Uniformly sized molecularly imprinted polymer for (*S*)-naproxen Retention and molecular recognition properties in aqueous mobile phase

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Abstract

A uniformly sized molecularly imprinted polymer (MIP) for (*S*)-naproxen has been prepared by a multi-step swelling and polymerization method using 4-vinylpyridine (4-VPY) and ethylene glycol dimethacrylate (EDMA) as a functional monomer and cross-linker, respectively. We optimized the preparation method of the MIP by changing the molar amounts of the template molecule and functional monomer. Further, we examined the effects of organic modifier type, column temperature and flow-rate on the retentivity and enantioselectivity for naproxen using a mixture of phosphate buffer and organic modifier (acetonitrile, ethanol and 2-propanol) as an eluent. When the amounts of (*S*)-naproxen, 4-VPY and EDMA used were 4, 6 and 25 mmol, respectively, the enantioselectivity and resolution for naproxen were good despite the shorter retention. When acetonitrile was used as an organic modifier, the highest column efficiency was obtained for the separation of naproxen enantiomers. With regard to the effects of column temperature and flow-rate, the column performance was improved by elevating a column temperature and decreasing a flow-rate. © 2001 Elsevier Science B.V. All rights reserved.

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1. Introduction

Molecularly imprinted polymers (MIPs), which give complementary binding site(s) for the template molecule, can be utilized for specific recognition of the target molecule when used as chromatographic media, sensors and artificial antibodies [1–4]. Generally, MIPs were prepared by a bulk polymerization method [5]. The disadvantage of the method is that the block polymers obtained had to be crushed, ground and sieved to produce packing materials. Recently, we prepared uniformly sized MIPs for (*S*)-naproxen [6,7], -ibuprofen [8] and -propranolol [9,10], where a typical multi-step swelling and polymerization method [11] was used, and evaluated these MIPs using a mixture of phosphate buffer and acetonitrile as an eluent. Our MIP for (*S*)-naproxen gave an enantioselectivity for naproxen similar to that prepared with non-aqueous bulk polymerization techniques by Kempe and Mosbach [12]. Further, we have prepared a RAM (restricted access media)-MIP material, a uniformly sized MIP for (*S*)-naproxen selectively modified with a hydrophilic external layer, through a combination of molecular imprinting and hydrophilic surface modification techniques [13].

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We showed the applicability of the RAM-MIP material for direct serum injection assay of (*S*)-naproxen.

In this study, we optimized the preparation of a uniformly sized MIP for (*S*)-naproxen using 4-vinylpyridine (4-VPY) and ethylene glycol dimethacrylate (EDMA) as the functional monomer and cross-linker, respectively, by a multi-step swelling and polymerization method. In addition, we examined the effects of the type of organic modifier, column temperature and flow-rate on the retentivity and enantioselectivity for naproxen enantiomers using a mixture of phosphate buffer and organic modifier (acetonitrile, ethanol or 2-propanol) as an eluent.

2. Experimental

2.1. Materials

4-VPY and 2-vinylpyridine (2-VPY) were purchased from Wako (Osaka, Japan), and 1-vinylimidazole (1-VI) and EDMA were from Tokyo Chemical (Tokyo, Japan). These monomers were purified by vacuum distillation to remove the polymerization inhibitor. 2,2′-Azobis(2,4-dimethylvaleronitrile)(V-65) was purchased from Wako, and used without further purification. (*S*)-(+)-Naproxen, racemic naproxen and 2-phenylpropionic acid were purchased from Tokyo Chemical. (*S*)-(+)-Ibuprofen and racemic ibuprofen were purchased from Sigma–Aldrich Japan (Tokyo, Japan). (*S*)-(+)-Flurbiprofen and racemic flurbiprofen were donated by Kaken Pharmaceutical (Tokyo, Japan). Racemic ketoprofen, pranoprofen and fenoprofen were donated by Chugai Pharmaceutical (Tokyo, Japan) and Yoshitomi Pharmaceutical (Osaka, Japan), Yamanouchi Pharmaceutical (Tokyo, Japan), respectively. 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

Uniformly sized, polystyrene seed particles utilized as shape template were prepared by emulsifier-free emulsion polymerization and purified by a previously reported method [14]. The size of the seed particles was ca. 1 μm in diameter. Preparation of the uniformly sized, macroporous MIPs as well as the non-imprinted polymers by a multi-step swelling and polymerization method was carried out as follows. A water dispersion of the uniformly sized, polystyrene seed particles (0.107 g/ml), 0.83 ml, was admixed with a micro-emulsion prepared from 0.48 ml of dibutyl phthalate as activating solvent [15], 0.02 g of sodium dodecyl sulphate and 10 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 the micro oil droplets completely disappeared. To the swollen particles, a micro-emulsion prepared from 0.375 g of 2,2′-azobis(2,4-dimethylvaleronitrile) as initiator, 5 ml of toluene as porogenic solvent, 12.5 ml of water and 10 ml of 4.8% poly(vinyl alcohol) (degree of polymerization=500, saponification value=86.5–89 mol%) solution as a dispersion stabilizer was added. This second-step swelling was carried out at room temperature, for 2 h, with stirring at 125 rpm. A dispersion of 12.5 ml of water and 10 ml of 4.8% poly(vinyl alcohol) solution including (*S*)-naproxen, 4-VPY and EDMA as shown in Table 1, was added to the dispersion of swollen particles. Similarly, non-imprinted polymers were prepared without addition of the template molecule. This third-step swelling was carried out at room temperature, for 2 h, with stirring at 125 rpm. 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 dispersion of polymerized materials was poured into 250 ml of water to remove the suspension stabilizer [poly(vinyl alcohol)], and

Table 1
Molar amounts of template, functional monomer and cross-linker used for the preparation of MIPs for (*S*)-naproxen

MIP	Amount (mmol)		
	Template	4-VPY	EDMA
1	1	3	25
2	2	3	25
3	2	6	25
4	2	12	25
5	2	18	25
6	4	6	25
7	4	12	25

the supernatant was discarded after sedimentation of the materials. The polymer was redispersed into methanol, and the supernatant was again discarded after sedimentation. This procedure was repeated 3 times in methanol and twice in tetrahydrofuran (THF). Then, the polymer was filtered on a membrane filter and washed with THF and acetone, followed by drying at room temperature.

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

2.3. Chromatography

The HPLC system used was composed of a PU-980 pump, a UV-970 spectrophotometer (both from Jasco, Tokyo, Japan), a Rheodyne 7125 injector with a 20-μl loop (Rheodyne, Cotati, CA, USA) and a C-R6A integrator (Shimadzu, Kyoto, Japan). The flow-rate was maintained at 0.2, 0.5, 0.8, 1.0 or 1.2 ml/min. Detection was performed at 220 or 200 nm. Acetone or tyrosine was used as an unretained solute. Separations were carried out at 30, 40, 50, 60 or 70°C using a column oven (TU-310, Jasco). The eluents were prepared by using phosphoric acid, sodium dihydrogenphosphate and organic modifier (acetonitrile, ethanol or 2-propanol). The eluent used was specified in the legends of tables and figures.

3. Results and discussion

3.1. Selection of a functional monomer

The MIPs for (*S*)-naproxen were prepared using 4-VPY, 2-VPY or 1-VI as a functional monomer and EDMA as a cross-linker by a multi-step swelling and polymerization technique. The molar amounts of (*S*)-naproxen, functional monomer and EDMA used were 2, 6 and 25 mmol, respectively. The MIPs were evaluated using 20 mM phosphoric acid and sodium dihydrogenphosphate (pH 3.2)–acetonitrile (50:50, v/v) as an eluent to examine the effect of a functional monomer on the retentivity and enantioselectivity for naproxen. Table 2 shows the retention factor, enantioseparation factor and resolution values

Table 2
Retention factor, enantioseparation factor and resolution for naproxen on the MIPs prepared using different functional monomers[a]

MIP	k_R	k_S	α	R_s
4-VPY–EDMA	9.69	16.7	1.73	0.73
2-VPY–EDMA	5.53	7.43	1.34	0.72
1-VI–EDMA	5.48	6.06	1.11	<0.3

[a] HPLC conditions: column size, 100×4.6 mm I.D.; column temperature, 25°C; eluent, 20 mM phosphoric acid and sodium phosphate (pH 3.2)–acetonitrile (50:50, v/v); flow-rate, 1.0 ml/min; loaded amount, 250 ng.

for naproxen on the MIPs. The 1-VIP–EDMA materials had little chiral recognition ability, while the 4-VPY–EDMA and 2-VPY–EDMA materials had excellent chiral recognition ability for naproxen. The 4-VPY–EDMA materials gave longer retentions and higher enantioseparation factors for naproxen than the 2-VPY–EDMA materials. In the following studies, we used 4-VPY as a functional monomer.

3.2. Effect of molar amounts of (S)-naproxen and 4-VPY on chiral resolution of naproxen

In previous studies [6,7], we prepared a MIP for (*S*)-naproxen using 2, 6 and 25 mmol of (*S*)-naproxen, 4-VPY and EDMA, respectively, and evaluated the retentivity and enantioselectivity of the MIP using a mixture of phosphate buffer and acetonitrile as an eluent. Constant retentivity and enantioselectivity were obtained with eluent pH between 2.3 and 4.6 [7,8]. In this study, we tried to optimize the preparation method of the MIP for (*S*)-naproxen and the separation of naproxen enantiomers on the MIP. Table 3 shows the effect of the molar amounts of

Table 3
Effect of molar amounts of (*S*)-naproxen and 4-VPY on retention factor, enantioseparation factor and resolution for naproxen[a]

MIP	k_R	k_S	α	R_s
1	5.19	7.51	1.48	0.56
2	6.32	10.2	1.61	0.72
3	9.69	16.7	1.73	0.73
4	11.9	18.8	1.58	0.58
5	12.0	16.7	1.39	0.45
6	11.5	20.5	1.79	0.97
7	15.8	27.8	1.75	1.01

[a] HPLC conditions as in Table 2.

(*S*)-naproxen and 4-VPY on the retention factor, enantioseparation factor and resolution for naproxen, when 25 mmol EDMA was used. The eluent used was 20 m*M* phosphoric acid and sodium dihydrogenphosphate (pH 3.2)–acetonitrile (50:50, v/v). When the template molecule, (*S*)-naproxen, used was 2 mmol, the retention factor for (*R*)-naproxen increased with an increase in the functional monomer amount used (see MIPs 2, 3, 4 and 5). However, maximum enantioselectivity was observed with the use of 6 mmol of 4-VPY. Excess functional monomer resulted in inferior chiral recognition for naproxen. When the molar ratio of (*S*)-naproxen to 4-VPY is 1:3, retentivity and enantioselectivity for naproxen increased with an increase of their amounts (see MIPs 1, 3, and 7). When (*S*)-naproxen and 4-VPY used were 4 and 6 mmol, respectively, enantioselectivity and resolution for naproxen were better despite the shorter retention times (see MIPs 6 and 7). In the following study, the amounts of (*S*)-naproxen, 4-VPY and EDMA used were 4, 6 and 25 mmol, respectively.

3.3. Effect of the type of the organic modifier on chiral resolution of naproxen

Table 4 shows the effect of the type of the organic modifier on the retention factor, enantioseparation factor and resolution for naproxen. The content of acetonitrile, ethanol and 2-propanol was 50, 60 and 55%, respectively, to get almost the same retention factor of naproxen. The enantioseparation factor for naproxen decreased in the order of 2-propanol > ethanol > acetonitrile, while the number of theoretical

plates for naproxen enantiomers decreased in the order of acetonitrile > ethanol > 2-propanol. The resolution for naproxen enantiomers decreased in the order of acetonitrile > ethanol > 2-propanol. In the following study, we used acetonitrile as the organic modifier.

3.4. Separation of naproxen and other 2-arylpropionic acid derivatives

Fig. 1 shows the separation of naproxen, ibuprofen, flurbiprofen, pranoprofen, 2-phenylpropionic acid and fenoprofen on the MIP for (*S*)-naproxen. The enantioseparation factors obtained were 1.79, 1.14, 1.27, 1.17, 1.09 and 1.06 for naproxen, ibuprofen, flurbiprofen, pranoprofen, 2-phenylpropionic acid and fenoprofen, respectively. The MIP showed the highest enantioselectivity for naproxen and moderate enantioselectivity for the other 2-arylpropionic acid derivatives. Previously, chiral resolution of fenoprofen was not attained [7]. However, by optimizing the preparation of the MIP for (*S*)-naproxen, chiral resolution of fenoprofen was attained, and all 2-arylpropionic acid derivatives tested gave higher enantioselectivities.

3.5. Effect of column temperature and flow-rate

Table 5 shows the retention factor, enantioseparation factor, resolution and the number of theoretical plates for the naproxen enantiomers at column temperatures of 30, 40, 50, 60 and 70°C. With an increase in column temperature, the retention factor and enantioseparation factor decreased; yet the highest resolution was obtained at a column temperature of 70°C. This is due to the suppression of the band-broadening of the second-eluted enantiomer, (*S*)-naproxen. Table 6 shows the retention factor, enantioseparation factor, resolution and the number of theoretical plates for the naproxen enantiomers at flow-rates of 0.2, 0.5, 0.8, 1.0 and 1.2 ml/min. With a decrease in the flow-rate, the retention times increased; the highest resolution was obtained at a flow-rate of 0.2 ml/min. This could be due to the slow mass transfer of naproxen enantiomers on the MIP.

Table 4
Effect of the type of the organic modifier on retention factor, enantioseparation factor, resolution and the number of theoretical plates for naproxen[a]

Organic modifier	k_R	k_S	α	R_s	N_R	N_S
Acetonitrile	4.74	6.89	1.45	1.40	628	211
Ethanol	3.70	5.69	1.54	1.35	443	151
2-Propanol	3.66	5.79	1.59	1.18	313	99

[a] HPLC conditions: eluent, 20 m*M* phosphoric acid and sodium dihydrogenphosphate (pH 3.2) with 50% acetonitrile, 60% ethanol or 55% 2-propanol; column size, 100×4.6 mm I.D.; column temperature, 70°C; flow-rate, 0.2 ml/min; loaded amount, 250 ng.

Naproxen

Ibuprofen

Flurbiprofen

Pranoprofen

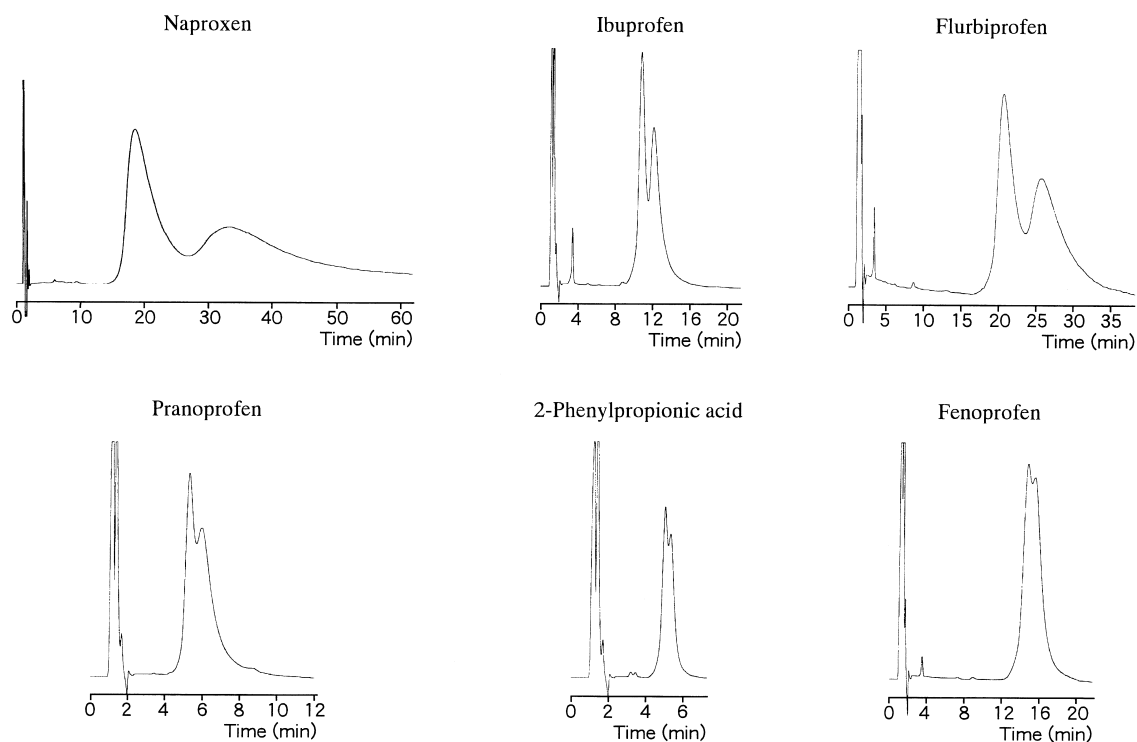2-Phenylpropionic acid

Fenoprofen

Fig. 1. Separation of naproxen, ibuprofen, flurbiprofen, pranoprofen, 2-phenylpropionic acid and fenoprofen on the MIP for (*S*)-naproxen. HPLC conditions: column size, 100×4.6 mm I.D.; column temperature, 25°C; eluent, 20 m*M* phosphoric acid and sodium dihydrogenphosphate (pH 3.2)–acetonitrile (50:50, v/v); detection, 220 or 200 nm; flow-rate, 1.0 ml/min. Loaded amount, 250 ng.

Table 5
Effect of column temperature on retention factor, enantioseparation factor, resolution and the number of theoretical plates for naproxen[a]

Column temp. (°C)	k_R	k_S	α	R_s	N_R	N_S
30	13.5	23.7	1.76	1.08	112	50
40	10.2	17.1	1.67	1.19	161	74
50	7.81	12.3	1.58	1.18	264	90
60	6.03	8.99	1.49	1.16	346	122
70	4.68	6.59	1.41	1.23	574	185

[a] HPLC conditions: column size, 100×4.6 mm I.D.; eluent, 20 m*M* phosphoric acid and sodium dihydrogenphosphate (pH 3.2)–acetonitrile (50:50, v/v); flow-rate, 0.5 ml/min; loaded amount, 500 ng.

Table 6
Effect of flow-rate on retention factor, enantioseparation factor, resolution and the number of theoretical plates for naproxen[a]

Flow-rate (ml/min)	k_R	k_S	α	R_s	N_R	N_S
0.2	4.61	6.48	1.41	1.38	699	260
0.5	4.68	6.59	1.41	1.23	574	185
0.8	4.94	7.04	1.42	1.14	436	145
1.0	5.10	7.32	1.44[b]	1.03	394	109
1.2	5.34	7.70	1.45[b]	0.92	266	88

[a] HPLC conditions: column size, 100×4.6 mm I.D.; column temperature, 70°C; eluent, 20 m*M* phosphoric acid and sodium phosphate (pH 3.2)–acetonitrile (50:50, v/v); loaded amount, 500 ng.

[b] Possibly artifacts due to poor peak shape and low resolution.

4. Conclusion

A uniformly sized molecularly imprinted polymer for (*S*)-naproxen was prepared using 4-VPY and EDMA as a functional monomer and cross-linker, respectively, and evaluated using a mixture of phosphate buffer and organic modifier as an eluent. When the amounts of (*S*)-naproxen, 4-VPY and EDMA used were 4, 6 and 25 mmol, respectively, the enantioselectivity and resolution for naproxen was good, despite the shorter retention. When acetonitrile was used as an organic modifier, the highest column efficiency was obtained for the separation of naproxen enantiomers. With regard to the effects of column temperature and flow-rate, the column performance was improved by elevating the column temperature and decreasing the flow-rate.

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