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Retentivity and enantioselectivity of uniformly sized molecularly imprinted polymers for *d*-chlorpheniramine and -brompheniramine in hydro-organic mobile phases

Jun Haginaka*, Chino Kagawa

Faculty of Pharmaceutical Sciences, Mukogawa Women's University, 11-68 Koshien Kyuban-cho, Nishinomiya, Hyogo 663-8179, Japan

Abstract

Uniformly sized molecularly imprinted polymers (MIPs) for *d*-chlorpheniramine (CP) and -brompheniramine (BP) have been prepared by a multi-step swelling and polymerization method using methacrylic acid (MAA) or 2-(trifluoromethyl)acrylic acid (TFMAA) and ethylene glycol dimethacrylate (EDMA) as a functional monomer and cross-linker, respectively. The retentive and enantioselective properties of CP, BP and their structurally related compounds on the MIPs were evaluated using hydro-organic mobile phases. CP and BP enantiomers were retained the most as a monovalent cation on MAA-*co*-EDMA polymers and a divalent cation on TFMAA-*co*-EDMA polymers. Ion exchange and hydrophobic interactions could mainly work for the retention and enantioseparation of CP and BP on both MAA-*co*-EDMA and TFMAA-*co*-EDMA polymers in hydro-organic mobile phases. Though the respective MIPs gave the highest enantioselectivity for the template molecule, cross-reactivity for CP and BP was observed with them.

Keywords: Enantiomer separation; Molecularly imprinted polymers; Chlorpheniramine; Brompheniramine

1. Introduction

Molecularly imprinted polymers (MIPs) have been used for selective recognition of a target molecule as chromatographic media, sensors and artificial antibodies [1–4]. When we use MIPs as chromatographic media, especially as HPLC packing materials, it is desirable to prepare spherical and monodispersed beads, whose particle sizes are 3–5 μm. Recently, we prepared uniformly sized, monodispersed MIPs for (*S*)-naproxen [5], (*S*)-ibuprofen [6], (*S*)-propranolol [7] and (*S*)-chlorpheniramine (CP) [8] using a multi-step swelling and polymerization method. Further, our MIP gave molecular recognition not only for the template molecule but also for its structurally related compounds such as drug metabolites in hydro-organic mobile phases [6,7].

CP and brompheniramine (BP) are antihistaminic H_1 drugs. Their antihistaminic activities exist predominantly in the dextro-isomers [9], and (*S*)-*d*-CP has been shown to be 200-fold more active than its enantiomer in vivo in protecting guinea-pig against histamine [10]. Recently, a sensitive enantioselective HPLC method has been developed for the simultaneous determination of CP and its metabolites in human plasma [11]. However, the method required a tedious sample preparation procedure; liquid–liquid extraction and back extraction to acidic aqueous layer. It is important to develop selective materials for CP and its metabolites for the purpose of a simple sample preparation.

Previously, we prepared the MIP for *d*-CP using methacrylic acid (MAA) and ethylene glycol dimethacrylate (EDMA) as a functional monomer and cross-linker, respectively, and evaluated the retentive and enantioselective properties for CP and its structurally related compounds on the MIP using hydro-organic mobile phases [8]. In this study, uniformly sized MIPs for *d*-CP and -BP have been prepared by a multi-step swelling and polymerization method using MAA or 2-(trifluoromethyl)acrylic acid (TFMAA) and EDMA as a functional monomer and cross-linker, respectively. The retentive and enantioselective properties of CP and BP on the respective MIPs were evaluated using hydro-organic mobile phases. Furthermore,

* Corresponding author. Tel.: +81-798-45-9949; fax: +81-798-41-2792.
E-mail address: haginaka@mwu.mukogawa-u.ac.jp (J. Haginaka).

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their retention and enantioseparation mechanisms on the MIPs are discussed.

2. Experimental

2.1. Materials

EDMA and TFMAA were purchased from Tokyo Chemical Industry (Tokyo, Japan), and MAA from Wako Pure Chemical Industry (Osaka, Japan). These monomers were purified by general distillation techniques in vacuo to remove the polymerization inhibitor. 2,2′-Azobis(2,4-dimethylvaleronitrile) was purchased from Wako Pure Chemical Industry (Tokyo, Japan). *d*-CP, -BP, and propranolol were purchased from Sigma-Aldrich Japan (Tokyo, Japan). Pheniramine was purchased from Nacalai Tesque (Kyoto, Japan). The structures of CP, BP and other compounds used in this study are illustrated in Fig. 1. 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 mobile phase and the sample solution.

2.2. Multi-step swelling and polymerization method

Preparation of the uniformly sized, macroporous MIPs for *d*-CP and -BP as well as non-imprinted polymers (NIPs) by a multi-step swelling and polymerization method was carried out as reported previously [12,13]. The molar amounts of a template molecule, functional monomer and cross-linker used for the preparation of the MIPs and NIPs are shown in Table 1. The prepared polymers were packed into a stainless-steel column (100 mm × 4.6 mm i.d.) by a slurry packing technique using methanol as the slurry and packing solvents to evaluate their chromatographic characteristics.

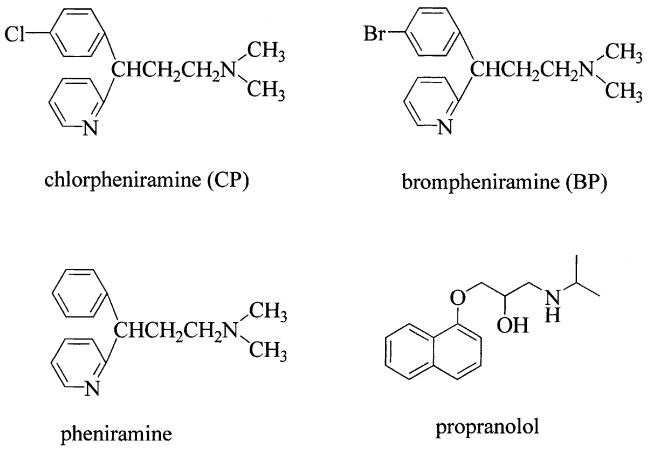

Fig. 1. Structures of CP, BP and other compounds used in this study.

Table 1
Molar amounts of template molecule, functional monomer and cross-linker used for the preparation of MIPs and NIPs in this study

Polymer	Template molecule		Functional monomer		Cross-linker
	Type	Amount (mmol)	Type	Amount (mmol)	–EDMA (mmol)
MIP 1	*d*-CP	2	MAA	7	25
NIP 1	No	0	MAA	7	25
MIP 2	*d*-CP	2	TFMAA	7	25
NIP 2	No	0	TFMAA	7	25
MIP 3	*d*-BP	2	MAA	7	25

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, Cotani, CA, USA), and a C-R6A integrator (Shimadzu, Kyoto, Japan). The flow rate was maintained at 0.5, 0.8, 1.0, 1.5 or 2.0 ml/min. Detection was performed at 200 nm. The retention factor was calculated from the equation $k = (t_R - t_0)/t_0$, where t_R and t_0 are retention times of retained and unretained solutes, respectively. The retention time of unretained solute, t_0, was measured by injecting acetone or tyrosine. The enantioseparation factor is calculated from the equation $\alpha = k_d/k_l$, where k_l and k_d are the retention factors of the first and second eluted enantiomers, respectively. Resolution is calculated from the equation $Rs = 2(t_d - t_l)/(w_l + w_d)$, where t_l and t_d are the retention times of the first and second eluted enantiomers, respectively, and w_l and w_d are the baseline peak widths of the first and second eluted enantiomers, respectively. Separations were carried out at 30, 40, 50, 60 or 70 °C using a column oven (TU-310, Jasco, Tokyo, Japan). The mobile phases are prepared by using phosphoric acid, potassium dihydrogen phosphate, dipotassium hydrogen phosphate or tripotassium phosphate and acetonitrile. The mobile phases used and their pH values were specified in the legends of tables and figures. However, the pH values were "apparent" because a mixture of phosphate buffer and acetonitrile was used as the mobile phase.

3. Results and discussion

3.1. Effect of a functional monomer on the retention of d-CP and propranolol

In a previous study [8], we tried to optimize the preparation of the MIP for *d*-CP and the separation of CP enantiomers on the MIP. When the amounts of *d*-CP, MAA and EDMA used were 2, 7 and 25 mmol, respectively, the resolution of CP enantiomers was good despite the shorter retention times. In this study, the molar ratio described above was

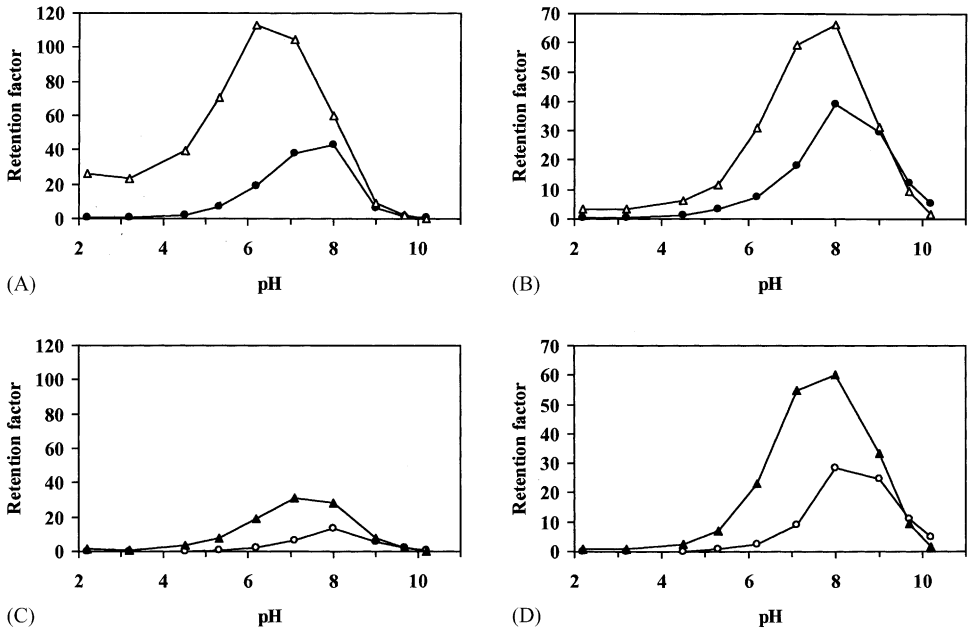

Fig. 2. Effect of mobile phase pH on the retention properties of *d*-CP and propranolol on MIPs and NIPs. (A) *d*-CP on MIPs 1 and 2; (B) propranolol on MIPs 1 and 2; (C) *d*-CP on NIPs 1 and 2; (D) propranolol on NIPs 1 and 2. Keys: (●) MIP 1; (△) MIP 2; (○) NIP 1; (▲) NIP 2. HPLC conditions: column size, 100 mm × 4.6 mm i.d.; column temperature, 25 °C; mobile phase, 50 mM phosphoric acid and/or potassium phosphate–acetonitrile (30/70; v/v); detection, 200 nm; flow rate, 1.0 ml/min. Loaded amount, 1000 ng.

used for the preparation of *d*-CP-imprinted MAA-*co*-EDMA and TFMAA-*co*-EDMA polymers (MIPs 1 and 2).

Fig. 2A and B shows the effects of mobile phase pH on the retention properties of *d*-CP and propranolol, respectively, on MIPs 1 and 2, and Fig. 2C and D shows those of *d*-CP and propranolol, respectively, on the respective NIPs. The mobile phase used was 50 mM phosphoric acid and/or potassium phosphate–acetonitrile (30:70; v/v). As shown in Fig. 2A and B, MIP 2 gave longer retentions for *d*-CP and propranolol than MIP 1. The differences in retention factors could be ascribable to differences in acidity of MIPs 1 and 2, because the pK_a values of MAA and TFMAA are 4.5 and 3.0, respectively [14].

The maximum retention factor of *d*-CP on MIPs 1 and 2 was observed at mobile phase pH of 8.0 and 6.2, respectively, while that of propranolol on both MIPs was at mobile phase pH of 8.0. These strange phenomena can be elucidated, taking account into the average pK_a values of MAA-*co*-EDMA (ca. 9) and TFMAA-*co*-EDMA polymers, and the pK_a values of CP (4.0 and 9.2) and propranolol (9.5) [15]. However, the apparent pK_a values of CP and propranolol could be different from those values. The average pKa value of TFMAA-*co*-EDMA polymers seems to be lower than that of MAA-*co*-EDMA polymers, because the pK_a values of MAA and TFMAA are 4.5 and 3.0, respectively. In a previous study [8], we reported that with regard to its retention on the MIP 1, *d*-CP was not retained at low mobile phase pH because of almost no dissociation of MAA-*co*-EDMA polymers. Furthermore, the maximum retention was observed at around its apparent pK_a value because of the ionic interaction of the amino group with the negatively charged polymers. Then, drastic decrease of the retentions was observed with further increase in the mobile phase pH, because of deprotonation of the amino group. On the other hand, *d*-CP was retained at low mobile phase pH on MIP 2. This means that at acidic mobile phase MIP 2 dissociates and that *d*-CP is present in a divalent cation because of protonations on its pyridyl and amino groups. With an increase in the mobile phase pH, the retention of *d*-CP was increased, and *d*-CP was retained the most as a divalent cation on MIP 2. Further increase in the mobile phase pH resulted in the drastic decrease of the retention because of deprotonation of the pyridyl and amino groups. This is the reason why the maximum retention factor of *d*-CP on MIPs 1 and 2 is observed at mobile phase pH of 8.0 and 6.2, respectively: that is, it is retained the most as the monovalent and divalent cations, respectively, on MIPs 1 and 2. In addition, the retention tendencies of *d*-BP and pheniramine, which have pyridyl and amino groups in the molecules, were similar to those of *d*-CP on MIPs 1 and 2. Contrary to these phenomena, propranolol (which has only an amino group in the molecule) showed its maximum retention factor on both MIPs 1 and 2 at mobile phase pH of 8.0. The maximum retention was observed at around its apparent pK_a value because of the ionic interaction of the amino group with the negatively charged polymers, independent of the average pK_a values of the polymers. The retention tendencies for *d*-CP and propranolol on NIPs 1 and 2 were similar to those on MIPs 1 and 2, except that *d*-CP was retained more on the MIPs 1 and 2 because of the molecular imprinting effect.

Table 2
Effects of mobile phase pH on the retention factor, enantioseparation factor and resolution of CP on MIPs 1and 2

Mobile phase pH	MIP 1			MIP 2		
	k_d	α	Rs	k_d	α	Rs
2.2	0.40	1.00	–	26.0	1.00	–
3.2	0.70	1.00	–	23.1	1.24	<0.3
4.5	2.26	1.30	<0.3	39.22	1.95	0.75
5.3	7.07	1.64	0.44	70.6	2.16	0.86
6.2	19.4	1.87	0.53	113	2.12	0.81
7.1	38.2	1.89	0.52	104	1.73	0.43
8.0	43.1	1.66	0.56	60.0	1.27	<0.3
9.0	6.51	1.00	–	9.16	1.28	
9.7	1.72	1.00	–	2.33	1.00	–
10.2	0.48	1.00	–	0.31	1.00	–

HPLC conditions: column size, 100 mm × 4.6 mm i.d.; mobile phase, 50 mM phosphoric acid and/or potassium phosphate/acetonitrile = 30/70 (v/v); flow rate, 1.0 ml/min; column temperature, 25 °C; loaded amount, 1000 ng; detection, 200 nm.

3.2. Separation of CP enantiomers on the imprinted MAA-co-EDMA and TFMAA-co-EDMA polymers

Table 2 shows the effect of mobile phase pH on the separation of CP enantiomers on MIPs 1 and 2. NIPs 1 and 2 had no chiral recognition ability toward CP, while MIPs 1 and 2 showed enantioselectivity for CP. As shown in Table 2, the highest enantioselectivity and resolution were obtained with MIP 1 at mobile phase pH between 6.2 and 8.0, and MIP 2 at mobile phase pH of 5.3 and 6.2. Furthermore, MIP 2 gave higher enantioselectivity and resolution for CP than MIP 1. As described above, in this pH range CP enantiomers were present as a monovalent or divalent cation, and MAA-*co*-EDMA and TFMAA-*co*-EDMA polymers were partially charged. The results obtained suggest that ionic interactions could play an important role in the enantioseparation of CP on MIPs 1 and 2. Fig. 3 shows the separation of CP enantiomers on MIPs 1 and 2. On MIP 1, the optimal enantioseparation was attained using neutral mobile phase, while on MIP 2 using acidic mobile phase. These results suggest that TFMAA is a more appropriate monomer for the preparation of MIP for CP than MAA.

Table 3
Retentivity and enantioselectivity of MIPs 1 and 3 toward CP, BP and pheniramine

	MIP 1			MIP 3		
	k_d	α	Rs	k_d	α	Rs
CP	19.4	1.87	0.53	30.2	2.06	0.66
BP	24.1	1.72	0.53	41.5	2.37	0.92
Pheniramine	9.69	1.33	0.32	14.0	1.41	0.34

HPLC conditions: column size, 100 mm × 4.6 mm i.d.; mobile phase, 50 mM phosphoric acid and/or potassium phosphate/acetonitrile = 30/70 (v/v) (final mobile phase pH 6.2); flow rate, 1.0 ml/min; column temperature, 25 °C; loaded amount, 1000 ng; detection, 200 nm.

3.3. Comparison of d-CP- and d-BP-imprinted MAA-co-EDMA polymers

Table 3 shows the retention factors, enantioseparation factors and resolution of CP, BP and pheniramine enantiomers on *d*-CP and -BP imprinted MAA-*co*-EDMA polymers (MIPs 1 and 3), respectively, at mobile phase pH of 6.2. The retention tendencies of BP on MIP 3 were similar with those of CP on MIP 1 except that BP was more retained. This could be ascribable to almost the same pKa values of CP, BP and pheniramine, and to that the log P_{ow} (P_{ow} = octanol–water partition coefficient) value is in order of BP (3.57), CP (3.39) and pheniramine (2.79) [16]. The latter means that their hydrophobicity is in order of BP, CP and pheniramine. Thus, it is concluded that for the retentions and enantioseparations of CP, BP and pheniramine, hydrophobic interactions work in addition to ion exchange interactions. As shown in Table 3, MIP 1 gave higher enantioseparation factor for CP than BP and the same resolution,

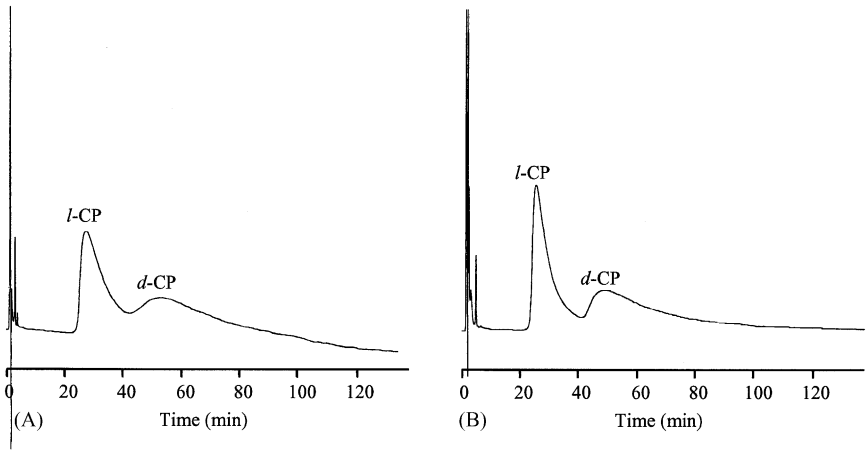

Fig. 3. Separation of CP enantiomers on MIPs 1 (A) and 2 (B). HPLC conditions: column size, 100 mm × 4.6 mm i.d.; column temperature, 70 °C; mobile phase, 50 mM potassium phosphate buffer-acetonitrile (30/70; v/v) (final pH 7.1 and 5.3 for A and B, respectively); detection, 200 nm; flow rate, 0.8 ml/min. Loaded amount, 700 ng.

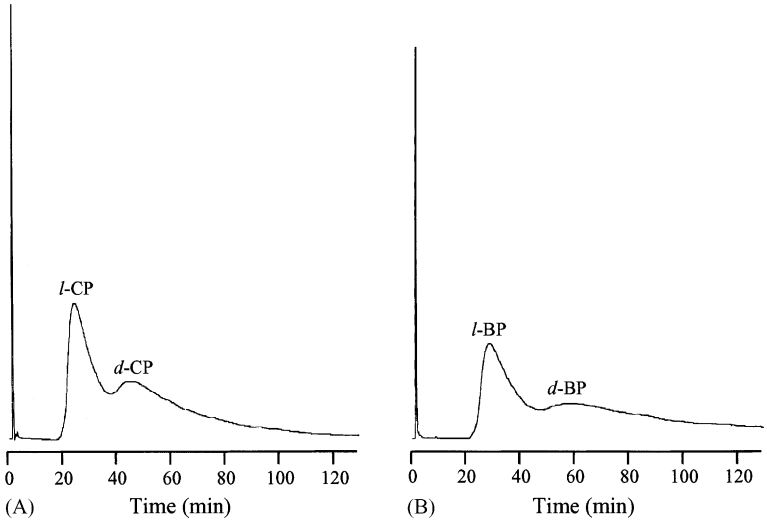

Fig. 4. Separation of CP (A) and BP (B) enantiomers on MIP 3. HPLC conditions as in Table 3.

while MIP 3 gave higher enantioseparation factor and resolution for BP than CP. Fig. 4A and B shows the separation of CP- and BP-enantiomers, respectively, on MIP 3. The respective MIPs showed high cross-reactivity for CP and BP. Leakage of a trace amount of the imprint molecule remaining in the MIP prevented the accurate and precise assays of the target analytes. This problem has been overcome by imprinting a structurally related analogue [17]. The results obtained above reveal that BP or CP could be used for such purposes.

3.4. Effect of column temperature and flow rate on the separation of CP enantiomers on the d-CP-imprinted TFMAA-co-EDMA columns

Fig. 5A–C shows the separation of CP enantiomers on MIP 2 at column temperatures of 30, 50 and 70 °C, respectively. Table 4 shows the retention factor, enantioseparation factor and resolution of CP enantiomers at column temperatures of 30, 40, 50, 60 and 70 °C. With an increase in the column temperature, the retention factor and enantioseparation factor decreased, while the resolution increased. This is due to the suppression of the band-broadening of the second-eluted enantiomer, *d*-CP. The retention factor and enantioseparation factor of CP decreased with an increase of column temperature. Thus, the enthalpy-driven interactions seem to be dominant at mobile phase pH of 4.5. Similar phenomena were observed with all mobile phases tested, and also on MIPs 1 and 3.

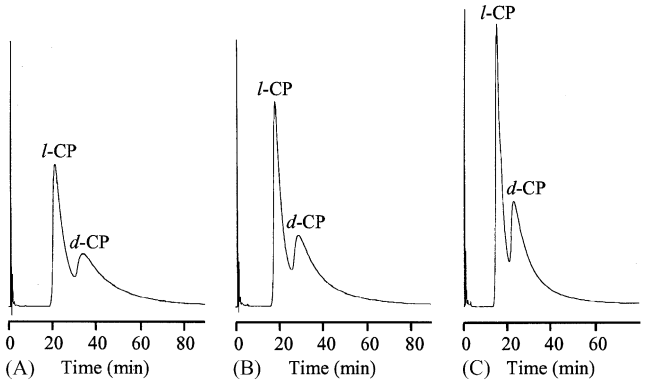

Fig. 5. Separation of CP enantiomers on MIP 2 at column temperatures of 30 °C (A), 50 °C (B) and 70 °C (C). HPLC conditions as in Table 4.

Table 4
Effect of column temperature on retention factor, enantioseparation factor and resolution of CP[a]

Column temperature (°C)	k_l	k_d	α	Rs
30	14.7	24.0	1.64	0.79
40	13.4	22.1	1.64	0.80
50	13.0	20.7	1.59	0.87
60	12.1	19.1	1.58	0.95
70	11.1	17.2	1.56	0.98

[a] HPLC conditions: column, MIP 2 (100 mm × 4.6 mm i.d.); flow rate, 1.0 ml/min; detection, 200 nm; eluent, 50 mM potassium phosphate buffer–acetonitrile (30/70; v/v) (final mobile phase pH 4.5); loaded amount, 3000 ng; detection, 200 nm.

Fig. 6A–C shows the separation of CP enantiomers on MIP 2 at flow rates of 0.5, 1.0 and 2.0 ml/min, respectively. Table 5 shows the retention factor, enantioseparation factor

Table 5
Effect of flow rate on retention factor, enantioseparation factor and resolution of CP[a]

Flow rate (ml/min)	k_l	k_d	α	Rs
0.5	11.4	17.8	1.57	0.98
0.8	11.3	17.5	1.55	0.98
1.0	11.1	17.2	1.56	0.98
1.5	11.1	17.0	1.53	0.90
2.0	10.9	16.5	1.52	0.81

[a] HPLC conditions: column, MIP 2 (100 mm × 4.6 mm i.d.); column temperature, 70 °C; eluent, 50 mM potassium phosphate buffer (pH 3.2)–acetonitrile (30/70; v/v); loaded amount, 3000 ng; detection, 200 nm.

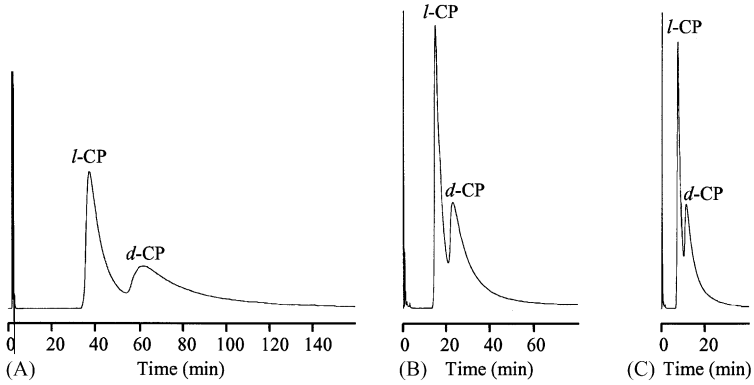

Fig. 6. Separation of CP enantiomers on MIP 2 at flow-rates of 0.5 ml/min (A), 1.0 ml/min (B) and 2.0 ml/min (C). HPLC conditions as in Table 5.

and resolution of CP enantiomers at flow rates of 0.5, 0.8, 1.0, 1.5 and 2.0 ml/min. With a decrease in the flow rate, the retention times increased, while the highest enantioseparation factor was obtained at a flow rate of 0.5 ml/min. This could be due to the slow mass transfer of CP enantiomers on the MIP. As described above, the column performance of the MIPs for *d*-CP and -BP was improved by elevating the column temperature and decreasing the flow rate as reported previously [8].

4. Conclusion

Uniformly sized MIPs for *d*-CP and -BP were prepared using MAA or TFMAA and EDMA as a functional monomer and cross-linker, respectively, and evaluated using a mixture of phosphate buffer and acetonitrile as an mobile phase. CP and BP enantiomers were retained the most as a monovalent and divalent cations, respectively, on MAA-*co*-EDMA and TFMAA-*co*-EDMA polymers. Ion exchange and hydrophobic interactions could mainly work for the retention and enantioseparation of CP and BP on both MAA-*co*-EDMA and TFMAA-*co*-EDMA polymers. Furthermore, MIPs for *d*-CP and -BP gave very similar enantioselectivity and resolution for CP and BP. Thus, CP or BP could be used as a structural analog each other to avoid the leakage of a template molecule.

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