Molecularly Imprinted Polymers for Catechin Recognition Prepared Using Dummy-Template Molecules

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Abstract
Molecular imprinting for (+)-catechin (CA) recognition was successfully demonstrated using naringerin (NG) and 7-hydroxyflavanon (7HF) derivatives as dummy-template molecules. Molecularly imprinted polymer (MIP) films were synthesized using methacryloyl NG (NGMA) by semi-covalent-type imprinting process on gold substrate. The selective binding property toward CA was confirmed by surface plasmon resonance measurements, and we clarified that the imprinting process was effective for the formation of the specific binding cavities from the results of binding experiments for non-imprinted polymers (NIPs), in which the binding amounts towards CA and its analogue were negligible. Moreover, we synthesized successfully the bulk MIPs for CA separation using methacryloyl 7HF (7HFMA) as dummy-template and methacrylamidyl β-cyclodextrin (βCD) as another functional monomers by combination of semi-covalent- and non-covalent-type molecular imprinting processes. For the bulk MIPs, the binding property of obtained βCD-MIP was confirmed by the fluorescent measurements derived from CA remained in the supernatant after interaction with obtained βCD-MIP. From these results, the molecular imprinting with dummy-template is effective technique for preparation of the CA recognition materials (MIPs). The technique is promising for the development of the separation materials for CA as well as the other important compounds.

Keywords: Molecular imprinting; Catechin; Molecular recognition; Dummy-template

1. Introduction
Recognition, extraction, and selective sensing of valiant components from beverages and foods are attracted attentions from wide industrial fields such as pharmaceutical and food industries [1]. Catechins such as (+)-catechin (CA) and (-)-epicatechin (EC) are polyphenol compounds and are well-known as antioxidant chemicals due to its radical scavenging ability derived from phenol moieties, thus, it is widely used as products of shelf-life enhancer for pharmaceuticals, nutraceuticals, and cosmetics [2,3]. In addition, catechins work beneficially in our bodies for maintaining human health [4]. To date, many researchers reported the effects of catechins on human body [5,6]. Several researchers clarified that the ingestion of catechins resulted in inhibition of several kinds of cancer growth [1,7,8].

With becoming recognized the importance of catechins, high-precision separation and collection techniques of catechins is desired. Until now, various extraction methods for catechin were reported such as water-, polar organic solvent-, microwave-assisted-, and high pressure-extraction methods [9-12]. The important process of catechins collection is catechins separation from the other compounds in the extractive. Use of polymeric resin for adsorption of catechins is one of effective techniques for separation of catechins because catechins could be separated from the mixture solution by only addition of the adsorbents [13]. However, the selective recognition property toward catechins was not enough in this case.

Molecular imprinting is one of the template polymerization technique, which is prepared by radical polymerization of functional monomer(s) interacting with or conjugating to the template molecules, cross-linking agents, and co-monomer(s), followed by the template removal, resulting in the specific binding cavities for target...
molecules in the polymer matrix [14-20]. From the pioneer’s works of Wulff and Mosbach groups, molecularly imprinted polymers (MIPs) bearing the recognition ability towards quite wide range of chemicals have been reported [21-26]. In our previous work, we demonstrated a series of studies for synthesis of MIPs towards pesticide, endocrine disrupter, neummessenger, as well as proteins [27-32]. We also clarified that MIPs could be used as an absorbance, recognition elements in biosensors, and separation materials [33].

One of the drawbacks of molecular imprinting is use of the sacrificial template molecules. If the template molecules are noble, highly expensive, and/or hazardous, the molecular imprinting technique faces a major obstacle due to its intrinsic problems that the use of the template molecules in the molecular imprinting process. In the case of catechins as a template molecule, degradation of tea catechins were accelerated at pH higher than 6.0 [34] and during thermal sterilizations [35]. To overcome the problems, using dummy-template, which has a similar chemical structure and size with target molecules but inexpensive and safety, has been developed [36,37]. The dummy-template method enables to form the binding cavities having the similar size and shape for the target molecules as well as the effective interaction sites towards objective target molecules in MIPs.

In this study, we prepared the MIPs for CA recognition using dummy-template strategy. In the first chapter, we attempted to prepare a MIP film for CA recognition using naringerin (NG) (see Fig. 1) as dummy-template on gold substrate, which enables us to measure the binding property of the MIPs toward catechin by surface plasmon resonance (SPR) measurements. In the later chapter, the CA recognition MIPs bearing the specific binding cavities, in which a β-cyclodextrin (βCD)-based functional monomer was copolymerized with methacryloyl 7-hydroxyflavanon (7HFMA) as dummy-template, to construct an interaction site based on inclusion complexation between βCD and CA in aqueous media [38-40]. Adsorption of CA towards MIPs was investigated by the fluorescence measurements of catechin having intrinsic fluorescence property remained in the supernatant after interaction of CA with MIPs.

2. Experimental part

2.1. Materials

(+)-Catechin hydrate, sodium hydroxide (NaOH), magnesium sulfate, hexane, methanol, 1-butanol, ethyl acetate, dichloromethane, acetone, tetrahydrofuran (THF) and β-cyclodextrin (βCD) were purchased from Nacalai tesque Co. (Kyoto, Japan). Methacrylic acid (MAA), methacryloyl chloride, N,N'-methylene bisacrylamide (MBAA), sodium hydrate and DM-TMM were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). 7-Hydroxyflavanone (7HF), naringerin (NG), N,N'-diisopropylethylamine (DIEA) and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC) were purchased from Tokyo Chemical Industries (Tokyo, Japan). 4,4’-Azobis(4-cyanovoleric acid) (V-501) was purchased from SIGMA-Aldrich Japan K. K. (Tokyo, Japan). Carboxymethyl N,N'-diethyldithiocarbamate as photo initiator was prepared utilizing previously reported procedure [33,41]. Mono-6-((N-methacrylamide)-6-deoxy-βCD (βCDMAm) was synthesized by coupling reaction between methacryloyl chloride and mono-6-amino-6-deoxy-βCD utilizing previously reported procedure [42,43].

2.2. Characterizations

1H NMR spectra were measured using a 300 MHz FT-NMR apparatus (JNM-LA300 FT NMR system, JEOL Ltd., Tokyo, Japan). Surface plasmon resonance (SPR) measurements were performed on a Biacore 3000 (GE Healthcare Japan, Tokyo, Japan). UV-Vis spectra measurements were carried out using a UV-Vis spectrophotometer (V560, JASCO, Japan). Fluorescence spectra measurements were carried out using a spectrofluorometer (F-2500, Hitachi, Japan).

2.3. Synthesis of methacryloyl naringerin (NGMA)

NG (500 mg, 1.8 mmol) and DMAP (785 mg, 6.4 mmol) were dissolved in DMF (2 mL). After mixing for 1 h in ice bath under N₂ atmosphere, methacryloyl chloride (0.62 mL, 6.4 mmol) dissolved in DMF (1 mL) were added to the mixture, and the mixture was reacted in ice bath for 1 h, followed by further reaction at r.t. for 24 h was carried out. After the reaction, the product was dissolved in ethyl acetate and washed with water (3 times). The oil phase was dried by MgSO₄, and the silica gel chromatography was carried out (eluent: ethyl acetate/hexane = 1:2), followed by further chromatography (eluent: ethyl
acetate/hexane = 1:3) was carried out. After evaporation, the yellow oil was obtained. \(^1\)H-NMR (300 MHz, DMSO-\(d_6\)), ppm = 7.62 (d, 2H, phenyl, \(J=8.8\) Hz), 7.26 (d, 2H, phenyl, \(J=11\) Hz), 7.00 (s, 1H, phenyl), 6.84 (s, 1H, phenyl), 6.30-5.90 (m, 6H, \(\mathrm{CH}_3\mathrm{C} = \mathrm{CH}_2\)), 5.75 (t, 1H, \(\mathrm{CHCH}_2\mathrm{C}=\mathrm{O}\), \(J= 8.1\) Hz), 2.03-1.99 (m, 9H, \(\mathrm{C}_3\mathrm{C}=\mathrm{CH}_2\)), 1.67 (m, 2H, \(\mathrm{CHCH}_2\mathrm{C}=\mathrm{O}\)).

2.4. Synthesis of methacrylamide group-immobilized gold substrate

Gold substrate for SPR measurement was washed by ethanol and water. After UV-O\(_3\) treatment, the substrate was immersed in bis(acryloyl)cysteamine (5 mM) in ethanol for 24 h.

2.5. Synthesis of MIP films on the gold substrate

NGMA (1 mL, 0.21 mmol), THMA (1.98 mg, 2.1 mmol), MBAA (4.8 mg, 3.15 mmol), and inifertor (2 wt \%) were dissolved in DMSO (1 mL). The prepolymerization mixture was polymerized on the methacrylamide group immobilized gold substrate by photo-irradiation (\(\lambda=365\) nm) for 1 h at 4 °C. After the polymerization, the hydrolysis for the template removal was carried out with KOH (0.5 M) on the gold substrate for overnight. The non-imprinted polymer (NIP) film was also obtained by the same procedure except for without using NGMA.

2.6. SPR measurements

Catechin dissolved in 10 mM HEPES buffer (pH 7.4), and reference compounds (NG, 7-HF, 6-HF) dissolved in 10 mM HEPES buffer (pH 7.4) with 5 \% acetonitrile (0-1.0 mM) were used for these experiments. Flow rate was 20 \(\mu\)L/min, and injection volume was 20 \(\mu\)L. Regeneration solutions were selected appropriately from the following candidates: 10 mM NaOH and 10 mM Gly-NaOH (pH 10.0). The amount of bound compounds was calculated from the signal intensity (resonance units, RU; 1 RU = 10.0). The amount of bound compounds was calculated from the signal intensity (resonance units, RU; 1 RU = 10.0). The amount of bound compounds was calculated from the signal intensity (resonance units, RU; 1 RU = 10.0). The amount of bound compounds was calculated from the signal intensity (resonance units, RU; 1 RU = 10.0).

2.7. Synthesis of methacryloyl 7-hydroxyflavanon (7HFMA)

7HF (200 mg, 833 \(\mu\)mol) and DMAP (152 mg, 1.24 mmol) were dissolved in dry THF (3 mL). After mixing for 30 min at r.t. under N\(_2\) atmosphere, methacryloyl chloride (0.13 mL, 1.37 mmol) dissolved in dry THF (1 mL) were added to the mixture, and the mixture was reacted at r.t. for 1 h. After filtration, the product was evaporated, and silica gel chromatography was carried out (eluent: ethyl acetate/hexane = 1:9). After evaporation, the clear oil was obtained. \(^1\)H-NMR (300 MHz, DMSO-\(d_6\)), ppm = 7.86 (d, 2H, phenyl, \(J=8.0\) Hz), 7.57 (d, 2H, phenyl, \(J=8.0\) Hz), 7.48-7.37 (m, 3H, phenyl), 7.01 (s, 1H, phenyl), 6.94 (d, 1H, phenyl, \(J=11\) Hz), 6.30 (s, 1H, \(\mathrm{CH}_3\mathrm{C}=\mathrm{CH}_2\)), 5.94 (s, 1H, \(\mathrm{CH}_3\mathrm{C}=\mathrm{CH}_2\)), 5.75 (t, 1H, \(\mathrm{CHCH}_2\mathrm{C}=\mathrm{O}\), \(J= 8.1\) Hz), 2.85 (m, 2H, \(\mathrm{CHCH}_2\mathrm{C}=\mathrm{O}\)), 2.00 (s, 3H, \(\mathrm{CH}_3\mathrm{C}=\mathrm{CH}_2\)).

2.8. Fluorescence measurements of mixture of CA and \(\beta CD\)

CA dissolved in 10 mM acetate buffer (pH 5.0) (CA concentration: 0.1 mM) was mixed with 10 mM acetate buffer (pH 5.0) dissolving various concentrations of \(\beta CD\) (0 – 1.0 mM), and the mixture was incubated for 30 min. Then, the fluorescence spectra of the mixture were measured (excitation wavelength: 280 nm). Relative fluorescence intensity, \(I/I_0\) was calculated, where \(I\) is fluorescence intensity at 620 nm after mixing with \(\beta CD\), \(I_0\) is initial fluorescence intensity at 620 nm.

2.9. Fluorescence measurements of mixture of 7HFMA and \(\beta CD\)

7HFMA dissolved in water with 1 vol\% DMSO (7HFMA concentration: 0.1 mM) was mixed with water with 1 vol\% DMSO dissolving various concentration of \(\beta CD\) Am (0 – 1.0 mM), and the mixture was incubated for 30 min. Then, the fluorescence spectra of the mixture were measured (excitation wavelength: 273 nm). In the same manner, relative fluorescence intensity, \(I/I_0\) was calculated, where \(I\) is fluorescence intensity at 400 nm after mixing with \(\beta CD\), \(I_0\) is initial fluorescence intensity at 400 nm.

2.10. Preparation of bulk MIP using 7HFMA and \(\beta CD\)-MIP

7HFMA (0.324 mg, 0.1 \(\mu\)mol) and \(\beta CD\) Am (12.3 mg, 1.0 \(\mu\)mol) were dissolved in water with 1 vol\% DMSO solution. For formation of the complex between 7HFMA and \(\beta CD\) Am, the prepolymerization mixture was heated at 80 °C for 1 h. After injection of MBAA (100 mg, 65 \(\mu\)mol) and V-501 (2.0 mg, 7.1 \(\mu\)mol) dissolved in water (40 \(\mu\)L), the polymerization was carried out 80 °C for 1 h. The obtained polymer was crushed and the remained monomer species were washed by Soxhlet extraction using methanol. The template removal was carried out by hydrolysis with 1 M KOH, and then neutralization was carried out with HCl. As a control, a MIP prepared by only 7HFMA was prepared by similar protocol without using \(\beta CD\) Am (MIP(\(\beta CD\)-MIP)). In this case, \(\beta CD\) was used instead of \(\beta CD\) Am to completely dissolve 7HFMA by inclusion complexation with \(\beta CD\).

2.11. Binding experiments of catechins on \(\beta CD\)-MIP and MIP(\(\beta CD\)-MIP)

\(\beta CD\)-MIP and MIP(\(\beta CD\)) (3.0 mg) were dispersed in various concentrations of CA aqueous solutions (2.0 mL) (pH 5.0, adjusted by adding HCl) (CA concentration: 0,
6.25, 12.5, 25, 50, 100 μM). After 12 h incubation at 40 °C, centrifugation was carried out for separation of βCD-MIP or MIP(-βCD) from the supernatant. The adsorbed amounts of CA were determined by the fluorescence measurements of supernatant containing unbound CA (excitation wavelength: 280 nm). A calibration curve of CA (0 – 100 μM) was obtained by the fluorescent intensity at 620 nm. By subtracting the initial fluorescence intensity (I₀) and the intensity after the incubation (I), the absorbed amounts of CA per 1g of MIPs or NIPs were calculated. The selectivity tests were also carried out by the same procedures using (-)-epigallocatechin (EC) and (-)-epicatechin gallate (ECG) as reference compounds.

3. Results and discussion

3.1. Preparation of catechin recognition polymer film on SPR sensor chip

We demonstrated the preparation of CA recognition polymers by molecular imprinting technique using dummy template molecules. First of all, the MIPs having CA recognition property were prepared using naringerin (NG)-derivatives as a template molecule, and the catechin adsorption ability was evaluated by SPR measurements. Methacryloyl naringerin (NGMA) as a dummy template molecule was prepared by the introduction of methacryloyl groups towards three hydroxyl groups of NG, which was confirmed by 1H-NMR spectra. A MIP film was prepared by photo-radical polymerization (λ= 365 nm) of NGMA, THMA, and MBAA as a functional monomer, a co-monomer, and a crosslinker, respectively, with carboxymethyl N,N'-diethyldithiocarbamate as photo initiator on the methaclylamide-functionalized gold substrate for 1 h at 4 °C, followed by hydrolysis of the methacryloyl groups by 0.5 M KOH to remove the template (naringerin moiety).

CA adsorption toward the MIP was evaluated by SPR measurements in 10 mM HEPES buffer (pH 7.4). The ΔRU values increased gradually with the CA concentration, indicating that MIP had CA binding ability (Fig. 2). To confirm the imprinting effect, CA adsorption toward non-imprinted polymer (NIP) film was also evaluated, where the NIP was prepared by the same procedure without the template monomer (NGMA). The CA adsorption toward the NIP film was almost negligible below 1.0 mM of CA, indicating that the non-specific bindings toward NIP were negligible at the concentration range. Therefore, the MIP film could be applicable for further selectivity tests in this concentration range. Since the binding isotherm of CA on the MIP was described by a liner fraction, the ΔRU values should increase with the concentration over 1 mM until reaching the saturated fraction of the binding sites. On the other hand, the rate of increase in the ΔRU on the NIP was significantly smaller than on the MIP (Fig. 2), suggesting that the MIP could be also applicable even above 1 mM of CA concentration.

Selectivity tests both for MIP and NIP were demonstrated with NG, 6-hydroxyflavanon and 7-hydroxyflavanon as reference compounds that bear similar flavan frame with CA (Fig. 3), where these reference compounds have similar molecular sizes with different number of phenol groups. Among these molecules, CA showed the largest adsorption as compared with the other reference compounds. Moreover, the adsorptions of these molecules toward NIP were significantly smaller than MIP. The largest binding amounts for catechin with MIP should be caused by the largest number of interaction groups (phenol groups) of catechin compared to the other molecules (catechin: four phenol groups; naringerin: three phenol groups; 6-hydroxyflavanon and 7-hydroxyflavanon: one phenol group). These results clearly indicate that the molecular imprinting using the dummy template is effective technique to make specific binding cavity for CA.

![Fig. 2. SPR measurements for CA adsorption behavior towards MIP and NIP films prepared on gold substrate by photo-radical polymerization of NGMA, THMA, and MBAA with iniferter as a photo-initiator for 1 h at 4 °C (n=3, Mean±S.D.).](image-url)

![Fig. 3. Selectivity tests for MIP and NIP film with naringerin (NG), 6-hydroxyflavanon, and 7-hydroxyflavanon by SPR measurement (n=3, Mean±S.D.). Concentration of all the compounds was 0.5 mM.](image-url)
3.2. Preparation of CA recognition polymer comprising \(\beta\)CD-based recognition sites by hybrid molecular imprinting process

We demonstrated to synthesize MIP for CA recognition by combination of semi-covalent- and non-covalent-types molecular imprinting processes. It is widely known that \(\beta\)CD has an interaction property with several compounds by hydrophobic interactions inside of \(\beta\)CD, resulting in the inclusion of these compounds [36]. Jullian et. al. have clarified that the interaction between CA and \(\beta\)CD was happened with 1:1 stoichiometry using 2D-NOESY NMR technique and molecular modeling [44]. It was clarified that the B-ring of CA (see Fig. 1) was interacted with \(\beta\)CD by hydrophobic interaction and several hydrogen bonding, but the interactions between A- or C-rings and \(\beta\)CD were not observed, resulting that the B-ring was deeply inserted into \(\beta\)CD cavity [44]. Herein, we designed the binding cavities in the MIPs having multiple interaction sites including carboxy group and \(\beta\)CD (\(\beta\)CD-MIP). We designed a dummy template molecule, methacryloyl 7-hydroxyflavanon (7HFMA), and methacrylamidyl \(\beta\)CD (\(\beta\)CDAm) as an another functional monomer because the methacryloyl group on A-ring of 7HFMA placed at the outside of \(\beta\)CDmAm after interaction of these molecules based on the above inclusion mechanism of CA in \(\beta\)CD. The 7HFMA was prepared by the condensation reaction between hydroxyl group of 7-hydroxyflavanon and methacryloyl chloride. \(\beta\)CDAm was also synthesized by conjugation reaction between mono-amino-functionalized \(\beta\)CD and methacryloyl chloride. The preparations of the compounds were confirmed by 1H-NMR spectra.

To investigate the interaction between CA and \(\beta\)CD, we measured the fluorescence spectra of CA (0.1 mM) with various concentrations of \(\beta\)CD (0 - 1.0 mM) in 10 mM acetate buffer (pH 5.0) at 25°C after 24 h incubation. The incubation time was enough because the fluorescence spectra were kept similar value even after 3 h incubation. CA shows maximum absorption wavelength at 220 and 280 nm, so that the following fluorescence spectra were measured with excitation wavelength at 280 nm. With increasing the \(\beta\)CD concentration, the fluorescence intensity at ca. 620 nm increased, which is caused by the decrease of energy dissipation of CA after excitation due to the inclusion of CA in \(\beta\)CD (Fig. 4). Langmuir binding isotherm was drown from the fluorescence intensity change, and the binding constant was calculated to be \(2.6 \times 10^4\) (M\(^{-1}\)). The results indicate that CA was included into \(\beta\)CD cavity, and thus, the \(\beta\)CD-based inclusion complexation is likely to be a good candidate as an interaction mechanism at binding sites in MIPs.

In addition, the interaction between 7HFMA and \(\beta\)CDAm was also investigated in water with 1 vol\% of DMSO, where DMSO was necessary for dissolving 7HFMA, at 25 °C by fluorescence measurements (excitation wavelength: 273 nm) derived from 7HFMA (Fig. 5). The fluorescence intensity at ca. 400 nm from 7HFMA (0.1 mM) increased with the \(\beta\)CDAm concentration (0 - 1 mM), which was similar phenomenon between CA and \(\beta\)CD and was caused by the inclusion of 7HFMA by \(\beta\)CDAm. From the corresponding Langmuir binding isotherm, \(K_a\) value was calculated to be \(7.2 \times 10^4\) (M\(^{-1}\)), which was slightly higher than that between CA and \(\beta\)CD. These results indicate that the \(\beta\)CDAm acts as a functional monomers toward the template molecule, 7HFMA, because the complexation between 7HFMA and \(\beta\)CDAm occurred during the radical polymerization for the MIP preparation over the concentration range, leading to constructing the effective binding cavities containing \(\beta\)CD as an interaction site based on the inclusion complexation.

![Fig. 4. Fluorescence spectra (Ex: 280 nm) (a) and the relative fluorescence intensity change at 620 nm (b) of CA as a function of various concentrations of \(\beta\)CD in 10 mM acetate buffer (pH 5.0) at 25°C.](image)

![Fig. 5. Fluorescence spectra (Ex: 273 nm) (a) and the relative fluorescence intensity change at 400 nm (b) of 7HFMA after interaction with various concentrations of \(\beta\)CDAm in water with 1 vol % DMSO at 25°C.](image)
cavities after washing MIPs because they were not covalently linked with polymer matrix.

The binding properties of βCD-MIP and MIP(-βCD) toward CA were confirmed by the fluorescence measurements (see Experimental section 2.11). The binding amount of CA toward βCD-MIP increased linearly with the CA concentration. Moreover, the binding amounts were much greater than that in MIP(-βCD) (Fig. 6). The results indicate that βCD moiety contributed to forming the binding sites toward CA in the binding cavities.

Finally, we performed the selectivity test of obtained βCD-MIP and MIP(-βCD). We selected (-)-epigallocatechin (EC) and (-)-epicatechin gallate (ECG) as reference compounds, where the EC and EGC have similar molecular size with five phenol groups and larger molecular size with eight phenol groups, respectively (Fig. 7). The adsorption amount of ECG toward βCD-MIP was significantly lower than that for CA, and the results might be caused due to the unfitting of ECG with binding cavities in βCD-MIP because of its larger molecular size. As mentioned above, CA was included in βCD cavity with hydrophobic interaction and several hydrogen bonding, and they clarified that the B-ring in CA (see Fig. 1) was deeply inserted into βCD [42]. EGC has another benzene ring compared to CA, and it seems that the steric hindrance due to the additional benzene ring was caused for the inclusion of ECG by βCD, leading that the βCD did not work as effective binding sites in the βCD-MIP. Actually, the MIP(-βCD) showed the negligible binding properties towards ECG as well. On the other hand, EC bound toward βCD-MIP was larger than that of CA. Presumably, the larger number of the interaction groups (phenol groups) of EC contributes to non-specific interaction at the binding sites that could interact with CA. However, the adsorption amounts of EC toward NIP were negligible, which also indicate that the molecular recognition cavities were successfully formed in βCD-MIP by the molecular imprinting process using the dummy-template molecule.

4. Conclusions
We demonstrated that preparation of CA recognition materials by molecular imprinting using dummy-template molecules. The MIP film was prepared by using NGMA, THMA, and MBAA with iniferter as photo-initiator on the methaclylamide-functionalized gold substrate. The molecular recognition property investigated by SPR indicate that CA was recognized by the binding cavities formed in the molecular imprinting process. Moreover, we successfully prepared the βCD-MIP for CA recognition by combination with the dummy-template and inclusion complexation of βCD. The binding cavities were formed due to low bound amounts of ECG as compared with CA. However, the formed binding cavities had a precisely prescript molecular size for CA and its analogue with similar size and shape. We hope CA recognition materials prepared by the molecular imprinting technique in conjunction with dummy-template methods develop the separation and collection of CA for various industrial fields such as pharmaceuticals and cosmetics.

Fig. 6. Binding isotherms of CA with βCD-MIP and MIP(-βCD) (n=3, Mean±S.D.).

Fig. 7. Selectivity tests for βCD-MIP and MIP(-βCD) with CA, EC and ECG by fluorescence measurements of these compounds remained in the supernatant after interaction with these polymers (n=3, Mean±S.D.).

References


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