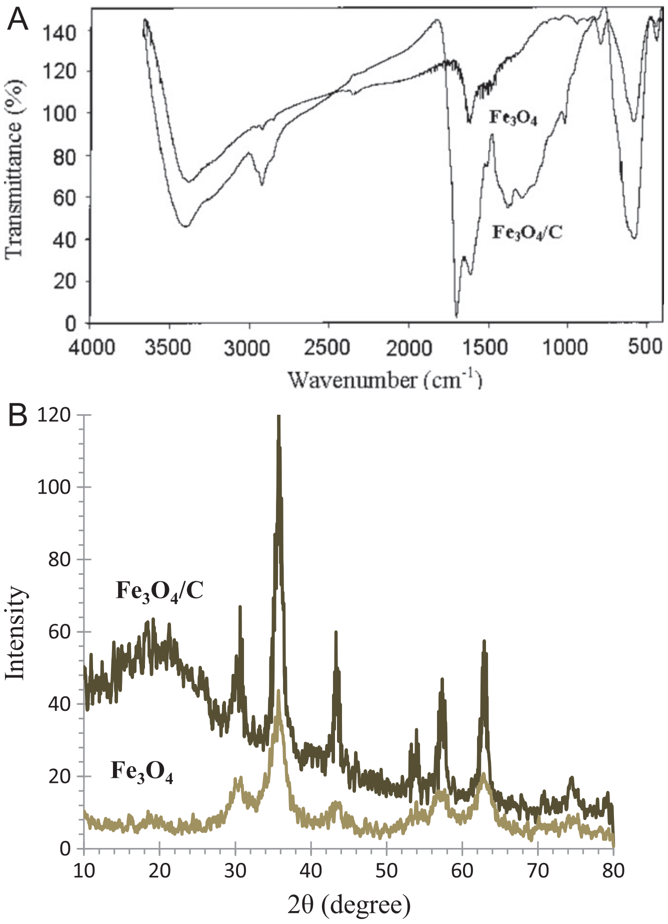


Fig. 2. FTIR spectra (A) and XRD pattern (B) of the Fe_3O_4 and Fe_3O_4/C MNPs.

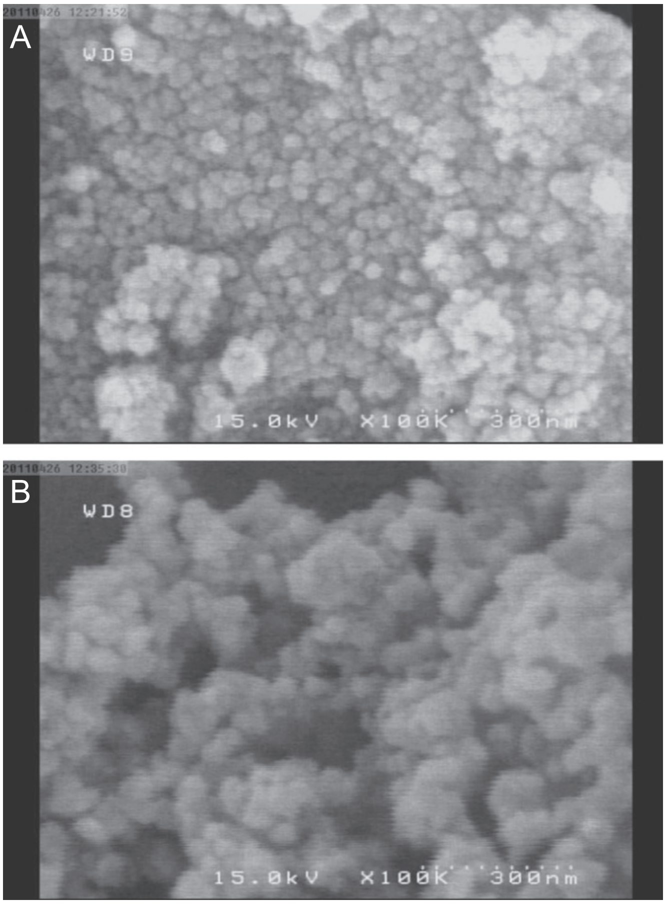


Fig. 3. FESEM images of Fe_3O_4 MNPs (A) and Fe_3O_4/C MNPs (B).

3.2. Optimization of extraction process

3.2.1. Extraction efficiency of Fe_3O_4 and Fe_3O_4/C MNPs adsorbents and desorption conditions

Fig. 4 shows comparatively the capabilities of Fe_3O_4 and Fe_3O_4/C MNPs for the extraction of four OPPs from the 500 mL sample volume. As can be seen, Fe_3O_4/C MNPs shows excellent extraction efficiency in comparison with bare Fe_3O_4 MNPs. These results confirm the enhancement of adsorption capability of Fe_3O_4/C MNPs against bare Fe_3O_4 MNPs and this can be attributed to the presence of carboxyl and hydroxyl groups on the surface of Fe_3O_4/C MNPs in comparison with Fe_3O_4 MNPs that makes it completely suitable for effective interaction with analytes and adsorb them.

Desorption solvent was optimized to achieve accurate quantification of the analytes. Desorption process of the analytes from the Fe_3O_4/C MNPs was studied using different kinds of organic solvents (methanol, acetonitrile) individually and desorption ability of acetonitrile was found to be superior to that of methanol. Also, different volumes (4, 6, 8, and 10 mL) of acetonitrile were tested. The minimum volume of acetonitrile required for a quantitative elution of the retained analytes was found to be 8.0 mL. In order to achieve complete desorption of analytes, Fe_3O_4/C MNPs were sonicated for 5 min in desorption solvent. No carryover was found after the Fe_3O_4/C MNPs were desorbed in acetonitrile (8 mL) for 5 min with sonication. Therefore, the Fe_3O_4/C MNPs particles can be reused without a significant decrease of the extraction capability.

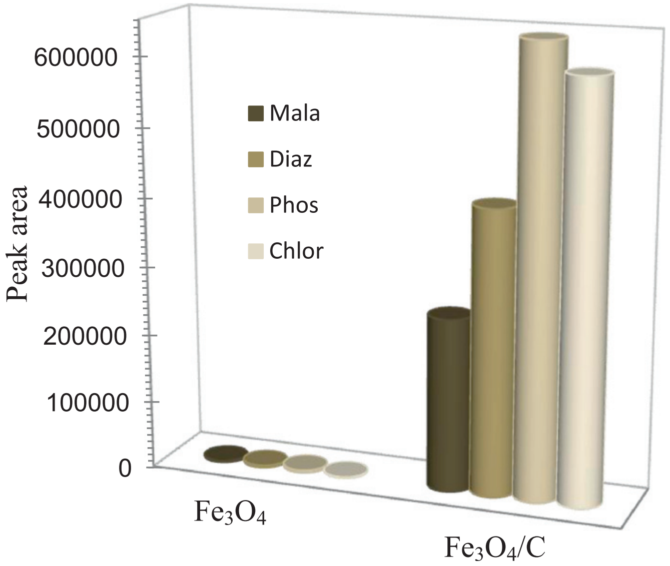


Fig. 4. Comparison of the extraction efficiencies of Fe_3O_4 MNPs with Fe_3O_4/C MNPs. Sample volume, 500 mL; concentrations of Mala, Diaz, Phos and Chlor are 30, 30, 15, 15 ng mL^{-1}, respectively; amount of adsorbent, 100 mg; no salt addition; pH not adjusted.

3.2.2. *Study of other experimental factors by multivariate optimization*

Different factors can affect the extraction yield in the MSPE procedure and in the most cases they are correlated. Therefore, their optimization through a multivariate approach is recommended. One of the experimental design techniques that are commonly used for process analysis and optimization is response surface methodology (RSM). RSM is a collection of statistical and mathematical methods that involves experimental designs to achieve an adequate and reliable measurement of the response of interest. Box–Wilson or CCD is one of the most used response surface designs, because it requires fewer experimental runs and provides sufficient information as compared to a factorial design. CCD consists of a factorial design (2^k) augmented with ($2k$) star points, where k is the number of factors to be optimized, and with a central point, which can be run n times [31]. Therefore, after performing some preliminary experiments, optimization of the extraction conditions by the proposed MSPE method was conducted using a CCD. Four factors including the sample solution pH (A), ionic strength (B), amount of the adsorbent (C) and equilibrium time (D) were considered in the design. The equilibrium time was defined the start time interval from the end of the sonication and beginning of the separation of MNPs from sample solution by magnet. The low (-1), central (0), and high ($+1$) levels of these factors, as well as the location of their star points (± 2), are given in Table 2. The Design-Expert statistical software program (7.0.0 version) was used to generate the experimental matrix and calculate the standardized main effects of the factors considered. The design involved 29 experiments with five replicates at the center point, which were performed in random order in order to protect against the effects of lurking variables.

The data obtained were evaluated by ANOVA test, and the effects were visualized using Pareto charts (Fig. 5). In Pareto charts the length of each bar indicates the standardized effect of that factor in quadratic model on the response and the vertical line on the plot judges the effects that are statistically significant with 95% probability. The results in Fig. 5 demonstrate that the pH of the sample, amount of the adsorbent and ionic strength were important parameters to obtain the maximum extraction yield in all of the tested OPPs. Amount of the adsorbent and ionic strength showed a positive effect, whilst pH of sample showed a negative effect on the extraction. The negative effect of pH of sample on extraction yield can be attributed to degradation of OPPs in alkaline medium [32]. The positive effect of the ionic strength can be attributed to decrease of analyte solubility in water samples and increases extraction efficiency. Besides, the ionic strength might not only affect the extraction efficiency of the target compounds, but also the stability of the MNPs colloidal suspension and the settling speed [33]. Hence, in the presence of NaCl, MNPs could be quantitatively settled whereas without salt addition, MNPs settling was not quantitative and clear solutions cannot be achieved [33]. Equilibrium time was the least influential factor and had a positive significant effect on extraction for diazinon. For other compounds equilibrium time showed a non-significant effect with a positive sign for malathion and negative sign for phosalone and chlorpyrifos. These results indicated that the equilibrium time has no impact on extraction efficiency indicating that the analytes can be adsorbed on the surface of Fe_3O_4/C MNPs within a short time and this can be attributed to the short diffusion route of nanosized sorbents. However, the negative sign of the equilibrium time for phosalone and chlorpyrifos may be attributed to aging effect on the interaction between these analytes and adsorbent resulting in a strong adsorption and thus insufficiency in desorption process after a given time. Pareto charts also reveal that the interactions between ionic strength and other parameters and also between amount of the adsorbent and equilibrium time were statistically significant.

Table 2
Factors and value levels used in the central composite design.

Factors	Level				
	α (-2)	Low (-1)	Center (0)	High ($+1$)	α ($+2$)
A: pH	4	6	8	10	12
B: Ionic strength (mmol L^{-1}; NaCl)	0	100	200	300	400
C: Amount of the adsorbent (mg Fe_3O_4/C)	5	28.75	52.5	76.25	100
D: Equilibrium time ($_{\text{min}}$)	0	15	30	45	60

3.2.3. *Response surface optimization using DF approach*

The numerical optimization of the software has been chosen in order to find the specific point that maximizes the DF. The desired goal was selected by adjusting the weight or importance that might alter the characteristics of a goal. The criteria for the optimization of all studied factors in correspondence with peak area were shown in Table 3. The goal fields have the following options: maximum, minimum, target and in range, none (for response only), and equal to (for factor only). A weight factor, which defines the shape of the DF for each response, is then assigned. Weights must be between 0.1 and 10, with larger weights corresponding to more important responses. A weight factor of 1 was chosen for all individual desirabilities in this work.

By using this DF with all pre-selected goal for each factors, the optimization procedure was carried out and the response surfaces obtained for the global DF are presented in Fig. 6(A–F). These plots were obtained for a given pair of factors, while maintaining the others fixed at their optimal values. As can be seen in Fig. 6C, E and F, when the equilibrium time is its upper level, the desirability is 0. This fact is caused by select minimize goal for equilibrium time. Fig. 6B, D and F show that the highest desirability is obtained at upper level of adsorbent and when the amount of the adsorbent is its lower level the D reduced to zero.

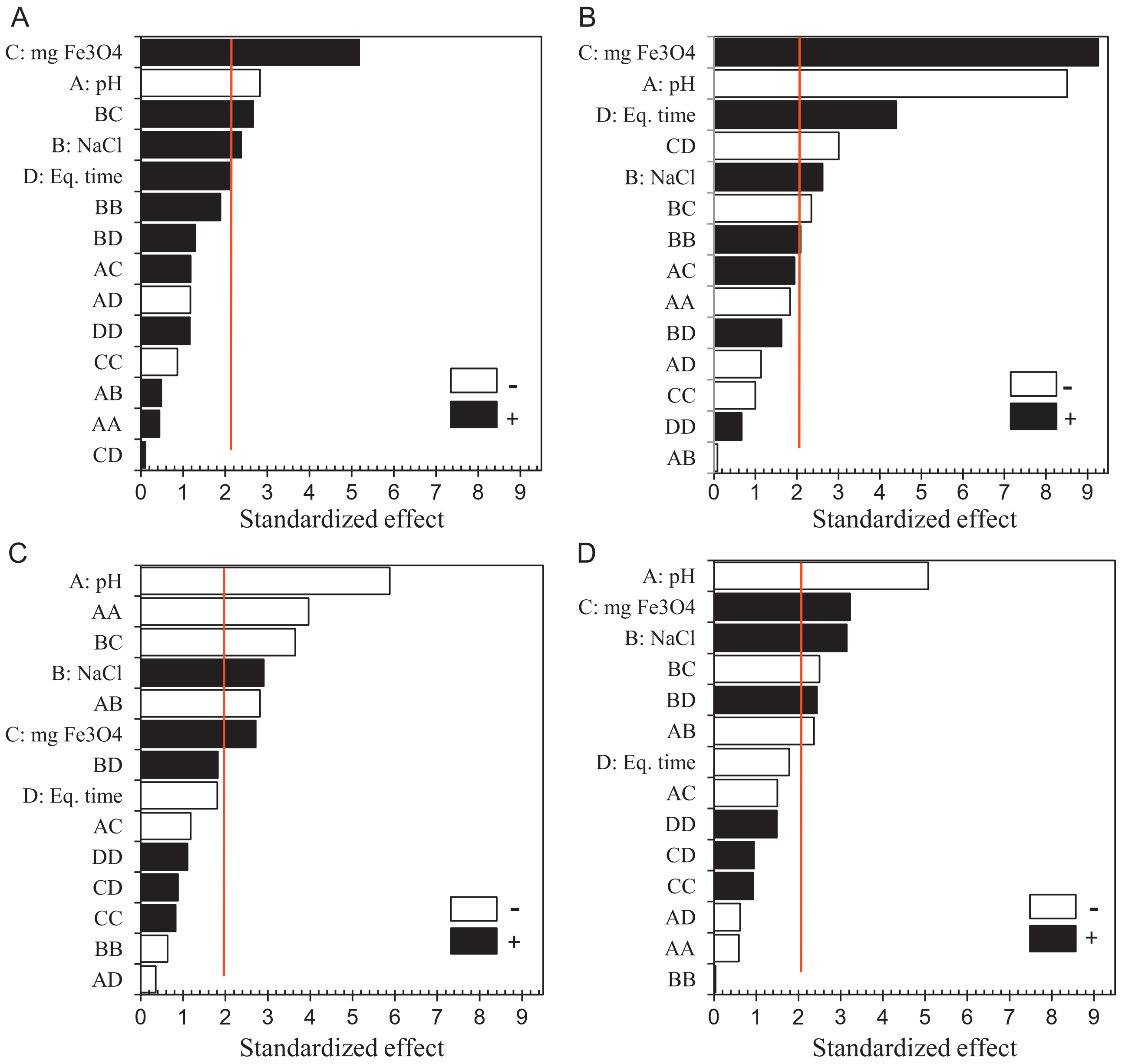


Fig. 5. Pareto charts of the main effects obtained from the CCD (the vertical lines define the 95% confidence interval): (A) Mala, (B) Diaz, (C) Phos, (D) Chlor.

Table 3
Constraints of factors and responses for optimization.

Name	Goal	Lower Limit	Upper Limit	Weight	Importance
pH	Is in range	4	12	1	–
NaCl	Is in range	0	400	1	–
mg Fe_3O_4	Is in range	5	100	1	–
Eq. time	Minimize	0	60	1	–
R_{Mala}	Maximize	12000	133308	1	5
R_{Diaz}	Maximize	10004	73964	1	5
R_{Phos}	Maximize	32498	470478	1	5
R_{Chlor}	Maximize	86136	429764	1	5

Under the mentioned optimization criteria, the experimental conditions corresponding to one maximum in the DF $(D=1)$ are: amount of adsorbent 97.4 mg, sample solution pH 9.16, equilibrium time 0 min and ionic strength 10 mmol L^{-1}. The suggested optimal conditions were then experimentally corroborated, the results are closely co-related with the data obtained from desirability optimization analysis using CCD, indicating that CCD with *DF* could be effectively used to optimize the MSPE procedures.

3.2.4. Breakthrough volume

Breakthrough volume (the maximum volume that can be preconcentrated with quantitative recovery of analyte) is a major parameter in SPE and preconcentration of samples. It significantly affects the preconcentration factor, the reproducibility and reliability of results. The breakthrough volume was determined by a series of different volume aqueous solutions (200–800 mL) spiked with fixed amount of each analyte (7.5 μg phos and chlor, 15 μg mala and diaz) at optimized conditions. The results showed that the recoveries of all the analytes do not decrease with sample volume increasing from 200 to 800 mL. By extracting analytes from 800 mL aqueous solution and after drying the eluent with a nitrogen stream at 25 °C and re-dissolving in 0.5 mL acetonitrile, the preconcentration factors achieved for

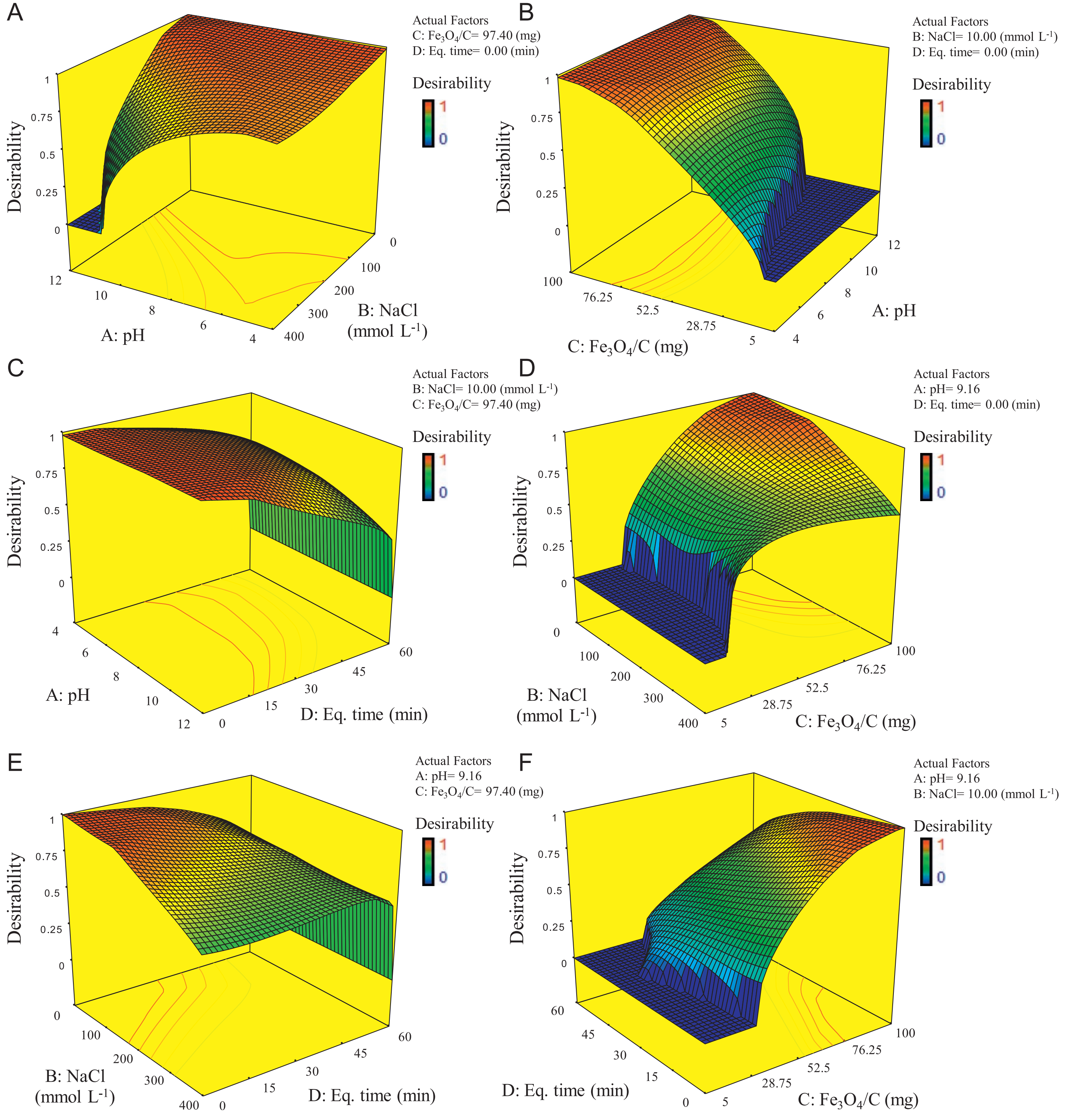


Fig. 6. Response surface plots corresponding to the DF when optimizing the following pair of factors, while maintaining constant the remaining ones at their optimum values: (A) pH–NaCl, (B) mg Fe_3O_4–pH, (C) pH–equilibrium time, (D) NaCl–mg Fe_3O_4, (E) NaCl–equilibrium time, and (F) equilibrium time–mg Fe_3O_4.

malathion, diazinon, phosalone, and chlorpyrifos are 330, 458, 1200 and 1002, respectively.

3.3. Validation of the method

Quantitative parameters of the proposed method, such as linear range (LR), coefficient of determination (R^2), limit of detection (LOD), limit of quantification (LOQ), enrichment factor (*EF*) and precision, were evaluated under optimum conditions (Table 4). The calibration curves were established using 800 mL deionized water spiked with different concentrations of each analyte. Calibration curves were prepared at six levels and each point on the calibration plot was the mean value of two area measurements. To obtain the precision of the method, replicated analysis of spiked water samples were carried out for three times, and relative standard deviation (R.S.D.) values were calculated by the obtained peak area of each analyte. All the analytes exhibited good linearity with the coefficient of determinations ranging from 0.9949–0. 9996. The LODs, based on signal-to-noise ratio (S/N) of 3, were 4.3–47.4 pg mL^{-1}, and the LOQs, based on signal-to-noise

Table 4
Linearity, precision, enrichment factors, LODs and LOQs obtained for target analytes.

Analytes	Regression equation	LR[a] (ng mL^{-1})	R^2	LODs[b] (pg mL^{-1})	LOQs[c] (pg mL^{-1})	R.S.D.(%) (n=3)	EF[d]
Malathion	Y=3052X+46681	0.4–400	0.9963	47.4	158	3.7	330
Diazinon	Y=4779X+78035	0.4–400	0.9949	41.8	139	6.4	458
Phosalone	Y=53221X+56509	0.05–60	0.9974	4.4	15	4.8	1200
Chlorpyrifos	Y=53517X+25067	0.05–100	0.9996	4.3	14	6.6	1002

[a] Linear range.
[b] Limits of detection (S/N=3).
[c] Limits of quantification (S/N=10).
[d] Enrichment factor.

Table 5
Results of determination and recoveries of water samples spiked with OPPs.

Samples	Mala	Diaz	Phosa	Chlor
Well water				
Initial	ND[a]	ND	ND	ND
Found[b]	5.010	4.200	0.891	1.014
RR[c,d] (%,R.S.D.%)[e]	100.2 (5.0)	84.0 (4.1)	89.1 (6.4)	101.4 (3.3)
Tap water				
Initial	ND	ND	ND	ND
Found	4.895	3.980	1.035	0.956
RR (%,R.S.D.%)	97.9 (4.7)	79.6 (2.7)	103.5 (6.5)	95.6 (7.3)
River water				
Initial	ND	ND	ND	ND
Found	4.100	4.215	1.009	0.987
RR (%,R.S.D.%)	82.0 (3.9)	84.3 (2.8)	100.9 (4.6)	98.7 (7.6)
Mineral water				
Initial	ND	ND	ND	ND
Found	5.035	4.060	0.922	0.906
RR (%,R.S.D.%)	100.7 (3.3)	80.2 (5.8)	92.2 (2.9)	90.6 (4.3)

[a] Not detected.
[b] Spiked concentrations of Mala, Diaz, Phos and Chlor are 5, 5, 2, 2 ng mL^{-1}, respectively.
[c] Relative Recovery.
[d] Percentage ratio of the found and spiked concentrations.
[e] Obtained for three determinations.

ratio (S/N) of 10, were 14–158 pg mL^{-1}. The LODs values easily and conveniently meets the MAC of 0.1 ng mL^{-1} for one organophosphorus pesticide and 0.5 ng mL^{-1} for the total concentration of these pesticides in drinking water set by EU.

3.4. Analysis of spiked samples

To further verify our proposed method, the method was applied to four environmental water samples (tap, well, river and mineral) with the results shown in Table 5. Initial analysis confirmed that they were free of target analytes. The accuracy of the method was evaluated by the recovery test carried out with spiked water samples. Relative recoveries (RR%) for the analysis of OPPs in spiked water samples using the proposed method based on three replicate extractions and determinations are shown on Table 5, which indicated that the recoveries for the four OPPs were in the range from 79.6%–103.5% with R.S.D.s between 2.7% and 7.6%. Typical chromatograms of spiked water samples were shown in Fig. 7.

3.5. Comparison of MSPE with other methods

In order to further demonstrate the superiority of our proposed method, a comparison of the important features of the proposed method with those reported in the literature

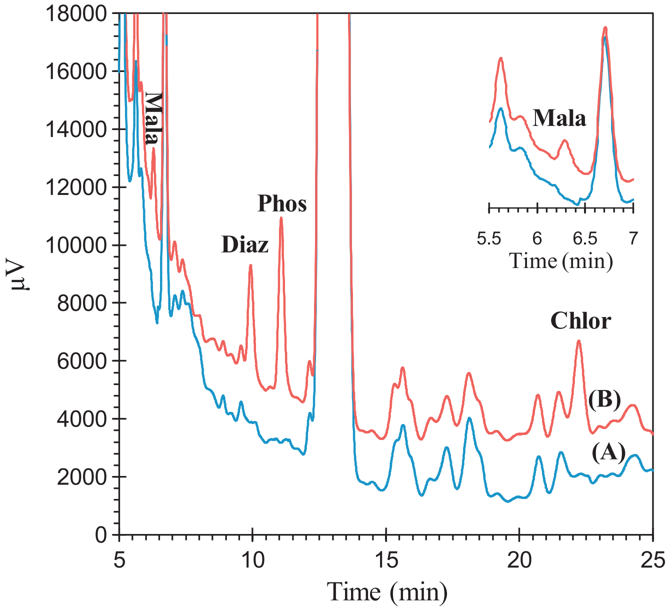


Fig. 7. MSPE–HPLC–UV chromatograms: (A) Aji chai River water sample; (B) Aji chai River water sample spiked with 5 ng mL^{-1} Mala and Diaz and 2 ng mL^{-1} Phos and Chlor.

Table 6
Comparison of the current MSPE method with other sample preparation techniques for the determination of the OPPs.

Methods	LR(ng mL^{-1})	LOD (pg mL^{-1})	*EF*	RSD(%)	Sample	Ref.
HF–MMLLE/ LC–MS-MS[a]	10–10000	3000–330000	–	4.7–7.6	Orange juice	[34]
SPME/GC–NPD	0.1–10	6–120	–	7–17	Water	[35]
MWCNT–SPE/HPLC–UV[b]	1–500	1–4	500	1.5–1.8	Water	[27]
SPME/GC–FPD	1–50	10–300	–	3–9	Water	[22]
IL-DLLME/HPLC–UV	10–1000	100–5000	>200	2.4–4.7	Water	[20]
SDME/GC–MS	0.5–100	10–70	–	8.6–93	Water	[23]
CPE–back extraction /GC–FPD	0.1–20	40–80	–	3.4–4.7	Urine	[24]
LPME/GC–MS[c]	0.1–100	6–200	–	5–8	Water	[36]
LSE/HPLC–DAD[d]	0.4–5	40–200	–	2–5	Water	[37]
VSLLME/GC-FPD[e]	0.1–50	10–50	282–309	2.3–8.9	Wine	[38]
MSPE/HPLC–UV	0.05–400	4–47	330–1200	3.7–6.6	Water	This work

[a] Hollow fiber microporous membrane liquid–liquid extraction.
[b] Multi-walled carbon nanotube-solid phase extraction.
[c] Liquid-phase microextraction.
[d] On-line liquid–solid extraction.
[e] Vortex-assisted surfactant-enhanced-emulsification liquid–liquid microextraction.

[20,22–24,27,34–38] is given in Table 6. According to the information in Table 6, it was clear that the enrichment factor obtained for Fe_3O_4/C MNPs made it an ideal adsorbent compared to the other given methods. As could be seen, the proposed method exhibits wider linear ranges, lower LODs and good reproducibility. The results revealed that the proposed method for the analysis of OPPs in water sample was rapid, simple, precise and sensitive.

4. Conclusions

In this research, Fe_3O_4/C MNPs were synthesized and successfully applied for the efficient enrichment of some trace OPPs from environmental water samples. The use of chemometric tools such as the experimental design and the multi-response optimization has shown to be of great help to achieve a fast and an efficient optimization of the extraction conditions. The results showed that the proposed method is very suitable for the rapid preconcentration and separation of OPPs from large volume of environmental samples. The comparison of current work with some other methods on the determination of OPPs (Table 6) reveals that this method is either comparable or has pronounced advantages over them. The MAC set by the EU for these pesticides can be determined without difficulty. The high breakthrough volume of water samples and the small volume of the elution permitted to get high enrichment factors.

Acknowledgment

The authors gratefully acknowledge The Research Council of Azarbaijan University of Tarbiat Moallem for financial support.

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