Polymer 51 (2010) 5417–5423

Contents lists available at ScienceDirect

Polymer

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

Molecularly imprinted polymers synthesized via semi-covalent imprinting with sacrificial spacer for imprinting phenols

Peipei Qi, Jincheng Wang, Liandi Wang, Yun Li, Jing Jin, Fan Su, Yuzeng Tian, Jiping Chen*

Dalian Institute of Chemical Physics, Chinese Academy of Science, Dalian 116023, China

ARTICLE INFO

Article history:
Received 26 May 2010
Received in revised form
12 August 2010
Accepted 15 September 2010
Available online 22 September 2010

Keywords:
Semi-covalent imprinting
Sacrificial spacer
Stationary phases
Molecularly imprinted polymer

ABSTRACT

Semi-covalent imprinting with carbonyl group as sacrificial spacer was employed to synthesize molecularly imprinted polymer (MIP) for phenols. A series of semi-covalently imprinted polymers were prepared by varying the templates and porogens. The MIP with 4-chlorophenyl (4-vinyl)phenyl carbonate as template was proved to be the best one, with ethylene glycol dimethacrylate (EGDMA) as cross-linker, 2,2-azobisisobutyronitrile(AIBN), and chloroform as initiator and porogen, respectively. Under such conditions, the corresponding non-covalently imprinted polymer was fabricated with 4-chlorophenol (4-CP) as template and 4-vinylpyridine (4-VP) as functional monomer. The polymer prepared by semi-covalent imprinting displayed superior selectivity to the non-covalently imprinted polymer for phenols. The peak broadening and tailing had been largely reduced on the column packed with semi-covalently imprinted polymer. Meanwhile, the constant retention for these phenols and the good linearity for phenol and 4-CP augured that the semi-covalently imprinted polymer had the potential application as stationary phase for quantitative determination of phenols.

1. Introduction

Molecularly imprinted polymer has drawn much attention for its superior selectivity, and it has become a potential material from an application's point of view. Recently, three different strategies have been employed to prepare molecularly imprinted polymer (MIP), that is, non-covalent, covalent and semi-covalent imprinting, depending on the molecular interaction between the template and functional monomer during both pre-polymerization and rebinding process. Non-covalent imprinting is by far the most widespread in the research on MIP, due to its relative simplicity of the synthesis process and wide range of chemical functionalities. However, the method is always compromised by the heterogeneity of binding site distribution to some extent. The covalent imprinting significantly lowers the non-specific interactions, but the complicated rebinding process makes it not practical for the vast majority of applications. An attractive option is referred to as semi-covalent imprinting. Semi-covalent imprinting approach differs from covalent imprinting in that the rebinding step is non-covalent in nature. Synthetic strategies for the generation of MIP has been extensively reviewed by A.G. Mayes [1].

Initially, (meth)acrylate ester of template is synthesized to form a semi-covalent MIP. After removal of the template by hydrolysis, the polymer can bind the unesterified template using non-covalent interaction. However, template hydrolysis is often not straightforward and the steric requirements of an acid and an alcohol in hydrogen-bonding contact are rather different from the corresponding ester, which largely hamper the imprinting. Then some of the limitations of the semi-covalent imprinting approach can be overcome by the use of a linker group between the template and the functional molecule, which is lost on template removal [1]. This linker group has dual role of attaching the template to the functional monomer during polymer formation and acting as spacer between the template and polymer-bound functionality to prevent steric crowding in the non-covalent rebinding step. The carbonyl group of a carbonate ester was the first sacrificial spacer group to be used in the imprinting of cholesterol [2]. Several groups have also adopted semi-covalent imprinting with sacrificial spacer to prepare MIP. Those MIPs have employed carbonyl spacer in template monomers linked through urea [3], carbonate [2,4–12] and carbamate [13–15] linkage. The dimethyl silyl group of silyl ether and silyl esters has also been introduced to the MIP preparation as a spacer for binding heterocycles [16]. Owing to the complicated synthesis process prior to polymerization, this method has been confined to imprint few compounds, such as cholesterol [2,4,6,8,9], estrone [15], propofol [5,11], menthol [10,12], DDT [13], 2,3,7,8-tetrachlorodibenzodioxin (TCDD) [3] and so on.

* Corresponding author. Tel./fax: +86 411 84379562.
E-mail address: chenjp@dicp.ac.cn (J. Chen).

0032-3861/$ – see front matter Crown Copyright © 2010 Published by Elsevier Ltd. All rights reserved.
doi:10.1016/j.polymer.2010.09.037

Phenolic compounds, especially the priority pollutants controlled by EPA, have aroused researchers' serious concern for their considerable detriment to humans and environment. Up to date, molecular imprinting technique has already been utilized for the pre-treatment and determination of these phenols, such as 2,4-dichlorophenol (2,4-DCP) [17,18], 2,4-dimethylphenol (2,4-DMP) [19], 2,4,6-trichlorophenol (2,4,6-TCP) [20–23], pentachlorophenol [24–26], 2,4-dinitrophenol [27,28], 4-nitrophenol [29–33] and phenol [34–36]. Among these works, most of the MIPs were fabricated by non-covalent imprinting, except that 4-nitrophenol-MIP was synthesized by semi-covalent imprinting using methacrylate of the target compounds [30]. To the best of our knowledge, semi-covalent imprinting with carbonyl group as sacrificial spacer has not been employed to produce MIP for phenols. Therefore, MIP preparation via semi-covalent imprinting may be a valuable attempt to acquire a more selective and sensitive methodology for determination of phenolic compounds.

In the present work, 4-chlorophenyl (4-vinyl)phenyl carbonate (4-CPC) and 4-methylphenyl (4-vinyl)phenyl carbonate (4-MPC) were synthesized as the template for bulk polymerization to imprint phenols. The bulk polymer presented superior recognition selectivity with 4-CPC as the template, ethylene glycol dimethacrylate (EGDMA) and 2,2-azobisisobutyronitrile(AIBN) as cross-linker agent and initiator, respectively. Meanwhile, the semi-covalently imprinted polymer showed potential application as stationary phase in the determination of phenols.

2. Experimental

2.1. Chemicals

2,4-DMP, 2,4-DCP, 2,4,6-TCP, 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 the standard procedure 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. *p*-Acetoxystyrene was purchased form TCI chemical (Tokyo, Japan). 4-Chlorophenyl chloroformate and 4-methylphenyl chloroformate were from Chemlin Chemical Industrial Co. (Nanjing, China). Dichloromethane and isopropanol were of HPLC grade. Phenol, 4-chlorophenol (4-CP), 4-methylphenol (4-MP), acetic acid, methanol, chloroform, toluene and other regents were of analytical grade. Water used was purified using a Milli-Q gradient A10 system (Millipore, Milford, MA, USA).

2.2. Polymer preparation

2.2.1. p-Vinylphenol

This compound was prepared by the hydrolysis of *p*-acetoxystyrene with aqueous potassium hydroxide according to the method of Corson et al. [37] and obtained as shiny colorless plates. The product was washed thoroughly with water, dried under vacuum and stored at −20 °C until used.

2.2.2. 4-Chlorophenyl (4-vinyl)phenyl carbonate (4-CPC)

To a cooled solution (ice bath) of 4-vinylphenol (2.0 g, 16.6 mmol) in dry THF (60 mL) and triethylamine (4 mL) containing a trace of 2, 6-di-tert-butyl-4- methylphenol (3 mg) was added dropwise a solution of 4-chlorophenyl chloroformate (3.17 g, 16.6 mmol) in THF (40 mL), and the mixture was stirred overnight at room temperature. The obtained solution was filtered and the filtrate was evaporated to yield the crude product. Recrystallization from methanol gave the product as colorless plates, mp 111–114 °C. IR (KBr) 3099 (CH), 1764 (C=O), 1602 (Ar), 1630 (C=C), 1507 (Ar), 1266 (C–O) cm^{-1}; 400 MHz ^1H NMR ($CDCl_3$)δ (ppm): 7.48(d, 2H, aryl), 7.42(d, 2H, aryl), 7.28(m, 4H, aryl), 6.70 (dd, 1H, CH=CH), 5.78 (d, $-CH=CH_2$, cis), 5.32 (d, $-CH=CH_2$, trans); 100 MHz ^{13}C NMR ($CDCl_3$)δ (ppm): 151.88(carbonyl), 150.45 (C1), 149.55 (C1′), 136.11 (C4′), 135.77 ($-CH$, $CH=CH_2$), 131.89 (C4), 129.78 (C3, 5), 127.47 (C3′, 5′), 122.42 (C2, 6), 121.02 (C2′, 6′), 114.71 ($-CH_2$, $CH=CH_2$).

2.2.3. 4-Methylphenyl (4-vinyl)phenyl carbonate (4-MPC)

The 4-MPC was prepared by the same method as the 4-CPC, from 4-vinylphenol (2.0 g, 16.6 mmol) and 4-methylphenyl chloroformate (2.83 g, 16.6 mmol). The compound was obtained as white crystal, following recrystallization from aqueous methanol, mp 84–85 °C. IR (KBr) 3044 (CH), 1773 (C=O), 1596 (Ar), 1630 (C=C), 1506 (Ar), 1257 (C–O) cm^{-1}; 400 MHz ^1H NMR ($CDCl_3$)δ (ppm): 7.47 (d, 2H, aryl), 7.44(d, 2H, aryl), 7.26(m, 4H, aryl), 6.75 (dd, 1H, CH=CH), 5.76 (d, $-CH=CH_2$, cis), 5.29 (d, $-CH=CH_2$, trans); 100 MHz ^{13}C NMR ($CDCl_3$)δ (ppm): 152.29 (carbonyl), 150.62 (C1), 148.95 (C1′), 136.11 (C4, 4′), 135.77 ($-CH$, $CH=CH_2$), 129.78 (C3, 5), 127.47 (C3′, 5′), 122.42 (C2, 6), 121.02 (C2′, 6′), 114.71 ($-CH_2$, $CH=CH_2$), 20.98 ($-CH_3$).

2.2.4. Polymer synthesis

For the non-covalently imprinted polymer, the template (4-CP, 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), cross-linking monomer (EGDMA) (3.8 mL, 20 mmol) and initiator (AIBN) (0.04 g) were then added to the above solution. For the semi-covalent imprinting, the template (4-CPC, 4-MPC or their mixture, 1 mmol) was dissolved in porogen (5.6 mL) in a 10-mL thick walled glass tube. The initiator (AIBN) (0.04 g) and the cross-linking monomer (EGDMA) (3.8 mL, 20 mmol) were then added to the above solution. Both the non-covalent and semi-covalent pre-polymerization solution were sonicated and purged with oxygen-free nitrogen for 10 min on an ice bath. The glass tubes were sealed under nitrogen and placed in a water bath at 60 °C. The reaction was allowed to proceed for 24 h. As a reference, non-imprinted polymers that did not contain any template were prepared simultaneously using the same protocol. The obtained hard polymers were crushed, ground, and wet-sieved using acetone to obtain regularl sized particles between 45 and 63 μm suitable for the chromatographic evaluations.

2.2.5. Template removal

For the semi-covalent polymers, the removal of template requires hydrolysis of polymers, while the template of the non-covalent polymers can be removed by extraction. The semi-covalent polymers were hydrolyzed using the method described previously by Whitcombe et al. [2]. The polymers were suspended in 1 mol L^{-1} sodium hydroxide in methanol and heated to reflux for 6 h. The cooled suspensions were added to an excess of dilute hydrochloric acid, and the products were filtered and washed with water and methanol. Then both the non-covalent and pre-hydrolyzed semi-covalent polymers were extracted in a Soxhlet extraction apparatus with methanol/acetic acid solution (9:1, v/v) followed by methanol. The polymers were dried in vacuum at 50 °C overnight.

2.3. Characterization of the prepared polymers

2.3.1. Nitrogen sorption porosimetry measurements

Nitrogen sorption porosimetry measurements were performed on a Nova Surface Area and Porosimetry Analyzer (Quantachrome Instrument Corporation, USA). The specific surface area was calculated using the standard BET method, with the specific pore volume and average pore diameter using BJH theory.

2.3.2. FTIR analysis

The spectra of the polymers were measured using a Spectrum GX spectrometer (PerkinElmer, USA) in the 4000–400 cm^{-1} region with a resolution of 4 cm^{-1}. The spectrum of each solution was obtained by averaging 8 consecutive scans.

2.3.3. X-ray fluorescence (XRF) spectroscopy

Residual chlorine content of the prepared semi-covalently imprinted polymer was determined by XRF spectroscopy on a Philips Magix XRF spectrometer. Samples were pressed as homogenous tablets for XRF analysis.

2.3.4. Solid-state ^{13}C NMR measurement

^{13}C CP/MAS NMR spectra were recorded on Varian Infinityplus-400 spectrometer using a 5 mm probe at a spinning speed of 6 kHz. 2000–3000 scans were accumulated with a $\pi/2$ pulse width of 4.2 μs, recycle delay of 2 s, and contact time of 7 ms.

2.4. 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 10 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. A Waters 515 ternary HPLC pump and a Waters 2487 dual λ absorbance detector were used.

2.4.1. Reversed-phase liquid chromatographic evaluation

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.

2.4.2. Normal liquid chromatographic evaluation

The HPLC columns packed with MIP and NIP particles were firstly conditioned with isopropanol and dichloromethane, and then evaluation were performed using dichloromethane as mobile phase at the flow rate of 1.0 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 and imprinting factor were calculated by the same equations as demonstrated in Section 2.4.1.

3. Results and discussion

3.1. Preparation and evaluation of semi-covalently imprinted polymers

A series of MIPs were synthesized via semi-covalent imprinting with carbonyl group as sacrificial spacer. Fig. 1 showed the synthesis process of the templates and the formation of those semi-covalent MIPs, and the compositions of the polymerization mixtures were described in Table 1. The corresponding NIPs were prepared by the same protocol without addition of the templates.

Semi-covalent MIPs 2–7 were hydrolyzed using the standard conditions of 6 h reflux with 1 mol L^{-1} NaOH in methanol [2]. The template removal was confirmed by FTIR spectroscopy. The aromatic carbonate carbonyl band of MIP 2 was clearly resolved as

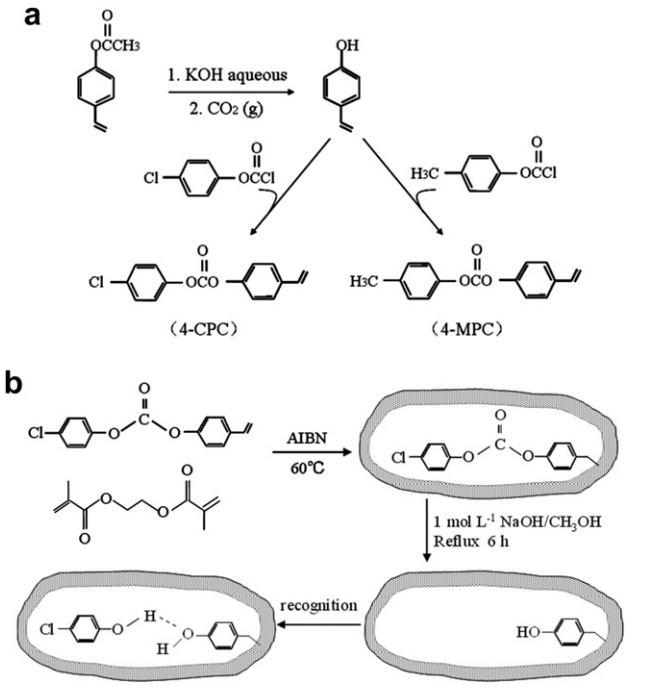

Fig. 1. Schematic representation of the sacrificial semi-covalent approach used in this research. (a) Template synthesis. (b) Polymer preparation of 4-chlorophenol- imprinted polymers by semi-covalent imprinting.

a shoulder in the IR spectrum at 1779 cm^{-1}, and its double-frequency absorption at 3445 cm^{-1}, which all disappeared on hydrolysis (Fig. 2, bottom curve). The broad and strong band appeared at 3527 cm^{-1} (Fig. 2, top curve), indicative of the formation of aromatic phenolic hydroxyl group on account of the polymer hydrolysis. Other semi-covalently imprinted polymers were also characterized by FTIR, with the same spectra obtained.

Residual chlorine content was determined to clarify the hydrolysis of the polymers. In the synthesized semi-covalently MIP 2, chlorine is introduced from the reactants, 4-CPC and chloroform. The porogen chloroform and unreacted template 4-CPC can be released by extraction with methanol, while the removal of the chlorine in the integrated 4-CPC required the hydrolysis of the polymer. Thus, the chlorine contents in the polymers with different treatments were measured. The chlorine content was 10.206 wt% in the synthesized polymer without any treatment, and then it decreased to 8.740 wt% after extraction with methanol, later to 2.575 wt% upon hydrolysis (Fig. S2). The decrease of the chlorine content fully proved that the hydrolysis of the semi-covalently imprinted polymer can remove the template, forming the functional site for imprinting phenols.

Solid-state ^{13}C NMR experiments were also carried out to demonstrate the change of the semi-covalent MIP through hydrolysis. The results displayed that the carbonate carbonyl group in 4-CPC at 151.0 ppm disappeared in the NMR spectrum of the polymer after hydrolysis, providing abundant evidence for the hydrolysis of the semi-covalent MIP. The detailed information was offered in Fig. S3.

Various templates and porogens were employed to explore their influence on selectivity of the obtained polymers and determine the optimum combination of the MIP for imprinting phenols. Three templates, 4-CPC, 4-MPC and 4-CPC/4-MPC (1:1, mol/mol) were compared. As for porogen, hexane and the mixture of hexane and toluene were used in the majority of the imprinted polymers described in literature by semi-covalent imprinting. Hexane and

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

Polymer	Template	Functional monomer 4-VP	Cross-linker EGDMA	Initiator AIBN	Porogen
MIP 1	4-CP	0.425 mL	20 mmol	0.04 g	Chloroform
MIP 2	4-CPC	–	20 mmol	0.04 g	Chloroform
MIP 3	4-CPC	–	20 mmol	0.04 g	Acetonitrile
MIP 4	4-MPC	–	20 mmol	0.04 g	Chloroform
MIP 5	4-MPC	–	20 mmol	0.04 g	Acetonitrile
MIP 6	4-MPC/4-CPC	–	20 mmol	0.04 g	Chloroform
MIP 7	4-MPC/4-CPC	–	20 mmol	0.04 g	Acetonitrile

hexane/toluene (9:1, v/v) were also employed in our initial study. Unsatisfactorily, the polymers were not rigid for further application. Thus, chloroform and acetonitrile were extensively investigated. Superficially, there was no obvious difference among these MIPs using different templates and porogens. All were white powders after grinding, except that the polymers prepared with chloroform as porogen were more rigid than those prepared with acetonitrile as porogen. The recognition selectivity of the polymers for phenols were characterized by IF values. The results were demonstrated in Fig. 3.

Using chloroform as porogen in the bulk polymerization, the polymers obtained from the different templates showed the same trends for the phenols with regard to IF value (Fig. 3). The IF values of these phenols decreased with increasing of their structure size. This result verifies the imprinting effect and the significant role of the imprinting cavities. As illustrated in Fig. 1b, the molecular recognition is based on the hydrogen bonding interaction between the phenolic hydroxyl group of the target analyte and the hydroxyl residue of the polymers. Prior to interaction, the test analyte is required to enter into the vacant imprinted cavities. That is, 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, resulting in the smaller IF values for 2,4,6-TCP and 2,4,6-TMP than other phenols. Large molecular size prevents their entrance into the imprinted cavities, and thus disrupts the specific interaction between the phenolic hydroxyl group of the target analyte and the hydroxyl residue of the polymers. However, 2,4-DCP and 2,4-DMP can give rise to slightly larger IF value than 2,4,6-TCP and 2,4,6-TMP, which may be attributed to the fact that the loss of carbonyl group during hydrolysis of the carbonate ester provides sufficient space to allow the analyte with slightly larger structure and vinylphenol-derived hydroxyl groups to form a hydrogen bond.

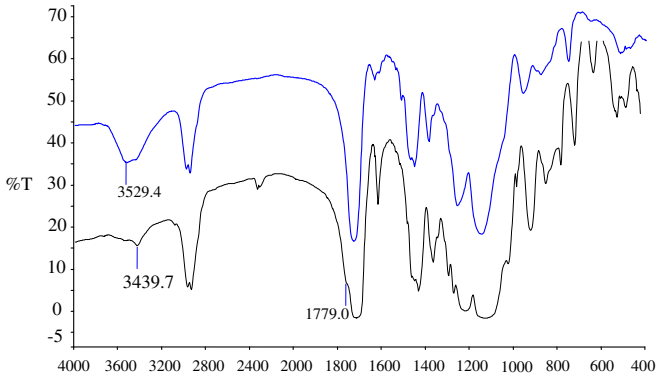

Fig. 2. IR spectra of polymers imprinted, before (bottom) and after (top) removal of template by hydrolysis.

With regard to the templates, the test phenols arouse larger IF values in the polymer column with 4-CPC as template than the other polymers. 4-CPC/4-MPC (1:1, mol/mol) was also served as the template in order to exploit the dual functional groups of chloro- and methyl- in 4-CPC and 4-MPC, in an effort to improve the selectivity of the polymers for both the methyl- and chloro-substituted phenols [38]. Unsatisfactorily, all the phenols yielded lower IF values in this polymer than the other two polymers.

Using acetonitrile as porogen, the effect of various templates on the selectivity of the MIPs was also investigated. Unlike the polymers prepared with chloroform as porogen, the IF values for these phenols did not show identical trends among these three polymers. On the contrary, the polymer with the mixture of 4-CPC and 4-MPC as template shows better selectivity for these phenols. Nevertheless, this polymer did not display better selectivity than MIP 2 with 4-CPC and chloroform as template and porogen, respectively. Hence, MIP 2 was the choice of the optimum polymers for further investigation.

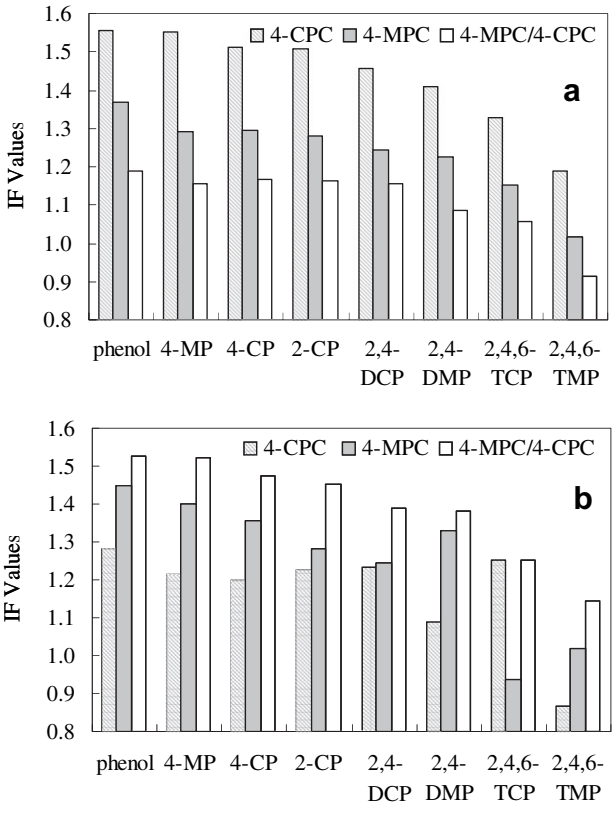

Fig. 3. IF values for the phenolic compounds in the columns packed with semi-covalently imprinted polymers under the chromatographic conditions described in Section 2.4.1.

3.2. Comparison of imprinting approaches

Based on the optimized synthesis condition for semi-covalently imprinted polymer, the corresponding non-covalently imprinted polymer was prepared with 4-CP as template, 4-VP, EGDMA, AIBN and chloroform as functional monomer, cross-linker, initiator and porogen, respectively. The differences of the polymers were investigated regarding their physical characteristics, recognition selectivity and chromatographic behaviors.

3.2.1. Physical characteristics

Nitrogen sorption porosimetry measurements were performed to evaluate the morphology of the imprinted polymers, and the possible difference resulting from the different imprinting methods. As illustrated in Fig. 4a, both the semi-covalent MIP 2 and non-covalent MIP 1 produced type IV isotherm, characterized by its hysteresis loop, which is associated with capillary condensation taking place in mesopores [39]. This phenomenon is similar to the finding by Holland et al. [40]. In the successfully fabricated MIPs, mesopores predominates and the hysteresis loop is not closed, implying incomplete removal of the gas adsorbate from narrow pores [39]. However, MIP 2 had a larger surface area than MIP 1 (Table 2). Higher surface areas indicated that phase separation occurred at later stages of the polymerization and the formed polymers were accompanied with smaller pore size distributions [41]. Fig. 4b also demonstrated that MIP 2 had a narrower pore size distribution than MIP 1.

Table 2
Physical properties of the semi-covalently imprinted polymer (MIP 2), the non-covalently imprinted polymer (MIP 1) characterized by nitrogen sorption porosimetry.

Polymers	Surface area ($m^2\ g^{-1}$)	Pore volume ($cm^3\ g^{-1}$)	Pore size (nm)
MIP 1	165.2	0.223	5.40
MIP 2	286.4	0.269	4.55

3.2.2. Difference in molecular imprinting selectivity

Evaluation of the two polymers was performed by the reversed-phase HPLC and normal HPLC, so as to compare the selectivity of the polymers for the phenols. In the reversed-phase HPLC system, acetonitrile was used as the mobile phase at 1 mL min^{-1}. Phenol, 4-CP, 2-CP, 4-MP, 2,4-DMP, 2,4-DCP, 2,4,6-TMP and 2,4,6-TCP were injected into the MIP columns and the corresponding NIP columns under the previously described chromatographic conditions. Imprinting factor was calculated to verify their difference in selectivity and imprinting effect. As depicted in Fig. 5a, it can be clearly seen that the IF values for these phenols are higher in MIP 2 column than MIP 1 column, that is, the semi-covalent imprinting method is more selective. Furthermore, the IF values for these phenols present different trends in the two MIP columns. For the non-covalently imprinted MIP 1, 4-CP gave rise to the largest IF value, followed by 2,4-DCP, 4-MP, phenol, 2,4-DMP, 2-CP, 2,4,6-TCP and 2,4,6-TMP successively. This result explains that the selectivity of the non-covalently imprinted polymer column for the test compounds is not only affected by the molecular structure, but also the acidity of the analytes [19,24]. With regard to semi-covalent imprinting, the molecular structure is more crucial for the recognition

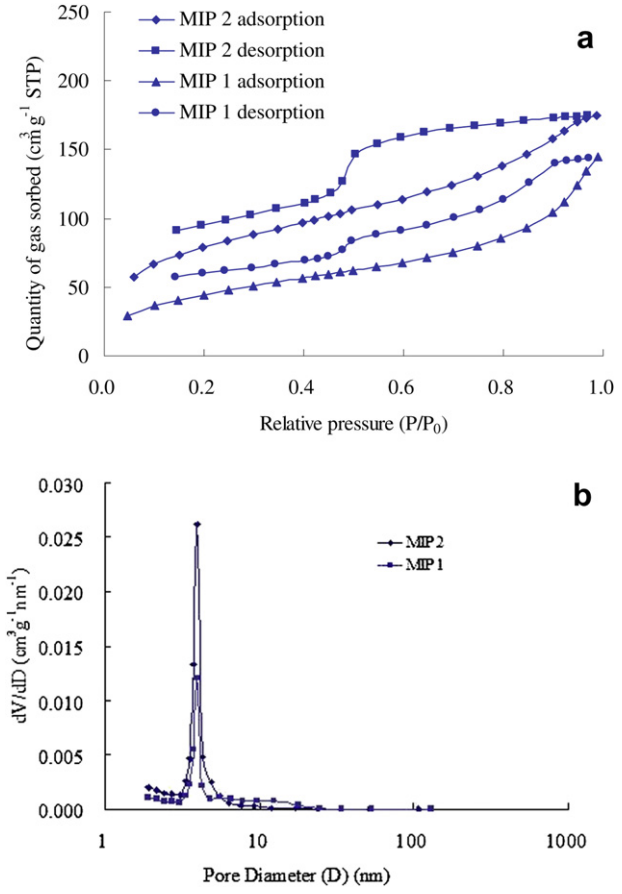

Fig. 4. Sorption isotherms (a) and pore volume distributions (b) for the imprinted polymers prepared by semi-covalent imprinting (MIP 2) and non-covalent imprinting (MIP 1).

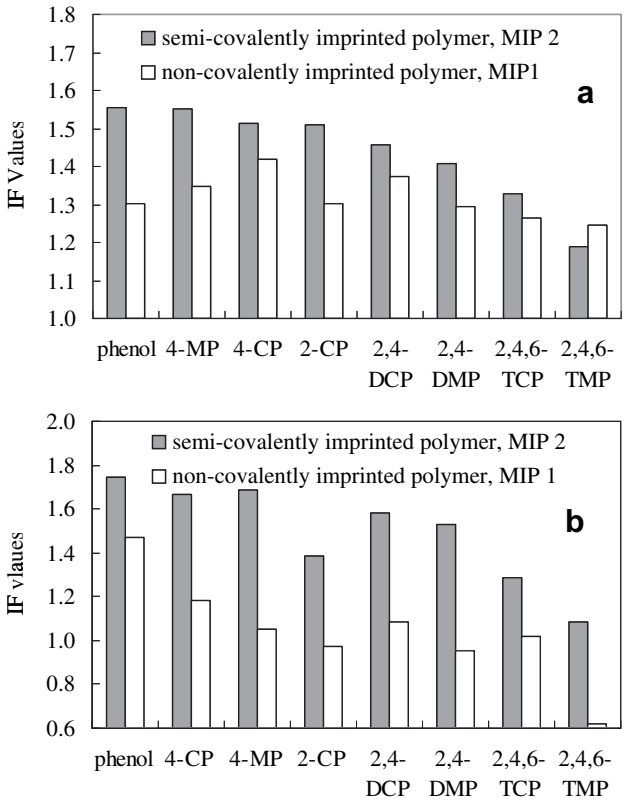

Fig. 5. Comparison of the recognition selectivity of the polymers prepared by semi-covalent imprinting and non-covalent imprinting approaches in the reversed-phase HPLC conditions (a) and normal HPLC system (b).

ability of the test analytes in the polymer columns, which has been extensively illustrated in 3.1.

In the normal HPLC, dichloromethane was used as the mobile phase. As described in Fig. 5b, all the phenols brought to higher IF values in the MIP column prepared by semi-covalent imprinting than that prepared by non-covalent imprinting method.

In conclusion, the semi-covalently imprinted polymer showed better selectivity than the non-covalently imprinted polymer evaluated by both the reversed-phase and normal HPLC.

3.2.3. Chromatographic behaviors in normal HPLC

Spiked standard solution involving phenol, 4-CP, 2,4-DCP and 2,4,6-TCP were injected into the polymer columns, and the elution profiles were shown in Fig. 6. It is undeniable that the imprinting effect exists in the polymers prepared by both non-covalent imprinting and semi-covalent imprinting approaches. The phenols have shorter retention times in the NIP columns than in the corresponding MIP columns, stemming from the presence of the imprinted cavities in the MIPs.

As far as the chromatographic separation is concerned, the two MIP columns are distinguished from each other. The four phenols had longer retention times in the column packed with non-covalently imprinted polymer, but they had a much sharper peak when they were chromatographed on the semi-covalently imprinted polymer. This result is in accordance with the result obtained by Hwang et al. [6]. This fact can be attributed to the presence of binding sites of different affinity in the non-covalently imprinted polymer and the uniform distribution of the binding sites in the semi-covalently imprinted polymers. In the semi-covalent imprinting, the template molecule is covalently bound to the monomer and after polymerization the template occupies exactly the position of the recognition site. Each recognition site formed after removal of the template was shown to bind one molecule of phenol through hydrogen bonding. In contrast, the complexes in the non-covalent imprinting were formed from template and functional monomer in a somewhat loose manner. Since the complexes were formed simply by just mixing these two together and stabilized only by weak interaction like hydrogen bonding during the polymerization, the construct of future recognition sites was looser in comparison with semi-covalent imprinting and contained aggregates of two or more of the template molecules. This inevitably leads to the heterogeneous distribution of recognition sites in the imprinted polymer, and finally peak broadening and tailing when the polymer was used as the stationary phase in liquid chromatography.

In addition, the phenols at various concentrations were injected into the two MIP columns, and the chromatograms were plotted. The phenols at 0.1 μg mL^{-1} can be clearly observed in the column packed with semi-covalently imprinted polymer, whilst these phenols at 1.0 μg mL^{-1} cannot be observed in the column packed with non-covalently imprinted polymer. This result can mainly be ascribed to the band broadening and peak tailing in the non-covalently imprinted polymers, and consequently the poor separation efficiency and low resolution. Although the four phenols still can not achieve complete baseline separation from each other in the MIP column packed with semi-covalently imprinted polymer, this result is still inspiring. To the best of our knowledge, the semi-covalently imprinted polymer has not been used as stationary phase to separate the priority phenolic compounds.

Then the emphasis was paid on the semi-covalently imprinted polymer MIP 2 column. The four phenols at 10 μg mL^{-1} were injected into the column alone, the retention times were 2.53, 3.18, 5.66, 7.84 min for 2,4,6-TCP, 2,4-DCP, phenol and 4-CP, respectively. The spiked solution of these phenols at the same concentration was analyzed under the identical chromatographic conditions, and their elution profiles were shown in Fig. 7. It can be clearly observed that every compound has the same retention time both when eluted in mixture and alone.

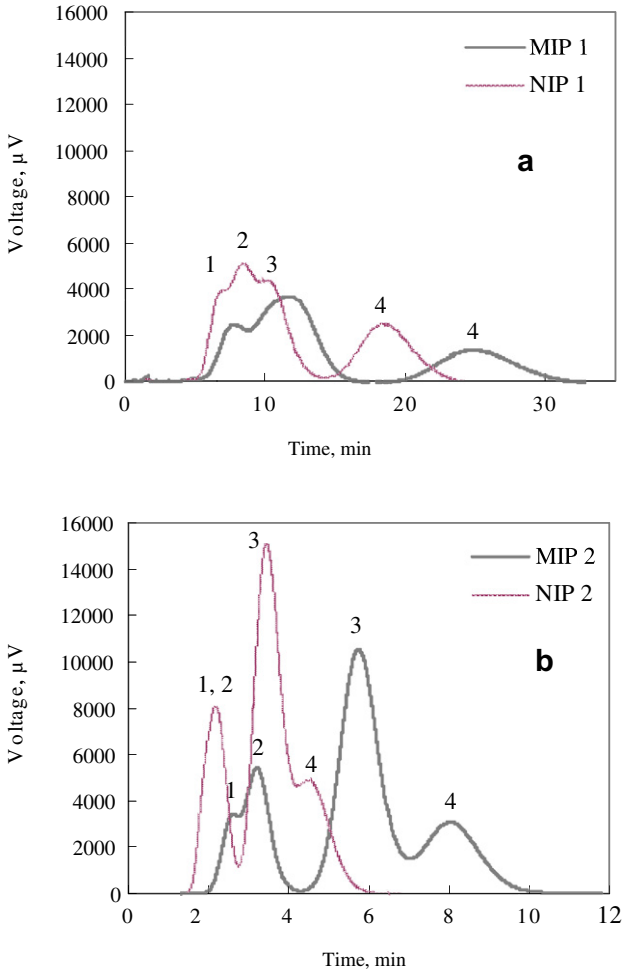

Fig. 6. Elution profiles of phenolic compounds on the HPLC columns packed with the non-covalently imprinted polymer (a) and the semi-covalently imprinted polymer (b). Peak designation: (1) 2,4,6-TCP, (2) 2,4-DCP, (3) phenol, (4) 4-CP.

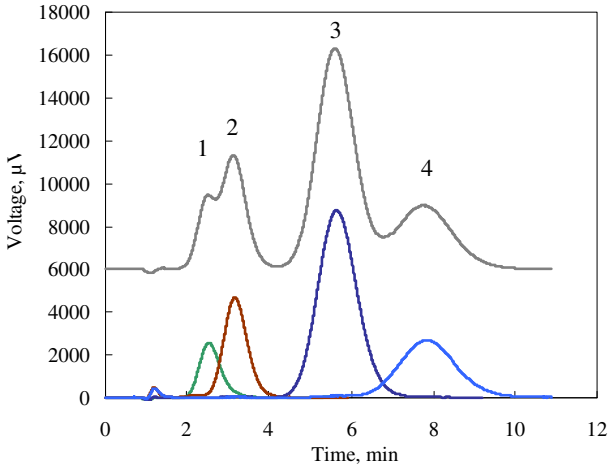

Fig. 7. Chromatograms of phenols on the semi-covalently imprinted polymer (MIP 2) column. Samples were analyzed by alone (bottom) or mixture (top). Peak designation: (1) 2,4,6-TCP, (2) 2,4-DCP, (3) phenol, (4) 4-CP.

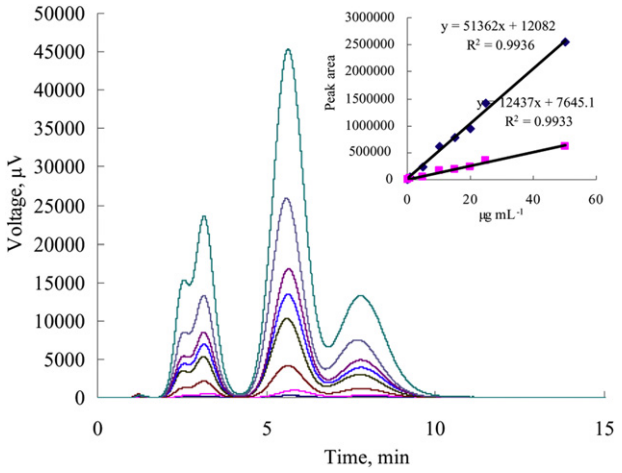

Fig. 8. Elution profiles on MIP 2 column of phenols with different concentrations, and the corresponding calibration curves of phenol and 4-CP. Peak designation: (1) 2,4,6-TCP, (2) 2,4-DCP, (3) phenol, (4) 4-CP.

The phenols mixture with concentrations from 0.1 to 50 μg mL^{-1} was tested in MIP 2 column. The chromatograms were depicted in Fig. 8. As for phenol and 4-CP, the calibration curve based on the peak area versus sample concentration was constructed, and the regression coefficients better than 0.993 were obtained. This result along with the constant retention implies its potential application as stationary phase for quantitative analysis, although baseline separation still needs to be resolved for these two compounds.

It should be noted that with the semi-covalent methods, the residual template remains covalently bound to the polymer, and is not subject to leaching under normal conditions of use. In fact, template leaching was not observed during the analysis. This is very important to attain the prefect application of molecular imprinting technique.

4. Conclusions

This research clearly demonstrates the value of the semi-covalent imprinting approach for fabricating imprinted polymer to determine the priority environmental pollutants, phenolic compounds involving phenol, 4-CP, 2,4-DCP, 2,4,6-TCP. As stationary phase, the semi-covalently imprinted polymer exhibits superior selectivity to non-covalently imprinted polymer from an application's point of view. In this semi-covalent imprinting approach, the carbonyl group of 4-vinylphenyl carbonate ester acts as a sacrificial spacer. The covalently-bound template monomer can be easily hydrolyzed with the loss of CO_2, which results in the formation of a non-covalent recognition site, bearing a phenolic residue capable of recognizing phenols with homogenous binding sites. Therefore, the use of semi-covalent imprinting significantly reduced the peak tailing and band broadening. This advantage along with the constant retention augurs that the semi-covalently imprinted polymer has the potential application as stationary phase for quantitative determination of phenols. Besides with the semi-covalent methods, the residual template remains covalently bound to the polymer, and is not subject to leaching under normal conditions of use. This is very important to attain the applications of molecular imprinting technique.

Acknowledgements

Financial supports from the National Basic Research Program of China (973 program) (2009CB421602), the National Natural Science Foundation of China (20775081) and National High Technology Research and Development Program of China (863 Program) (2007AA061601) are gratefully acknowledged.

Appendix. Supplementary material

Supplementary data related to this article can be found online at doi:10.1016/j.polymer.2010.09.037.

References

[1] Mayes AG, Whitcombe MJ. Adv Drug Deliv Rev 2005;57:1742–78.
[2] Whitcombe MJ, Rodriguez ME, Villar P, Vulfson EN. J Am Chem Soc 1995;117:7105–11.
[3] Lubke M, Whitcombe MJ, Vulfson EN. J Am Chem Soc 1998;120:13342–8.
[4] Boonpangrak S, Prachayasittikul V, Bulow L, Ye LJ. J Appl Polym Sci Symp 2006;99:1390–8.
[5] Petcu M, Cooney J, Cook C, Lauren D, Schaare P, Holland P. Anal Chim Acta 2001;435:49–55.
[6] Hwang CC, Lee WC. J Chromatogr A 2002;962:69–78.
[7] Joshi VP, Kulkarni MG, Mashelkar RA. J Chromatogr A 1999;849:319–30.
[8] Flores A, Cunliff D, Whitcombe MJ, Vulfson EN. J Appl Polym Sci Symp 2000;77:1841–50.
[9] Perez N, Whitcombe MJ, Vulfson EN. J Appl Polym Sci Symp 2000;77:1851–9.
[10] Patel A, Fouace S, Steinke JHG. Anal Chim Acta 2004;504:53–62.
[11] Petcu M, Schaare PN, Cook CJ. Anal Chim Acta 2004;504:73–9.
[12] Patel A, Fouace S, Steinke JHG. Chem Commun; 2003:88–9.
[13] Graham AL, Carlson CA, Edmiston PL. Anal Chem 2002;74:458–67.
[14] Katz A, Davis ME. Nature 2000;403:286–9.
[15] Ki CD, Oh C, Oh S-G, Chang JY. J Am Chem Soc 2002;124:14838–9.
[16] Kirsch N, Alexander C, Lubke M, Whitcombe MJ, Vulfson EN. Polymer 2000;41:5583–90.
[17] Feng QZ, Zhao LX, Yan W, Ji F, Wei YL, Lin JM. Anal Bioanal Chem 2008;391:1073–9.
[18] Li Y, Li X, Li YQ, Qi JY, Bian J, Yuan YX. Environ Pollut 2009;157:1879–85.
[19] Qi P, Wang J, Jin J, Su F, Chen J. Talanta 2010;81:1630–5.
[20] Feng QZ, Zhao LX, Lin JM. Anal Chim Acta 2009;650:70–6.
[21] Feng QZ, Zhao LX, Yan W, Lin JM, Zheng ZX. J Hazard Mater 2009;167:282–8.
[22] Feng QZ, Zhao LX, Chu BL, Yan W, Lin JM. Anal Bioanal Chem 2008;392:1419–29.
[23] Schwarz L, Holdsworth CI, McCluskey A, Bowyer MC. Aust J Chem 2004;57:759–64.
[24] Han DM, Fang GZ, Yan XP. J Chromatogr A 2005;1100:131–6.
[25] Nicholls C, Karim K, Piletsky S, Saini S, Setford S. Biosens Bioelectron 2006;21:1171–7.
[26] Baggiani C, Anfossi L, Giovannoli C, Tozzi C. J Chromatogr B Analyt Technol Biomed Life Sci 2004;804:31–41.
[27] Luo W, Zhu LH, Yu C, Tang HQ, Yu HX, Li X, et al. Anal Chim Acta 2008;618:147–56.
[28] Zakaria ND, Yusof NA, Haron J, Abdullah AH. Int J Mol Sci 2009;10:354–65.
[29] Masque N, Marce RM, Borrull F, Cormack PAG, Sherrington DC. Anal Chem 2000;72:4122–6.
[30] Caro E, Masque M, Marce RM, Borrull F, Cormack PAG, Sherrington DC. J Chromatogr A 2002;963:169–78.
[31] Caro E, Marce RM, Cormack PAG, Sherrington DC, Borrull F. J Chromatogr A 2003;995:233–8.
[32] Say R, Ersoz A, Sener I, Atilir A, Diltemiz S, Denizli A. Sep Sci Technol 2004;39:3471–84.
[33] Ersoz A, Denizli A, Sener I, Atilir A, Diltemiz S, Say R. Separ Purif Tech 2004;38:173–9.
[34] Joshi VP, Karode SK, Kulkarni MG, Mashelkar RA. Chem Eng Sci 1998;53:2271–84.
[35] Lv YQQ, Lin ZX, Feng W, Tan TW. Chromatographia 2007;66:339–47.
[36] An FQ, Gao BJ, Feng XQ. J Hazard Mater 2008;157:286–92.
[37] Corson BB, Heintzelman WJ, Schwartzman LH, Tiefenthal HE, Lokken RJ, Nickels JE, et al. J Org Chem 1958;23:544–9.
[38] Krupadam RJ, Bhagat B, Wate SR, Bodhe GL, Sellergren B, Anjaneyulu Y. Environ Sci Technol 2009;43:2871–7.
[39] Sing KSW. Pure Appl Chem 1982;54:2201–18.
[40] Holland N, Frisby J, Owens E, Hughes H, Duggan P, McLoughlin P. Polymer 2010;51:1578–84.
[41] Sherrington DC. Chem Commun; 1998:2275–86.