# Uniform-sized Molecularly Imprinted Polymers for β-Estradiol

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A uniform-sized molecularly imprinted polymer (MIP) for β-estradiol has been prepared using 4-vinylpyridine and ethylenedimethacrylate as a functional monomer and cross-linker, respectively. The molecular recognition properties of the MIP were evaluated by HPLC using a mixture of phosphate buffer and acetonitrile as the eluent. The MIP showed the highest selectivity for β-estradiol. The MIP could efficiently separate estrogens from other steroids such as testosterone and corticosterone, and could separate estrogens one another.

Since the molecular imprinting techniques can afford specific recognition against an imprint (template) molecule, the molecularly imprinted polymer (MIP) is used for chromatographic separations, solid phase extractions, membranes, antibody-mimics and sensors.[1,2] In the molecular imprinting techniques, the functional monomers, which allow interactions with the functional groups of an imprint molecule, are copolymerized with cross-linkers in the presence of the imprint molecule. Usually, non-aqueous bulk polymerization techniques[3] are utilized to obtain MIPs. Recently, suspension polymerization methods using water[4-8] or perfluorocarbon[9] as an suspension medium were utilized for the preparation of MIPs. It is thought that water is to weaken the interaction between the imprint molecule and functional monomers.[10] We prepared uniform-sized MIPs for isomers of diaminonaphthalene or a chiral amide derived from (S)-α-methylbenzylamine,[4,5] (S)-naproxen[6,7] and propranolol[8], where a typical multi-step swelling and polymerization method[11] with water as the suspension medium was used. However, the MIP for (S)-naproxen prepared by us gave higher enantioselectivity for naproxen than that prepared with non-aqueous bulk polymerization techniques by Kempe and Mosbach.[12]

With regard to the MIPs for steroids, those for cholesterol,[13-15] testosterone,[15,16] corticosterone[17] and castasterone[18] have been prepared. There is no report for the preparation of the MIPs for estrogens. It is reported that some of xenoestrogens disrupt development and reproduction in wildlife.[19] In this study, we prepared uniform-sized MIPs for β-estradiol and evaluated its molecular recognition ability by HPLC using a mixture of phosphate buffer and acetonitrile as the eluent. Our goal is to apply the MIP for β-estradiol, which is an estrogen receptor mimic, to the screening of the endocrine disrupting chemicals.

Uniform-sized MIPs were prepared through a typical multi-step swelling and polymerization method as reported previously.[7] 4-Vinylpyridine and ethylenedimethacrylate were used as a functional monomer and cross-linker, respectively. The mole ratio of template, monomer and cross-linker was 2:14:25. Thermal polymerization was carried out at 50 °C for 24 h using

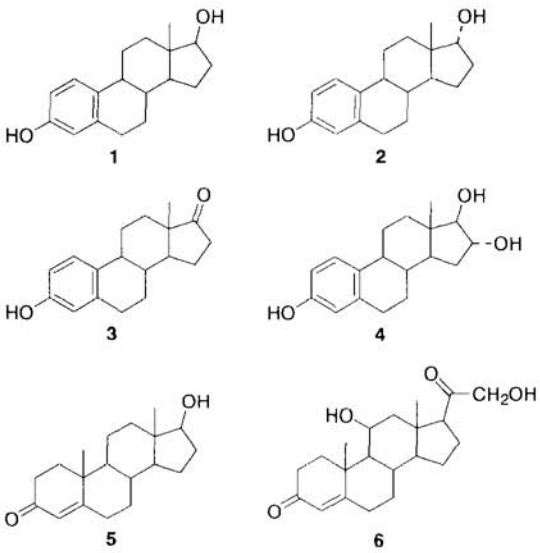


**Figure 1.** Structures of steroids: **1**, β-estradiol; **2**, α-estradiol; **3**, estrone; **4**, estriol; **5**, testosterone; **6**, corticosterone.

**Table 1.** Effect of eluent pH on the retention factors of various solutes on the MIP for β-estradiol and its selectvities[a]

| Solute | Eluent pH | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | pH 2.4 | | pH 4.0 | | pH 6.0 | | pH 7.8 | | pH 9.5 | |
| | $k_{imprinted}$ | S | $k_{imprinted}$ | S | $k_{imprinted}$ | S | $k_{imprinted}$ | S | $k_{imprinted}$ | S |
| β-Estradiol (**1**) | 11.7 | 2.43 | 12.1 | 2.41 | 12.9 | 2.42 | 12.2 | 2.43 | 12.1 | 2.37 |
| α-Estradiol (**2**) | 9.58 | 1.84 | 9.97 | 1.84 | 10.6 | 1.85 | 10.1 | 1.85 | 10.2 | 1.86 |
| Estrone (**3**) | 6.95 | 1.69 | 7.28 | 1.67 | 7.79 | 1.68 | 7.39 | 1.68 | 7.37 | 1.65 |
| Estriol (**4**) | 2.51 | 1.86 | 2.56 | 1.86 | 2.66 | 1.86 | 2.57 | 1.89 | 2.57 | 1.86 |
| Testosterone (**5**) | 1.39 | 1.37 | 1.52 | 1.34 | 1.60 | 1.32 | 1.54 | 1.34 | 1.56 | 1.34 |
| Corticosterone (**6**) | 0.91 | 1.38 | 0.98 | 1.35 | 1.03 | 1.34 | 0.99 | 1.36 | 1.01 | 1.36 |
| Phenol | 2.68 | 1.02 | 2.73 | 1.03 | 2.84 | 1.03 | 2.75 | 1.04 | 2.74 | 1.04 |
| 1-Naphthol | 12.6 | 1.02 | 12.6 | 1.04 | 13.1 | 1.02 | 12.6 | 1.04 | 12.6 | 1.06 |

[a]HPLC conditions: column size, 4.6 mm I.D. x 10 cm; eluent, 20 mmol $dm^{-3}$ phosphate buffer/$CH_3CN$ = 50/50 (V/V); flow-rate, 1.0 mL $min^{-1}$; detection, 200 nm. The $k_{imprinted}$ is the retention factor of a solute on the MIP. The S is selectivity, $k_{imprinted}/k_{base}$, where $k_{base}$ is the retention factor of a solute on the base (non-imprinted) polymer.

2,2'-azobis(2,4-dimethylvaleronitrile) as an initiator under argon atmosphere with slow stirring. The obtained MIPs were washed with methanol and tetrahydrofurane to remove the template molecule, and were packed into a stainless-steel column (4.6 mm I.D. X 10 cm) using a slurry packing procedure. For comparison, base (non-imprinted) polymers were prepared without the imprint molecule, β-estradiol.

Table 1 shows effects of eluent pH on the retention factors of steroids (Figure 1), phenol and 1-naphthol on the MIP for β-estradiol and its selectivity, S, which is the ratio of the retention factors on the MIP and base polymer, $k_{imprinted}/k_{base}$. The eluents used were a 1:1 mixture of 20 mmol $dm^{-3}$ phosphate buffer and acetonitrile. The retention factors and selectivities of all solutes tested were not so much affected by the change of the eluent pH despite the protonation of the stationary phases in low eluent pH. The highest selectivity for **1** was 2.43, and those for other estrogens, **2**, **3** and **4**, were 1.86, 1.69 and 1.89, respectively. Though the selectivities for **5** and **6** were around 1.3-1.4, these steroids were little retained. On the other hands, phenol and 1-naphthol were not selectively recognized on the MIP. These results indicate that the MIP shows high selectivity for **1** and moderate selectivity for other estrogens.

The effects of the acetonitrile content in eluents on the retention factors of steroids on the MIP were examined. With an increase in the acetonitrile content, retentivity of steroids tested decreased. This result suggests that hydrophobic interactions play an important role in the retentivity. When the MIP for **1** was prepared with methacrylic acid as a functional monomer or with no functional monomer, the obtained MIP had little selectivity for **1**. Further study is required to clarify the recognition mechanism of **1** on the MIP prepared using 4-vinylpyridine as the functional monomer.

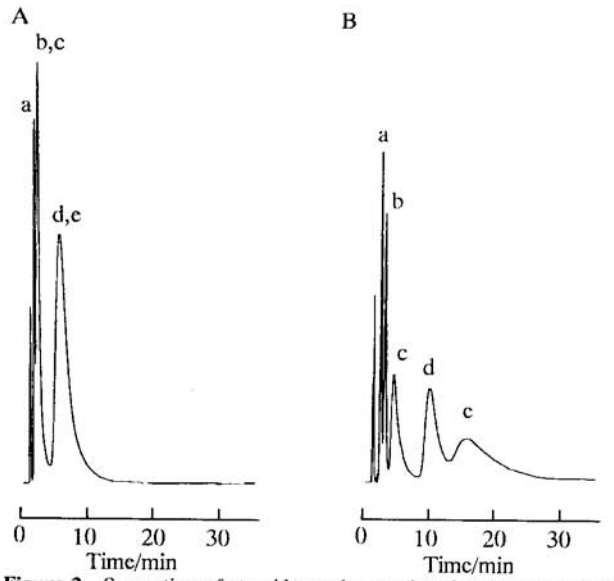


**Figure 2.** Separation of steroids on the non-imprinted polymer (A) and MIP (B) for β-estradiol. Peak assignments: a, corticosterone **6**; b, testosterone **5**; c, estriol **4**; d, estrone **3**; e, β-estradiol **1**. HPLC conditions as in Table 1 except that the eluent is 20 mmol $dm^{-3}$ phosphate buffer/$CH_3CN$=50/50 (V/V)(pH 6.0). Loaded amounts: **6**, 580 ng; **5**, 880 ng; **4**, 80 ng; **3** and **1**, 230 ng.

Figure 2, parts A and B, shows the separation of **1**, **3**, **4**, **5** and **6** on the base polymer and MIP, respectively. On the base polymer, **1** and **3**, and **4** and **5** were overlapped, while these were completely separated on the MIP. This result indicates that the MIP for **1** could efficiently separate estrogens from other steroids such as testosterone and corticosterone and could separate estrogens one another.

The results obtained above indicate that the MIP prepared shows high selectivity for β-estradiol and moderate selectivity for other estrogens. The MIP, which is an estrogen receptor mimic, might be able to recognize the endocrine disrupting chemicals such as diethylstilbestrol and bisphenol A. The detailed study is now under progress in our laboratory.

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