# Using Polystyrene-*co*-maleic Acid for Molecularly Imprinted Membranes Prepared in Supercritical Carbon Dioxide

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**ABSTRACT:** Supercritical $CO_2$ ($ScCO_2$) has been used to prepare molecularly imprinted polymers of poly(styrene-*co*-maleic acid) (PSMA) for targeted uracil (URA). The condition of $ScCO_2$ was on 16 MPa between 35 and 50°C. The resultant imprinted membranes prepared at 35 and 50°C bound URA with 9.2 ± 0.10 and 12.6 ± 0.06 μmol $g^{-1}$, respectively. Competitive binding studies were undertaken in binary substrate solution containing each of URA/DMURA, URA/Thymine, and URA/Cytosine with 2 μ*M*. The URA imprinted membrane showed high separation factor (α) with 17 for both URA/DMURA and URA/Thymine and for URA/Cytosine, α = 13. Results strongly suggested that the URA imprinted membrane had effective selectivity hydrogen bonding to separately bind in the binary components to the template. Effect of organic solvents on the URA imprinting in $ScCO_2$ was also studied, in addition to comparison of properties with those obtained in both $ScCO_2$ and water. Evidence presented that $ScCO_2$ medium was effective to prepare the URA imprinted membrane. We discussed that $ScCO_2$ fluid was efficient to fix the shape of URA template into the PSMA membrane through hydrogen bonding. © 2008 Wiley Periodicals, Inc. J Appl Polym Sci 108: 757–768, 2008

**Key words:** molecular imprinted membrane; molecular recognition; supercritical $CO_2$

## INTRODUCTION

Various applications of supercritical fluids can be found in broad range of areas such as natural product extraction, cleaning of fiber and textiles, purifications, chemical synthesis, minerals recovery, and polymerization.[1] This is due to unique abilities of supercritical fluids having high diffusivity like gas and dissolvability like a liquid.[2] Thus, the low viscosity and absence of surface tension of supercritical fluids gives it a clear advantage over traditional organic solvents. Therefore, several techniques based on supercritical fluids have been developed, because rapid expansion has been performed using supercritical solutions,[3,4] in a microcellular foaming process[5–7] and precipitation with compressed fluid antisolvent (PCA).[8–10] From these processes, polymeric materials such as micropheres, microparticles, porous fibers, and porous foams were formed. However, the potential to obtain unique thin film and membrane morphologies suggest the utility of casting dissolved polymeric solutions in the presence of a compressed antisolvent. In this process, supercritical carbon dioxide ($ScCO_2$) induces the phase separation of the polymer solution. Since $ScCO_2$ is miscible toward the polymer solvent and antisolvent to the polymer, it is useful to prepare the polymeric membrane by phase separation in the fluid. Membrane formation using $ScCO_2$ is analogous to traditional immersion precipitation of polymers.[11] Advantages of the phase separation process of polymer in $ScCO_2$ are as follows: First, the $ScCO_2$ can dry the polymer membrane rapidly without collapse of the structure due to the absence of a liquid–vapor interface. This means that the dry membrane can be obtained without additional posttreatment because of no traces of organic solvents. Another advantage was on an easy recovery of solvent, since the solvent dissolved in the $ScCO_2$ was removed from the gaseous $CO_2$ after the pressure was diminished. In immersion precipitation, variables are traditionally used to control membrane morphology such as the influence of the composition of the casting solution, choice of nonsolvent, and the precipitation temperature.[11]

Microporous polystyrene membranes were prepared by phase separation using $ScCO_2$ as nonsolvent.[12] Effects of several experimental conditions (temperature, pressure, and polymer concentration) on the microporous structure were investigated. Average pore size ranged from 8 to 35 μm was observed by changing pressure from 75 to 150 bar, polymer concentration from 15 to 30% (w/w) and temperature in the range of 20 to 70°C. They also studied

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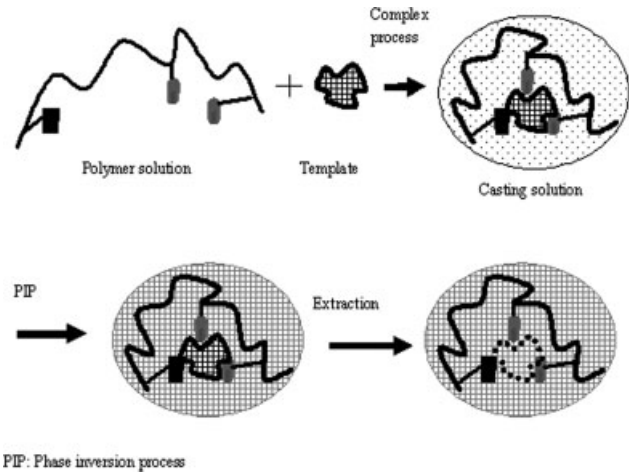


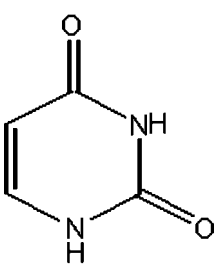

**Figure 1** Illustration of preparation imprinting membrane in $ScCO_2$.

effect of organic solvent on the formation of cellulose acetate membranes.[13] In our previous work, we have studied molecularly imprinted membranes by phase inversion method, which display selective adsorption to target molecule.[14–16] Such molecular imprinting processes used water as a coagulation medium to build tailor-made imprinted sites of the template. However, problems like high solubility of template into the water medium reduces the number of imprinted sites for the resultant imprinted membrane. Therefore, it has been expected that complementary template sites in imprinted membrane is easily formed in $ScCO_2$ fluid as an alternative medium.[17] As known, $ScCO_2$ fluid has superior merits for membrane formation as mentioned earlier; the rapid drying of polymer membrane makes it suitable for imprinting process, because collapse of membrane structure was a serious problem in imprinting. Furthermore, commonly imprinted polymers having no membrane shape are difficult to handle in practical application because of its powder shape. For these reasons, $ScCO_2$ was considered to be generally applicable for the preparation of molecularly imprinted polymers (MIP). Besides its nontoxic and additional tuning properties, $ScCO_2$ represents a particular type of inert reaction medium that was unlikely to interrupt noncovalent template-functional monomer interactions.

In the present article, $ScCO_2$ fluid was utilized to prepare artificial receptor by molecular imprinting technique for poly(styrene-*co*-maleic acid) (PSMA) membranes. It has been known that application of $ScCO_2$ is little known in the field of MIP. In general, molecular imprinting is a powerful method for preparing substrate-selective recognition sites in a polymer matrix; using a molecular template was applicable in a casting procedure of polymer.[18,19] The molecularly imprinted process was illustrated as shown in Figure 1. The template molecule was first allowed to form solution interactions with functional groups and subsequent locking-in of these interactions leads to the formation of a matrix accommodates as recognition sites, which showed selectivity for the template. By this procedure, natural binding sites can be mimicked synthetically complementary in size and shape to the template. Because of the easy preparation and high recognition ability as a man-made receptor, MIPs have been used in increasing number of applications such as in the fields of solid phase extraction, metal ion selective, enzyme mimics for catalytic applications, and recognition elements in biosensor.[18]

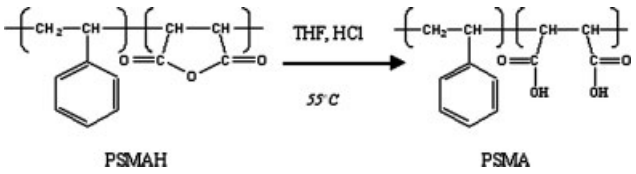
The present work reports preparation of uracil (URA) imprinted membranes by using PSMA in $ScCO_2$ as shown in Scheme 1. PSMA has some good properties such as solubility, filming, miscibility, and is widely used in industry. In addition, the easy modification of the COOH groups and the ability of styrene to form membrane make it suitable for MIP preparation by noncovalent bonding. Herein, URA was used as template because nitrogenous heterocyclic nucleic acid base presents in RNA molecules and in nucleotides. The URA imprinted polymer selective binding to URA also capable of being one application for biomimetic components that compose RNA in biological organisms. Interest in URA is growing as the knowledge of its biochemical importance and applications. For instance, Baker et al. used URA as a model compound to understand the effect of agrochemicals on foliar penetration.[20] Zielenkiewicz et al. studied URA and its halo and amino derivatives that are of special interest because of their antimetabolic and antitumor properties.[21] Therefore, studying and developing of URA recognition materials were meaningful for artificial receptors and applications are envisaged in biosensors, drug therapy, diagnostics for drug assays, and separation science using synthetic antibodies and polymers imprinting nucleotides bases.

We describe effect of $ScCO_2$ fluid to study the URA imprinted membrane formation of PSMA by phase separation in the fluid. Influence of tempera-




URA

**Scheme 1** Chemical structure for URA.




**Scheme 2** Hydrolysis of PSMAH to PSMA.

ture change of $ScCO_2$ fluid on the property of the imprinted membranes would be investigated. Comparing the resultant membrane characteristics and substrate selectivity of the MIP prepared in $ScCO_2$ and that prepared in water, the advantage of $ScCO_2$ was also discussed.

## EXPERIMENTAL

### Materials

The polymer used was Poly(styrene-*co*-maleic anhydride) (PSMAH) (Aldrich, $M_w$: 224,000, 7 wt % maleic anhydride). URA was purchased from Nacalai Tesque. 1,3-Dimethyluracil (DMURA), thymine and cytosine were purchased from Tokyo Kasei Kogyo. Organic solvents of *N*,*N*-dimethylformamide (DMF) and dimethyl sulfoxide (DMSO) were distilled before used. *N*-methyl-2-pyrrolidone (NMP, purity $>99.5\%$) was bought from Nacalai Tesque and used as received. Utilized carbon dioxide ($CO_2$) (Nagata, Japan) for production of compressed $CO_2$ was 99.999%.

### Preparation of membrane in $ScCO_2$

To obtain interaction sites to URA template in PSMA, hydrolysis of PSMAH was carried out as follows: 4 g of PSMAH was added into 50-mL of THF in a three-neck flask. The solution was stirred until all PSMAH dissolved. Then, concentrated HCl (36 wt %) was added slowly into the solution and refluxed at 55°C for overnight. The solution was precipitated in water, and then the resultant white polymer was washed with large amount of distilled water for 3–4 times. Then, the polymer was vacuum dried to obtain PSMA. Here, the hydrolysis reaction of PSMAH to PSMA containing change of chemical structure is shown in Scheme 2.

The schematic procedure for the preparation of URA imprinted membrane using PSMA is shown in Figure 2. Both 15 wt % of PSMA and 2 wt % of URA were dissolved in DMF, DMSO, and NMP. Three kinds of organic solvents were used to study the effect of organic solvents on membrane formation by phase separation in $ScCO_2$. The cast solution was mixed at 50°C on hot stirrer for 1 day. Then, the solution was spread onto a glass plate ($10 \times 10$ cm$^2$) at 50°C. The glass plate was rapidly put inside $CO_2$ reactor, which was preheated to desired temperature

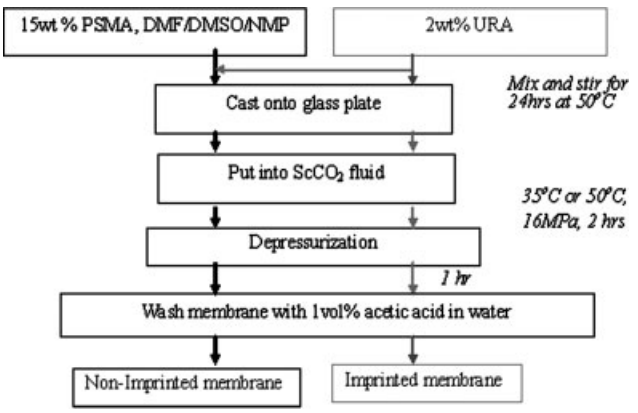



**Figure 2** Procedure for preparation of PSMA membrane.

at 35°C. Then, following operation for the $ScCO_2$ instrument (Fig. 3) was performed. (1) Valve 1 was opened and liquid $CO_2$ was pumped into the reactor from gas cylinder through a high-pressure pump. (2) The reactor was then filled with pumping $CO_2$ up to the desired pressure of 16 MPa and maintaining its temperature at 35°C. (3) When the inside pressure of the $CO_2$ reached to 16 MPa, the input valve 1 of the reactor was closed. (4) The system was maintained for 2 h to form polymer solid. (5) Then, the system was slowly depressurized by opening the valve 2 for about 1 h at the experimental temperature. As soon as the depressurization process was completed, the resulting white membrane in the reactor was taken out. Since the resultant membranes involved the URA template, the extraction was carried out with 1 vol % acetic acid solution and then large quantity of water for 1 week at 30°C. After complete removal of template, the membranes were vacuum dried. As a control, a nonimprinted membrane was prepared

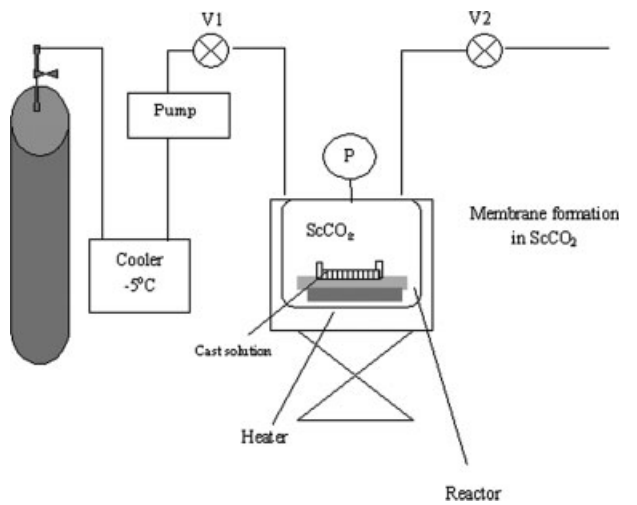



**Figure 3** Flow diagram of experimental apparatus for preparation of imprinted membrane in $ScCO_2$ fluid.

in the same way except for the presence of URA template in the cast solution.

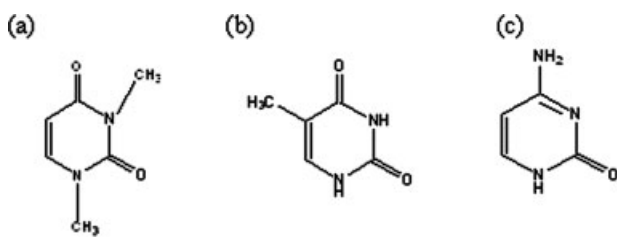
Effect of temperature of $ScCO_2$ fluid on the imprinted membrane was also investigated as follows: For example, both 15 wt % of PSMA and 2 wt % of URA were dissolved in NMP as cast solvent. Then, the solution was cast onto a glass plate and put into $ScCO_2$ fluid at 50°C with 16 MPa for 2 h. Finally, depressurization process was carried out by extraction of template as mentioned earlier.

### Membrane characterization

The imprinted membranes prepared in $ScCO_2$ were characterized by FTIR Prestige-21 (Shimadzu Corp.) in transmittance setup with 20 integrations. The surface and cross section morphology of the membrane were observed by using SEM (JEOL, JSM-5300 LV). The cross section was obtained by fracturing the membrane in liquid nitrogen temperature and then sputter-coating was carried out with gold. SEM with accelerating voltage set to 20 kV was used to view the morphology of the membrane surface and cross section. Tensile strength of the resultant membranes ($3 \times 0.5$ cm$^2$) was measured by using PT-200N (Minebea).

### Binding experiment

To study the substrate uptake of URA to the imprinted membrane, binding experiment was carried out as follows: 5 mg of imprinted or nonimprinted membrane was placed inside 15-mL container having 100 μ*M* URA solution or other analog solutions. The analogs for URA were DMURA, thymine, and cytosine as shown in Scheme 3. The URA imprinted membrane was equilibrated in the aqueous solution of URA or other analogs at 30°C for 6 h. Then, the concentration of the substrate in the solution was determined by UV–vis spectrophotometer after the substrate bound. The reduction of absorbance of the substrate was observed by the UV measurement. The value of substrate bound to the URA imprinted membrane, [S] (μmol g$^{-1}$), was calculated from the following equation,




**Scheme 3** Chemical structures of (a) DMURA, (b) thymine, and (c) cytosine.

$$[S] = (C_b - C_a)V/W$$

where $C_b$ and $C_a$ represented the molar concentrations (μ*M*) of URA or other substrates before and after equilibration, respectively. $V$ and $W$ were volume of substrate solution and weight of imprinted membrane, respectively. The selectivity of the URA imprinted membrane was compared by using the values of binding amount of URA to the analogs. The maximum absorption of URA was monitored at 258 nm and $\varepsilon$ used was $8200M^{-1}$ cm$^{-1}$.

To estimate the selective binding to URA imprinted membranes, selectivity factor to URA and analogs was further more analyzed by binary separation experiment. To estimate each substrate concentration, reverse phase HPLC system was used. Here, reference solution used was 80 vol % 50 m*M* sodium dihydrogenphosphate anhydrous (pH 2.5) and 20 vol % acetonitrile. The binary separation of URA/DMURA, URA/thymine, and URA/cytosine was carried out by using the mixture solution of each 2, 4, 7, and 14 μ*M* concentration; 5 mg of imprinted membrane was immerged into the 15-mL mixture solution and incubated at 30°C for 6 h. Each substrate concentration before and after binding was evaluated at 261 nm with a UV–vis detector connected to HPLC apparatus with TSKgel ODS-80Ts QA column (4.6 mm I.D × 15 cm). Here, the separation efficiency of URA/DMURA was defined as, for example,

$$\alpha = [S]_{URA}/[S]_{DMURA}$$

where $[S]_{URA}$ and $[S]_{DMURA}$ were binding amounts of each substrate.

## RESULTS AND DISCUSSION

### Characteristics of imprinted membranes

The hydrolysis reaction of PSMAH to PSMA was monitored by FTIR measurement. The FTIR spectra of PSMAH and PSMA obtained after hydrolysis reaction are shown in Figure 4. In (a), the bands at 1780 and 1857 cm$^{-1}$ were characteristic to PSMAH, which showed the asymmetrical and symmetrical carbonyl absorption of anhydride groups, respectively. The IR bands at 1600, 1492, and 1452 cm$^{-1}$ were stretching of C=C of phenyl groups on the backbone. The 1217 cm$^{-1}$ band was attributed to the C—O—C stretch of maleic anhydride units, having as five-membered cycloanhydride for $\upsilon_{C-O-C}$ band at 1310–1210 cm$^{-1}$. The IR bands at 758 and 698 cm$^{-1}$ were those of the bending of C=C of the phenyl groups. After hydrolysis was completed, it was clearly shown on (b) spectrum that the carbonyl stretch of anhydride band was changed into carbox-

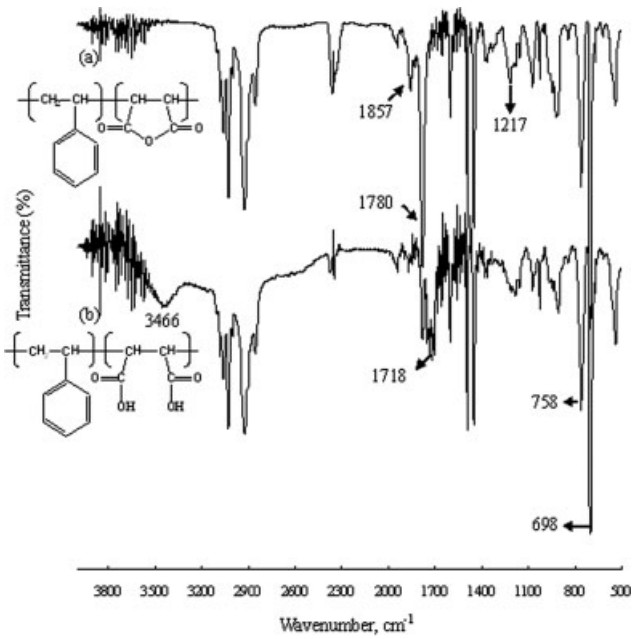



**Figure 4** FTIR spectra of (a) PSMAH and (b) PSMA.

ylic acid band at 1718 $cm^{-1}$. The peaks due to the unreacted anhydride groups clearly disappeared at 1780 and 1857 $cm^{-1}$.

It is known in acetic acid that carboxylic acids often exist as dimmers because of strong hydrogen bonds in liquid or solid state, and not a very dilute solution in nonpolar solvents.[22] The carboxylic acid dimmer displayed as an intense O—H stretching in broaden region of 3300–2500 $cm^{-1}$. These broaden bands differed from the strong absorption of a free hydroxyl stretching vibration near 3560–3500 $cm^{-1}$. Therefore, from (b) spectrum, the O—H stretch indicated the presence of hydrogen bonding between the COOH groups in PSMA. Hence, the IR absorption band at 2600 $cm^{-1}$ in PSMA was for O—H stretching vibration caused by the strong hydrogen bonding between carboxylic acid groups. In addition, a free OH stretching vibration was observed around 3446 $cm^{-1}$. These assignments of observed functional groups for PSMAH and PSMA are summarized in Table I.

**TABLE I**
**Assignment of Functional Groups for PSMAH and PSMA**

| Absorption band | Wavenumber ($cm^{-1}$) |
|---|---|
| $\upsilon_{as}$ CH, $\upsilon_{s}$ CH | 2924, 2850 |
| Aromatic $\upsilon_{C-H}$ | 3082, 3059, 3024 |
| Cylic anhydride $\upsilon_{C=O}$ | 1857, 1780 |
| Cyclic anhydride $\upsilon_{C-O-C}$ | 1217 |
| Phenyl $\upsilon_{C=C}$ | 1600, 1492, 1452 |
| Phenyl $\delta_{C=C}$ | 758, 698 |
| Carboxylic acid $\upsilon_{C=O}$ | 1718 |
| Carboxylic acid $\upsilon_{O-H}$ | 3446, 2600 |

To examine the presence of the interaction between the URA template and PSMA, comparison of FTIR spectra before and after the imprinting process was made as shown in Figure 5. The resultant spectra of the URA-imprinted PSMA membranes prepared from DMF cast solvent (a) without and (c) with URA, and (d) after extraction of URA were obtained. The spectrum (b) was for the URA template, which was measured with KBr method. From spectrum (b), the NH stretch was observed with multiple bands in 3330–3060 $cm^{-1}$ regions. Also, two bands due to the C=O stretching could be observed at 1718 and 1670 $cm^{-1}$. The band at 1238 $cm^{-1}$ was resulted from interaction between the N—H bending and C—N stretching. The medium intensity band occurs at 862 and 825 $cm^{-1}$ were due to absorption of the out of plane NH wagging. In the URA imprinted membrane of (c), characteristic IR peaks of the out of plane band could be clearly seen in wavenumber at 842 and 821 $cm^{-1}$. As compared with the URA spectrum (b), the band shift toward lower wavenumber side indicated that the wagging bond of the N—H group of URA had hydrogen bonding interaction with the COOH segment of PSMA. Furthermore, it was interesting that the bond in (d) disappeared after the URA was extracted. Another evidence was observed in the OH stretch band of COOH group in PSMA. In (c), the IR inten-

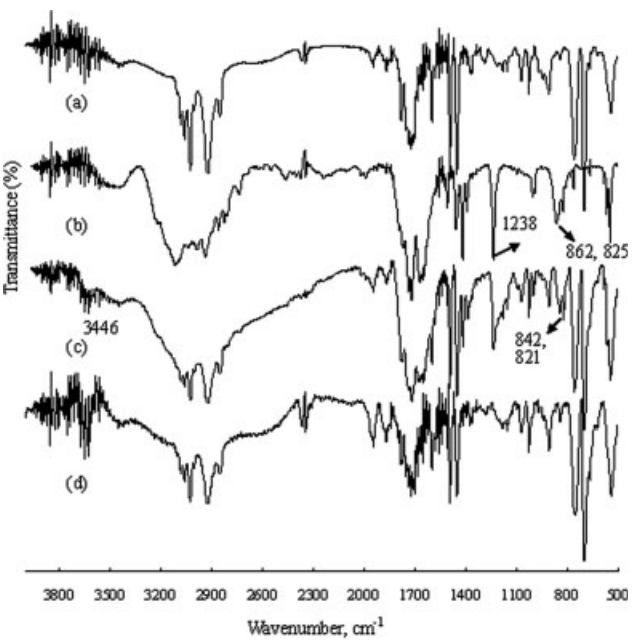
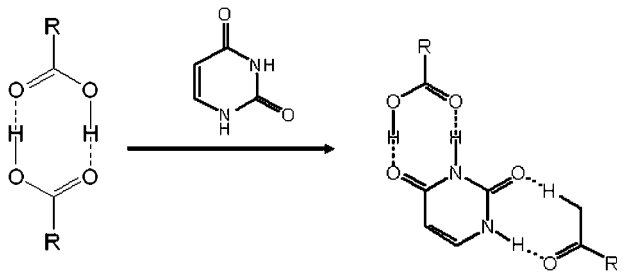



**Figure 5** FTIR spectra of URA imprinted membranes prepared from DMF cast solvent: (a) Nonimprinted membrane, (b) URA template, (c) imprinted with URA, and (d) after extraction.



**Scheme 4** Illustration of hydrogen bonds between URA template and COOH segment of PSMA.

sity of the 3446 $cm^{-1}$ band was lower than that observed in the spectrum (d). This implied that the added URA template disrupted the inter- or intramolecular hydrogen bonding between COOH segments of the PSMA. As shown in Scheme 4, hydrogen bonds between URA and the COOH segment of PSMA were observed in the present case. In addition, the appearance of C=O stretch of URA in spectrum (c) was confirmed because formation of hydrogen bonding between URA and PSMA. Similar results were obtained for URA imprinted membrane prepared in DMSO and NMP as used as cast solvents.

To be clear, the effect of $ScCO_2$ fluid on membrane formation, the data of the imprinted membranes prepared in $ScCO_2$ were compared with those of water. Herein, each DMF, DMSO and NMP solvent was similarly utilized. Absence of N—H band wagging out of plane and the C=O band for URA were observed for the FTIR spectra obtained in water. The disappearance of the URA band might be due to high solubility of URA into the water medium when the coagulation process of PSMA was performed. The URA escaped in water layer from polymer layer during the phase inversion process. Therefore, these data indicated that loss of the URA template prevented the formation of the imprinted sites in PSMA. Whereby, when the polymer was solidified in $ScCO_2$ fluid, comparison of these data suggested that the loss of URA was smaller in the $ScCO_2$. This was due to low solubility of URA in $ScCO_2$ rather than in water. Eventually in the $ScCO_2$ fluid, the URA imprinted sites were formed inside PSMA. This was evidence of an effective method to prepare the URA imprinted membrane in $ScCO_2$.

### Morphology characterization of URA imprinted membranes

Observation of the morphology of resultant membranes is meaningful for understanding membrane formation by phase inversion method. The whole cross section structure with the presence of top and bottom surface of URA imprinted membrane prepared in $ScCO_2$ at 35°C and water using DMF as cast solvent are shown in Figure 6. A homogeneous and regular cellular structure was obtained for URA imprinted membrane prepared in $ScCO_2$ as presented in Figure 6(a). We also observed URA crystals on this imprinted membrane. Meanwhile, asymmetric porous structure with a dense layer on top of the surface and a supporting layer formed with macrovoids was presented for membrane prepared in water. Similar structure was obtained when DMSO and NMP were used as cast solvent. Over the whole cross section in the PSMA membrane, the macrovoid layer was observed in the water system as shown in Figure 6(b). It was known that macrovoids usually appeared in systems exhibiting instantaneous demixing during the membrane formation.[11] Such macrovoid observed in the PSMA membranes for all three solvents was due to high mutual affinity of water with DMF, DMSO, and NMP. It was known that the development of macrovoids was initiated by some of the nuclei of the polymer poor phase, which were formed directly under the top layer of membrane. Since diffusion of solvent and nonsolvent occurred into the first nuclei, growth continued until the poly-

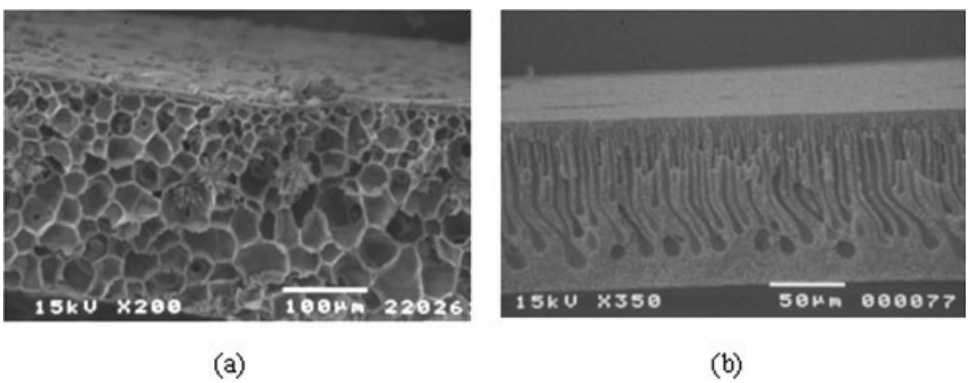
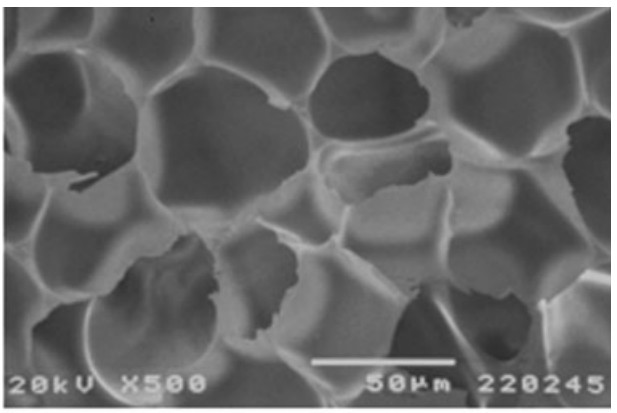



**Figure 6** SEM pictures of cross section of URA imprinted membrane prepared in (a) $ScCO_2$ at 35°C and (b) in water using DMF as cast solvent.

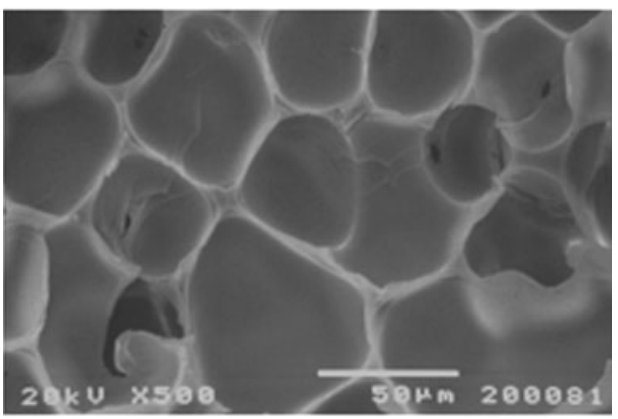
mer concentration at the macrovoid/solution interface was so high with the solidification of polymer. Therefore, macrovoids were observed in the PSMA membranes when water was used as a coagulation medium.

Compared with water, the membrane prepared in $ScCO_2$ showed cellular structure and no fingers like morphology. Figure 7 shows effect of cast solvents on the cross section structure of nonimprinted membrane when (a) DMF, (b) DMSO, and (c) NMP were used in $ScCO_2$. It was found that the morphology were different dependence on the solvent used. Large pore size was for the NMP, and the pores were slightly isolated to each other [Fig. 7(c)]. This indicated that lower mutual affinity was existed in the NMP system. When the mutual affinity was low, the outflow of solvent into the $ScCO_2$ phase decreased. This meant that there was enough time for polymer-lean phase formed by the phase separation to grow and produce their larger pores. When the outflow of solvent was low, the membrane solution contained more solvent. This brought about lower membrane porosity with low interconnectivity and increase in thickness.[23] As for the membrane prepared using DMF and DMSO, the pore size was not isolated and connected to each other. This implied that the phase separation of the polymer occurred instantaneously after the $CO_2$ fluid was introduced, resulting in higher membrane porosity with high interconnectivity. Systems exhibiting instantaneous demixing often showed macrovoids formation as explained in the water system mentioned earlier. However, in these membranes, no macrovoid was observed in spite of the instantaneous demixing. The presence of macrovoids was not generally favorable, because they might lead to a weak spot in the membrane. From the SEM pictures, it was confirmed that the morphology of the membranes was highly effected by the coagulant and organic solvent used in the phase inversion process.

To evaluate resultant membranes, mechanical properties were measured. The values of tensile strength of URA imprinted membranes prepared in $ScCO_2$ and water were compared in each membrane prepared from different organic solvent, DMF, DMSO, and NMP. The value of tensile strength was expressed in unit of force per unit area (N $mm^{-2}$). As shown in Table II, the values of tensile strength meant that membranes prepared in $ScCO_2$ had a tendency to be higher than that of water. The values of 1.0 N $mm^{-2}$ for DMSO and NMP were larger than those of 0.4 and 0.5 N $mm^{-2}$ for the water system. These verified that the use of $ScCO_2$ fluid enhanced the tensile strength for the $ScCO_2$ system of membrane as a mechanical property. Table II contains surface area ($A_s$) of the resultant membranes. The values of $A_s$ were 4.6 and 23.2 $m^2$ $g^{-1}$ for the imprinted membranes prepared in the $ScCO_2$ and water, respectively, when DMF was used as cast solvent. In case of other DMSO and NMP, the values of $A_s$ for membranes prepared in $ScCO_2$ were also lower than those prepared in water. As a result, the use of $ScCO_2$ made lesser porous structure of the resultant membranes rather than that of water. This




(a)

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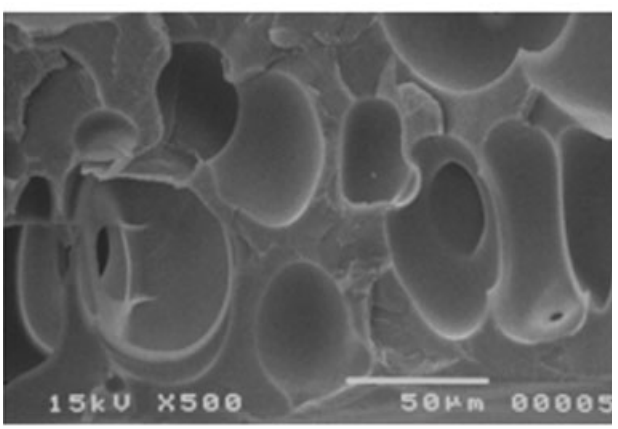


(c)

**Figure 7** Effect of cast solvents on nonimprinted membranes structure in $ScCO_2$ at 35°C with 16 MPa: (a) DMF, (b) DMSO, and (c) NMP.

**TABLE II**
**Tensile Strength of URA Imprinted Membrane Prepared in $ScCO_2$ and Water from Different Organic Solvent**

| | $ScCO_2$ | | Water | |
|---|---|---|---|---|
| | Tensile strength (N $mm^{-2}$) | Surface area ($m^2$ $g^{-1}$) | Tensile strength (N $mm^{-2}$) | Surface area ($m^2$ $g^{-1}$) |
| DMF | 0.9 | 4.6 | 0.6 | 23.2 |
| DMSO | 1.0 | 4.6 | 0.4 | 23.1 |
| NMP | 1.0 | 3.0 | 0.5 | 23.7 |

meant that the resultant membrane prepared in $ScCO_2$ showed dense structure compared to membranes prepared in water, as suggested by the data of tensile strength.

### Substrate uptake for URA imprinted membrane

Comparisons of binding amounts of URA substrate to the nonimprinted and the imprinted membrane prepared in $ScCO_2$ and water by different cast solvent are presented in Figure 8. Herein, the data of the membrane prepared in different cast solution were contained. Results clearly showed that the binding amounts of URA to the imprinted membrane prepared in $ScCO_2$ were much higher than those in water. This indicated that little imprinted sites were successfully formed if water was used as coagulation medium. The reason was that most of URA easily dissolved in water during the polymer coagulation process, preventing the formation of a rigid polymer matrix with PSMA.

For $ScCO_2$ system, the amounts of URA bound to imprinted membrane for DMF, DMSO, and NMP were 7.4, 9.2, and 9.1 μmol $g^{-1}$, respectively. Meanwhile, the values of the URA bound to the nonimprinted membranes were 1.7 μmol $g^{-1}$ for both DMF and DMSO systems and 1.5 μmol $g^{-1}$ for NMP system. Results clearly showed that the binding amounts of URA to the imprinted membranes were higher than those of the nonimprinted membrane. This indicated that the PSMA imprinted membrane recognized the URA molecule because of efficient formation of the URA imprinted sites. On the contrary, the low binding amounts in the nonimprinted system indicated that little binding sites existed in the membranes. As noted, the imprinted membrane prepared by DMF showed somewhat lower crystal formation relative to that of DMSO and NMP. As shown in Figure 9, crystals formed on the surface of the imprinted membrane could be observed for (a) DMF, (b) DMSO, and (c) NMP. If more URA crystals were present on the imprinted membrane, the ability to imprint the URA template in PSMA might be reduced. Actually, the value of $[S]_{URA}$ for the imprinted membrane prepared in DMF was lower than that of DMSO and NMP. This meant that less imprinted sites were available in the case of DMF. Therefore, it seemed that the crystal formation influences the URA binding.

Therefore, reducing crystal in the membrane is very important strategy for formation of URA imprinted membrane. In reducing formation of crystals, further experimental analysis was carried out by increasing the temperature from 35 to 50°C during the membrane formation in $ScCO_2$. Effect of temperature change on the binding amount of the imprinted membrane as NMP cast solvent was used. It was found that the value of [S] of the imprinted membrane prepared increased from 9.2 ± 0.10 to 12.6 ± 0.06 μmol $g^{-1}$, when the temperature of the $CO_2$ fluid became higher from 35 to 50°C (Fig. 10). This indicated that in the membrane prepared with high temperature, the binding ability of URA to the imprinted membrane increased because no crystal formation made homogeneity of the template in the membrane. Figure 11 presents the SEM pictures of surface nonimprinted and imprinted membrane prepared at different temperature in $ScCO_2$. Comparing Figure 11(a,b), the crystals formed at 50°C on the surface of the imprinted membrane was very lesser than at 35°C. This meant that most of the URA mole-

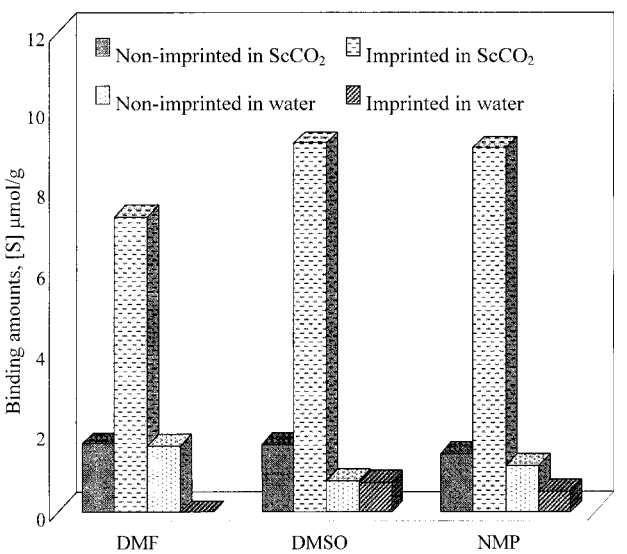


**Figure 8** Binding amounts of URA to PSMA membranes prepared from different cast solutions in ScCO2 at 35°C and water.

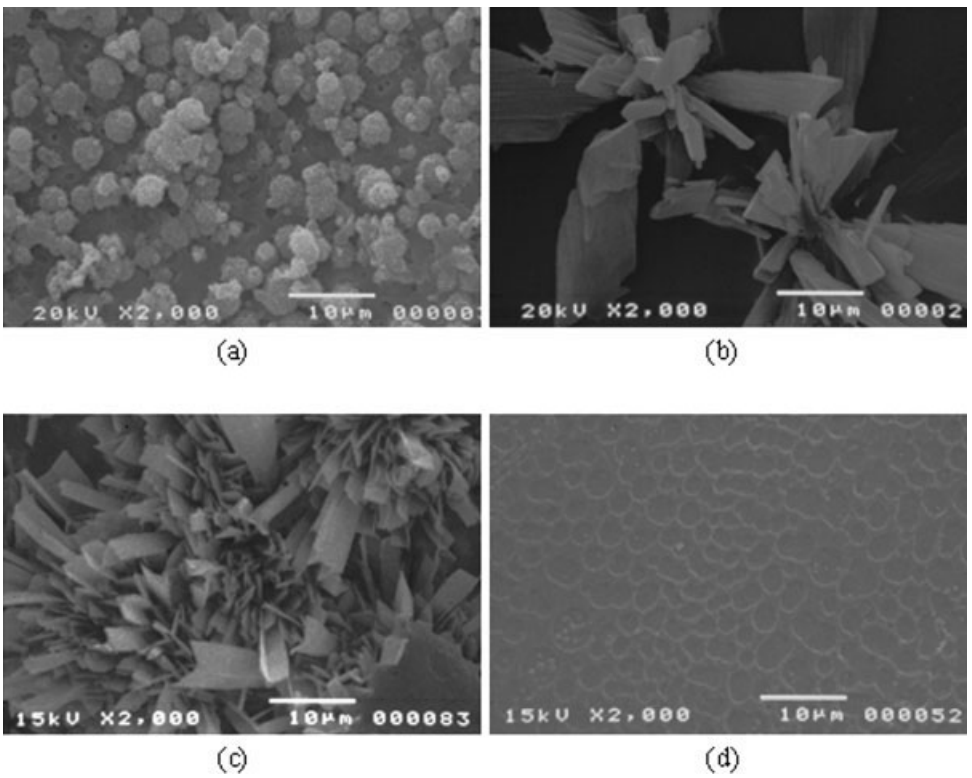


**Figure 9** SEM pictures of surface URA imprinted membranes before extraction using (a) DMF, (b) DMSO, (c) NMP as cast solvent, and (d) was by the nonimprinted membrane. The temperature of $ScCO_2$ fluid was 35°C.

cule was mainly formed through the noncovalent bonding with PSMA membrane and related to formation of the imprint sites. Binding sites for URA in the imprinted membrane were effectively formed in the $CO_2$ fluid. Therefore, the increased number of the imprinted sites led to the increased capacity of URA binding when the temperature was 50°C in the fluid. Furthermore, after extraction of URA template [Fig. 11(b)], the membrane prepared at 50°C in $ScCO_2$ showed smooth surface. The higher temperature enhanced the solubility of URA in PSMA homogenously without varying the morphology of the membrane.

To verify the specificity of the URA imprinted membrane, the substrates binding experiments were extended to DMURA, thymine, and cytosine for both temperatures. These substrates were structurally analogous to URA. Figure 10 also shows comparison of substrate binding of such URA analogs. Results presented that the binding amounts of URA to the imprinted membrane were higher than that compared to DMURA, thymine, and cytosine for both temperatures. The resultant values of $[S]_{DMURA,}$ $[S]_{thymine}$, and $[S]_{cytosine}$ for the URA imprinted membranes prepared at 35°C were 1.2, 0.2, and 1.2 μmol $g^{-1}$, respectively. For the imprinted membranes prepared at 50°C, the values of $[S]_{DMURA,}$ $[S]_{thymine}$, and $[S]_{cytosine}$ were 1.4, 0.1, and 1.1 μmol $g^{-1}$, respectively. All these values showed lower binding capacity that were observed in these analogs. As compared to URA binding prepared at 35 and 50°C, 9.2 ± 0.10 and 12.6 ± 0.06 μmol $g^{-1}$ were observed, respectively. These values indicated that the URA imprinted membranes showed high selectivity to URA rather than DMURA, thymine and cytosine, especially in case of higher temperature. Furthermore, the values of $[S]_{DMURA}$, $[S]_{thymine}$, and $[S]_{cytosine}$ of the binding amounts for the resultant imprinted membranes were lower than that of nonimprinted membrane with binding capacity of 2.0, 1.0, and

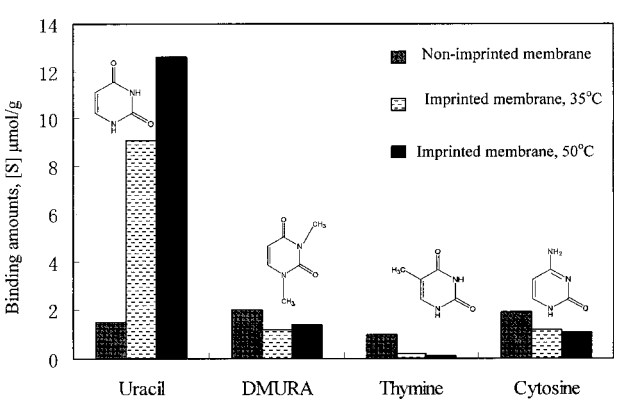


**Figure 10** Binding amounts of different substrates to the PSMA membranes prepared in $ScCO_2$ at 35 and 50°C.

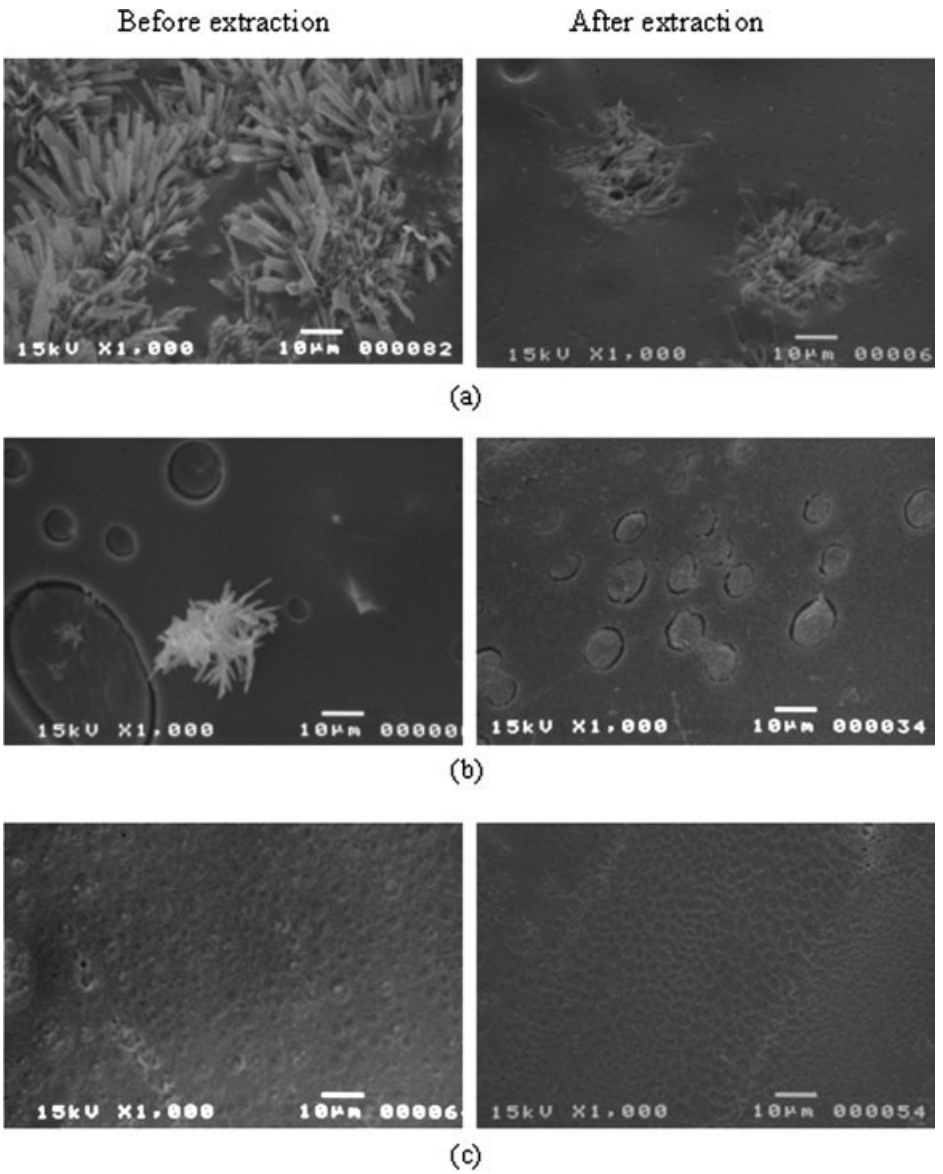


**Figure 11** SEM pictures of surface (a) imprinted membrane at 35°C, (b) imprinted membrane at 50°C, and (c) nonimprinted membrane prepared in $ScCO_2$ using NMP cast solvent.

1.9 μmol $g^{-1}$, respectively. There was similar tendency at 35 and 50°C for different imprinted and nonimprinted membranes. Evidence of the binding results was the fact that phase inversion of PSMA was capable of imprinting the URA shape into the polymer in the fluid. It is shown that the $ScCO_2$ fluid was efficient to fix the shape of URA template into the PSMA membrane. In addition, the extraction manner left URA imprinting sites in the PSMA matrix. As a result, the recognized URA was shown efficiently in the imprint membrane.

### Separation of URA and other analogs by URA imprinted membrane

To estimate the selective binding to the URA imprinted membranes prepared in $ScCO_2$ at 35 and 50°C, competitive binding studies were undertaken in batch binding experiment. In binary substrate solution containing each 14 μ*M* concentration of URA/DMURA, URA/Thymine, and URA/Cytosine, each concentration of the substrate before and after binding in the mixture was measured with HPLC. Separation effect of URA from the binary mixture solution was evaluated with α ratio of binding amounts of DMURA, thymine, cytosine to that of URA as described in experimental section. It was found that the values of α obtained for the imprinted membrane prepared at 50°C were 4.1, 5.2, and 3.3 for URA/DMURA, URA/Thymine, and URA/Cytosine, respectively, which was almost twice to that of the imprinted membrane prepared at 35°C. Since membrane prepared at 50°C showed higher selectivity to URA than that of 35°C, further evaluation

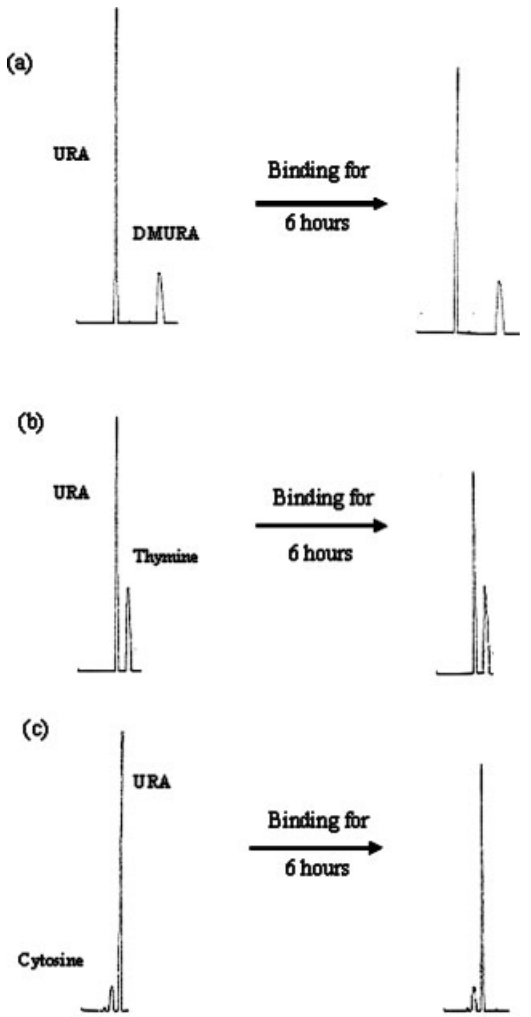


**Figure 12** HPLC charts of 2 μ*M* binary mixture solutions of (a) URA/DMURA, (b)URA/Thymine, (c) URA/Cytosine for before and after binding by imprinted membrane prepared in $ScCO_2$ at 50°C.

was carried in binary mixture solutions containing 7, 4, and 2 μ*M*, respectively. Figure 12 shows representative HPLC charts for the binary experiment before and after binding by imprinted membrane prepared at 50°C. The resultant HPLC peaks for the retention time of URA, DMURA, thymine, and cytosine were observed at 1.4, 2.9, 1.9, and 0.9 min, respectively. Since the column packed content had octadecylated silica, these data suggested that the substrate having hydrophilic properties become longer than that of hydrophobic one. In the separation, apparently, the URA imprinted membrane selectively bound URA rather than other substrates. The plots of α versus various concentrations of binary mixture solutions for separation experiment are shown in Figure 13. From this figure, the value of α increased as the concentration of the binary mixture solutions decreased. Figure 14 illustrates the effect of concentration on separation. The resultant imprinted membrane con-

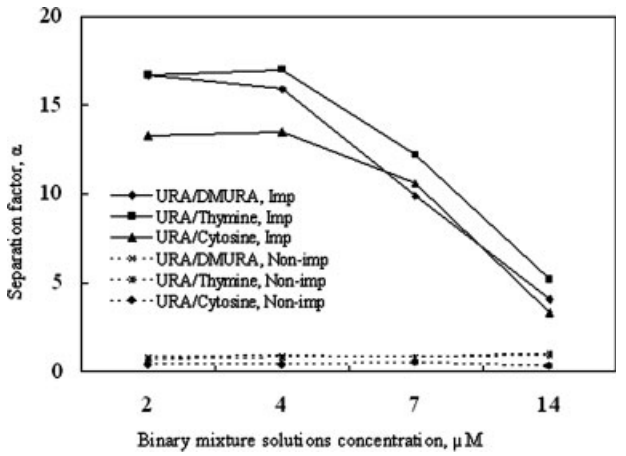


**Figure 13** Separation factor of binary mixture solutions in various concentrations.

tained limited binding sites for the template URA, the separation behavior seems to influence on substrate concentration. When separation experiment was carried out in low concentration of binary solution, most of URA template was bonded to imprinted membrane. Therefore, high separation factor was obtained as presented in Figure 14(a). For

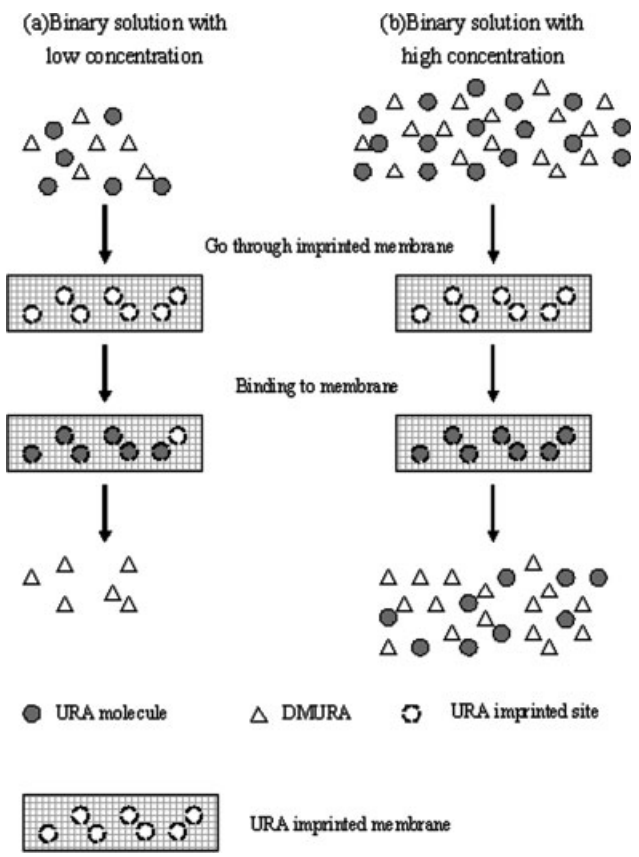


**Figure 14** Illustration of separation process in binary solution with (a) low concentration and (b) high concentration.

mixture solution with high concentration, some template molecule could not bind to the imprinted sites where this case resulted in low separation factor [Fig. 14(b)]. As shown in Figure 13, higher selectivity is shown for the URA/Thymine compared to the URA/DMURA and the URA/Cytosine in all concentrations. Although thymine was structurally close to the URA template, difference of the chemical structure was only on the presence of methyl group in the URA framework. At 2 $\mu M$ concentration, the value of $\alpha$ for both system of the URA/DMURA and the URA/Thymine were $\alpha_{URA/DMURA,Thymine} = 17$ and URA/Cytosine system became slightly to be low with $\alpha_{URA/cytosine} = 13$. Interestingly, the URA/Cytosine system decreased the separation ability of the membrane, meaning that the amino group on the URA framework interfered the selectivity. Therefore, these data strongly suggested that the URA imprinted membrane had effective selectivity by hydrogen bonding to separately bind in the binary components.

## CONCLUSIONS

URA imprinted polymers were successfully prepared by phase inversion in $ScCO_2$. The $ScCO_2$ fluid was efficient to fix the shape of URA template into the PSMA membrane through hydrogen bonding. However, when water was used as coagulation medium, it showed no effect on imprinting. Effect of cast solvents of DMF, DMSO, and NMP on the membrane structure was studied with SEM. It was found that the morphology of membranes was highly effected by the coagulant and organic solvent used for the phase inversion of PSMA. Results showed that URA imprinted membrane prepared at 50°C was highly recognized and selectively bound to URA rather than case at 35°C. The URA imprinted membrane had effective separation property of the binary components. Therefore, developing URA recognition materials was meaningful and was applied in the field such as drug therapy, biosensors, and separation science.

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