EXCLI Journal 2006;5:103-117 – ISSN 1611-2156
Received: 5. September 2006, accepted: 10. September 2006, published: 26. September 2006

Original article:

In silico design for synthesis of molecularly imprinted microspheres specific towards bisphenol A by precipitation polymerization

Chanin Nantasenamat[1], Chartchalerm Isarankura-Na-Ayudhya[1], Leif Bülow[2], Lei Ye[2], Virapong Prachayasittikul[1*]

[1]Department of Clinical Microbiology, Faculty of Medical Technology, Mahidol University, Bangkok 10700, Thailand. [2]Pure and Applied Biochemistry, Chemical Center, Lund University, Box 124, 221 00, Lund, Sweden. *Corresponding author. Telephone: 662-849-6318, Fax: 662-849-6330, E-mail: mtvpr@mahidol.ac.th

ABSTRACT

Bisphenol A (BPA), a ubiquitous chemical used in industries, has attracted great attention due to its widespread leakage to the environment and foodstuff. This has spurred great interest in the preparation of synthetic polymers capable of selectively sequestering BPA. In this study, theoretical calculation was utilized to confirm the selection of suitable functional monomer capable of strong interaction with BPA. It was found that 4-vinylpyridine was the optimal functional monomer as demonstrated in the literature. A series of molecularly imprinted polymers (MIPs) were prepared by varying the functional monomer, cross-linker, and porogen. After template removal, rebinding with the original template molecule was carried out in acetonitrile, acetonitrile/water (50/50 – 20/80, v/v). 4-vinylpyridine co-polymerized with ethylene glycol dimethacrylate (4VPY-co-EDMA) and 4-vinylpyridine co-polymerized with trimethylolpropane trimethacrylate (4VPY-co-TRIM) were found to exhibit good binding performance towards bisphenol A in acetonitrile. However, only 4VPY-co-EDMA was able to maintain its imprinting effect in acetonitrile/water (50/50 v/v) whereas 4VPY-co-TRIM totally lost its imprinting effect.

Keywords: Bisphenol A, estrogen disruptor, precipitation polymerization, microspheres, molecular imprinting

INTRODUCTION

Bisphenol A (BPA), also known as 2,2-bis(4-hydroxyphenyl)propane, is a common organic compound used in the production of epoxy resins, phenol resins, flame-retardants, polycarbonates, polyacrylates, polyesters, lacquer coatings on food cans. BPA is an estrogen disruptor which can be attributed to the structural similarity with estrogen. The physicochemical properties of BPA suggest that it is stable in aqueous environment due to its low volatility (Henry's constant of 1.0 x 10^{-10} atm-m^3/mol) and solubility in water (120 mg/L) (Howard, 1993). It has been reported that BPA can leak from food and drink packagings (Goodson et al., 2002), waste landfills (Yamamoto et al., 2001), and plastic wastes (Yamamoto and Yasuhara, 1999).

Molecular imprinting is a technique that affords the production of synthetic polymers that are capable of selective recognition of target molecules of interest. These MIPs are produced by allowing the complexation between template and functional monomer molecules, either by the covalent or non-covalent approach. MIPs have common application as separation media for chromatography (Tamayo and Martin-Esteban, 2005) and solid phase extraction (Caro et al., 2004). Furthermore, there are a plethora of other applications for MIPs namely as recognition elements for

biological and chemical sensors (Lin et al., 2004; Piacham et al., 2005), synthetic receptors for drug assays (Vlatakis et al., 1993), enzyme mimetics (Piacham et al., 2003), and nanoreactors for combinatorial synthesis of new enzyme inhibitors (Mosbach et al., 2001).

There has been a significant interest in the molecular imprinting of bisphenol A as seen by the extensive publications found in the literature. This includes the preparation of polymers specific for bisphenol A by the non-covalent approach with different polymerization methods, particularly bulk polymerization (Navarro-Villoslada et al., 2004; San Vicente et al., 2004), emulsion polymerization (Watabe et al., 2005a), suspension polymerization (Kawaguchi et al., 2005), precipitation polymerization (Jiang et al., 2006; Joshi et al., 1999), multi-step swelling and polymerization (Watabe et al., 2005a; Sanbe and Haginaka, 2003; Sanbe et al., 2003; Watabe et al., 2005b; Watabe et al., 2004), restricted access media surface modification (RAM-MIP) (Sambe et al., 2005), immobilized template grafting (Lee and Takeuchi, 2005), self-assembled monolayer (Tsuru et al., 2006), in situ polymerization (Ou et al., 2006), phase inversion method (Yang et al., 2005), phase inversion scaffold membrane (Takeda and Kobayashi, 2006), and amylose-based host matrix (Kanekiyo et al., 2002; Kanekiyo et al., 2003) for the preparation of bisphenol A imprinted polymers. Furthermore, bisphenol A imprinted polymers has also been prepared by the covalent approach using bulk polymerization (Ikegami et al., 2004a; Ikegami et al., 2004b; Takeda and Kobayashi, 2005).

In this study, an extensive investigation was performed utilizing both quantum chemical calculation and precipitation polymerization to obtain molecularly imprinted microspheres capable of selective recognition of bisphenol A. The formulation for the preparation of the molecularly imprinted microspheres was optimized by varying the functional monomer, cross-linker, porogen, and rebinding solvent. The results from our computer simulation confirmed the use of 4VPY as the optimal functional monomer for the recognition of bisphenol A. Furthermore, the role of water on the recognition of bisphenol A via hydrophobic interaction was also investigated.

MATERIALS AND METHODS

Materials

Bisphenol A (BPA), divinylbenzene (DVB, technical grade, 80%), ethylene glycol dimethacrylate (EDMA), trimethylolpropane trimethacrylate (TRIM), and 2,2'-azobisisobutyronitrile (AIBN) were purchased from Aldrich. 4-vinylpyridine (4VPY), methacrylic acid (MAA), and toluene (analytical grade) were obtained from Merck. Acetonitrile (HPLC grade) was purchased from BDH. Trifluoroacetic acid was acquired from Fluka.

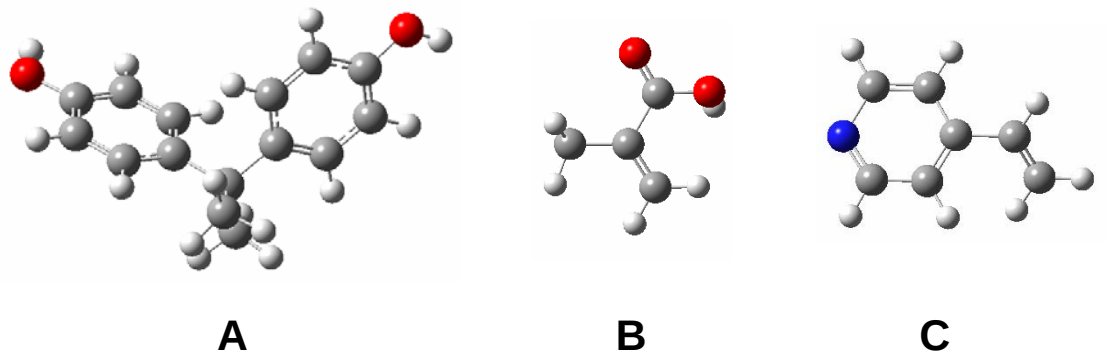
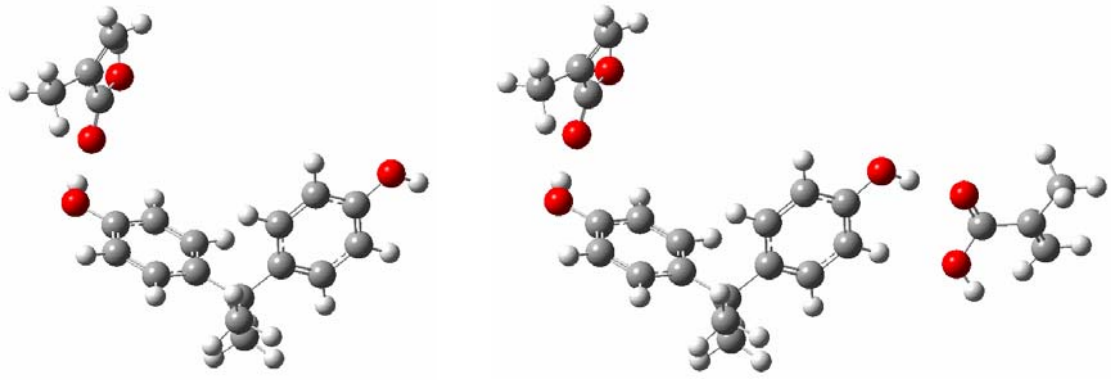


Figure 1: The optimized conformation of bisphenol A (BPA) (A), methacrylic acid (MAA) (B), and 4-vinylpyridine (4VPY) (C) at HF/3-21G(d).



A B

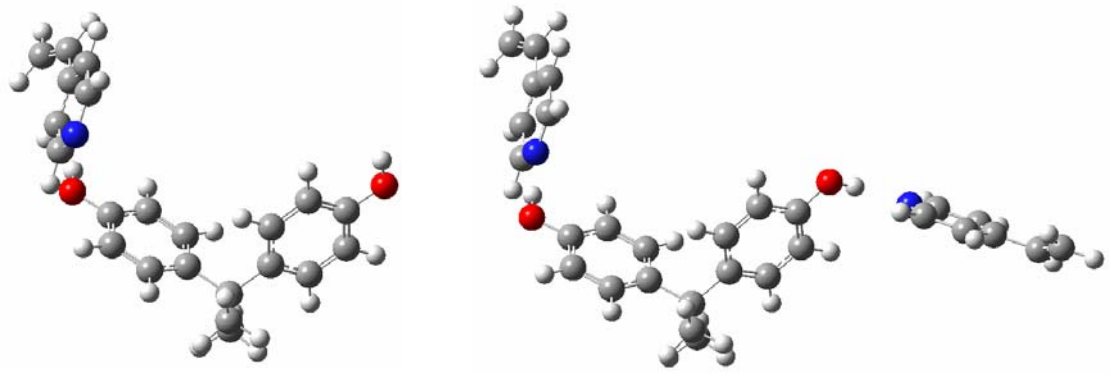
Figure 2: The simulated interaction of bisphenol A (BPA) with methacrylic acid (MAA) as 1:1 complex (A) and 1:2 complex (B).

Quantum Chemical Calculation

The chemical structures of the template and functional monomer molecule (see Figure 1) was drawn into the computer using GaussView (Dennington II et al., 2003). The three-dimensional structure of the compounds was refined by full geometry optimization at the Hartree-Fock (HF) level of theory using the 3-21G(d) basis set with Gaussian 03W (Frisch et al., 2004). The energy of the molecules were derived from single point calculation at the Density Functional Theory (DFT) level using Becke's three-parameter Lee-Yang-Par (B3LYP) functional and 6-31G(d) basis set. The template-monomer complex was drawn as 1:1 and 1:2 complexes in which the template bisphenol A molecule is in complexation with 1 molecule of the functional monomer and 2 molecules of the functional monomer, respectively (see Figures 2 and 3). The interaction energy (Table 1) was derived from the following equation:

$$\Delta E = \left| E_{template-monomer} - E_{template} - E_{monomer} \right| \quad (1)$$

where ΔE is the interaction energy, $E_{template-monomer}$ is the energy of template-monomer complex, $E_{template}$ is the energy of template molecule, and $E_{monomer}$ is the energy



A B

Figure 3: The simulated interaction of bisphenol A (BPA) with 4-vinylpyridine (4VPY) as 1:1 complex (A) and 1:2 complex (B).

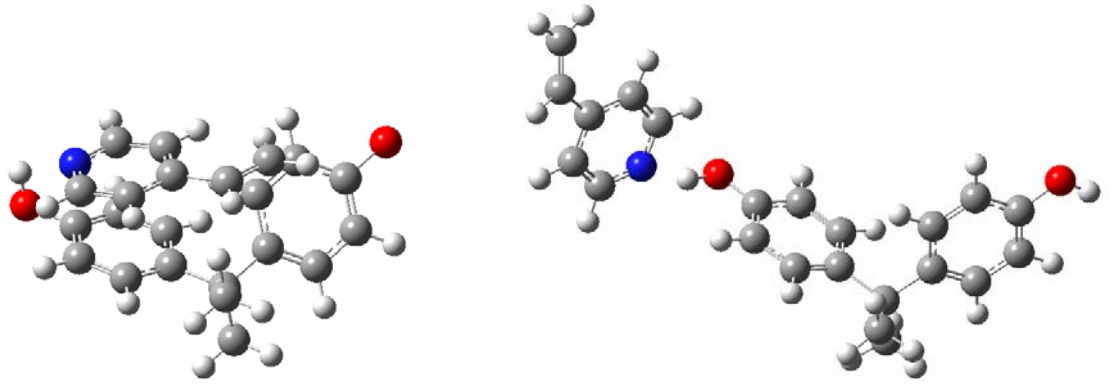


Figure 4: The input structure (A) and the optimized structure (B) for calculation of bisphenol A-4-vinylpyridine (BPA-4VPY) as 1:1 complex via ring stacking.

of functional monomer molecules.

Artificial Neural Network

The molecular descriptors used as input for prediction via artificial neural network (Nantasenamat et al., 2005a; Nantasenamat et al. 2005b; Nantasenamat et al., 2006) were calculated using RECON and the descriptors were reduced with Unsupervised Forward Selection (UFS) (Whitley et al., 2000) as described previously (Nantasenamat et al., 2005b). The training set were derived from the data set used in our previous study where data samples of bisphenol A were removed and used as the testing set. Calculations were performed for 10 runs using Weka 3.4.8 (Witten and Frank, 2000).

Calculation of octanol/water partition coefficient (logP)

The logP for EDMA, TRIM, and DVB was calculated using MarvinSketch (ChemAxon).

Polymer Preparation

The compositions of molecularly imprinted and non-imprinted microspheres prepared by precipitation polymerization are detailed in Table 2. Bisphenol A, the functional monomer, the crosslinker and AIBN were dissolved in the different imprinting solvents in a borosilicate glass tube. The solution was purged with a gentle flow of Ar for 5 min and sealed under Ar. The borosilicate glass tube was fixed horizontally in a Stovall HO-10 hybridization oven (Greensboro, NC, USA), and rotated at a speed of 20 rpm. The temperature was ramped from 20 °C to 70 °C within 20 min, thereafter kept for 24 h. A non-imprinted control polymer (CP) was prepared under the same condition except for the omission of the template.

Template Removal

The microspheres obtained were collected by centrifugation at 18,000 rpm for 20 min using an RC5C superspeed refrigerated centrifuge from Beckman (Palo Alto, CA). The template molecule was removed from the microspheres by washing repeatedly with 30 mL of methanol containing 10% acetic acid (v/v) followed by washing twice with methanol. The polymer was dried overnight under vacuum.

Rebinding Studies

The rebinding experiments were performed by incubating 20 mg of imprinted or non-imprinted polymers with 0.05 mg of bisphenol A in 1 mL of acetonitrile, acetonitrile/water (50/50 v/v), or acetonitrile/water (20/80 v/v) with gentle agitation at room temperature for 20 h. The solution was centrifugated and the free concentration of bisphenol A was quantitated by RP-HPLC on a PuroSpher RP-18e column from Merck (Darmstadt, Germany).

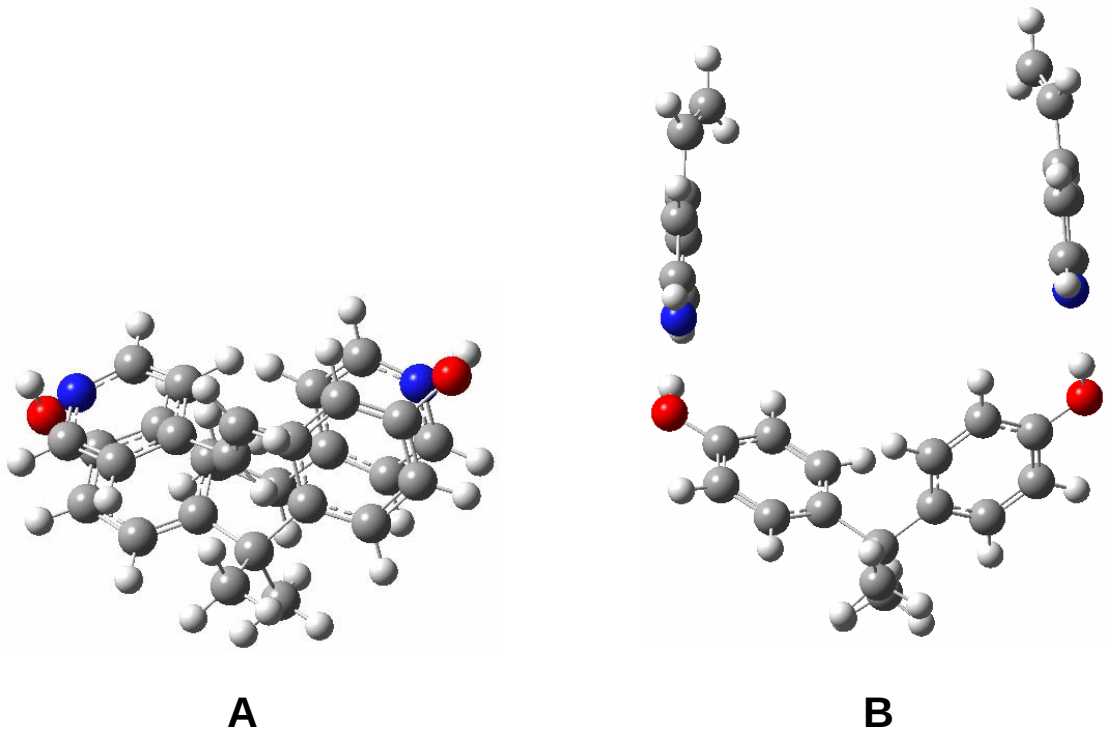


Figure 5: The input structure (A) and the optimized structure at HF/3-21G(d) (B) for calculation of bisphenol A-4-vinylpyridine (BPA-4VPY) as 1:2 complex via ring stacking.

The LaChrom HPLC system (diode array detector L-7455, autosampler L-7200, pump L-7100, and interface D-7000) was from Hitachi-Merck (Darmstadt, Germany). BPA was detected at 280 nm using as the mobile phase acetonitrile/water (40/60 v/v) containing 0.1% trifluoroacetic acid. The flow rate was set to 1 mL/min and carried out at room temperature. The injection volume was 10 μL.

Scanning Electron Microscopy

Scanning electron microscopy (SEM) imaging was carried out on a JEOL JSM-6700F Field Emission Scanning Electron Microscope (Tokyo, Japan). Polymer microspheres were sputter coated with gold prior to the SEM measurement.

RESULTS AND DISCUSSION

Computer simulation of template-monomer interaction

The optimized conformation of the individual molecules of template BPA and functional monomers MAA and 4VPY are shown in Figure 1. The proposed conformation of template-monomer as 1:1 and 1:2 complexes as elucidated from computer simulation is shown in Figures 2 and 3 for BPA-MAA and BPA-4VPY, respectively. Possible ring stacking interaction of 4VPY with BPA was investigated by placing the heterocylic ring of 4VPY in close proximity to the phenol rings of BPA. This was performed for 1:1 (Figure 4) and 1:2 (Figure 5) complexes of BPA-4VPY. The computer simulation pointed out that BPA did not engage in ring stacking interaction with 4VPY as 4VPY displaces from the initial ring stacking placement to interact with the hydroxyl group of BPA via its heterocyclic nitrogen, which is observed for both 1:1 and 1:2 BPA-4VPY complexes.

The template-monomer interaction energy is calculated from equation 1 and the results are shown in Table 1. The interaction energies of BPA-MAA and BPA-4VPY as 1:1 complex are 38.74889 kJ mol-1 and 42.96882 kJ mol-1, respectively. Furthermore, the interaction energies of

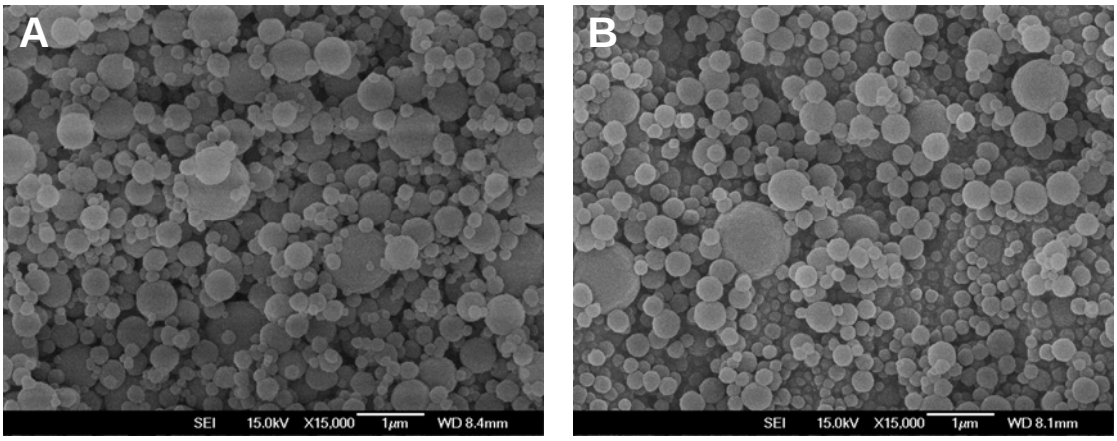


Figure 6: Scanning electron micrograph of imprinted (A) and non-imprinted (B) 4-vinylpyridine co-polymerized with divinylbenzene (4VPY-co-DVB) prepared in acetonitrile/toluene (75/25 v/v).

BPA-MAA and BPA-4VPY as 1:2 complex are 71.96327 kJ mol-1 and 83.25323 kJ mol-1, respectively. Thus, it is observed that the computed interaction energy is in good accordance with the experimental results that 4VPY is the better functional monomer than MAA since BPA-4VPY gave binding capacity and interaction energy values greater than that of BPA-MAA.

Artificial neural network calculation of template-monomer interaction

In our previous work, artificial neural network was used for the quantitative prediction of the imprinting factor of MIPs. The imprinting factors were derived from retention factor values taken from HPLC measurements. The strength of template-monomer interaction is positively correlated with the magnitude of the imprinting factor. Therefore, in this study the predicted imprinting factor is used as a measure of the strength of template-monomer interaction. Our calculation corroborate the selection of 4-vinylpyridine as the optimal functional monomer as revealed by the greater predicted imprinting factor value of 3.2059 for 4-vinylpyridine to 2.0854 of methacrylic acid.

Scanning electron micrographs

It should be noted that the SEM images of DVB-based microspheres were those prepared with a mixture of toluene and acetonitrile as porogen. On the other hand, EDMA and TRIM-based microspheres were prepared with acetonitrile as porogen. It is observed that there is no apparent differences between imprinted and non-imprinted 4VPY-co-DVB microspheres as both were found to be polydispersed (Figure 6). Non-imprinted MAA-co-DVB microspheres (Figure 7B) are found to be larger in size than 4VPY-co-DVB microspheres (Figure 6B). In terms of morphology, 4VPY-co-DVB microspheres were polydispersed while MAA-co-DVB microspheres were near monodispersed (Figure 7). There was no significant difference in the morphology of imprinted and non-imprinted 4VPY-co-EDMA microspheres (Figure 8). It is observed that non-imprinted 4VPY-co-TRIM microspheres were near monodispersity. On the other hand, imprinted 4VPY-co-TRIM microspheres were polydispersed with the presence of larger particles (Figure 9).

Influence of functional monomer and porogen on particle size

As observed under light microscope, MAA-co-DVB polymers gave no significant difference in particle size when prepared in acetonitrile and acetonitrile/toluene 75/25 v/v. On the other hand, 4VPY-co-DVB polymers were monodispersed when prepared in acetonitrile and polydispersed in acetonitrile/toluene 75/25 v/v. This can possibly be explained by the different polarity of the functional monomers. In terms

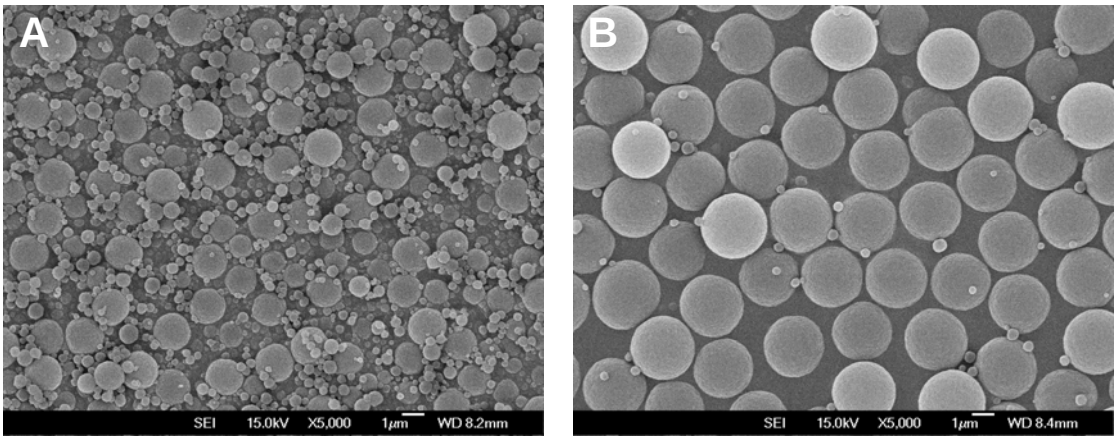


Figure 7: Scanning electron micrograph of imprinted (A) and non-imprinted (B) methacrylic acid co-polymerized with divinylbenzene (MAA-co-DVB) prepared in acetonitrile/toluene (75/25 v/v).

of the theta solvent, introduction of toluene possibly makes the porogen mixture a poor solvent for the 4VPY-co-DVB polymers as observed by the polydispersity in which there is the presence of a subpopulation of small particles. This is probably due to the minimization of their interaction with the solvent resulting in a collapse of the polymer.

Influence of cross-linker on particle size

The size of DVB-based polymers was larger than those of EDMA- and TRIM-based polymers as observed under optical microscope. This is in general agreement with previous results where crosslinked polymer microspheres were prepared with precipitation polymerization using the different crosslinkers.

Influence of functional monomer on imprinting effect

It is observed that 4VPY is the better functional monomer as it gives an imprinting effect whereas MAA displayed no imprinting effect. The same phenomenon was observed by the computer simulation in which 4VPY gave higher interaction energy value than that of MAA as previously mentioned.

Influence of cross-linker on imprinting effect

4VPY-co-EDMA polymers displayed the greatest imprinting effect as observed from the total binding capacity of 21.2269 % and 11.4676 % in acetonitrile for MIP and CP, respectively. The imprinting effect of 4VPY-co-DVB polymers was not significant. 4VPY-co-TRIM gave some imprinting effect with total binding capacity of 21.7232 and 16.962 % for MIP and CP, respectively. No imprinting effect was observed for 4VPY-co-DVB/TRIM.

Effect of EDMA and TRIM as crosslinkers

In terms of the molecular structures, there are two apparent differences between the cross-linkers EDMA and TRIM. First, the number of vinyl groups for EDMA is 2 while for TRIM had 3. Second, the number of esters for EDMA is 2 while TRIM had 3. Results indicated that the performance of polymers based on EDMA and TRIM were good when the porogen acetonitrile was used as the rebinding solvent. As the water composition of the rebinding solvent increased, the performance of TRIM was heavily affected whereas the performance of EDMA was left unaltered. Perhaps this can be explained by the molecular hydrophobicity.

The calculated molecular hydrophobicity in order of increasing octanol/water partition coefficient (logP) has been established as follows: EDMA < TRIM < DVB. The more hydrophobic crosslinkers (TRIM and DVB) has been shown experimentally to perform poorly in water-containing rebinding solvents. We can ascribe this to the hydrophobic nature of the crosslinkers as

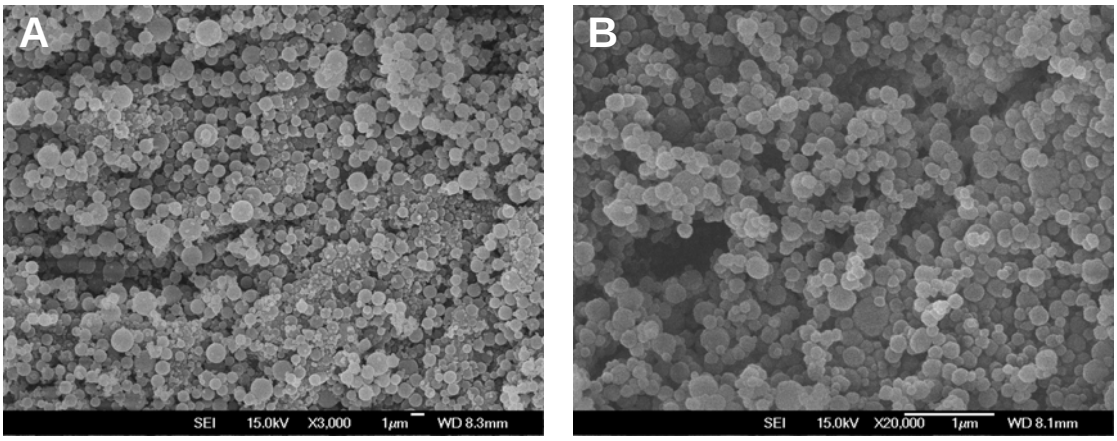


Figure 8: Scanning electron micrograph of imprinted (A) and non-imprinted (B) 4-vinylpyridine co-polymerized with ethylene glycol dimethacrylate (4VPY-co-EDMA) prepared in acetonitrile.

TRIM and DVB has logP of 3.32 and 3.84, respectively. Hypothetically, the addition of water may have exerted a collapsing effect (e.g. collapse of the polymer as hydrophobic crosslinkers would tend to aggregate and avoid water molecules) on the more hydrophobic polymer. The less hydrophobic EDMA-based polymer with logP of 1.61 were unaffected by the presence of water.

Influence of water on imprinting effect

It is observed that for 4VPY-co-EDMA, the total binding capacity was higher in acetonitrile/water (50/50 v/v) than in acetonitrile. In both rebinding solvent, good imprinting effect was observed, particularly, inclusion of 50% water in acetonitrile enhanced the total binding capacity while maintaining the imprinting effect. However, when rebinding is carried out in acetonitrile/water (20/80 v/v) the total binding capacity of both imprinted and non-imprinted polymers increased at the cost of a loss of imprinting effect. 4VPY-co-TRIM possessed slight imprinting effect in acetonitrile; however, when rebinding was performed in acetonitrile/water (50/50 v/v) the imprinting effect disappeared albeit an increase in the total binding capacity. Other polymers followed a general trend in which the total binding capacity increased in relation to the percentage of water present in acetonitrile.

Based on the notion that the inclusion of

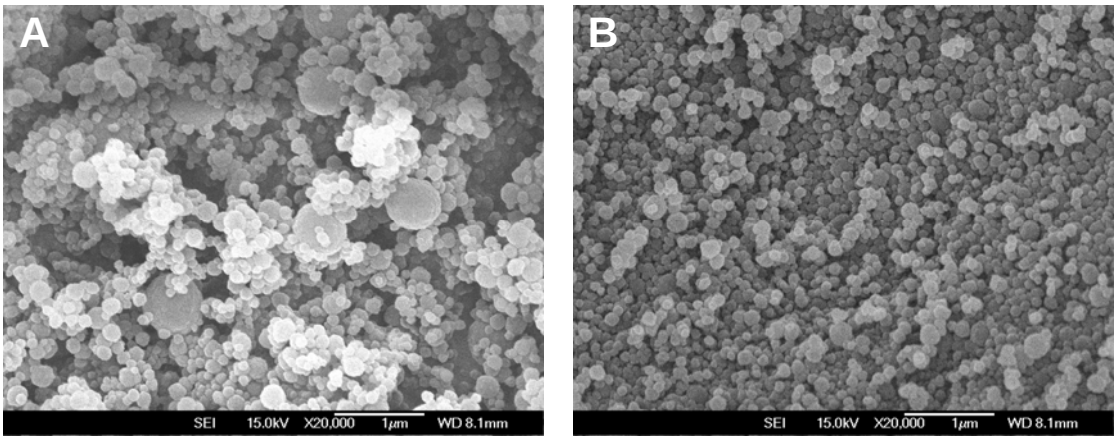


Figure 9: Scanning electron micrograph of imprinted (A) and non-imprinted (B) 4-vinylpyridine co-polymerized with trimethylolpropane trimethacrylate (4VPY-co-TRIM) prepared in acetonitrile.

water could enhance the binding performance by means of hydrophobic interaction, a set of polymers was prepared in the presence of water. However, the inclusion of water as porogen did not enhance the binding performance but rather led to the loss of the imprinting effect (Table 3, Entries 19-22, 63-66).

Influence of template-monomer molar ratio

To test the influence of template-monomer molar ratio used for the preparation of imprinted polymers on the binding performance, the molar ratio of template-monomer was increased from 2:7 to 1:1. The template-monomer molar ratio of 1:1 gave no imprinting effect suggesting that an excess of functional monomer is crucial for good imprinting effect (Table 3, Entries 15-18, 37-40, 59-62).

Cross-binding with β-estradiol

Results indicate that the BPA-imprinted polymers did not show preferential binding for β-estradiol as compared to non-imprinted reference polymers (data not shown), i.e. the BPA-imprinted sites are highly specific to recognize the template structure.

CONCLUSION

In summary, we have tested the influence of functional monomer, template-monomer ratio, cross-linker, porogen, and rebinding solvent on the binding performance of bisphenol A imprinted polymers. In this study, it was observed that 4VPY was the optimal functional monomer as also confirmed independently by our computer simulations. Furthermore, EDMA and TRIM were good cross-linkers with EDMA performing better than TRIM. It was observed that the presence of toluene in the porogen gave rise to polydispersity in the size of the 4VPY-based microspheres and no significant difference for the MAA-based microspheres. It was shown that an excess of the functional monomer was essential for good binding performance. The introduction of water in the rebinding solvent in the molar ratio of 50/50 v/v with acetonitrile seemed to enhance the total binding capacity by approximately 2 fold for some of the polymers. Addition of water to 80% caused total loss of the imprinting effect. Therefore, the presence of water in the porogen did not enhance the binding performance but eliminated the imprinting effect.

Acknowledgements: C.N., under V.P. supervision, is a recipient of the Royal Golden Jubilee Ph.D. Scholarship from the Thailand Research Fund. This work was supported in part by the Thailand Toray Science Foundation (TTSF), Swedish Strategic Research Foundation (Biomimetic Materials Sciences), and a grant from the annual budget of Mahidol University (B.E.2546-2550).

Table 1: Interaction energies of bisphenol A (BPA) with methacrylic acid (MAA) or 4-vinylpyridine (4VPY).

Molecules	Energy (a.u.)	ΔE (a.u.) [a]	ΔE (kJ mol^{-1}) [b]
BPA	–731.66226	—	—
MAA	–306.46712	—	—
4VPY	–325.68191	—	—
BPA-MAA 1:1	–1038.14414	0.01476	38.74889
BPA-MAA 1:2	–1344.62390	0.02740	71.96327
BPA-4VPY 1:1	–1057.36054	0.01636	42.96882
BPA-4VPY 1:2	–1383.05779	0.03170	83.25323

[a] ΔE is the interaction energy derived from equation 1.
[b] ΔE was converted from a.u. to kJ mol^{-1} by multiplying the energy value in a.u. by the conversion factor 2.626 x 10^{-3}.

Table 2: Preparation of molecularly imprinted microspheres.

No.	Polymer	BPA (mmol)	4-VPY (mmol)	MAA (mmol)	DVB (mmol)	EDMA (mmol)
1	MIP 4VPY-co-DVB	0.4	1.4	—	5	—
2	NIP 4VPY-co-DVB	—	1.4	—	5	—
3	MIP 4VPY-co-DVB (ACN/ TOL 75/25 v/v)	0.4	1.4	—	5	—
4	NIP 4VPY-co-DVB (ACN/TOL 75/25 v/v)	—	1.4	—	5	—
5	MIP MAA-co-DVB	0.4	—	1.4	5	—
6	NIP MAA-co-DVB	—	—	1.4	5	—
7	MIP MAA-co-DVB (ACN/ TOL 75/25 v/v)	0.4	—	1.4	5	—
8	NIP MAA-co-DVB (ACN/ TOL 75/25 v/v)	—	—	1.4	6	—
9	MIP 4VPY-co-EDMA	0.4	1.4	—	—	5.6
10	NIP 4VPY-co-EDMA	—	1.4	—	—	5.6
11	MIP 4VPY-co-TRIM	0.4	1.4	—	—	—
12	NIP 4VPY-co-TRIM	—	1.4	—	—	—
13	MIP 4VPY-co-DVB/TRIM	0.4	1.4	—	1.95	—
14	NIP 4VPY-co-DVB/TRIM	—	1.4	—	1.95	—
15	MIP 4VPY-co-EDMA (T:M 1/1)	1.4	1.4	—	—	5.6
16	NIP 4VPY-co-EDMA (T:M 1/1)		1.4	—	—	5.6
17	MIP 4VPY-co-TRIM (T:M 1/1)	1.4	1.4	—	—	—
18	NIP 4VPY-co-TRIM (T:M 1/1)		1.4	—	—	—
19	MIP 4VPY-co-EDMA (ACN/H_2O 75/25 v/v)	0.4	1.4	—	—	5.6
20	NIP 4VPY-co-EDMA (ACN/H_2O 75/25 v/v)	—	1.4	—	—	5.6
21	MIP 4VPY-co-TRIM (ACN/H_2O 75/25 v/v)	0.4	1.4	—	—	—
22	NIP 4VPY-co-TRIM (ACN/H_2O 75/25 v/v)	—	1.4	—	—	—

Table 2 (Continued):

No.	Polymer	TRIM (mmol)	AIBN (mg)	Acetonitrile (mL)	Toluene (mL)	Water (mL)
1	MIP 4VPY-co-DVB	—	44.5	40	—	—
2	NIP 4VPY-co-DVB	—	44.5	40	—	—
3	MIP 4VPY-co-DVB (ACN/ TOL 75/25 v/v)	—	44.5	30	10	—
4	NIP 4VPY-co-DVB (ACN/ TOL 75/25 v/v)	—	44.5	30	10	—
5	MIP MAA-co-DVB	—	44.5	40	—	—
6	NIP MAA-co-DVB	—	44.5	40	—	—
7	MIP MAA-co-DVB (ACN/ TOL 75/25 v/v)	—	44.5	30	10	—
8	NIP MAA-co-DVB (ACN/ TOL 75/25 v/v)	—	44.5	30	10	—
9	MIP 4VPY-co-EDMA	—	26.97	40	—	—
10	NIP 4VPY-co-EDMA	—	26.97	40	—	—
11	MIP 4VPY-co-TRIM	2.1	18.98	40	—	—
12	NIP 4VPY-co-TRIM	2.1	18.98	40	—	—
13	MIP 4VPY-co-DVB/TRIM	0.75	14.92	40	—	—
14	NIP 4VPY-co-DVB/TRIM	0.75	14.92	40	—	—
15	MIP 4VPY-co-EDMA (T:M 1/1)	—	26.97	40	—	—
16	NIP 4VPY-co-EDMA (T:M 1/1)	—	26.97	40	—	—
17	MIP 4VPY-co-TRIM (T:M 1/1)	2.1	18.98	40	—	—
18	NIP 4VPY-co-TRIM (T:M 1/1)	2.1	18.98	40	—	—
19	MIP 4VPY-co-EDMA (ACN/H_2O 75/25 v/v)	—	26.97	30	—	10
20	NIP 4VPY-co-EDMA (ACN/H_2O 75/25 v/v)	—	26.97	30	—	10
21	MIP 4VPY-co-TRIM (ACN/H_2O 75/25 v/v)	2.1	18.98	30	—	10
22	NIP 4VPY-co-TRIM (ACN/H_2O 75/25 v/v)	2.1	18.98	30	—	10

MIP, molecularly imprinted polymer; NIP, non-imprinted polymer; T:M, template-monomer molar ratio; 4VPY, 4-vinylpyridine; MAA, methacrylic acid; DVB, divinylbenzene; EDMA, ethylene glycol dimethacrylate; TRIM, trimethylolpropane trimethacrylate; TOL, toluene; ACN, acetonitrile.

Table 3: Binding results of the prepared polymers.

No.	Polymer	Rebinding solvent	% Bound
1	MIP 4VPY-co-DVB ACN	ACN	29.6287
2	NIP 4VPY-co-DVB ACN	ACN	23.3740
3	MIP 4VPY-co-DVB TOL ACN	ACN	32.7595
4	NIP 4VPY-co-DVB TOL ACN	ACN	25.6024
5	MIP MAA-co-DVB ACN	ACN	4.3438
6	NIP MAA-co-DVB ACN	ACN	2.8285

7	MIP MAA-co-DVB TOL ACN	ACN	5.1877
8	NIP MAA-co-DVB TOL ACN	ACN	5.5948
9	MIP 4VPY-co-EDMA	ACN	21.2269
10	NIP 4VPY-co-EDMA	ACN	11.4676
11	MIP 4VPY-co-TRIM	ACN	21.7232
12	NIP 4VPY-co-TRIM	ACN	16.9620
13	MIP 4VPY-co-DVB TRIM	ACN	24.4495
14	NIP 4VPY-co-DVB TRIM	ACN	24.2785
15	MIP 4VPY-co-EDMA T:M 1:1	ACN	28.5480
16	NIP 4VPY-co-EDMA T:M 1:1	ACN	24.2590
17	MIP 4VPY-co-TRIM T:M 1:1	ACN	40.4230
18	NIP 4VPY-co-TRIM T:M 1:1	ACN	38.0820
19	MIP 4VPY-co-EDMA ACN:H_2O 3:1	ACN	29.3810
20	NIP 4VPY-co-EDMA ACN:H_2O 3:1	ACN	25.9050
21	MIP 4VPY-co-TRIM ACN:H_2O 3:1	ACN	39.6290
22	NIP 4VPY-co-TRIM ACN:H_2O 3:1	ACN	41.5690
23	MIP 4VPY-co-DVB ACN	ACN/H_2O (50/50)	30.0781
24	NIP 4VPY-co-DVB ACN	ACN/H_2O (50/50)	25.2874
25	MIP 4VPY-co-DVB TOL ACN	ACN/H_2O (50/50)	29.7349
26	NIP 4VPY-co-DVB TOL ACN	ACN/H_2O (50/50)	26.1202
27	MIP MAA-co-DVB ACN	ACN/H_2O (50/50)	-6.6080
28	NIP MAA-co-DVB ACN	ACN/H_2O (50/50)	-6.3614
29	MIP MAA-co-DVB TOL ACN	ACN/H_2O (50/50)	-3.8906
30	NIP MAA-co-DVB TOL ACN	ACN/H_2O (50/50)	-1.9523
31	MIP 4VPY-co-EDMA	ACN/H_2O (50/50)	28.9600
32	NIP 4VPY-co-EDMA	ACN/H_2O (50/50)	14.6563
33	MIP 4VPY-co-TRIM	ACN/H_2O (50/50)	37.8835
34	NIP 4VPY-co-TRIM	ACN/H_2O (50/50)	36.3139
35	MIP 4VPY-co-DVB TRIM	ACN/H_2O (50/50)	42.5938
36	NIP 4VPY-co-DVB TRIM	ACN/H_2O (50/50)	42.8569
37	MIP 4VPY-co-EDMA T:M 1:1	ACN/H_2O (50/50)	33.5850
38	NIP 4VPY-co-EDMA T:M 1:1	ACN/H_2O (50/50)	32.0030
39	MIP 4VPY-co-TRIM T:M 1:1	ACN/H_2O (50/50)	96.4430
40	NIP 4VPY-co-TRIM T:M 1:1	ACN/H_2O (50/50)	96.2860
41	MIP 4VPY-co-EDMA ACN:H_2O 3:1	ACN/H_2O (50/50)	32.6000
42	NIP 4VPY-co-EDMA ACN:H_2O 3:1	ACN/H_2O (50/50)	34.9640
43	MIP 4VPY-co-TRIM ACN:H_2O 3:1	ACN/H_2O (50/50)	96.1000
44	NIP 4VPY-co-TRIM ACN:H_2O 3:1	ACN/H_2O (50/50)	96.0720
45	MIP 4VPY-co-DVB ACN	ACN/H_2O (20/80)	96.2830
46	NIP 4VPY-co-DVB ACN	ACN/H_2O (20/80)	94.0159
47	MIP 4VPY-co-DVB TOL ACN	ACN/H_2O (20/80)	96.2350
48	NIP 4VPY-co-DVB TOL ACN	ACN/H_2O (20/80)	96.0181
49	MIP MAA-co-DVB ACN	ACN/H_2O (20/80)	97.0117

50	NIP MAA-co-DVB ACN	ACN/H_2O (20/80)	96.8464
51	MIP MAA-co-DVB TOL ACN	ACN/H_2O (20/80)	97.3354
52	NIP MAA-co-DVB TOL ACN	ACN/H_2O (20/80)	95.0551
53	MIP 4VPY-co-EDMA	ACN/H_2O (20/80)	97.0456
54	NIP 4VPY-co-EDMA	ACN/H_2O (20/80)	97.2715
55	MIP 4VPY-co-TRIM	ACN/H_2O (20/80)	79.7722
56	NIP 4VPY-co-TRIM	ACN/H_2O (20/80)	83.2327
57	MIP 4VPY-co-DVB TRIM	ACN/H_2O (20/80)	89.1997
58	NIP 4VPY-co-DVB TRIM	ACN/H_2O (20/80)	90.2698
59	MIP 4VPY-co-EDMA T:M 1:1	ACN/H_2O (20/80)	29.1750
60	NIP 4VPY-co-EDMA T:M 1:1	ACN/H_2O (20/80)	33.0120
61	MIP 4VPY-co-TRIM T:M 1:1	ACN/H_2O (20/80)	98.4240
62	NIP 4VPY-co-TRIM T:M 1:1	ACN/H_2O (20/80)	97.4680
63	MIP 4VPY-co-EDMA ACN:H_2O 3:1	ACN/H_2O (20/80)	35.6940
64	NIP 4VPY-co-EDMA ACN:H_2O 3:1	ACN/H_2O (20/80)	33.5890
65	MIP 4VPY-co-TRIM ACN:H_2O 3:1	ACN/H_2O (20/80)	97.6640
66	NIP 4VPY-co-TRIM ACN:H_2O 3:1	ACN/H_2O (20/80)	98.1460

All measurements were based on duplicate measurement unless otherwise noted.
*Triplicate measurement
**Single point measurement (the duplicate gave very low to unquantifiable peak, while the other gave low peak area and is represented here)

MIP, molecularly imprinted polymer; NIP, non-imprinted polymer; T:M, template-monomer molar ratio; 4VPY, 4-vinylpyridine; DVB, divinylbenzene; EDMA, ethylene glycol dimethacrylate; TRIM, trimethylolpropane trimethacrylate; TOL, toluene; ACN, acetonitrile.

REFERENCES

Caro E, Marce RM, Cormack PAG, Sherrington DC, Borrull F. Molecularly imprinted solid-phase extraction of naphthalene sulfonates from water. *J Chromatogr A*. 2004;1047:175-80.

Dennington II R, Keith T, Millam J, Eppinnett K, Hovell WL, Gilliland R. GaussView, Version 3.09, Semichem, Inc., Shawnee Mission, KS. 2003.

Goodson A, Summerfield W, Cooper I. Survey of bisphenol A and bisphenol F in canned foods. *Food Addit Contam*. 2002;19:796-802.

Frisch MJ, Trucks GW, Schlegel HB, Scuseria GE, Robb MA, Cheeseman JR, et al. Gaussian 03, Revision C.02, Gaussian, Inc., Wallingford CT, 2004.

Howard PH. Handbook of environmental fate and exposure data for organic chemicals. Boca Raton: Lewis Publishers; 1993.

Ikegami T, Mukawa T, Nariai H, Takeuchi T. Bisphenol A-recognition polymers prepared by covalent molecular imprinting. *Anal Chim Acta*. 2004a;504:131-5.

Ikegami T, Lee W-S, Nariai H, Takeuchi T. Synthetic polymers adsorbing bisphenol A and its analogues prepared by covalent molecular imprinting using bisphenol A dimethacrylate as a template molecule. *Anal Bioanal Chem*. 2004b;378:1898-902.

Jiang M, Zhang J-H, Mei S-R, Shi Y, Zou L-J, Zhu Y-X, Dai K, Lu B. Direct enrichment and high performance liquid chromatography analysis of ultra-trace Bisphenol A in water samples with narrowly dispersible Bisphenol A imprinted polymeric microspheres column. *J Chromatogr A*. 2006;1110:27-34.

Joshi VP, Karmalkar RN, Kulkarni MG, Mashelkar RA. Effect of Solvents on Selectivity in Separation Using Molecularly Imprinted Adsorbents: Separation of Phenol and Bisphenol

A. *Ind Eng Chem Res*. 1999;38:4417-23.

Kanekiyo Y, Naganawa R, Tao H. Molecular imprinting of bisphenol A and alkylphenols using amylose as a host matrix. *Chem Commun*. 2002;21:2698-9.

Kanekiyo Y, Naganawa R, Tao H. pH-Responsive Molecularly Imprinted Polymers. *Angew Chem Int Ed*. 2003;42:3014-6.

Kawaguchi M, Hayatsu Y, Nakata H, Ishii Y, Ito R, Saito K, Nakazawa H. Molecularly imprinted solid phase extraction using stable isotope labeled compounds as template and liquid chromatography–mass spectrometry for trace analysis of bisphenol A in water sample. *Anal Chim Acta*. 2005;539:83-9.

Lee W-S, Takeuchi T. Bisphenol A Analog-Imprinted Polymers Prepared by an Immobilized Template on a Modified Silica Microsphere Matrix. *Anal Sci*. 2005;21:1125-8.

Lin TY, Hu CH, Chou TC. Determination of albumin concentration by MIP-QCM sensor. *Biosens Bioelectron*. 2004;20:75-81.

Mosbach K, Yu Y, Andersch J, Ye L. Generation of new enzyme inhibitors using imprinted binding sites: the anti-idiotypic approach, a step toward the next generation of molecular imprinting. *J Am Chem Soc*. 2001;123:12420-1.

Nantasenamat C, Naenna T, Isarankura Na Ayudhya C, Prachayasittikul V. Quantitative prediction of imprinting factor of molecularly imprinted polymers by artificial neural network. *J Comput Aid Mol Des*. 2005a;19:509-24.

Nantasenamat C, Naenna T, Isarankura-Na-Ayudhya C, Prachayasittikul V. Recognition of DNA Splice Junction via Machine Learning Approaches. *EXCLI J*. 2005b;4:114-29.

Nantasenamat C, Isarankura-Na-Ayudhya C, Tansila N, Naenna T, Prachayasittikul V. Prediction of GFP spectral properties using artificial neural network. *J Comput Chem*. 2006;In press.

Navarro-Villoslada F, Vicente BS, Moreno-Bondi MC. Application of multivariate analysis to the screening of molecularly imprinted polymers for bisphenol A. *Anal Chim Acta*. 2004;504:149-62.

Ou J, Hu L, Hu L, Li X, Zou H. Determination of phenolic compounds in river water with on-line coupling bisphenol A imprinted monolithic precolumn with high performance liquid chromatography. *Talanta*. 2006;69:1001-6.

Piacham T, Isarankura Na Ayudhya C, Prachayasittikul V, Bülow L, Ye L. A polymer supported manganese catalyst useful as a superoxide dismutase mimic. *Chem Commun*. 2003;(11):1254-5.

Piacham T, Josell A, Arwin H, Prachayasittikul V, Ye L. Molecularly imprinted polymer thin films on quartz crystal microbalance using a surface bound photo-radical initiator. *Anal Chim Acta*. 2005;536:191-6.

Sambe H, Hoshina K, Hosoya K, Haginaka J. Direct injection analysis of bisphenol A in serum by combination of isotope imprinting with liquid chromatography-mass spectrometry. *Analyst*. 2005;130:38-40.

San Vicente B, Navarro Villoslada F, Moreno-Bondi MC. Continuous solid-phase extraction and preconcentration of bisphenol A in aqueous samples using molecularly imprinted columns. *Anal Bioanal Chem*. 2004;380:115-22.

Sanbe H, Haginaka J. Uniformly sized molecularly imprinted polymers for bisphenol A and β-estradiol: retention and molecular recognition properties in hydro-organic mobile phases. *J Pharm Biomed*. 2003;30:1835-44.

Sanbe H, Hosoya K, Haginaka J. Preparation of Uniformly Sized Molecularly Imprinted Polymers for Phenolic Compounds and Their Application to the Assay of Bisphenol A in River Water. *Anal Sci*. 2003;19:715-9.

Takeda K, Kobayashi T. Bisphenol A imprinted polymer adsorbents with selective recognition and binding characteristics. *Science and Technology of Advanced Materials*. 2005;6:165-71.

Takeda K, Kobayashi T. Hybrid molecularly imprinted membranes for targeted bisphenol derivatives. *J Memb Sci*. 2006;275:61-9.

Tamayo FG, Martin-Esteban A. Selective high performance liquid chromatography imprinted-stationary phases for the screening of phenylurea herbicides in vegetable samples. *J Chromatogr A*. 2005;1098:116-22.

Tsuru N, Kikuchi M, Kawaguchi H, Shiratori S. A quartz crystal microbalance sensor coated with MIP for ‘‘Bisphenol A’’ and its properties. *Thin Solid Films*. 2006;499:380-5.

Vlatakis G, Andersson LI, Muller R, Mosbach K. Drug assay using antibody mimics made by molecular imprinting. *Nature*. 1993;361:645-7.

Watabe Y, Kondo T, Morita M, Tanaka N, Haginaka J, Hosoya K. Determination of bisphenol A in environmental water at ultra-low level by high-performance liquid chromatography with an effective on-line pretreatment device. *J Chromatogr A*. 2004;1032:45-9.

Watabe Y, Hosoya K, Tanaka N, Kondo T, Morita M, Kubo T. LC/MS determination of bisphenol A in river water using a surface-modified molecularly-imprinted polymer as an on-line pretreatment device. *Anal Bioanal Chem*. 2005a;381:1193-8.

Watabe Y, Hosoya K, Tanaka N, Kubo T, Kondo T, Morita M. Novel surface modified molecularly imprinted polymer focused on the removal of interference in environmental water samples for chromatographic determination. *J Chromatogr A*. 2005b;1073:363-70.

Whitley DC, Ford MG, Livingstone DJ. Unsupervised Forward Selection: A Method for Eliminating Redundant Variables. *J Chem Inf Model*. 2000;40:1160-8.

Witten IH, Frank E. Data Mining: Practical Machine Learning Tools and Techniques with Java Implementations. San Francisco: Morgan Kaufmann Publishers; 2000.

Yamamoto T, Yasuhara A. Quantities of bisphenol a leached from plastic waste samples. *Chemosphere*. 1999;38:2569-76.

Yamamoto T, Yasuhara A, Shiraishi H, Nakasugi O. Bisphenol A in hazardous waste landfill leachates. *Chemosphere*. 2001;42:415-8.

Yang K, Liu Z, Maoa M, Zhang X, Zhao C, Nishi N. Molecularly imprinted polyethersulfone microspheres for the binding and recognition of bisphenol A. *Anal Chim Acta*. 2005;546:30-6.