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Uniformly sized molecularly imprinted polymers for *d*-chlorpheniramine: Influence of a porogen on their morphology and enantioselectivity

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Abstract

Uniformly sized molecularly imprinted polymers (MIPs) for *d*-chlorpheniramine have been prepared by a multi-step swelling and polymerization method using methacrylic acid (MAA) or 2-(trifluoromethyl)acrylic acid (TFMAA) as a functional monomer and toluene, phenylacetonitrile, benzylacetonitrile or chloroform as a porogen. From measurement of their scanning electron microscopy images and physical properties in the dry state, the MIP prepared using TFMAA and chloroform as the functional monomer and porogen, respectively, seemed to be non-porous and had extremely low specific surface areas and pore volumes, while the other MIPs were porous beads with high specific surface areas and pore volumes. All the MIPs prepared were evaluated using hydro-organic mobile phases in HPLC. As a result, they showed the similar retentive and enantioselective properties for chlorpheniramine, brompheniramine and pheniramine. This result suggests the presence of enantioselective binding sites in the swollen state for all the MIPs.

Keywords: Molecular imprinting; Molecularly imprinted polymer; Chiral separation; Chlorpheniramine; Pheniramine; Porogen

1. Introduction

Molecular imprinting techniques are very attractive because specific recognition sites for a target molecule could be easily molded in synthetic polymer networks [1–3]. The obtained molecularly imprinted polymers (MIPs) have been utilized as chromatographic media, sensors, artificial antibodies and catalysts [4]. Typically, MIPs were prepared by a bulk polymerization method, where the resultant monoliths had to be crushed, ground and sieved to produce microparticles for their applications. When we use MIPs as HPLC stationary phases, it is desirable to prepare the spherical and monodispersed beads. Recently, we prepared uniformly sized MIPs for (*S*)-naproxen [5], (*S*)-ibuprofen [6] and (*S*)-propranolol [7] using a multi-step swelling and polymerization method. Furthermore, the MIPs for *d*-chlorpheniramine [8] and *d*-brompheniramine [8,9] were similarly prepared using methacrylic acid (MAA) or 2-(trifluoromethyl)acrylic acid (TFMAA) as a functional monomer, ethylene glycol dimethacrylate (EDMA) as a cross-linker and toluene as a porogen.

It is well-known that a lot of factors relating the stability of monomer–template complexes and the polymerization reaction affect the surface morphology and molecular recognition properties of MIPs [10]. The influence of a porogen on them was intensively examined [11–14]. In this study, in order to clarify the influence of a porogen on their surface morphology and molecular recognition properties, uniformly sized MIPs for *d*-chlorpheniramine have been prepared by a multi-step swelling and polymerization method using MAA or TFMAA as a functional monomer and toluene, phenylacetonitrile, benzylacetonitrile or chloroform as a porogen. The surface morphology of the respective MIPs was precisely examined by measurement of their scanning electron microscope (SEM) images and surface porosity. The molecular recognition properties of chlorpheniramine, brompheniramine and pheniramine on the respective MIPs were evaluated using hydro-organic mobile phases.

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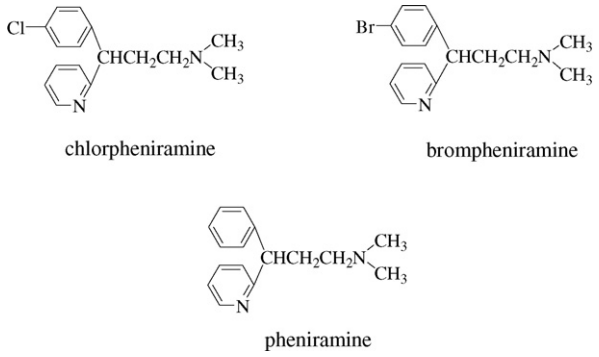

Fig. 1. Structures of chlorpheniramine, brompheniramine and pheniramine.

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) (ADVN) was purchased from Wako Pure Chemical Industry (Tokyo, Japan). *d*-Chlorpheniramine, *dl*-chlorpheniramine and *dl*-brompheniramine were purchased from Sigma–Aldrich Japan (Tokyo, Japan). Racemic pheniramine was purchased from Nacalai Tesque (Kyoto, Japan). The structures of chlorpheniramine, brompheniramine and pheniramine 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 MIPs for *d*-chlorpheniramine as well as non-imprinted polymers (NIPs) by a multi-step swelling and polymerization method was carried out as reported previously [15,16]. Briefly, a water dispersion of 0.17 mL of uniformly sized, polystyrene seed particles (0.497 g/mL) was admixed with a microemulsion prepared from 0.48 mL of dibutyl phthalate as an activating solvent, 0.02 g of sodium dodecyl sulfate and 10 mL of water by sonication. This first-step swelling was carried out at room temperature for 15 h with stirring at 125 rpm until oil microdrops completely disappeared. A dispersion of 0.375 g of ADVN as an initiator, 5 mL of toluene (T), phenylacetonitrile (PA), benzylacetonitrile (BA) or chloroform (C) as a porogenic solvent, 10 mL of 4.8% polyvinyl alcohol aqueous solution as a dispersion stabilizer, and 12.5 mL of water was added to the dispersion of swollen particles. This second-step swelling was carried out at room temperature for 2 h with stirring at 125 rpm. A dispersion of 2 mmol of *d*-chlorpheniramine as a template, 25 mmol of EDMA as a cross-linker, 7 mmol of TFMAA or MAA as a functional monomer, 10 mL of 4.8% polyvinyl alcohol aqueous solution and 12.5 mL of water was added to the dispersion of swollen particles. 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 stirring at 160 rpm for 24 h. After polymerization, the dispersion of polymerized beads was poured into 200 mL of methanol and the supernatant was discarded after sedimentation of the beads. The polymer beads were redispersed into methanol, and this procedure was repeated three times in methanol, once in water, and twice in tetrahydrofuran. The resulting 5–6 μm polymer beads were collected using a glass filter, washed with tetrahydrofuran and dried at room temperature.

The NIPs were similarly prepared without a template molecule. The obtained MIP was abbreviated as $MIP_{MAA/T}$, which stands for the use of MAA and toluene as a functional monomer and porogen, respectively. The prepared polymers were packed into a stainless-steel column by a slurry packing 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, Cotani, CA, USA), and a C-R6A integrator (Shimadzu, Kyoto, Japan). 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. 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. The mobile phases are prepared using phosphoric acid, potassium dihydrogen phosphate or dipotassium hydrogen phosphate and acetonitrile. The mobile phases used and their pH values were specified in the legends of tables and figures.

2.4. Scanning electron microscopy images

Scanning electron microscopy (SEM) images of the MIPs for *d*-chlorpheniramine and NIPs were obtained at an S-4300 instrument (Hitachi, Tokyo, Japan).

2.5. Porosity measurements

Surface areas and porosity of MIPs and NIPs were measured by nitrogen sorption porosimetry using a TriStar surface area and porosity analyzer (Micrometrics Instrument, Norcross, GA, USA). Prior to measurement, 200 mg of the beads were heated at 80 °C for 5 h in vacuo. The specific surface areas were calculated using the BET method, and the specific pore volumes and

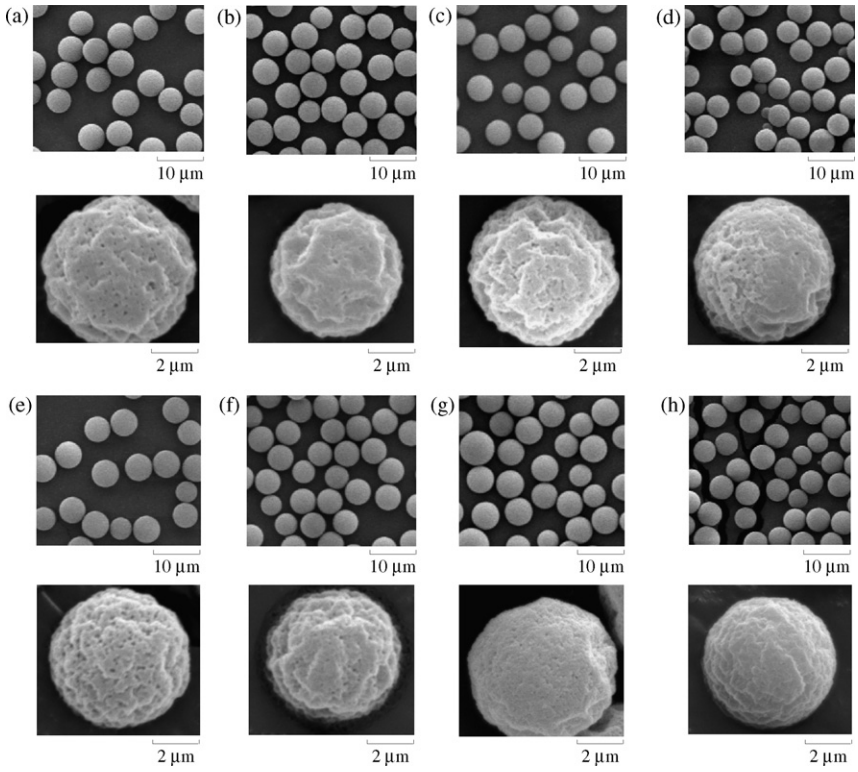

Fig. 2. SEM images of the respective MIPs: (a) $MIP_{MAA/T}$; (b) $MIP_{MAA/PA}$; (c) $MIP_{MAA/BA}$; (d) $MIP_{MAA/C}$; (e) $MIP_{TFMAA/T}$; (f) $MIP_{TFMAA/PA}$; (g) $MIP_{TFMAA/BA}$; (h) $MIP_{TFMAA/C}$.

average pore diameters were calculated according to the BJH method.

3. Results and discussion

3.1. Morphology of MIPs for d-chlorpheniramine

In this study, we prepared uniformly sized MIPs for *d*-chlorpheniramine using MAA or TFMAA as the functional monomer, and toluene, phenylacetonitrile, benzylacetonitrile or chloroform as the porogen by a multi-step swelling and polymerization method. Fig. 2 shows the SEM images of the respective MIPs with different functional monomers and porogens. The good size uniformity was obtained for all the MIPs prepared. Moreover, the outward appearances were relatively similar to each other except for $MIP_{TFMAA/C}$, which seems to be non-porous. Table 1 shows the physical properties (specific surface area, specific pore volume and average pore diameter) of the MIPs and NIPs, measured by nitrogen sorption porosimetry. Furthermore, both MIPs and NIPs prepared with the same porogen have similar morphology. These data indicate that the obtained MIPs and NIPs have high specific surface areas associated with microporous structures except for $MIP_{TFMAA/C}$ and $NIP_{TFMAA/C}$. The SEM images and physical properties of the MIPs and NIPs indicate that $MIP_{TFMAA/C}$ and $NIP_{TFMAA/C}$ seem to be non-porous and to have extremely low specific surface areas and pore volumes in the dry state, while the other MIPs and NIPs were porous beads with high specific surface areas and pore volumes.

3.2. Separation of chlorpheniramine, brompheniramine and pheniramine enantiomers on the MIPs

Table 2 shows the retention factors, enantioseparation factors and resolution of chlorpheniramine, brompheniramine and pheniramine enantiomers on MIP_{MAA} and MIP_{TFMAA}, where the mobile phases used were 50 mM potassium phosphate buffer (pH 4.5)–acetonitrile (30:70, v/v) and 50 mM potassium phosphate buffer (pH 3.2)–acetonitrile (30:70, v/v), respectively. The mobile phase pH was adjusted for *d*-chlorpheniramine to have similar retentions on $MIP_{MAA/C}$ and $MIP_{TFMAA/C}$. In a previous paper [9], we reported that chlorpheniramine and brompheniramine enantiomers were retained the most as a mono- or di-valent cation, respectively, on *d*-chlorpheniramine-imprinted MAA-*co*-EDMA and TFMAA-*co*-EDMA polymers. Thus, *d*-chlorpheniramine was more retained on $MIP_{TFMAA/C}$ than $MIP_{MAA/C}$ at the same mobile phase pH. The differences in retention factors could be ascribable to differences in acidity of $MIP_{MAA/C}$ and $MIP_{TFMAA/C}$, because the pK_a values of MAA and TFMAA are 4.5 and 3.0, respectively [9]. We selected different mobile phase pHs for evaluation of $MIP_{MAA/C}$ and $MIP_{TFMAA/C}$. As shown in Table 2, MIP_{TFMAA} gave higher resolution for chlorpheniramine, brompheniramine and pheniramine than MIP_{MAA}. In the following study, we used MIP_{TFMAA}.

As described above, $MIP_{TFMAA/C}$ gave the non-porous SEM images and extremely low specific surface areas and pore volumes in the dry state. However, $MIP_{TFMAA/C}$ as well as the other MIPs could separate chlorpheniramine, brompheniramine and pheniramine enantiomers using hydro-organic mobile phases

Table 1
Physical properties of MIPs for *d*-chlorpheniramine and NIPs[a]

Functional monomer/porogen[b]	MIP			NIP		
	S (m^2/g)	V_p (cm^3/g)	d_p (nm)	S (m^2/g)	V_p (cm^3/g)	d_p (nm)
MAA/T	269	0.66	9.67	231	0.55	8.99
MAA/PA	303	0.48	6.02	279	0.48	6.43
MAA/BA	300	0.63	7.95	313	0.58	7.08
MAA/C	186	0.28	5.83	104	0.26	8.58
TFMAA/T	214	0.54	8.91	206	0.58	10.3
TFMAA/PA	323	0.51	6.06	349	0.59	6.45
TFMAA/BA	312	0.54	6.48	317	0.51	6.17
TFMAA/C	10	0.04	11.1	8.0	0.04	12.9

[a] S: specific surface area; V_p: specific pore volume; d_p: average pore diameter.
[b] MAA: methacrylic acid; TFMAA: 2-(trifluoromethyl)acrylic acid; T: toluene, PA: phenylacetonitrile; BA: benzylacetonitrile; C: chloroform.

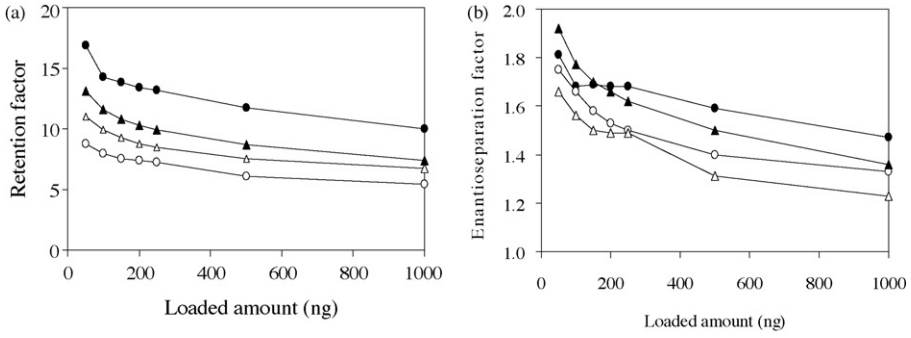

Fig. 3. Effect of sample loading amounts on the retention factor (a) and enantioseparation factor (b) of chlorpheniramine. Keys: (●) $MIP_{TFMAA/T}$; (△) $MIP_{TFMAA/PA}$; (◯) $MIP_{TFMAA/BA}$; (▲) $MIP_{TFMAA/C}$. HPLC conditions: column size, 100 mm × 2.0 mm i.d.; mobile phase, 50 mM potassium phosphate buffer (pH 3.2)–acetonitrile (30:70, v/v); flow rate, 0.4 mL/min; column temperature, 70 °C; detection, 200 nm.

in HPLC. This result suggests the presence of enantioselective binding sites in its swollen state for all the MIPs.

With regard to the number of accessible binding sites for *d*-chlorpheniramine, the loadability of MIP_{TFMAA} was checked. Fig. 3 shows dependence of the retention factor of *d*-chlorpheniramine (k_d) and enantioseparation factor (α) of chlorpheniramine on the loading amount of chlorpheniramine. In the case of $MIP_{TFMAA/C}$, the decrement of the retention factor at a higher loading was the largest among MIP_{TFMAA}. Furthermore, though $MIP_{TFMAA/C}$ gave the highest enantioseparation factor at a 50 ng loading of chlorpheniramine, it decreased drastically with an increase of the loading amount. These results suggest that the number of accessible binding sites for *d*-chlorpheniramine on $MIP_{TFMAA/C}$ could be smaller than the other MIP_{TFMAA}.

$MIP_{TFMAA/C}$ gave the highest enantioseparation factor for chlorpheniramine at a lower sample loading. Thus, $MIP_{TFMAA/C}$ was applied for enantiomeric purity test of *d*-chlorpheniramine. The optimized enantiomer separation of chlorpheniramine on $MIP_{TFMAA/C}$ is shown in Fig. 4a, while Fig. 4b shows a chro-

Table 2
Retention factors, enantioseparation factors and resolution of chlorpheniramine, brompheniramine and pheniramine enantiomers on MIP_{MAA} and MIP_{TFMAA}[a]

	Chlorpheniramine			Brompheniramine			Pheniramine		
	k_d[b]	α[b]	Rs[b]	k_d	α	Rs	k_d	α	Rs
$MIP_{MAA/T}$	9.00	1.80	0.42	9.93	1.77	0.44	4.19	1.28	–
$MIP_{MAA/PA}$	11.8	1.91	0.52	14.8	2.04	0.65	5.06	1.31	–
$MIP_{MAA/BA}$	12.5	1.79	0.68	16.1	1.93	0.71	6.32	1.32	0.37
$MIP_{MAA/C}$	23.4	1.98	0.52	22.0	1.75	0.40	11.3	1.49	0.37
$MIP_{TFMAA/T}$	26.8	1.91	0.75	28.9	1.30	0.78	15.7	1.12	0.51
$MIP_{TFMAA/PA}$	27.1	1.86	0.85	29.9	1.86	0.88	14.7	1.43	0.67
$MIP_{TFMAA/BA}$	19.2	1.82	0.96	23.4	1.96	1.08	11.3	1.43	0.77
$MIP_{TFMAA/C}$	24.0	1.86	0.80	27.8	1.90	0.75	13.9	1.48	0.66

[a] HPLC conditions: column size, 100 mm × 4.6 mm i.d.; mobile phase, 50 mM potassium phosphate buffer (pH 4.5)–acetonitrile (30:70, v/v) for MIP_{MAA} and 50 mM potassium phosphate buffer (pH 3.2)–acetonitrile (30:70, v/v) for MIP_{TFMAA}; column temperature, 25 °C; flow rate, 1.0 mL/min detection, 200 nm; loaded amount, 1000 ng.
[b] k_d: retention factor of the second eluted enantiomer; α: enantioseparation factor; Rs: resolution.

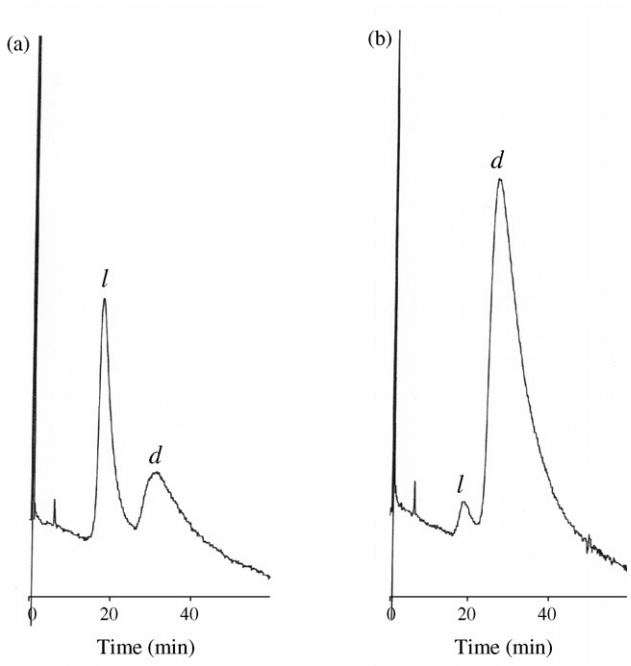

Fig. 4. Separation of chlorpheniramine racemate (a) and *d*-chlorpheniramine (b). HPLC conditions: column, $MIP_{TFMAA/C}$ (50 mm × 4.6 mm i.d.); mobile phase, 50 mM potassium phosphate buffer (pH 4.8)–acetonitrile (30:70, v/v); flow rate, 1.0 mL/min; column temperature, 70 °C; detection, 200 nm; loaded amount, 150 ng for chlorpheniramine racemate and 300 ng for *d*-chlorpheniramine.

matogram of *d*-chlorpheniramine. Using $MIP_{TFMAA/C}$, 1.44% of the *l*-antipode could be separated and determined precisely with a relative standard deviation of 1.8% ($n = 3$).

4. Conclusions

Uniformly sized MIPs for *d*-chlorpheniramine have been prepared by a multi-step swelling and polymerization method using MAA or TFMAA as a functional monomer and toluene, phenylacetonitrile, benzylacetonitrile or chloroform as a porogen. From measurement of their scanning electron microscopy images and physical properties in the dry state, $MIP_{TFMAA/C}$ seemed to be non-porous and had extremely low specific surface areas and pore volumes, while the other MIPs were porous beads with high specific surface areas and pore volumes. All the MIPs prepared showed the similar retentive and enantioselective properties for chlorpheniramine, brompheniramine and pheniramine in HPLC. This result suggests the presence of enantioselective binding sites in its swollen state for all the MIPs. However, it was suggested that the number of accessible binding sites for *d*-chlorpheniramine on $MIP_{TFMAA/C}$ could be smaller than the other MIP_{TFMAA}. Using $MIP_{TFMAA/C}$, 1.44% of the *l*-antipode could be separated and determined precisely with a relative standard deviation of 1.8% ($n = 3$).

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