Molecular Imprinting of Theophylline in Acrylonitrile-acrylic Acid Copolymer Membrane

Takaomi Kobayashi,* Hong Ying Wang, and Nobuyuki Fujii
Department of Chemistry, Nagaoka University of Technology, 1603-1 Kamitomioka, Nagaoka 940-21

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Molecular size of Theophylline was imprinted in ultrafiltration membrane of poly(acrylonitrile-co-acrylic acid), when the membrane was prepared by phase inversion method. Evidence was presented by the permeation experiments that the Theophylline solute binds more strongly to the imprinted membrane.

Molecular imprinting has practical application in chromatograph materials for enantiometric separation and drug assay.[1-4] In this way, progress has been made in building specific binding sites into cross-linked polymer. The imprinting techniques involve the polymerization of monomer with the print molecule, which interacts with the monomer. After the polymerization, the removal of the print molecule from the polymer gives the sites in the matrix. But, little is known concerning the membranes with the imprinting functionality.

We report here the preliminary result of our investigations on the molecular imprinting in ultrafiltration membrane of poly(acrylonitrile-co-acrylic acid) (**1**). The copolymer contains both acrylic acid residue as the functional sites and acrylonitrile residue as membrane formation sites.[5] As illustrated in Figure **1**, the imprinting of Theophylline (**2**) in the membrane was taken place during the cast process for the membrane preparation.

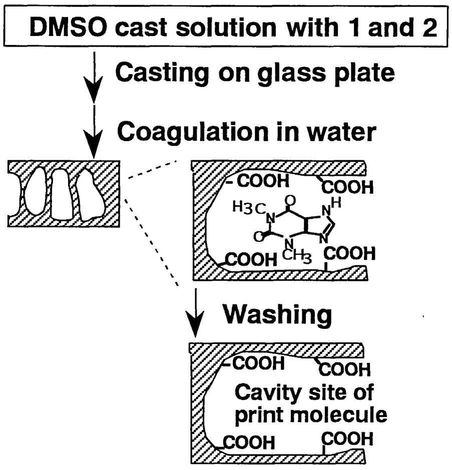
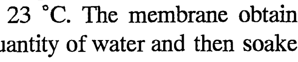
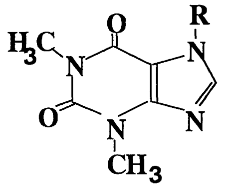


Figure 1. Schematic illustration of imprint process of **2** in copolymer membrane **1** by phase inversion method.[9]



$$-(CH_2-\underset{CN}{CH})-(CH_2-\underset{COOH}{CH})-$$

1



2: R=H
3: R=CH$_3$

The experimental procedure is as followed; Dimethylsulfoxide solution having 10 wt% of **1** and 2.5 wt% of **2** was well mixed for overnight at 50 °C. Then, the polymer solution was cast on glass plate and the solution was immediately coagulated in water at 23 °C. The membrane obtained was well washed with large quantity of water and then soaked in water containing acetic acid (Ac) to remove the solvent and the template molecule. The removal of **2** template from the membrane was checked by IR measurements of the membrane.[8] Scanning micrographs of cross-section of the membranes indicated that the membrane has an asymmetric porous structure like a typical ultrafiltration membrane,[9] consisting of dense layer and the support one with many pores in μm order. We prepared two types of membranes from the cast solution with and without 2.5 wt% of the template **2**. The porous membranes prepared for 100 μm thickness were cut for 43 mm diameter to set it in ultrafiltration cell. The apparatus and procedure of the filtration were similar to those previously used.[10] To characterize the ultrafiltration properties, we permeated dextran solutions through the membranes. These results indicated that the membranes from the cast solution with and without the molecule **2** have similar molecular weight cutoff properties. The membranes have water flux values of 4×10^{-6} (m^3/m^2s) and, for example, permeate dextrans with molecular weights (MW) of 1×10^4 and 7×10^4 by the rejection of 5 and 30%, respectively. This means that the pore size of membranes is larger than the dextran molecular size.

By using the membranes, permeation experiments of aqueous solution of **2** were carried out.[11] Figure 2 shows plots of the amounts of the solute taken in the membrane, [S] (μmol/g-membrane), versus filtration time. The values of [S] increase with an increase in the filtration time and then becomes constant after about 2 and 12 h for the membrane prepared from the cast solution without and with **2**, respectively. In the filtration time, the total amounts of solute, $[S]_b$, for the membrane imprinted with **2** are about 0.36. We noted that the value becomes large relative to the value of 0.05 for the unimprinted membrane. This result means that the membrane records the shape of the **2** molecule in it and the solute molecules are taken in the sites. Figure 2 also has plots for Caffeine **3** using the membrane imprinted with **2**. The $[S]_b$ value of **3** becomes constant after about 5 h with 0.06 value. As shown in the text, the molecule **3** has methyl group on the nitrogen atom instead of H group of molecule **2**. Therefore, the comparison suggests that the hydrogen bonding between **2** and **1** is important on the solute uptake in the membrane. As mentioned,[8] the IR data of the membranes without and with the Ac treatment strongly suggest the presence of the hydrogen bonding between **2** and COOH group of the imprinting membrane. Further study on the

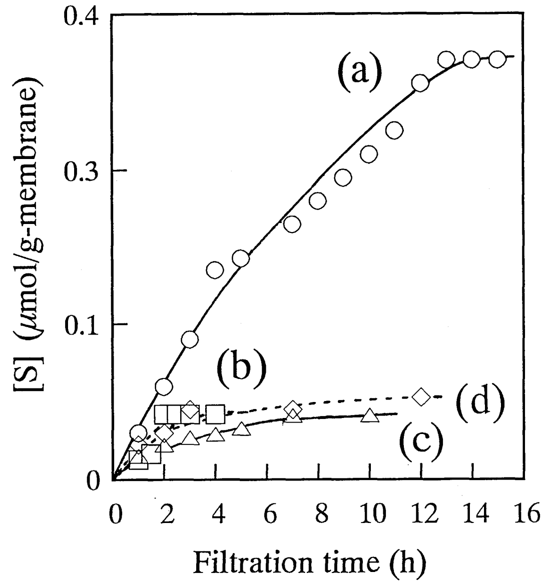


Figure 2. Plots of solute amounts taken in the membrane at various filtration times. The membranes for the permeation of **2** were prepared from DMSO cast solution (a) with and (b) without the print molecule **2**. (c) and (d) for the permeation experiments of **3** by the membranes from the cast solution with and without the template **2**, respectively.

molecular imprinting in the membrane and its permeation behavior is now under progress and the details will be reported elsewhere.

References and Notes

1 G. Vlatakio, L. I. Andersson, R. Muller, and K. Mosbach, *Nature*, **361**, 645 (1993) and references therein.

2 M. Lepisto and B. Sellergren, *J. Org. Chem.*, **54**, 6010 (1989).

3 G. Walff, B. Heide, and G. Helfmeier, *J. Am. Chem. Sci.*, **108**, 1089 (1986).

4 L. Fischer, R. Muller, B. Ekberg, and K. Mosbach, *J. Am. Chem. Sci.*, **113**, 358 (1991).

5 Copolymerization of acrylonitrile (AN) and acrylic acid (AA) was carried out in DMSO solution as follows; In reaction vessel of 500 ml capacity, 30.4 g (566 mmol) of purified AN, 7.51 g (104 mmol) of AA, 110.5 g of DMSO and 0.22 g (1.3 mmol) of AIBN were introduced. Polymerization was carried out at 60 °C for 6 h in nitrogen flow. The mixture was poured into large quantity of water to precipitate the crude copolymer **1**. Finally, the copolymer was washed with methanol and then dried *in vacuo* (55% conversion). The AA content in **1** was determined by acid-base titration for 0.3 mmol/g-polymer.[6] Also, the copolymerization was checked by measuring FT-IR spectra and ^{1}H-NMR spectra in d$_6$-DMSO. Viscosity of the copolymer was measured at 30 °C using Ubbelohde viscometer as $[\eta]=1.22$ (cm^3/g). The MW of the copolymer was estimated as $7\text{x}10^4$ according to the literature.[7]

6 H. Miyama, H. Yoshida, and Y. Nosaka, *Makromol. Chem., Rapid Commun.*, **9**, 57 (1988).

7 P. F. Onyon, *J. Phys. Soc.*, **37**, 315 (1959).

8 IR (KBr pellet): 2925 and 2870 cm^{-1} (CH stretching), 2240 cm^{-1} (CN stretching), 1650 cm^{-1} (C=O stretching); The values of the IR peak ratio of the CH stretch at 2925 cm^{-1} to that of the CN stretch are 0.28 and 0.38 for the membranes prepared by the casting solution without and with **2** template, respectively. The value for the imprinting membrane was decreased to the value of 0.27 by washing it with 0.1 wt% of Ac aqueous solution.

9 M. Mulder, "Basic Principles of Membrane Technology," Kluwer Academic Publishers, Netherlands (1991), pp.96-109.

10 T. Kobayashi, T. Nagai, T. Suzuki, Y. Nosaka, and N. Fujii, *J. Membrane Sci.*, **86**, 47 (1994) and other references therein.

11 The permeations of the solute solution containing 3.6 μM concentration of **2** or **3** at pH 7.5 were carried out by the hydraulic pressure of 2.5 kPa with starring 400 rpm. The concentration for **2** and **3** was analyzed by HPLC (Toso CCPD, UV8000 with column of TSK$_{gel}$ ODS80TM). The permeation experiments were repeated three times for each run. The average value of [S] is plotted in Figure 2.