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Journal of Chromatography A, 828 (1998) 91–94

JOURNAL OF CHROMATOGRAPHY A

Molecular recognition towards coplanar polychlorinated biphenyls based on the porogen imprinting effects of xylenes

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Abstract

Molecularly imprinted, uniformly sized polymeric separation media were prepared using *o*-, *m*- or *p*-xylene as a porogenic template to investigate recognition ability towards coplanar polychlorinated biphenyls (PCBs). PCBs having chlorine atoms at *meta* positions of biphenyl were preferably retained on stationary phase with *m*-xylene as porogenic template and PCBs having chlorine atoms at *para* positions of biphenyl were found to be retained longer on the stationary phase imprinted by the porogenic template, *p*-xylene. It was found that positional relationship between substituted chlorine atoms was also important for chromatographic recognition.

Keywords: Molecular imprinting; Molecular recognition; Stationary phases, LC; Xylene stationary phases; Polychlorinated biphenyls

1. Introduction

The molecular imprinting technique has interested many researchers working on separation or analysis based on molecular recognition, however this technique has been hardly expanded into practical method to prepare really useful separation medium, especially commercial separation medium so far. One of big problems of this method is that the molecule which would be separated or recognized should be used as the template molecule in nearly gram scale. However, if template molecules are expensive, unstable, or toxic, we can hardly realize molecularly imprinted polymers for those compounds.

Recently, Takeuchi and co-workers reported a molecularly imprinted polymer prepared using atrazine as the template and this polymer can recognize molecules having a similar structure to atrazine [1,2]. This kind of group recognition is an interesting way to realize practical molecular imprinting. However a template molecule is still utilized to be molecularly imprinted in this case.

We have also proposed namely porogen imprinting effects [3]. In this paper, porogenic solvents which produce macroporous structure into a cross-linked polymer network can be somehow imprinted onto a cross-linked polymer. For example, toluene or benzene as porogenic solvent can be molecularly imprinted on the cross-linked polymer network to result in preferential molecular recognition towards the solute utilized as the porogenic solvent. The imprinted recognition sites formed by the porogen imprinting effects is found to be very stable and the loading capacity is large enough because porogenic template can be used almost the same amount of the cross-linking monomers. We will call these porogenic solvents porogenic templates in this work because they work as template molecules in molecular imprinting.

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0021-9673/98/$ – see front matter
PII: S0021-9673(98)00591-3

In addition, the imprinted sites can recognize structurally similar compounds as well, for example the polymer imprinted with *o*-xylene as porogenic template can recognize 1,2-dichlorobenzene, conversely, that imprinted with *p*-xylene preferably retained 1,4-dichlorobenzene. Interestingly, isomers of dinitrobenzenes as well as dibromobenzenes are poorly recognized on the polymer imprinted with corresponding isomers of xylenes as porogenic templates with even larger retention time, probably due to the slightly larger molecular shapes as well as another electron properties. The similar poor recognition is also found in the separation of molecularly smaller solutes, difluorobenzenes. These findings suggest that the isomer of xylenes can be utilized as a potential analogue to more toxic chlorine-substituted benzenes based on the porogen imprinting effects.

The aim of this paper is to extend this analogous recognition ability of the porogen imprinting effects to toxic congeners such as polychlorinated biphenyls (PCBs). PCBs involve chlorine-substituted benzene units and are of course good targets to be separated due to their many structural isomers, however those can hardly be utilized as template molecules directly due to the toxicity and unavailability of single isomers in the gram scale. In this report we wish to demonstrate preliminary results of molecular recognition towards PCBs on the analogously imprinted stationary phases by xylene isomers as porogenic templates in high-performance liquid chromatography (HPLC).

2. Experimental

2.1. Preparation of stationary phases

Porogen imprinting stationary phases, which were uniformly sized, were prepared using two-step swelling and polymerization method using *o*-, *m*- or *p*-xylenes as porogenic templates [3,4]. In this study, we utilized ethylene dimethacrylate (EDMA) as a cross-linking monomer and no other functional monomers were utilized.

A typical preparation method is as follows; uniform sized polystyrene seed particles were prepared by an emulsifier-free emulsion polymerization method. The diameter of the polystyrene seed particles was ca. 1 μm.

The polystyrene seed particles ($7.0\cdot10^{-2}$ g/ml), ($7.8\cdot10^{-1}$ ml) were admixed with a microemulsion prepared from dibutyl phthalate ($2.5\cdot10^{-1}$ ml), sodium dodecyl sulfate ($3.5\cdot10^{-2}$ g), 2,2′-azobis(2,4-dimethylvaleronitrile) ($5.0\cdot10^{-2}$ g) and distilled water (40 ml) by sonication. This suspension was stirred at 125 rpm until oil droplets of the added emulsion were completely absorbed on the seed particles at room temperature. A suspension of cross-linking agent (5.0 ml), porogen (5.0 ml), polyvinyl alcohol (degree of polymerization, DP=500, saponification degree=96 mol%) ($7.2\cdot10^{-1}$ g) and distilled water (45 ml) were added to the swollen polystyrene seed particles. This suspension was stirred at 125 rpm for 2 h at room temperature. The polymerization was carried out at 50°C for 24 h under an argon atmosphere. The resulting polymer particles were washed with methanol and tetrahydrofuran by a repeated sedimentation–redispersion process, and were quantitatively yielded. The final particle size was 5.5 μm in diameter, and relative standard deviation (R.S.D.) values of the prepared particles were around 5%.

2.2. Chromatography

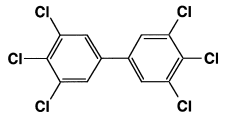
The polymeric stationary phases prepared were packed into a stainless steel column (150 mm×4.6 mm I.D.) by slurry method using a mixture of methanol, 2-propanol and glycerol as slurry medium. HPLC was performed using a Shimadzu LC-4A pump equipped with a Rheodyne 7125 valve loop injector, a Shimadzu SPD-2A UV detector and a Shimadzu C-R4A integrator. All the solvents utilized for HPLC were of the highest grade available from Nacalai Tespue (Kyoto, Japan).

The samples of PCBs were gifts from Centers for Disease Control and Prevention (CDC) (Atlanta, GA, USA) however, unfortunately, exact concentration of the samples in methanol or toluene was not mentioned. We injected 0.5–3 μl of each sample to get appropriate peak area. The reproducibility observed in retention time was very good and was not dependent on the volume of the injected sample.

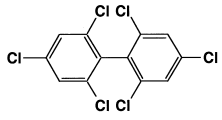
3. Results and discussion

Polymer-based stationary phases involving non-imprinted EDMA stationary phases have potential advantages for separation of halogenated compounds and in addition, have preferential retention towards relatively plane solutes compared with sterically bulky solutes based on a contribution of so called micropores within the cross-linked polymer network [5,6]. Therefore polymer-based stationary phases potentially have great advantages in the recognition of coplanar PCBs. In fact, the PCBs having chlorine substituents on *ortho* positions are retained with much shorter retention times compared with coplanar PCBs without any *ortho* chlorine atoms. A separation of PCB 155 and 169 is demonstrated in Fig. 1. A coplanar PCB, PCB 169 afforded much larger k' values compared with those of PCB 155 on three kinds of EDMA stationary phases prepared with isomers of xylene as porogenic template. Interestingly, the EDMA stationary phase prepared with *o*-xylene as the porogenic template showed largest α value. This might be due to *ortho*-substituted chlorine atoms on PCB 169, which could be preferably retained on the *o*-xylene-imprinted EDMA stationary phase with the largest k' for PCB 169, while PCB 155 involves only *meta*-substituted chlorine atoms on the phenyl rings where the *m*-xylene-imprinted stationary phase afforded the largest k' for PCB 155.

Since non-*ortho*-PCBs (coplanar PCBs) are reported to be more toxic, we try to recognize positional isomers of the PCBs having no *ortho* substituents,



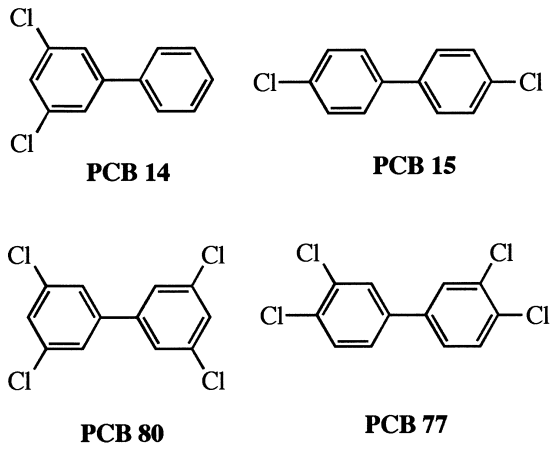
PCB 169



PCB 155

Porogenic template	k'	α	k'
o-Xylene	5.40	**4.82**	1.12
m-Xylene	5.31	**4.57**	1.16
p-Xylene	5.19	**4.59**	1.13

Fig. 1. Separation of PCB 169 (coplanar) and PCB 155. Mobile phase, 100% methanol; flow-rate, 1 ml/min; detection, UV 254 nm, sample injected, 3 μl.



PCB 14 PCB 15 PCB 80 PCB 77

Fig. 2. Structures of coplanar PCBs utilized for this study.

chromatographically. We selected the following PCBs in this study to evaluate recognition ability of the prepared porogen imprinting stationary phases. These structures and PCB numbers are depicted in Fig. 2. In top pair (PCB 14 and PCB 15), PCB 14 was found to be retained slightly longer than PCB 15 on a non-imprinted EDMA stationary phase, but both peaks of PCB 14 and 15 were overlapped.

The observed molecular recognition ability of the prepared stationary phases towards PCBs in methanol as the mobile phase are summarized in Table 1. PCB 14 has two chlorine atoms at *meta* positions based on biphenyl linkage and was retained longer on *m*-xylene imprinting stationary phase than PCB 15 which has no chlorine atoms at *meta* positions. On the contrary, PCB 15 was found to be retained longer on the *p*-xylene imprinting stationary phase compared with PCB 14. This is understandable because PCB 15 has two chlorine atoms at *para* positions based on the biphenyl linkage. The ob-

Table 1
Molecular recognition ability towards PCBs

PCB	Retention time (min)	
	m-Xylene imprinting	*p*-Xylene imprinting
14	5.03	5.02
15	4.88	5.22
80	7.75	7.43
77	7.38	7.43

Mobile phase: methanol, flow-rate: 1.0 ml/min, detection: UV 254 nm.

tained inversion of the retention times between PCBs 14 and 15 indicates that even the porogen imprinting stationary phase with xylenes as porogenic template can somehow recognize PCB isomers based on structural differences.

Typical chromatograms of the separation of PCB 14 and PCB 15 in an aqueous methanol as the mobile phase are shown in Fig. 3. Due to relatively poor column efficiency, almost no resolution with comparable peak height to those of the separated peaks in (A) was unfortunately found on the stationary phases with *p*-xylene as the porogenic template, however, contrastive chromatograms were really obtained on ''chemically equal'' EDMA stationary phases prepared with different isomers of xylene as porogenic templates.

Furthermore, PCB 80 has four chlorine atoms at *meta* positions of biphenyl and was retained longer on the *m*-xylene imprinting stationary phase than PCB 77 having two *meta* chlorines but another two chlorines at *para* positions from the biphenyl linkage. In PCB 77, chlorine atoms on each benzene rings are in fact positioned at *ortho* positions from each other. On the stationary phase prepared using *p*-xylene as the porogenic template showed almost identical retention times toward both PCBs 80 and 77. Although PCB 77 has two chlorine atoms at *para* positions based on biphenyl linkage but in this case structural positions of substituted two chlorine atoms on the each benzene rings are dominant. In fact, the stationary phase prepared using *o*-xylene as the porogenic template retained PCB 77 longer than PCB 80, where t_R of PCBs 77 and PCB 80 were 7.34 and 7.26, respectively. This is also understandable, because PCB 77 involves two chlorine atoms at *ortho* positions on each phenyl rings.

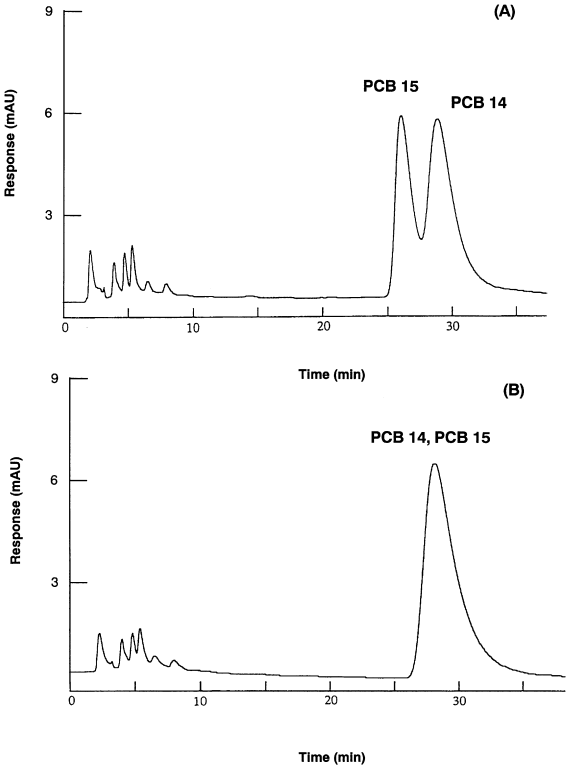


Fig. 3. Chromatograms of separations between PCBs 14 and 15 on *m*- or *p*-xylene as the porogenic template. (A) *m*-Xylene as porogenic solvent, (B) *p*-xylene as porogenic solvent. Mobile phase, 90% aq. methanol; flow-rate 0.8 ml/min; detection, UV 254 nm, sample injected, 1 μl.

4. Conclusions

The observed recognition ability in this study is not so drastic, however, analogous molecular recognition using molecularly imprinted stationary phases using analogous template, especially porogenic template is one possible way to obtain the separation media proving excellent stability and reproducibility which can be hardly prepared using molecular imprinting technique with real molecule as the template. Improvement of this method is under progress.

References

[1] J. Matsui, Y. Miyoshi, O. Doblhoff-Dier, T. Takeuchi, Anal. Chem. 67 (1995) 4404.
[2] M. Matsui, Y. Miyoshi, T. Takeuchi, Chromatographia 17 (1996) 332.
[3] K. Yoshizako, K. Hosoya, Y. Iwakoshi, K. Kimata, N. Tanaka, Anal. Chem. 70 (1998) 386.
[4] K. Hosoya, J.M.J. Fréchet, J. Polym. Sci. Polym. Chem. Educ. 31 (1993) 2129.
[5] K. Hosoya, E. Sawada, K. Kimata, T. Araki, N. Tanaka, J. Chromatogr. A 662 (1994) 37.
[6] K. Hosoya, Y. Kageyama, K. Yoshizako, K. Kimata, T. Araki, N. Tanaka, J. Chromatogr. A 711 (1995) 24.