Available online at www.sciencedirect.com
SCIENCE DIRECT
Journal of Chromatography A, 1100 (2005) 131–136
JOURNAL OF CHROMATOGRAPHY A
www.elsevier.com/locate/chroma

Preparation and evaluation of a molecularly imprinted sol–gel material for on-line solid-phase extraction coupled with high performance liquid chromatography for the determination of trace pentachlorophenol in water samples

De-Man Han [a,b], Guo-Zhen Fang [a,c], Xiu-Ping Yan [a,*]

[a] *Key Laboratory of Functional Polymer Materials, The Ministry of Education of China, Research Centre for Analytical Sciences, Department of Chemistry, Nankai University, 94 Weijin Road, Tianjin 300071, China*
[b] *Department of Chemistry, Taizhou University, Linhai 317000, China*
[c] *Tianjin Key Laboratory of Food Nutrition and Safety, Faculty of Food Engineering and Biotechnolgy, Tianjin University of Science and Technology, Tianjin 300222, China*

Received 13 July 2005; received in revised form 30 August 2005; accepted 14 September 2005
Available online 26 September 2005

Abstract

A highly selective imprinted amino-functionalized silica gel sorbent was prepared by combining a surface molecular imprinting technique with a sol–gel process for on-line solid-phase extraction–HPLC determination of trace pentachlorophenol (PCP) in water samples. The PCP-imprinted amino-functionalized silica sorbent was characterized by FT-IR, SEM, nitrogen adsorption and the static adsorption experiments. The imprinted functionalized silica gel sorbent exhibited high selectivity and offered a fast kinetics for the adsorption and desorption of PCP. The prepared sorbent was shown to be promising for on-line solid-phase extraction for HPLC determination of trace levels of PCP in environmental samples. With a sample loading flow rate of 5 ml min^{-1} for 2 min, an enhancement factor of 670 and a detection limit (S/N = 3) of 6 ng l^{-1} were achieved at a sample throughput of five samples h^{-1}. The precision (RSD) for nine replicate on-line sorbent extractions of 10 μg l^{-1} PCP was 3.8%. The sorbent also offered good linearity ($r = 0.9997$) for on-line solid-phase extraction of trace levels of PCP. The method was applied to the determination of PCP in local lake water, river water and wastewater samples.

Keywords: Pentachlorophenol; Solid-phase extraction; Molecular imprinting; High performance liquid chromatography

1. Introduction

Pentachlorophenol (PCP) is used as a general herbicide in agriculture and as an insecticide for termite control in the preservation of wood [1–3]. Because of its toxicity and unpleasant organoleptic properties (concentrations as low as a few μg/l of phenol affect the taste and odour of water and fish), PCP has been included in the list of priority pollutants by the US Environmental Protection Agency (EPA) [4]. Consequently, the development of new sorbents for selective removal and separation of pentachlorophenol in environmental matrices is of particular significance.

Molecular imprinting is an attractive method for the preparation of selective sorbents [5,6]. Recently, molecularly imprinted sol–gel materials (MISGMs) have been extensively studied [7–10]. MISGMs are fabricated by a conventional sol–gel process and incorporation of the template molecules into rigid inorganic or inorganic–organic networks. After removal of the template, molecular cavities with distinct pore size, shape or chemical functionality remain in the cross-linked host. These "molecularly designed cavities" show an affinity for the template molecule over other structurally related compounds. However, most of these materials exhibit high affinity and selectivity but poor site accessibility to the target molecules. So, the kinetics of the sorption/desorption process is unfavorable, as the template and functionality are totally embedded in the polymer matrices and the mass transfer is slow. A promising solution to this problem is the development of surface molecular imprinting [11]. The

* Corresponding author. Fax: +86 22 23506075.
E-mail address: xpyan@nankai.edu.cn (X.-P. Yan).

0021-9673/$ – see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.chroma.2005.09.035

materials with binding sites situated at the surface show many advantages including high selectivity, more accessible sites, fast mass transfer and binding kinetics [12,13].

The purpose of this work is to prepare a new molecularly imprinted amino-functionalized silica gel sorbent with binding sites situated at the surface with respect to PCP by a surface imprinting technique in combination with a sol–gel process, and to apply it to on-line selective solid-phase extraction (SPE) coupled with HPLC for the determination of trace PCP in water samples.

2. Experimental

2.1. Materials and chemicals

Silica gel (80–120 mesh, Qingdao Ocean Chemical Co., Qingdao, China) was used as the support to prepare the PCP-imprinted functionalized sorbent. Tetraethoxysilicane (TEOS), 3-aminopropyltriethoxysilane (APTES) (Wuhan University Chemical Factory, Wuhan, China), pentachlorophenol (PCP), phenol (Phe), 2,4-dichlorophenol (2,4-DCP), acetic acid (HAc) (Tianjin Chemical Co., Tianjin, China) were used in this study. Doubly deionized water (DDW, 18 MΩ cm^{-1}) obtained from a WaterPro water system (Labconco Corporation, Kansas City, MO, USA) was used throughout the experiments. The mobile phase used for HPLC experiments was a mixture of methanol (Concord Technology Co. Ltd., Tianjin, China), HAc and water (90:0.3:9.7), and was filtered through 0.45-μm filter prior to use. All reagents used were of at least analytical grade.

2.2. Samples

Water samples were collected from local lake, river and wastewater of timber factory. The glass bottles for sample storage were thoroughly washed with detergents, water, methanol and doubly deionized water, and dried before use. The samples were filtered through 0.45-μm Supor filters, stored in precleaned glass bottles. Water samples were adjusted to pH 7.0–7.6 with HCl or NaOH to insure the efficient solid-phase extraction of the analytes by the sorbent, and analyzed immediately.

2.3. Instrumentation

The chromatographic system consisted of a Model 600 HPLC pump and a Waters 2996 photodiode array detector (Waters, Milford, MA, USA). All separations were achieved on an analytical reversed-phase column (Symmetry-C_{18} 5 μm, 4.6 mm i.d. × 25 cm long, Waters, USA) at a mobile flow rate of 1.0 ml min^{-1} under isocratic conditions at room temperature. The Empower software was used to acquire and process spectral and chromatographic data. The photodiode array detector was operated between 210 and 400 nm.

A Model FIA-3100 flow injection system (Vital Instruments, Beijing, China) was used for solid-phase extraction preconcentration. Tygon pump tubes were used for delivering the sample solution. Small-bore (0.5 mm i.d.) PTFE tubings were adapted for all connections, which were kept the shortest possible length to minimize the dead volume. The SEM micrographs of the sorbents were obtained at 20.0 kV on a Hitachi S-4100 field emission scanning electron microscopy. FT-IR spectra (4000–400 cm^{-1}) in KBr were recorded using a Magna-560 spectrometer (Nicolet, USA). Average pore diameter and surface area of the sorbents were measured by nitrogen adsorption with a Model CHEMBET-3000 Sorptometer (Quantachrome, USA).

2.4. Procedures for the preparation of the PCP-imprinted amino-functionalized silica gel sorbent

To activate the silica gel surfaces, 8 g of silica gel (80–120 mesh) was mixed with 60 ml of 33% methanesulfonic acid and refluxed under stirring for 8 h. The solid product was recovered by filtration, washed with DDW to neutral and dried under vacuum at 70 °C for 8 h. To prepare the PCP-imprinted amino-functionalized silica gel sorbent, 1 g of PCP was dissolved in 5 ml of ethanol, and mixed with 2 ml of APTES. The mixture was stirred for 20 min, then 4 ml of TEOS was added. After stirring for 5 min, 1 g of activated silica gel and 1 ml of 1 mol l^{-1} HAc (as catalyst) were added. The mixture began to co-hydrolyse and co-condense after stirring for a few minutes, then incubated for 10 h at room temperature. The product was filtrated and dried in a vacuum oven at 100 °C for 8 h. Thus, the activated silica gel surface was grafted with the complex. The sorbent was extracted with 25 ml of ethanol and 25 ml of 1 mol l^{-1} HCl under stirring for 2 h to remove PCP (Fig. 1). The product was isolated by filtration, washed with ethanol + 6 mol l^{-1} HCl (1:1), neutralized with 0.1 mol l^{-1} NaOH, and washed with pure water. Finally, the sorbent was dried under vacuum at 80 °C for 12 h. For comparison, the non-imprinted functionalized silica gel sorbent was also prepared using an identical procedure, but without the addition of PCP.

2.5. Static adsorption test

To measure adsorption capacity, 50 mg of PCP-imprinted or nonimprinted sorbents was equilibrated with 10 ml of various concentrations of PCP dissolved in ethanol. The mixtures were mechanically shaken for 1 h at room temperature and separated centrifugally. The supernatants were measured for the unextracted PCP by UV spectrometry. Adsorption and competitive recognition studies were performed with PCP and structurally related compounds, phenol and 2,4-DCP at the 100 mg l^{-1} level.

Uptake kinetics of PCP by the imprinted functionalized silica gel sorbent was also examined. Fifty milligrams of the sorbent was added to 10 ml of 100 mg l^{-1} of PCP ethanol solution. The mixture was mechanically shaken for 5, 10, 30, 60, 90 and 120 min at room temperature, respectively, then separated centrifugally. The supernatants were measured for the unextracted PCP by UV spectrometry.

2.6. Procedures for selective on-line SPE–HPLC determination of PCP using the imprinted sorbent

To evaluate the applicability of the imprinted functionalized silica gel sorbent for on-line SPE–HPLC determination of trace

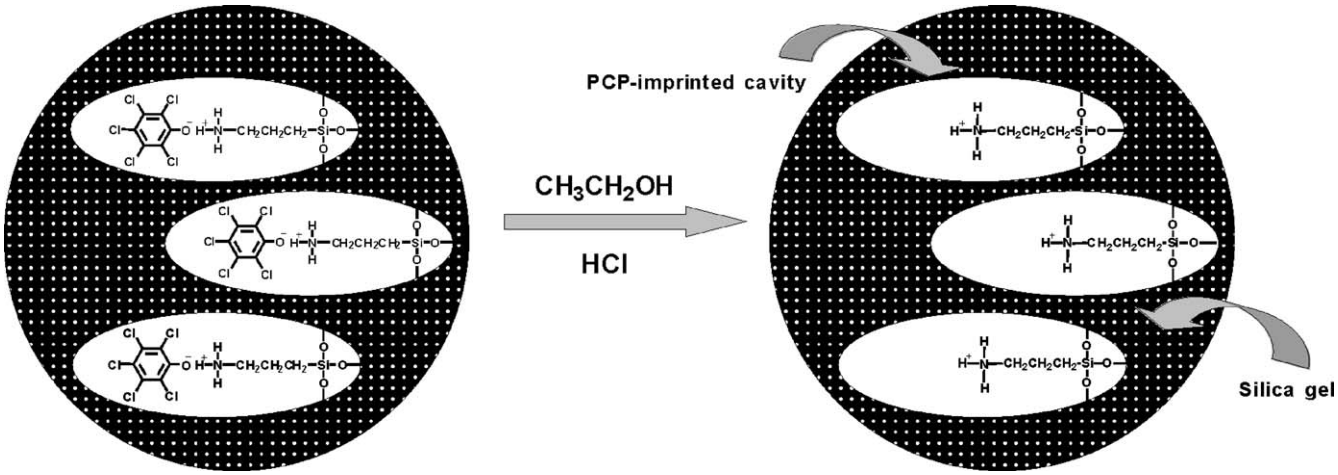

Fig. 1. Protocol for template imprinting of PCP.

PCP, a cylindrically shaped microcolumn (1.5 cm × 4 mm i.d.) packed with 50 mg of the imprinted functionalized silica gel sorbent was prepared. A schematic diagram for the on-line SPE preconcentration coupled to HPLC for determination of trace PCP in water is shown in Fig. 2. First, the sample solution was introduced onto the SPE microcolumn at a flow rate of 5 ml min^{-1} for 2 min while the HPLC injector valve was in the load position, so that the PCP were preconcentrated by the sorbent packed precolumn and the unwanted water went to waste (W) (Fig. 2a). Second, the analyte adsorbed on the SPE microcolumn were eluted in the backflush mode by the HPLC mobile phase at a flow rate of 1.0 ml min^{-1} into the chromatographic separation column for 2 min by switching HPLC valve from "load" to "inject" position (Fig. 2b). As such, the sample band in the microcolumn was compressed into a narrow band before entering the analytical column and the band broaden effect was reduced [14]. Third, the HPLC injector valve was turned to the "load" position for next sample preconcentration while the analytes were separated in the chromatographic separation column to improve sample throughput. In this way, a complete cycle of the on-line SPE preconcentration and HPLC separation of the PCP lasted 12 min. Chromatograms were recorded and stored on the hard disk of the computer. The peak areas were calculated at 304 nm and used for data evaluation.

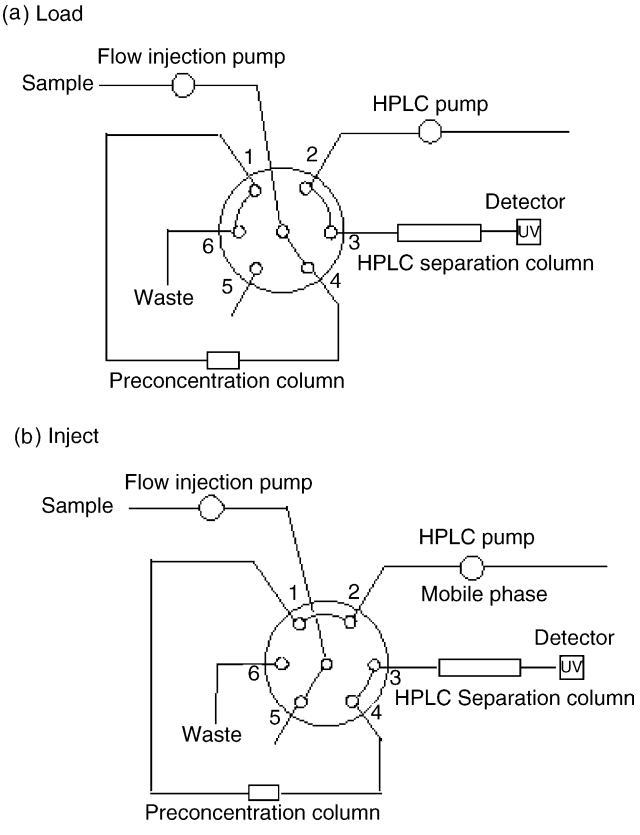

Fig. 2. Schematic diagram of the on-line solid-phase extraction preconcentration coupled with HPLC. HPLC injector valve position: (a) load; (b) inject.

3. Results and discussion

3.1. Characteristic of the FT-IR spectra, SEM image and N_2 adsorption

To ascertain the presence of APTES in the functionalized silica gel sorbents, FT-IR spectra for activated silica gel, PCP-imprinted and nonimprinted amino-functionalized silica gel sorbents are compared in Fig. 3. The observed features around 1100 and 976 cm^{-1} indicate Si–O–Si and Si–O–H stretching vibrations, respectively. OH vibration was reflected at 3442 and 1636 cm^{-1}. The bands around 780 and 470 cm^{-1} resulted from Si–O vibrations. A characteristic feature of the imprinted and nonimprinted sorbents when compared with activated silica gel is N–H bond around 1560 cm^{-1} and C–H bond around 2935 cm^{-1}. These results suggest that –NH_2 be grafted onto the surface of activated silica gel after modification. Imprinted and nonimprinted sorbent showed similar location and appearance of the major bands. SEM image of PCP-imprinted sol–gel material (Fig. 4) shows that the surface is multied-porous by being bond and coated with molecularly imprinted complex while the surface of activated silica gel is much smoother. The imprinted sorbent has surface area of 177 m^2 g^{-1} with an average pore

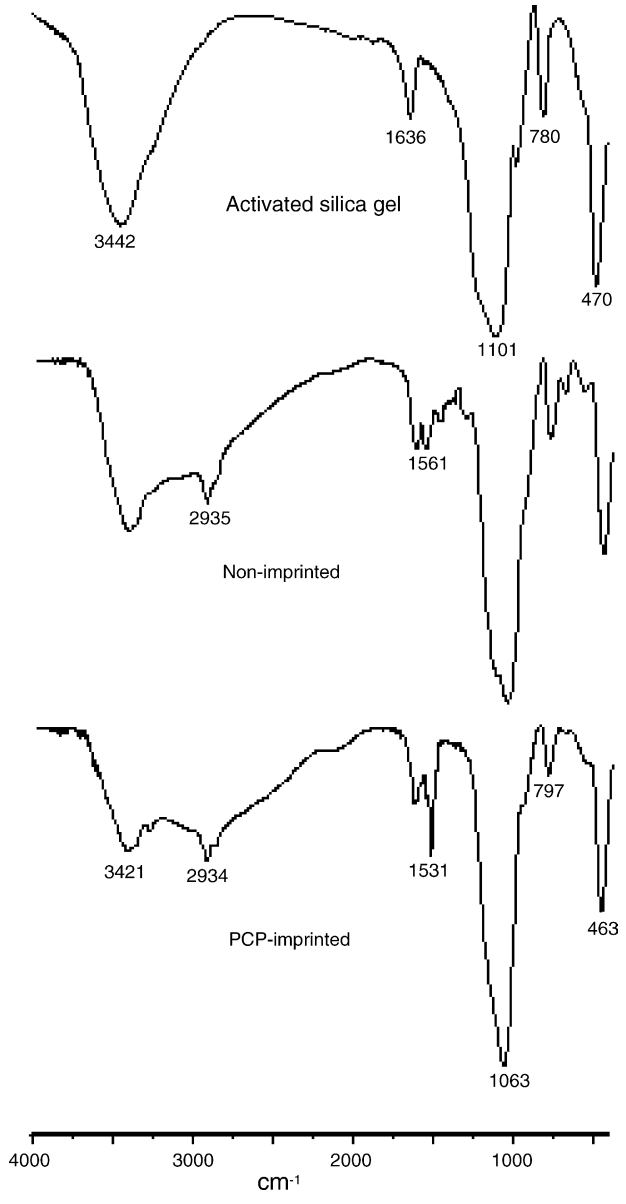

Fig. 3. FT-IR spectra of the activated silica gel, PCP-imprinted and nonimprited sorbent.

size of 8.8 nm while the activated silica gel has surface area of 355 $m^2\ g^{-1}$ with an average pore size of 8.6 nm.

3.2. Evaluation of static adsorption

To measure adsorption capacity, 50 mg of PCP-imprinted or nonimprinted sorbent was equilibrated with 10 ml of various concentrations of PCP dissolved in ethanol. The mixture was mechanically shaken for 1 h at room temperature, then separated centrifugally. The supernatant was measured for the unextracted PCP by UV spectrometry. Fig. 5 shows the binding isotherm of PCP onto sorbents. Obviously, the binding capacity of the imprinted sorbent is larger than that of the nonimprinted sorbent. Adsorption and competitive recognition studies were performed with PCP and structurally related compounds, phenol and 2,4-dichlorophenol (2,4-DCP). Table 1 summarizes the data for

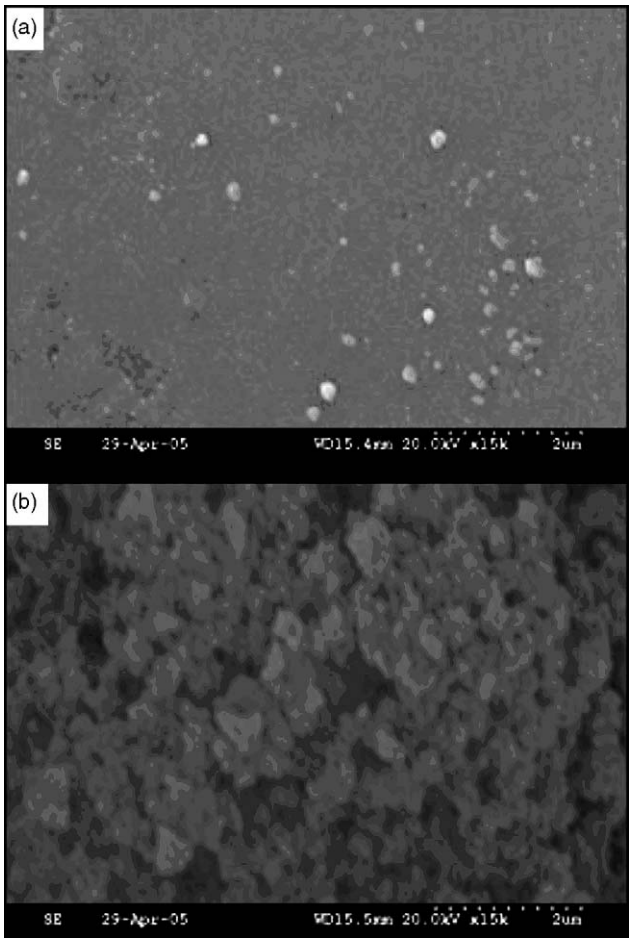

Fig. 4. SEM image of the surface of (a) the activated silica gel; (b) the PCP-imprinted sorbent.

uptake capacity, distribution coefficient (K_d), selectivity coefficient of the sorbent (k) and the relative selectivity coefficient (k') obtained in these competitive binding experiments. Comparison of the k values for the imprinted sorbent with the corresponding nonimprinted sorbent reveals a significant increase in k for PCP through imprinting. The k (PCP/2,4-DCP) value of the imprinted sorbent (89.2) is 10-fold that of nonimprinted sorbent (8.9) while

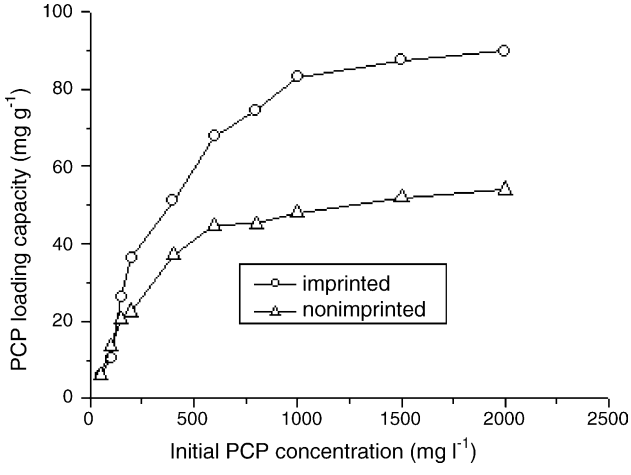

Fig. 5. Loading isotherm of PCP onto the imprinted and nonimprinted sorbents.

Table 1
Competitive loading of PCP, 2,4-DCP and phenol (Ph) by the imprinted and nonimprinted sorbents

Sorbent	Initial solution (mg l^{-1})			Capacity (mg g^{-1})			K_d (ml g^{-1})			k		k'	
	PCP	2,4-DCP	Ph	PCP	2,4-DCP	Ph	PCP	2,4-DCP	Ph	PCP/2,4-DCP	PCP/Ph	PCP/2,4-DCP	PCP/Ph
Imprinted	200	200	200	80.4	4.4	2.0	2051	23	10	89.2	205.1	10.0	17.8
Nonimprined	200	200	200	46.6	9.0	7.0	436	49	38	8.9	11.5		

Note: K_d, distribution coefficient; $K_d = \{(C_i - C_f)/C_f\} \times \{\text{volume of solution [ml]}/\{\text{mass of gel [g]}\}$, where C_i and C_f represent the initial and final concentrations, respectively; k, selectivity coefficient, $k = K_{d1}/K_{d2}$; k', relative selectivity coefficient, $k' = k_{\text{imprinted}}/k_{\text{nonimprinted}}$.

the k (PCP/Ph) value of the imprinted sorbent (205) is more than 17-fold that of the nonimprinted sorbent (11.5). The large k value of the imprinted sorbent is an indicative of its high selectivity for PCP over the related compounds. This may result from the imprinting effect and the difference of the molecular interactions. During the preparation of the imprinted sorbent, the template of PCP was incorporated into inorganic–organic networks. After the removal of PCP, the imprinted cavities and specific binding sites of $-NH_2$ group in a predetermined orientation was formed, whereas the nonimprinted sorbent has no such imprinted cavities and specific binding sites. On the other side, the acidity of PCP ($pK_a = 4.93$) is larger than 2,4-DCP ($pK_a = 7.85$), so the interaction of PCP with the $-NH_2$ group is stronger than that of 2,4-DCP (or Ph) with the $-NH_2$ group.

Uptake kinetics of PCP by the imprinted functionalized silica gel sorbent was also examined. The results indicate that the imprinted sorbent has a fast uptake kinetics; 72% of binding capacity (200 mg l^{-1} PCP onto 50 mg of the imprinted sorbent) was obtained within 5 min. If the concentration of PCP was lower, the time to saturation became shorter. This means that the surface imprinting greatly facilitate diffusion of the analyte to the binding sites.

3.3. Application of the imprinted sorbent to selective on-line SPE–HPLC determination of PCP

The applicability of the imprinted amino-functionalized silica gel to on-line SPE–HPLC determination of trace PCP was evaluated. The chemical and flow variables, such as sample acidity, sample loading flow rate and loading time, eluent and its concentration and flow rate, were optimized to achieve good sensitivity and precision for the extraction and elution of PCP.

The influence of sample acidity on the on-line extraction of 10 μg l^{-1} PCP was tested at a sample flow rate of 5 ml min^{-1} for 2 min extraction. The result shows that the maximum chromatographic peak area of PCP was achieved in the pH range of 6.8–7.6. Out of the optimum pH range, the chromatographic peak area of PCP decreased. These results show that PCP can be effectively adsorbed by the imprinted amino-functionalized silica gel-packed column in the pH range of 6.8–7.6.

The effect of sample loading time on the on-line solid-phase extraction of 10 μg l^{-1} PCP was tested at a sample loading flow rate of 5 ml min^{-1}. The chromatographic peak area increased almost linearly as sample loading time increased up to at least 12 min. Studies on the effect of sample loading flow rate on the on-line solid-phase extraction of 10 μg l^{-1} PCP for 2 min showed that the chromatographic peak area increased linearly with increase of the sample loading flow rate up to 6.0 ml min^{-1}. These results also indicate that the kinetics for the adsorption of PCP by the imprinted sorbent was very fast. The wide range of linearity for the chromatographic peak area against sample loading time and sample loading flow rate in the present on-line solid-phase extraction system offered great potentiality for achieving high enhancement factors by increasing sample loading rates and sample loading time without losing extraction efficiency.

For simplicity, the optimum HPLC mobile phase (MeOH:HAc:H_2O = 90:0.3:9.7) was used for desorption of the adsorbed PCP from the imprinted amino-functionalized silica gel-packed column. The time required for quantitative desorption of the adsorbed PCP when the HPLC injector valve was in the "inject" position was evaluated in order to determine when the HPLC injector valve should turn to the "load" position for next on-line solid-phase extraction during the HPLC separation of the analytes this cycle. It was found that the chromatographic peak area of the PCP increased remarkably as the desorption time increased from 0 to 0.3 min, increased slightly (~6%) as the desorption time increased from 0.5 to 1.0 min, then leveled off in the range of 1.0–10.0 min. Accordingly, 2.0-min desorption was selected to ensure the complete stripping of the adsorbed PCP from the imprinted amino-functionalized silica gel-packed column. Once the adsorbed PCP was quantitatively stripped from the imprinted amino-functionalized silica gel-packed column, the HPLC injector valve turned to the "load" position for next preconcentration so that the current HPLC separation and the next preconcentration proceeded in parallel. The results indicate that the optimum HPLC mobile phase (methanol, HAc and water = 90:0.3:9.7) at a flow rate of 1.0 ml min^{-1} for 2 min was quite efficient for quantitative elution of the adsorbed PCP from the microcolumn packed with PCP-imprinted functionalized silica gel sorbent.

The selectivity of the imprinted silica gel sorbent for on-line solid-phase extraction of PCP was tested by passing 10 ml standard aqueous solution containing 10 μg l^{-1} of phenol, 2,4-DCP and PCP each through the precolumn (Fig. 6). Only PCP appeared in the chromatogram after eluting by mobile phase, indicating that PCP was selectively extracted onto the imprinted sorbent whereas phenol, 2,4-DCP did not retained on the sorbent.

3.4. Figures of merit for the present on-line SPE coupled with HPLC using the developed imprinted functionalized silica gel sorbent

The analytical figures of merit of the present on-line solid-phase extraction using the imprinted functionalized silica gel

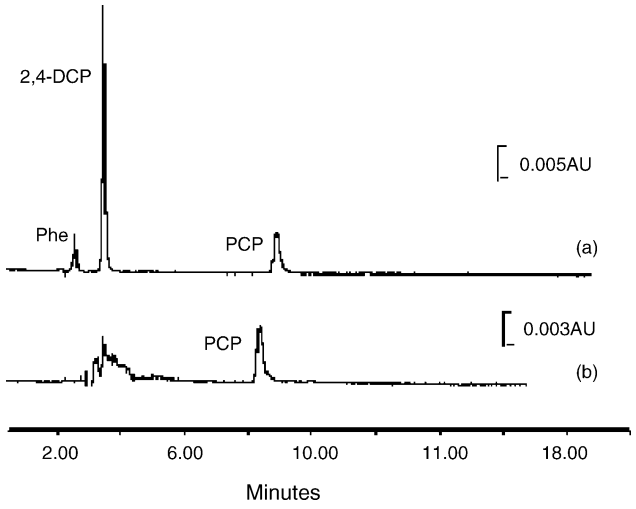

Fig. 6. Chromatograms of (a) direct injection of 20 μl standard mixture solution containing 10 mg l^{-1} of phenol, 2,4-DCP and PCP each; (b) 10 ml standard mixture solution of 10 μg l^{-1} of phenol, 2,4-DCP and PCP with on-line solid-phase extraction preconcentration.

Table 2
Figures of merit for the on-line solid-phase extraction coupled with HPLC for determination of trace PCP

Enrichment factors[a]	670
Detection limit (S/N = 3) (ng l^{-1})	6
Peak area precision[b] (n = 9) (%, RSD)	3.8
Linear range of the calibration graph (μg l^{-1})	0.05–500
Sample consumption (ml)	10

[a] Compared with direct injection of 20 μl sample solution.
[b] For 0.2 μg l^{-1} PCP.

Table 3
Analytical results for the determination of PCP in real water samples

Sample	Concentration determined (mean ± σ, n = 3) (μg l^{-1})	Recovery of 2 μg l^{-1} PCP spiking (%)
Wastewater	0.16 ± 0.02	91 ± 3
Lake water	0.09 ± 0.01	93 ± 2
River water	0.08 ± 0.01	92 ± 1

sorbent coupled with HPLC for the determination of trace PCP were evaluated under optimal experimental conditions (Table 2). With a sample loading flow rate of 5 ml min^{-1} for 2 min extraction, the enrichment factor (EF) obtained by comparing the slopes of the linear portion of the calibration curves before and after the on-line extraction was 670. A detection limit (S/N = 3) of 6 ng l^{-1} was achieved at a sample throughput of five samples h^{-1}. The precision (RSD) for nine replicate on-line SPE of 10 μg l^{-1} PCP was 3.8%. The sorbent also offered good linearity (r = 0.9997) for on-line SPE of trace PCP. The developed methodology was also applied to the determination of local water samples. The analytical results are given in Table 3. The recoveries of PCP ranged from 91 to 93%.

4. Conclusions

A simple procedure was developed to synthesize a highly selective PCP-imprinted amino-functionalized silica gel sorbent by combining a surface molecular imprinting technique with a sol–gel process. The prepared material shows high affinity, selectivity, capacity and good site accessibility of the target, being promising for selective absorption and determination of PCP from environmental matrices by on-line SPE–HPLC.

Acknowledgements

This work was supported by the National Basic Research Program of China (No. 2003CB415001) and the National Natural Science Foundation of China (No. 20437020).

References

[1] T.B. Gaines, Toxicol. Appl. Pharmacol. 14 (1969) 515.
[2] G.W. Muna, N. Tasheva, G.M. Swain, Environ. Sci. Technol. 38 (2004) 3674.
[3] M. Li, S.F. Tsai, S.M. Rosen, R.S. Wu, K.B. Reddy, J.D. Cesare, S.J. Salamone, J. Agric. Food Chem. 49 (2001) 1287.
[4] Toxic Substance Control Act, U.S. Environmental Protection Agency, Washington, DC, 1979.
[5] A. Martin-Esteban, Fresenius J. Anal. Chem. 370 (2001) 795.
[6] L.I. Andersson, Bioseparation 10 (2001) 353.
[7] F.L. Dickert, O. Hayden, Anal. Chem. 74 (2002) 1302.
[8] S. Dai, M.C. Burleigh, Y.H. Ju, H.J. Gao, J.S. Lin, S.J. Pennycook, C.E. Barnes, Z.L. Xue, J. Am. Chem. Soc. 122 (2000) 992.
[9] A. Katz, M.E. Davis, Nature 403 (2000) 286.
[10] M.K.-P. Leung, C.-F. Chow, M.H.-W. Lam, J. Mater. Chem. 11 (2001) 2985.
[11] S. Dai, M.C. Burleigh, Y. Shin, C.C. Morrow, C.E. Barnes, Z. Xue, Angew. Chem. Int. Ed. Engl. 38 (1999) 1235.
[12] H.-H. Yang, S.-Q. Zhang, F. Tan, Z.-X. Zhuang, X.-R. Wang, J. Am. Chem. Soc. 127 (2005) 1378.
[13] G.-Z. Fang, J. Tan, X.-P. Yan, Anal. Chem. 77 (2005) 1734.
[14] N. Masqué, R.M. Marcé, F. Borrull, J. Chromatogr. A 793 (1998) 257.