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2,4-Dimethylphenol imprinted polymers as a solid-phase extraction sorbent for class-selective extraction of phenolic compounds from environmental water

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ABSTRACT

A molecularly imprinted polymer (MIP) was prepared using 2,4-dimethylphenol (2,4-DMP) as template. The synthesis is optimized by using three different porogens, chloroform, acetonitrile and toluene. The MIP was used as a class-selective sorbent in molecularly imprinted solid-phase extraction (MIP-SPE) for pre-concentration and determination of phenolic compounds from the environmental water. The difference in recognition selectivity of the polymer columns was observed in HPLC system. The variables affecting the extraction efficiency of MIP-SPE procedure were systematically investigated to facilitate the class-selective extraction of phenols from spiked water samples. The spiked aqueous solution was adjusted to pH 6.0 before being percolated through the MIP-SPE cartridge at the flow rate of 0.5 mL min^{-1}. After rinsing with dichloromethane, the bound phenolic compounds were desorbed with acetonitrile containing 5% aqueous ammonia. The developed MIP-SPE method was demonstrated to be applicable to the analysis of phenolic compounds in the environmental water.

1. Introduction

Many analytical techniques, e.g. high performance liquid chromatography (HPLC) [1,2], capillary electrophoresis (CE) [3–6] and gas chromatography (GC) [7,8], have been developed to determine the phenolic compounds. However, the analysis of phenols in complex matrices at low level of concentration usually requires a sample pre-treatment procedure before chromatographic analysis. Solid-phase extraction (SPE) is an efficient sample pre-treatment method, routinely used for the extraction of compounds from liquid or solid matrices. The classical SPE sorbents retain analytes by nonselective hydrophobic or polar interactions that lead to a partial co-extraction of interfering substances. In order to facilitate the trace enrichment of analytes and enhance the selectivity of the extraction, many new functionalized polymeric sorbents and highly cross-linked polymers have recently been developed as the alternatives to the classical SPE materials. Molecularly imprinted polymer (MIP) is one of these novel polymeric sorbents for selective SPE of target compounds from complex matrices. In recent years, several reviews have been published demonstrating the advantages of this strategy for developing selective sample pre-treatment methods [9–11].

MIPs are synthetic polymeric macromolecules with specific recognition sites complementary in shape, size and functional groups to template molecule, involving an interaction mechanism based on molecular recognition. The polymers are prepared by the copolymerization of a functional monomer with a cross-linker agent in the presence of a template molecule via non-covalent and covalent imprinting. Removal of the template molecule results in a functional polymeric matrix with recognition sites, which enables the template or template analogue to interact with the functional matrix in the rebinding process. Accordingly, the selectivity of a MIP for a target compound or a group of compounds can be pre-determined by the choice of the template employed for its preparation, which is also the most attractive advantage of molecular imprinting technique.

Recently, much concern has been devoted to synthesize the MIPs for the priority phenolic pollutants, such as 2,4-dichlorophenol (2,4-DCP) [12,13], 2,4,6-trichlorophenol (2,4,6-TCP) [14–17], pentachlorophenol [18–20], 2,4-dinitrophenol [21,22], 4-nitrophenol [23–27] and phenol [28–30]. Most of those synthesized MIPs have been used as SPE sorbents for selective extraction of the analytes, including on-line and off-line SPE. However, those research mainly aim at selectively isolating the template molecule from the complex matrix, and little report has been published to prepare MIPs as SPE sorbent for class-selective extraction of a group of phenolic compounds, except that 2,4,6-TCP imprinted polymer has been applied to selective pre-concentration of phenolic compounds from the environmental water [14,15]. In addition, to the best of our knowledge, 2,4-dimethylphenol (2,4-DMP) has scarcely been used

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as template to prepare MIP sorbent. Among the phenolic compounds, the methyl-substituted phenols exhibits smaller toxicity than the chloro- or nitro-substituted phenols according to Free-Wilson approach, one of the main Quantitative Structure–Activity Relationships (QSARs) approaches. As shown in the QSAR toxicity data, 2,4-DCP and other chlorophenols are confirmed to possess much larger toxicity than the methyl-substituted phenols. More interestingly, 2,4-DMP is extremely similar to 2,4-DCP in terms of molecular size and position of the substituted group. Therefore, it may be a good way to prepare a MIP with 2,4-DMP as template for class-selective extraction of phenolic compounds, especially 2,4-DCP. In this way, the possible detriment to the environment can be fairly reduced during the preparation process.

The present paper describes the synthesis and the evaluation of a non-covalently imprinted MIP with 2,4-DMP as template, 4-vinylprydine (4-VP), ethylene glycol dimethacrylate (EGDMA) and 2,2-azobisisobutyronitrile (AIBN) as functional monomer, cross-linker agent and initiator, respectively. The obtained MIP was applied as a class-selective SPE sorbent to pre-concentrate and determine the phenolic compounds in the environmental water.

2. Experimental

2.1. Reagents and chemicals

2,4-DMP, 2,4-DCP, 2,4,6-trimethylphenol (2,4,6-TMP), 4-VP, EGDMA and AIBN were all from Acros Organics (Geel, Belgium); acetonitrile was from Fisher (Loughborough, UK). The monomer 4-VP was purified by standard procedures to remove stabilizers. EGDMA was extracted with 10% aqueous sodium hydroxide and water; after drying over $MgSO_4$, it was filtered and distilled under reduced pressure. AIBN was recrystallized in ethanol prior to use. Phenol, 4-chlorophenol (4-CP), 4-methylphenol (4-MP), α-naphthol, acetic acid, methanol, chloroform, toluene and other regents were of analytical grade.

2.2. Preparation of the imprinted polymer with bulk polymerization

For the preparation of the 2,4-DMP imprinted polymer, the template (2,4-DMP) (0.124 g, 1 mmol) was dissolved in porogen (5.6 mL) in a 10-mL thick walled glass tube. The functional monomer (4-VP) (0.425 g, 4 mmol), the cross-linking monomer (EGDMA) (3.8 mL, 20 mmol), and the initiator (AIBN) (0.04 g) were then added to the above solution. The solution was sonicated and purged with oxygen-free nitrogen for 10 min on an ice bath, and then the glass tube was sealed under nitrogen and then placed in a water bath at 60 °C. The reaction was allowed to proceed for 24 h. A reference, non-imprinted, polymer that did not contain any template was prepared simultaneously using the same protocol. The obtained hard polymers were crushed, ground, and wet-sieved using acetone to obtain regularly sized particles between 38.5 and 63 μm suitable for the chromatographic and SPE evaluations.

2.3. Chromatographic evaluation of the polymers

To evaluate the polymers in analytical columns, ground polymer particles were suspended in acetonitrile by sonication and then slurry packed into 15 cm × 0.46 cm i.d. stainless steel HPLC columns at 3000 psi using an air-driven fluid pump (Haskel) with ethanol as the solvent.

To remove template and eliminate interfering compounds from the synthetic procedure, the analytical columns were washed online with methanol/acetic acid (90/10, v/v) and methanol until a stable baseline was obtained. A Waters 515 HPLC pump and a Waters 2487 dual λ absorbance detector were used. The chromatographic evaluation of the polymers was carried out using acetonitrile as the mobile phase at 1 mL min^{-1}. The injection volume was 20 μL, the detector was set at 280 nm, and the analyses were performed at room temperature. Acetone was injected as the void marker. Capacity factor, k', was calculated by the equation $k' = (t_R - t_0)/t_0$, where t_R and t_0 are the retention time of the analyte being investigated and the void marker, respectively. The molecular imprinting factor (IF) proposed for the evaluation of the recognition selectivity was calculated by the equation $IF = k'_{MIP}/k'_{NIP}$, where k'_{MIP} was the capacity factor of the analyte on the MIP and k'_{NIP} was that on the NIP.

2.4. MIP-SPE procedure

MIP particles (100 mg) were packed in a syringe barrel and used for MIP-SPE. The cartridges were conditioned with 15 mL acetonitrile and then 15 mL water. After percolating the spiked aqueous solution at 0.5 mL min^{-1}, 30 min dryness of the cartridge was operated by vacuum, and then 0.5 mL dichloromethane was employed to rinse the cartridge. At the end, elution of phenolic compounds was achieved with 3 mL Acetonitrile/aqueous ammonia (95:5, v/v). The eluent was purged with nitrogen to remove the solvent and 2 mL acetonitrile/water solution (40:60, v/v) was added to the conical bottle. After filtering through the 0.45 μm filter, the sample was analyzed by HPLC.

Real river water samples were collected from the Liao River in Yingkou, and filtered through a 0.45 μm filter. The samples were acidified to pH 6.0 with hydrochloric acid before MIP-SPE.

2.5. HPLC

To determine the obtained eluent of phenolic compounds, the analysis was performed on a Waters alliance system equipped with Waters 996 photodiode array detector. Chromatographic separations were carried out with a Hydrsil ODS2 column 25 cm × 0.46 cm i.d., 5 μm, supplied by Elite Analytical Instrument Co. (Dalian, China). The mobile phase consisted of Milli-Q-quality water (acidified to pH 2.5 with hydrochloric acid) as solvent A and acetonitrile as solvent B. The ratio of solvent A to solvent B was 4:6 (v/v), and the flow rate of the mobile phase was 1 mL min^{-1}. The injection volume was 20 μL, the column temperature was set at 40 °C and all compounds were detected at 280 nm.

3. Results and discussion

3.1. Synthesis and chromatographic evaluation of the polymers

Solvent plays an important role in the formation of the porous structure of MIPs, and the morphological properties of porosity and surface area are determined by the type of solvent, referred to as "porogen", used in the polymerization. Meanwhile, porogen has effect on the complexation of functional monomers with the template during pre-polymerization. Especially in the non-covalent polymerization, the extent of the pre-polymer complex is affected by the polarity of the porogen solvent. Less polar solvents usually will increase formation of the polymer complex by Le Chatelier's principle, facilitating polar non-covalent interactions such as hydrogen bonding [31]. On the other hand, the polar solvents tend to dissociate the non-covalent interaction in the pre-polymer complex, especially protic solvents that afford a high degree of disruption to hydrogen bonds [32]. Thus, three common used imprinting porogen, chloroform, acetonitrile and toluene were chosen to evaluate its influence on the recognition ability of the obtained polymers.

Table 1
Compositions of the polymerization mixtures used for the preparation of the MIPs and NIPs.

Polymers	Template 2,4-DMP	Functional monomer 4-VP	Cross-linker EGDMA	Initiator AIBN	Porogen[a]
P1	1 mmol	4 mmol	20 mmol	40 mg	Chloroform
P2	1 mmol	4 mmol	20 mmol	40 mg	Acetonitrile
P3	1 mmol	4 mmol	20 mmol	40 mg	Toluene
N1	–[b]	4 mmol	20 mmol	40 mg	Chloroform
N2	–	4 mmol	20 mmol	40 mg	Acetonitrile
N3	–	4 mmol	20 mmol	40 mg	Toluene

[a] Porogen volume is 5.6 mL.
[b] No template added.

Table 2
Chromatographic evaluation of the MIPs synthesized using different porogen.

Analytes	Capacity factor, k'_{MIP}			Capacity factor, k'_{NIP}			Imprinting factor, k'_{MIP}/k'_{NIP}		
	P1	P2	P3	N1	N2	N3	P1	P2	P3
2,4-DMP	0.416	0.744	0.973	0.320	0.425	0.629	1.300	1.751	1.547
2,4-DCP	1.716	3.043	3.115	1.415	1.575	2.030	1.213	1.932	1.534

P1, P2 and P3 were three non-covalent molecularly imprinted polymers using chloroform, acetonitrile and toluene as porogen, respectively (Table 1). Three non-imprinted polymers (NIP), N1, N2 and N3, which did not contain any template, were prepared simultaneously using the same protocol.

The obtained polymers were evaluated under the chromatographic conditions previously described by injecting 2,4-DMP and 2,4-DCP as test analytes into the columns. Table 2 gives the k' and the IF values for the 2,4-DMP and 2,4-DCP in the three different MIP columns and the corresponding NIP columns. Both 2,4-DMP and 2,4-DCP show better retention behavior in the MIP columns than in their corresponding NIP columns. This result confirms the imprinting effect. In addition, P2 was shown to be superior to P1 and P3 in terms of imprinting factor. Therefore, acetonitrile was selected as porogen in the future preparation of MIPs.

In order to demonstrate the retention capacity of phenolic compounds in the P2 and prepare for the MIP-SPE evaluation, 2,4-DMP, 2,4-DCP, phenol, 4-CP, 4-MP and 2,4,6-TMP, α-naphthol and toluene were evaluated on the imprinted polymer column P2 and the reference column N2. Fig. 1 shows the k' values for the test compounds in P2 column and the corresponding IF values. Among the test analytes, all phenolic compounds gave rise to larger imprinting factor and capacity factor values than toluene. This result indicates that the recognition mechanism is based on the hydrogen bonding between the phenolic hydroxyl group and the pyridine nitrogen atom of the MIP polymer.

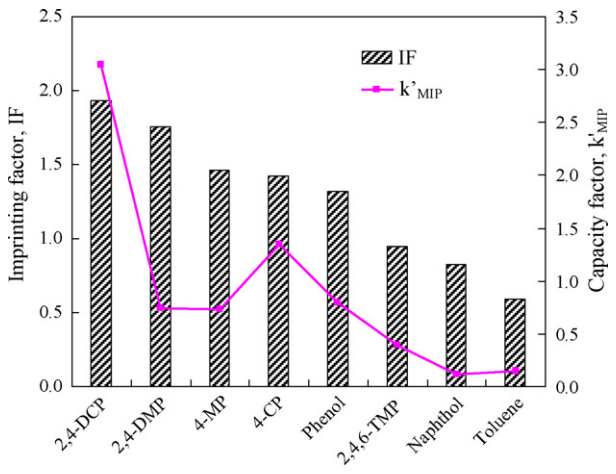

Fig. 1. Capacity factor (k'_{MIP}) and IF values of the test analytes in P2 column under the previously described chromatographic conditions.

As far as the phenolic compounds are concerned, 2,4-DCP and 2,4-DMP show larger IF value than the other phenolic compounds. This result verifies the imprinting effect and the molecular interaction. During the preparation of the MIPs the template was incorporated into the highly cross-linked polymer network. After the removal of template 2,4-DMP, the imprinted cavities and specific binding sites of pyridine nitrogen atom in a pre-determined orientation was formed, whereas the NIPs have no such imprinted cavities and specific binding sites. Consequently, to achieve efficient recognition in the binding process, the compounds need to possess the phenolic hydroxyl group and similar molecular size and structure to the template. The phenolic compounds with larger molecular size than the template cannot be recognized efficiently, which results in the smaller k' and IF values for 2,4,6-TMP and α-naphthol than other phenols. The large molecular size prevents their entrance into the imprinted cavities, and thus disrupts the specific interaction of the phenolic hydroxyl group with the pyridine nitrogen atom.

Furthermore, the molecular interaction between the hydroxyl group and the pyridine nitrogen atom is also affected by the acidity of the phenolic compounds [18]. The acidity of 2,4-DCP ($pK_a = 7.85$) is larger than 2,4-DMP ($pK_a = 10.6$) (NTP, 1992), leading to a stronger interaction of 2,4-DCP with the pyridine nitrogen atom than that of 2,4-DMP. Consequently, a larger IF value was obtained when 2,4-DCP was injected into the MIP column. Compared to the reported MIP matrix based on phenolic compounds, the prepared 2,4-DMP-MIP shows better selectivity by chromatographic evaluation [15,23,25].

3.2. MIP-SPE optimization

The purpose of MIP-SPE is to evaluate the feasibility of the prepared 2,4-DMP-MIPs sorbent for class-selective extraction of phenolic compounds. A generic SPE protocol was employed with the following steps: (1) the MIPs sorbent was conditioned in an appropriate environment to make sure that the treated sample retained by the sorbent; (2) the sample is loaded in the same solvent; (3) the sorbent was rinsed with an organic solvent of low polarity to disrupt the nonspecific interactions between the analytes and the polymeric matrix or the possible interferences; (4) the sorbent was eluted with a pure solvent, solvent mixtures, or a solvent containing a small amount of acid or basic additive that disrupt the strong interaction of the analytes with the polymers.

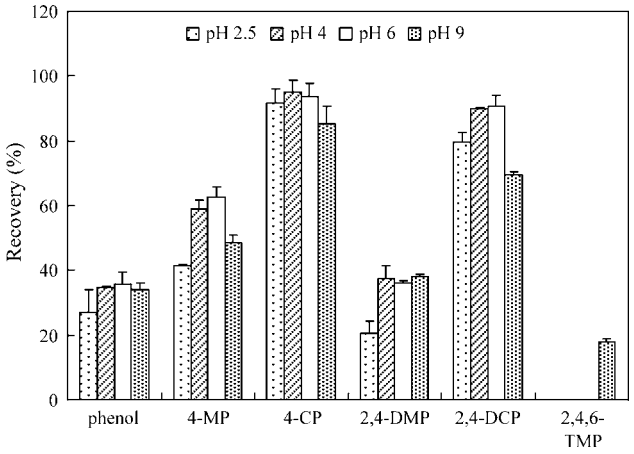

Fig. 2. Recoveries of phenolic compounds obtained by percolating 50 mL spiked aqueous solution at concentration of 0.1 μg mL^{-1} with each analyte at different pH values.

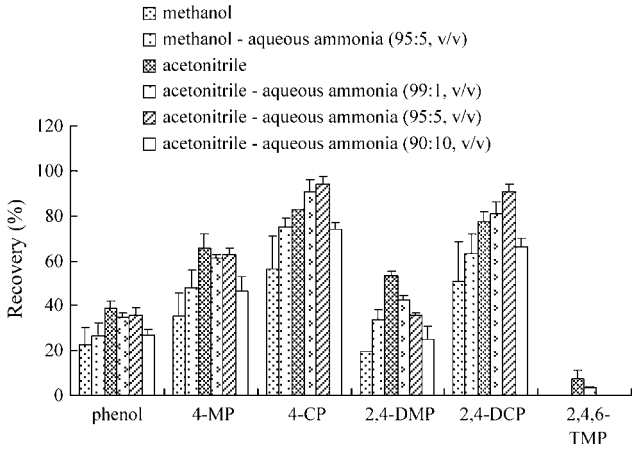

Fig. 3. Recoveries of phenolic compounds using different elution solvents after percolating through MIP cartridge 50 mL spiked aqueous solution at 0.1 μg mL^{-1} with each phenolic compound.

In the preliminary experiments, the flow rates ranging from 0.2 mL min^{-1} to 1 mL min^{-1} were compared. The results demonstrated that higher flow rate led to the decreasing interaction time between the analytes and the sorbent, and consequently, the analytes recovery decreased. Too low flow rate caused low efficiency, thus, flow rate of 0.5 mL min^{-1} was chosen. Herein, six phenolic compounds, 2,4-DMP, 2,4-DCP, phenol, 4-CP, 4-MP and 2,4,6-TMP, were spiked at 0.1 μg mL^{-1} in Milli-Q-quality water as the analytes to evaluate the effect of sample pH, eluting solvent and the breakthrough volume on the extraction process.

3.2.1. *Effect of pH values*

The aqueous solution was adjusted to pH 2.5, 4.0, 6.0 and 9.0 by dilute hydrochloric acid or sodium hydroxide. 50 mL of 0.1 μg mL^{-1} spiked aqueous solution at various pH values were percolated through the MIP cartridge at the flow rate of 0.5 mL min^{-1}, followed by rinsing with 0.5 mL dichloromethane, and eluting with acetonitrile containing 5% (v/v) aqueous ammonia. The obtained eluent was analyzed by HPLC, and consequently, the recoveries of the phenolic compounds were calculated to evaluate the extraction efficiency.

As can be seen in Fig. 2, when the pH of aqueous solution was adjusted to 4.0 or 6.0, all the phenolic compounds displayed equivalent advantageous extraction recoveries. But when the pH of aqueous solution was adjusted to 2.5 or 9.0, the recoveries of the analytes decreased to some extent. This is mainly related to the acidity (pK_a) of the phenolic compounds. The pK_a of the six test phenolic compounds are larger than 7. Therefore, at pH 4.0 or 6.0 all the phenolic compounds cannot be dissociated. The hydrogen bonding interaction between the phenolic hydroxyl group and the nitrogen atoms in the pyridine residues of the polymer was enhanced, resulting in the stronger retention of the phenolic compounds on the MIP sorbent. The recovery decrease at pH 2.5 may be due to the very high acidity. The free hydrogen is competitive with the hydroxyl group of the phenolic compounds and forms stronger hydrogen bonding with the nitrogen atoms in the pyridine residue. As a result the hydrogen bonding interaction between the analytes and the MIP sorbent is disrupted. The pH of the sample was adjusted to 9.0 in order to exploit potential ionic interactions between the phenolate forms of the analytes and the nitrogen atoms in the pyridine residues of the polymer [23], in an attempt to increase the recoveries of the analytes. Although the largest recoveries of the analytes did not attained at pH 9.0, the result also verified the significant role of the ionic interaction played in the extraction process. Therefore, the subsequent extraction processes were all performed at pH 6.0.

In addition, it was worth noting that recoveries of 2,4,6-TMP is lower than the other phenols, which was in accordance with its smaller IF value by chromatographic evaluation. The pH value of the loading solution also affected its retention on the sorbent, leading to the different recovery of 2,4,6-TMP in the different conditions.

3.2.2. *Elution solvent optimization*

50 mL of 0.1 μg mL^{-1} spiked aqueous solution was percolated through the MIP cartridge at the flow rate of 0.5 mL min^{-1}, followed by rinsing with 0.5 mL dichloromethane. Then various elution solvents were added to assess whether the elution solvent had an influence on the extraction recovery, especially the desorbing efficiency.

Pure methanol and acetonitrile were firstly used as elution solvent, and acetonitrile showed stronger desorbing ability than methanol (Fig. 3). It is likely that favorable interactions between the target analytes and acetonitrile molecules lead to desorbing the analytes from the MIP sorbents [17,28]. Furthermore, the aqueous ammonia was added to acetonitrile as the elution solvent mixture, and the additive amount of aqueous ammonia in acetonitrile was also evaluated. The results demonstrated that the addition of aqueous ammonia in acetonitrile enabled the recoveries of the analytes to increase, in particular, the 4-CP and 2,4-DCP. When the aqueous ammonia content changed from 1% to 5% (v/v), the extraction recovery had slight increase for the phenolic compounds, with exception of 2,4,6-TMP and 2,4-DMP. However, increasing the ammonia content to 10% (v/v) led to the lower recovery. Therefore, acetonitrile containing 5% (v/v) aqueous ammonia was selected as the elution solvent.

3.2.3. *Breakthrough volume*

The breakthrough volume for phenolic compounds in the MIP cartridge was determined under the optimum conditions. This was carried out by percolating through the MIP sorbent different volumes (25, 50, 100 and 200 mL) of spiked aqueous solutions at various concentration of each analyte, with the amount of each analyte in the samples constant (5 μg).

For 4-CP and 2,4-DCP, no obvious decrease in recoveries was observed from 25 to 200 mL (Fig. 4), while for phenol, 4-MP and 2,4-DMP, the recovery decreased significantly, especially when percolating 200 mL spiked aqueous solution through the MIP cartridge. From these results, it can be concluded that 50 mL is a suitable sample volume because the recovery decreased with higher sample volumes.

Table 3
The analytical performance of the methodology for determination of phenolic compounds with HPLC.

Phenolic compounds	*a*	*b*	R^2	Linear scope (μg mL^{-1})	LOD (ng mL^{-1})
Phenol	108,941	−210.61	0.9997	0.04–10.0	4.0
4-MP	166,324	−4728.9	0.9996	0.04–10.0	2.5
4-CP	124,976	−4785.6	0.9993	0.04–10.0	3.3
2,4-DMP	188,934	−7897.3	0.9992	0.04–10.0	2.2
2,4-DCP	114,754	−6641.4	0.9989	0.04–10.0	3.7
2,4,6-TMP	117,595	−13478	0.9971	0.04–10.0	3.6

Calibration curves are expressed as regression lines ($y = ax + b$), where y is the integrated peak area and x is the concentration of phenolic compounds (μg mL^{-1}), a is the slope, b is the intercept and R^2 is the relative coefficient, LOD is limit of detection at 3:1 signal-to-noise ratio.

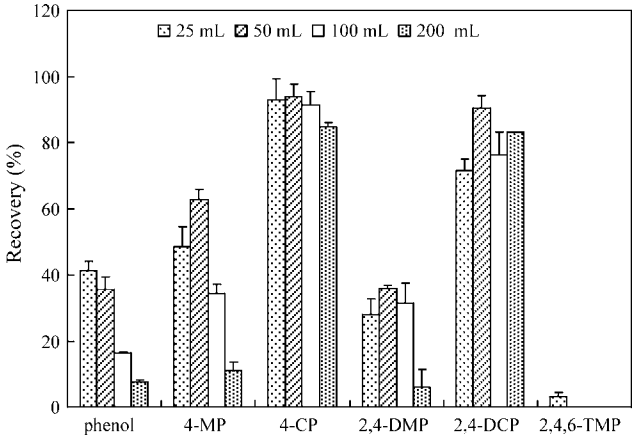

Fig. 4. Recoveries of phenolic compounds after percolating through the MIP sorbent different volumes (25, 50, 100 and 200 mL) of spiked aqueous solution with each analyte at concentration of 0.2, 0.1, 0.05 and 0.025, respectively.

The low standard deviation of the method facilitates the optimized MIP-SPE procedure to determine the phenolic compounds in the real environmental water. Though the low recoveries of phenol, 4-MP may be not a real limitation, further studies to increase the retention capacity of the analytes on the polymer, and thus the higher recoveries, are still in progress.

3.3. Determination of phenolic compounds with HPLC

Determination of phenolic compounds with HPLC was carried out as described in Section 2.5. The method performance was evaluated by the determination of the linearity and sensitivity of the method.

The linearity of the calibration curves were obtained by the determination of the peak areas from analysis of 0.04 to 10.0 μg mL^{-1} of each analyte and all the R^2-values were higher than 0.997 (Table 3).

The limits of detection (LOD), defined as the lowest analyte concentration with a signal-to-noise ratio of 3, were also investigated through the detection of spiked aqueous solution at serial concentrations. The results show that the LODs ranges from 2.2 to 4.0 ng mL^{-1}, which indicates this method could be used to detect some trace-level of phenolic compounds from the environmental water.

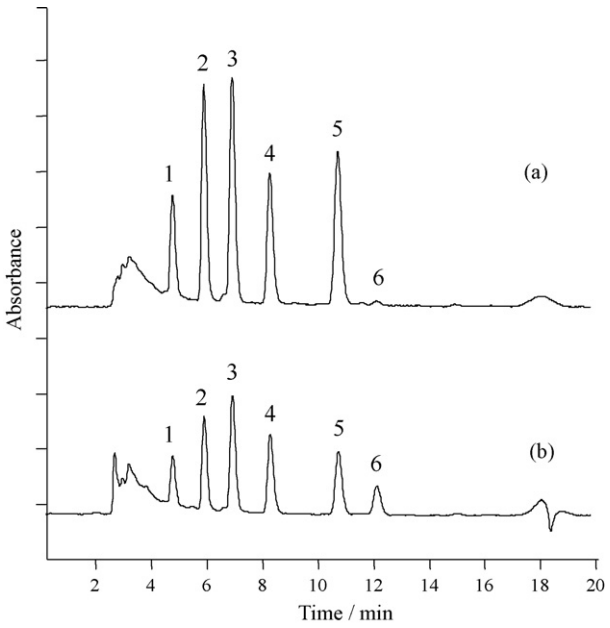

Fig. 5. Chromatograms obtained by MIP-SPE with the 2,4-DMP imprinted polymer (a) and non-imprinted polymer (b) of 50 mL Yingkou river water spiked at 0.1 μg mL^{-1} with each phenolic compound. Peak designation: (1) phenol, (2) 4-MP, (3) 4-CP, (4) 2,4-DMP, (5) 2,4-DCP and (6) 2,4,6-TMP.

3.4. Analysis of real samples

The developed procedure was applied to analyze phenolic compounds in the real samples. The river water commonly contains humic acids, and humic acids can prevent detection of polar compounds because a broad band due to the presence of these acids elutes early in chromatograms [33]. To address this matrix interference problem, the Liao river (Yingkou, Liaoning) water was chosen as real sample, and the water samples were pre-treated with the MIP and the reference polymer NIP. Fig. 5 shows the difference in performance between the MIP and NIP in the analysis of 50 mL of Yingkou river water spiked at 0.1 μg mL^{-1} with each phenolic compound. SPE was carried out under the optimum conditions for both sorbents. As can be seen in Fig. 5, both pre-treatment methods reduce the humic acid band to some extent. In this way, the humic acid does not exert obvious interference on the determina-

Table 4
Recoveries of phenolic compounds from river water samples spiked at different concentrations.

Samples concentrations	Recoveries of phenolic compounds, %[a]					
	Phenol	4-MP	4-CP	2,4-DMP	2,4-DCP	2,4,6-TMP
0.05 μg mL^{-1}	34.9	57.4	93.3	35.8	89.8	2.9
0.10 μg mL^{-1}	35.7	58.8	93.8	36.9	90.5	3.1
0.20 μg mL^{-1}	37.0	58.2	92.6	37.7	90.4	2.5

[a] RSDs (relative standard deviations) were lower than 8% in all instances ($n = 3$).

tion of phenolic compounds. However, using MIP as SPE sorbent the recoveries of the phenol, 4-MP, 4-CP, 2,4-DMP and 2,4-DCP (Fig. 5a) are nearly two times higher than using NIP (Fig. 5b). This result confirms the existence of specific binding sites, leading to the specific interactions between phenolic compounds and the MIP. Meanwhile, the recovery for 2,4,6-TMP is better using NIP than MIP-SPE. This result also illustrates the existence of the imprinted cavities in MIP, and consequently, inhabiting the interaction between the 2,4,6-TMP and MIP.

Furthermore, the river water samples were spiked with each phenolic compound at different concentrations to demonstrate reliability of the system. As shown in Table 4, recoveries of phenolic compounds were slightly varied between different concentrations. Hence, the developed MIP-SPE can be applied to real water analysis.

4. Conclusions

This research clearly demonstrates the value of the molecular imprinting strategy for fabricating class-selective sorbent for SPE to pre-concentrate the environmental pollutants, phenolic compounds. 2,4-DMP is chosen as the template to synthesize the MIP sorbent because of its smaller toxicity than other chlorophenols and structure similarity to the 2,4-DCP. The resulting 2,4-DMP imprinted polymer shows good selectivity for 2,4-DMP, 2,4-DCP, 4-CP, 4-MP and phenol, which provides basis for the following SPE. The SPE methodology was then developed and used to pre-concentrate phenolic compounds in the environmental water. The application of MIP-SPE to environmental water samples confirms the ability of the MIP sorbent to class-selectively isolate phenolic compounds from the complex matrix. This is very important in environmental analysis since in many cases substituted phenols are present as mixture of several compounds.

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