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Molecularly imprinted uniform-sized polymer-based stationary phase for naproxen
Comparison of molecular recognition ability of the molecularly imprinted polymers prepared by thermal and redox polymerization techniques

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Abstract

A molecularly imprinted uniform-sized polymer-based stationary phase for (*S*)-naproxen has been prepared by a multi-step swelling and thermal or redox polymerization method using 4-vinylpyridine and ethylenedimethacrylate as a host functional monomer and cross-linker, respectively. The obtained molecularly imprinted polymers were evaluated using aqueous-rich mobile phases. The molecularly imprinted polymer materials could separate naproxen enantiomers. Further, the materials showed high selectivity for naproxen and moderate selectivity for other 2-arylpropionic acid derivatives. On the other hand, the molecularly imprinted polymer materials showed little selectivity for other acidic compounds, and basic and neutral compounds. With regard to comparison of the molecularly imprinted polymers prepared by thermal and redox polymerization techniques, the latter materials gave higher retentivity and enantioselectivity than the former materials, while the former materials gave higher column efficiency than the latter materials. Thus, naproxen enantiomers were resolved much better on the molecularly imprinted polymer material prepared by a thermal polymerization method.

Keywords: Stationary phases, LC; Enantiomer separation; Molecularly imprinted polymer; Naproxen

1. Introduction

Molecularly imprinted polymers have considerable molecular recognition ability, and can be used as separation media, immunoassay-like probes and sensing devices [1]. In the molecular imprinting technique, the functional monomers, which allow interactions with the functional groups of a target molecule, are polymerized in the presence of the target molecule. The obtained polymer can afford specific recognition toward the imprint molecule. The molecularly imprinted polymers prepared thus far have been for sugars, amino acids, peptides, proteins, nucleosides, nucleotides, drugs and pesticides [1]. Usually, non-aqueous bulk polymerization techniques [2] are utilized to obtain molecularly

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imprinted polymers, which are followed by smashing, sieving and classifying of the block polymer to produce the packing material [3]. Although 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-sized 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 ability to that of continuous rod-type polymers prepared by non-aqueous bulk polymerization methods [8].

Previously, Kempe and Mosbach [9] prepared a molecularly imprinted polymer stationary phase for (*S*)-naproxen, a 2-arylpropionic acid non-steroidal anti-inflammatory drug, through bulk polymerization, and evaluated its chiral recognition ability using non-aqueous mobile phases. In a previous communication [10], we prepared a molecularly imprinted uniform-sized polymer-based stationary phase for (*S*)-naproxen by a multi-step swelling and redox polymerization method with water as the suspension medium. The molecularly imprinted polymer prepared by the present authors gave higher enantioselectivity for naproxen than that prepared by Kempe and Mosbach [9]. In this study, we prepared molecularly imprinted uniform-sized polymer-based stationary phases for (*S*)-naproxen by a multi-step swelling and thermal or redox polymerization method, and compared the molecular recognition ability of the molecularly imprinted polymers prepared by both methods. Although evaluation of the molecularly imprinted polymer-based stationary phase was usually carried out using non-aqueous mobile phases, we evaluated the obtained polymer using aqueous-rich mobile phases.

2. Experimental

2.1. Materials

Ethylene dimethacrylate (EDMA) and 4-vinylpyridine were purchased from Tokyo Chemical Industry (Tokyo, Japan) and Wako Pure Chemical Industry (Osaka, Japan), respectively. Both monomers were purified by general distillation techniques in vacuo to remove the polymerization inhibitor. Benzoyl peroxide and 2,2′-azobis(2,4-dimethylvaleronitrile) were purchased from Nacalai Tesque (Kyoto, Japan) and Wako Pure Chemical Industry (Tokyo, Japan), respectively, and used without further purification. (*S*)-(+)-Naproxen and racemic naproxen were purchased from Tokyo Chemical Industry. (*S*)-(+)-Ibuprofen and racemic ibuprofen were purchased from Aldrich Chemical (Milwaukee, WI, USA). Racemic flurbiprofen and ibuprofen, and (*S*)-(+)-flurbiprofen were donated by Kaken Pharmaceutical Co. (Tokyo, Japan). Racemic ketoprofen and pranoprofen were donated by Chugai Pharmaceutical Co. (Tokyo, Japan) and Yoshitomi Pharmaceutical Co. (Osaka, Japan), respectively. The structures of the 2-arylpropionic acid derivatives used in this study are illustrated in Fig. 1. Racemic hexobarbital was donated by Teikoku Chemical Industry

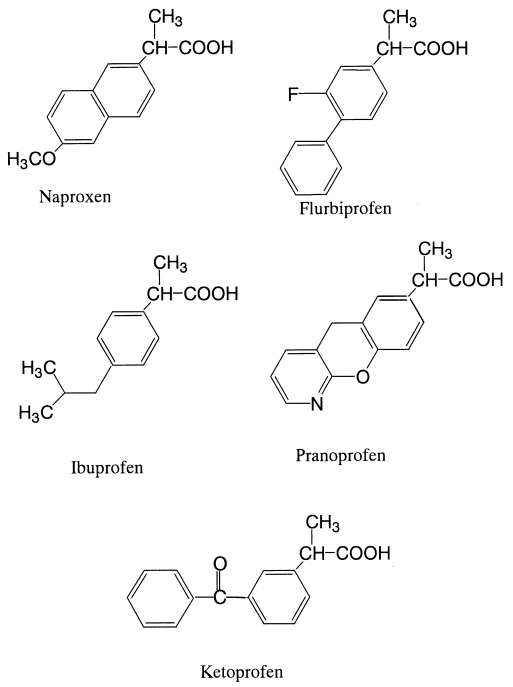

Fig. 1. Structures of 2-arylpropionic acid derivatives used in this study.

(Osaka, Japan). Other reagents and solvents were used without further purification.

Water purified with a Nanopure II unit (Barnstead, Boston, MA, USA) was used for the preparation of the eluent and the sample solution.

2.2. Multi-step swelling and polymerization method

Uniform-sized polystyrene seed particles utilized as the shape template were prepared by emulsifier-free emulsion polymerization and purified by a method reported previously [11]. The size of the seed particle was ca. 1 μm in diameter. The preparation of uniform-sized, macroporous, molecularly imprinted polymer beads as well as non-imprinted polymer beads by a multi-step swelling and polymerization method was carried out as follows. For redox polymerization, a water dispersion of the uniform-sized polystyrene seed particles (0.107 g/ml), 0.83 ml, was admixed with a micro-emulsion prepared from 0.48 ml dibutyl phthalate as activating solvent [12], 0.02 g of sodium dodecyl sulphate and 5 ml of distilled water by sonication. This first-step swelling was carried out at room temperature for 15 h with stirring at 125 rpm until micro oil droplets completely disappeared. To the swollen particles was added a micro-emulsion prepared from 0.335 g benzoyl peroxide, 5 ml toluene as porogenic solvent, 12.5 ml water and 10 ml 4.8% polyvinylalcohol (dp=500, saponification value 86.5–89 mol.%) solution as a dispersion stabilizer. This second-step swelling was carried out at room temperature for 2 h with stirring at 125 rpm. To the dispersion of swollen particles was added a dispersion of 4.5 ml EDMA, 0.63 g 4-vinylpyridine as a host molecule, 12.5 ml water and 10 ml 4.8% polyvinylalcohol solution. This third-step swelling was carried out at room temperature for 2 h with stirring at 125 rpm. When the template molecule was added, 0.46 g (*S*)-naproxen was admixed with the monomers utilized to prepare the dispersion for the third-step swelling. After the third-step swelling was completed, the polymerization procedure was started at 0°C by addition of a micro-emulsion of 0.1 ml *N*,*N*-dimethylaniline, 0.5 ml EDMA, 0.02 g sodium dodecyl sulfate and 5 ml distilled water under argon atmosphere with slow stirring for 24 h. The dispersion of polymerized beads was poured into 250 ml water to remove the suspension stabilizer (polyvinylalcohol), and the supernatant was discarded after sedimentation of the beads. The polymer beads were redispersed into methanol, and the supernatant was again discarded after sedimentation. This procedure was repeated three times in methanol and twice in tetrahydrofuran (THF), then the polymer beads were filtered on a membrane filter and washed with THF and acetone followed by drying at room temperature.

For thermal polymerization, the first-step swelling was carried out in the same manner as the redox polymerization method. To the swollen particles was added a micro-emulsion prepared from 0.375 g 2,2′-azobis(2,4-dimethylvaleronitrile), 5 ml toluene, 12.5 ml water and 10 ml 4.8% polyvinylalcohol solution. This second-step swelling was carried out at room temperature for 2 h with stirring at 125 rpm. To the dispersion of swollen particles was added a dispersion of 5 ml EDMA, 0.63 g 4-vinylpyridine, 0.02 g sodium dodecyl sulphate, 12.5 ml water and 10 ml 4.8% polyvinylalcohol solution. This third-step swelling was carried out at room temperature for 2 h with stirring at 125 rpm. When the template molecule was added, 0.46 g (*S*)-naproxen was admixed with the monomers utilized to prepare the dispersion for the third-step swelling. After the third-step swelling was completed, the polymerization procedure was started at 50°C under argon atmosphere with slow stirring for 24 h. The obtained polymerized beads were treated in the same manner as described above.

The prepared beads were packed into a stainless-steel column (100 mm×4.6 mm I.D.) by a slurry technique using methanol as the slurry medium to evaluate their chromatographic characteristics.

2.3. Chromatography

The HPLC system used was composed of an LC-9A pump, an SPD-6A spectrophotometer, a Rheodyne 7125 injector with a 20-μl loop and a C-R6A integrator (all from Shimadzu, Kyoto, Japan). The flow-rate was maintained at 1.0 ml/min. Detection was performed at 220 or 254 nm. Retention factors were calculated from the equation $k' = (t_R - t_0)/t_0$, where t_R and t_0 are the retention times of the retained and unretained solutes, respectively. The retention time of unretained solute, t_0, was measured

by injecting a solution whose organic modifier content was slightly different from that of the eluent used. The enantioseparation factor is calculated from the equation $\alpha = k_2'/k_1'$, where k_1' and k_2' are the retention factors of the first and second eluted enantiomers, respectively. Resolution is calculated from the equation $R_s = 2(t_{R2} - t_{R1})/(w_1 + w_2)$, where t_{R1} and t_{R2} are the retention times of the first and second eluted enantiomers, respectively, and w_1 and w_2 are the baseline peak widths of the first and second eluted enantiomers, respectively. The number of theoretical plates (N) was calculated by the equation $N = 16(t_R/w)^2$. All separations were carried out at 25°C using a water bath (Thermo Minder Lt-100, Taitec Co., Saitama, Japan). The eluents were prepared using phosphoric acid, sodium dihydrogenphosphate, disodium hydrogenphosphate and acetonitrile. The eluent used is specified in the legends of tables and figures.

3. Results and discussion

3.1. Retention properties of acidic, neutral and basic compounds on the molecularly imprinted polymers

Table 1 shows the effects of eluent pH on the retention properties of (*S*)-naproxen, benzoin and propranolol on the molecularly imprinted uniform-sized polymer-based stationary phases for (*S*)-naproxen prepared by a multi-step swelling and thermal or redox polymerization technique with water as suspension medium. Ethylene dimethacrylate and 4-vinylpyridine were used as cross-linker and host monomers, respectively. As shown in Table 1, the retention of a neutral compound, benzoin, was not affected by pH, suggesting it was retained by hydrophobic interactions with the polyethyleneglycol backbones. The retention factors of an acidic compound, (*S*)-naproxen, gave a maximum value at an eluent pH of ca. 4. Taking into account the pK_a value of (*S*)-naproxen (4.2), this indicates that, at this pH, dissociated (*S*)-naproxen was retained by both hydrophobic and electrostatic interactions. The retention factor of undissociated (*S*)-naproxen could be decreased by decreasing the eluent pH because of lack of electrostatic interactions. On the other hand, the decrease in the retention factor of (*S*)-naproxen by increasing the eluent pH is attributable to ion exclusion. These results reveal that hydrophobic and electrostatic interactions play an important role in the retention of (*S*)-naproxen. Thus, the molecularly imprinted polymer materials could retain an acidic compound, (*S*)-naproxen, by electrostatic and hydrophobic interactions, and a neutral compound, benzoin, by hydrophobic interactions, while a basic compound, propranolol, was not retained because of ionic repulsion and/or ion exclusion. Similar results were obtained with other acidic, neutral and basic compounds.

With regard to comparison of the retention properties of acidic, neutral and basic solutes on the

Table 1
Effect of eluent pH on retention factors of (*S*)-naproxen, benzoin and propranolol on the molecularly imprinted polymers by thermal and redox polymerization methods[a]

Solute	Retention factor				
	pH 2.4	pH 4.0	pH 4.8	pH 6.0	pH 7.0
Thermal polymerization					
(*S*)-Naproxen	14.8	15.2	15.2	9.30	2.84
Benzoin	2.36	2.54	2.63	2.62	2.55
Propranolol	–[b]	–	–	–	–
Redox polymerization					
(*S*)-Naproxen	21.2	23.0	21.2	13.2	3.53
Benzoin	3.04	3.27	3.34	3.37	3.14
Propranolol	–	–	–	–	–

[a]HPLC conditions: column, 100 mm×4.6 mm I.D.; eluent, 20 m*M* phosphate buffer–CH_3CN (50:50, v/v); flow-rate, 1.0 ml/min.
[b]Not retained.

molecularly imprinted polymer materials prepared by thermal and redox polymerization techniques, all solutes tested were less retained on the former than the latter materials. However, similar retention properties were observed between the two molecularly imprinted polymer materials.

3.2. Separation of naproxen enantiomers on the molecularly imprinted polymer

Table 2 shows the effect of eluent pH on the separation of naproxen enantiomers on the molecularly imprinted polymers for (*S*)-naproxen prepared by thermal and redox polymerization methods. For comparison, non-imprinted polymer materials were prepared without the template molecule, (*S*)-naproxen. The non-imprinted polymer materials had no chiral recognition ability toward naproxen, while the molecularly imprinted polymer materials showed enantioselectivity for naproxen. The highest retention was obtained with eluent pH 4.0, and higher enantioselectivity was obtained at lower eluent pH. As described above, hydrophobic and electrostatic interactions take place between acidic solutes and molecularly imprinted polymer materials. Table 3 shows the effects of acetonitrile content on the separation of naproxen enantiomers on the molecularly imprinted polymers. With an increase in the acetonitrile content, retentivity and enantioselectivity of naproxen enantiomers decreased. This result suggests that hydrophobic interactions play an important role in the retention and enantioselectivity of naproxen enantiomers. The results obtained above suggest that hydrophobic and electrostatic interactions could play an important role in the retention and enantioselectivity of naproxen enantiomers on molecularly imprinted polymers [13].

Table 3
Effect of acetonitrile content on separation of naproxen enantiomers on the non-imprinted and imprinted polymers prepared by thermal and redox polymerization methods[a]

Template	Acetonitrile content (%)					
	40		50		60	
	k'	α	k'	α	k'	α
Thermal polymerization						
None	11.2	1.00	4.46	1.00	2.16	1.00
(*S*)-Naproxen	37.3	1.57	15.2	1.57	6.79	1.49
Redox polymerization						
None	15.3	1.00	6.32	1.00	2.86	1.00
(*S*)-Naproxen	54.7	1.58	23.0	1.74	10.0	1.61

[a]HPLC conditions: column, 100 mm×4.6 mm I.D.; eluent, a mixture of 20 m*M* phosphate buffer (pH 3.2) and CH_3CN; flow-rate, 1.0 ml/min.

With regard to comparison of the separation of naproxen enantiomers on the molecularly imprinted polymers prepared by thermal and redox polymerization methods, the molecularly imprinted polymer prepared by a redox polymerization method gave a higher enantioseparation factor than that prepared by a thermal polymerization method, as shown in Table 2. However, the latter material gave a higher resolution than the former. In addition, the numbers of theoretical plates of the molecularly imprinted poly-

Table 2
Effect of eluent pH on separation of naproxen enantiomers on the non-imprinted and molecularly imprinted polymers prepared by thermal and redox polymerization methods[a]

Template	Eluent pH														
	pH 2.4			pH 4.0			pH 4.8			pH 6.0			pH 7.0		
	k'	α	*Rs*	k'	α	*Rs*	k'	α	*Rs*	k'	α	*Rs*	k'	α	*Rs*
Thermal polymerization															
None	4.34	1.00	–[b]	4.46	1.00	–	4.52	1.00	–	3.44	1.00	–	1.15	1.00	–
(*S*)-Naproxen	14.8	1.60	0.94	15.2	1.57	0.84	15.2	1.58	0.80	9.30	1.42	0.58	2.84	1.00	–
Redox polymerization															
None	5.97	1.00	–	6.32	1.00	–	6.10	1.00	–	4.76	1.00	–	2.44	1.00	–
(*S*)-Naproxen	21.2	1.70	0.56	23.0	1.74	0.44	21.2	1.62	0.48	13.2	1.51	0.43	3.53	1.00	–

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

mer prepared by a thermal polymerization method are 108 and 36 for (*R*)- and (*S*)-naproxen, respectively, at eluent pH 4.0, while those of the molecularly imprinted polymer prepared by a redox polymerization method are 34 and 18, respectively. The redox polymerization is performed at 0°C, while the thermal polymerization is at 50°C. It is expected that the polymerization at lower temperature produces a large molecular imprinting effect [14]. In a previous paper [15], we reported that polymerization techniques at different polymerization temperatures affects the physical and chromatographic properties of molecularly imprinted polymer materials. However, we do not know why the molecularly imprinted polymer for (*S*)-naproxen prepared by a thermal polymerization method gives a higher column efficiency than that prepared by a redox polymerization method.

3.3. Selectivity of the molecularly imprinted polymer

The selectivities of the molecularly imprinted polymer toward 2-arylpropionic acid derivatives and acidic and neutral compounds were examined. Fig. 2A,B show the retention factors of 2-arylpropionic acid derivatives on the non-imprinted and molecularly imprinted polymer materials prepared by the thermal polymerization method, and the ratio of the retention factors, selectivity [k'(imprinted)/k'(non-imprinted)], respectively. Selectivity for (*S*)-naproxen is 3.40, and those for other 2-arylpropionic acid derivatives, flurbiprofen, ibuprofen, pranoprofen and ketoprofen, are 1.80, 1.65, 1.60, and 1.48, respectively. The molecularly imprinted polymer gave the highest selectivity for (*S*)-naproxen. Separation of naproxen enantiomers was attained, as described above. Further, flurbiprofen and ibuprofen, which gave higher selectivity, showed partial resolution of those enantiomers with enantioselectivity of 1.17 and 1.09, respectively. The elution order of flurbiprofen and ibuprofen enantiomers was (*R*)/(*S*). On the other hand, no chiral resolution of pranoprofen and ketoprofen, which gave lower selectivity, was observed. Fig. 3A,B shows the retention factors and selectivities of 2-arylpropionic acid derivatives on the non-imprinted and molecularly imprinted polymer materials, respectively, prepared by the redox polymerization method. Higher retentivity and selectivity of naproxen were obtained with the molecularly imprinted polymer materials prepared by the redox polymerization method than that prepared by the thermal polymerization method. Higher retentivity of other 2-arylpropionic acid derivatives was obtained with the former material, while selectivities for other 2-arylpropionic acid were slightly different between the molecularly imprinted materials prepared by both polymerization techniques. In addition, no resolution of other 2-arylpropionic acid derivatives' enantio-

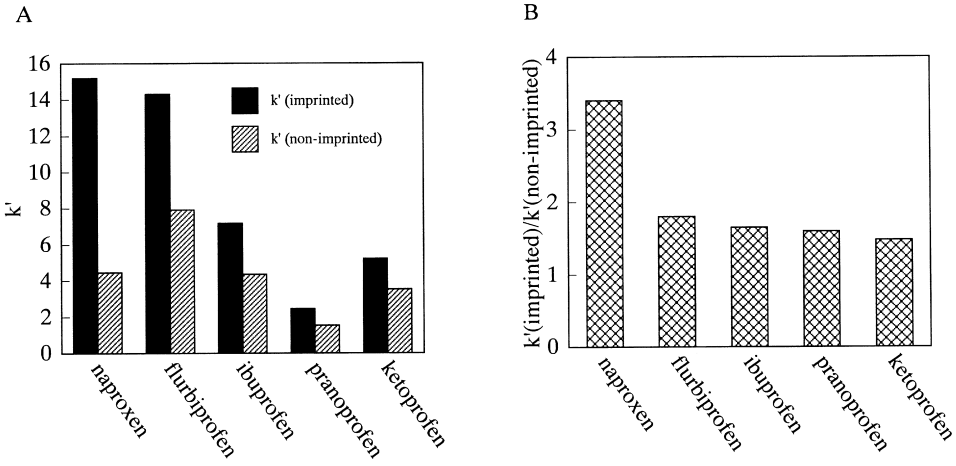

Fig. 2. Selectivity of the molecularly imprinted polymer prepared by thermal polymerization toward 2-arylpropionic acid derivatives. HPLC conditions: mobile phase, 20 m*M* phosphate buffer (pH 3.2)–CH_3CN (50:50, v/v); flow-rate, 1.0 ml/min; loaded amount, 0.5 μg.

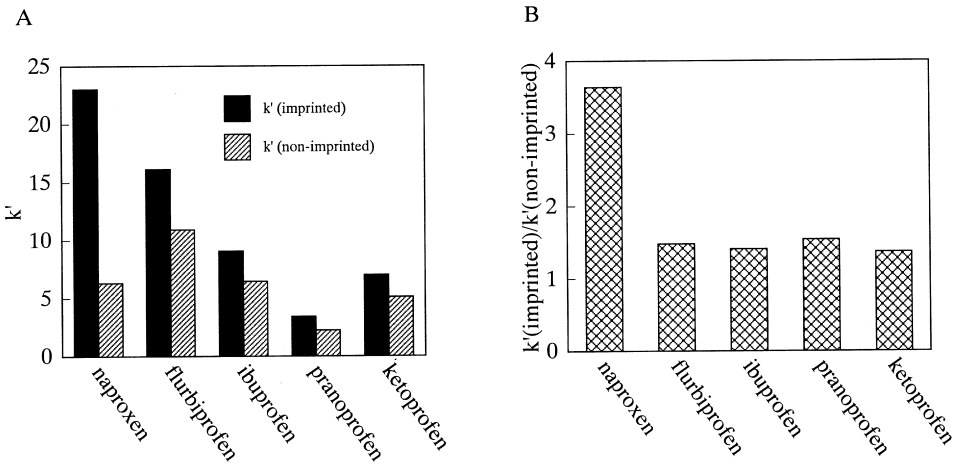

Fig. 3. Selectivity of the molecularly imprinted polymer prepared by redox polymerization toward 2-arylpropionic acid derivatives. HPLC conditions as in Fig. 2.

mers was observed with the molecularly imprinted polymer prepared by the redox polymerization method.

Selectivities for acidic compounds, aspirin and benzoic acid, were 1.34 and 1.13, respectively, on the molecularly imprinted polymer materials prepared by the thermal polymerization method. Fig. 4 shows the retention factors and selectivities of neutral compounds on the molecularly imprinted and non-imprinted polymer materials prepared by the thermal polymerization method. Selectivities for neutral compounds, hexobarbital, benzoin, acetophenone and benzophenone, were 1.51, 1.18, 1.15 and 1.13, respectively. Similar results were obtained with the molecularly imprinted polymer for naproxen prepared by the redox polymerization method.

These results reveal that the molecularly imprinted polymer materials showed high selectivity for naproxen and moderate selectivity for other 2-

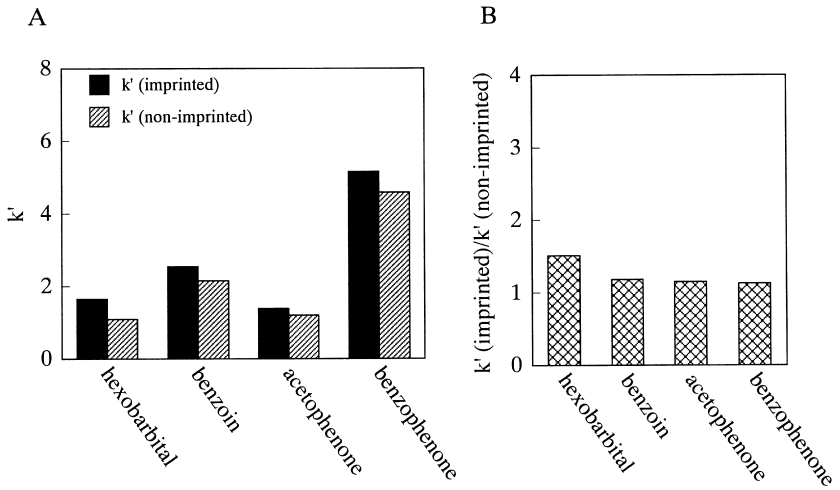

Fig. 4. Selectivity of the molecularly imprinted polymer prepared by thermal polymerization toward neutral compounds. HPLC conditions as Fig. 2.

arylpropionic acid derivatives. On the other hand, the molecularly imprinted material showed little selectivity for other acidic compounds, and basic and neutral compounds.

3.4. Separation of naproxen enantiomers and other 2-arylpropionic acid derivatives on the molecularly imprinted polymer

Fig. 5A,B show the separation of ketoprofen, ibuprofen and (*R*)- and (*S*)-naproxen on the non-imprinted and molecularly imprinted polymer materials, respectively, prepared by the thermal polymerization method. Fig. 5C,D show the separation on the non-imprinted and molecularly imprinted polymer materials, respectively, prepared by the redox polymerization method. The molecularly imprinted polymer materials prepared by both polymerization techniques could separate naproxen enantiomers and other 2-arylpropionic acid derivatives. Since the molecularly imprinted polymers prepared by the thermal polymerization method gave higher column efficiency than that prepared by the redox polymerization method, naproxen enantiomers were resolved much better on the former than the latter material. With regard to separation of ketoprofen and ibuprofen, it is interesting to compare the materials prepared by the thermal and redox polymerization methods. Ketoprofen and ibuprofen were completely resolved on the non-imprinted polymer prepared by the thermal polymerization method, while their separation was incomplete on that prepared by the redox polymerization method. The molecularly imprinted polymer materials prepared by both polymerization techniques gave moderate selectivity for ketoprofen and ibuprofen. Thus, separation of ketoprofen and ibuprofen was complete on the molecularly imprinted polymers prepared by the thermal polymerization method, and incomplete on that prepared by the redox polymerization method.

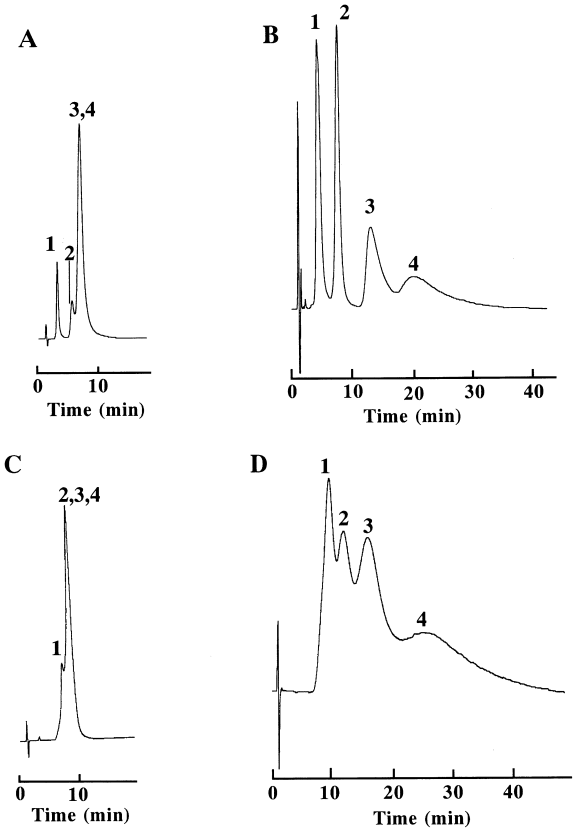

Fig. 5. Separation of 2-arylpropionic acid derivatives on the non-imprinted and molecularly imprinted polymer materials prepared by thermal and redox polymerization methods. (A) Non-imprinted polymer materials prepared by thermal polymerization; (B) molecularly imprinted polymer materials prepared by thermal polymerization; (C) non-imprinted polymer materials prepared by redox polymerization; (D) molecularly imprinted polymer materials prepared by redox polymerization. Key: (1) racemic ketoprofen; (2) racemic ibuprofen; (3) (*R*)-naproxen; (4) (*S*)-naproxen. HPLC conditions as in Fig. 2.

In conclusion, the results obtained above indicate that the prepared molecularly imprinted polymer shows high selectivity for naproxen and moderate selectivity for other 2-arylpropionic acid derivatives. This means that a molecularly imprinted polymer showing selectivity for a series of compounds such as a drug and its metabolites could be prepared.

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