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Chiral separation of drugs using cyclodextrins in capillary zone electrophoresis

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ABSTRACT

Chiral separation in capillary zone electrophoresis was investigated employing four kinds of cyclodextrins (CDs). The effects of the type and amount of CDs added to the background electrolyte and the pH of the buffer solution on the resolution of enantiomers were examined. The best enantioselectivity was obtained by employing heptakis(2,6-di-O-methyl)-β-cyclodextrin in acidic solution. In particular, the enantiomeric separation of denopamine, which is a potent cardiotonic agent, the direct enantiomeric separation of which had not been successful, was achieved by the method. The effects of buffer, urea and an organic solvent on the enantioselectivity of three enantiomeric drugs were also investigated.

INTRODUCTION

Capillary electrophoresis (CE) has become a powerful separation technique during the past decade because of its separation efficiency and high resolution, and several different separation modes, from capillary gel electrophoresis to micellar electrokinetic chromatography (MEKC), have been developed [1,2]. In particular, MEKC permits the separation of electrically neutral solutes by the CE system [3,4] and has been used in the determination of almost every kind of analyte.

The enantiomeric separation of drugs is an important subject of interest in the pharmaceutical and medical fields, because stereochemistry can have a significant effect on the biological activity of a drug. Further, it is important to develop chiral separation methods for the determination of the optical purity of drugs from the viewpoint of quality control, because the antipode of a chiral drug is regarded as one of the impurities.

A variety of chromatographic approaches, particularly those using high-performance liquid chromatography (HPLC), have been developed [5,6]. Recently much work has been reported on the direct resolution of enantiomers by chiral stationary phases and a wide variety of such HPLC stationary phases are now commercially available [7,8].

Chiral separation by CE, which includes both MEKC and capillary zone electrophoresis (CZE), is a relatively recent technique [9]. There are many advantages of CE compared with HPLC. Direct chiral separation has been performed easily using CE by adding chiral surfactants or chiral compounds, which interact with the enantiomeric solute, to the buffer solution without changing the capillary tube. High resolution is achieved within a short time.

In MEKC, chiral separation has been achieved by using a chiral surfactant such as bile salts [10–12] or sodium N-dodecanoyl-L-valinate [13–16]. Other than using chiral surfactants, the

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chiral separation of some dansyl-DL-amino acids has been achieved by electrokinetic chromatography with cyclodextrin derivatives, which have both chirality and an ionic group within a molecule, as a carrier [17]. Chiral separation in MEKC with sodium dodecyl sulphate (SDS) solution was also successful by adding cyclodextrins (CDs) to the buffer solutions [18–20]. This method is called cyclodextrin-modified MEKC (CD-MEKC) and was first developed for the determination of highly hydrophobic solutes such as aromatic hydrocarbons [21,22]. CDs have also been successfully used for enantiomeric separation in CZE [23–27] or CGE [28] under acidic conditions. Differential inclusion complex formation of the CD with the solute provides differential solute migration and chiral recognition.

This paper describes the chiral separation of drugs by CZE using CDs (CD-CZE). The effect of the type of CD on the chiral recognition of about 30 enantiomeric compounds was investigated. The influence of the amount of CD added to the background electrolyte, the pH value, buffer concentrations, addition of urea and an organic solvent on the resolution of enantiomers was also examined by using three enantiomeric drugs and heptakis(2,6-di-O-methyl)-β-cyclodextrin (DM-β-CD). CD-MEKC and CD-CZE are briefly compared.

EXPERIMENTAL

Apparatus

A fused-silica capillary of 55 cm length (effective length 40 cm) × 0.05 mm I.D. (Scientific Glass Engineering, Ringwood, Victoria, Australia) was used as a separation tube. An HCZE-30PN0.25 high-voltage d.c. power supply (Matsusada Precision Devices, Kusatsu, Shiga, Japan) delivering from −25 to +25 kV was used to drive the separation. The migrating solutes were detected by the on-column measurement of UV absorption at 210–220 nm with an SPD-6A spectrophotometer (Shimadzu, Kyoto, Japan) with a time constant of 0.05 s using a laboratory-made cell holder and a slit. A Chromatopac C-R5A (Shimadzu) was used for data processing. Other apparatus and experimental procedures were as reported previously [10].

Reagents

Four types of CD, β-cyclodextrin (β-CD), DM-β-CD, heptakis(2,3,6-tri-O-methyl)-β-cyclodextrin (TM-β-CD) and γ-cyclodextrin (γ-CD), were obtained from Nacalai Tesque (Kyoto, Japan). All other reagents and solvents were of analytical-reagent grade from Katayama Kagaku Kogyo (Osaka, Japan). Water was purified with a Milli-RO 60 water system (Millipore Japan, Tokyo, Japan). Each CD solution was prepared by dissolving the CD in a 25 m*M* phosphate buffer solution containing urea; the solution was passed through a membrane filter of 0.45-μm pore size (Gelman Science, Tokyo, Japan) and degassed by sonication with a Branson B-2200 ultrasonic cleaner (Yamato, Tokyo, Japan) prior to use.

About 30 enantiomeric drugs or compounds, including acidic and basic solutes, were used as test solutes for the investigation of the chiral recognition of CDs. The structures of some of them, which were successfully separated by the method, are shown in Fig. 1. Enantiomers of denopamine and trimetoquinol hydrochloride were obtained from Tanabe Seiyaku (Osaka, Japan). Etilefrine hydrochloride (Japanese Pharmacopoeia grade) was purchased from Iwaki Seiyaku (Tokyo, Japan). Other compounds were purchased from Wako (Osaka, Japan), Nakalai Tesque and Aldrich (Milwaukee, WI, USA). The sample solutions were prepared by dissolving each solute in methanol at an approximate concentration of 1 mg/ml so that adequate peak heights could be obtained.

RESULTS AND DISCUSSION

Chiral recognition of CDs

The effect of the type of CDs on the chiral recognition of about 30 enantiomeric drugs was investigated by using 25 m*M* phosphate buffer solution (pH 3.0) containing 2 *M* urea and 20 m*M* of each CD. Urea was added to increase the solubility of CDs in the aqueous phase [29]. An acidic buffer was selected from preliminary studies, because no chiral separation was observed under the neutral and alkaline conditions (the concentration of CD used was 20 m*M*).

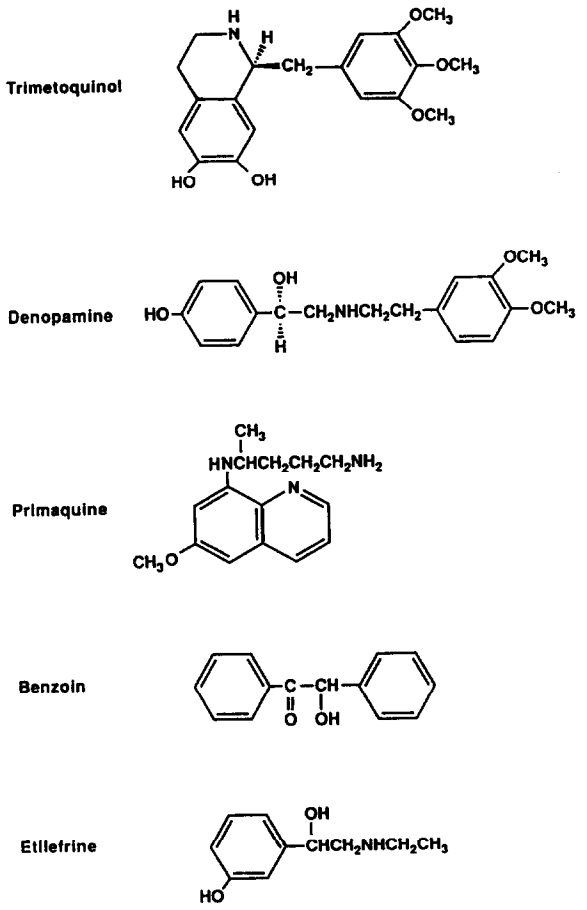

Fig. 1. Structures of the solutes.

Chiral recognition was observed in all CDs, and basic solutes were more effectively enantioresolved than acidic solutes in the CD-CZE mode employing acidic buffers. The numbers of enantiomerically resolved solutes were as follows: β-CD, six solutes (trimetoquinol, its derivatives, chlorpheniramine, propranolol); DM-β-CD, eleven solutes (trimetoquinol, its derivatives, primaquine, denopamine, phenylephrine, etilefrine, pindolol, atenolol, 2,2′-dihydroxy-1,1′-dinaphthyl); TM-β-CD, four solutes (trimetoquinol, primaquine, pindolol, chlorpheniramine); and γ-CD, four solutes (promethazine, laudanosine, benzoin, primaquine). The enantiomeric separation of an acidic solute (naproxen), diltiazem, its derivatives and warfarin was unsuccessful.

Chiral recognition depended on the type of CD. Among the four CDs employed, DM-β-CD was the most effective for the chiral recognition of the solutes. Typical electropherograms of primaquine and benzoin using γ-CD are shown in Fig. 2. These two compounds were optically resolved within 12 min better than all others in a γ-CD buffer system of pH 3.0. Other solutes were poorly enantioresolved with buffer solution of pH 3.0.

In MEKC using SDS solution and CDs, that is, in the CD-MEKC mode, chiral separation was most successful when employing γ-CD [18]. The difference in the type of CD offering the best enantioselectivity between CD-MEKC and CD-CZE can probably be interpreted by the presence of a surfactant monomer, as reported previously [18]. In CD-CZE, the cavity size of β-type CDs (seven glucose units) conforms well with the size of the solutes having aromatic rings.

Concentration effects of CDs on migration and chiral recognition

The effects of CD concentration on the migration times and chiral recognition were investigated by using 25 m*M* phosphate buffer solution (pH 2.5) containing 2 *M* urea and DM-β-CD

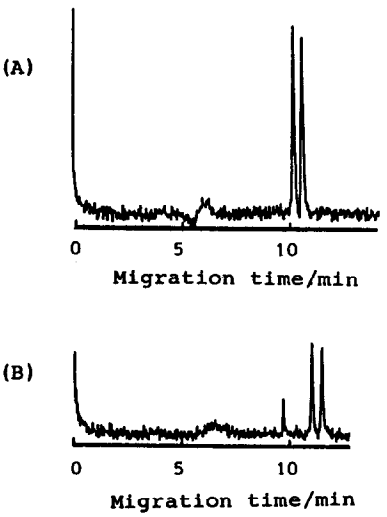

Fig. 2. Chiral separation of (A) primaquine and (B) benzoin. Conditions: buffer, 25 m*M* phosphate buffer (pH 3.0) containing 2 *M* urea and 20 m*M* γ-CD; separation tube, 0.05 mm I.D. × 550 mm (effective length 400 mm); applied voltage, 20 kV; detection, 220 nm (0.08 AUFS); temperature, ambient.

over the concentration range 10–40 m*M*. DM-β-CD was selected on the basis of the widest enantioselectivity, and was also used in subsequent investigation. Denopamine, trimetoquinol and primaquine were used as the test sample because these were completely separated from one another in the DM-β-CD buffer system, although most of other enantioresolved solutes (described above) eluted around at the peak of trimetoquinol under the conditions used. The results are shown in Figs. 3 and 4. The migration times of all solutes increased with an increase in the CD concentration.

The relationship between the migration times and CD concentration can be expressed by the equation [28]

$$t_R = \frac{l}{\mu E} = \frac{l(1+K[C])}{\mu_f E} \quad (1)$$

assuming $\mu_f \gg \mu_c$, where μ_f is the mobility of the free solute and μ_c is the complexed solute, t_R = migration time, l = effective length of capillary, E = electric field, $[C]$ = concentration of CD and K = complex equilibrium constant. Eqn. 1 shows that a linear relationship exists between the migration time of the solute and the concentration of CD. This relationship was certainly observed with primaquine, which migrated first among the three solutes. The migration times of trimetoquinol and denopamine, which migrated more slowly than primaquine, however, did not increase linearly with increasing concentration of CD. The assumption $\mu_f \gg \mu_c$ probably did not apply with these solutes.

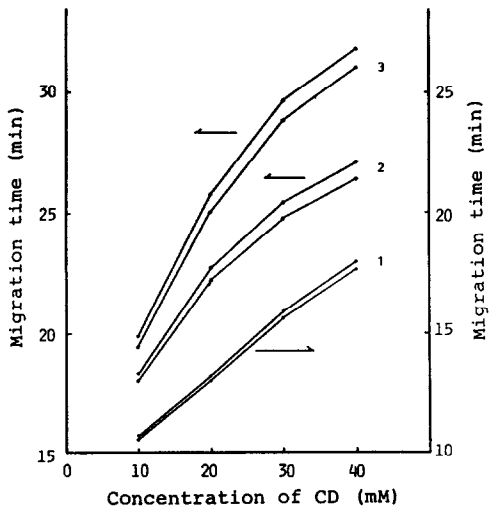

Fig. 3. Dependence of the migration times and chiral recognition on CD concentration. Solutes: 1 = primaquine; 2 = trimetoquinol; 3 = denopamine. Buffer, 25 m*M* phosphate buffer (pH 2.5) containing 2 *M* urea and DM-β-CD. Other conditions as in Fig. 2.

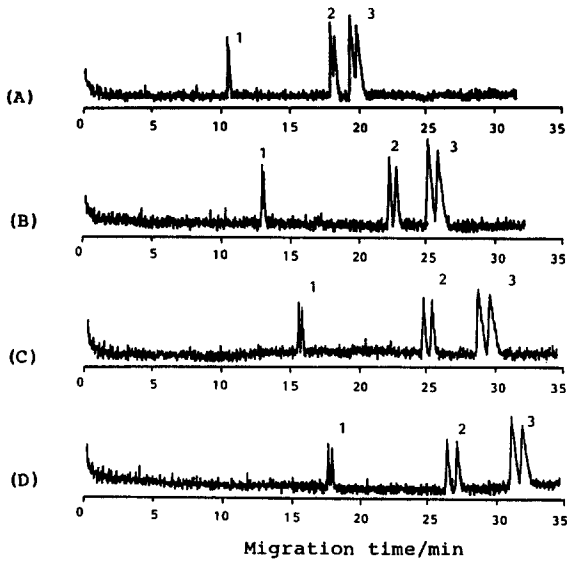

Fig. 4. Effects of CD concentration on the migration times and chiral recognition: (A) 10; (B) 20; (C) 30; (D) 40 m*M*. Conditions as in Fig. 3.

Tables I and II show the differences in the migration times and the ratios of the migration times of enantiomeric pairs. These values were used instead of the resolution R_s for the evaluation of enantioselectivity because exact plate numbers of the solute could not be calculated because of the noisy baseline and non-Gaussian peak shape. It was found that there is an optimum concentration of CD, $[C]$, which gives a maximum separation of the two enantiomers. This has been reported by Wren and Rowe [30]. The optimum concentration depends inversely on the affinity of the enantiomeric solutes for CD, that is, solutes having large K values require a low CD concentration for optimum separation. The optimum CD concentration for primaquine, trimetoquinol and denopamine were *ca.* 40, 40 and 30 m*M*, respectively.

TABLE I

EFFECT OF CD CONCENTRATION ON THE DIFFERENCE IN MIGRATION TIMES OF ENANTIOMERS

Buffer, 25 m*M* phosphate buffer (pH 2.5) containing 2 *M* urea and DM-β-CD. Applied voltage, 20 kV. Ambient temperature.

Concentration of DM-β-CD (m*M*)	Difference in migration times (min)		
	Primaquine	Trimetoquinol	Denopamine
10	0.12	0.29	0.49
20	0.14	0.51	0.75
30	0.25	0.65	0.84
40	0.27	0.72	0.79

TABLE II

EFFECT OF CD CONCENTRATION ON THE RATIOS OF MIGRATION TIMES OF ENANTIOMERS

Buffer, 25 m*M* phosphate buffer (pH 2.5) containing 2 *M* urea and DM-β-CD. Applied voltage, 20 kV. Ambient temperature.

Concentration of DM-β-CD (m*M*)	Ratio of migration times		
	Primaquine	Trimetoquinol	Denopamine
10	1.011	1.016	1.025
20	1.011	1.023	1.030
30	1.016	1.026	1.029
40	1.015	1.027	1.025

Effects of buffer pH on migration and chiral recognition

The effects of buffer pH on the migration times and chiral recognition were investigated using 25 m*M* phosphate buffer solution containing 2 *M* urea and 20 m*M* DM-β-CD (pH range 2.5–3.5). The solutes tested were denopamine, trimetoquinol and primaquine. The results are shown in Fig. 5. The migration times of denopamine and trimetoquinol decreased with increase in pH. This can be interpreted by an increase in the velocity of electroosmotic flow (EOF) at high pH [31]. On the other hand, the migration time of primaquine increased with increase in pH. At pH 3.5 (not shown in Fig. 5), it eluted with almost the same migration time as denopamine. Primaquine is probably positively charged at pH 2.5, and this causes the solute to have a fast migration time at pH 2.5.

Migration time differences and migration time ratios are summarized in Tables III and IV. The

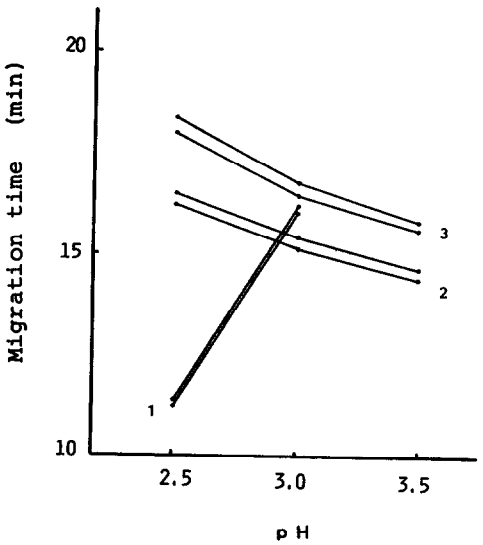

Fig. 5. Dependence of the migration times and chiral recognition on buffer pH. Buffer, 25 m*M* phosphate buffer containing 2 *M* urea and 20 m*M* DM-β-CD. Detection, 220 nm (0.04 AUFS). Other conditions as in Fig. 3.

TABLE III

EFFECT OF BUFFER pH ON THE DIFFERENCE IN MIGRATION TIMES OF ENANTIOMERS

Buffer, 25 m*M* phosphate buffer containing 2 *M* urea and 20 m*M* DM-β-CD. Applied voltage, 20 kV. Ambient temperature.

Buffer pH	Difference in migration times (min)		
	Primaquine	Trimetoquinol	Denopamine
2.5	0.13	0.26	0.38
3.0	0.15	0.22	0.32
3.5	–	0.22	0.24

enantioselectivity decreased with increase in pH. Lower pH values gave a higher enantioseparation for the solutes tested. An example of the enantiomeric separation of denopamine and trimetoquinol using buffer solution of pH 2.2 is presented in Fig. 6, showing good peak shapes and enantioseparation in comparison with separation at pH 2.5 or 3.0 (see Fig. 4B). In both solutes, active enantiomers [(*R*)-trimetoquinol and (*S*)-denopamine] migrated more slowly than the corresponding inactive enantiomers.

Effects of concentration of buffer on migration and chiral recognition

The effects of buffer concentration on the migration times and chiral recognition were investigated using phosphate buffer solution (25–100 m*M*) of pH 2.5 containing 2 *M* urea and 20 m*M* DM-β-CD. The solutes tested were denopamine, trimetoquinol and primaquine. Separation of the enantiomers was impaired with increasing concentration of the buffer, as shown in Fig. 7. This result conflicts with that of Kuhn

TABLE IV

EFFECT OF BUFFER pH ON THE RATIOS OF MIGRATION TIMES OF ENANTIOMERS

Buffer, 25 m*M* phosphate buffer containing 2 *M* urea and 20 m*M* DM-β-CD. Applied voltage, 20 kV. Ambient temperature.

Buffer pH	Ratio of migration times		
	Primaquine	Trimetoquinol	Denopamine
2.5	1.011	1.016	1.021
3.0	1.010	1.015	1.019
3.5	–	1.015	1.016

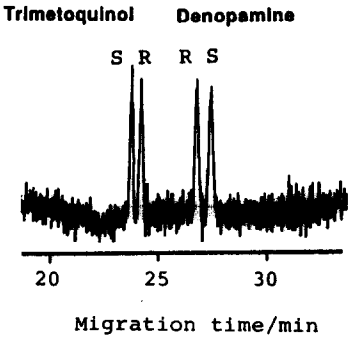

Fig. 6. Chiral separation of enantiomers of trimetoquinol and denopamine. Buffer, 25 m*M* phosphate buffer (pH 2.2) containing 2 *M* urea and 20 m*M* DM-β-CD. Other conditions as in Fig. 3.

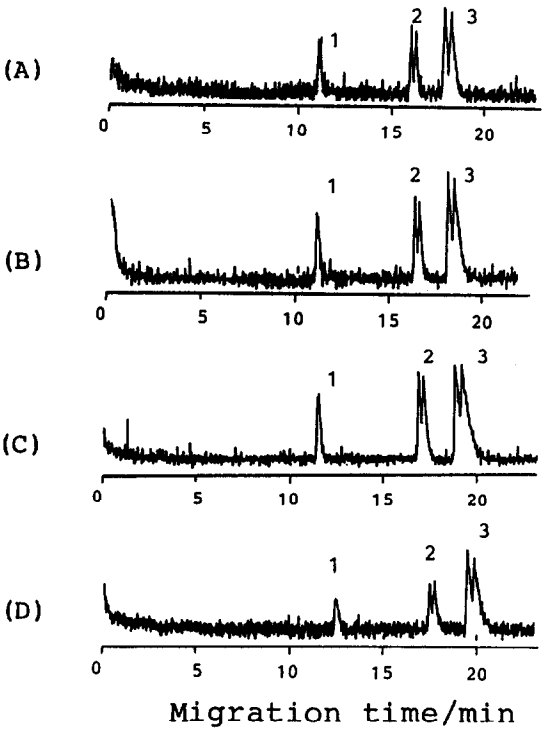

Fig. 7. Effects of buffer concentration on the migration times and chiral recognition: (A) 25; (B) 50; (C) 75; (D) 100 m*M*. Buffer, phosphate buffer (pH 2.5) containing 2 *M* urea and 20 m*M* DM-β-CD. Other conditions as in Fig. 3.

et al. [27]. This opposite result may be explained by the capillary temperature. In our apparatus, the capillary temperature rises rapidly owing to Joule heating as the buffer concentration increases through the increase in current. The resolution decreases with increasing temperature owing to enhanced band broadening. In the apparatus without a cooling system, the concentration of the phosphate buffer should be kept low.

Effects of the addition of urea or organic solvent on chiral recognition

Urea increases the solubility of lipophilic solutes in the aqueous phase by diminishing the water structure around the alkyl group [32]. The addition of urea would be expected to decrease the affinity of the solute for CD and increase it for bulk buffer in a similar manner to the addition of an organic solvent, although urea was initially used to promote the dissolution of CDs [29].

The effects of urea concentration on the migration times and chiral recognition were investigated using 25 m*M* phosphate buffer solution (pH 2.5) containing urea and 20 m*M* DM-β-CD. The results are summarized in Tables V and VI and Fig. 8. The migration times increased with increase in urea concentration, probably owing to the decrease in the velocity of EOF [33]. The enantiomeric separation of denopamine and trimetoquinol was impaired with increase in urea concentration. The enantioselectivity of primaquine was improved with increase of urea concentration. It was found that there is an optimum concentration of urea, similarly to CD concentration, as expected.

The effect of the addition of an organic solvent

TABLE V

EFFECT OF UREA CONCENTRATION ON THE DIFFERENCE IN MIGRATION TIMES OF ENANTIOMERS

Buffer, 25 m*M* phosphate buffer (pH 2.5) containing urea and 20 m*M* DM-β-CD. Applied voltage, 20 kV. Ambient temperature.

Urea concentration (*M*)	Difference in migration times (min)		
	Primaquine	Trimetoquinol	Denopamine
2	0.14	0.51	0.70
4	0.21	0.42	0.64
6	0.22	0.29	0.50
8	0.22	0.27	0.45

TABLE VI

EFFECT OF UREA CONCENTRATION ON THE RATIOS OF THE MIGRATION TIMES OF ENANTIOMERS

Buffer, 25 m*M* phosphate buffer (pH 2.5) containing 2 *M* urea and 20 m*M* DM-β-CD. Applied voltage, 20 kV. Ambient temperature.

Urea concentration (*M*)	Ratio of the migration times		
	Primaquine	Trimetoquinol	Denopamine
2	1.011	1.023	1.028
4	1.015	1.019	1.026
6	1.015	1.013	1.020
8	1.014	1.011	1.018

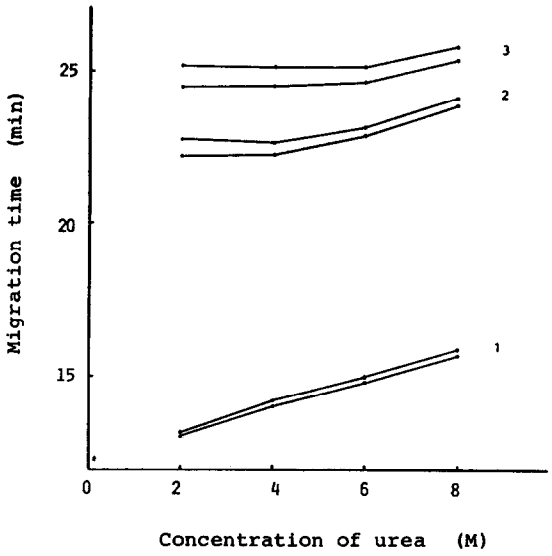

Fig. 8. Effects of urea addition on the migration times and chiral recognition. Buffer, 25 m*M* phosphate buffer (pH 2.5) containing urea and 20 m*M* DM-β-CD. Other conditions as in Fig. 3.

was investigated by using methanol. Methanol (up to 20%) was added to the above buffer solution. Organic solvents are expected to play a similar role as in urea addition. As expected from the investigation of urea addition, the enantioseparation of denopamine and trimetoquinol was impaired through the addition of methanol, compared with the separation in the absence of methanol, and the migration times of the solutes were increased owing to the decrease in the velocity of EOF.

From the result that the enantioselectivity of denopamine and trimetoquinol was impaired with increase in urea concentration or in the presence of methanol, *i.e.*, a decrease in the equilibrium constant K, it was thought that 20 m*M* DM-β-CD was lower than the optimum concentration for denopamine and trimetoquinol.

In conclusion, it was found that the type and concentration of CDs, the pH, the concentration of buffer solutions and addition of urea or an organic solvent affect the enantioselectivity in CD-CZE. The best enantioselectivity was obtained by employing DM-β-CD, although γ-CD was the most successful in CD-MEKC. It was also found that there is an optimum CD concentration. Acidic buffer solutions were well suited for enantiomeric separations of drugs, especially basic drugs, in the CD-CZE mode. An enantiomeric separation can be optimized by changing the type and concentration of the CD, the pH of the buffer and adding urea or an organic solvent.

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