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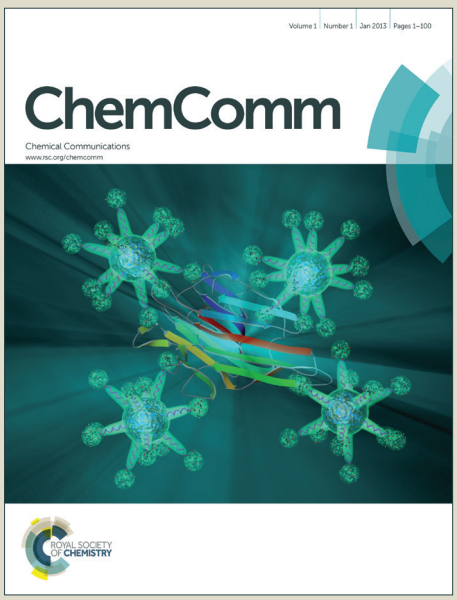

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Target Molecule-responsive Hydrogels Designed by Molecular Imprinting Using Bisphenol A as a Template

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Target molecule-responsive hydrogels with β-cyclodextrin (β-CD) were prepared by molecular imprinting using bisphenol A (BPA) as a template. BPA-imprinted hydrogels showed greater shrinkage than non-imprinted hydrogels because CD ligands arranged at suitable positions formed CD-BPA-CD complexes that acted as crosslinks.

Stimuli-responsive hydrogels exhibit reversible volume changes in response to environmental changes such as changes in pH,[1] temperature,[2] electric field[3] and light.[4] The stimuli-responsiveness of such hydrogels is mainly based on changes in the hydrophilicity of polymer networks or the osmotic pressure by charged groups. These stimuli-responsive hydrogels have potential applications as smart soft materials in the fabrication of sensors and drug delivery systems. However, a few studies on stimuli-responsive hydrogels that exhibit volume changes in response to a biomolecule, i.e., biomolecule-responsive hydrogels, have been undertaken. Differing from the general strategies for preparing stimuli-responsive hydrogels, we proposed a novel strategy to prepare biomolecule-responsive hydrogels[5] and gel particles.[6] Our strategy utilizes biomolecular complexes as dynamic crosslinks that dissociate and associate in the presence and absence of a target biomolecule, respectively. The biomolecule-responsive hydrogels prepared on the basis of this strategy exhibit volume changes in response to a target biomolecule because their crosslinking density changes with the association and dissociation of biomolecular complexes acting as dynamic crosslinks.

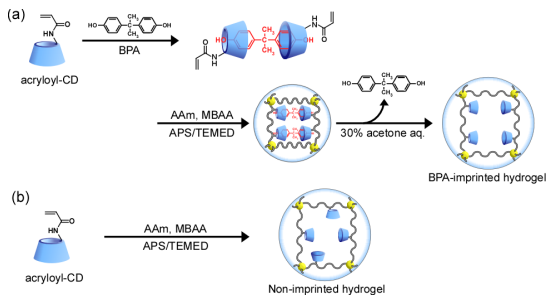
Molecular imprinting has attracted considerable attention as a useful technique for constructing sensors and separation systems because it enables convenient formation of molecular recognition sites in materials.[7] In molecular imprinting, after polymerizable functional groups as ligands are prearranged around a template molecule by non-covalent interactions, they are copolymerized with a large amount of crosslinker. Molecularly imprinted polymers with molecular recognition sites are then obtained by extraction of the template molecule from the resultant polymer networks to create a complementary molecular cavity. Typically, in standard molecular imprinting, the resulting polymer networks are highly crosslinked by a large amount of crosslinker to fix functional groups at a strictly defined and optimal position within the molecular recognition site. Furthermore, we prepared tumour marker-responsive hydrogels by biomolecular imprinting with a minute amount of crosslinker, an approach that differs from standard molecular imprinting that uses a large amount of crosslinker.[5d, 5e] In biomolecular imprinting, biomolecules such as antibodies and lectins are used as ligands for a template biomolecule. The resultant biomolecule-imprinted hydrogel selectively recognizes a target biomolecule and shrinks with an increase in its concentration because the gel networks formed by biomolecular imprinting memorize a target biomolecule and form dynamic crosslinks of biomolecular complexes.

In addition to hydrogen bonding and electrostatic interactions, host–guest interactions are used to create molecular recognition sites in molecular imprinting. For example, molecularly imprinted polymers with recognition sites for steroids, such as cholesterol and stigmasterol, have been synthesized using cyclodextrins (CDs) as ligands.[8] CDs are amphiphilic molecules that form an apolar cavity and hydrophilic exterior. They can form inclusion complexes with a variety of guest molecules such as phenol derivatives. For example, bisphenol A (BPA) forms an inclusion complex with β-CD.[9] BPA is an important industrial compound that is used as a monomer in the manufacture of polycarbonate plastics and epoxy resins. However, BPA may be able to mimic the effects of estrogens, thereby disrupting endocrine systems.[10] Therefore, methods to detect and remove BPA are in considerable demand.

In this study, BPA-responsive hydrogels that shrink in response to BPA were strategically prepared by molecular imprinting using β-CD as a ligand and a minute amount of crosslinker. This paper focuses on the effect of molecular imprinting on the shrinkage behaviour of BPA-responsive hydrogels in an aqueous BPA solution. This fundamental research into the responsive behaviour of molecularly

imprinted hydrogels will significantly contribute to the development of smart materials required for sensors and separation materials.

BPA-imprinted hydrogel was prepared as shown in Scheme 1. After complexation between β-CD with a polymerizable acryloyl group (acryloyl-CD)[11] and BPA, the resulting sandwich-like CD-BPA-CD complexes with acryloyl groups were copolymerized with acrylamide (AAm) and *N,N'*-methylenebisacrylamide (MBAA). To obtain BPA-imprinted hydrogels with molecular recognition sites for BPA, the template BPA was removed from the resultant networks by immersing in acetone solution. Furthermore, non-imprinted hydrogel also was prepared by copolymerization of AAm, MBAA and acryloyl-CD in a similar manner without template BPA.



Scheme 1. Synthesis of BPA-imprinted (a) and non-imprinted hydrogel (b).

The interaction of CDs with aromatic guest molecules can be studied by measurement using UV-Vis spectroscopy. The inclusion complexation between aromatic compounds and CD induces a red shift of the specific absorption related to the aromatic groups. The stoichiometry of the inclusion complex between CD and BPA was determined by UV titration measurements (Fig. S1). The maximum absorption of BPA observed at 276 nm was shifted to longer wavelengths in accordance with an increase in CD concentration. The maximum absorption wavelength of BPA became almost constant at a CD/BPA ratio greater than 2.0. This result indicates that the CDs and BPA formed an inclusion complex with a stoichiometry of 2:1.

To examine the BPA-responsive behaviours of BPA-imprinted, non-imprinted and PAAm hydrogels, changes in their swelling ratios in aqueous BPA solution were measured after swelling equilibria were achieved in water (Fig. 1). The PAAm hydrogel exhibited no volume change in the presence of BPA because it had no ligands to recognize BPA. In contrast, non-imprinted and BPA-imprinted hydrogels showed a gradual decrease in swelling ratio after immersion in aqueous BPA solution. The BPA-imprinted hydrogel showed greater shrinkage than the non-imprinted hydrogel. In contrast, the BPA-imprinted hydrogel remained unchanged in the presence of phenylethyl acetate which has a single aromatic group (data not shown). While molecular recognition ability of the BPA-imprinted hydrogel still requires further research, they are likely to recognize two aromatic groups of BPA and induce its structural change.

To better understand the shrinkage behaviour of BPA-imprinted and non-imprinted hydrogels in response to BPA, their crosslinking densities were determined by measuring their compressive moduli. The relationships between BPA concentration and apparent crosslinking density of the BPA-imprinted, non-imprinted and PAAm hydrogels are shown in Fig. 2(a). The apparent crosslinking density of PAAm hydrogel was not influenced by BPA concentration. In contrast,

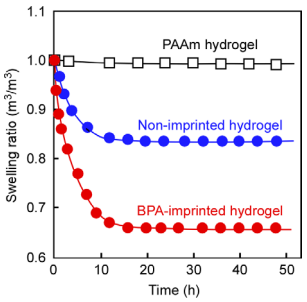


Fig. 1. Changes in the swelling ratios of BPA-imprinted, non-imprinted and PAAm hydrogels in an aqueous BPA solution (0.12 mg/mL) as a function of time.

the apparent crosslinking density of BPA-imprinted and non-imprinted hydrogels increased with increasing BPA concentration. In addition, Fig. 2(a) demonstrates that the change in apparent crosslinking density of the BPA-imprinted hydrogel was greater than that of the non-imprinted hydrogel.

The BPA-imprinted and non-imprinted hydrogels had CDs as ligands for BPA in their networks, which associate with BPA to form sandwich-like CD-BPA-CD complexes upon immersion of the hydrogels in aqueous BPA solution. Because the resultant CD-BPA-CD complexes act as crosslinks, formation of these complexes leads to an increase in apparent crosslinking density of the BPA-imprinted and non-imprinted hydrogels, followed by shrinkage in response to BPA. The more drastic BPA-responsive shrinkage of the BPA-imprinted hydrogel compared with that of the non-imprinted hydrogel is attributed to a greater change in the crosslinking density.

Although the same amount of CDs were used in the preparation of BPA-imprinted and non-imprinted hydrogels, the BPA-imprinted hydrogel showed greater shrinkage and crosslinking density than non-imprinted hydrogel in the presence of BPA. The adsorption of BPA into the hydrogel networks was examined to investigate the difference in the molecular recognition sites of BPA-imprinted and non-imprinted hydrogels. Fig. 2(b) shows the amount of BPA adsorbed into BPA-imprinted, non-imprinted and PAAm hydrogels in an aqueous BPA solution. A minute amount of BPA was adsorbed into the PAAm hydrogel without CD ligands. In contrast, large amounts of BPA were adsorbed into both the BPA-imprinted and non-imprinted hydrogels. Furthermore, the BPA-imprinted hydrogel showed more effective adsorption of BPA than the non-imprinted hydrogel. Notably, the BPA adsorption behaviour of the BPA-

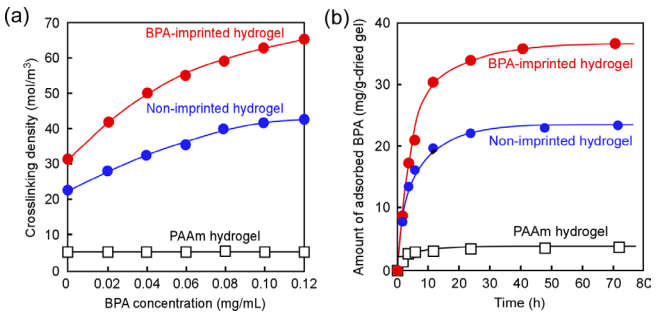


Fig. 2. (a) Effect of BPA concentration on the crosslinking densities of BPA-imprinted, non-imprinted and PAAm hydrogels in an aqueous BPA solution. (b) BPA adsorption into BPA-imprinted, non-imprinted and PAAm hydrogels immersed in an aqueous BPA solution (0.12 mg/mL) as a function of time.

imprinted hydrogel was quite different from that of the non-imprinted hydrogel, in spite of the use of the same amount of CD ligands in the preparation of these hydrogels. In previous studies,[5d, 5e] we reported that tumour maker (α-fetoprotein: AFP)-imprinted hydrogels with its antibody (anti-AFP) and lectin (concanavalin A: ConA) as ligands shrank in response to target AFP. ConA and anti-AFP optimally arranged in gel networks by biomolecular imprinting enabled the AFP-imprinted hydrogel to shrink by the effective formation of sandwich-like ConA-AFP-anti-AFP complexes, which acted as dynamic crosslinks. On the other hand, the non-imprinted hydrogel, in which ConA and anti-AFP were randomly distributed, swelled slightly in response to AFP because of changes in osmotic pressure associated with the binding of AFP to ConA or anti-AFP. Based on the mechanism for the responsive behaviour of the AFP-imprinted hydrogels, the highly BPA-responsive shrinkage of the BPA-imprinted hydrogel can be explained by the tentative model schematically illustrated in Fig. 3. In the BPA-imprinted hydrogel, CDs as ligands for BPA are arranged at suitable positions for the formation of 2:1 complexes between CD and BPA because gel networks are formed by molecular imprinting using template BPA. Thus, molecular imprinting enables the creation of BPA recognition sites with optimally arranged CDs. Therefore, upon immersion in an aqueous BPA solution, BPA is more effectively adsorbed into the BPA-imprinted hydrogel than the non-imprinted hydrogel. In contrast, CD ligands are randomly distributed in the non-imprinted hydrogel. Although a few CD ligands in the non-imprinted hydrogel are stochastically arranged at optimal positions for the recognition of BPA to form CD-BPA-CD complexes, the other CD ligands cannot form sandwich-like complexes with BPA because of their random arrangement. Furthermore, there is a very small amount of 1:1 complexes between CD and BPA in the non-imprinted hydrogel owing to their relatively low binding constant, i.e. 1.33×10^3 $L\cdot mol^{-1}$, which was determined by Klotz plot. Therefore, the amount of BPA adsorbed into the non-imprinted hydrogel and its swelling ratio change were smaller than that of the BPA-imprinted hydrogel.

In conclusion, a BPA-responsive hydrogel with CDs as ligands was prepared by molecular imprinting. The BPA-imprinted and non-imprinted hydrogels with CD ligands showed gradual shrinkage in response to BPA because of an increase in crosslinking density based on the formation of CD-BPA-CD complexes. Furthermore, the BPA-imprinted hydrogel showed greater shrinkage in response to BPA than the non-imprinted hydrogel. This is attributed to the arrangement of CD ligands within the imprinted network at suitable positions for the formation of the 2:1 CD and BPA complexes by molecular imprinting. In contrast, because the CD ligands are randomly distributed in the network of the non-imprinted hydrogel, this hydrogel shrank less than the BPA-imprinted hydrogel in the presence of BPA. Finally, as smart functional materials, BPA-imprinted hydrogels can provide a useful platform for constructing materials for use in molecular sensors and separation technology, among many other applications. However, as acetone is required to remove BPA adsorbed in the hydrogels, their applicability may be limited. Although BPA-imprinted hydrogels require further research, they are likely to become important functional materials in the future.

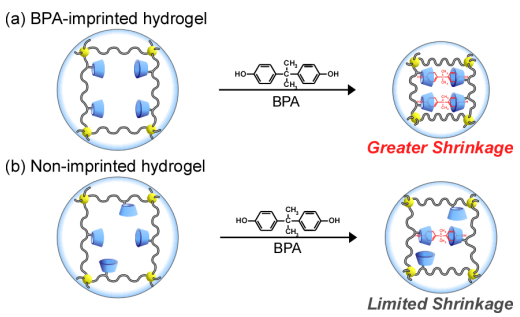


Fig. 3. Schematic of swelling behaviours of BPA-imprinted (a) and non-imprinted hydrogel (b).

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Notes and references

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† Electronic Supplementary Information (ESI) available: detailed synthesis of hydrogels and determination of stoichiometry of the inclusion complexes between CD and BPA. See DOI: 10.1039/c000000x/

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