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Molecularly Imprinted Nylon Membranes Having Amino Acid Selective Binding Properties. Effect of Phase Inversion Condition on Membrane Characteristics

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Nylon 6 was used in modified phase inversion imprinting of L-phenylalanine (L-PhA). Effect of the imprinting process on membrane morphology and binding selectivity of D-PhA and L-PhA was studied. The process of solvent evaporation and nylon coagulation in water resulted in imprinted membranes with both dense and aggregated-sphere segments of nylon. The solvent evaporation process before nylon coagulation also led to an increase of L-PhA binding capacity with high selectivity.
Key words: Molecule imprinting, Selective binding, Nylon, Phase inversion, Selective binding

1. INTRODUCTION

Molecularly imprinting technologies are a useful strategy in design of functional macromolecules for application of molecular recognition[1] and separation systems[2,3]. Design and synthesis of artificial host polymers have been studied and based on pre-organization, complementarily to maintain topological volumetric spaces in molecularly imprinted polymers (MIPs). These materials have specific recognition ability to selectively take target molecules into the polymer[3,4]. Most studies of imprinted polymers have been carried out using rigid cross-linked matrixes in particles and films, can be made of template cross-linked copolymerization[4-7]. In recent molecularly imprinted works, an attractive challenge has paid to membrane[5,8]. Functionality of membranes has been an attractive research subject to separation fields. However, little is still known about polymeric membrane having molecular imprinted characteristics. We believe that membrane can promise a challenging technology for the selective transport of molecules and for the selective binding of target molecules by imprinting function. Thus, the technology provides a powerful tool for creation of novel functional polymer membrane, which can separate and concentrate target molecule from mixture media.

We have investigated molecular imprinting membranes for development of novel functional membranes, which can selectively separate and concentrate a target molecule[9,10]. Since molecular imprinting technique, which we proposed in phase inversion imprinting process (PIP) (Fig 1), has been found to be an effective means of encoding template molecule information in polymeric membranes. In the technique, coagulation process of polymer was applied to encode an information of template molecules in polymeric porous membranes. It was shown that the technique can build tailor-made volumetric sizes for the target molecules.

This study shows imprinted membranes having recognition ability of amino acid, L and D-phenylalanines (PhA) by Nylon-6. Nylon was composed of amide segments can be formed inter-polymer chain through hydrogen networks (Scheme 1)[11].

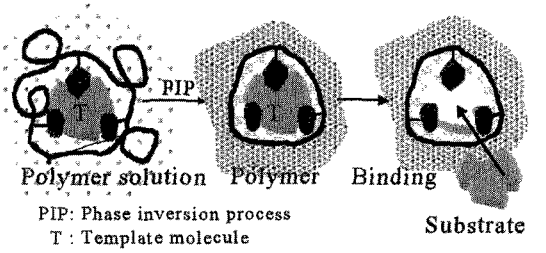


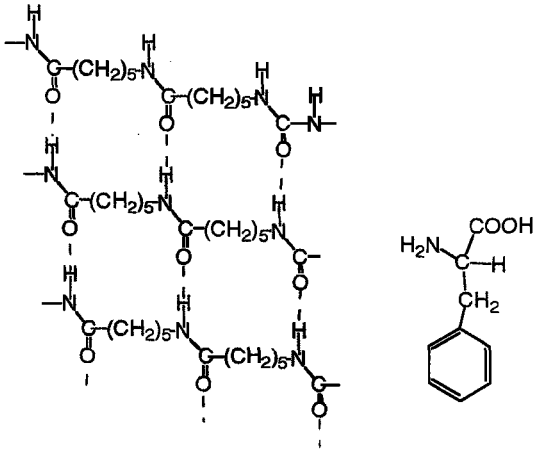
Fig. 1 Illustration of MIPs preparation process for phase inversion imprinting.

2. EXPERIMENTAL

2.1 Membrane preparation

Nylon 6 (Mitsubish Kasei) was used to imprint L-PhA. The average molecular weight of Nylon 6 was $7x10^4$ and was soluble in formic acid. Molecular imprinted membranes for enatio-separation of L or D-PhA were prepared by phase inversion imprinting[14,15] with partial modification including solvent evaporation before nylon coagulation (Fig. 2). At first, polymer-formic acid solution containing the amino acid template was prepared at 30 °C. The polymer concentration was 17wt% concentration in formic acid (20 ml) and then 4g (30.3 mmol) of L or D-PhA was added. For overnight, the viscous solution was stirring before casting. In PIP, we coagulated the polymer-template solution in water after the solution was cast on flat plate. On the other hand, in solvent evaporation-phase inversion (SEPI) the

solution was cast on a flat glass plate warmed at 50 °C for 1 min. Then, the polymer solution was



Scheme 1 Inter-molecule hydrogen bonding networks of Nylon 6 and template L-PhA.

coagulated in water at 30 °C. After coagulation, the polymeric membrane solidified was kept overnight in water in order to remove formic acid and the template. Further water washing was performed with an excess of water. As references, the nylon membranes were prepared without the template.

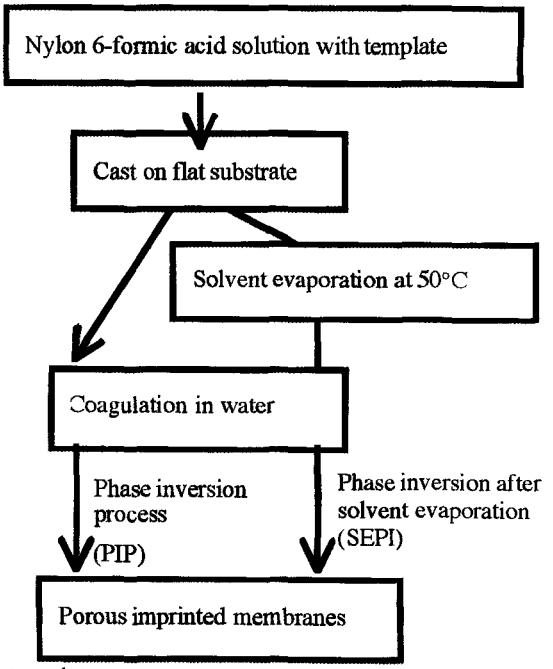


Fig. 2 Schematic illustration of nylon imprinted membrane through PIP and SEPI.

Also, imprinted membranes were obtained by common phase inversion imprinting, which has no solvent evaporation process before the coagulation.

2.2 PhA binding to L-PhA imprinted membranes

In order to study substrate uptake of PhA, heterogeneous batch experiments were carried out in 5μM concentration. The L-PhA imprinted membrane with weight (W) (g) was equilibrated in the PhA aqueous solution at 30 °C. Then, the PhA concentration of bulk solution was estimated at various times by UV-Visible detector ((UV 8000) monitored at 210 nm) with a crown pack column (Disel co). Partition factor (α) was calculated from L and D form concentration ratio,

$$\alpha = [\text{L-PhA}]/[\text{D-PhA}]$$

Value of the substrate binding of L-PhA to the L-PhA-imprinted membrane, $[S_{L\text{-PhA}}]$ (μmol/g-polymer), was calculated by following equation:

$$[S_{L\text{-PhA}}] = (C_b - C_a)V/W$$

where C_b and C_a are mole concentration (μM) of PhA before and after equilibrium time (h), respectively: V (L) is the volume of bulk PhA solution for binding experiments.

3. RESULTS AND DISCUSION

3.1 Effect of solvent evaporation on membrane morphology

Imprinting procedure involves incorporation of small amounts of template molecules in a polymer solution and phase separation process for phase separation from liquid phase to solid phase. As is advantageous for methods without the polymerization step in the imprinting process, polymer solidification obtains imprinted membranes. So, the coagulation process of polymer is important.

In order to evaluate the membrane morphology, we measured scanning electron micrograph (SEM) of the imprinted membrane for Nylon 6 in Fig. 3 (a) and (b), which were for PIP and SEPI, respectively. The SEPI process was of experience in solvent evaporation for 1 min and then coagulated in water (Fig.2). It was characteristics that globular nylon aggregates having few micrometer size composed porous structure in SEPI. In case of the PIP membrane, the cross-section showed porous structure with about 30μm thickness. The morphology of the PIP membranes, which was prepared without the solvent evaporation before the coagulation, had porous sponge structure without such globular aggregates. Therefore, the solvent-water exchange was taken place slowly in water medium for PIP. It was noted that the membrane morphology was dramatically changed after solvent evaporation for 1 and 3 min. The surface morphology of the membrane (b) showed grain structure with about few μm height and 3-5 μm diameter. The cross-section thickness of SEPI membranes was about 30 and 20 μm for 1min and

3 min, respectively. With increase of the evaporation time at 50 °C, the cross-section morphology became a dense in top surface and the thickness was reduced by the evaporation process. This was due to nylon concentration increasing by the evaporation process. Therefore, the membrane morphology changed the solvent exchange between water and surface quickly occurred in SEPI.

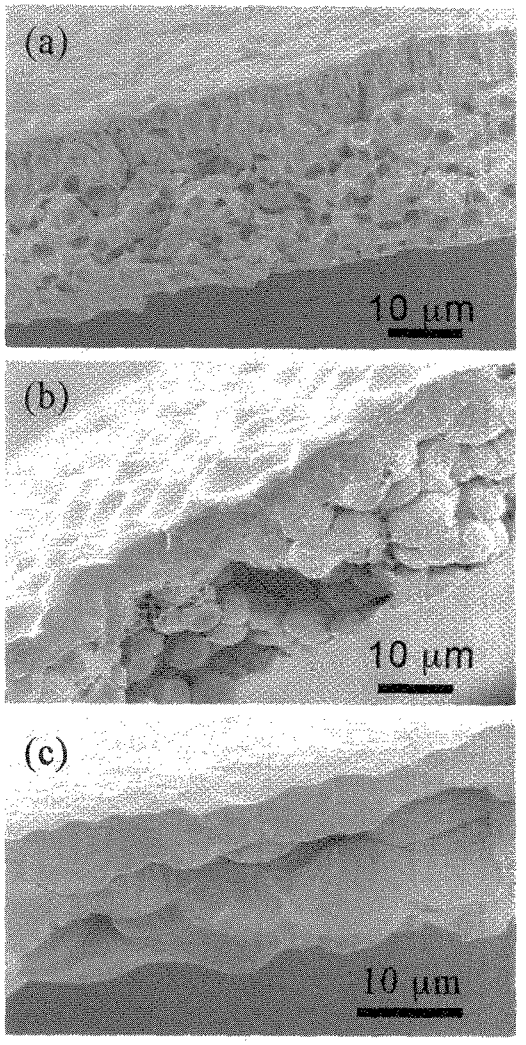
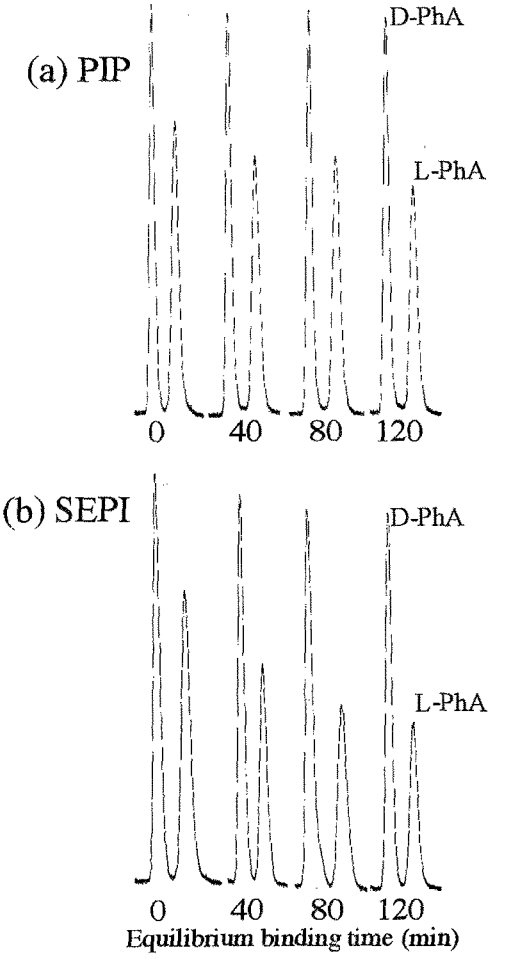


Fig. 3 SEM pictures of nylon imprinted membranes prepared by (a) PIP and (b) SEPI for 1 min and (c) 5 min at 50 °C.

3.2 PhA bindning to the imprinted membranes

Selective binding characteristics of the L-PhA imprinted membranes were carried out using heterogeneous batch experiment in 5 mM total concentration of L and L PhA. Up to the time of saturation binding of PhA to the membrane, the experiments were kept for 120 min. The concentration of L and D form of PhA was determined by optical resolution column chromatography. We observed two peaks having 5 min and 7 min retention times in the chromatogram for D and L-PhA, respectively.

Figures 4 show resultant chromatograms measured at various saturation times; 0, 40, 80 and 120 min. It could be observed in (a) PIP that the peak intensity of D-form was almost constant at each equilibrium time. Contrary to D-form, the L-form intensity was decreased with increase of the equilibrium time. Note that the degree of the peak reduction of L-PhA was higher in SEPI than that of PIP as shown in (b) chromatographs. This indicated that the SEPI membrane showed high



Figs 4 Time change of chromatogram of D and L-mixture of PhA aqueous solution. (a) PIP and (b) SEPI imprinted membranes. Flow rate eluent, 1 ml/min, with operation pressure, 117 kgf/cm^2 was operated.

selectivity to L-PhA in the binding processes. When the solvent evaporation was for 3 min, but, the degree of the peak reduction of the L-form decreased relative to the SEPI imprinted membrane prepared by 1min evaporation. This was due to dense morphology of resultant membrane prepared by the 3min solvent evaporation.

Table I lists L-PhA binding capacity and partition factor α for each membrane. The PIP

membrane had the value of L-PhA binding capacity, $[S_{L\text{-}PhA}]$ = 0.20 and 0.07 μmol/g and that of partition factor was 5.1 and 1.0 for the imprinted and non-imprinted membranes. In SEPI membranes prepared by the 1min and 3 min evaporation, $[S_{L\text{-}PhA}]$ = 2.45 and 1.37 μmol/g. Since the SEPI membrane prepared by the 1min

Table 1 Binding capacity of L-PhA and partition factor for L-PhA imprinted and non-imprinted membranes.

	SE time (min)	$[S_{L\text{-}PhA}]$ (μmol/g)	α
PIP imprint	0	1.20	5.1
Non-imprint	0	0.07	1.0
SEPI imprint	1	2.45	26.7
	3	1.37	2.2

evaporation showed high binding capacity of the L-form target and low in the D-form, the value of partition factor was obtained. Because the concentration of polymer was increase at the interface between air and the polymer solution by the solvent evaporation process, leakage of the template molecule wad retarded during the coagulation process. Therefore, the molecular structure of L-PhA could be encoded into the membranes during the SEPI imprinting processes.

4. CONCLUSION

It was found that in phase inversion imprinting of Nylon 6-L-PhA, both solvent evaporation and coagulation (SEPI) process were effective for efficient formation of L-PhA imprinting. In the L-PhA imprinted membranes, globular nylon aggregates having few micrometer size composed porous structure, which was characteristics in addition to high imprinting efficiency of L-PhA by the imprinted membrane.

5. ACKNOWLEDGEMENTS

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