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Uniformly sized molecularly imprinted polymers for bisphenol A and β-estradiol: retention and molecular recognition properties in hydro-organic mobile phases

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Dedicated to Professor Terumichi Nakagawa on the occasion of his retirement and 63rd birthday.

Abstract

Uniformly sized molecularly imprinted polymers (MIPs) for bisphenol A (BPA) have been prepared using ethylene glycol dimethacrylate (EDMA) as a cross-linker and methacrylic acid, 2-diethylaminoethyl methacrylate or 4-vinylpyridine (4-VPY) as a functional monomer or without use of a functional monomer. The MIPs obtained for BPA were evaluated using a mixture of phosphate buffer (or water) and acetonitrile or only acetonitrile as the mobile phase. Among the MIPs prepared, that using 4-VPY showed the highest retentivity and selectivity for BPA. The highest selectivity factor, which is defined as the ratio of the retention factors (k) on the molecularly imprinted and non-imprinted polymers, $k_{\text{imprinted}}/k_{\text{non-imprinted}}$, was 9.4 for BPA on the BPA-imprinted 4-VPY–co-EDMA polymers, while that for β-estradiol on the β-estradiol-imprinted 4-VPY–co-EDMA polymers was 2.4. The differences in the selectivity factors between BPA and β-estradiol on the respective MIPs could be ascribable to differences in the number of interaction sites. It is plausible that the phenol groups of BPA could interact with two pyridyl groups of the MIP by hydrogen bonding interactions, while there is only one such site for β-estradiol. Furthermore, the results suggest that hydrophobic and hydrogen bonding interactions can play an important role in the retention and recognition of BPA and β-estradiol in the hydro-organic mobile phase, while hydrogen bonding interactions seem to be useful for the retention and recognition when acetonitrile is used as the mobile phase.

Keywords: Molecularly imprinted polymer; Bisphenol A; β-Estradiol; Endocrine disruptors; Molecular recognition

1. Introduction

Molecular imprinting techniques can afford complementary binding site(s) for an imprint molecule, and molecularly imprinted polymers (MIPs) are used for chromatographic separations, solid phase extractions, membranes, antibody-

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mimics and sensors for the purpose of specific recognition of target molecules [1–3]. Usually, non-aqueous bulk polymerization methods [4] have been utilized to obtain MIPs. Recently, suspension polymerization methods using water [5,6] or perfluorocarbon [7] as a suspension medium were employed for the preparation of MIPs. However, water has been thought to weaken interactions such as the hydrogen bonding interaction between the imprint molecule and functional monomers [8]. We prepared uniformly sized MIPs by a typical multi-step swelling and polymerization method [9] with water as the suspension medium. However, the MIP for (*S*)-naproxen [10–12] that we prepared gave similar enantioselectivity for naproxen to that prepared by non-aqueous bulk polymerization techniques by Kempe and Mosbach [13]. Furthermore, the MIPs for (*S*)-ibuprofen and -propranolol that we prepared showed molecular recognition abilities not only for the template molecule but also for structurally related compounds in hydro-organic mobile phases [14–16].

Recently, it has been reported that many chemicals released into the environment disrupt the endocrine system in wildlife and humans [17]. Thus, it is necessary to develop screening and assay methods for endocrine disruptors, some of which possess estrogen-like effects, in environmental and biological samples. In previous communications [18,19], we have reported preparation methods of MIPs for β-estradiol and bisphenol A (BPA), which are a major estrogen and a xenoestrogen, respectively, by a multi-step swelling and polymerization method using 4-vinylpyridine (4-VPY) as a functional monomer and ethylene glycol dimethacrylate (EDMA) as a cross-linker, respectively. Many β-estradiol-imprinted polymers have been prepared using methacrylic acid (MAA) [20,21] or 2-(methacryloyloxy)ethylphosphate [22] as a functional monomer by a bulk polymerization method and using MAA as a functional monomer by a precipitation polymerization method [23,24]. Also, BPA-imprinted polymers have been prepared using 4-VPY and divinylbenzene as a functional monomer and cross-linker, respectively, by a multi-step swelling and polymerization method [25]. BPA-imprinted polyamide membranes have also been prepared [26].

In this study, we prepared the MIPs for BPA and β-estradiol using a multi-step swelling and polymerization method, and evaluated the retention and recognition properties of BPA, β-estradiol and other structurally related compounds, steroidal and non-steroidal estrogens, and estrogen disruptors on the MIPs using a mixture of phosphate buffer (or water) and acetonitrile or only acetonitrile as the mobile phase. The retention and molecular recognition mechanisms of BPA and β-estradiol on the MIPs are discussed.

2. Experimental

2.1. Materials

EDMA was purchased from Tokyo Chemical Industry (Tokyo, Japan). MAA, 2-diethylaminoethyl methacrylate (DAEM) and 4-VPY were purchased from Wako Pure Chemical Industry (Osaka, Japan). These monomers were purified by general distillation techniques in vacuo to remove the polymerization inhibitor. 2,2′-Azobis(2,4-dimethylvaleronitrile), estrone and *p*-*t*-octylphenol were purchased from Wako Pure Chemical Industry (Osaka). Dienestrol, hexestrol and 16-epiestriol were purchased from Sigma–Aldrich Japan (Tokyo, Japan). β-Estradiol, α-estradiol, ethinylestradiol, estriol, testosterone, corticosterone, diethylstilbestrol and BPA were purchased from Nacalai Tesque (Kyoto, Japan). The structures of compounds used in this study are illustrated in Fig. 1. Other reagents and solvents were used without further purification.

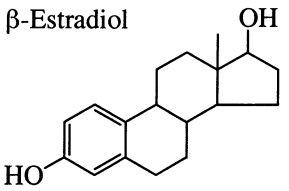
Water purified with a Nanopure II unit (Barnstead, Boston, MA) was used to prepare the eluent and the sample solution.

2.2. Preparation of uniformly sized MIPs

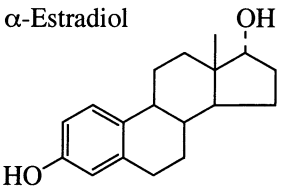
Preparation of uniformly sized, macroporous, MIPs for BPA and β-estradiol as well as non-imprinted polymer by a multi-step swelling and polymerization method was carried out as reported previously [11,18]. Table 1 shows the molar

Steroidal estrogens

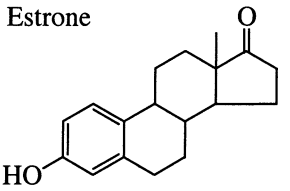
β-Estradiol



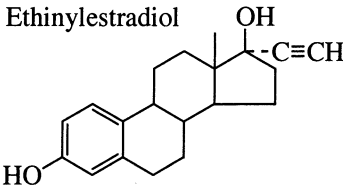
α-Estradiol



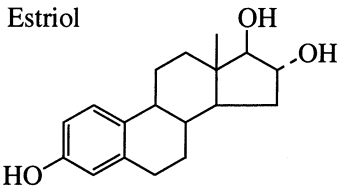
Estrone



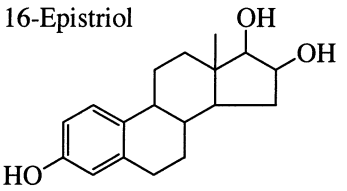
Ethinylestradiol



Estriol

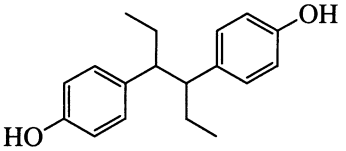


16-Epistriol

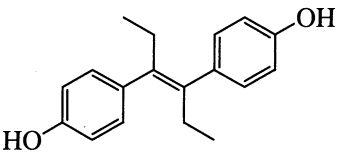


Non-steroidal estrogens

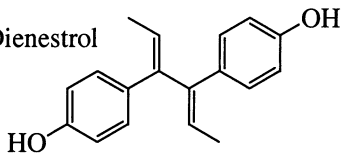
Hexestrol



Diethylstilbestrol

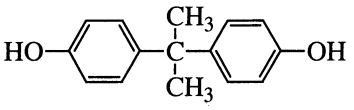


Dienestrol



Estrogen disruptors

Bisphenol A



p-t-Octylphenol

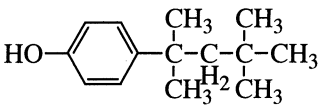


Fig. 1. Structures of the compounds used in this study.

Table 1
Molar amounts of template molecule, functional monomer and cross-linker used for the preparation of MIPs in this study

Polymer	Template molecule		Functional monomer		Cross-linker
	Type	Amount (mmol)	Type	Amount (mmol)	EDMA (mmol)
MIP 1	Bisphenol A	2	–	–	25
MIP 2	Bisphenol A	2	MAA	7	25
MIP 3	Bisphenol A	2	DAEM	7	25
MIP 4	Bisphenol A	2	4-VPY	7	25
MIP 5	Bisphenol A	1	4-VPY	3.5	25
MIP 6	Bisphenol A	4	4-VPY	7	25
MIP 7	Bisphenol A	4	4-VPY	14	25
MIP 8	β-Estradiol	2	4-VPY	14	25

amounts of template molecule, functional monomer and cross-linker used for the preparation of MIPs in this study. The obtained polymers were packed into a stainless-steel column (4.6 mm i.d. × 100 mm) by a slurry packing technique using methanol–2-propanol (2:1, v/v) as the slurry solvent and methanol as the packing solvent to evaluate their chromatographic characteristics.

2.3. Chromatography

The HPLC system used was composed of a PU-980 pump, a UV-970 spectrophotometer (both from Jasco, Tokyo, Japan), a Rheodyne 7125 injector with a 20-μl loop (Rheodyne, Cotani, CA), and a C-R6A integrator (Shimadzu, Kyoto, Japan). The flow rate was maintained at 1.0 ml/min. Detection was performed at 200 nm. All separations were carried out at 25 °C using a water bath (Thermo Minder Lt-100, Taitec, Saitama, Japan). The eluents are prepared using phosphoric acid, sodium dihydrogen phosphate, disodium hydrogen phosphate, trisodium phosphate and acetonitrile. The eluents used were specified in the legends of tables and figures.

The retention factor was calculated from the equation $k = (t_R - t_0)/t_0$, where t_R and t_0 are the retention times of retained and unretained solutes, respectively. The retention time of the unretained solute, t_0, was measured by injecting the solution whose organic modifier content was slightly different from that of the eluent. The selectivity factor was calculated from the equation $S = k_{imprinted}/k_{non\text{-}imprinted}$, where $k_{imprinted}$ and $k_{non\text{-}imprinted}$ are the retention factors of a solute on the molecularly imprinted and non-imprinted polymers, respectively.

3. Results and discussion

3.1. Selection of a functional monomer

As shown in Table 1, we prepared the MIPs for BPA without using a functional monomer (MIP 1), but using MAA, DAEM and 4-VPY (MIPs 2, 3 and 4, respectively). The MIPs were evaluated using hydro-organic mobile phases to examine the effect of a functional monomer on the retention and selectivity factors for BPA. As shown in Table

Table 2
Retention factors and selectivity factors of BPA on the BPA-imprinted polymers prepared using different functional monomers[a]

Polymer	Retention factor		Selectivity factor
	Imprinted	Non-imprinted	
MIP 1	3.87	2.09	1.85
MIP 2	2.77	1.31	2.12
MIP 3	5.44	3.31	1.64
MIP 4	31.9	4.48	7.12

[a] HPLC conditions: column size, 4.6 mm i.d. × 100 mm; column temperature, 25 °C; eluent, sodium dihydrogen phosphate and disodium hydrogen phosphate (pH 5.1; 20 mM)–acetonitrile (1:1, v/v); flow rate, 1.0 ml/min; detection, 200 nm; loaded amount, 240 ng.

2, BPA-imprinted 4-VPY–co-EDMA polymer (MIP 4) gave the highest retention and selectivity factors for BPA among the MIPs prepared. MAA has been used for the preparation of MIPs for β-estradiol [20,21,23,24] as a functional monomer, largely due to the potential for hydrogen bonding and ionic interactions with carboxyl groups. However, our results reveal that MAA is not a good functional monomer for the preparation of MIP for BPA. This could be because the hydrogen bonding interactions between BPA and MAA may be weak in hydro-organic mobile phases. On the other hand, the hexestrol-imprinted polymers prepared using basic DAEM as a functional monomer showed excellent molecular recognition ability for hexestrol [27]. The low selectivity of MIP 3, prepared using DAEM as a functional monomer, could be ascribable to the high solubility of DAEM in aqueous phases. In the following experiments, we used 4-VPY as the functional monomer for the preparation of the MIP for BPA. Similarly, we prepared β-estradiol-imprinted 4-VPY–co-EDMA polymer (MIP 8) for comparison with the MIP for BPA (MIP 4).

Table 3 shows the retention and selectivity factors for BPA on BPA-imprinted 4-VPY–co-EDMA polymers (MIPs 4–7) prepared with different molar amounts of BPA and 4-VPY, where the eluent used was H_2O–acetonitrile (2:3, v/v). The retention factors of BPA increased with an increase of molar amounts of BPA and 4-VPY. The maximum selectivity factor of BPA, which is 9.4, was observed with the use of 4 mmol of BPA and 7 mmol of 4-VPY, MIP 6. Thus, we could control the retentivity and selectivity of the MIPs by changing the molar amounts of BPA and 4-VPY. It could be useful for the preparation of BPA-imprinted 4-VPY–co-EDMA polymers used as an adsorbent.

Table 3
Retention factors and selectivity factors of BPA on the BPA-imprinted 4-VPY–co-EDMA polymers prepared with different molar amounts of BPA and 4-VPY[a]

Polymer	Retention factor		Selectivity factor
	Imprinted	Non-imprinted	
MIP 4	15.0	2.22	6.74
MIP 5	5.74	1.59	3.62
MIP 6	21.0	2.22	9.44
MIP 7	24.6	2.89	8.51

[a] HPLC conditions as in Table 2 except that the eluent used was H_2O–acetonitrile (2:3, v/v).

3.2. Retention properties of various compounds on EDMA and 4-VPY–co-EDMA polymers

The effects of eluent pH and acetonitrile content on the retention properties of various compounds were investigated to clarify the retention and molecular recognition mechanism of BPA and β-estradiol on the EDMA and 4-VPY–co-EDMA polymers. Fig. 2A–C show the effects of eluent pH on the retention properties of BPA, phenol and benzene on non-imprinted EDMA, non-imprinted 4-VPY–co-EDMA, and BPA-imprinted 4-VPY–co-EDMA (MIP 4) polymers, respectively, where the eluent used is phosphoric acid and/or sodium phosphate (20 mM)–acetonitrile (2:3, v/v). The retention factor of a neutral compound, benzene, was not affected by the eluent pH tested on the EDMA polymers. Since EDMA polymers had no ionizable groups in the polymer backbone, solutes could be mainly retained by hydrophobic interactions. In spite of the protonation of a pyridyl group on the 4-VPY–co-EDMA polymers in the low eluent pH region, the retention of benzene was not affected by the eluent pH tested on the 4-VPY–co-EDMA polymers. This could be due to the fact that the amount of the pyridyl group is low on the 4-VPY–co-EDMA polymers and/or that the degree of protonation of a pyridyl group is not so large at low eluent pH, as reported previously [14]. The retention factors of BPA and phenol on the EDMA and 4-VPY–co-EDMA polymers remained unchanged in the eluent pH range of 2–10, while they decreased in the range of 10–13. Decreases in the retention factors of phenolic compounds on the EDMA and 4-VPY–co-EDMA polymers are due to dissociation of the phenolic hydroxyl group. The retention factors of phenolic compounds on non-imprinted 4-VPY–co-EDMA polymers were twice those on the EDMA polymers. This could be ascribable to the hydrogen bonding interactions between the phenolic hydroxyl group and the pyridyl group on the

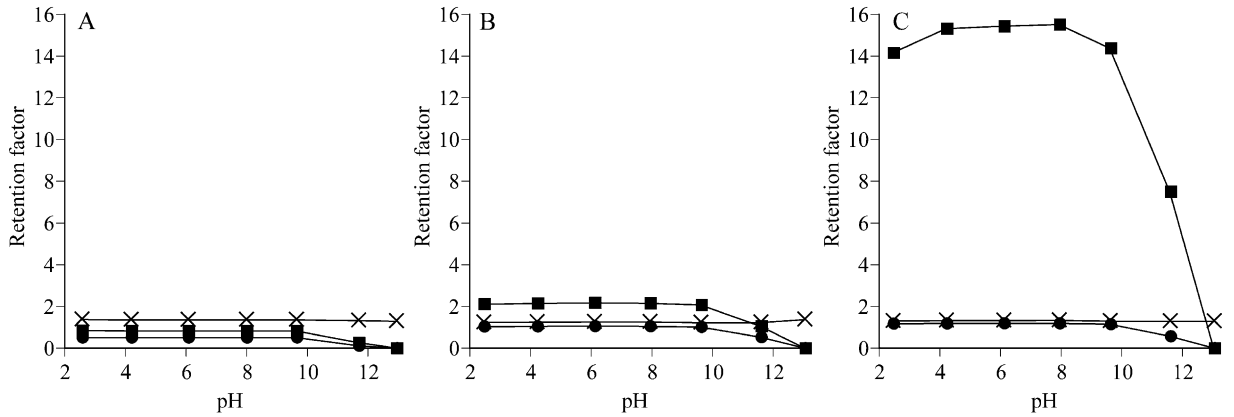


Fig. 2. Effects of eluent pH on the retention factors of BPA, phenol and benzene on the non-imprinted EDMA (A), non-imprinted 4-VPY–co-EDMA (B), and BPA-imprinted 4-VPY–co-EDMA (MIP 4) (C) polymers. Symbols: ■–■, BPA; ●–●, phenol; × – ×, benzene. HPLC conditions: column size, 4.6 mm i.d. × 100 mm; column temperature, 25 °C; eluent, phosphoric acid and/or sodium phosphate (20 mM)–acetonitrile (2:3, v/v); flow rate, 1.0 ml/min; detection, 200 nm; loaded amount, 240 ng.

4-VPY–co-EDMA polymers. Thus, phenolic compounds are retained on the 4-VPY–co-EDMA polymers by hydrogen bonding interactions with a pyridyl group on the 4-VPY–co-EDMA polymers in addition to the hydrophobic interactions with the polymer backbone. Phenol showed almost the same retention on both the BPA-imprinted and non-imprinted 4-VPY–co-EDMA polymers, while BPA showed about seven times longer retention on the imprinted 4-VPY–co-EDMA polymers. This could be explained by a molecular imprinting effect.

Fig. 3A–C show the effects of acetonitrile content on the retention properties of BPA, phenol and benzene on non-imprinted EDMA, non-imprinted 4-VPY–co-EDMA, and BPA-imprinted 4-VPY–co-EDMA (MIP 4) polymers, respectively, where the eluent used is a mixture of H_2O and acetonitrile. The retention of benzene on the EDMA and 4-VPY–co-EDMA polymers decreased with an increase in acetonitrile content. The retention factors of phenolic compounds on the EDMA and 4-VPY–co-EDMA polymers decreased with an increase in the acetonitrile

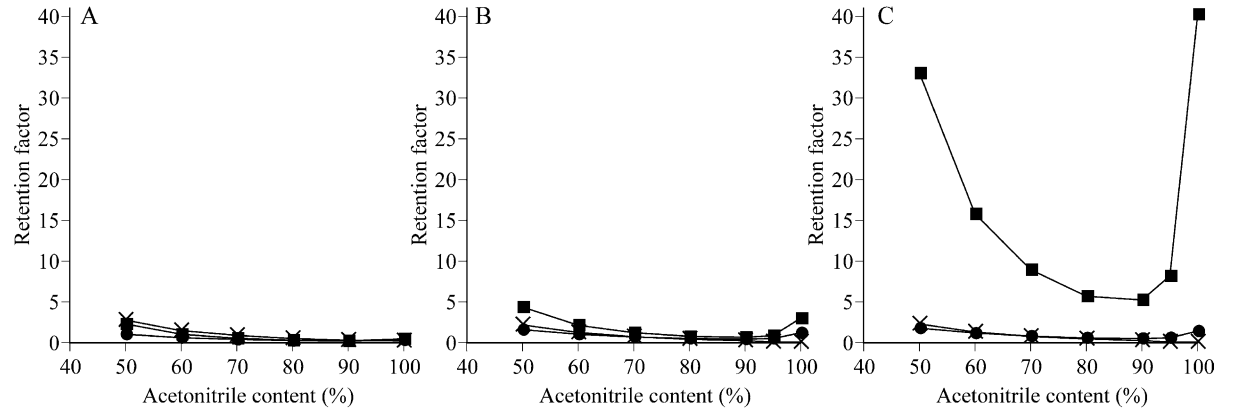


Fig. 3. Effects of acetonitrile content on the retention factors of BPA, phenol and benzene on the non-imprinted EDMA (A), non-imprinted 4-VPY–co-EDMA (B), and BPA-imprinted 4-VPY–co-EDMA (MIP 4) (C) polymers. Symbols as in Fig. 2. HPLC conditions as in Fig. 2 except that the eluent used was a mixture of H_2O and acetonitrile.

content from 50 to 90%. With a further increase in the acetonitrile content from 90 to 100%, the retentions on the EDMA polymers slightly increased. However, the retention factors on the 4-VPY–co-EDMA polymers in 100% acetonitrile were nearly equal to those in 50% acetonitrile. In hydro-organic mobile phases, the retention of phenolic compounds on the 4-VPY–co-EDMA polymers was mainly due to hydrophobic interactions in addition to the hydrogen bonding interactions of the OH-groups with 4-VPY. In 100% acetonitrile, in the absence of water, the hydrogen bonding interactions became dominant.

Fig. 4 shows the effects of eluent pH and acetonitrile content on the retention properties of β-estradiol on non-imprinted 4-VPY–co-EDMA and β-estradiol-imprinted 4-VPY–co-EDMA (MIP 8) polymers. The retention properties of β-estradiol on β-estradiol-imprinted 4-VPY–co-EDMA polymers were similar to those of BPA on BPA-imprinted 4-VPY–co-EDMA polymers. These results reveal that hydrophobic and hydrogen bonding interactions can play an important role in the retention and recognition of β-estradiol in hydro-organic mobile phases, while with acetonitrile as the mobile phase, hydrogen bonding interactions can work for the retention and recognition.

3.3. Selectivity of BPA- and β-estradiol-imprinted 4-VPY–co-EDMA polymers for various compounds

The molecular recognition abilities of BPA- and β-estradiol-imprinted 4-VPY–co-EDMA polymers toward various compounds were evaluated using the selectivity factor, $k_{\text{imprinted}}/k_{\text{non-imprinted}}$. Table 4 shows the selectivity factors of steroidal and non-steroidal estrogens, and estrogen disruptors on BPA-imprinted 4-VPY–co-EDMA poly-

Table 4
Retention factors and selectivity factors of steroidal and non-steroidal estrogens, and estrogen disruptors on BPA-imprinted 4-VPY–co-EDMA polymers (MIP 4)[a]

Solute	Retention factor		Selectivity factor
	Imprinted	Non-imprinted	
Steroidal and non-steroidal estrogens			
β-Estradiol	2.63	1.83	1.44
Diethylstilbestrol	5.84	3.42	1.71
Dienestrol	5.62	2.28	1.71
Hexestrol	5.34	3.09	1.73
Estrogen disruptors			
Bisphenol A	15.0	2.22	6.74
p-t-Octylphenol	4.33	2.91	1.49

[a] HPLC conditions as in Table 2 except that the eluent used was H_2O–acetonitrile (2:3, v/v).

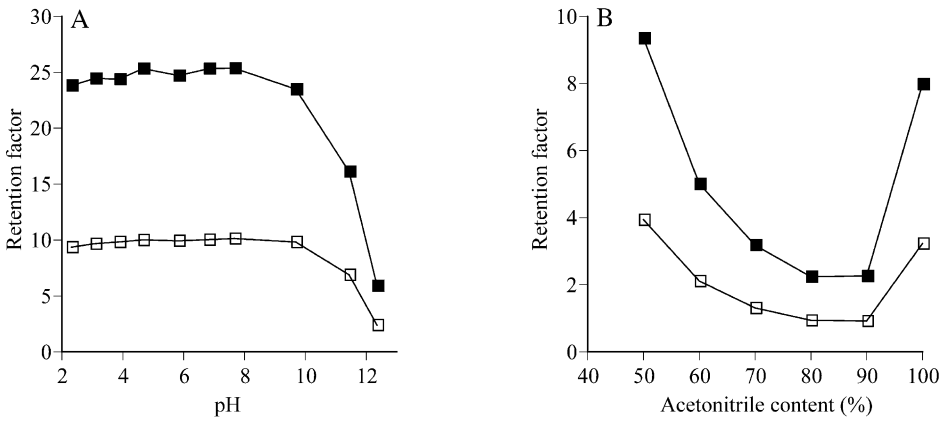


Fig. 4. Effects of eluent pH (A) and acetonitrile content (B) on the retention properties of β-estradiol on non-imprinted 4-VPY–co-EDMA and β-estradiol-imprinted 4-VPY–co-EDMA (MIP 8) polymers. Symbols: ■–■, β-estradiol-imprinted 4-VPY–co-EDMA polymers (MIP 8); □–□, non-imprinted 4-VPY–co-EDMA polymers. The eluent used for part A was phosphoric acid and/or sodium phosphate (20 mM)–acetonitrile (3:2, v/v), and that the eluent used for part B was a mixture of H_2O and acetonitrile. Other HPLC conditions as in Fig. 2.

mers (MIP 4), where the eluent used was H_2O–acetonitrile (2:3, v/v). The highest selectivity factor of 6.74 was obtained for BPA. On the other hand, the MIP 4 gave slight selectivity factors for non-steroidal estrogens (ranging from 1.71 to 1.73) and for β-estradiol and *p*-*t*-octylphenol of 1.44 and 1.49, respectively. Recently, hexestrol-imprinted polymer was prepared by a bulk polymerization method [27]. The obtained MIP showed high cross reactivity with diethylstilbestrol and dienestrol, which structurally resemble BPA. However, the BPA-imprinted 4-VPY–co-EDMA polymers that we prepared could selectively recognize BPA among the phenolic compounds tested. This reveals that the distance between the two phenol groups is also an important factor for the recognition of BPA on the BPA-imprinted 4-VPY–co-EDMA polymers.

Table 5 shows the selectivity factors of steroidal and non-steroidal estrogens, estrogen disruptors and other compounds on β-estradiol-imprinted 4-VPY–co-EDMA polymers (MIP 8), where the eluent used was sodium dihydrogen phosphate and disodium hydrogen phosphate (pH 5.1; 20 mM)–acetonitrile (1:1, v/v). Over the eluent pH range of 2–10, the selectivity factors of the compounds tested were not much affected by the eluent pH change. The highest selectivity factor of 2.36 was obtained for β-estradiol. On the other hand, the MIP 8 gave moderate selectivity factors for other steroidal estrogens (ranging from 1.64 to 1.97) and for non-steroidal estrogens (ranging from 1.32 to 1.42), and slight selectivity factors for BPA and *p*-*t*-octylphenol of 1.22 and 1.29, respectively. Other compounds, benzene, phenol, naphthalene and 1-naphthol, were not recognized on the MIP 8.

Why are there large differences in the selectivity factors of BPA and β-estradiol between the respective imprinted 4-VPY–co-EDMA polymers? It has been reported that increasing the number of interaction sites on the template leads to sites of higher specificity [28]. Thus, the phenol groups of BPA may interact with the two pyridyl groups by hydrogen bonding interactions, while there is only one hydrogen bonding interaction site for β-estradiol. The differences in the selectivity factors between the BPA- and β-estradiol-imprinted 4-VPY–co-EDMA polymers could be ascribable to differences in the number of interaction sites on the template molecule.

Table 5
Retention factors and selectivity factors of steroidal estrogens, non-steroidal estrogens, estrogen disruptors and other compounds on β-estradiol-imprinted 4-VPY–co-EDMA polymers (MIP 8)[a]

Solute	Retention factor		Selectivity factor
	Imprinted	Non-imprinted	
Steroidal estrogens			
β-Estradiol	11.2	4.76	2.36
α-Estradiol	9.16	5.13	1.79
Estriol	2.37	1.31	1.81
16-Epiestriol	4.83	2.59	1.86
Estrone	6.72	4.09	1.64
Ethinylestradiol	13.8	7.03	1.97
Non-steroidal estrogens			
Diethylstilbestrol	15.5	11.2	1.39
Dienestrol	14.3	10.8	1.32
Hexestrol	13.5	9.53	1.42
Estrogen disruptors			
Bisphenol A	8.67	7.08	1.22
p-*t*-Octylphenol	9.34	7.26	1.29
Other compounds			
Benzene	2.43	2.31	1.05
Phenol	2.56	2.53	1.01
Naphthalene	7.46	7.20	1.04
1-Naphthol	11.6	11.4	1.02

[a] HPLC conditions as in Fig. 4 except that the eluent used was sodium dihydrogen phosphate and disodium hydrogen phosphate (pH 5.1; 20 mM)–acetonitrile (1:1, v/v).

3.4. Separation of BPA or β-estradiol on BPA- or β-estradiol-imprinted 4-VPY–co-EDMA polymers

Fig. 5A–B show the separation of BPA, hexestrol, diethylstilbestrol, dienestrol and β-estradiol on the non-imprinted 4-VPY–co-EDMA and BPA-imprinted 4-VPY–co-EDMA (MIP 4) polymers, respectively. On the non-imprinted 4-VPY–co-EDMA polymers, there was overlapping of all compounds, while BPA was completely separated from the other compounds on the BPA-imprinted 4-VPY–co-EDMA polymers. Fig. 6A–B shows the separation of β-estradiol, estrone, estriol, testosterone and corticosterone on the non-im-

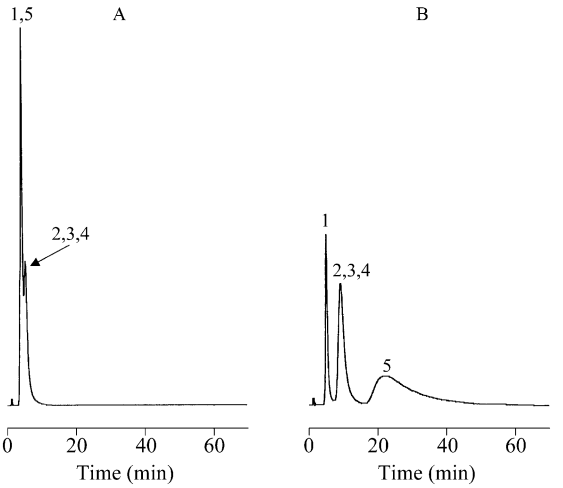


Fig. 5. Separation of phenolic estrogenic and xeno-estrogenic compounds on the non-imprinted 4-VPY–co-EDMA (A) and BPA-imprinted 4-VPY–co-EDMA polymers (MIP 4) (B). Peak assignments: 1, β-estradiol; 2, hexestrol; 3, dienestrol; 4, diethylstilbestrol; 5, BPA. HPLC conditions as in Fig. 2 except that the eluent used was H_2O–acetonitrile (2:3, v/v). Loaded amounts: BPA, 400 ng; hexestrol, dienestrol and diethylstilbestrol, 80 ng; β-estradiol, 160 ng.

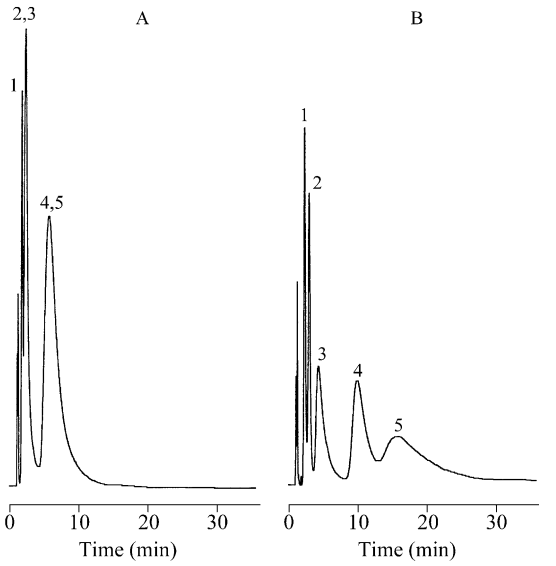


Fig. 6. Separation of steroids on the non-imprinted 4-VPY–co-EDMA (A) and β-estradiol-imprinted 4-VPY–co-EDMA polymers (MIP 8) (B). Peak assignments: 1, corticosterone; 2, testosterone; 3, estriol; 4, estrone; 5, β-estradiol. HPLC conditions as in Fig. 4 except that the eluent used was sodium dihydrogen phosphate and disodium hydrogen phosphate (pH 5.1; 20 mM)–acetonitrile (1:1, v/v). Loaded amounts: corticosterone, 580 ng; testosterone, 880 ng; estriol, 80 ng; estrone and β-estradiol, 230 ng.

printed 4-VPY–co-EDMA and β-estradiol-imprinted 4-VPY–co-EDMA (MIP 8) polymers, respectively. On the non-imprinted 4-VPY–co-EDMA polymers, there was overlapping of β-estradiol and estrone, and estriol and testosterone, but complete separation of these compounds on the β-estradiol-imprinted 4-VPY–co-EDMA polymers. These results indicate that the MIPs for BPA and β-estradiol can efficiently separate the target molecule from other compounds.

4. Conclusions

We prepared uniformly sized MIPs for BPA and β-estradiol by a multi-step swelling and polymerization method. The obtained MIPs were evaluated using hydro-organic mobile phases. The highest selectivity factors of BPA and β-estradiol, obtained with the respective imprinted 4-VPY–co-EDMA polymers, were 9.4 and 2.4. The results suggest that the phenol groups of BPA can interact with two pyridyl groups by hydrogen bonding interactions, while there is one hydrogen bonding interaction site for β-estradiol. The differences in the selectivity factors between the BPA- and β-estradiol-imprinted 4-VPY–co-EDMA polymers may be ascribable to differences in the number of interaction sites on the template. The results also suggest that hydrophobic and hydrogen bonding interactions can play an important role in the retention and recognition of BPA and β-estradiol in hydro-organic mobile phases, while hydrogen bonding interactions seem to be useful for the retention and recognition when acetonitrile is used as the mobile phase.

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