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Molecularly imprinted copolymer membranes functionalized by phase inversion imprinting for uracil recognition and permselective binding

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Abstract

Uracil (URA) was selected as a template for preparing molecularly imprinted membranes of poly(acrylonitrile-co-methylacrylic acid) [P(AN-co-MAA)] using the phase inversion technique. This study used Fourier transform infra-red (FT-IR) and ^{1}H nuclear magnetic resonance (NMR) spectroscopic studies to characterize the polymer-template interaction and scanning electron microscopy (SEM) and atomic force microscopy (AFM) for morphology of the URA imprinted membrane. Resultant membranes had typical ultrafiltration structure with porous morphology and showed a permeation flux of 3.5×10^{-5} m^3/(m^2 s) for 32 μM URA aqueous solution. Permselective binding to the target molecule was observed in permeation experiments with 7.9 μmol/g binding capacity of URA. Binding selectivity was discussed for URA and its analogs, dimethyluracil (DMURA) and caffeine (CAF), with 0.6 and 0.8 μmol/g binding capacity, respectively.
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1. Introduction

Molecular imprinting is an effective method to create selective recognition sites in synthetic polymers [1]. As tailor-made polymer materials for recognition of a template molecule, molecularly imprinted polymers (MIPs) have been applied in chromatography [2], chiral compound separation [3], and solid-phase extraction [4]. Such molecular imprinting processes mainly comprised polymerization of a functional monomer and a template molecule, which pre-arranged monomers and the template by covalent or non-covalent binding technique. After template polymerization, the template molecule was removed from the polymer to form imprint sites; the resultant polymers mimicked the template molecule in both shape and functionality [1,5].

As a molecule recognition material, most MIPs were suitable for chromatographic stationary phase because the rigid gel matrix was useful for preparation of the purpose. On the other hand, little was known about the imprinted membrane with recognition functionality and permselective

binding to the target molecule. We have anticipated that this kind of imprint membrane material is a useful technique for solid-phase extraction in the permeation experimental mode. Recently, Kobayashi and colleagues have developed theophylline (THO) molecularly imprinted copolymer membranes using the phase inversion imprinting technique of poly(acrylonitrile-co-acrylic acid) [P(AN-co-AA)] [6–9]. Porous membranes were formed by imprinting THO during the polymer solidification process. Thus far, phase inversion imprinting membranes have been made of various polymers for permselective binding of amino acids [2], caffeine (CAF) [10], and dibenzofuran [11,12]. The present work describes preparation of copolymer having methylacrylic acid (MAA) segments [P(AN-co-MAA)] for uracil (URA) (Scheme 1) recognition and binding. Here, URA has a smaller chemical structure than THO, CAF, and other xanthine derivatives. Application of the phase inversion imprinting method for URA template is meaningful because phase inversion precipitation has been certified as effective for preparation of molecularly imprinting membranes. Aside from great importance as a synthetic receptor to study permselective binding of URA by the imprinting membrane, URA selective binding behavior is one application for bio-mimetic artificial components that compose
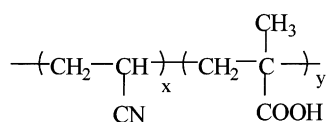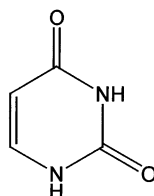
Scheme 1. Chemical structures of copolymer P(AN-co-MAA) and URA template.

RNA in biological organisms. Therefore, we have taken an interest in development of a novel imprinting membrane that is targeted to URA. As shown in Fig. 1, our approach to prepare URA-imprinted membranes involves solidification of P(AN-co-MAA) and URA template during the polymer precipitation process in water non-solvent. The imprinted P(AN-co-MAA) membranes were characterized from the viewpoint of recognition to discriminate URA and other analogs, CAF and dimethyluracil (DMURA). We describe results of preparation and properties of URA imprinted membranes for URA recognition and permselective binding.
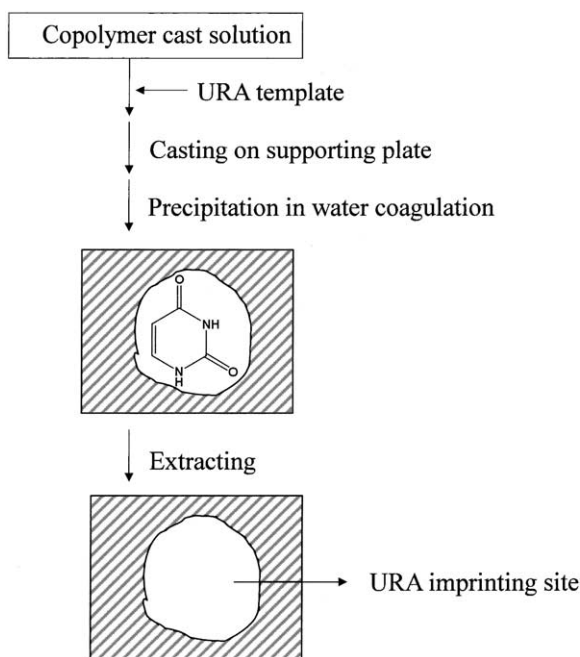

Fig. 1. Schematic illustration of URA imprint process by phase inversion process of P(AN-co-MAA) and URA.

2. Experimental

2.1. Materials and reagents

Acrylonitrile (AN) was distilled twice in atmosphere and dimethyl sulfoxide (DMSO) and MAA were distilled twice under vacuum condition. We used URA (Beijing Fuxing Chemical Reagents Factory, Beijing, PR China), CAF (Farco Chemical Supplies, Hong Kong, PR China) and DMURA (Tokyo Kasei Co.) without purification. All other reagents were used without further purification.

2.2. Imprinted membrane preparation

As a membrane material, poly(acrylonitrile-co-methylacrylic acid) (P(AN-co-MAA), Scheme 1) was synthesized with AN and MAA monomers (mole ratio AN:MAA = 90:10) by radical polymerization. For copolymerization of AN and MAA, DMSO and azobisisobutyronitrile (AIBN) were used as a polymerization solvent and initiator, respectively. Polymerization was carried in the following manner under a nitrogen atmosphere. In a reaction vessel of 500 ml capacity, 17.8 g (0.318 mol) AN, 3.2 g (0.038 mol) MAA, 70 ml DMSO, and 0.153 g AIBN were mixed; then N_2 gas was introduced. The copolymerization process was carried out at 60 °C for 7 h. The resultant viscous mixture of P(AN-co-MAA) copolymer was precipitated in water and then washed with ethanol. The copolymer was obtained with 54.1% conversion after room temperature evacuation. The MAA mole ratio in the copolymer was characterized by FT-IR and ^1H NMR as $y = 11.7$ and 10.7 mol%, respectively.

The URA imprinted membrane was prepared by phase inversion imprinting in a previously reported manner [6,13]. In DMSO-cast solvent, 10 wt.% of P(AN-co-MAA) and 1–3 wt.% of URA template were contained. Then, phase inversion of the DMSO solution formed a white P(AN-co-MAA) membrane in water. In the P(AN-co-MAA) copolymer used, the AN segment was for membrane formation and MAA for functional segment, which bound URA via a hydrogen bond. The resultant membrane was washed with 0.1 wt.% acetic acid (AcOH) aqueous solution after imprinting; then a large quantity of water was used to remove both URA and solvent. We stored resultant membranes in distilled water at room temperature after the imprinted membrane preparation until use for binding experiments.

### 2.3. Membrane properties and binding experiments

Interaction between the copolymer membrane and URA template was studied by means of Fourier transform infra-red spectrometer (FT-IR, Prestige-21; Shimadzu Corp.) and 400 MHz nuclear magnetic resonance spectrometer (^1H NMR, Avance 300; Bruker Analytik, GmbH). The morphological structure of URA imprinted membranes was observed using scanning electron microscopy

(SEM, JSM-5600; JEOL Co. Ltd.) and Atomic Force Microscopy (AFM, Nanopics 1000; Seiko Instruments, Inc.). AFM image analysis was performed with the Nanopics 1000 analysis program (Version 1.0); all images were analyzed with contacting mode using a silicon tip (DFM Cantilever-NPX1CTP004). Samples used in FT-IR spectrometry were prepared with a few micrometers' thickness for transmittance mode (20 times integration). Samples for SEM were prepared by fracturing the P(AN-co-MAA) membrane in liquid nitrogen and coating it with gold before measurement. To estimate the membrane characteristics, volume flux and molecule rejection were tested by ultrafiltration (UF) set of 100 ml dead-ends (type 8050; Amicon, Inc., Beverly, MA) under applied pressure of 100 Pa gradient across the membrane. The filtration experiment was performed similarly according to previous work [6]. During permeation of URA solution or URA analog solution through the membrane, substrate concentration in feed (32 μM) and permeation solution was measured using UV-Vis (UV-2401; Shimadzu Corp.) at 258 nm. Then, amounts of substrate binding $[S]_b$ (μmol/g membrane) were evaluated with mole binding per unit weight of imprinted polymer.

3. Results and discussion

3.1. Characteristics of MIP membranes

It was reported for THO imprinting that a carboxylic acid (COOH) group in the P(AN-co-AA) membranes showed hy-
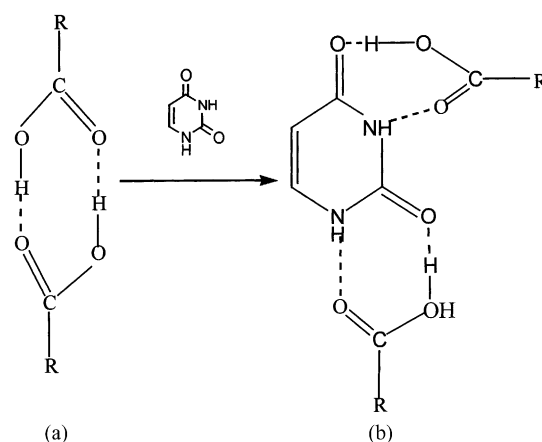
Scheme 2. Illustration of hydrogen bonds between URA template and COOH segment of P(AN-co-MAA) membrane.

drogen bonding with the THO template [6]. In this case, interaction force between the copolymer and THO template played an important role in imprinting and binding of substrate. In the present work, as shown in Scheme 2b, hydrogen-bonding interaction was expected between COOH segments and the URA template. Thus, formation of hydrogen bonding between P(AN-co-MAA) and URA template was observed using FT-IR and ^{1}H NMR, as well as THO [7].

Fig. 2 shows FT-IR spectra of URA-imprinted P(AN-co-MAA) membranes prepared from a cast solution of 2 wt.% URA (a) before and (b) after URA extraction, and (c) for unimprinted membrane. After the wet sample was frozen by liquid nitrogen, lyophilization was carried out for overnight
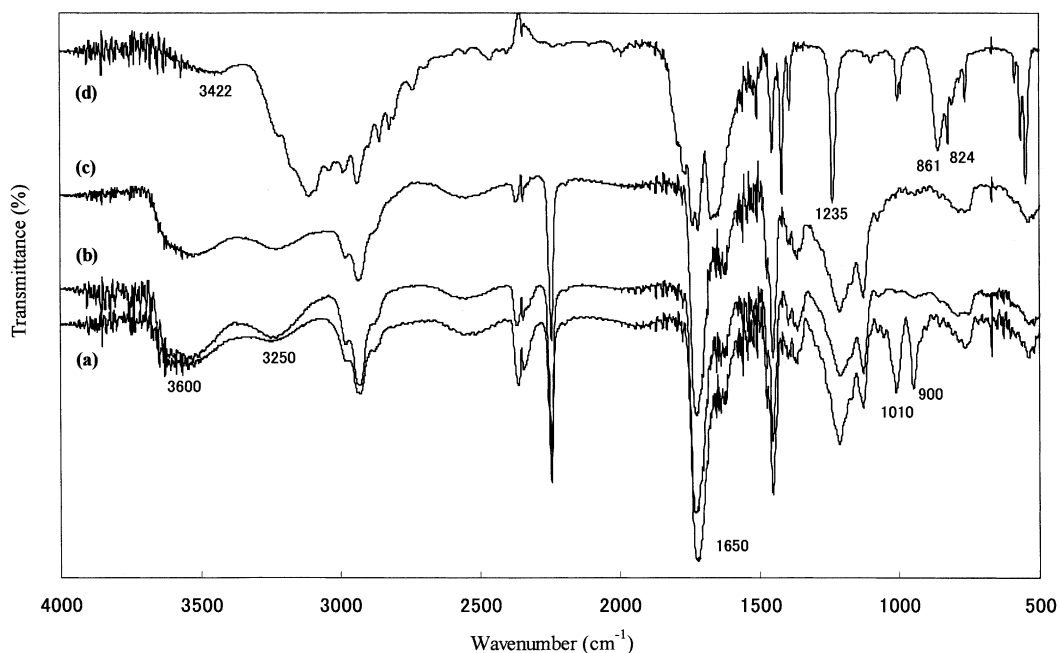

Fig. 2. FT-IR spectra of the URA imprinted membrane prepared from the cast solution (a) with 2 wt.% URA template, (b) after washing with 0.1 wt.% AcOH aqueous solution, and (c) without URA. Spectrum (d) was measured with KBr method for URA template.

to prepare an FT-IR sample of P(AN-co-MAA). The resultant FT-IR spectrum of URA is shown on trace (d). We found a URA spectrum N–H band peak at $3422 \, cm^{-1}$ and two C=O groups peaks at 1750 and $1650 \, cm^{-1}$ for URA. Stretching vibration of C–O was also found at $1235 \, cm^{-1}$ and strong out-of-plane wagging peaks of N–H at 861 and $824 \, cm^{-1}$. In trace (a) for URA imprinted P(AN-co-MAA) without extraction, we found characteristic IR peaks of the N–H group of URA near 900 and $1010 \, cm^{-1}$. Two peaks in the wavenumber region were observed only in the IR spectrum of trace (a) for the unextracted membrane. These were attributed to N–H bonds of URA, which shifted toward 1010 and $900 \, cm^{-1}$ from 861 and $824 \, cm^{-1}$ when URA was present in the P(AN-co-MAA) environment. This change was caused by wagging bonds of the N–H group of URA shifting by hydrogen bonding with the COOH segment [14]. In addition, we confirmed that these bonds disappeared after URA extraction in trace (b). We found that the IR band of C=O stretching shifted to a slightly lower wavenumber of $1650 \, cm^{-1}$ in trace (a). These facts suggest that hydrogen bonds were formed between URA and the COOH segments of the copolymer. Further evidence is shown in $3250–3600 \, cm^{-1}$ regions for OH stretch bands of COOH group of P(AN-co-MAA). In trace (a), the $3250 \, cm^{-1}$ band for dimmer COOH groups (Scheme 2a) [10] showed somewhat low intensity relative to that of trace (b). This fact means that the dimer COOH was consumed in formation of hydrogen bonding with URA template (Scheme 2b).

Fig. 3 shows ^{1}H NMR spectra of (a) P(AN-co-MAA), (b) URA template, and (c) P(AN-co-MAA) with URA. Spectrum (c) shows a noticeable proton peak of –COOH of P(AN-co-MAA) slightly shifted to 13.00 ppm from 12.96 ppm, which was for P(AN-co-MAA) without URA in d_6-DMSO. This shift confirmed that the –COOH group bound to URA via hydrogen bonding. We observed that two proton peaks of –NH group of URA also shifted from 11.06 and 10.86 ppm to 11.04 and 10.84 ppm, respectively, when both URA and P(AN-co-MAA) were present in the DMSO. These shifts were further evidence for hydrogen bonding between URA and P(AN-co-MAA).

3.2. Morphology characterization of URA imprinted membrane

Fig. 4 shows AFM images of the surface of unimprinted membranes with sizes of $40 \, \mu m \times 40 \, \mu m$ for (a) unimprinted membrane and (b) imprinted ones prepared from 2 wt.% URA cast solution. Both membrane samples had many grains on the membrane surface, indicating a porous membrane surface. As shown by (c) and (d) for unimprinted and imprinted membranes, respectively, surface cross-section of the AFM image indicated that the imprinting manner made slightly the surface rough. The image of the imprinted P(AN-co-MAA) exhibited rough surface with mean surface roughness, $R_a = 90 \, nm$, while the surface of the non-imprinted membrane with $R_a = 79 \, nm$ slightly

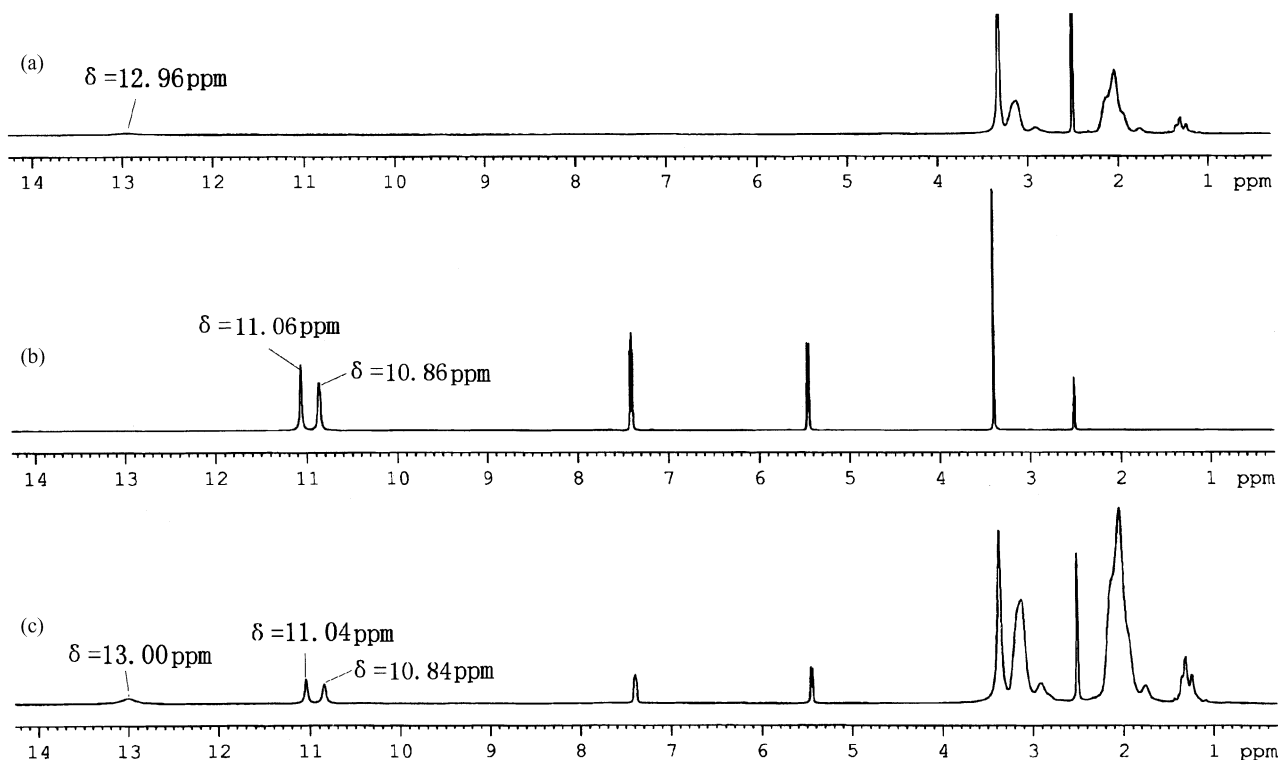Fig. 3. ^{1}H NMR spectra of: (a) P(AN-co-MAA) in DMSO-d_6, measured at 60 °C: (a) 10 wt.% copolymer without template; (b) URA; (c) copolymer with 2 wt.% URA concentration.

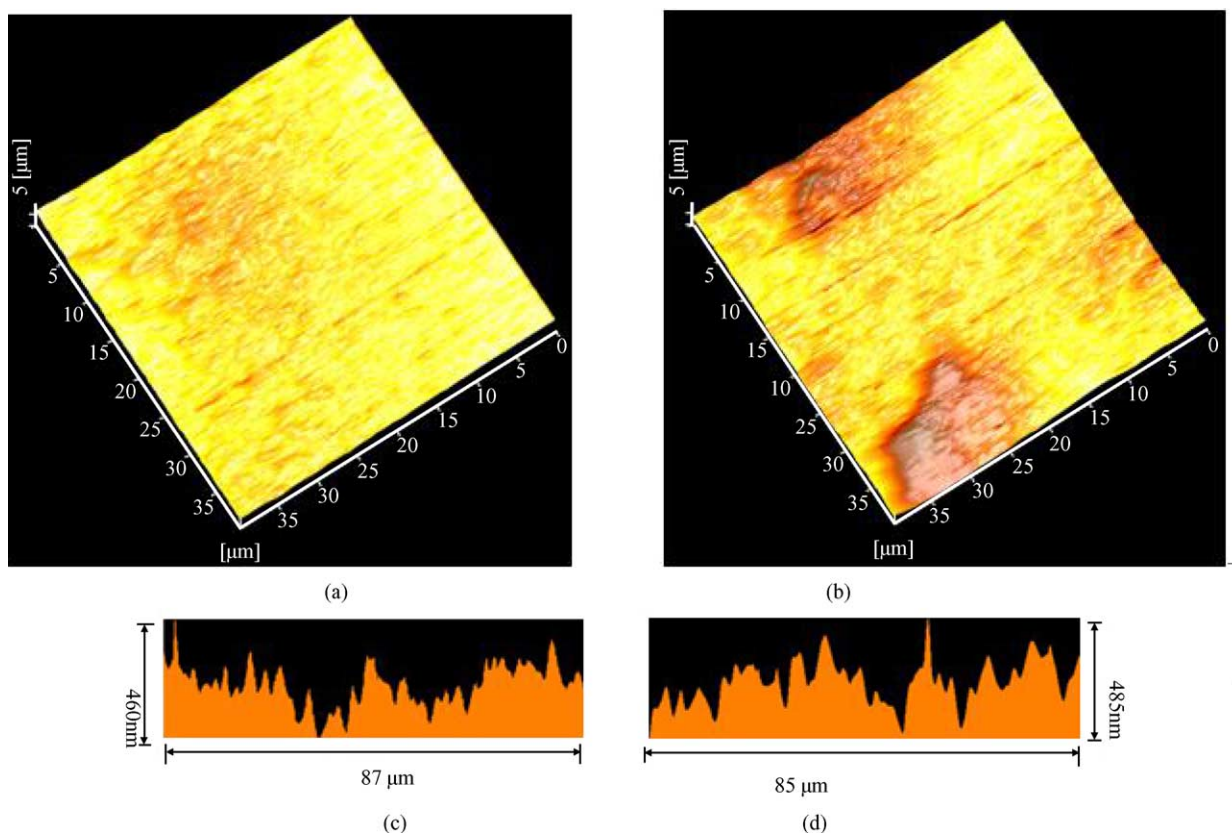Fig. 4. 40 μm × 40 μm AFM images of top surface of: (a) unimprinted and (b) URA imprinted membrane prepared from 2 wt.% URA concentration. AFM images (c) and (d) were for cross-section view of the unimprinted and the imprinted membranes, respectively.

decreased in the roughness. It was obviously noticed in the 20 μm × 20 μm that the AFM view of the imprinted P(AN-co-MAA) membrane demonstrated the surface having depression places, which look like craters with about 20 μm diameter. This fact indicated that the hydrogen bonds between the P(AN-co-MAA) membrane and URA roughed the surface slightly. That is, P(AN-co-MAA) and URA assembled was formed such characteristic morphology in the imprinted membrane. Also, the appearance of a somewhat rough surface on the top layer may be attributable to high liquid–liquid demixing of the copolymer in the presence of template molecules [8]. Therefore, the results may be caused by that the interaction between URA template and the COOH groups of P(AN-co-MAA) enhanced the solvent exchange between DMSO and water.

For solute solution permeation, high permeation flux is known to be preferred in these porous membranes. This preference is attributable to important characteristics to treat large amounts of volume solution with a porous membrane [15]. An SEM photograph of the 2 wt.% URA imprinted membrane was measured (Fig. 5) to evaluate the cross-section side of the P(AN-co-MAA) membrane morphology. The resultant imprinted membrane had ca. 250 μm thickness and the SEM picture showed an asymmetric porous structure of the cross-section with a dense layer on top of the surface in μm scale and a supporting layer formed
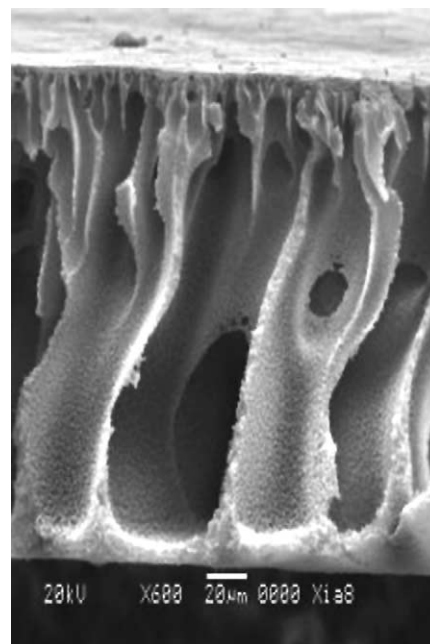

Fig. 5. SEM photograph of cross-section of URA imprinted membrane prepared from 2 wt.% URA concentration cast solution.

with many void pores in the cross-section. We also observed similar cross-section morphology of other membranes prepared without and with 1 or 3 wt.% URA, respectively, in the cast solution. Hence, the porous structure of the membranes is expected to be very suitable for permeation characteristics of URA solution with high volume flux.

3.3. Permselective binding of URA by permeation experiments

Because the P(AN-co-MAA) membrane morphology observed in Fig. 5 was typical for UF membrane [15], the resultant membranes prepared from cast solution containing 1–3 wt.% URA were characterized with water volume flux of 3.7×10^{-5} m^3/(m^2 s) under 100 Pa applied pressure. In the filtration condition, the membrane was observed to have 93% rejection for bovine serum albumin (MW = 68,000) and 18.8% for polyethylene glycol (MW = 1540). These data also suggested that the P(AN-co-MAA) membrane had similar permeation character to the UF membrane and had size exclusion characteristics of macromolecules.

In addition to the size exclusion of high-molecular-weight molecules, the present imprinted P(AN-co-MAA) membrane was expected to have permselective binding of URA, which was of molecular weight of 112. Figs. 4 and 5 show that porous surface imprinted sites of URA are present in the porous surface. Thus, using the imprinted membranes, permeation experiments of URA solution would be efficient membrane adsorbent for URA. Aqueous URA in 32 μM concentration was used in the present experiments. Under 100 Pa applied pressure, these membranes had 3.5×10^{-5} m^3/(m^2 s) volume flux, which was slightly lower than that of 3.7×10^{-5} m^3/(m^2 s) for water. This indicated that URA in the solution interacted with P(AN-co-MAA) when the URA solution was permeated through the imprinted membranes. Fig. 6 shows plots of URA and substrate analogs of DMURA and CAF for binding amounts uptaken in different membranes versus permeation time. Fig. 6a shows URA binding plots observed in different membranes. Values of $[S]_b$ increased with increased permeation time, then became constant at about 1.2, 3.3, 7.9, and 6.9 μmol/g membranes for URA imprinted membranes prepared from cast solutions of 0, 1, 2, and 3 wt.% of URA concentration. Here, the membrane without template was for unimprinted membrane. It is noteworthy that the saturated value of $[S]_b$ was obtained depending on URA concentration in P(AN-co-MAA)-DMSO cast solution used in phase inversion imprinting. That is, in the range of 0–2 wt.% concentration of URA, the binding capacity measured at
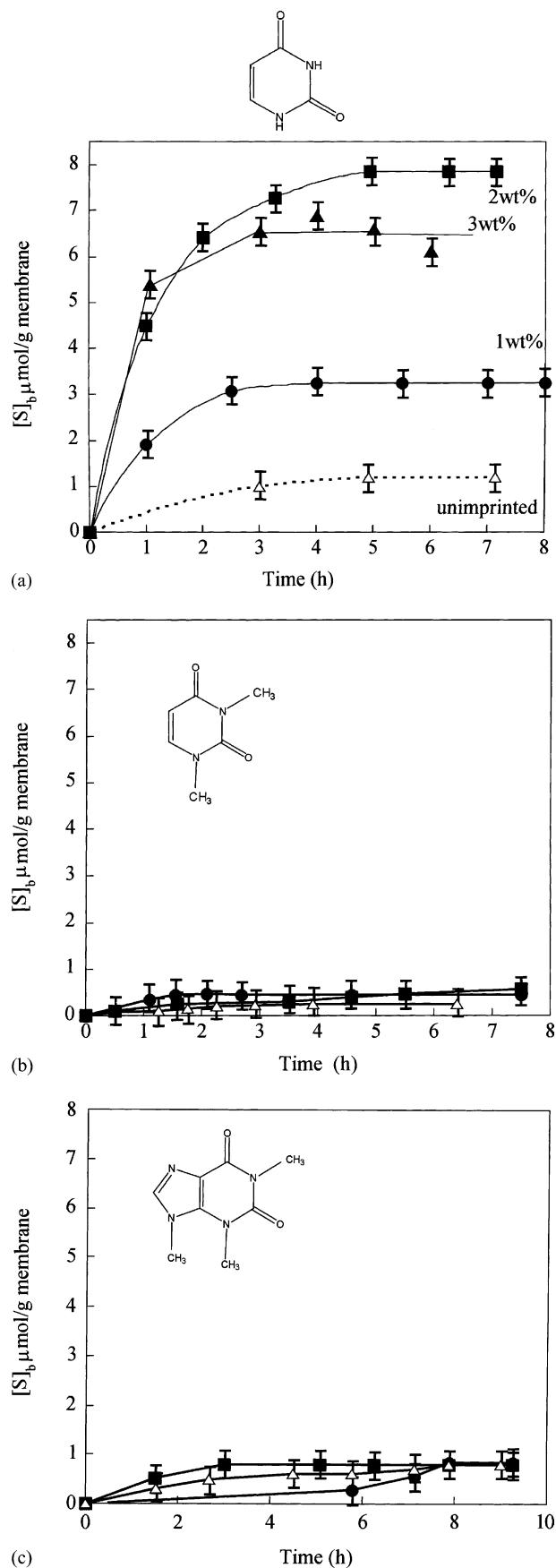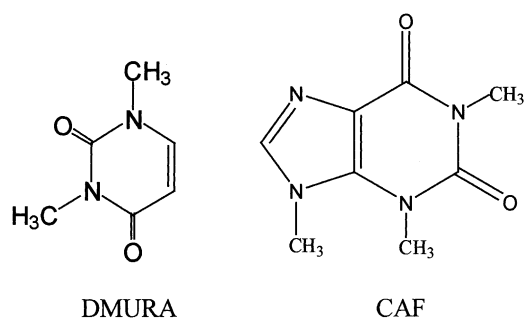

Fig. 6. Time course of: (a) URA, (b) DMURA and (c) CAF binding to P(AN-co-MAA) membranes prepared from cast solutions having various concentrations of URA template. Symbols of the binding amounts of substrate described the membranes prepared from 0 wt.% (△), 1 wt.% (■), 2 wt.% (●) and 3 wt.% (▲) URA concentration. Permeation experiments were carried out in 32 μM solute concentration.

Scheme 3. Chemical structures of URA analogous.

Table 1
Saturated substrate amounts and imprint factor of different template concentration membranes

URA concentration in DMSO cast solution	0 wt.%	1 wt.%	2 wt.%
$[S]_{b,URA}$	1.2	3.3	7.9
$[S]_{b,CAF}$	0.8	0.8	0.8
$[S]_{b,DMURA}$	0.3	0.5	0.6
$\alpha_{U/C}/\alpha_{U/D}$	1.4/4.8	4.1/6.8	9.9/13.6

$[S]_b$: binding amounts of substrate (μmol/g membrane) at saturation binding time.

saturated time was increased by increased template concentration. However, the URA binding amount was found to be 6.9 μmol/g at saturation time for the 3 wt.% URA imprinted membrane. It was lower than that of the 2 wt.% URA imprinted membrane. These results suggested that the imprint ability of the phase inversion process became an optimum condition when the [COOH]/[URA] = 1 at 2 wt.% concentration. We confirmed stability of the URA-imprinted membrane prepared from 2 wt.% URA concentration. The binding capacity remained constant over 2 months with storage at room temperature in a dark place. In addition, the resultant membranes demonstrated good reproducibility in its preparation and URA binding capacity. This may be attributable to hydrophobicity of methyl group on MAA sites being supported for stable polyacrylonitrile segments, which form the CN dipole–dipole coagulation forces [16,17].

We also compared results of permselective binding of DMURA and CAF for the URA imprinted membranes to confirm template effects on the URA imprinting. Here, as presented in Scheme 3, DMURA has a similar URA structure with the template molecule, whereas DMURA contains dimethyl groups on N atoms of the URA framework. CAF also contains a URA unit in the molecule. Fig. 6 also shows (b) DMURA and (c) CAF uptake curves of the URA imprinted membranes. Together, these showed that the saturate binding amounts of DMURA were 0.58 and 0.48 μmol/g membrane for 2 and 1 wt.% URA imprinted membranes and 0.25 μmol/g for the unimprinted membrane. The saturate binding amounts of CAF were about 0.80 μmol/g for both imprinted membranes and the unimprinted one. These implied that both CAF and DMURA bindings were less than those of URA for the URA-imprinted membranes. Resultant binding amounts of CAF in the imprinted membranes were slightly larger than those for DMURA, but CAF had a larger molecule sites relative to DMURA. Nevertheless, the values of $[S]_b$ for URA in the imprinted membrane were higher than those observed in DMURA and CAF. Table 1 lists saturation binding and imprint factor α_i for the imprinted and unimprinted membranes. The value of α_i was defined as the following equation

$$\alpha_i = \frac{[S]_{b,URA}}{[S]_b}$$

where $[S]_{b,URA}$ and $[S]_b$ represent saturate binding amounts of URA and CAF or DMURA, respectively. Values of α_i of URA imprinted membrane were 9.9 for URA to CAF and 13.6 for URA to DMURA, when prepared from 2 wt.% URA concentration. Values of $\alpha_{U/C}$ and $\alpha_{U/D}$ of 4.1 and 6.8 were observed for the imprinted membrane prepared from 1 wt.% concentration. These values indicate that the URA-imprinted P(AN-co-MAA) membranes showed high selectivity of URA in permselective binding. Evidence of this binding was the fact that phase inversion imprinting of URA-P(AN-co-MAA) was capable of imprinting the URA molecule shape in the copolymer membranes. In addition, the extraction manner left URA imprinting sites that were able to rebound URA into the sites as the URA solution was permeated through the membrane. This evidence indicates that resultant recognition properties of URA imprinted membranes were efficient for URA recognition and useful for membrane extraction in permeation mode.

4. Conclusion

We prepared URA imprinted membranes by phase inversion imprinting of P(AN-co-MAA) copolymer. FT-IR and ^{1}H NMR spectra confirmed that hydrogen bonding was formed between P(AN-co-MAA) membrane and the template molecule. The imprinted membrane was characterized with AFM and SEM photographs in addition to permeation properties of volume flux and rejections of BSA and PEG. Permeation experiments addressing URA or DMURA and CAF showed that the resultant imprinted membranes recognized the template molecule effectively and bound it with efficient permselective manner to URA.

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