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Rational design of heteropolyacid-based nanosorbent for hollow fiber solid phase microextraction of organophosphorus residues in hair samples

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ABSTRACT

A novel heteropolyacid-based supported ionic liquid (IL) mediated sol–gel hybrid organic–inorganic material is presented for effective use in hollow fiber solid phase microextraction (HF-SPME). We examined a Keggin-based IL that was evaluated in conjunction with sol–gel. This study shows that Keggin-based IL sol–gel generated porous morphology pro effective extraction media. The method was developed for the extraction of the organophosphorus pesticides (OPs); diazinon, fenitrothion and malathion from human hair samples. The OPs were subsequently analyzed with high performance liquid chromatography and photodiode array detection (HPLC–PDA). In the basic condition (pH 10–11), the gel growth process in the presence of IL was initiated. Afterward, this sol was injected into a polypropylene hollow fiber segment for in situ-gelation process. Parameters affecting the efficiency of HF-SPME were thoroughly investigated. Linearity was observed over a range of 0.02–50,000 μg/g and 0.0001–25,000 ng/mL with detection limits between 0.0074–1.3000 μg/g and 0.00034–0.84 ng/mL for the OPs in hair and aqueous matrices, respectively. The relative recoveries in the real samples, for OPs in the storekeeper hair ranged from 86 to 95.2%.

1. Introduction

The term organophosphorus pesticides (OPs) is often used to describe organic phosphorus containing compounds, especially when it deals with neurotoxic compounds. Many of the so-called OPs contain C—P bonds. OPs irreversibly inactivate acetylcholinesterase, which is essential to nerve function in insects, humans, and many other animals. OPs affect this enzyme in varied ways and thus, in their potential for poisoning.

OPs degrade rapidly by hydrolysis on exposure to sunlight, air, and soil, although small amounts can be detected in food and drinking water. Their ability to degrade made them an attractive alternative to the persistent organochloride pesticides, such as DDT. Although organophosphates degrade faster than the organochlorides, they have greater acute toxicity; posing risks to people who may be exposed to large amounts [1,2].

Commonly used OPs have included parathion, malathion, methyl parathion, chlorpyrifos, diazinon, dichlorvos, phosmet, fenitrothion, and azinphos methyl. Malathion is widely used in agriculture, residential landscaping, public recreation areas, and in public health pest control programs. It is the most commonly used organophosphate insecticide [2].

Scientists concerned about OPs because even at relatively low levels, they may be the most hazardous substances to the brain development of fetuses and young children. The United States Environmental Protection Agency (EPA) banned most residential uses of organophosphates in 2001, but they are still sprayed on agricultural lands, on fruit and vegetables or are also used to control pests such as mosquitoes in many countries including Iran. They can be absorbed through the lungs and skin and by eating them on food or as you could find in this research through human hair [3,4].

The use of hair as biological samples to biomonitoring of exposure to (PPs) by hair analysis has been pioneered by Tsatsakis and co-workers and has been extensively employed since [3–6].

The European Union (EU) allows a maximum concentration of 0.1 ng/mL of each individual pesticide and 0.5 ng/mL of the sum of OPs in drinking water [7]. Determination of OPs in water is usually carried out by methods involving gas chromatography (GC), gas chromatography–mass spectrometry (GC–MS) or high-performance liquid chromatography (HPLC) [8–10]. Due to the low OPs concentrations and complex matrices, water samples are not directly analyzed using these approaches. Currently sample

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preparation is performed by liquid–liquid extraction (LLE) and solid-phase extraction (SPE) [11,12]. These techniques are time-consuming, expensive and especially related to LLE, hazardous for health due to the high volume of potentially toxic solvents used [13].

Solid-phase microextraction (SPME) is a solvent free process, developed by Arthur and Pawliszyn [14] that features simultaneous extraction and preconcentration of analytes directly from an aqueous or from the headspace of aqueous and solid sample [15,16]. This technique is fast, portable, easy to use and is applied for the determination of OPs in water samples [17–19]. SPME suffers from some drawbacks: its fiber is fragile and has limited lifetime and desorption temperature, and also sample carry-over is another problem [20]. Among the different developments for coating of SPME fibers, Malik and co-workers established a suitable method using sol–gel technology to overcome other important drawbacks of conventional SPME coatings such as: operating temperature problems, instability and swelling in organic solvents [21–23].

Recently, some potential utilization of ionic liquids (ILs) has been reported in various separation processes and several articles have been reviewed to get analytical applications of those solvents in detail [24,25]. ILs have some unique properties, such as negligible vapor pressure, desired thermal stability, tunable viscosity and miscibility with water and organic solvents, as well as good extractability for various organic compounds.

The reason for great applications of ILs in the field of solid phase extraction is that IL facilitates particles incorporation into the extractor phase and increases the homogeneity of the active adsorbent sites [26,27].

In our previous works the significant enhancement of analyte extraction which was performed in LPME and SPME by providing an integrated method of hollow fiber solid/liquid and solid phase microextraction had been proposed and studied [28–31]. The details of the HF-SPME method were described previously.

Following previous studies, we have tried to develop the researches in order to improve method efficiency though access to lower detection limits for the determination of OPs. In our last work, the sol containing IL along with a kind of nanoparticles prepared based on sol–gel technique was injected into a piece of polypropylene hollow fiber and the process of in situ gelation was occurred into the fiber [32]. To continue our previous works on the applications of IL in the extraction of OPs compounds we used Keggin; $H_3PW_{12}O_{40}$ as a Bronsted acid in preparation of an IL; $(PY\ BS)_3PW_{12}O_{40}$ that was synthesized, by Bamohharam et al. [33].

SO_3H-functionalized ILs have received a great deal of attention in the last few years due to their broad range of potential uses. Typically, for synthesis of these ILs, first of all a N-alkylimidazole is reacted with 1,4-butan (or 1,3-propan) sultone to obtain a solid zwitterion. This zwitterion is then protonated with a Bronsted acid such as sulfuric acid or phosphoric acid to obtain the corresponding IL. This method involves applying the hazardous acids, although restricting the use of these materials is one of the purposes of green chemistry. It could be possible by using of environmentally friendly materials involving the application of solid acids. It shows that heteropolyacids (HPAs) in the solid state are pure Bronsted acids and are stronger than conventional acids such as H_3PO_4, H_2SO_4, HNO_3. These solid acids are "green" by corrosiveness, safety, quantity of waste, and separability [34,35].

Keggin is the best known structural form for heteropolyacids. It is the structural form of α-Keggin anions with a general formula of $[XM_{12}O_{40}]^{n-}$, where X is the heteroatom (most commonly are Si^{IV}, Ge^{IV}, P^{V},...), M is the addenda atom (most common are molybdenum and tungsten), and O represents oxygen.

It must be mentioned that IL incorporation into extractor phase occurs more homogeneously, and also the number of active adsorbent sites is another important factor. Thereupon the preconcentration factors are higher than what we previously reported.

2. Experimental

2.1. Chemicals and materials

The Accurel Q 3/2 polypropylene hollow fiber membrane used here was obtained from Membrana (Wuppertal, Germany). The wall thickness of the fiber was 200 μm, the inner diameter was 600 μm, and the pore size was 0.2 μm.

Tetraethylorthosilicate (TEOS, 97%) and teris hydroxyl methyl amino methane were purchased from Alfa Aesar (Ward Hill, MA, USA). Ethanol, cyclohexane, 1-octanol, acetonitrile, toluene, and pyridine that all had HPLC grade were obtained from Merck (Darmstadt, Germany). 1,4-Butansultone was obtained from Sigma–Aldrich (Saint Louis, MO, USA).

Target OPs: Diazinon, fenitrothion and malathion were purchased from Riedel-de Haen (Seelze, Hannover, Germany). Stock solutions of OPs (2000 μg/mL) were prepared by dissolving calculated amounts of them in methanol. Fresh working solutions were prepared daily by diluting the stock solution in distilled water. All experiments were carried out at room temperature, 22 ± 0.5 °C.

2.2. Instrumentation

The HPLC system used in this work was a Knauer (Germany, D-14163) containing photo diode array detector; S2600, a port sample injection valve equipped with a 20-μL loop. Separation was accomplished using a 100/5-C_{18} column with; 4.6 mm diameter, 250 mm length; from Knauer. An RP-18 guard column was fitted upstream of the analytical column. The mobile phase was a mixture of water–methanol–acetonitrile, optimized on (25:20:55, v/v) and degassed by own system degasser and delivered two pumps S1000. The flow rate of the mobile phase was 1.0 mL/min. The PDA detector was set at the wavelength ranging from 190 to 400 nm. The system also was equipped with a computer system and software EZ-Chrom Elite with integration capability.

The FT-IR instrument used for recording the infrared spectrum was Buck Scientific M-500 Fast-Scan IR Spectrometer (East Norwalk, CT 06855, USA). The microstructure of samples was investigated by scanning electron microscopy (SEM) (LEO, Model 1450VP, Germany). A Metrohm 780 pH-meter (Herisau, Switzerland) equipped with a combined glass electrode was used to determine pH values during the experiment.

2.3. Synthesis of ionic liquid based on Keggin heteropoly acid

For the synthesis of the mentioned IL, pyridine (PY; 0.02 mol) and 1,4-butane sultone (BS; 0.02 mol) were charged into an appropriate round-bottom flask and were dissolved in toluene (100 mL). Then, the mixture was stirred at 40 °C for 10 h under nitrogen atmosphere. The white precipitate zwitterion was washed repeatedly with ether to remove non-ionic residues and dried in vacuum. Then, aqueous solution of $H_3PW_{12}O_{40}$; Keggin heteropoly acid (0.007 mol) was added and the mixture was stirred for 20 min at 60 °C to form the viscose IL; $(PY\ BS)_3PW_{12}O_{40}$. The schematic diagram of reaction and FT-IR spectra of the IL; $(PY\ BS)_3PW_{12}O_{40}$, based on Keggin heteropoly acid has been shown in Figs. 1 and 2, respectively.

2.4. Preparation of the sol solution

640 μL TEOS, 130 μL teris hydroxyl methyl amino methane in de-ionized water (5%, v/v) as alkali catalyst and 500 μL methanol were transferred into a 5 mL vial and sonicated for 10 min for

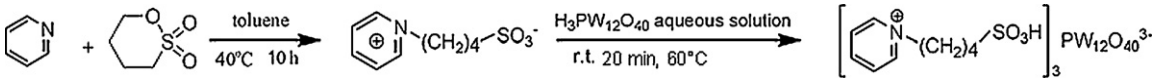

Fig. 1. Schematic diagram of reaction steps involved in the preparation of Keggin based ionic liquid.

hydrolysis operation. Then 50 μL NH_3, 30 mg $(PY\ BS)_3PW_{12}O_{40}$ and 30 μL HCl (25%) were added. The mixture was sonicated for 10 min in the glass vial and stay 24 h for gel aging processes, which enhance the porosity of nanocomposite. After the optimal aging time, the mixture was centrifuged at 5000 rpm for 5 min. The solution was transferred to another tube to be ready for next step. Scanning electron microscopy (SEM) was used to characterize the products of the synthesis sol–gel (see Fig. 3).

2.5. Fabrication of the HF-SPME fiber

The polypropylene hollow fibers were cut into small segments with a length of 2.0 cm. The fiber segments were cleaned by acetone to remove impurities and residue monomers of polypropylene which was accumulated during preparation stage and then, the fibers were dried in air. Finally 6 μL of the sol–gel solution was gradually injected into the hollow fiber using a syringe. The fibers were left to dry in ambient condition.

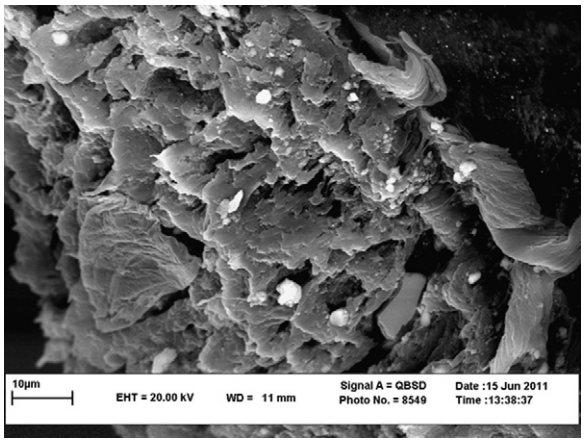

Fig. 3. The scanning electron microscopy (SEM) of sol–gel composite.

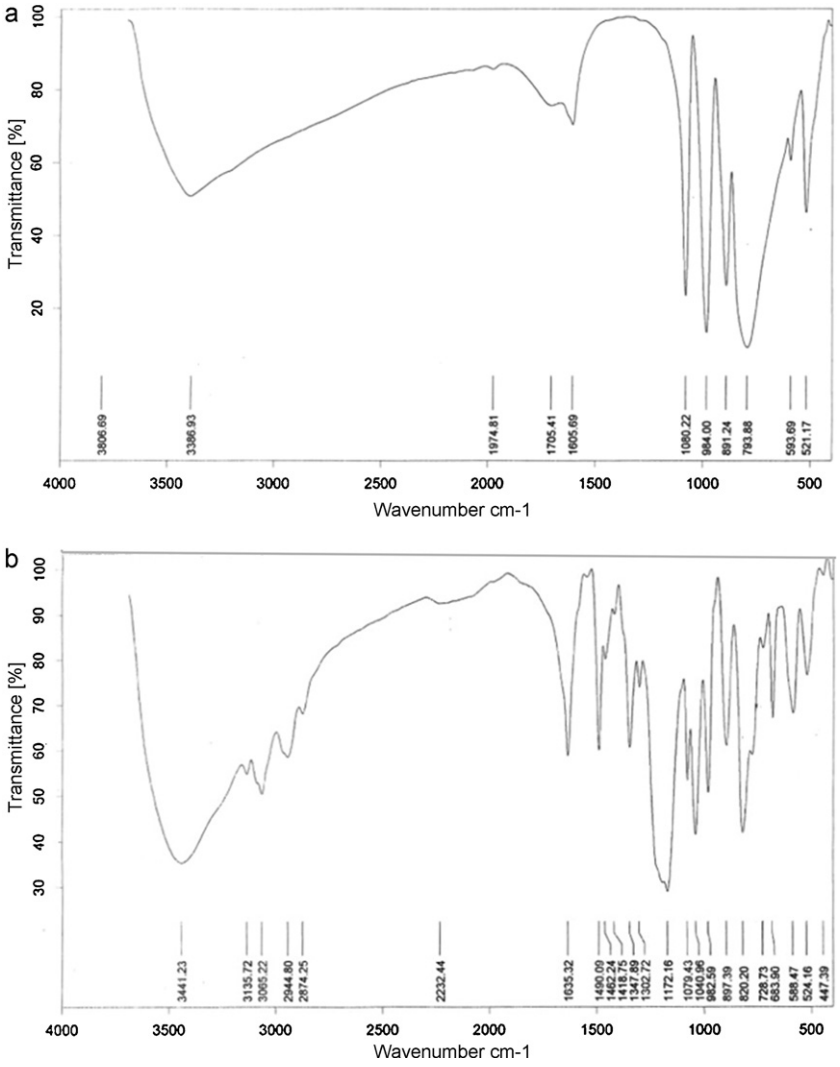

Fig. 2. FT-IR spectrum of: (a) Keggin, H_3 [PW_{12} O_{40}] and (b) Keggin based ionic liquid, $(PY\ BS)_3PW_{12}O_{40}$.

2.6. HF-SPME procedure

HF-SPME process was carried out according to the following steps: optimized volume of aqueous solution containing the OPs was added to the sample vial with a 8 mm × 4 mm magnetic stirring bar. The HF-SPME fiber segment was then placed in the aqueous solution. The vial was sealed and the stirrer was turned on. The extraction was done for a certain period of time at room temperature and then the fiber was removed from the extraction vial and transferred into another glass vial containing optimal organic solvent (1000 μL methanol). The analytes were desorbed from the fiber with sonication for 5 min.

Finally, 10.00 μL of the organic solvent was withdrawn into the HPLC microsyringe and then injected into the HPLC for further analysis. Due to low cost, and to prevent the carryover effect, each hollow piece of fiber was used only once in the experiments to reduce the carry over effect.

2.7. Hair samples treatment

A bulk of blank hair, necessary for method development and validation, was obtained from persons who referred to a barber shop in Mashhad, Iran. We did not have any materials as reference. We only had a hair sample as reference which was confirmed to have no pesticide by using HPLC analysis as authentic hair samples. A reference material is a standard sample that is measured in water, soil, inorganic and biological samples precisely in standard laboratories and is bought as reference. The hair of a pesticide storekeeper was collected as the biological samples of suspected contamination with the OPs. Both blank and samples hair were cut with round-point scissors about 5 mm in diameter from the vertex posterior region of the scalp. Samples of 2–4 cm long were selected for analysis.

Fat and other surface contaminations on the hair had to be removed. The hairs were washed by following solvents on a hierarchy basis:

Washing was conducted by 20 mL dichloromethane, 20 mL acetone and 15 mL methanol, 10 mL methanol, respectively; then hair samples were dried at room temperature. The absence of OPs was verified by using HPLC analysis at the last washing solvent.

The washed and dried hair samples were finally cut into approximately 1 mm pieces and digested by the following procedure: 2.0 mL methanol as an extracting solvent was added to 50 mg of hair in a 10 mL screw-cap tube. The pH was adjusted to 7.4 by 2 mL phosphate buffer solution. The samples were incubated at 55 °C for 5 h. In case of a remaining solid matrix, extracts were filtered. The remaining was rinsed with 0.5 mL ethanol and both fractions were evaporated to dryness at 40 °C under a steam of nitrogen. The remaining was diluted with appropriate deionized water [36].

3. Results and discussion

3.1. FT-IR spectrums

In this study, neat $H_3PW_{12}O_{40}$ FT-IR spectra has been illustrated in Fig. 2A, comparing with that of the (PY BS)$_3PW_{12}O_{40}$ (Fig. 2B).

As it can be seen from IR spectra, in wavenumber region of 750–1100 cm^{-1}, $H_3PW_{12}O_{40}$ gave four featured peaks 1000–1080 (P—O_a), 960–1000 cm^{-1} (W—O_d), 850–890 cm^{-1} (W—O_b—W) (corner-sharing), and 760–800 cm^{-1} (W—O_c—W) (edge-sharing) assigned to the Keggin structure [37].

For the hybrid compound, (PY BS)$_3PW_{12}O_{40}$, in spite of the decrease of peak intensities and slight shift of peak positions, the four peaks appeared distinctively indicating that the Keggin structure of heteropoly anion was well reserved after the protons in the heteropolyacid were substituted by the organic cation.

Comparing the IR spectrums of the (PY BS)$_3PW_{12}O_{40}$ with $H_3PW_{12}O_{40}$ (Fig. 2A and B), the vibrational band of the W—O_c—W of the Keggin is split from 793.88 into two bands, including 728.73 and 820.20 cm^{-1}, due to difference in the W—O_c—W bonds. The vibrational band of the P—O is split from 1080.22 into two bands, including 1040.96 and 1079.43. This means that, the inter bridges between edge-sharing octahedra (800–760 cm^{-1}) and the P—O stretchings have been affected.

On the other hand, S=O stretching vibration at 1172 cm^{-1} were detected to verify the presence of sulfonic groups in IL. Furthermore, the hybrids showed a band at 684 cm^{-1} that is attributed to the bending vibration of the C—H bond in pyridine.

3.2. Experimental optimization for the HF-SPME

In order to obtain high pre-concentration and extraction efficiency of the analytes using HF-SPME, the main parameters were optimized as indicated below.

3.2.1. The effect of the time on the extraction efficiency

HF-SPME is a slow equilibrium process, and mass transfer depended on time. Over the extraction time solute molecules have sufficient chance to be transferred from donor phase to interface between the feed and HF-SPME device and for collection in it. Therefore, extraction time is a significant factor that affects the method efficiency. Extraction was performed from 10 to 40 min to determine the effect of extraction time on the method efficiency. The results shown in Fig. 4 illustrate the peak area versus extraction time profiles for the analytes. However, the increase on the peak areas for these analytes after 20 min extraction did not vary significantly, but the results shows that there is a degeneration on the method precision for longer extraction times. Therefore, the extraction time was fixed in 20 min.

3.2.2. The effect of the stirring rate

The extraction efficiency of the method is enhanced by stirring due to an increase in the mass transfer rate and also reduces the time required to reach thermodynamic equilibrium. Moreover, in HF-SPME the sorbent was protected by the hollow-fiber segments and faster stirring rates may lead to fiber collision with the wall of the vial and air bubbles formation around the fiber side wall.

The response of instrument was recorded for several stirring rates ranging from 250 to 1000 rpm for an extraction time of 20 min of 15 mL aqueous samples with each target analyte concentration of 10 μg/mL. The results confirmed that agitation of the sample greatly enhances extraction. However, violent stirring (>500 rpm) resulted in massive air bubbles and decreased the pre-concentration factors. Therefore, 500 rpm was selected for extraction at the subsequent experiments.

3.2.3. The effect pH and adding of salt to aqueous sample

The pH value of aqueous feed-phase plays an essential role in the extraction process. Considering the feed solution pH is also one of the major factors that it progresses the transfer of analytes from the feed to the HF-SPME device. Therefore, after survey of the pH effect in the pH range 2–11, by adding the appropriate hydrocholoric acid or sodium hydroxide solution to the aqueous donor phase.

The results confirmed that the OPs extraction performance reached a better level at pH 6 (see Fig. 5). When pH rose above 6, the peak areas of malathion and diazinon decreased rapidly. It is due to the occurrence of degradation under high alkaline condition. Based on thorough consideration, pH 6 was selected for further experiments.

The salt has two effects on the extraction phenomenon: (1) it ties up H_2O molecules in the aqueous phase (forming hydrated ions) so that less free water is available for solvation of analyte;

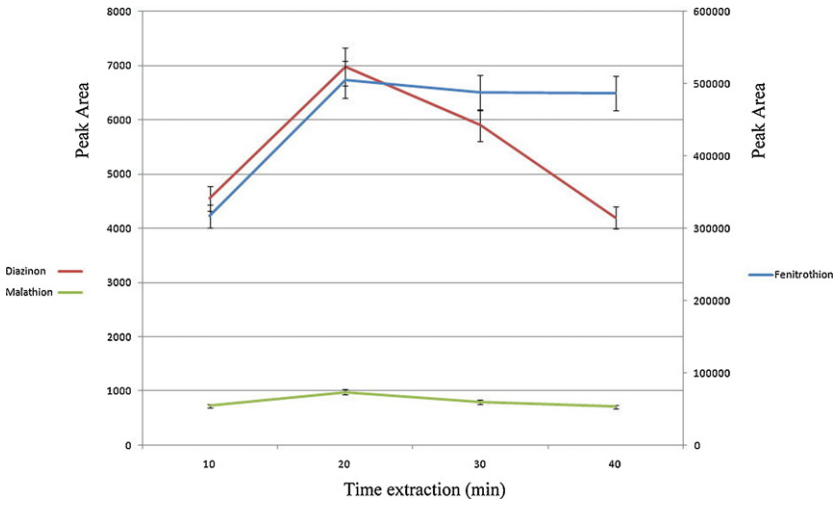

Fig. 4. The effect of extraction time on the extraction efficiency of OPs compounds under HF-SPME. Analytes concentration 10 μg/mL.

and (2) it breaks down the hydrogen bond of the water structure which makes it easier for analyte to extract into the acceptor phase.

This effect is called salting-out effect. For the most part salting-out is the commonly observed pattern, but many researchers described the reverse as a salting-in effect (decreasing the extraction efficiency).

In some cases by increasing of salt concentration and ionic strength, salting-in effect occurred. Whereby, polar molecules may participate in electrostatic interactions with the salt ions in solution. Therefore, the mass transfer is reduced. By adding salt, at first the predominant process is the interaction of salt with water (salting-out effect). With increasing salt concentration salt molecules start to interact by analytes (salting-in effect). Salting-in effects could influence detection limits and selectivity of HPLC system [38].

The effect of adding NaCl to aqueous sample was studied in the range of 0–15% (w/v); however, adding NaCl decreased the response of OPs. This may be due to competitive interaction of Na (I) with active sites on the sorbent surface which is a decrease in sorption capacity of analytes by the sol–gel. In addition, the presence of salt caused a second effect; the physical properties of the aqueous-solid extraction film were changed [39]. So, further extractions were carried out without adding NaCl.

3.2.4. Effect of the donor phase volume

The length of the hollow fiber segment (indeed, volume of the acceptor phase) was fixed at 2.0 cm and the reduced length was compatible with small sample volumes, which are highly relevant in some analytes in the biomedical and environmental applications. In addition, the enrichment of the analyte increases with rising the volume ratio of sample solution to acceptor solution [40]. The pre-concentration factor in HF-SPME basically depends upon the phase's volume of the sample and the acceptor. As the volume of the sample increases, the pre-concentration factor also increases [41].

In the present work, the phase ratio of donor and acceptor solutions was optimized by changing the volume of the donor phase between 5 and 20 mL while the volume of acceptor phase was kept constant at 6 μL. As can be seen in Fig. 6 the extraction results obtained for the analytes were most favorable to suggest a phase ratio of 2490 (15 mL donor phase volume). Repeatability was decreased in the donor phase volumes more than 20 mL. While with an increase in the aqueous donor phase volume, gel phase

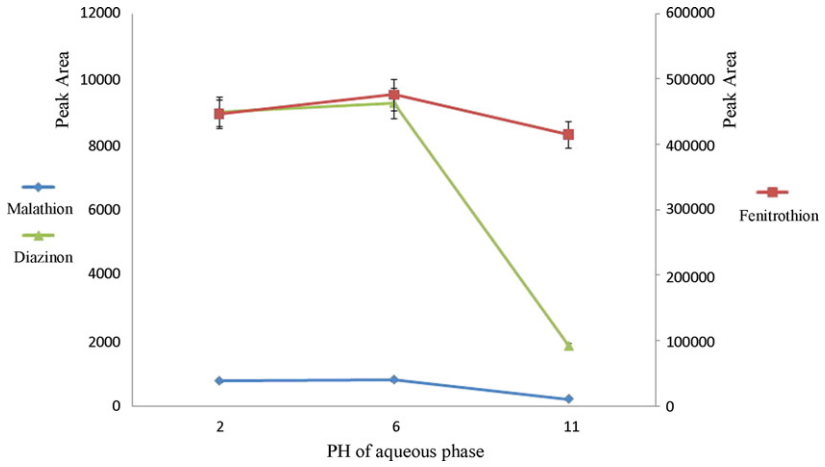

Fig. 5. The effect of pH of aqueous feed on the extraction.

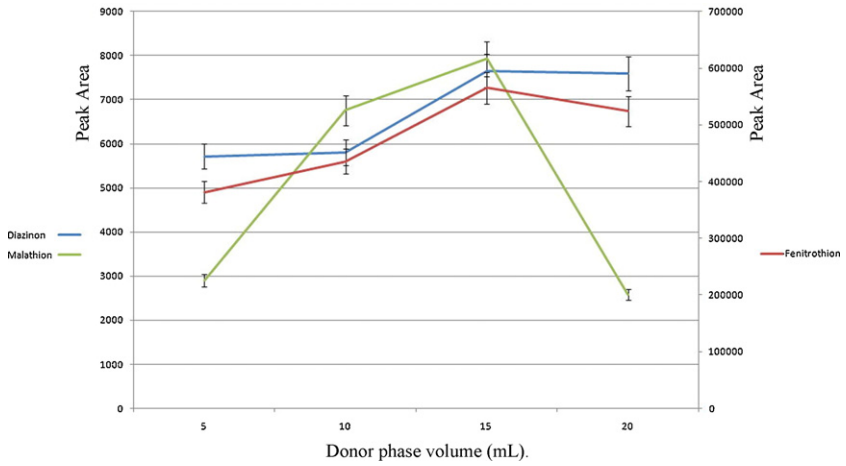

Fig. 6. The effect of aqueous feed volume on the extraction.

dissolution may also be a concern. This would lead to a decrease in the extraction efficiency. Therefore, we selected a volume of 15.0 mL as the optimized donor phase volume.

3.2.5. Desorption solvent selection

Offline desorption performed using minimum volume of desorption solvent required to completely immerse the SPME fiber segment. Different desorption solvents such as, cyclohexane, acetonitrile, methanol and 1-octanol were employed. Based on the obtained results, methanol was found to get the best extraction efficiency, while its chromatographic peak was easily separated from the analyte peaks. Meanwhile, the extract was stable at the extraction period due to its low vapor pressure at the extraction conditions. Therefore, methanol was selected as the desorption solvent. Pesticide compounds at the concentration level of 10 μg/mL, were used in the extraction studies.

3.2.6. The effect of deorption solvent volume and desorption time

As it was mentioned in Section 3.2.5, methanol was the best desorbtion solvent. Volume of methanol is important on the desorption capacity of HF-SPME device. So, desorption volumes ranging from 0.5 to 4.0 mL for three OPs were examined. Concentration of each target analyte was 10 μg/mL. The highest extraction efficiency was observed when 1 mL desorption solvent volume was used. A decrease in peak areas was observed in the larger ones. This might be due to the dilution target analytes effect.

Repeatability decreased in the desorption solvent volumes less than 1.0 mL, thus 1.0 mL was used as the optimal volume. On the other hand, it is understood that sonication of the sample enhances desorption.

The effect of desorption time on the back extraction was assessed by varying desorption times ranging from 2 to 10 min. It was observed that the peak areas of the analytes are increased with the increase of desorption time up to 5 min. Above this value the amount of extracted analyte remained unchanged. Therefore, 5 min was set as the rate for subsequent experiments.

3.2.7. The role of ionic liquids

Here, a novel porous nanocomposite was prepared via incorporation of the Keggin based IL; $(PY\ BS)_3PW_{12}O_{40}$.

IL can act as catalyst, porogenous agent and solvent or co-solvent in the sol–gel systems. The IL mediated sol–gel sorbents provided enhanced efficiency extraction because they were more porous than those that are IL-free [41,42]. Scanning electron microscopic investigation of the sol–gel morphology revealed this fact. Enhanced efficiency extraction provided by the IL mediated sol–gel sorbents is indicated as higher surface area of this matrix compared to the IL-free sol–gels.

As the doping level of the Keggin based IL increased, aggregation among the product particles became more serious; at the same time, the particle sizes increased somewhat. Interestingly, it seems that $(PY\ BS)_3PW_{12}O_{40}$ exhibited almost monodisperse morphology. In addition SEM analysis implies that the $(PY\ BS)_3PW_{12}O_{40}$ units homogeneously dispersed within the sol–gel framework. This homogeneous dispersion of Keggin based IL within the sol is important to increase their sorbent capacity.

The amount of $(PY\ BS)_3PW_{12}O_{40}$ used in this pre-concentration procedure is a critical factor to obtain the extraction efficiency. Therefore, the extraction system was carefully studied in order to define the lowest IL-phase mass necessary for achieving the highest possible pre-concentration factor. The variation in the extraction efficiency of the amount of IL, which was added to 1.5 mL sol, was investigated in the range of 0–40 mg. It was observed that the extraction efficiency of the proposed system was remarkably affected by the IL amount. No significant changes were observed on the extraction efficiency of sol with an amount higher than 30 mg $(PY\ BS)_3PW_{12}O_{40}$. Therefore, in order to achieve a good pre-concentration factor, 30 mg IL was chosen as optimum level of it.

3.3. Evaluation of the method performance

3.3.1. Figures of merit

Validation procedures were performed using spiked de-ionized water and blank hair samples. The HF-SPME method was evaluated for linear range, limits of detection (LODs), limits of quantification (LOQs), correlation coefficients (R) and linear dynamic range (LDR) under the best conditions.

Limits of detection were calculated experimentally as the minimum concentration providing chromatographic signals three times higher than background noise (S/N = 3).

Calibration curves in aqueous and human hair samples were plotted against the concentration levels of the OPs compounds. For each level, four replicate extractions were performed.

In the case of hair samples, standard calibration curves were obtained by adding calculated amounts of the OPs standards into the mixture of 2.0 mL methanol (extracting solvent) and 50 mg of finely cut washed blank hair. These spiked samples were digested according to the instruction of Section 2.7, extracted with the mentioned HF-SPME method, subsequently analyzed with the HPLC system, and then calibration curves were plotted.

Table 1
Figures of merit of the proposed method in the determination of organophosphorus pesticides in aqueous matrices.

Analyte	LDR[a] (ng/mL)	R^2[b]	LOD[c] (ng/mL)	LOQ[d] (ng/mL)	RSD%($n=4$)[e]	Equation[f]
Diazinon	0.01–25,000	0.9983	0.006	0.02	2.25	$Y=7483X+315$
Fenitrothion	0.001–25,000	0.9981	0.00034	0.001	2.55	$Y=67220X+25{,}445$
Malathion	10–25,000	0.9975	0.840	2.8	3.24	$Y=95.479X+1090$

[a] Linear dynamic range.
[b] Correlation coefficient.
[c] Limit of detection.
[d] Limit of quantification.
[e] Relative standard deviation.
[f] Y and X are the peak area and concentration of the analytes (ng/mL), respectively.

Table 2
Figures of merit of the proposed method in the determination of the organophosphorus pesticides in hair matrices.

Analyte	LDR[a] (μg/g)	R^2[b]	LOD[c] (μg/g)	LOQ[d] (μg/g)	P.F[e]	RSD% ($n=4$)[f]	Equation[g]
Diazinon	2.00–50,000	0.9982	0.6200	2.000	368.5	3.36	$Y=264.72X+853.07$
Fenitrothion	0.02–50,000	0.9980	0.0074	0.024	1506.8	2.64	$Y=79728X+32{,}696$
Malathion	4.00–50,000	0.9983	1.3000	4.200	389.1	4.10	$Y=171X+1060$

[a] Linear dynamic range.
[b] Correlation coefficient.
[c] Limit of detection.
[d] Limit of quantification.
[e] Pre-concentration factor.
[f] Relative standard deviation.
[g] Y and X are the peak area and concentration of the analytes (μg/g), respectively.

Table 3
Detected concentrations (μg/g) of organophosphorus pesticides in the hair of pesticides store seller.

Analtye	Concentration (μg/g)	RSD% ($n=5$)
Diazinon	2.04	2.6
Fenitrothion	10.6	3.6
Malathion	Under LOD	–

The results are tabulated in Table 1, for aqueous solution matrix, and Tables 2 and 3, for hair matrix. The pre-concentration factors (P.F.) were calculated based on the following equation [40]:

$$\text{P.F.} = \frac{A_{\text{RP,final}}}{A_{\text{PS,initial}}} \times \left(\frac{V_{\text{aq}}}{V_{\text{In}}}\right)$$

where $A_{\text{RP,final}}$ and $A_{\text{SP,initial}}$ are the final and initial peak areas after and before extraction of the OPs compounds in organic solvent, respectively that were obtained based on direct injection of the OPs solutions in methanol into the HPLC for analysis. V_{aq} and V_{In} are volume aqueous sample and internal volume of hollow fiber. The method was compared with the other works (Table 4). In comparison with the other conventional sample preparation methods, the developed method has the merits of considerable analysis speed, good separation efficiency and elevated pre-concentration, notable precision and high sensitivity.

3.3.2. Real samples

The developed IL mediated HF-SPME method has also been evaluated for the determination of the analytes in the OPs from hair samples. The analytical results of hair matrix are given in

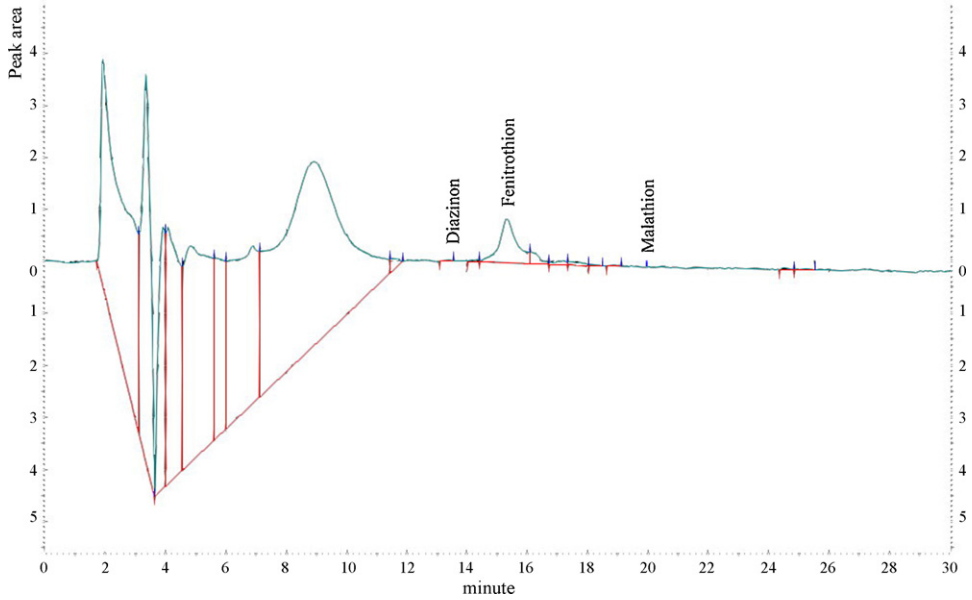

Fig. 7. HPLC Chromatogram of OPs in hair of the pesticide storekeeper under IL mediated HF-SPME.

Table 4
Comparison of some methods which were used for determination of pesticides compounds.

No.	Date	Matrices	Extraction technique	LOD	*R*	RSD%	Reference
1	2004	Honey	SPME	0.08–20 mg/kg	0.996	3.6–7.6	[43]
2	2004	Herbal infusions	SPME	0.13–1.1 mg/mL	0.974	1.3–12.1	[44]
3	2004	Food	SPME	0.01–0.1 ng/g	0.998	2.1–12.1	[45]
4	2006	Cork	SPME	0.1–1.7 μg/L	0.95	6.1–11.5	[46]
5	2008	Water	SPME	0.17–0.29 μg/L	0.998	2.–2.7	[47]
6	2008	Soil	SPME	0.004–1.2 ng/g	0.994	1.1–14.1	[48]
7	2010	Water	SPME	0.1–1 pg/mL	0.996	2–10	[49]

Table 3 and Fig. 7. The obtained results showed the linear range 1–25,000 ng/mL for diazinon; 0.01–25,000 ng/mL for fenitrothion and 10–25,000 ng/mL for malathion with RSDs% about 2.64–4.1% for all OPs compounds in the hair blank matrices.

The method was successfully applied to assay OPs in human hair for a pesticide storekeeper as real samples. Due to daily use of various OPs compounds in the mentioned store the accumulation of OPs components in hair are possible. The chromatograms of hair sample pesticide storekeeper were depicted in Fig. 7. The blank human hair was collected from a local men barbershop (Mashhad, Iran) and absence of analytes in the blank sample was confirmed by using HPLC analysis. It can be seen that the relative recovery for spiked samples was in the range of 83–92%.

4. Conclusion

In the present study, novel SPME sorbent based on Keggin based IL mediated was fabricated via sol–gel technique. One obstacle to materializing IL mediated arises from high viscosity of ILs significantly slowing down sol–gel reactions. In this work, we developed a method that overcomes this hurdle and provides heteropoly acid-based supported IL mediated advanced sol–gel materials for HF-SPME. As-prepared composites were successfully used in the process of pre-concentration of organophosphorus residue in hair samples.

The enhanced sorbent capacity was originated from the properties of the composites, including porous structure, small particle size, and homogeneous dispersion of the ILs within the sol–gel framework. The above composite also had the advantage of the strong interaction between analyte and sorbent.

Polypropylene wall pores are the channels which the analytes molecules (in the feed solution) and the adsorbent (sol–gel inside the fiber) were in contact with each other. Meanwhile, the pores can cause a kind of dimensional selectivity to the analyte molecules.

Flexibility, simplicity, disposable nature of the device that eliminates the possibility of sample carry over, more convenient handling than the other traditional SPME fibers and high preconcentration factors are among the advantages of this method.

Excellent clean-up of OPs in hair matrices as very complicated matrices. Also good linearity and reasonable relative recovery were also achieved. The developed method to isolate OPs, diazinon, fenitrothion and malathion from hair samples has many advantages over conventional methods.

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References

[1] L.G. Sultatos, J. Toxicol. Environ. Health 43 (1994) 271.
[2] M.R. Bonner, J. Coble, A. Blair, L.E. Beane Freeman, J.A. Hoppin, D.P. Sandler, M.C.R. Alavanja, Am. J. Epidemiol. 166 (2007) 1023.
[3] http://www.webmd.com/baby/news/20110421/pesticide-exposure-in-womb-linked-to-lower-iq.
[4] A.M. Tsatsakis, Environ. Res. 109 (2009) 350.
[5] A.M. Tsatsakis, M.G. Barbounis, M. Kavalakis, M. Kokkinakis, I. Terzi, M.N. Tzatzarakis, J. Chromatogr. B 878 (2010) 1246.
[6] A.M. Tsatsakis, M.N. Tzatzarakis, M. Tutudaki, Forensic Sci. Int. 176 (2008) 67.
[7] Drinking Water Guideline, 98/83/EEC, European Union, Brussels, 1998.
[8] D. Barcelo, J. Chromatogr. 643 (1993) 117.
[9] I. Liska, J. Slobodnik, J. Chromatogr. A 733 (1996) 235.
[10] S. Dasgupta, K. Banerjee, S.H. Patil, M. Ghaste, K.N. Dhumal, P.G. Assule, J. Chromatogr. A 1217 (2010) 3881.
[11] R.E. Wagner, Guide to Environmental Analytical Methods, fourth ed., Benium Publ., Schenectady, NY, 1998.
[12] C. Aquilar, I. Ferrer, F. Borrull, R.M. Marce, D. Barcelo, Anal. Chim. Acta 386 (1999) 237.
[13] Q. Xiao, B. Hu, C. Yu, L. Xia, Z. Jiang, Talanta 69 (2006) 848.
[14] C.L. Arthur, J. Pawliszyn, Anal. Chem. 62 (1990) 2145.
[15] D. Vuckovic, X. Zhang, E. Cudjoe, J. Pawliszyn, J. Chromatogr. A 1217 (2010) 4041.
[16] H. Kataoka, Curr. Pharm. Anal. 1 (2005) 65.
[17] T.K. Choudhury, K.O. Gerhordt, T.P. Mawhinney, Environ. Sci. Technol. 30 (1996) 3259.
[18] R. Eisert, K. Levsen, G. Wunsch, J. Chromatogr. A 683 (1994) 175.
[19] S. Magdic, A. Bayd-Boland, K. Jimo, J. Pawliszyn, J. Chromatogr. A 736 (1996) 219.
[20] L. Hou, H.K. Lee, J. Chromatogr. A 1038 (2004) 37.
[21] S.L. Chong, D. Wang, J.D. Hayes, B.W. Wilhite, A. Malik, Anal. Chem. 69 (1997) 3889.
[22] D. Wang, S.L. Chong, A. Malik, Anal. Chem. 69 (1997) 4566.
[23] A. Kabir, C. Hamlet, K.S. Yoo, G.R. Newkome, A. Malik, J. Chromatogr. A 1034 (2004) 1.
[24] J.-F. Liu, G.-B. Jiang, J.-F. Liu, J.A. Jönsson, Trends Anal. Chem. 24 (2005) 20.
[25] J.-F. Liu, N. Li, G.-B. Jiang, J.-M. Liu, J.Å.M.-J. Jönsson, J. Chromatogr. A 1066 (2005) 27.
[26] D.-H. Kim, I.-H. Baek, S.-U. Hong, H.-K.J. Lee, Membr. Sci. 372 (2011) 346.
[27] Y. Tao, J.-F. Liu, X.-L. Hu, H.-C. Li, T. Wang, G.-B. Jiang, J. Chromatogr. A 1216 (2009) 6259.
[28] Z. Es'haghi, M. Ahmadi Golsefidi, A. Saify, A. Tanha, Z. Rezaeifar, Z. Alian-Nezhadi, J. Chromatogr. A 1217 (2010) 2768.
[29] Z. Es'haghi, M.M. Ebrahimi, S. Hosseini, J. Chromatogr. A 1218 (2011) 3400.
[30] Z. Es'haghi, Z. Rezaeifar, G.H. Rounaghi, Z. Alian-Nezhadi, M. Ahmadi-Golsefidi, Anal. Chim. Acta 689 (2011) 122.
[31] Z. Es'haghi, M. Khalili, A. Khazaeifar, G.H. Rounaghi, Electrochim. Acta 56 (2011) 3139.
[32] M. Ebrahimi, Z. Es'haghi, F. Samadi, M.S. Hosseini, J. Chromatogr. A 1218 (2011) 8313.
[33] M.M. Heravi, F.F. Bamohharam, N. Tavakoli-Hoseini, Synth. React. Inorg. Met.: Org. Nano-Met. Chem. 41 (2011) 616.
[34] E.F. Kozhevnikova, J. Quartararo, I.V. Kozhevnikov, Appl. Catal. A 245 (2003) 69.
[35] M.M. Heravi, N. Javanmardi, H.A. Oskooie, B. Baghernejad, M. Heidari, F.F. Bamoharram, J. Chin. Chem. Soc. 56 (2009) 589.
[36] A. Sarafraz Yazdi, Z. Es'haghi, J. Chromatogr. A 1094 (2005) 1.
[37] C. Rocchiccioli-Deltcheff, R. Thouvenot, M. Dabbabi, Spectrochim. Acta 33 (1977) 143.
[38] J. Xiong, B. Hu, J. Chromatogr. A 1193 (2008) 7.
[39] J. Yu, C. Wu, J. Xing, J. Chromatogr. A 1036 (2004) 101.
[40] E. Psillakis, N. Kalograkis, TrAC, Trends Anal. Chem. 22 (2003) 565.
[41] Z. Es'haghi, Anal. Chim. Acta 641 (2009) 83.
[42] Y. Zhou, J.H. Schattka, M. Antonietti, Nano Lett. 4 (2004) 477.
[43] Y. Liu, M. Wang, J. Li, Z.Y. Li, P. He, H.T. Liu, J.H. Li, Chem. Commun. 13 (2005) 1778.
[44] C. Blasco, M. Fernández, Y. Picó, G. Font, J. Chromatogr. 1030 (2004) 77.
[45] V.G. Zuin, A.L. Lopes, J.H. Yariwake, F. Augusto, J. Chromatogr. A 1056 (2004) 21.
[46] M. Riu, M. Mestres, O. Busto, J. Guasch, J. Chromatogr. A 1107 (2006) 240.
[47] Q. Zhou, H. Bai, G. Xie, J. Xiao, J. Chromatogr. A 1188 (2008) 148.
[48] M. Fernandez-Alvarez, M. Llompart, J.P. Lamas, M. Lores, C. Garcia-Jares, R. Cela, T. Dagnac, J. Chromatogr. A 1188 (2008) 154.
[49] H. Bagheri, Z. Ayazi, E. Babanezhad, Microchem. J. 94 (2010) 1.