Uniform-sized Molecularly Imprinted Polymers for Bisphenol A

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A uniform-sized molecularly imprinted polymer for bisphenol A (**1**) has been prepared by a multi-step swelling and thermal polymerization method using 4-vinylpyridine (4-VPY) and ethylene glycol dimethacrylate (EDMA) as a functional monomer and cross-linker, respectively. Hydrophobic and hydrogen bonding interactions between **1** and 4-VPY-EDMA materials could play an important role in the recognition of **1**. The **1**-imprinted 4-VPY-EDMA materials selectively recognized the template molecule among the phenolic estrogenic and xenoestrogenic compounds tested.

Molecularly imprinted polymers (MIPs), which can afford specific recognition against a template molecule, are used for chromatographic separations, solid phase extractions, membranes, antibody-mimics and sensors.[1,2] Among those, molecularly imprinted solid phase extractions are very promising, and have been used for selective enrichment and pretreatment of analytes in complex matrices such as biological fluids and environmental samples.[1,2] Generally, a non-aqueous bulk polymerization method[3] is utilized to obtain MIPs. The disadvantage of the method is that the block polymers should be crushed, ground and sieved to produce packing materials. We prepared uniform-sized MIPs for (S)-naproxen,[4,5] propranolol[6] and β-estradiol,[7] where a typical multi-step swelling and polymerization method[8] was used. The method had advantages that monodispersed beads could be obtained with simple washing, and that in situ surface modification could be made to introduce another functionality to the materials.[9,10] It is difficult to make such surface modification to the block polymer prepared by a bulk polymerization method.

This paper deals with preparation of the uniform-sized MIP for bisphenol A (**1**) using 4-vinylpyridine (4-VPY) and ethylene glycol dimethacrylate (EDMA) as a functional monomer and cross-linker, respectively, and preliminary evaluation of its molecular recognition ability by HPLC using a mixture of water and acetonitrile as the eluent. Further, the retention and recognition properties of **1**, and other phenolic estrogenic and xenoestrogenic compounds (Figure 1) on the MIP were discussed.

The uniform-sized MIP for **1** was prepared through a typical multi-step swelling and thermal polymerization method as reported previously.[5] The mole ratio of template, monomer and cross-linker was 2:7:25. Thermal polymerization was carried out at 50 °C for 24 h using 2,2'-azobis(2,4-dimethylvaleronitrile) as an initiator under argon atmosphere with slow stirring. The obtained MIP was washed with methanol and tetrahydrofurane to remove the template molecule, and was packed into a stainless-steel column (4.6 mm I.D. X 10 cm) using a slurry packing procedure. For comparison, non-imprinted polymers were prepared without the template molecule, **1**.

Table 1 shows effects of eluent pH on the retention factors ($k_{imprinted}$) of various solutes on the MIP. The eluents used were a 2:3 mixture of 20 mmol dm^{-3} phosphoric acid and/or sodium phosphate, and acetonitrile. The retention factor of a neutral compound, benzene, was not affected by eluent pH, while the

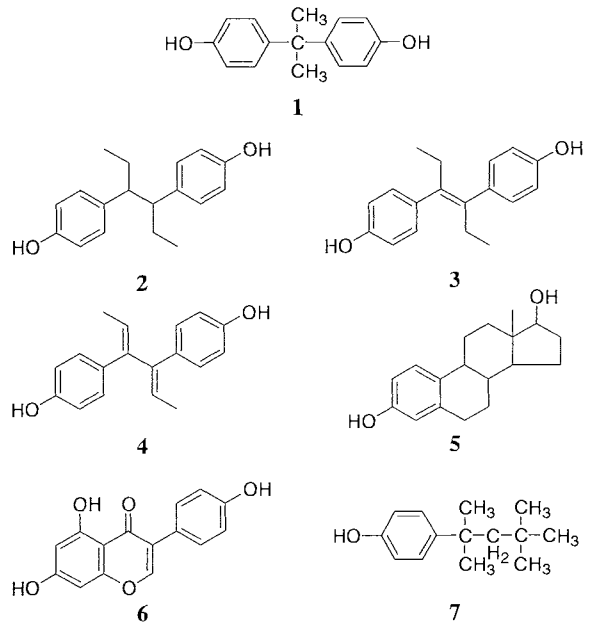

Figure 1. Structures of phenolic estrogenic and xenoestrogenic compounds: **1**, bisphenol A; **2**, hexestrol; **3**, diethylstilbestrol; **4**, dienestrol; **5**, 17β-estradiol; **6**, genistein; **7**, *p*-*t*-octylphenol.

Table 1. Effects of eluent pH on the retention factors of bisphenol A, diethylstilbestrol, phenol and benzene on the MIP for bisphenol A[a]

Solute	pH 2.5	pH 4.2	pH 6.1	pH 7.9	pH 9.5	pH 11.3	pH 12.4
Bisphenol A (**1**)	12.51	12.77	12.85	12.97	12.81	8.89	1.39
Diethylstilbestrol (**3**)	5.52	5.56	5.60	5.61	5.59	3.86	0.51
Phenol	1.13	1.13	1.13	1.14	1.13	0.86	0.12
Benzene	1.22	1.24	1.23	1.25	1.24	1.26	1.23

[a]HPLC conditions: column size, 4.6 mm I.D. x 10 cm; eluent, 20 mmol dm^{-3} phosphoric acid and/or sodium phosphate/CH_3CN = 40/60 (v/v); flow-rate, 1.0 mL min^{-1}; detection, 200 nm.

retention factors of phenolic compounds were decreased in the eluent pH ranges 11 - 12. The retentions of phenolic compounds were explained by hydrophobic interactions with polymer backbones and hydrogen bonding interactions with a pyridyl group(s) on 4-VPY-EDMA materials. Decreases in the retention factors in the eluent pH ranges 11 - 12 are due to dissociation of phenolic compounds. The protonation of pyridyl groups on the low eluent pH regions did not affect the retention of benzene and phenolic compounds. As reported previously,[11] this is due to that the extent of pyridyl groups is low on the 4-VPY-EDMA materials, and/or that the degree of protonation of pyridyl groups is not so large in the eluent pHs tested.

Table 2 shows the selectivity factor (S) of either estrogenic or xenoestrogenic compound, which is the ratio of the retention factors on the MIP and non-imprinted polymer, $k_{imprinted}/k_{non\text{-}imprinted}$. Selectivity factors of the compounds tested were not so much affected by the change of the eluent pH. The highest selectivity factor was obtained with **1**, which was 6.32, and those for other phenolic estrogenic and xenoestrogenic compounds were in the ranges 1.64 - 2.30. Previously, we prepared the **5**-imprinted 4-VPY-EDMA materials, which showed the highest selectivity factor, 2.43, for **5**.[7] It is reported that increasing the number of interaction sites on the template will lead to sites of higher specificity.[12] It is plausible that the phenol groups of **1** could interact with two pyridyl groups by hydrogen bonding interactions. The differences in the selectivity factors between the **1**- and **5**-imprinted 4-VPY-EDMA materials could be ascribable to the differences in the number of interaction sites on the template. Recently, the MIP for **2** was prepared by a bulk polymerization method.[13] The obtained MIP showed high cross reactivity with **3** and **4**, which structurally resemble **1**. However, the MIP prepared by us could selectively recognize **1** among the phenolic estrogenic and xenoestrogenic compounds tested. This reveals that the distance of two phenol groups is also an important factor for the recognition of **1** on the MIP.

Table 2. Selectivity factors of phenolic estrogenic and xenoestrogenic compounds on the MIP for bisphenol A[a]

Solute	$k_{imprinted}$	$k_{non\text{-}imprinted}$	S
Bisphenol A (**1**)	12.65	2.00	6.32
Hexestrol (**2**)	5.24	2.72	1.93
Diethylstilbestrol (**3**)	5.82	3.03	1.92
Dienestrol (**4**)	5.48	2.91	1.88
17β-Estradiol (**5**)	2.58	1.68	1.54
Genistein (**6**)	7.89	3.43	2.30
p-t-Octylphenol (**7**)	4.21	2.57	1.64

[a]HPLC conditions as in Table 1 except for that the eluent used is H_2O/CH_3CN = 40/60 (v/v). The $k_{imprinted}$ and $k_{non\text{-}imprinted}$ are the retention factors of a solute on the MIPs and non-imprinted polymers, respectively. The S is the selectivity factor, $k_{imprinted}/k_{non\text{-}imprinted}$.

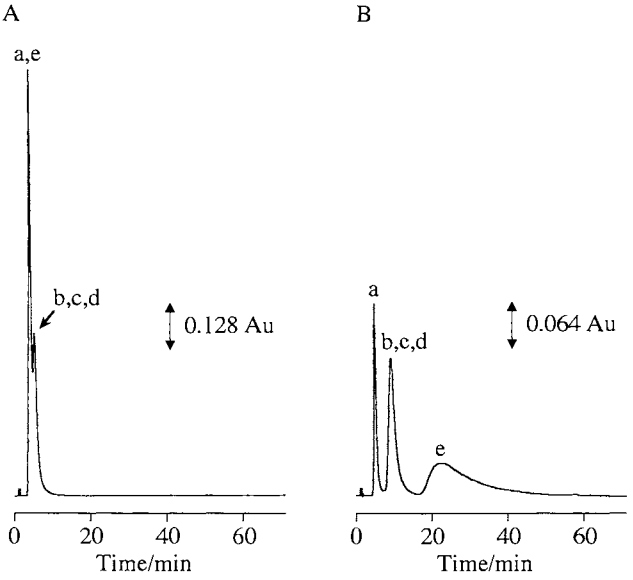

Figure 2. Separation of phenolic estrogenic and xenoestrogenic compounds on the non-imprinted polymer (A) and the MIP (B) for bisphenol A. Peak assignments: a, 17β-estradiol (**5**); b, hexestrol (**2**); c, dienestrol (**4**); d, diethylstilbestrol (**3**); e, bisphenol A (**1**). HPLC conditions as in Table 2. Loaded amounts: **1**, 400 ng; **2**, **3** and **4**, 80 ng; **5**, 160 ng.

Figure 2, parts A and B, shows the separation of **1**, **2**, **3**, **4** and **5** on the non-imprinted polymer and MIP, respectively. On the non-imprinted polymer, all compounds were overlapped, while **1** was completely separated from other compounds on the MIP.

The results obtained above indicate that the MIP prepared selectively recognizes **1** among the phenolic estrogenic and xenoestrogenic compounds, and that the MIP might be applied to solid phase extractions of **1** in biological fluids and environmental samples. The detailed study is now under progress in our laboratory.

References

1 B. Sellergren, *Trends Anal. Chem.*, **16**, 310 (1997).
2 A.G. Mayes and K. Mosbach, *Trends Anal. Chem.*, **16**, 321 (1997).
3 G. Wulff and A. Sarhan, *Angew. Chem.*, **84**, 364 (1972).
4 J. Haginaka, H. Takehira, K. Hosoya, and N. Tanaka, *Chem. Lett.*, **1997**, 555.
5 J. Haginaka, H. Takehira, K. Hosoya, and N. Tanaka, *J. Chromatogr. A*, **816**, 113 (1998).
6 J. Haginaka, Y. Sakai, and S. Narimatsu, *Anal. Sci.*, **14**, 829 (1998).
7 J. Haginaka and H. Sanbe, *Chem. Lett.*, **1998**, 1089.
8 K. Hosoya and J. M. J. Fréchet, *J. Polym. Sci., Part A, Polym. Chem.*, **31**, 2129 (1993).
9 K. Hosoya, Y. Kishii, K. Kimata, T. Araki, N. Tanaka, F. Svec, and J.M.J. Fréchet, *J. Chromatogr. A*, **690**, 21 (1995).
10 K. Hosoya, Y. Shirasu, K. Kimata, and N. Tanaka, *Anal. Chem.*, **70**, 943 (1998).
11 J. Haginaka, H. Sanbe, and H. Takehira, submitted for publication in *J. Chromatogr., A*.
12 B. Sellergren, "Molecular and Ionic Recognition with Imprinted Polymers," ed by R.A. Bartsch and M. Maeda, American Chemical Society, Washington, D.C., USA (1998), pp. 49-80.
13 J.A. Tarbin and M. Sharman, *Anal. Commun.*, **36**, 105 (1999).