Bisphenol A imprinted polymer adsorbents with selective recognition and binding characteristics

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Abstract

Imprinted copolymers, which highly recognized and bound bisphenol A (Bis A), were synthesized by using covalent imprinting technique. Bisphenol A dimethacrylate (BADM) was used as a template monomer in the copolymerization with a crosslinkable monomer of divinyl benzene (DVB), ethylene glycol dimethacrylate (EGDM) or N,N'-methylenebisacrylamide (MBAA). The resultant copolymer was hydrolyzed in acidic or basic condition. It was found that the Bis A imprinted copolymers of EGDM and MBAA had no selectivity to Bis A, because the crosslinker dissociated by the hydrolysis reaction and thus comprehensive imprinted sites were not formed. On the other hand, imprinted copolymer of DVB showed excellent selectivity to differentiate Bis A from bisphenol E and bisphenol F (Bis F). Characterization of the copolymers suggested that the DVB copolymer had resistance to the acid and alkali conditions. Effect of recognition by the Bis F imprinted polymer was also compared with that of the Bis A imprinted polymer, when DVB and bisphenol F dimethacrylate were copolymerized. The Bis F imprinted copolymer was able to recognize Bis F in ethanol solution, however showed higher binding capacity for both Bis A and Bis F in water solution without recognition. Therefore, hydrophobic interaction between Bis A and the imprinted site enhanced the binding capacity with high selectively for the BADM-co-DVB imprinted copolymer.

Keywords: Molecular imprinting; Molecular recognition; Polymer adsorbent; Bisphenol A

1. Introduction

Bisphenol A (Bis A), which is known as endocrine disruptor, affects the reproduction and development of animal organism in extra-diluted concentration. Therefore, exposures to Bis A even in the ppb concentration range occur in human and animal body cause serious problems. In general, Bis A is eluted from plastics into foods or beverage [1,2], because Bis A is widely used for production of polycarbonates and epoxy raisins in an industrial scale, and consumed in large amounts. Therefore, the material, which recognizes and selectively absorbs Bis A, is surely required from the viewpoint of environmental conservation. For development of Bis A adsorbents, molecular imprinting has been very useful as alternative methods [3–5]. The imprinting techniques have found various applications in fields of separation and concentration. However, imprinted polymers with highly selective and largely adsorbed abilities to such endocrine disruptor still have been on progress. Therefore, in the near future, it is certainly necessary for such environmental conservation to utilize Bis A imprinted polymers, which selectively bind this chemical with high binding capacity. It is known that imprinting method gives both molecule recognition and binding polymer materials. Thus, resultant imprinting polymers memorizes the target molecule in shape and functionality. The non-covalent approach for Bis A imprinting mainly employed hydrogen bonding between a polymer and a Bis A molecule as the driving force for imprinting a target molecule [6]. Also, in covalent imprinting approach, recognition sites in imprinted polymers are constructed by means of common cross-linking polymerization in the presence of a template [4–7]. According to such template polymerization including a monomer-template complex, the resultant polymer matrix can be complementary to the template molecule. Recently, Bis A imprinted polymer was

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prepared by Takeuchi et al by applying to covalent technique [7]. They reported that copolymerization of functional monomer containing Bis A framework structure and ethylene glycol dimethacrylate (EGDM) as a cross-linking monomer gave Bis A imprinting materials. In this case, after the template was removed by alkali conditions, the Bis A imprinted polymer was prepared. However, recently, we confirmed the technique reported was unsuitable for highly recognizing the template molecules because the alkali treatment caused hydrolysis breakage of ester bonds for the crosslinked EGDM segments in the imprinted polymers. As a result, this was for cause and effect on increase of non-selective absorption by the imprinted polymers. Therefore, in the present work, we focused on selection of the crosslinkers and bisphenol monomers to discriminate bisphenol derivatives. Bisphenol A dimethacrylate (BADM) and Bisphenol F dimethacrylate (BFDM) (Scheme 1) were used as functional monomers. When the ester bonds of BADM copolymer are hydrolyzed, methacrylic acid segments and Bis A can be formed as shown in Scheme 2. The manner leaves the template trace as imprinted sites in the polymer matrix, which is capable to recognizing and binding only Bis A (Scheme 3). For Bis A imprinted polymers with EGDM, N,N'-methylenebisacrylamide (MBAA) and divinyl benzene (DVB), the stability of the copolymer was compared under the hydrolysis condition. We discussed binding and recognition of several Bis A analogs by using various imprinted copolymers cross-linked with DVB, EGDM and MBAA in order to discriminate Bis A analogs and phenol derivatives (Scheme 4).

2. Experiments

2.1. Materials

Bisphenol A (Bis A), methacrylic acid (MAA), N,N'-methylenebisacrylamide (MBAA) and divinyl benzene (DVB) were purchased from Nacalai Tesque Inc. (Kyoto, Japan). Bisphenol A dimethacrylate was obtained from Aldrich Chemical Co. (Milwaukee, WI) and used without purification. Ethylene glycol dimethacrylate (EGDM), 4,4'-ethylidenebisphenol (Bis E), 4,4'-dihydroxydiphenyldimethane (Bis F), 2-(4-hydroxyphenyl)ethyl alcohol (HPEA) and resorcinol (RSO) were purchased from Tokyo Kasei Industry Co. (Tokyo, Japan).

2.2. Preparation of Bis A imprinted and non-imprinted polymers

Table 1 lists monomer feeds for copolymers of BADM synthesized with various crosslinked monomers. Copolymerization was carried out in toluene except for cases of MBAA. Due to non-soluble in toluene for MBAA, the polymerization was carried out in N-methyl-2-pyrrolidone (NMP). In polymerization, the monomer solution prepared was purged with nitrogen for 10 min and then 2,2'-azobis(isobutyronitrile) was added into the solution. The copolymerization was carried out at 55 °C for 12 h. The resulting copolymer was ground by pestle and mortar. Resultant copolymer granules were washed by solvent used in the copolymerization and then tetrahydrofuran (THF). Then, dried under vacuum in desiccator was for copolymer granules. The content of BADM in each copolymer was estimated by using IR measurements of copolymers. The measurements of FT-IR spectrum were carried out by IR Prestige-21 FTIR-8400s (Shimadzu co.) using the KBr method. For example, the BADM content of 9.0 mol% in BADM-co-DVB was determined from the IR spectra of mixtures of PolyBADM and PolyDVB. Herein, the data of

![Scheme 1. Chemical structures of BADM and BFDM.](image1)

![Scheme 2. Synthetic reaction scheme of imprinted polymer including hydrolysis process of BADM segment.](image2)
IR peak height appeared at 1750 and 700 cm\(^{-1}\) for C=O and phenyl groups, respectively, were used for calibration.

Hydrolysis of ester linkage of MBAA copolymer was mainly carried out in aqueous solution containing 1.0 M sulfuric acid (H\(_2\)SO\(_4\)) or 1.0 M sodium hydroxide (NaOH) at 50 °C for 12 h with stirring. Then, the hydrolyzed polymer was washed with water until neutral pH. The copolymer, which was treated under acid and base condition, was signed (A) or (B) in polymer symbols. The hydrolysis reaction was confirmed by measurements of weight loss and FT-IR spectrum of the resultant copolymer.

The value of weight loss (WL) was evaluated by the following equation:

\[
WL = \left(\frac{W_{\text{after}}}{W_{\text{before}}}\right) \times 100
\]

where \([W]_{\text{before}}\) and \([W]_{\text{after}}\) represent weight of the copolymer before and after hydrolysis reaction.

2.3. Substrate binding with heterogeneous batch experiments to imprinted polymers

As substrates for heterogeneous binding, Bis A and analog molecules were selected (Scheme 4). The batch experiments for binding Bis A with the Bis A imprinted and non-imprinted polymers were carried out in 40 ml aqueous or ethanol solution of containing 100 μM Bis A at 30 °C. The value of binding amount of Bis A was determined by monitoring UV absorbance for Bis A concentration before and after experiment. Then, the binding amount of Bis A by the imprinted polymer was estimated as unit of μmol/g. Similar manner was carried out for Bis E, Bis F, HPEA and RSO. Here, the absorbance of each water solution was monitored at 277, 276 and 275 nm for Bis A, Bis E and Bis F with extraction coefficient \(\varepsilon = 3.08 \times 10^3, 2.98 \times 10^4\) and \(3.01 \times 10^4\) (M\(^{-1}\)cm\(^{-1}\)), respectively. Amounts of substrates bound to imprinted polymer \([S]\) (μmol/g) were calculated by the following equation:

\[
[S] = (C_0 - C_t)V/W
\]

where \(C_0\) and \(C_t\) represent molar concentrations of the substrate measured at an initial time and saturated binding time, \(V\) and \(W\) represent volume of substrate solution and weight imprinted polymer, respectively. In addition, imprint coefficient (\(\alpha_i\)) was calculated by the following equation:

\[
\alpha_i = \frac{[S]_s}{[S]_t}
\]

where \([S]_s\) and \([S]_t\) were binding amount of each substrate and imprinted template substance. When \(\alpha_i < 1\), it was meant that the substrate bound to imprinted polymer was recognized.

3. Results and discussion

3.1. Bis A binding ability of imprinted polymers

Binding ability of non-imprinted polymers was compared for each imprinted polymer and substrate. Here, as listed in Table 1 copolymers of BADM with DVB, EGDM
and MBAA were singed as BADM-co-DVB, BADM-co-EGDM and BADM-co-MBAA, respectively. The contents of BADM in BADM-co-DVB, BADM BADM-co-EGDM and BADM-co-MBAA were estimated as 9.0, 8.8 and 9.0 mol%, respectively, by using IR measurements of their copolymers. Herein, the IR peaks ratio of 1750 cm\(^{-1}\) of C=O stretching to 1650 cm\(^{-1}\) of amide C=O stretching for BADM-co-MBAA and 1010 cm\(^{-1}\) aromatic ester bond of BADM and 1200 cm\(^{-1}\) of C–O stretching of EGDM were used and applied for the calibration curves. As reference, each homopolymer of DVB, EGDM and MBAA was prepared. In order to examine imprinting function, we measured binding amount of Bis A for their homopolymers having crosslinked monomer segments. It was conformed that PolyDVB, PolyMBAA and MAA-co-DVB had no binding ability for Bis A. However, PolyEGDM had binding capacity of 16.2 \(\mu\)mol/g. This may be due to the presence of ester group of EGDM segments. Fig. 1 shows binding amounts of Bis A for each Bis A imprinted polymer (a) before and (b) after hydrolysis process. In these experiments, hydrolysis reaction was carried out in acidic and basic aqueous solution. For copolymer without the hydrolysis process, as presented in Fig. 1 (a), resultant copolymer had no binding to Bis A as well as homopolymer. However, binding ability of Bis A appeared in their copolymers after hydrolysis reaction (Fig. 1(b)). In addition, we noted that the value of binding amount of Bis A into the imprinted copolymers cross-linked with EGDM was high and about 16 \(\mu\)mol/g. The enhancement of binding amount of Bis A for about 12 \(\mu\)mol/g was also caused in copolymer of MBAA when it was treated with acidic solution. However, the binding amount of Bis A for the imprinted copolymer of BADM-co-DVB was about 6 \(\mu\)mol/g. Therefore, it was considered that the hydrolysis surely formed binding sites of Bis A in the copolymers via dissociation of ester group of BADM. Also, these data indicated that difference of binding amounts in each imprinted polymer hydrolyzed in \(\text{H}_2\text{SO}_4\) and \(\text{NaOH}\) was negligibly small in BADM-co-EGDM and BADM-co-DVB. For example, BADM-co-EGDM(A) and (B), which were treated with \(\text{H}_2\text{SO}_4\) and \(\text{NaOH}\), had Bis A binding capacity of 15.8 and 15.7 \(\mu\)mol/g, respectively. However, the resultant binding behavior of BADM-co-MBAA(A) and (B) considerably differed in the hydrolysis treatment in acidic and basic solution. This may be caused by hydrolytic cleavage of crosslinked polymer segments of the ester or amide group. Therefore, for instance, cleaved EGDM segments became loose in the binding sites of Bis A.

In order to evaluate properties of copolymers before and after the hydrolysis process, characterization of imprinted polymers was carried out by means of FT-IR measurement. Fig. 2 show FT-IR spectra of resultant copolymers, which were obtained by hydrolysis reaction under acidic and basic condition for (a) BADM-co-EGDM, (b) BADM-co-MBAA and (c) BADM-co-DVB. In BADM-co-EGDM and BADM-co-MBAA, because the IR bands of BADM segments mostly overlapped with those of crosslinker segments, it was thus difficult to compare structural change of copolymer by the hydrolysis reaction. However, the data showed that chemical structure of BADM-co-EGDM had a little change after acid and base tereatments. In basic condition, the bands at 3000–3800 cm\(^{-1}\) assigned from OH stretching of carboxylic acid were appeared by decomposition of ester group in EGDM as shown in Fig. 2 (a). Also, the band intensity at about 1250 cm\(^{-1}\) was strong in copolymers hydrolyzed (BADM-co-EGDM(B)). For BADM-co-MBAA (Fig. 2(b)), the bands in the region of about 1700 and 1600 cm\(^{-1}\) assigned as amide group of MBAA became broaden by the hydrolysis reaction. The band of about 1280 cm\(^{-1}\) for BADM-co-MBAA(B) was also intense relative to that before hydrolysis. This suggested that the hydrolysis process in alkali solution influenced the chemical structure of polymer especially alkali condition. As compared with FT-IR spectra in BADM-co-DVB before and after hydrolysis, IR band intensity at about 730 cm\(^{-1}\) assigned form BADM ester bond decreased after hydrolysis as shown in Fig. 2(c).

Table 2 lists weigh loss (WL) of polymer, which was measured before and after hydrolysis reaction. The value of WL was 1.0% and 0.8% in BADM-co-DVB(A) and BADM-co-DVB(B), respectively. However, the value of BADM-co-MBAA was about 17–29%. Therefore, this suggested that
crosslinked MBAA polymer was unstable under acid and base condition, since it was decomposed effectively via hydrolysis. In BADM-co-EGDM, the value of WL was enhanced in alkali treatment. Namely, the resultant values were 0.8 and 9.2% for BADM-co-EGDM(A) and (B). In addition, the low value of BADM-co-DVB was well corresponded to the data of FT-IR (Fig. 2(C)). As a result, the hydrolysis process in BADM-co-EGDM cleaved the ester bond of EGDM segments. As shown in Fig. 1(a), the resultant BADM-co-EGDM bound highly Bis A molecule. On the other hand, the copolymer of BADM-co-DVB showed low value of Bis A binding. It was reasonable to consider that the value of WL was in the range of 0.8–1.0%. In these conditions, little was changed in the FT-IR spectra for the resultant BADM-co-DVB(A) and BADM-co-DVB(B). Therefore, both data suggested that the copolymer decomposition under hydrolysis condition was low relative to that of other copolymers. Therefore, the binding sites of Bis A imprinting in BADM-co-DVB surely were formed by the hydrolysis reaction (Scheme 3). We confirmed that the estimated theoretical value of weight loss was 11.5% for BADM-co-DVB, if whole BADM was completely hydrolyzed. Therefore, most of DVB segments in BADM-co-DVB was remained without cleavage. To make effectively such the hydrolysis condition, we furthermore examined the detail of hydrolysis as concentration H$_2$SO$_4$ in the range of 1–9 M at 50 °C for 24 h. These indicated that the weight loss and the resultant IR spectra showed little change in resultant Fig. 2. FT-IR spectra of (a) BADM-co-EGDM, (b) BADM-co-MBAA and (c) BADM-co-DVB. The symbols of (A) and (B) were similar with those shown by Fig. 1.

| Polymer     | Hydrolysis condition | WL  |
|-------------|----------------------|-----|
| BADM-co-DVB | H$_2$SO$_4$          | 1.0 |
|             | NaOH                 | 0.8 |
| BADM-co-EGDM| H$_2$SO$_4$          | 0.8 |
|             | NaOH                 | 9.2 |
| BADM-co-MBAA| H$_2$SO$_4$          | 18.0|
|             | NaOH                 | 28.1|
BADM-co-DVBs and also similar binding capacity of Bis A was observed. Therefore, it was considered that only polymer surface was contributed to formation of imprinted sites of Bis A. This was due to nature of PolyDVB segments, which had strongly tight and rigid property.

3.2. Recognition of Bis A for imprinted polymers

As shown in Scheme 4, analogous substrates of Bis A were used for investigation on selectivity of Bis A imprinted copolymers. Herein, Bis E and Bis F are bisphenol molecules, which have mono-methyl group and no methyl group on the molecule framework. HPEA and RSO are phenols derivatives, which have different chemical structures.

Fig. 3 shows binding capacity of these substrates for the resultant imprinted and non-imprinted polymers, respectively. Here, these values of binding amounts of substrate were saturated binding amounts when measured after 24 h. The binding amounts of Bis A, Bis E and Bis F were 6.2, 5.0 and 4.0 \( \mu \text{mol/g} \) for BADM-co-DVB(A) and 6.8, 5.8 and 4.7 \( \mu \text{mol/g} \) for BADM-co-DVB(B), respectively. In these cases, there were no binding to HPEA and RSO, although BADM-co-EGDM showed binding with 7 and 9 \( \mu \text{mol/g} \) for HPEA and RSO, respectively. The binding capacity of these substrates was highly found in the EGDM-copolymers. That is, the BADM-co-EGDM had binding capacity of about 16 \( \mu \text{mol/g} \) for Bis A, Bis E and Bis F and about 8–9 \( \mu \text{mol/g} \) for HPEA and RSO, respectively. The binding amounts of Bis A, Bis E and Bis F were 6.8, 5.8 and 4.7 \( \mu \text{mol/g} \) for BADM-co-EGDM(A) and 5.9, 5.9 and 4.9 \( \mu \text{mol/g} \) for BADM-co-EGDM(B), respectively.

Table 3 also lists imprint coefficient \( \alpha_i \) for various imprinted polymers. The results represented that EGDM homopolymer had the almost same values of binding amount for Bis A and its analog substrates. Thus, the high binding capacity of BADM-co-EGDM was not due to imprinting manner. This indicated non-selective binding in the copolymer. Such results of IR analysis and weight loss of the copolymers implied that the formation of COOH group was enhanced by hydrolysis cleavage of EGDM segments in the copolymer. Similar tendency was observed in BADM-co-MBAA(B), which was treated with alkali condition. On the other hand, the DVB copolymer exhibited recognition binding of Bis A against Bis E and Bis F. Thus, the values of \( \alpha_i \) were 0.85 and 0.69 for Bis E and Bis F, respectively, even though the value of binding capacity was lower than that of BADM-co-EGDM. We guessed that this was due to low yield of that hydrolysis reaction in BADM-co-DVB, but the imprinted DVB copolymer demonstrated that high molecule recognition of Bis A against other substrates.

3.3. Comparison of recognition ability of BADM and Bisphenol F dimethacrylate (BFDM)

Since BADM-co-DVB showed recognition on molecular difference of bisphenol without and with methyl group, we compared BADM and BFDM in covalent imprinting. Herein, BFDM has no methyl group in the chemical structure of dimethacrylate framework (Scheme 1). The BFDM monomer was prepared as followed by ester coupling reaction between Bis F and methacryloyl chloride. In THF (30 ml) in the presence of triethylamine (6.07 g, 60.0 mmol), methacryloyl chloride (6.27 g, 60 mmol) was added dropwise to Bis F (4.00 g, 20.0 mmol). Then, the contents were stirred for 12 hours under room temperature. After the reaction, insoluble precipitation of triethylamine hydrochloride salt was removed by filtration. Then, the THF solution was evaporated to extract unreacted Bis F. The obtained crude monomer was soluble in toluene (300 ml) and washed with water to extract the amine salt. Then, the toluene layer was dried and concentrated. The crystallization of the monomer was carried out in refrigerator from

![Fig. 3. Binding amounts of substances for various imprinted polymers. The binding amounts were saturated value, which measured at 24 h. The symbols of (A) and (B) meant hydrolyzed condition using H\textsubscript{2}SO\textsubscript{4} and NaOH, respectively.](image-url)
the toluene solution (86.4% yield). The copolymerization of BFDPM with DVB was carried out similar to BADM-co-DVB. The hydrolysis process of BFDPM segment was taken place in acid condition.

Table 4 shows saturated binding amount of Bis A, Bis E and Bis F for BADM-co-DVB and BFDM-co-DVB. When the binding experiment was carried out in water solution, the binding amount of Bis A was higher than that of Bis E and Bis F for both imprinted polymers. Namely, the imprinted BFDPM-co-DVB had no selectivity to Bis F template. As shown in those data measured in ethanol solution, both polymers with BADM and BFDPM were able to selectively bind Bis A and Bis F in the solution, respectively. However, the binding capacity in ethanol was smaller than that of water. Therefore, it was considered that in water the increase of binding amount was caused by hydrophobic interaction. That is, low value of binding amount in ethanol indicated that hydrophobic association of Bis A or Bis F with the imprinted copolymer was decreased in the solvent. This was because of disconstruction of iceberg structure of water network [8] in such organic solvent. On the other hand, the selectivity of BADM-co-DVB for Bis A seemed to be surely higher than that of BFDPM-co-DVB, as compared with $\alpha_i$ for both polymers. As shown in Table 4, the value of $\alpha_i$ of Bis F and Bis E for BADM-co-DVB was 0.65 and 0.81, while that for BFDPM-co-DVB was 0.47 and 0.86 in water, respectively. This indicated that the covalent imprinting of BFDPM was difficult to recognize both chemical structures of Bis A and Bis F with and without methyl groups. Therefore, in the crosslinked DVB segments the covalent imprinting technique with BADM encoded the shape of Bis A having dimethyl group on the chemical structure.

4. Conclusions

Bis A imprinted polymers were synthesized by copolymerization of BADM and DVB. Effect of hydrolysis reaction of copolymer with BADM and several crosslinked monomers of EGDM, MBAA and DVB were compared on binding capacity of various substrates. Although the imprinted polymers of EGDM and MBAA showed high capacity, the selectivity was low relative to polymers of DVB. This was caused by additional hydrolysis of the crosslinked EGDM and MBAA. In addition, Bis F imprinted polymer was synthesized by BFDPM and DVB to compare imprinted manner of BADM-co-DVB. The resultant imprint efficiency of BFDPM-co-DVB was lower than that of BADM-co-DVB. We concluded that the optimum environment of covalent Bis A imprinting was cross linked DVB segments for BADM-co-DVB.

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