Molecularly Imprinted Uniform-Sized Polymer-Based Stationary Phase for Naproxen

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A molecularly imprinted uniform-sized polymer-based stationary phase for (S)-naproxen has been prepared using 4-vinylpyridine and ethylenedimethacrylate as a host functional monomer and cross-linker, respectively. The imprinted polymer material could separate naproxen enantiomers with separation factor of 1.74. Further, the materials showed high selectivity for naproxen and moderate selectivity for other 2-arylpropionic acid derivatives. On the other hand, the imprinted polymer material showed little selectivity for other acidic, basic and neutral compounds.

The molecular imprinting technique has considerable potential in the area of separations. In this technique, the functional monomers, which allow interactions with the functional groups of an imprint molecule, are polymerized in the presence of the imprint molecule. The obtained polymer can afford specific recognition toward the imprint molecule. Template molecules so far employed for imprinted polymers have included sugars, amino acids, peptides, proteins, nucleosides, nucleotides, drugs and pesticides.[1] Usually, non-aqueous bulk polymerization techniques[2] are utilized to obtain molecularly imprinted polymers, which are followed by crashing, grinding and sieving of the block polymer to produce the packing material.[3] Though suspension polymerization methods, which require an aqueous suspension medium, can produce spherical polymer beads, water is thought to weaken the interaction between the imprint molecule and functional monomers.[4] Recently, we prepared molecularly imprinted, uniform-size, polymer-based stationary phases for isomers of diaminonaphthalene or a chiral amide derived from (S)-α-methylbenzylamine,[5,6] where a typical two-step swelling and polymerization method[7] with water as the suspension medium was used. However, the prepared stationary phase showed an equivalent molecular recognition to that with the previously reported, continuous, rod-type, polymeric separation medium prepared by a kind of non-aqueous bulk polymerization methods.[8]

Previously, Kempe and Mosbach[9] prepared a molecularly imprinted polymer stationary phase for (S)-naproxen, 2-arylpropionic acid non-steroidal anti-inflammatory drug, through bulk polymerization, and evaluated its chiral recognition ability using non-aqueous mobile phases. In this study, we prepared a molecularly imprinted uniform-sized polymer-based stationary phase for (S)-naproxen, and evaluated it by using aqueous-rich mobile phases. Usually, evaluation of the imprinted polymer-based stationary phase was carried out by using non-aqueous mobile phases. Since our goal is to develop a molecularly imprinted polymer for simultaneous trapping of a drug and its metabolites in biological fluids, we evaluated the obtained polymer by using aqueous-rich mobile phases.

Molecularly imprinted, uniform-sized stationary phases were prepared through a typical two-step swelling and polymerization method from polystyrene seed polymer prepared using an emulsifier-free emulsion polymerization as reported previously.[5] 4-Vinylpyridine and ethylenedimethacrylate were used as a host functional monomer and cross-linker, respectively. Polymerization was carried out at 0 °C for 24 h using benzoyl peroxide-dimethylaniline as a redox initiator under argon atmosphere with slow stirring. The obtained polymer materials were washed with methanol and tetrahydrofurane to remove the template molecule, and were packed into a stainless-steel column (4.6 mm I. D. X 10 cm) using a slurry packing procedure. For comparison, base polymer materials were prepared without the template molecule, (S)-naproxen.

Table 1. Effect of eluent pH on separation of naproxen enantiomers[a]

Eluent pH	Template			
	None		(S)-Naproxen	
	k'	α	k'	α
3.0	5.97	1.00	21.2	1.70
4.0	6.32	1.00	23.0	1.74
4.8	6.10	1.00	21.2	1.62
6.0	4.76	1.00	13.2	1.51
6.9	2.44	1.00	3.53	1.00

[a]HPLC conditions: mobile phase, 20 mmol dm^{-3} phosphate buffer/CH_3CN = 50/50 (v/v); detection, 254 nm; flow rate, 1.0 ml/min.

Table 1 shows effect of eluent pH on the separation of naproxen enantiomers, where k' is the capacity factor of (S)-naproxen, and α is the separation factor of racemic naproxen. The base polymer materials had no chiral recognition ability toward naproxen, while the imprinted polymer materials showed enantioselectivity for naproxen. No resolution of 2-arylpropionic acid derivative enantiomers tested were observed on the imprinted polymer material. The highest retentivity and enantioselectivity were obtained with eluent pH 4.0. This suggests that electrostatic and hydrophobic interactions could play an important role in retentivity and enantioselectivity of naproxen enantiomers.[10] The imprinted polymer prepared by us gave higher enantioselectivity for naproxen than that prepared by Kempe and Mosbach.[9]

As shown in Table 2, the imprinted polymer materials could retain acidic compounds, (S)-naproxen and benzoic acid, by electrostatic and hydrophobic interactions, and a neutral compound, benzoin, by hydrophobic interaction, while a basic compound, propranolol, was not retained because of ionic repulsion.

Selectivity of the molecularly imprinted polymer toward other 2-arylpropionic acid derivatives, and acidic and neutral compounds was examined. Figure 1, parts A and B, shows the capacity factors of 2-arylpropionic acid derivatives, and acidic and neutral compounds on the imprinted and base polymer materials, and the

ratio of the capacity factors, selectivity (= k'(imprinted)/k'(base)), respectively. Selectivity for (S)-naproxen is 3.64, and those for other 2-arylpropionic acid derivatives are 1.37 - 1.48. High retentivity of flurbiprofen on the base material might be due to the high hydrophobicity of flurbiprofen. On the other hand, those for acidic compounds, aspirin and benzoic acid, were 1.27 and 1.17, respectively, while those for neutral compounds, benzoin and benzophenone, were 1.11. These results revealed that the imprinted polymer materials showed high selectivity for naproxen and moderate selectivity for other 2-arylpropionic acid derivatives. On the other hand, the imprinted polymer material showed little selectivity for other acidic, basic and neutral compounds.

Table 2. Effect of eluent pH on retentions of acidic, neutral and basic compounds on the imprinted polymer material[a]

Compound	Capacity factor				
	pH 3.0	pH 4.0	pH 4.8	pH 6.0	pH 6.9
(S)-Naproxen	21.2	23.0	21.2	13.2	3.53
Benzoic acid	3.41	3.59	3.45	2.14	0.97
Benzoin	3.04	3.27	3.34	3.37	3.14
Propranolol	-[b]	-	-	-	-

[a]HPLC conditions as in Table 1. [b]Not retained.

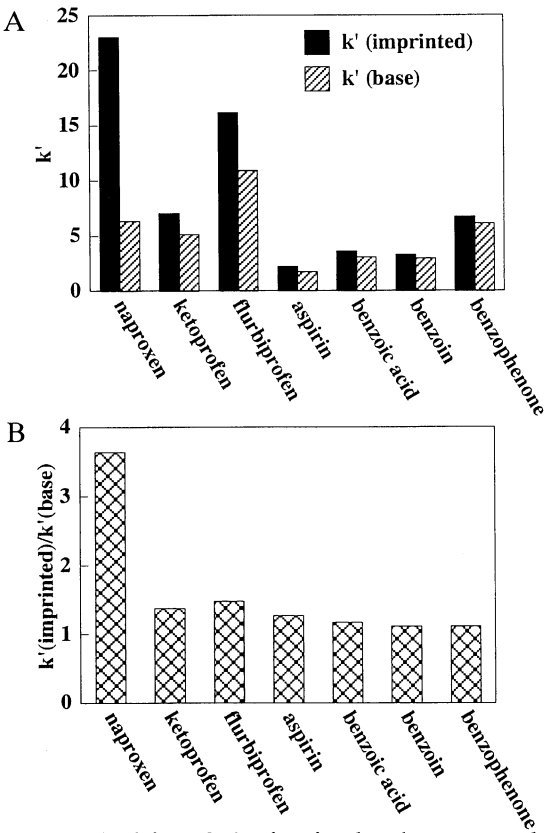

Figure 1. Selectivity of the imprinted polymer toward 2-arylpropionic acid derivatives, and acidic and neutral compounds. HPLC conditions: mobile phase, 20 mmol dm^{-3} phosphate buffer (pH 4.0)/CH_3CN = 50/50 (v/v); detection, 254 nm; flow rate, 1.0 ml/min; loaded amount, 0.5 μg.

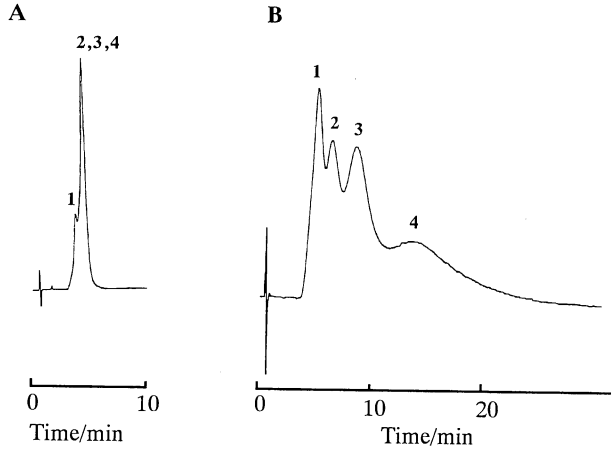

Figure 2. Separation of 2-arylpropionic acid derivatives on the base (A) and molecularly imprinted (B) polymer materials. HPLC conditions as in Figure 1. Keys: 1, ketoprofen; 2, ibuprofen; 3, (R)-naproxen; 4, (S)-naproxen.

Figure 2 shows the separation of ketoprofen, ibuprofen and (R)- and (S)-naproxen ón the base and imprinted polymer materials. The imprinted polymer material could separate naproxen enantiomers and other 2-arylpropionic acid derivatives.

The results obtained above indicate that the imprinted polymer prepared shows high selectivity for naproxen and moderate selectivity for other 2-arylpropionic acid derivatives. This means that an imprinted polymer showing selectivity for a series of compounds such as a drug and its metabolites could be prepared. The detailed study is now under progress in our laboratory.

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References

1 G. Wulff, *Angew. Chem., Int. Ed. Engl.*, **34**, 1812 (1995) and references cited therein.
2 G. Wulff and A. Sarhan, *Angew. Chem.*, **84**, 364 (1972).
3 G. Wulff, A. Sarhan, and K. Zabrocki, *Tetrahedron Lett.*, **14**, 4329 (1973).
4 B. Sellergren, *J. Chromatogr. A*, **673**, 133 (1994).
5 K. Hosoya, K. Yoshizako, N. Tanaka, K. Kimata, T. Araki, and J. Haginaka, *Chem. Lett.*, **1994**, 1437.
6 K. Hosoya, K. Yoshizako, Y. Shirasu, K. Kimata, T. Araki, N. Tanaka, and J. Haginaka, *J. Chromatogr. A*, **728**, 139 (1996).
7 K. Hosoya and J. M. J. Fréchet, *J. Polym. Sci., Part A, Polym. Chem.*, **31**, 2129 (1993).
8 J. Matsui, T. Kato, T. Takeuchi, M. Suzuki, K. Yokoyama, E. Tamiya, and I. Karube, *Anal. Chem.*, **65**, 2223 (1993).
9 M. Kempe and K. Mosbach, *J. Chromatogr. A*, **664**, 276 (1994).
10 J. Haginaka, C. Seyama, H. Yasuda, H. Fujima, and H. Wada, *J. Chromatogr.*, **592**, 301 (1992).