Nanofluid of zinc oxide nanoparticles in ionic liquid for single drop liquid microextraction of fungicides in environmental waters prior to high performance liquid chromatographic analysis

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ABSTRACT

Using a nanofluid obtained by dispersing ZnO nanoparticles (ZnO NPs) in 1-hexyl-3-methylimidazolium hexafluorophosphate, new single drop microextraction method was developed for simultaneous extraction of three fungicides (chlorothalonil, kresoxim-methyl and famoxadone) in water samples prior to their analysis by high performance liquid chromatography (HPLC-VWD). The parameters affecting the extraction efficiency such as amount of ZnO NPs in the nanofluid, solvent volume, extraction time, stirring rate, pH and ionic strength of the sample solution were optimized. Under the optimized conditions, the limits of detection were in the range of 0.13–0.19 ng/mL, the precision of the method assessed with intra-day and inter-day relative standard deviations were <4.82% and <7.04%, respectively. The proposed method was successfully applied to determine the three fungicides in real water samples including lake water, river water, as well as effluent and influent of wastewater treatment plant, with recoveries in the range of 74.94–96.11% at 5 ng/mL spiking level. Besides to being environmental friendly, the high enrichment factor and the data quality obtained with the proposed method demonstrated its potential for application in multi residue analysis of fungicides in actual water samples.

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1. Introduction

Pesticides are used at various stages in agricultural process to boost the harvesting products. Therefore, contamination of matrices such as soil, water and even the agricultural products with various agrochemicals is a serious threat to human health [1,2]. Owing to properties like high stability and resistant to a wide range of pH, temperature and light, fungicides, one type of pesticides, are widely used for the protection and preservation of a variety of crops, fruits, and vegetables [3]. Fungicides are directly applied to the agricultural soils or sprayed on the field crops [4,5], and hence can easily release away from the application sites by air or rain water and contaminate water bodies. European Union Directive set the maximum allowed concentration of individual and total pesticides to be 0.1 and 0.5 ng/mL, respectively on the quality of water intended for human consumption [6]. Therefore, preconcentration techniques are required to investigate fungicides at trace levels.

There are numerous preconcentration techniques like solid-phase microextraction (SPME) [7–9], dispersive liquid–liquid microextraction (DLLME) [10–12], hollow fiber liquid phase microextraction (HF-LPME) [13], salting-out assisted liquid–liquid extraction [14], ultrasound-assisted emulsification microextraction [15,16], cloud point extraction [17] for the determination of fungicides in water samples. SPME is simple, solvent free and a technique in which efficient extraction is possible. The draw backs of SPME are cost of analysis, analyte carry-over, limited life time due to fiber-fragility and long analysis time. DLLME is the other microextraction technique which is cheap, simple, fast and present high enrichment factor. But difficulty to automation and requirement of environmentally unfriendly extraction solvents are its limitations. HF-LPME is also a simple and inexpensive technique in which high analyte preconcentration and excellent sample cleanup is possible. But, its limitations like long analysis time and memory effect when reusing the hollow fiber could be stated. Even though the use of microextraction techniques decrease environmental impact and the exposure of personnel to solvent vapors [18,19] most of these approaches directly or indirectly consume significant amount of organic solvents which can cause different toxicological problems. Hence, using either relatively green solvents

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or further reducing the amount of these solvents for the intended purpose is still interesting scientific research. Single drop microextraction (SDME) is liquid–liquid microextraction technique in which the amount of extraction solvent is drastically reduced. It is simple, inexpensive, easy to operate, versatile, and has additional qualities like different extraction modes, high enrichment factor and can be fully automated [18]. However, instability and ease of dislodgment of the micro-drop, limited drop volume, and evaporation of the micro-drop due to its volatility and low viscosity are the main problems associated to SDME. To overcome these limitations, investigation of suitable mode of SDME and appropriate solvent (viscous and non-volatile) which can form stable, relatively large drop volume with high extraction quality is required.

Metal oxide nanoparticles (NPs) have attracted considerable attention for their potential application in many technologies [20]. ZnO nanoparticles (ZnO NPs), one of the metal oxide NP, was employed for extraction purpose. Ghaedi et al. [21] used ZnO NPs loaded on activated carbon for efficient extraction of brilliant green dye and for removal of bromophenol red [22] in aqueous solutions. It was also applied as solid-phase adsorbent and as efficient solar photo-catalyst [23]. Wang and his co-authors employed silver NPs decorated ZnO NP sheets as effective surface enhanced Raman scattering substrate and applied for rapid detection of organic pollutants [24]. Besides, preparation and characterization of ZnO nanofluids (ZnO-NFs) and its applications have been reported [25–28].

Nanofluids (NFs) are materials comprising of stable suspensions of NPs (<100 nm) in base fluids [29] which can be prepared in different ways [30]. NFs have wide applications in various scientific areas. Suspending small amount of NPs in liquid–liquid system can cause considerable augmentation in mass transfer coefficient, and hence expected to improve the extraction efficiency which is of great interest in recent. A recent review provided the unique liquid–liquid properties, fundamental technologies and NFs application in chemical and bio-analysis methods and devices [31]. Krishnamurthy et al. obtained faster diffusion of a dye droplet in water based NF than in pure water [32]. Fang et al. also observed mass diffusion of rhodamine B to be about 10.71 times in 0.5% Cu NFs than in water alone [33]. The application of NFs to modify the mass transfer coefficient in extraction processes has been reported in recent. Above 121% enhancement in the rate of mass transfer was reported upon adding 0.002% modified Fe_3O_4 and Al_2O_3 NPs to a single drop in liquid–liquid extraction process [34]. The use of NFs to enhance the performance of a pulsed liquid–liquid extraction column was investigated and addition of 0.01–0.1% (v/v) SiO_2 NPs to kerosene increased the mass transfer by a factor of 4–60% [35]. The same research group obtained 60% improvement in mass transfer performance by adding 0.1% (v/v) SiO_2 to kerosene–acetic acid–water chemical system in pulsed liquid–liquid extraction column [36].

Ionic liquids (ILs) are compounds containing exclusively of ions, usually organic cations and organic/inorganic anions [37]. Even though ILs are considered as green alternates to volatile and toxic organic solvents, the issue of their potential biological toxicities has been reported [38–40]. However, due to their astonishing characteristics, ILs have wide application in several aspects of scientific work including as advantageous solvents for sample pretreatment [41]. The application of nanocomposites of different types of NPs and ILs has been reported in recent. Pena-Pereira et al. used nanoconfined IL-rich fibers in headspace SPME of volatile aromatic compounds and obtained high enrichment factors (up to 7400) and low LOD values (0.03–1.27 ng/mL) [42]. Ternary composites of nanocellulose, carbonnanotubes and ILs exhibited excellent extraction efficiency than the corresponding pure ILs in direct immersion SDME of heterocyclic amine [43]. Therefore, combination of ILs and

NPs in the form of IL-NFs may impart certain additional properties to the ILs. In addition, the aforementioned studies are indicators for the possibility of ILs based NFs consumption as extraction solvents for trace analysis of environmental pollutants.

The purpose of this study was to prepare stable 1-hexyl-3-methylimidazolium hexafluorophosphate ([HMIM][PF₆]) based ZnO-NF for its application in a modified direct immersion single drop microextraction (SDME), namely ZnO-NF-based SDME (ZnO-NF-SDME), for enrichment of fungicides in water samples. The preferences of ZnO-NF for the investigation were initiated from the fact that unlike many metallic NFs, ZnO-NF form stable suspension without additional stabilizers due to the surface charges on ZnO NPs. The stability is crucial for its application in different areas like in extraction procedures. In addition to these, the use of ZnO-NF as a replacements of other organic solvents is relatively non-toxic and it allow the use of large drop volume due to its high viscosity and stability. Furthermore, ZnO-NF-SDME would exhibit higher enrichment factor which would be practically helpful to extract the target analytes at very trace level.

2. Experimental

2.1. Chemicals and reagents

Zinc nitrate hexahydrate ($\text{Zn}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$) (AR grade), sodium hydroxide (NaOH) (AR grade) and sodium chloride (NaCl) (GR grade) were obtained from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). 1-Butyl-3-methylimidazolium tetrafluoroborate ([BMIM][BF₄]) and 1-hexyl-3-methylimidazolium hexafluorophosphate ([HMIM][PF₆]) were purchased from Aladdin Industrial Corporation (Shanghai, China). Kresoxim-methyl and famoxadone standards (Purity > 98%) were purchased from Dr. Ehrenstorfer GmbH (Augsburg, Germany), while chlorothalonil (Purity, 99.7%) was obtained from AccuStandard, Inc. (New Haven, USA). The physicochemical characteristics of these fungicides are shown in Table 1. HPLC grade acetonitrile and methanol solvents were obtained from Fisher Scientific (New Jersey, USA). Ultrapure water prepared by a Milli-Q Gradient system (Millipore, Bedford, MA, USA) was used throughout the experiments

2.2. Preparation and characterization of ZnO NPs

For the preparation of ZnO NPs, the sol-gel process reported by Gandhi et al. [44] was followed with minor modifications. In detail, 50 mL of 0.4 mol/L $\text{Zn}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ solution was prepared in water and mixed with 1 mL of [BMIM][BF₄] to obtain colorless and transparent solution up on vigorous stirring. Freshly prepared 30 mL of NaOH (0.8 mol/L) was added in drops under magnetic stirring to obtain precursor $\text{Zn}(\text{OH})_2$ sol and further stirred for another 2 h at room temperature. The obtained $\text{Zn}(\text{OH})_2$ sol was allowed to stand overnight and the supernatant liquid was discarded. The settled precursor was recovered by centrifugation at 10,000 rpm for 15 min followed by washing with water and ethanol several times to remove any aggregates and organic impurities and then vacuum dried at 80 °C. The dried $\text{Zn}(\text{OH})_2$ precursor was grinded and calcined at 300 °C to obtain crystallized ZnO NPs.

The size and morphology of the ZnO NPs were characterized by transmission electron microscopy (TEM; H-7500, Hitachi). X-ray diffraction (XRD) analysis was performed with an X'pert PRO instrument (PANalytical) using Cu-K α radiation. Effective removal of the NPs from the extractant before HPLC system was confirmed by recording UV–vis spectra with Shimadzu UV-3600 (Kyoto, Japan) in separate experiment.

Table 1
Physicochemical properties of the target fungicides.

Common name	Abbr.	Chemical structure	CAS no.	Molecular weight (g/mol)	Water solubility (mg/L)	log K_{ow} (25 °C)
Chlorothalonil	CLT		1897-45-6	265.91	0.6–1.2 ^a	2.92
Kresoxim-methyl	KSM		143390-89-0	313.36	2 ^b	3.40
Famoxadone	FMX		131807-57-3	374.39	0.052 ^b	4.65

^a Values at 25 °C.^b Values at 20 °C.

2.3. Preparation of IL-ZnO-NFs

Two-step NF preparation method, which is suitable for metal oxide NPs based NF [34], was used in the preparation of ILs-ZnO-NFs. Accordingly, the synthesized ZnO NPs powder was dispersed in [HMIM][PF₆] and hand shaken for homogeneous distribution of the NPs. The NFs were sonicated for 90 min to break up any potential clusters of the NPs. To monitor the stability and extraction performance of the NFs which might be affected due to variation of the amount of NPs, ZnO NPs were used with concentrations of 0.005, 0.01, 0.1, 0.5, and 1.0% (m/m).

2.4. ZnO-NF-SDME procedure

For the ZnO-NF-SDME procedure, a syringe tip cap (tip protector) of 5 mL disposable syringe (Shandong Weigao Group, Medical Polymer Company Limited, Shandong, China) passed through screw capped vial cap, were used in place of syringe needle and the rest procedure is same as conventional SDME. In detail, 10 μ L IL-ZnO-NFs micro-drop was carefully placed on tip of the syringe cap, initially made after passing through the vial cap, and immersed in to 20 mL extraction vial containing 10 mL of the sample solutions spiked with the target analytes and magnetic stirring bar. All vials were tightly sealed, placed in a stirring plate and were kept stirring (500 rpm) at 25 °C for 40 min. After the extraction, the micro-drop was carefully deposited in an Eppendorf vial containing 100 μ L methanol followed by sonication for 10 min, and posterior centrifugation (7000 rpm, 10 min) for complete removal of the NPs which was confirmed by recording its UV–vis spectra in separate experiment (data not shown). Then, 20 μ L of the extracted solution was injected to HPLC for determination of the fungicides. The whole ZnO-NF-SDME procedure is schematically shown in Fig. 1.

2.5. HPLC analysis

An Agilent 1200 HPLC system (Agilent Technologies, Santa Clara, CA, USA) equipped with a quaternary pump, vacuum degasser, variable wavelength detector and manual injector was employed to perform chromatographic analysis. An Agilent TC-C18 column (250 mm $\times$ 4.6 mm i.d., particle size 5 μ m) was used for separation

of the analytes. Data acquisition and processing were achieved using Agilent LC ChemStation software (Rev.B.03.01) throughout the analysis. Acetonitrile and water (60:40, v/v) mixture was delivered at a flow rate of 1.0 mL/min in isocratic mode as the mobile phase. The detection wavelength was initially held at 229 nm (0–12.5 min) for chlorothalonil, 225 nm (12.5–14.5 min) for kresoxim-methyl, and finally 229 nm (14.5–20 min) for famoxadone. The sample volume of 20 μ L was manually injected, and all the analytes were eluted over an 18 min and a 2 min post run time to allow the system to equilibrate prior to the next analysis. For all the target analytes, baseline separation was obtained under these chromatographic conditions and the peak area was used as an instrumental response for quantification. The quantification of the pesticides was performed by external calibration with mixed standard solutions, using 10 calibration points.

Fig. 1. Schematic representation of ZnO-NF-SDME.

2.6. Sample collection

Lake water sample was collected from Weiming Lake located in Peking University (Beijing, China), river water was collected from Chaobai River (Beijing, China), and sewage waste effluent and influent water samples were collected from Qinghe wastewater treatment plant (Beijing, China). The samples were filtered with 0.22 μm micropore membrane and stored at 4 °C until analysis time.

2.7. Quality assurance/quality control (QA/QC)

The sample solutions were stored in amber glass bottles to protect the possible loss of target analytes during storage. All the glassware and the magnetic stir bars were washed, oven dried and

rinsed with acetone before use. Blank samples were investigated passing through the whole extraction procedure parallel to the samples in all experiments. The retention times of the target analytes in each of the standard solutions were used for HPLC peak identification and the analytes in the sample solutions. Method validation was investigated under optimal extraction conditions. The target fungicides were quantitatively determined using 10 external calibration points. Linearity of the method was obtained by investigating water samples spiked with concentration range of the fungicides from 0.5 to 100 ng/mL. The limits of detections (LODs) and limits of quantifications (LOQs) were calculated on the basis of signal to noise ratios of 3 ($S/N=3$) and 10 ($S/N=10$), respectively. Intra-day and inter-day relative standard deviation (RSDs) at eight replicates ($n=8$) investigated with water samples

Fig. 2. Effect of ZnO NPs composition on the extraction efficiency ($n=4$). Error bars, standard deviation. Extraction conditions: volume of the sample solution, 10 mL; solvent volume, 10 μL ; extraction time, 40 min; and stirring rate, 500 rpm.

Fig. 3. Effect of the volume of NF on the extraction efficiency ($n=4$). Error bars, standard deviation. Extraction conditions: volume of the sample solution, 10 mL; extraction time, 40 min; and stirring rate, 500 rpm.

containing 5 ng/mL of the fungicides were used to evaluate the precision of the method. The robustness of the method in different waters was reflected by the recoveries determined for four different water samples (lake, river, effluent and influent) spiked at 5 and 100 ng/mL.

3. Results and discussion

3.1. Characterization of the ZnO NPs

TEM images of the synthesized ZnO NPs were collected to characterize the size distribution of the materials as presented in supporting information (Fig. S1a). The particles sizes were computed with Nano Measurer software (version, 1.2) from the TEM images. The average size of the calcinated product was 30.41 ± 4.01 nm. The size distribution of ZnO NPs for about 86 particles is shown in Fig. S1b.

The phase and purity of the ZnO NPs were characterized by XRD and the diffraction pattern is shown in Fig. S1c. The observed peaks can be indexed to the known hexagonal wurtzite structure of ZnO according to Joint Committee on Powder Diffraction Standards (JCPDS). No intense peaks of other phases of ZnO and impurities were found, indicating that the precursor is completely oxidized to the NPs. The high crystallinity of the materials can be manifested from the strong intensity and narrow width of the diffraction peaks.

3.2. Optimization of the extraction conditions

Experimental parameters like composition of the NF, volume of the NF, extraction time, stirring rate, pH of the sample solution and salting out affecting the extraction performance were optimized. The three fungicides were considered to evaluate the extraction capability of the ZnO-NF-SDME and extraction efficiency (%) of the

Fig. 4. Effect of the extraction time on the extraction efficiency ($n=4$). Error bars, standard deviation. Extraction conditions: volume of the sample solution, 10 mL; solvent volume, 10 μ L; and stirring rate, 500 rpm.

Fig. 5. Effect of the stirring rate on the extraction efficiency ($n=4$). Error bars, standard deviation. Extraction conditions: volume of the sample solution, 10 mL; solvent volume, 10 μ L; and extraction time, 40 min.

analytes was evaluated under different conditions and all experiments were performed in replicate ($n = 4$).

3.2.1. Effect of composition of the nanofluid

Four IL-ZnO-NFs were prepared by dispersing various amounts of ZnO NPs in the IL to investigate the effect of NP composition on the extraction efficiency. Taking [HMIM][PF₆] as base fluid, 0.005, 0.01, 0.05 and 0.1% (m/m) IL-ZnO-NFs were prepared and applied as extraction solvent. The extraction efficiency achieved with the NFs and IL alone is shown in Fig. 2. Among the solvents, 0.01 and 0.005% NFs exhibited comparable stability and high extraction efficiency than the others. Addition of the NPs to the IL enhanced the extraction efficiency which might be associated to increment in the coefficient of mass transfer due to Brownian motion [32–35]. The high extraction efficiency at lower ZnO NPs composition may be attributed to saturation of the ILs at higher composition which may thwarts the extraction of the fungicides which is in line with the work of Ruiz-Palomero et al. [43]. Therefore, 0.01% (m/m) IL-ZnO-NF was selected as extraction solvent in the subsequent experiments.

3.2.2. Effect of volume of the extraction solvent

To examine the effect of volume of the NF on the extraction efficiency, 2, 4, 6, 8 and 10 μ L of 0.01% IL-ZnO-NF were subjected to the ZnO-NF-SDME procedure. As shown in Fig. 3, the extraction efficiencies of the fungicides increased with the volume of NF in the range of 2–10 μ L, and then further increase in the solvent volume was found to be incompatible with the current method due to dislodging of the micro-drop. Accordingly, 10 μ L of the solvent was used as optimal extraction solvent volume in further experiments.

3.2.3. Effect of the extraction time

The extraction time was optimized using 10 μ L of 0.01% IL-ZnO-NF by increasing the extraction time from 10 to 60 min at 10 intervals. In liquid–liquid microextraction, like the current method, extraction efficiency increases with time until equilibrium point [10,13]. However, long extraction time could also result in the dissolution of the NF and decrease the extraction efficiency. The effect of extraction time on the extraction efficiency is shown in Fig. 4. As

Table 2

Analytical performances of ZnO-NF-SDME.

Fungicides	Linear range (ng/mL)	R^2	LOD (ng/mL)	LOQ (ng/mL)	EF ($n = 8$)	RSD (%) ($n = 8$)	
						Intra-day	Inter-day
CLT	0.5–100	0.998	0.19	0.64	984	4.7	6.1
KSM	0.5–100	0.997	0.13	0.44	846	4.8	7.0
FMX	0.5–100	0.996	0.19	0.63	764	4.7	6.2

depicted in the figure, the extraction efficiency do not improve or even decrease after 40 min of the extraction time which might be due to the dissolution of the NF. Therefore, 40 min was adopted as the optimized extraction time.

3.2.4. Effect of the stirring rate

In general, stirring rate improve extraction efficiency by facilitating exposure of the analytes present in the aqueous solution to the micro-drop [43]. Accordingly, different stirring rates were assessed from 0 to 700 rpm for extraction time of 40 min and its effect on the extraction efficiency is shown in Fig. 5. It was observed that the extraction efficiency increased with the stirring rate up to 500 rpm. After 500 rpm, the efficiency decreased due to instability of the micro-drop. Therefore, 500 rpm was selected in the following experiments.

3.2.5. Effect of the pH of sample solution

The pH effect was investigated in the range of 2–10 by adjusting pH of the sample solution with sodium hydroxide or hydrochloric acid solutions. The results showed that the pH of sample solution demonstrated insignificant effect, though slight increment of extraction efficiency was acquired in 4–7 pH range (Fig. S2). Due to the fact that the analytes under study are stable in neutral and weak acidic media, the fungicides were efficiently extracted to the NF in this pH interval. At higher pH values, destruction of the fungicides were observed which could be attributed to hydrolysis of the

Fig. 6. Effect of the ionic strength on the extraction efficiency ($n = 4$). Error bars, standard deviation. Extraction conditions: volume of the sample solution, 10 mL; solvent volume, 10 μ L; extraction time, 40 min; and stirring rate, 500 rpm.

analytes in alkaline media [13]. On the basis of these results, pH 7 was selected in the subsequent experiments.

3.2.6. Effect of ionic strength

The addition of salt plays a vital role in conventional extraction processes because it can increase an aqueous solution's ionic strength, which decreases the solubility of the analytes in the sample solution as well as improves the extraction efficiency [14]. The influence of ionic strength on ZnO-NF-SDME performance was investigated by adding different amounts of NaCl (0–10%, w/v) to the sample solution. It was found that salt concentration had an opposite effect on the extraction efficiency for the fungicides (Fig. 6). This is possibly due to the instability of the vesicular droplet probably because of bubble formation at higher salt concentrations, and high chloride concentration in the solution may also facilitate the dissolution of the IL which decreases the extraction efficiency [11]. It was also reported that decrease in extraction efficiency at high ionic strength could be caused by precipitation of the fungicides [13]. In addition to these, competitive adsorption might be also the reason for negative effect of the salting out. Hence, no salt was added in the subsequent experiments.

3.3. Method validation

After the optimal experimental parameters were determined, the figures of merit were investigated and the results are enumerated in Table 2. It was found that the proposed method exhibits excellent linearity, $R^2 \geq 0.996$, between the peak area and concentration over the range of 0.5–100 ng/mL for all the target fungicides. The calculated LODs and LOQs were in the range of 0.13–0.19 ng/mL and 0.44–0.64 ng/mL, respectively. High enrichment factor (EF), determined as the ratio of the analyte concentration in the microdrop to its concentration in the sample solution, was obtained. The intra-day and inter-day precisions (RSDs, $n = 8$) investigated using

Table 4

Recovery (%) values of ZnO-NF-SDME based analysis in real water samples ($n = 6$, mean $\pm$ SD).

Sample	Fungicide	Recovery (%)	
		5 ng/mL spiked	100 ng/mL spiked
River	CLT	86.8 $\pm$ 6.8	83.8 $\pm$ 3.8
	KSM	81.1 $\pm$ 5.4	75.9 $\pm$ 4.9
	FMX	81.8 $\pm$ 6.3	76.7 $\pm$ 3.5
Lake	CLT	96.1 $\pm$ 7.8	86.9 $\pm$ 2.3
	KSM	86.9 $\pm$ 6.4	80.8 $\pm$ 2.9
	FMX	81.0 $\pm$ 2.3	76.5 $\pm$ 3.5
Effluent	CLT	88.5 $\pm$ 6.3	91.0 $\pm$ 3.3
	KSM	80.3 $\pm$ 3.9	81.7 $\pm$ 5.0
	FMX	81.5 $\pm$ 3.8	80.5 $\pm$ 4.3
Influent	CLT	85.9 $\pm$ 6.8	81.2 $\pm$ 2.3
	KSM	74.9 $\pm$ 6.9	76.6 $\pm$ 4.5
	FMX	75.2 $\pm$ 4.4	79.8 $\pm$ 5.7

10 mL sample solution containing 5 ng/mL of each of the fungicides were obtained in the range of 4.7–4.8% and 6.1–7.0%, respectively.

3.4. Comparison with other methods

The current method proposed for extraction and determination of fungicides in various water samples were compared with those of other methods based on HPLC separation (Table 3). As shown, the advantages of the method described here over the other methods include the use ZnO-NF as extraction solvent in place of volatile organic solvents, high enrichment factor, and comparable or lower LODs. Therefore, ZnO-NF-SDME is a simple, effective, and relatively environmental benign technique with low solvent and sample consumption that can be used for the preconcentration of fungicides from aqueous samples.

Table 3

Comparison of the current method with other HPLC based methods proposed for the extraction of fungicides in water samples.

Analytes	Method	Instrument	Extraction solvent (volume μ L)	Extraction time (min)	EFs	LODs (ng/mL)	Ref.
Hexaconazole, procymidone, quinalphos and vinclozolin	CSM-LPME ^a	LC-UV	Hexane (200 μ L)	20	55–59	1.1–1.9	[45]
Thiram, metalaxyl, diethofencarb, myclobutanil and tebuconazole	μ SPME ^b	HPLC-UVD	–	40	–	0.016–0.086	[9]
Myclobutanil, tebuconazole, triadimenol, hexaconazole	DLLME-SFO ^c	HPLC-DAD	1-Dodecanol (12 μ L) and 200 μ L methanol (dispersive solvent)	1	190–450	0.06–0.1	[10]
Thiram, metalaxyl, diethofencarb, myclobutanil, and tebuconazole	TC-DLLME ^d	HPLC-UVD	[C ₈ MIM][PF ₆] (55 μ L)	30	–	0.32–0.79	[11]
Azoxystrobin, diethofencarb and pyrimethanil	ISD-DLLME ^e	HPLC-MS	Toluene (20 μ L) and 1000 μ L methanol (dispersive and demulsified solvent)	2	195–239	0.026–0.071	[12]
Carbendazim, fuberidazole, thiophanate-methyl and thiophanate	SA-LLE ^f	HPLC-UVD	Acetonitrile (2000 μ L)	5	–	0.14–0.38	[14]
Azoxystrobin, diethofencarb, pyrimethanil and kresoxim-methyl	IL-USA-EME ^g	HPLC-VWD	[C ₈ MIM][PF ₆] (40 μ L)	15	88–137	0.73–2.2	[16]
Chlorothalonil, kresoxim-methyl and famoxadone	ZnO-NF-SDME	HPLC-VWD	ZnO-NF (10 μ L)	40	764–984	0.13–0.19	This study

^a Cone-shaped membrane liquid phase microextraction.

^b Micro-solid phase extraction.

^c Dispersive liquid–liquid microextraction based on solidification of floating organic droplet.

^d Temperature controlled dispersive liquid–liquid microextraction.

^e In-syringe demulsified dispersive liquid–liquid microextraction.

^f Salting-out assisted liquid–liquid extraction.

^g Ultrasound-assisted emulsification microextraction.

Fig. 7. Typical chromatograms of blank and spiked real water sample analysis. Extraction conditions: volume of the sample solution, 10 mL; solvent volume, 10 μ L; extraction time, 40 min; stirring rate, 500 rpm; pH of the sample solution, 7; and ionic strength, no salt addition.

3.5. Real sample analysis

The proposed ZnO-NF-SDME method was applied to the determination of the three analytes in four different types of actual (lake, river, effluent and influent) water samples. In the assayed samples, the target analytes were not detected. Therefore, the samples were spiked with mixed standard detected and the extraction performance was evaluated to assess the matrix effect. The analytical results are listed in Table 4, and the typical chromatograms of the blank and spiked real water samples are shown in Fig. 7. The recoveries of present method were satisfied in the range of 81.2–96.1%, 74.9–86.9% and 75.2–81.8% for chlorothalonil, kresoxim-methyl and famoxadone, respectively. Hence, the developed method was successfully applied and can be used as alternative technique for analysis of multi residue fungicides in water samples.

4. Conclusions

In the current study, effective and risk-free ZnO-NF-SDME pre-concentration technique for HPLC-VWD analysis was developed for the determination of fungicides in water samples. ZnO-NF obtained through dispersion of ZnO NPs in [HMIM][PF₆] is proposed as an extraction solvent in this microextraction approach. Based on the developed method, effective extraction of chlorothalonil, kresoxim-methyl and famoxadone fungicides in real water samples

(river, lake, effluent and influent) were achieved with reasonable linearity, good precisions, and satisfactory relative recoveries. The potentials of the method described here include instead of volatile organic solvents, NF was used as the extraction solvent, which is relatively safe and environmentally compatible, high EF, low LOD, precise, and acceptable recovery values were obtained which indicate its high utility. In conclusion, IL-ZnO-NF based ZnO-NF-SDME presents a simple, effective, and relatively environmental benign technique with low sample consumption that can be used for the preconcentration of fungicides from water samples.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.chroma.2015.03.049>.

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