

Synthesis and adsorption properties of carbamazepine imprinted polymer by dispersion polymerization in supercritical carbon dioxide

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Abstract—We synthesized molecularly imprinted polymers (MIPs) which can selectively separate carbamazepine (CMZ) as a pharmaceutically active compound by using supercritical fluid technology in supercritical carbon dioxide (scCO₂), and also evaluated the adsorption properties of the prepared CMZ imprinted polymers (CMZ-IPs). CMZ-IPs is prepared with methacrylic acid (MAA) as a functional monomer, CMZ as a template, and ethylene glycol dimethacrylate (EGDMA) as a crosslinking agent. The binding characteristics of CMZ-IPs are evaluated using equilibrium binding experiments. The adsorption ability in aqueous solution of CMZ-IPs was investigated by HPLC analysis, measuring the adsorbed amounts for the template and its structural analogue, the selectivity factor (α), and the imprinting-induced promotion of binding (IPB). The adsorption properties with the change of pH and temperature of aqueous solution were also examined. The results of the evaluation analysis indicate that the prepared CMZ-IPs have high selectivity (102, 94, 75 and 44 $\mu\text{mol/g}$) and separation abilities.

Keywords: Molecularly Imprinted Polymers (MIPs), Carbamazepine, Supercritical Fluid Carbon Dioxide, Adsorption Properties, Separation Technology

INTRODUCTION

Supercritical carbon dioxide (scCO₂) has emerged as the most extensively studied supercritical fluid for polymerization reactions for its high density, high diffusivity and low viscosity [1-3]. Furthermore, the matrices can have a controlled morphology, and since they are obtained as dry powders that have no organic solvents residues there is no need for further purification and drying steps. This is a major advantage in applications such as in pharmaceutical and biomedical fields where purity is the key parameter. Recently, scCO₂ has been demonstrated to be a clean, one-step synthetic route for the preparation of affinity polymeric materials, with attested in chromatography and drug delivery [4-6]. The high diffusivity and low viscosity of scCO₂ reduce the mass transfer limitations found in the conventional synthesis. In addition, since scCO₂ is a nonpolar and aprotic medium, the hydrogen bonds between the template and the monomers are more stabilized than in a protic solvent, which leads to more stable complexes and consequently to polymers potentially with higher affinity and selectivity. For this reason, scCO₂ is a very attractive and promising medium for developing these affinity materials.

The molecular imprinting technique (MIT) began with the hypothesis of antibody formation by Pauling in 1940 [7]. It was a novel separation technique that prepared polymers with selectivity for specific molecular (template). After Pauling's hypothesis for the formation of antibodies, Wulff showed the possibility of effective molecular recognition to allow binding site by introducing functional groups in the early 1970s [8,9]. The studies about of molecular imprinting

actively have continued enabling molecularly imprinted polymers (MIPs) to facilitate the synthesis, to secure good physical properties, and to give them economic advantages [10-16]. Subsequently, it has been applied for chromatographic fillers [17], artificial enzymes [18,19], and high-efficiency bio-sensor [20]. MIPs are synthesized with template which is selectively combined with the functional monomer and crosslinking agents. After the template is removed with proper solvent from MIP, formed was the three-dimensional molecular structure that is able to recognize the purpose template (Fig. 1) [21,22]. The binding sites that were generated have suitable sizes and shapes complementary to a target molecule (template), resulting in strong adsorption of the template [23].

Carbamazepine (CMZ) is widely used for the treatment of epilepsy and bipolar disorder. In addition to many other pharmaceuticals [24,25], CMZ has been found in effluent water because it is not eliminated efficiently from wastewater. No other treatments are available to completely remove this compound from water, so CMZ can easily reach the surface water where it is discharged to other media when this water is reused. CMZ is thus becoming a significant hazard to the general population as well as to the environment.

Many authors have reported on preparation of CMZ-imprinted polymers using various polymerization methods. Dai et al. [26] investigated the adsorption properties of MIPs synthesized by precipitation polymerization. CMZ-imprinted polymers were prepared by a mixture of acetonitrile and toluene, 75 : 25, v/v as a porogen, and its separation abilities were measured using HPLC analysis. Beltran et al. [27,28] prepared CMZ-imprinted polymers by bulk and solution polymerizations. According to their results, the adsorption experiments of MIPs using various polymerization methods did not show the amount of quantitative adsorption. Although the separation abilities of imprinted and non-imprinted polymers were investigated by HPLC analysis, HPLC analysis or adsorption experiments were not

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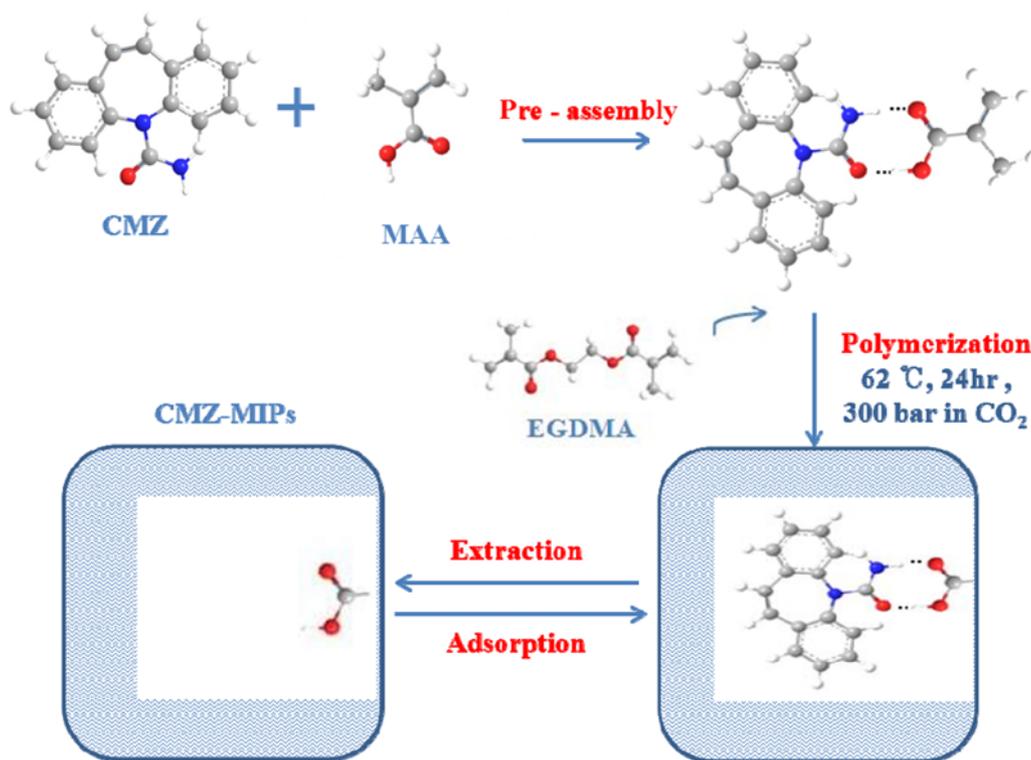


Fig. 1. Schematic diagram on synthesis processes of CMZ-MIPs.

performed for selective separation of structural analogue of CMZ.

In this study, we synthesized MIPs for CMZ as an endocrine disrupting material using the dispersion polymerization method in scCO_2 , called a green solvent. The binding characteristics of the prepared MIPs were analyzed by adsorption kinetics, adsorption isotherms, and Scatchard plot analysis. In addition, the selective separation abilities of MIPs were investigated applying the selectivity factor (α), the imprinting-induced promotion of binding (IPB), and the adsorption of materials with structures similar to templates (CMZ), such as 2,4-dichlorophenoxyacetic acid (2,4-DCPAA), aspirin (AS), acetaminophen (AAP). We also evaluated the effect of pH (2 to 10) and temperature range of (298 to 318) K on the selective separation from CMZ.

EXPERIMENTAL

1. Materials

Carbamazepine (CMZ), 2,4-dichlorophenoxyacetic acid (2,4-DCPAA), aspirin (AS), acetaminophen (AAP), ethylene glycol dimethacrylate (EGDMA), methacrylic acid (MAA) and 3, 3, 4, 4, 5, 5, 6, 6, 7, 7, 8, 8, 9, 9, 10, 10, 10-heptadecafluorodecyl methacrylate (HDFDMA) were purchased from Aldrich Chemical Company, Inc. (Milwaukee, WI, USA). The compound *a,a*-azobis(isobutyronitrile) (AIBN) was purchased from Junsei Chemical Co., Ltd (Tokyo, Japan). Water and acetonitrile (HPLC grade) were obtained from Wako Pure Chemicals (Osaka, Japan). Ethanol and *n*-hexane were purchased from Duksan (Pharmaceutical Co., Ltd Korea). Carbon

Table 1. Specifications of the chemical used

| Chemical name | Mass fraction purity | Source | CAS RN |
|--|----------------------|------------------------------|-----------|
| CO_2 | >0.998 | Daesung Industrial Gases Co. | 124-38-9 |
| Carbamazepine | | Sigma-Aldrich Co. | 298-46-4 |
| 2,4-Dichlorophenoxyacetic acid | >0.970 | Sigma-Aldrich Co. | 94-75-7 |
| Aspirin | >0.990 | Sigma-Aldrich Co. | 50-78-2 |
| Acetaminophen | >0.990 | Sigma-Aldrich Co. | 103-90-2 |
| Ethylene glycol dimethacrylate | >0.980 | Sigma-Aldrich Co. | 97-90-5 |
| 3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,10-Heptadecafluorodecyl methacrylate | >0.970 | Sigma-Aldrich Co. | 1996-88-9 |
| Acetonitrile | >0.998 | Wako Pure Chemical Co. | 75-05-8 |
| Ethanol | >0.995 | Duksan Co. | 64-17-5 |
| <i>n</i> -Hexane | >0.950 | Duksan Co. | 110-54-3 |
| <i>a,a</i> -Azobis(isobutyronitrile) | >0.980 | Junsei Chemical Co. | 78-67-1 |

Table 2. Composition for the synthesis of CMZ imprinted polymers

| Samples | EGDMA (mmol) | MMA (mmol) | CMZ (mmol) | AIBN (wt%) | Dispersion agent (wt%) |
|------------|--------------|------------|------------|------------|------------------------|
| CMZ-IP 10 | 50 | 25 | 10 | 2 | 4 |
| CMZ-IP 20 | 100 | 25 | 10 | 2 | 4 |
| CMZ-IP 40 | 200 | 25 | 10 | 2 | 4 |
| CMZ-NIP 10 | 50 | 25 | | 2 | 4 |

dioxide (CO₂, 99.8% minimum purity) was obtained from Daesung Industrial Gases Co. (Yeosu, Korea) and used as received. MAA and EGDMA were distilled under vacuum to remove the inhibitors before polymerization. The specifications of all chemicals used in this work are summarized in Table 1.

2. Preparation of the CMZ-imprinted Polymers in scCO₂

First, the CMZ-MIPs were prepared by dispersion polymerization in scCO₂ with CMZ as a template, MAA as a functional monomer, EGDMA as a cross-linker, poly (heptadecafluorodecyl methacrylate) (PHDFDMA) as a dispersion agent, and AIBN as an initiator. PHDFDMA was prepared by dispersion polymerization in scCO₂ at 338 K and 300 bar. Table 2 shows the composition of MIPs. The polymerization apparatus for the preparation of MIPs is shown in Fig. 2. 2.36 g CMZ (10 mmol) was weighed into a 50 mL vial, and then 2.15 g MAA (25 mmol), 19.82 g EGDMA (100 mmol), 0.44 g AIBN, and 0.88 g dispersion agent (PHDFDMA) were added into the vial in sequence. The mixed solution was poured into a 200 mL stainless steel reactor equipped with a mechanical stirrer. Dissolved oxygen was removed from the solutions by purging with dry nitrogen for 10 min, and it was pressurized at 100 bar by using a syringe pump (ISCO Series D, USA) which contains compressed CO₂. The reaction mixture was heated to 333 K at 300 rpm, and the remaining CO₂ was added to the system until the desired pressure of 300 bar and the temperature of 335 K were reached. The polymerization was then performed at 300 bar for 24 hrs. At the end of polymer-

ization, the reactor was cooled. After that, the residual monomer and the remaining CO₂ was slowly vented off to obtain dry MIP, and the product was collected and weighed. Non-imprinted polymers (NIP) were also prepared by the same procedure above without the addition of templates. The templates were removed by washing repetitively in ethanol. The removal degree of templates was verified by the UV-vis. spectrophotometer. The results showed that more than 95% of templates were removed.

3. Scanning Electron Microscope (SEM) Images of Prepared CMZ-IPs

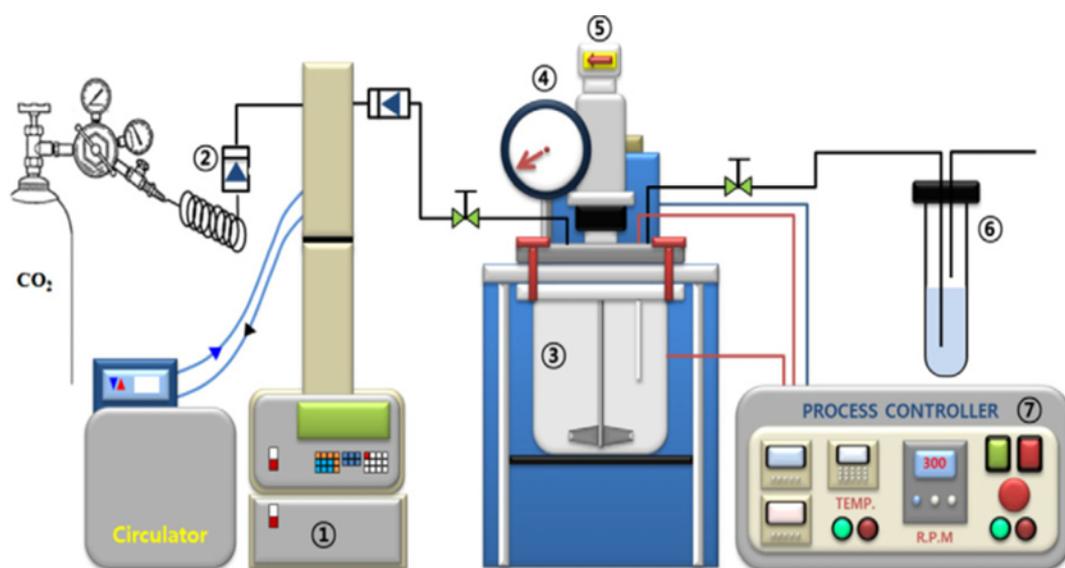
Microscopic images of polymeric particles were obtained from a scanning electron microscope (SEM, S-4700, Hitachi, Japan). The number-average particle size was determined by averaging the diameters of 100 particles measured on SEM images.

4. HPLC Analysis of Prepared CMZ-IPs

High performance liquid chromatography (HPLC) analysis was carried out using a Shimadzu HPLC system that is composed of a pump (LC-20AD, Japan) and a UV-VIS detector (SPD-20A, Japan). The synthesized polymer (CMZ-IP 10) was the slurry packed into blank column (150 mm×4.6 mm) using water. UV detection was carried out at the wavelength of 285 nm before the experiments were carried out at 298 K. In the mobile phase, water was used by volume at a flow rate of 1.0 ml min⁻¹. Samples of 0.2 mM were injected into HPLC for analysis using a loop volume of 20 μl.

5. Evaluation of Adsorption Properties of Prepared MIPs

Adsorption experiments including the adsorption kinetics, the binding isotherms, and the adsorption of materials with structures similar to templates were performed to evaluate the recognition properties of the MIPs imprinted templates. Fig. 3 shows the chemical structure of adsorption materials used in this work. Water was used as the adsorption solution. Kinetic studies were carried out with the initial concentration of 0.2 mmol/L and 10 mg of the dried MIPs. After stirring the mixture of 15 mL of CMZ aqueous solution (0.2 mmol/L) and 10 mg of MIP contained in a 30 mL vial using an isothermal water-bath shaker at 200 rpm and 298 K, the samples were

**Fig. 2. Polymerization apparatus for the preparation of molecularly imprinted polymers.**

① ISCO pump ② Check valve ③ Reactor ④ Pressure gauge ⑤ Mechanical stirrer ⑥ Trap ⑦ Process controller

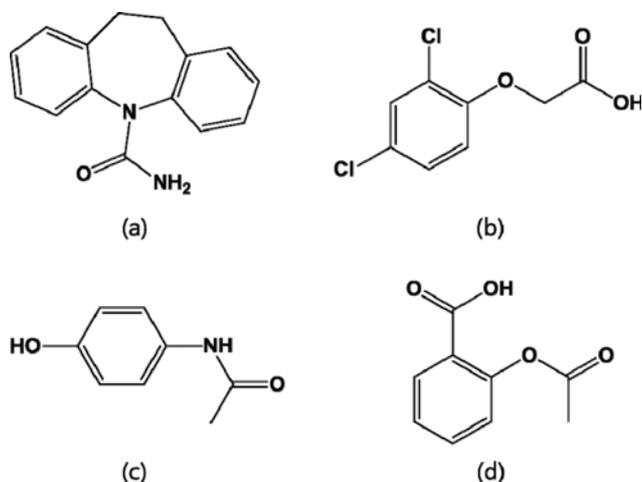


Fig. 3. Chemical structure of the adsorption materials used in this work. (a) CMZ; (b) 2,4-DCPAA; (c) AAP; (d) AS.

withdrawn at suitable time intervals, filtered through a 0.45 mm membrane filter (Millipore Corp., Bedford, Massachusetts, USA) and then analyzed for CMZ concentrations using a UV-vis. spectrophotometer. The binding isotherms studies were performed by adding a fixed amount of 10 mg of the dried MIPs into 30 mL vials containing 15 mL adsorption solution of different concentrations (0.03–1.3) mmol/L including target molecules. The vials were agitated in an isothermal water-bath shaker at 200 rpm and 298 K for 24 hrs until the equilibrium was reached.

Adsorption experiments, which include the adsorption kinetics,

the binding isotherms, and the adsorption of materials with structures similar to templates, were conducted using the UV-vis. spectrophotometer (Optizen 2120UV, Mecasys Co., Ltd., Korea).

The adsorbed amount (Q) of CMZ bound to the imprinted polymer was calculated by the following Eq. (1):

$$Q(\mu\text{mol/g}) = \frac{(C_i - C_e) \cdot V}{W} \quad (1)$$

where C_i and C_e are the concentrations (mmol/L) measured at the initial and equilibrium, V is the volume of the solution (L), and W is the mass of the dry MIPs used (g). To estimate the binding affinity of the MIPs for templates, a saturation-binding experiment and Scatchard analysis were carried out. The Scatchard equation is Eq. (2) below:

$$Q/[\text{Template}] = \frac{(Q_{\max} - Q)}{K_D} \quad (2)$$

where Q is the amount of templates bound to MIPs at equilibrium, Q_{\max} is the apparent maximum number of binding sites, $[\text{Template}]$ is the free CMZ concentration at equilibrium, and K_D is the equilibrium dissociation constant of binding sites.

Selectivity factor (α) of the imprinted polymer is the relative value of CMZ, 2,4-DCPAA, AAP, AS bound to the imprinted polymer compared with that of the CMZ as template. The α value was calculated by the following equation:

$$\alpha = \frac{Q(\text{CMZ}, 2,4\text{-DCPAA}, \text{AAP}, \text{AS})}{Q(\text{CMZ})} \quad (3)$$

where $Q(\text{CMZ}, 2,4\text{-DCPAA}, \text{AAP}, \text{AS})$ is the binding amount

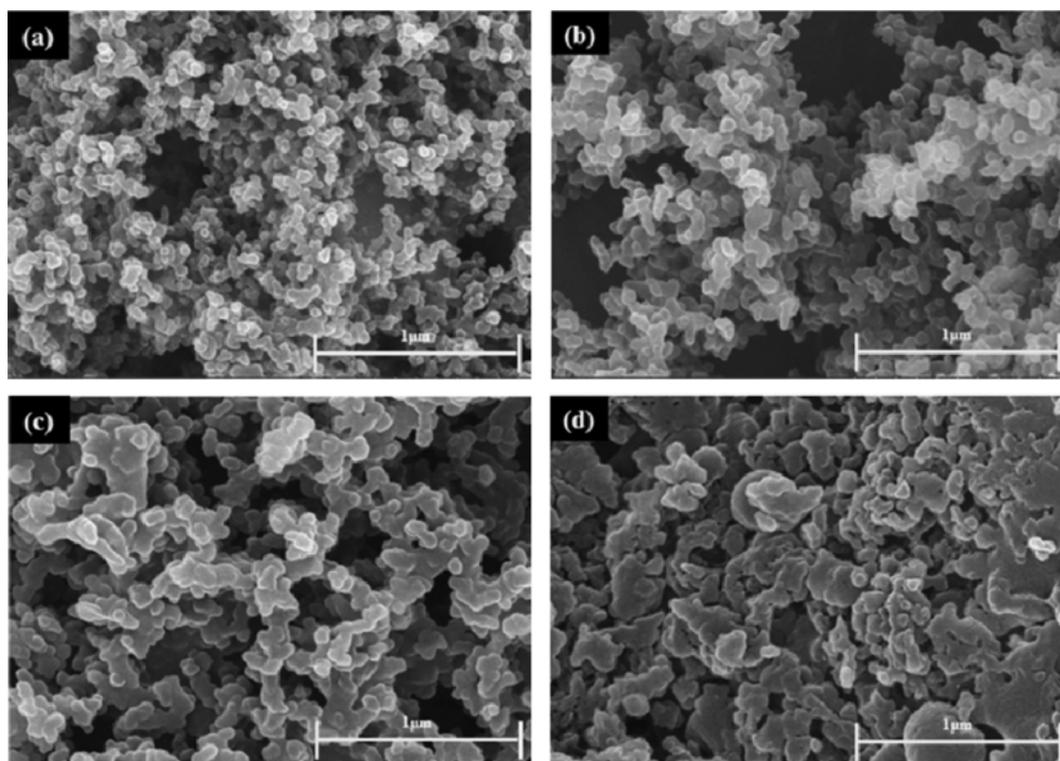


Fig. 4. SEM images of CMZ-IPs and CMZ-NIP prepared using supercritical polymerization. (a) CMZ-IP 10; (b) CMZ-IP 20; (c) CMZ-IP 40; (d) CMZ-NIP 10.

of CMZ, 2,4-DCPAA, AAP, AS for CMZ-IP, and Q (CMZ) is the binding amount of CMZ for CMZ-IP. In addition, the magnitude of the molecular imprinting effect was evaluated in terms of the imprinting-induced promotion of binding (IPB). This value is defined by Eq. (4) below:

$$IPB = \frac{(Q_{IP} - Q_{NIP})}{Q_{NIP}} \quad (4)$$

where Q_{IP} is the amount of the guest molecule that is bound by the imprinted polymer under the conditions described above, and Q_{NIP} is the corresponding value for the control polymer.

RESULTS AND DISCUSSION

1. Scanning Electron Microscope (SEM) Images of MIPs

The SEM images of CMZ-IPs and NIP prepared by using dispersion polymerization in $scCO_2$ are shown in Fig. 4. The SEM images show that nano-sized particles are successfully synthesized by dispersion polymerization in $scCO_2$. The CMZ-IPs particles were not spherical as in other polymerization methods such as suspension and emulsion polymerization. However, we obtained nano-size particles with the shape of grapes. The average size of CMZ-IPs particles prepared by using dispersion polymerization in $scCO_2$ is about 200 nm. When compared with the reported CMZ-IPs particles synthesized using solution polymerization [28], the particle size of CMZ-IPs synthesized by using dispersion polymerization in $scCO_2$ was about 10 times smaller than that of solution polymerization. Therefore, supercritical dispersion polymerization is more advantageous than other polymeric method in synthesizing smaller MIPs particles. In addition, the results of SEM indicate that the particles increase in size of as the mole ratio of crosslinking agent increases (see Fig. 4).

2. Equilibrium Binding Experiments

Adsorption kinetics was used to identify the equilibrium with interval of adsorption time in aqueous solution. As shown in Fig. 5, the adsorbed amount (Q) decreases as EGDMA content increases because the adsorption capacity reduces the density of the polymer network by the effect of cross-linking. The adsorption rate reached

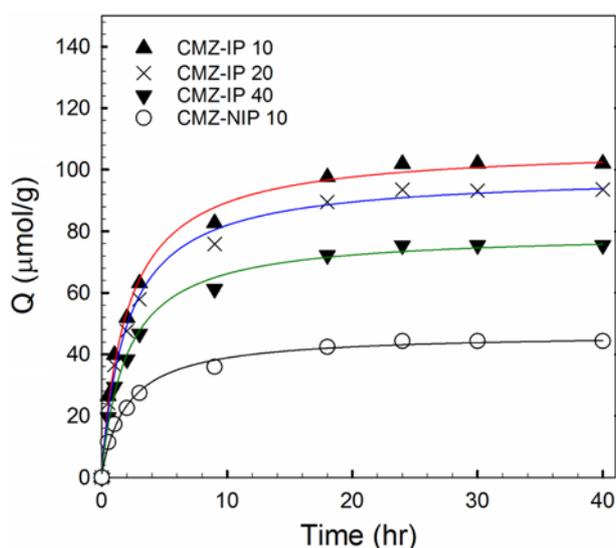


Fig. 5. Adsorption kinetics of CMZ-IPs for CMZ.

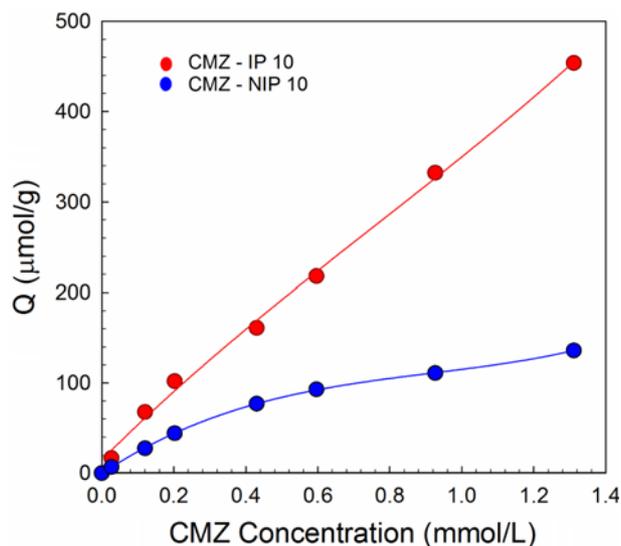


Fig. 6. Binding isotherm of CMZ-IP 10 and CMZ-NIP 10.

the equilibrium after 24 hrs.

The binding capacity of templates on each MIP is an important parameter for determining how much MIPs is required to quantitatively bind a specific amount of imprinted templates from solution. For this reason, the binding capacity of each MIP was investigated using the binding isotherms and Scatchard analysis. Fig. 6 shows the results of binding isotherms for templates on the CMZ imprinted polymers and on the non-imprinted polymers. The binding amount increased with the aqueous concentration of templates in the initial solution, but the binding amount of templates on the imprinted polymers was more than that on NIP. This difference may be ascribed to the effect of molecular imprinting. The adsorbed binding amount can reach a stable value because of some non-specific adsorption.

3. Scatchard Analysis

Scatchard analysis is a significant factor that provides affinity and number of binding sites on prepared MIPs. The obtained binding isotherm data was plotted in accordance with the method of Scat-

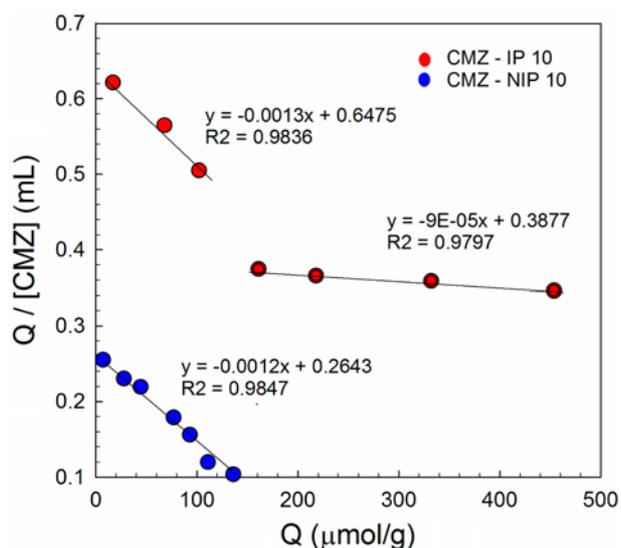


Fig. 7. Scatchard plot of binding for CMZ-IP 10 and CMZ-NIP 10.

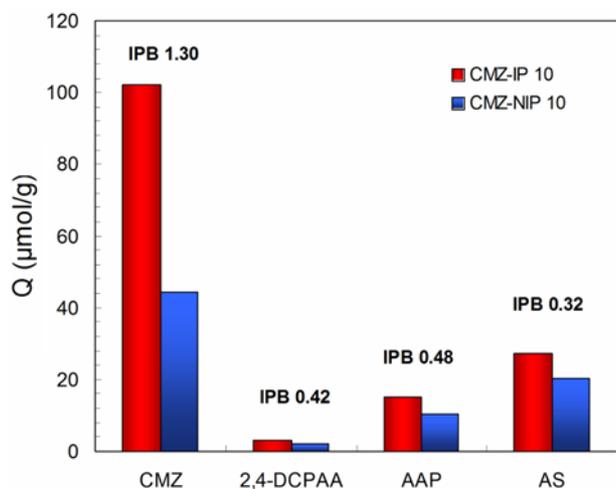


Fig. 8. Adsorption amount and IPB of CMZ-IP 10 and CMZ-NIP 10.

chard analysis. As shown in Fig. 7, there are two distinct sections within the plot which could be regarded as straight lines. The results indicate that there are two classes of binding sites in the CMZ-IP. It is possible to calculate the equilibrium dissociation constant K_D and the apparent maximum number Q_{max} of the affinity binding sites from the slope and intercept of the plot. In the case of CMZ-IP, K_{D1} and Q_{max1} of the higher affinity binding sites are 2.0×10^2 $\mu\text{mol/L}$ and 101.61 $\mu\text{mol/g}$, respectively. K_{D2} and Q_{max2} of the lower affinity binding sites are 1.3×10^3 $\mu\text{mol/L}$ and 453.77 $\mu\text{mol/g}$, respectively.

4. Evaluation of MIPs

The prepared MIPs were evaluated by comparing the adsorption of rebinding template and materials with the molecular structures similar to it on the MIPs, and also imprinting-induced promotion of binding (IPB), and selectivity factor (α) of MIPs was evaluated.

Fig. 8 shows the results of Q and IPB values for CMZ-IP and NIP prepared by applying dispersion polymerization in scCO_2 . The results indicated that Q values of CMZ-IP are higher than that of NIP.

Fig. 9 shows the adsorbed amount (Q) of CMZ as templates and materials, structurally similar to them, for the prepared MIPs. In these results, all the MIPs showed selective separation. This allowed us to determine the abilities of the MIPs because the Q values of the MIPs using the templates were higher than those materials with structures similar to imprinted templates.

In addition, as shown in Table 3, we verified that the MIPs prepared in this study have recognition capabilities based on the selectivity factor (α) and IPB values. The α values clearly show the characteristics of selective separation of prepared MIPs particles. The

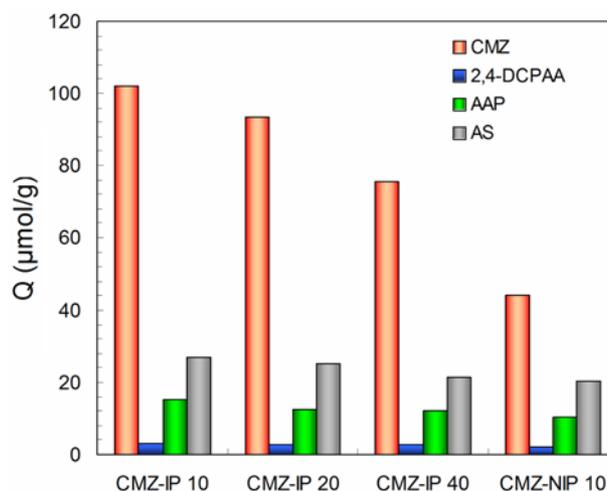


Fig. 9. Adsorption selectivity of CMZ onto CMZ-IPs.

results of the α value indicate that the value of an MIP synthesized using a particular template is smaller than 1 when materials that are structurally similar to that template are adsorbed on it. The IPB values reflect the imprinting efficiency more accurately than the Q_{ip} 's themselves, because the difference in the intrinsic binding activities of various guest molecules toward functional monomer residues is normalized. Compared to IPB values for each MIP, IPB value for MIPs synthesized using templates was higher than other values. These findings led us to conclude that the prepared MIPs particles have selective adsorption and molecule recognition capabilities.

5. HPLC Analysis

We conducted an HPLC analysis to CMZ-IP 10 with solutions of CMZ and similar materials. Chromatographic analyses were carried out using a Shimadzu LC-20AD HPLC system (Japan). UV detection was made at 285 nm. The HPLC experiments were at 298 K. In the mobile phase, water was used by volume at a flow rate of 1.0 mL/min.

Fig. 10 shows the chromatograms of CMZ-IP 10 by injecting CMZ and similar materials (2,4-DCPAA, AAP, AS) at 293 K using a column (150 mm \times 4.6 mm). The results indicated that the CMZ-IP10 show selective separation, and for the abilities of the MIPs, the Q values of the MIPs using the CMZ template were higher than those of templates and materials with similar structures.

6. Effects of pH and Temperature

To verify the effect of pH and temperature for the selective separation of CMZ solution, adsorption experiments were carried out in the range of pH (2 to 10) and (293 to 318) K. Fig. 11(a) and (b) show the adsorption amount of CMZ on CMZ-IPs and UV-spectrum of CMZ solutions for different pH (2, 4, 7 and 10). As shown in Fig.

Table 3. Selectivity factor (α) and the imprinting-induced promotion of binding (IPB) for prepared CMZ-IPs

| Samples | α | | | | IPB CMZ-IP 10 |
|-----------|-----------|-----------|-----------|------------|------------------|
| | CMZ-IP 10 | CMZ-IP 20 | CMZ-IP 40 | CMZ-NIP 10 | |
| CMZ | 1 | 1 | 1 | 1 | 1.30 |
| 2,4-DCPAA | 0.03 | 0.03 | 0.04 | 0.05 | 0.42 |
| AAP | 0.15 | 0.13 | 0.16 | 0.23 | 0.48 |
| AS | 0.27 | 0.27 | 0.28 | 0.46 | 0.32 |

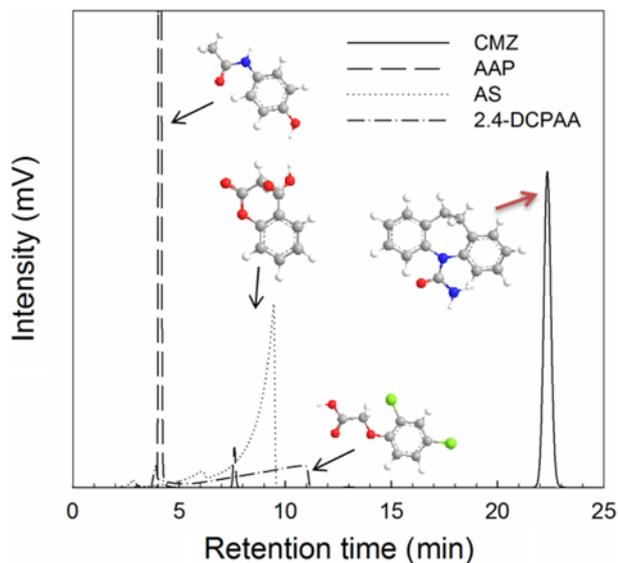


Fig. 10. HPLC analysis of CMZ-IP 10.

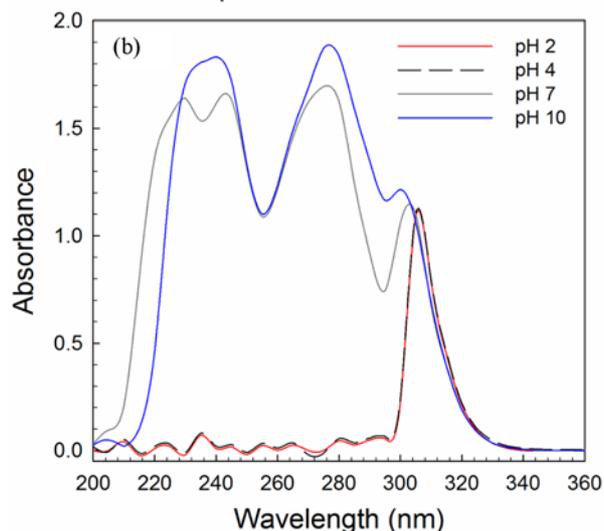
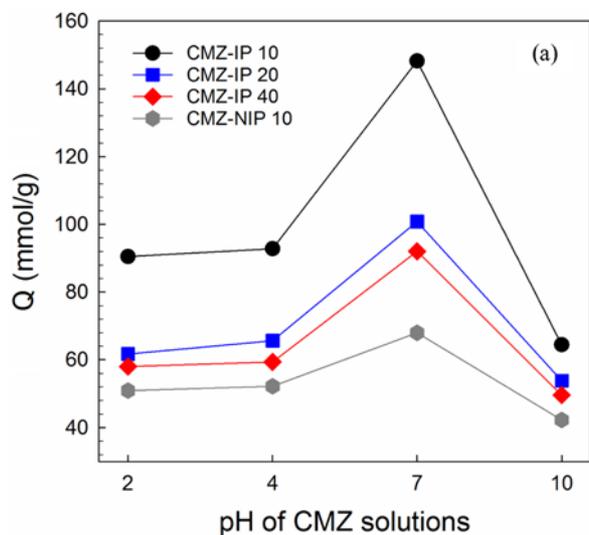


Fig. 11. Effects of adsorption with the change in pHs. (a) Adsorption amounts of CMZ on CMZ-IP with the increase of pH; (b) UV-Spectrum of CMZ with the increase of pH.

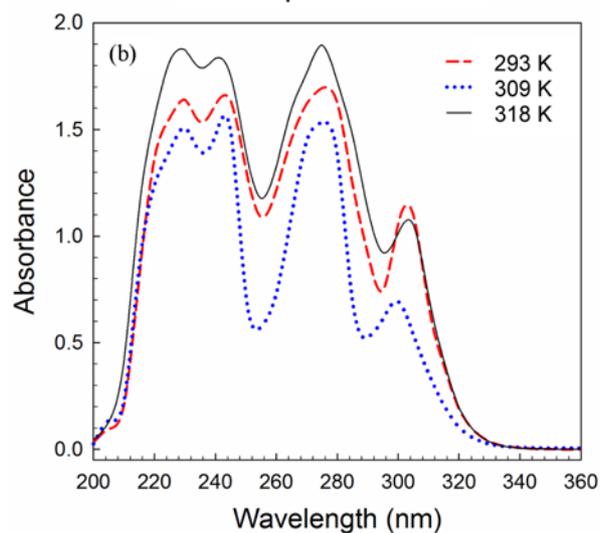
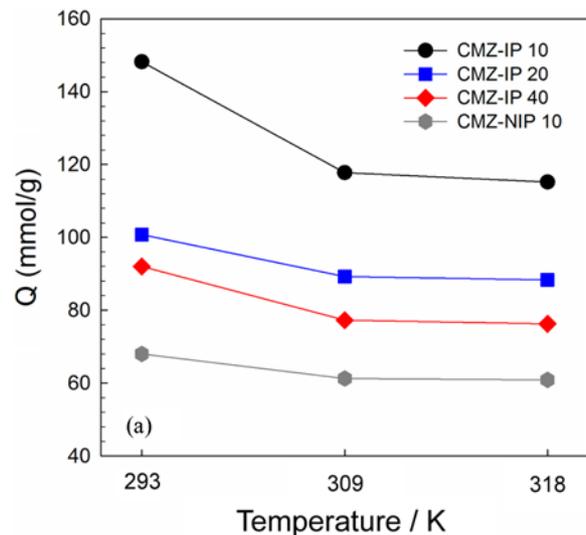


Fig. 12. Effects of adsorption with the change in each temperature. (a) Adsorption amounts of CMZ on CMZ-IP with the increase of temperature; (b) UV-Spectrum of CMZ with the increase of temperature.

11(a), the adsorption capacity in pH 7 was higher than that in pH 2, 4 and 10. The reason for these changes in adsorption amounts is that the change in the structure of CMZ occurs quickly in this range. To identify the changes in the structure of CMZ with pH, the UV absorption spectrum was investigated by UV-vis. spectrophotometer, as shown in Fig. 11(b). With an increase in pH, the shift of wavelength occurs to about 285.0 nm. The UV-spectrum of acidic and basic solutions shows a very different result in the neutral solution [29]. Thus, the changes in pH had a strong influence on the selective separation of CMZ using MIPs.

Figs. 12(a) and (b) represent the adsorption amount of CMZ on CMZ-IPs and UV-spectrum of CMZ solutions for different temperature of (293 to 318) K. Fig. 12(a) show that the adsorption amount at 309.7 K or at higher temperature decreases more than at 293 K. As shown in Fig. 12(b), the changes in UV-spectrum in the CMZ solution were not confirmed in other temperatures of (293 to 318) K. However, the adsorption amount of CMZ gradually decreased as the temperature increased. A possible explanation for this phe-

nomenon is that it is difficult for the specific recognition to be made by the swelling of prepared MIPs and expansion of imprinting cavities as the temperature increases. These findings led us to conclude that CMZ may be applied to the study of the biological reaction mechanism.

CONCLUSIONS

MIPs were successfully prepared for selective separation of carbamazepine (CMZ) from aqueous solutions. We applied supercritical polymerization in scCO₂ with methacrylic acid (MAA) as the functional monomer and ethylene glycol dimethacrylate (EGDMA) as the crosslinker. Nano-sized fine MIPs particles were obtained by dispersion polymerization in supercritical fluid carbon dioxide as a green solvent. The average size of the MIPs particles is about 200 nm. The adsorption abilities of the prepared CMZ-IPs were evaluated by the adsorption kinetics, the binding isotherms, Scatchard analysis, HPLC analysis, and the adsorption of materials with structures similar to the imprinted template, the selectivity factor (α) and the imprinting-induced promotion of binding (IPB). The analytical results of these evaluations for MIPs verified that the adsorption properties are superior to those of the parent and other materials. The analysis of the effect of pH and temperature indicated that the adsorption amount is high at pH 7 and 293 K. The dispersion polymerization process in scCO₂ may be applied for a new method of preparing highly functional polymers.

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