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Journal of Chromatography A, 864 (1999) 163–171

JOURNAL OF CHROMATOGRAPHY A

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Chiral resolution of basic drugs by capillary electrophoresis with new glycosaminoglycans

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Received 13 November 1998; received in revised form 6 September 1999; accepted 7 September 1999

Abstract

New glycosaminoglycans, fucose-containing glycosaminoglycan (FGAG) and depolymerized holothurian glycosaminoglycan (DHG), were investigated as chiral additives for the separation of drug enantiomers by capillary electrophoresis. The average molecular masses of FGAG and DHG were estimated to be about 59 000 and 14 000, respectively. A variety of basic drug enantiomers were resolved using 10 m*M* phosphate buffer, pH 5.0, containing 3% FGAG or DHG. Since chiral recognition properties of FGAG and DHG are different, some drug enantiomers were only separated by using FGAG or DHG. With regard to comparison of chiral recognition abilities of FGAG and DHG with other chiral selectors, tolperisone and eperisone enantiomers were not separated with α- or β-cyclodextrin, or heparin as the chiral additives, but were separated with FGAG and DHG. The results obtained reveal that FGAG and DHG are useful as the chiral selectors for separations of drug enantiomers by CE, and that they could be complementarily used with other chiral additives.

Keywords: Enantiomer separation; Chiral selectors; Glycosaminoglycans; Basic drugs

1. Introduction

Since there are differences in biological activity and toxicity between enantiomers, chiral resolution is an important subject in the development of an enantiomeric drug [1]. A lot of chromatographic and spectroscopic methods have been developed for the analysis of enantiomers. Among the chromatographic methods so far developed, high-performance liquid chromatographic (HPLC) methods based on chiral stationary phases are widely employed for the assays of drug enantiomers in pharmaceutical preparations and biological fluids [2,3]. However, one column can only separate a limited number of enantiomeric compounds. Recently, capillary electrophoretic (CE) methods using a chiral selector as the running buffer additive have been used for the above purposes [4–10]. The chiral additives so far employed have included polysaccharides, proteins, bile salts and chiral mixed micelles. Glycosaminoglycans such as heparin, chondroitin sulfates A and C, and dermatan sulfate were used for the chiral separation of basic and neutral drugs by CE [11–14]. A lot of enantiomers, which contain at least two nitrogens with one of the nitrogens incorporated in a heterocyclic aromatic ring, were resolved using heparin as the chiral additive [11]. Chondroitin sulfate C was successfully applied to the enantiomer purity test of an enantio-

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0021-9673/99/$ – see front matter
PII: S0021-9673(99)00969-3

meric drug [12]. Though some basic solutes were enatioseparated using chondroitin sulfate A as the chiral additive, chondroitin sulfate C gave higher enantioselectivity than chondroitin sulfate A for the basic solutes tested because of the strong ionic interaction [13]. Dermatan sulfate was useful as the chiral selector for the enantioseparations of a number of basic drugs, particularly for structures characterized by a phenolic moiety [14].

A new glycosaminoglycan, fucose-containing glycosaminoglycan (FGAG), was isolated from the body wall of sea cucumber *Stichopus japonicus*, and depolymerized holothurian (holothurian means sea cucumber) glycosaminoglycan (DHG) was obtained by oxidative depolymerization of FGAG with hydrogen peroxide [15]. DHG is now in clinical trials as an anticoagulant for human use [16,17]. As shown in Fig. 1, DHG was a mixture of types-I, -II and -III in the ratio 5:3:1 [15]. Since FGAG and DHG contained fucose in the molecule, it was expected that their chiral recognition abilities could be different from those of other glycosaminoglycans such as heparin, and chondroitin sulfates A and C. In a previous communication [18], we reported chiral separations of basic drugs by CE using FGAG and DHG as chiral selectors. In this study, we precisely investigated the effects of concentrations of FGAG and DHG, and running buffer concentration and pH on the migration times and resolution of basic drug enantiomers to optimize the proposed method.

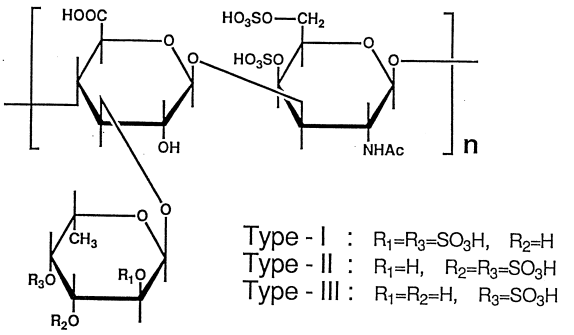

Fig. 1. Structure of DHG.

2. Experimental

2.1. Materials

FGAG and DHG were obtained as reported previously [15]. The average molecular masses of FGAG and DHG were estimated to be about 59 000 and 14 000, respectively, by size-exclusion chromatography with low-angle laser light-scattering detection. The molecular mass distributions (>90%) of FGAG and DHG were 50 000–65 000 and 8000–24 000, respectively. In order to examine the effects of the molecular mass of DHG on the migration times and resolution of various solutes, five kinds of DHG, differing in their molecular masses, were isolated by size-exclusion chromatography. The average molecular masses of the DHGs were estimated to be about 5300, 11 700, 16 000, 22 000 and 25 000. Heparin was obtained from Seikagaku Kogyo (Tokyo, Japan); α- and β-cyclodextrins (CDs) were obtained from Wako (Osaka, Japan).

Tolperisone and propranolol were purchased from Wako. Laudanosine, laudanosorine, tetrahydropapaverine and pindolol were obtained from Sigma-Aldrich (Tokyo, Japan). Trimetoquinol, denopamine and trimebutine were obtained from Tanabe Seiyaku (Osaka, Japan). Epinastine was obtained from Japan Berlinger (Tokyo, Japan). Eperisone and homochlorcyclizine were obtained from Eisai (Tokyo, Japan). Mesityl oxide was obtained from Nacalai Tesque (Kyoto, Japan). The structures of solutes used in this study are shown in Fig. 2. Other reagents used were of analytical reagent grade.

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

2.2. Capillary electrophoresis

CE separations were performed with a Beckman P/ACE system 5500 equipped with a photodiode array detector (Fullerton, CA, USA). Fused-silica capillaries (GL Science, Tokyo, Japan) [57 cm (effective length 50 cm)×75 μm I.D.] were used for separation. All capillaries were thermostated at 23°C by using a liquid coolant.

The electrophoretic buffer (running buffer) solutions used in this study were phosphate buffers

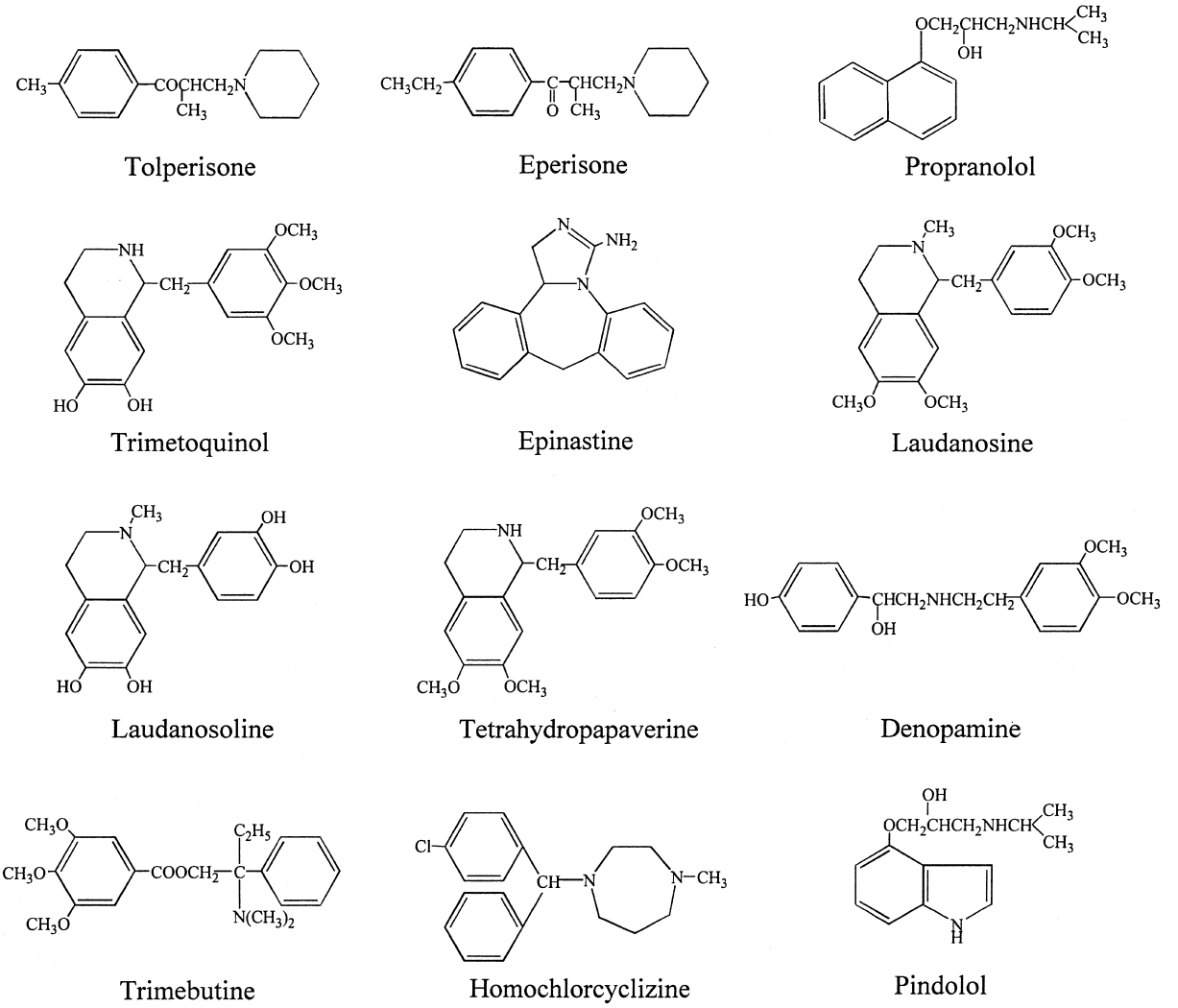

Fig. 2. Structures of the solutes used in this study.

including appropriate concentrations of FGAG or DHG. The running buffer solutions were filtered through a 0.45-μm membrane filter (Gelmann, Tokyo, Japan) and degassed with a Branson model B-2200 ultrasonic bath (Yamato, Tokyo, Japan) prior to use. Stock solutions of samples were prepared in water or methanol. Sample solutions for injection were prepared by dilution of the stock solution with water. The capillary was rinsed with water for 1 min, 0.1 *M* NaOH for 3 min, water for 1 min and the running buffer for 2 min prior to the run. The sample solution was injected at about 3450 Pa for 1 s. Both ends of the capillary were dipped into the running buffer solution, and a constant voltage of 12.0 kV was applied for the separation. Detection was performed at 254 or 214 nm.

Resolution is calculated from the equation $R_s = 2(t_2 - t_1)/(w_2 + w_1)$, where t_1 and t_2 are the migration times of the first- and second-eluted enantiomers, respectively, and w_1 and w_2 are the peak widths of the first- and second-eluted enantiomers, respectively.

Table 1
Effect of running buffer pH on the migration times and resolution of various solutes by CE with FGAG[a]

Solute	Running buffer pH							
	4.0		5.0		6.0		7.0	
	t_R (min)[b]	R_s[c]	t_R (min)	R_s	t_R (min)	R_s	t_R (min)	R_s
Tolperisone	23.36 23.94	2.31	15.06 15.30	1.93	10.94 11.06	1.33	10.63 10.74	1.29
Eperisone	27.22 27.87	2.07	14.69 14.86	1.40	11.17 11.26	1.02	10.42 10.50	0.90
Propranolol	32.41 32.72	0.57	16.73 16.86	0.84	12.24 12.28	<0.5	11.27	–
Trimetoquinol	–	–	22.63 22.98	1.41	14.78 14.93	0.89	13.36 13.43	<0.5
Mesityl oxide	36.89	–	18.84	–	12.68	–	11.19	–

[a] The running buffer solution used is 10 m*M* phosphate buffer containing 3% FGAG; other conditions as in Section 2.
[b] The migration times of the first- and second-eluted enantiomers.
[c] The resolution factor, $R_s = 2(t_2 - t_1)/(w_2 + w_1)$, where t_1 and t_2 are the migration times of the first- and second-eluted enantiomers, respectively, and w_1 and w_2 are the peak widths of the first- first- and second-eluted enantiomers, respectively.

3. Results and discussion

3.1. Effect of running buffer concentration and pH on the migration times and resolution of basic drugs

With regard to buffer species, we checked borate and phosphate buffer. There are no differences in the migration times and resolution of solutes tested between both buffers, when they are used with the same concentration and pH. Since FGAG and DHG are polyanions and sodium salts, the buffer concentration above 10 m*M* gave high values of the current. In this study, we used 10 m*M* phosphate buffer as the running buffer.

Tables 1 and 2 show the effects of running buffer pH on the migration times and resolution of tolperisone, eperisone, propranolol and trimetoquinol, where 10 m*M* phosphate buffer solutions of pH 4.0–7.0 containing 3% FGAG and DHG, respective-

Table 2
Effect of running buffer pH on the migration times and resolution of various solutes by CE with DHG[a]

Solute	Running buffer pH							
	4.0		5.0		6.0		7.0	
	t_R (min)	R_s	t_R (min)	R_s	t_R (min)	R_s	t_R (min)	R_s
Tolperisone	27.12 27.54	1.93	14.38 14.60	2.09	11.33 11.47	1.50	10.62 10.75	1.49
Eperisone	30.97 31.62	2.13	14.61 14.81	1.60	11.46 11.58	1.21	10.28 10.37	1.03
Propranolol	33.19 33.52	<0.5	16.60 16.71	<0.5	12.72 12.76	<0.5	11.18	–
Trimetoquinol	–	–	22.02 22.20	<0.5	15.53	–	13.39	–
Mesityl oxide	31.79	–	16.19	–	12.44	–	10.77	–

[a] The running buffer used is10 m*M* phosphate buffer containing 3% DHG; other conditions as in Section 2.

ly, are used. Additionally, the migration time of mesityl oxide is shown as a tracer of the electroosmotic flow. The currents were constant at about 60 μA under the conditions described above. Since the pK_a values of solutes tested were around 9, the solutes were almost completely protonated under the running buffer pH used in this study. Both the migration times of the solutes and mesityl oxide were decreased with an increase in the running buffer pH. These are due to that the electroosmotic mobility is increased with an increase in the buffer pH as shown in Tables 1 and 2. Higher resolution for the solutes was observed with acidic buffer pH than with neutral buffer pH. Thus, acidic conditions were more effective than neutral conditions for the separation of cationic enantiomers. However, trimetoquinol had not migrated within 60 min using the buffer solution of pH 4.0. We selected pH 5.0 as the running buffer pH. Typical electropherograms of tolperisone, eperisone, propranolol and trimetoquinol obtained with 10 m*M* phosphate buffer, pH 5.0 containing 3% FGAG are shown in Fig. 3.

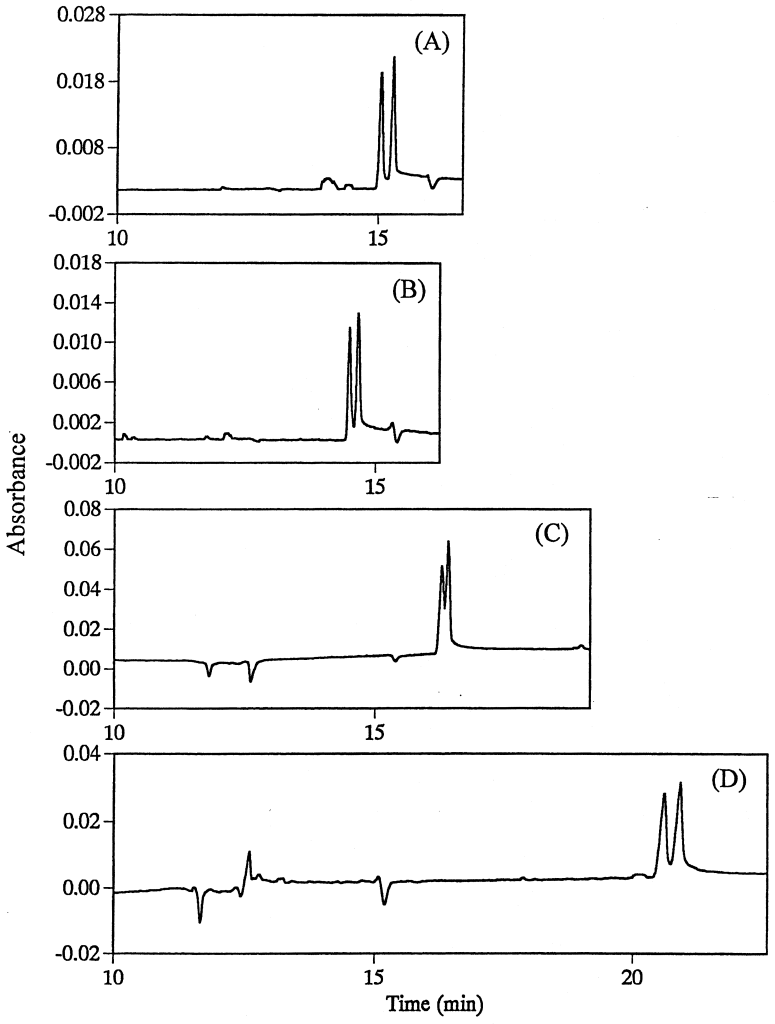

Fig. 3. Electropherograms of tolperisone (A), eperisone (B), propranolol (C) and trimetoquinol (D) obtained with the running buffer, pH 5.0, containing 3% FGAG; other conditions as in Section 2.

Table 3
Effect of FGAG concentration on the migration times and resolution of various solutes[a]

Solute	Concentration of FGAG (%)							
	0		1		3		5	
	t_R (min)	R_s	t_R (min)	R_s	t_R (min)	R_s	t_R (min)	R_s
Tolperisone	5.54	–	11.24 11.35	0.85	15.06 15.30	1.93	15.03 15.27	1.72
Eperisone	5.58	–	10.71 10.79	0.62	14.69 14.86	1.40	14.59 14.76	1.41
Propranolol	5.81	–	11.93	–	16.73 16.86	0.84	16.50 16.62	0.56
Trimetoquinol	5.75	–	13.35 13.46	<0.5	22.63 22.98	1.41	20.71 20.99	1.21

[a] The running buffer solution used is 10 m*M* phosphate buffer, pH 5.0, containing FGAG; other conditions as in Section 2.

3.2. Effect of the concentrations of FGAG and DHG on the migration times and resolution of basic drugs

Tables 3 and 4 show the effects of concentrations of FGAG and DHG, respectively, on the migration times and resolution of tolperisone, eperisone, propranolol and trimetoquinol, where 10 m*M* phosphate buffer, pH 5.0, containing FGAG or DHG is used as the running buffer solution. The concentration range of FGAG or DHG was from 0 to 5%. The electropherograms of tolperisone obtained with 10 m*M* phosphate buffer solution of pH 5.0 containing 0–5% FGAG are shown in Fig. 4. Without addition of FGAG or DHG, no chiral resolution was observed. With an increase in the concentration of FGAG from 0 to 3%, the migration times and resolution of the solutes were increased. However, no change or a slight decrease of the migration time and resolution was observed with 5% FGAG, compared with 3% FGAG. On the other hand, with an increase in the concentration of DHG, the migration times and resolution were increased. These suggest that the interaction of DHG and the solute increases with an increase of the concentration of DHG, but that excess FGAG interferes with the interaction of FGAG and the solute. The molecular mass of FGAG is about 5 times larger than that of DHG. Inter-

Table 4
Effect of DHG concentration on the migration times and resolution of various solutes[a]

Solute	Concentration of DHG (%)							
	0		1		3		5	
	t_R (min)	R_s	t_R (min)	R_s	t_R (min)	R_s	t_R (min)	R_s
Tolperisone	5.54	–	9.98 10.06	<0.5	14.38 14.60	2.09	15.86 16.17	2.22
Eperisone	5.58	–	8.93 9.05	0.85	14.61 14.81	1.60	16.11 16.36	1.76
Propranolol	5.81	–	9.74	–	16.60 16.71	<0.5	18.40 18.54	<0.5
Trimetoquinol	5.75	–	11.93 11.97	<0.5	22.02 22.20	<0.5	25.78 26.05	<0.5

[a] The running buffer solution used is 10 m*M* phosphate buffer, pH 5.0, containing DHG; other conditions as in Section 2.

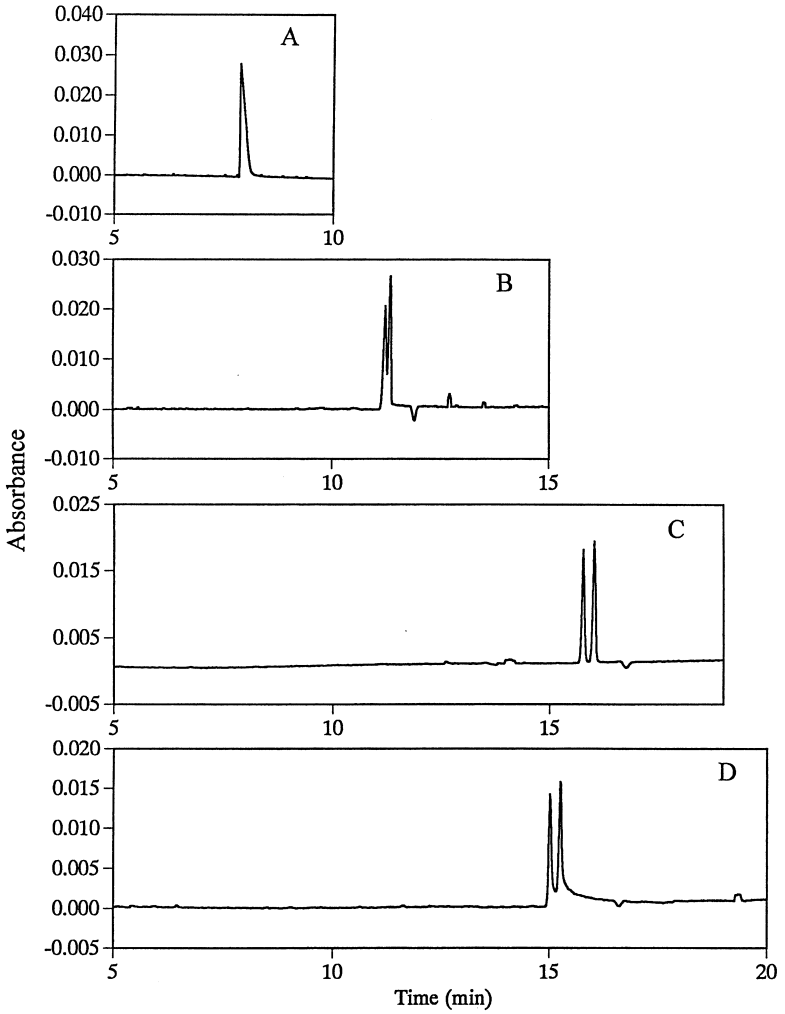

Fig. 4. Electropherograms of tolperisone obtained with the running buffer, pH 5.0, containing 0–5% FGAG. FGAG concentration: (A) 0%; (B) 1%; (C) 3%; (D) 5%; other conditions as in Section 2.

molecular interaction might occur at higher FGAG concentration. Thus, the 3% FGAG or DHG was selected as the concentration of the chiral additive.

3.3. Effect of molecular mass of DHG on enantioselectivity

Table 5 shows the effects of molecular mass of DHG on the migration times and resolution of tolperisone, eperisone, propranolol and trimetoquinol, where 10 m*M* phosphate buffer, pH 5.0, containing 30 m*M* DHG, different in its molecular mass, is used as the running buffer. The average molecular mass range of DHG is from about 5300 to about 25 000. With an increase in the molecular mass of DHG, the migration times of all solutes were increased. The resolution of the solutes except for propranolol was increased with an increase in the molecular mass of DHG. The DHG whose average molecular mass is about 5300, gave the highest enantioselectivity for propranolol. As described above, there are little differences in the migration times and resolution of tolperisone, eperisone and propranolol between FGAG and DHG, when 1 or

Table 5
Effect of molecular mass of DHG on the migration times and resolution of various solutes[a]

Solute	Molecular mass									
	5300		11 700		16 000		22 000		25 000	
	t_R (min)	R_s	t_R (min)	R_s	t_R (min)	R_s	t_R (min)	R_s	t_R (min)	R_s
Tolperisone	10.54	1.30	12.92	1.45	13.84	1.75	16.12	1.81	17.86	2.51
	10.67		13.11		14.07		16.41		18.23	
Eperisone	10.48	1.05	12.89	1.26	14.68	1.54	16.57	1.58	16.97	1.77
	10.59		13.05		14.89		16.82		17.24	
Propranolol	11.06	1.13	14.44	<0.5	16.32	<0.5	18.66	<0.5	19.05	0.70
	11.40		14.49		16.40		18.77		19.22	
Trimetoquinol	12.97	–	18.10	<0.5	20.04	<0.5	25.61	<0.5	26.51	0.95
			18.18		20.22		25.86		26.96	

[a] The running buffer solution used is 10 m*M* phosphate buffer, pH 5.0, containing 30 m*M* DHG; other conditions as in Section 2.

3% of FGAG and DHG was used as the chiral additive. On the other hand, FGAG gave higher enantioselectivity for trimetoquinol than DHG. It is interesting that propranolol is better separated with DHG, whose molecular mass is about 5300, than with the other DHGs tested, and that trimetoquinol is better separated with FGAG, whose molecular mass is about 59 000. The above results suggest that chiral recognition properties of FGAG and DHG are different.

The enantioseparation mechanism in CE with FGAG or DHG is not clear. Nishi et al. discussed the enantioseparation mechanism of the cationic solutes with mucopolysaccharide, where ionic and hydrophobic interactions worked between the solute and chiral selector [12]. With regard to the chiral recognition mechanism with DHG and FGAG, the data described above support that ionic and hydrophobic interactions should work between the cationic solute and anionic chiral selector. In addition to ionic and hydrophobic interactions, inclusion within chiral caviti(es) or pocket(s) of FGAG or DHG, and/or hydrogen bonding interactions with FGAG or DHG might play an important role in chiral recognition of these drug enantiomers, as reported previously [18].

Table 6
Resolution of basic drugs by CE with FGAG or DHG[a]

Solute	R_s	
	FGAG	DHG
Epinastine	1.22	1.00
Laudanosine	–	<0.5
Laudanosorine	–	<0.5
Tetrahydropapaverine	0.69	<0.5
Denopamine	–	<0.5
Trimebutine	–	0.92
Homochlorcyclizine	0.94	1.81
Pindolol	0.87	–

[a] The running buffer solution used is 10 m*M* phosphate buffer, pH 5.0, containing 3% FGAG or DHG.

3.4. Enantioseparation of basic drugs by FGAG and DHG

Chiral resolution of basic drug enantiomers was investigated by using 10 m*M* phosphate buffer, pH 5.0, containing 3% FGAG or DHG. The results are summarized in Table 6. The enantioseparations of epinastine, tetrahydropapaverine and homochlorcyclizine were achieved by FGAG and DHG in addition to tolperisone, eperisone, propranolol and trimetoquinol. DHG gave higher enantioselectivity than FGAG for homochlorcyclizine. On the other hand, FGAG gave higher enantioselectivity for pindolol. Chiral resolution of laudanosine, laudanosorine, denopamine and trimebutine was only successful by using DHG, and that of pindolol was only attained by using FGAG. It is plausible that some drug enantiomers could be only separated by using DHG or FGAG. Because their chiral recognition properties are different, as described above.

In conclusion, a variety of basic drug enantiomers were resolved using 10 m*M* phosphate buffer, pH 5.0, containing 3% FGAG or DHG. The results

obtained reveal that FGAG and DHG are useful as the chiral selectors for separations of drug enantiomers by CE, and that they could be complementarily used with other chiral additives.

Acknowledgements

The authors wish to thank Ms. Y. Miyano and Ms. N. Kanasugi for their skillful technical assistance.

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