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Chiral separation by cyclodextrin-modified micellar electrokinetic chromatography

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ABSTRACT

Chiral separation by micellar electrokinetic chromatography (MEKC), which permits the separation of uncharged or electrically neutral compounds by the electrophoretic technique, was achieved through the addition of cyclodextrins (CDs) to sodium dodecyl sulphate (SDS) micelle solution. In this cyclodextrin-modified MEKC (CD-MEKC), CDs cannot be solubilized to the SDS micelle and migrate with the same velocity as that of the electroosmotic flow. The solutes are distributed among three phases, an aqueous phase, the micelle and the CD. Chiral recognition depended on the type of CD; in particular, γ-CD was effective for the chiral separation in this method. The addition of an organic solvent or a chiral compound such as sodium *d*-camphor-10-sulphonate or *l*-menthoxyacetic acid to the SDS micelle solution containing CDs improved the enantioselectivity. The addition of CDs reduced the capacity factors of solutes; in contrast, chiral additives increased them. The resolution was optimized by changing the concentrations of CDs and chiral additives. The chiral separation mechanism is also briefly discussed.

INTRODUCTION

Micellar electrokinetic chromatography (MEKC) [1] is a recently developed high-resolution separation method [2–5]. MEKC, which uses the capillary zone electrophoretic (CZE) technique, permits the separation of uncharged or electrically neutral compounds [2,3] and offers greatly improved selectivity for the separation of ionic compounds [4–7].

Stereochemistry can have a significant effect on the biological activity of a drug. Racemic drugs exhibit pharmacological activities and/or side-effects different from those of the optically pure drugs [8]. It is therefore important to develop a chiral separation method for the determination of optical purity. A variety of chromatographic approaches, particularly those using high-performance liquid chromatography (HPLC), have been developed [9]. Recently, much work has been reported on the direct resolution of enantiomers by chiral stationary phases and a wide variety of the stationary phases are now commercially available.

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In MEKC, chiral separation has been achieved by using a chiral surfactant such as bile salts [10–12] or sodium N-dodecanoyl-L-valinate [13,14]. A mixed micelle of chiral surfactants as mentioned above or chiral compounds such as digitonin [13] with achiral sodium dodecyl sulphate (SDS) [12–15] has also been successfully used for the chiral separation of some DL-amino acid derivatives.

The resolution of enantiomers by cyclodextrin-modified MEKC (CD-MEKC) with SDS [16] was investigated in this work. CDs are widely used in analytical applications, especially as a mobile phase additive or a chiral moiety of the stationary phase in HPLC [9]. CDs cannot be solubilized into the SDS micelle employed in MEKC, because of the hydrophilic nature of the external portion. In CD-MEKC the solutes are distributed among three phases, an aqueous phase, the micelle and the CD. Differential inclusion-complex formation of a CD with the solute provides the differential solute migration and chiral recognition. CDs were used successfully for chiral separation in high-performance capillary gel electrophoresis by Guttman *et al.* [17] and in CZE by Fanali [18]. Chiral separation by cyclodextrin EKC, in which cyclodextrin derivatives are employed as a carrier, was reported by Terabe [1].

Chiral separation by high-performance capillary electrophoresis (HPCE), to which both MEKC and CZE belong, is a relatively new technique. There are many advantages of the HPCE mode compared with HPLC. Chiral separation has been achieved easily in the HPCE mode 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. HPCE should have other capabilities and advantages for chiral separations which are now being investigated.

This paper describes the chiral separation of enantiomers of some drugs and chemicals by employing five types of CDs with SDS solutions. The effects of CD concentration, organic solvent addition and chiral compound addition on chiral recognition are discussed. A possible chiral separation mechanism is suggested.

EXPERIMENTAL

Apparatus for MEKC

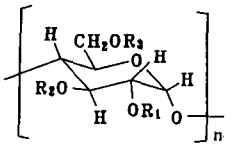
A fused-silica capillary of length 65 cm (effective length 50 cm) and I.D. 0.05 mm (Scientific Glass Engineering, Ringwood, Australia) was used as a separation tube. A Model HJLL-25PO high-voltage d.c. power supply (Matsusada Precision Devices, Kusatsu, Shiga, Japan) delivering from 0 to 25 kV was used to drive the MEKC separation. The migrating solutes were detected by the on-column measurement of UV absorption at 220 nm with an SPD-6A spectrophotometer (Shimadzu, Kyoto, Japan) at a time constant of 0.05 s using a laboratory-made cell holder and a slit. A Chromatopac C-R5A instrument (Shimadzu) was used for data processing. Other apparatus and experimental procedures were the same as described previously [6].

Reagents

SDS and five types of CDs, α-cyclodextrin (α-CD) β-cyclodextrin (β-CD), 2,6-di-O-methyl-β-cyclodextrin (DM-β-CD), 2,3,6-tri-O-methyl-β-cyclodextrin (TM-β-CD) and γ-cyclodextrin (γ-CD), were obtained from Nacalai Tesque (Kyoto, Japan).

TABLE I

PHYSICO-CHEMICAL PROPERTIES OF CYCLODEXTRINS USED



Parameter	α-CD	β-CD	DM-β-CD	TM-β-CD	γ-CD
n	6	7	7	7	8
R_1	H	H	CH_3	CH_3	H
R_2	H	H	H	CH_3	H
R_3	H	H	CH_3	CH_3	H
Molecular weight	973	1135	1331	1430	1297
Cavity diameter (Å)	5–6	7–8	7–8	7–8	9–10

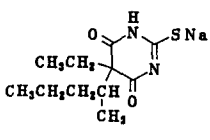
Some physico-chemical properties of CDs are summarized in Table I [19]. Sodium *d*-camphor-10-sulphonate and *l*-menthoxyacetic acid were obtained from Nacalai Tesque. *l*-Menthoxyacetic acid was converted to the sodium salt form by titrating the solution with 1 *M* sodium hydroxide solution. All other 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). The micellar solution was prepared by dissolving 0.05 *M* SDS in 0.02 *M* phosphate–borate buffer solution of pH 9.0 and the solution was passed through a membrane filter of 0.45-μm pore size (Gelman Science, Japan, Tokyo) and degassed by sonication with a Branson Model B-2200 ultrasonic cleaner (Yamato, Tokyo, Japan) prior to use.

Five enantiomeric drugs or compounds and two achiral barbiturates were used as test solutes. Thiopental (sodium salt) and pentobarbital (calcium salt) were obtained from the research laboratories at Tanabe Seiyaku (Osaka, Japan). Phenobarbital and sodium barbital were purchased from Wako (Osaka, Japan). 2,2′-Dihydroxy-1,1′-dinaphthyl and 1,1′-binaphthyl-2,2′-diyl hydrogenphosphate were purchased from Aldrich (Milwaukee, WI, U.S.A.). 2,2,2-Trifluoro-1-(9-anthryl)ethanol was purchased from Nacalai Tesque. Chemical structures of the solutes are shown in Fig. 1. The sample solutions were prepared by dissolving each solute in methanol at concentration of *ca.* 1 mg/ml so that adequate peak heights could be obtained. Sudan IV from Aldrich or diltiazem derivative from the research laboratory at Tanabe Seiyaku were used as tracers of the micelle. These solutes migrated last in all the experiments, although these may be partially included in the CD.

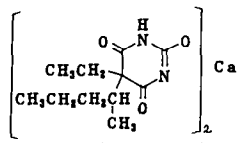
RESULTS AND DISCUSSION

Chiral recognition of CDs

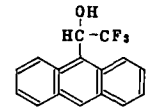
The effect of the type of CD on the chiral recognition of five enantiomeric solutes was first investigated by using a buffer solution of 0.05 *M* SDS and 4 *M* urea, to which each CD was added. Calculated capacity factors ($\tilde{k}'$), according to the



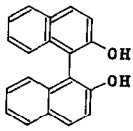
1. Thiopental (sodium)



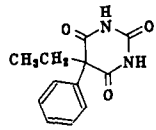
2. Pentobarbital (calcium)



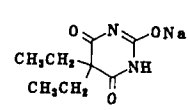
3. 2,2,2-Trifluoro-1-(9-anthryl)ethanol



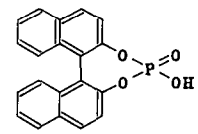
4. 2,2'-Dihydroxy-1,1'-dinaphthyl



5. Phenobarbital



6. Barbital (sodium)



7. 1,1'-Binaphthyl-2,2'-diyl hydrogen phosphate

Fig. 1. Structures of the solutes.

equation in ref. 2, and separation factors (α) are summarized in Table II. The concentrations of CDs were 15 mM for DM-β-CD, TM-β-CD and γ-CD and 40 mM for α-CD and β-CD to give $\tilde{k}' \approx 1$ for two enantiomeric barbiturates. The concentration effects (extent of reduction of migration times) of the former were larger than those of the latter [20].

Chiral recognition of the solutes was observed with four CDs, except for α-CD. Enantiomers of thiopental and of pentobarbital were only resolved by using γ-CD, although their resolution was not sufficient. A typical chromatogram of four enantiomeric solutes using a 0.05 M SDS solution containing 30 mM γ-CD is shown in Fig. 2. Chiral recognition depended on the type of CD. Among the five CDs, γ-CD was the most effective for the chiral recognition of the solutes. These results are

TABLE II

CHIRAL RECOGNITION BY FIVE CYCLODEXTRINS

Buffer, 0.05 M SDS buffer solution of pH 9.0 containing 4 M urea and CDs. Applied voltage, 20 kV. Ambient temperature.

Solute (No.)	α-CD (40 mM)		β-CD (40 mM)		DM-β-CD (15 mM)		TM-β-CD (15 mM)		γ-CD (15 mM)	
	$\tilde{k}'$	α	$\tilde{k}'$	α	$\tilde{k}'$	α	$\tilde{k}'$	α	$\tilde{k}'$	α
Thiopental (1)	0.92	1	0.78	1	1.01	1	1.38	1	1.06	1.03
	–		–		–		–		1.09	
Pentobarbital (2)	0.92	1	0.88	1	1.06	1	1.28	1	1.13	1.02
	–		–		–		–		1.15	
Binaphthyl phosphate (7)	1.49	1	1.19	1.04	2.64	1.09	3.65	1.09	1.45	1.03
	–		1.24		2.88		3.97		1.50	
Dinaphthyl (4)	11.84	1	9.87	1	14.62	1	15.32	1.08	20.06	1.08
	–		–		–		16.51		21.66	
Anthrylethanol (3)	15.05	1	10.94	1.04	16.62	1	48.57	1	23.26	1.48
	–		11.42		–		–		34.32	

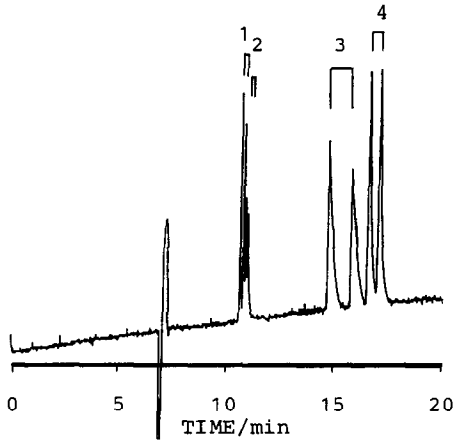


Fig. 2. Chiral separation of four enantiomers. Samples are identified by the same numbers as given in Fig. 1. Conditions: buffer, 0.02 *M* phosphate–borate buffer of pH 9.0 containing 0.05 *M* SDS and 30 m*M* γ-CD; separation tube, 650 mm × 0.05 mm I.D. (effective length 500 mm); applied voltage, 20 kV; detection, 220 nm; temperature, ambient.

different, however, from the results in HPLC using CDs as chiral additives to the mobile phase or as a chiral moiety of the stationary phase [9]. The cavity diameter (fitness of the solute) and hydrophobic nature of the internal portion of CDs probably influenced the effective or differential inclusion-complex formation of the enantiomers, leading to differential migration and successful chiral separation.

In a micellar solution, a monomeric surfactant, which is in equilibrium with the micelle, exists in an aqueous phase and it can be included by the CD because of the presence of the lipophilic hydrocarbon chain. This may prevent the solute from inclusion. CDs having wider cavity such as γ-CD have the capability of including the solute together with the surfactant monomer. A schematic illustration is shown in Fig. 3. The cavity size that is most suitable for chiral recognition is different between

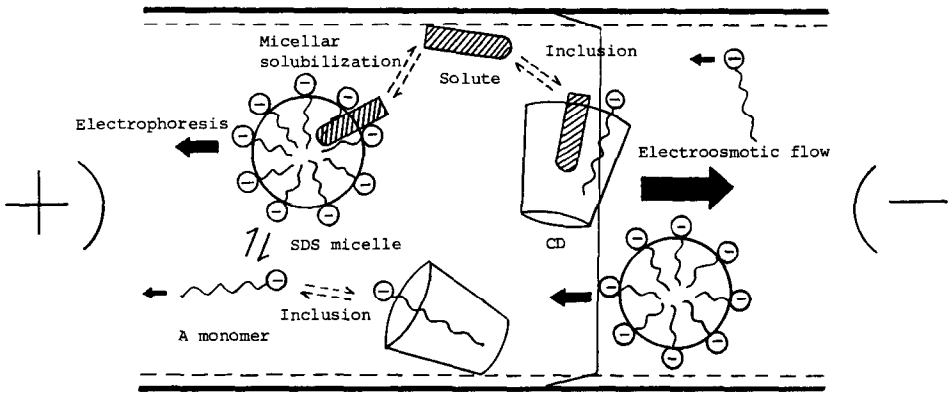


Fig. 3. Schematic illustration of the solute interaction in MEKC using SDS and CD.

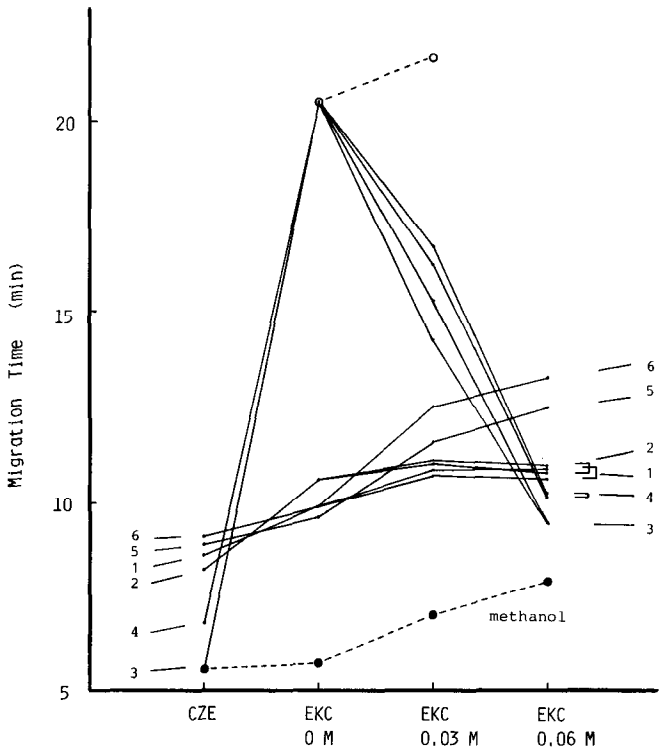


Fig. 4. Effects of γ-CD concentrations on the migration times and chiral recognition. Solute numbers as in Fig. 1. CZE, 0.02 *M* phosphate–borate buffer of pH 9.0. EKC, 0.05 *M* SDS added to CZE buffer. Open and closed circles represent the migration times of the micelle and methanol, respectively. Other conditions as in Fig. 2.

CD-MEKC and HPLC with CDs, where β-type CDs are the most effective [9]. The difference is interpreted in terms of the presence of the surfactant monomer in CD-MEKC.

Concentration effects of CDs on migration and chiral recognition

The effects of CD concentration on the migration times and chiral recognition were investigated using γ-CD over the concentration range 0–0.06 *M*. The results are shown in Fig. 4, with the results of CZE and MEKC without addition of CDs. The migration times or capacity factors of the solutes, especially of aromatic compounds except two achiral barbiturates, were considerably reduced with an increase in the CD concentration. It is therefore useful to use CDs in MEKC of lipophilic compounds, which migrate near the migration time of the micelle and cannot be resolved by the SDS solution alone, as reported previously [16,20].

In SDS solution containing CDs, a solute is distributed among three phases as mentioned above. A CD will not be solubilized by the micelle and migrate with the same velocity as that of the electroosmotic flow, except for methylation-type CDs. The stable inclusion-complex formation of the solute with the CD thus brings about a faster migration of the solute under the experimental conditions (pH 9.0), where the

electroosmotic flow is stronger than the electrophoretic mobility of the micelle [3].

As for the enantiomers, inclusion-complex formation with the CD can provide diastereomeric pairs of complexes. The difference in the stability of the diastereomeric inclusion complexes permits successful chiral separation. Theoretically, the optimum $\tilde{k}'$ for the highest resolution in MEKC can be calculated by the equation $\tilde{k}' = (t_{mc}/t_0)^{1/2}$, where t_{mc} and t_0 are the migration time of the micelle and that of an unincorporated solute, respectively [21]. The migration velocity of the solute in CD-MEKC should then be adjusted through the CD or micelle concentration to give the optimum resolution.

Effect of organic modifiers

Selectivity in MEKC can be manipulated through modification of micellar solution [1], in addition to varying the type of surfactant [22]. An organic modifier is useful for separations of aromatic amines [23] and cold medicine ingredients, in which ionic and non-ionic solutes are involved [24]. The effects of addition of methanol (10%) on the chiral recognition or capacity factors of four enantiomeric solutes were investigated using a 0.05 *M* SDS solution containing 30 m*M* γ-CD. The results are summarized in Table III. A typical chromatogram of the enantiomers of 2,2'-dihydroxy-1,1'-dinaphthyl and 2,2,2-trifluoro-1-(9-anthryl)ethanol is shown in Fig. 5. For these lipophilic compounds, the addition of an organic modifier was effective in changing the capacity factors and peak shapes. The capacity factors of the solutes were reduced by the addition of methanol, although their migration times increased because of the reduction of the electroosmotic flow. Chiral recognition of the enantiomers became poor and a single peak was observed from the enantiomers of pentobarbital. Methanol was probably included in the CD cavity, which will be unfavourable for the chiral recognition of the enantiomers of these barbiturates.

Effects of chiral additives

To enhance the chiral recognition of the solutes (from insufficient separation as shown in Fig. 2), the effect of some chiral additives, which can be included in the CD cavity, on the chiral recognition of the enantiomers was investigated. The effects of

TABLE III

EFFECTS OF METHANOL ADDITION ON SEPARATION FACTOR AND CAPACITY FACTOR

Buffer, 0.05 *M* SDS buffer solution of pH 9.0 containing 30 m*M* γ-CD.

Solute	No methanol		10% methanol	
	$\tilde{k}'$	α	$\tilde{k}'$	α
Thiopental	1.048	1.06	0.922	1.03
	1.114		0.950	
Pentobarbital	1.150	1.03	0.980	1
	1.194		–	
Dinaphthyl	3.055	1.34	2.486	1.27
	4.089		3.150	
Anthrylethanol	5.269	1.15	3.890	1.10
	6.055		4.297	

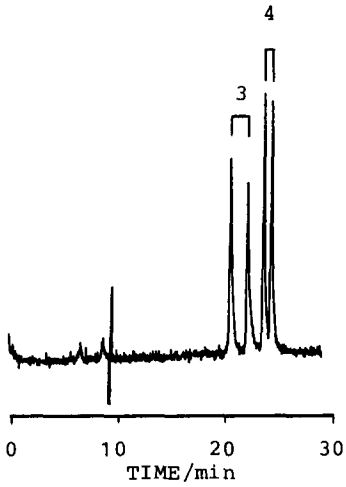


Fig. 5. Chiral separation of two lipophilic compounds by the addition of methanol. Buffer, 10% methanol in the same buffer solution as in Fig. 2. Other conditions as in Fig. 2.

addition of sodium *d*-camphor-10-sulphonate to the SDS solution containing γ-CD on the chiral recognition of four enantiomeric solutes are summarized in Table IV. A typical chromatogram is shown in Fig. 6. The separation factor and resolution of the enantiomers were improved with increasing concentration of the chiral additive, except for 2,2'-dihydroxy-1,1'-dinaphthyl, whose capacity factors are the largest among the four solutes. An increase in $\bar{k}'$ will reduce the resolution because of deviation from the optimum capacity factor mentioned above.

The effects of *l*-menthoxyacetic acid (sodium salt form) on the chiral recognition of the same solutes are summarized in Table V. The resolution increased with

TABLE IV

EFFECT OF SODIUM *d*-CAMPHOR-10-SULPHONATE ON THE ENANTIOSELECTIVITY

Buffer, 0.05 *M* SDS containing 30 m*M* γ-CD and *d*-camphor-10-sulphonate.

Solute	*d*-Camphor-10-sulphonate								
	0 m*M*			20 m*M*			40 m*M*		
	$\bar{k}'$	α	R_s	$\bar{k}'$	α	R_s	$\bar{k}'$	α	R_s
Thiopental	1.048	1.06	1.76	1.052	1.06	1.87	1.167	1.07	2.18
	1.114			1.117			1.247		
Pentobarbital	1.150	1.03	0.83	1.225	1.03	1.06	1.493	1.04	1.29
	1.194			1.265			1.549		
Anthrylethanol	3.055	1.34	3.08	3.442	1.20	4.22	4.362	1.22	4.40
	4.089			4.145			5.315		
Dinaphthyl	5.269	1.15	2.29	9.033	1.12	2.08	56.0	1.33	1.34
	6.055			10.07			74.3		

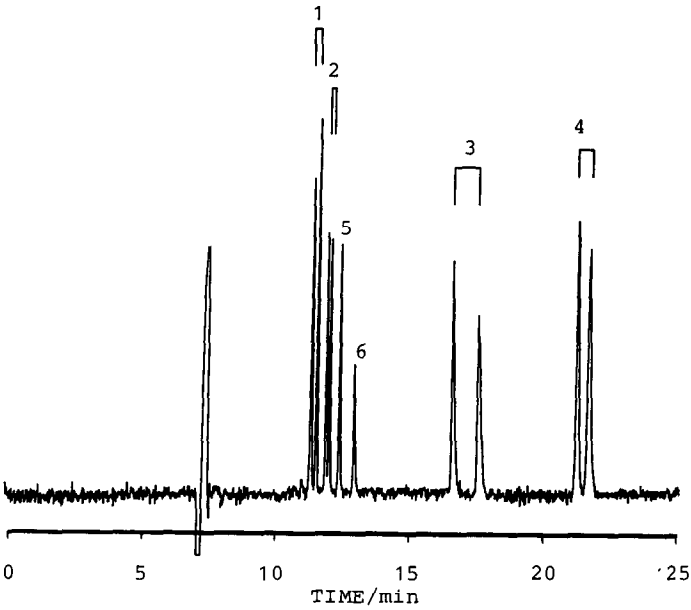


Fig. 6. Chiral separation by the addition of sodium *d*-camphor-10-sulphonate. Buffer, 20 m*M* sodium *d*-camphor-10-sulphonate in the same buffer solution as in Fig. 2. Other conditions as in Fig. 2.

the addition of the chiral compound up to 60 m*M*. In particular, the separation of two barbiturates and the peak shapes of the solutes were substantially improved. However, a high concentration of the chiral additive (90 m*M*) reduced resolution because of an increase in $\tilde{k}'$. A suitable concentration is selected to obtain the optimum resolution for the separation of each enantiomeric solute. A typical chroma-

TABLE V

EFFECT OF *l*-MENTHOXYACETIC ACID ON THE ENANTIOSELECTIVITY

Buffer, 0.05 *M* SDS containing 30 m*M* γ-CD and *l*-menthoxyacetic acid.

Solute	*l*-Menthoxyacetic acid								
	30 m*M*			60 m*M*			90 m*M*		
	$\tilde{k}'$	α	R_s	$\tilde{k}'$	α	R_s	$\tilde{k}'$	α	R_s
Thiopental	1.103	1.07	1.77	1.095	1.07	1.97	1.320	1.06	1.99
	1.178			1.167			1.398		
Pentobarbital	1.391	1.03	0.91	1.461	1.04	1.18	1.843	1.03	0.96
	1.436			1.515			1.903		
Anthrylethanol	9.751	1.49	3.21	11.99	1.27	3.66	17.27	1.24	3.14
	14.56			15.17			21.47		
Dinaphthyl	21.04	1.25	2.31	21.26	1.26	2.40	29.03	1.38	3.05
	26.40			26.70			40.20		

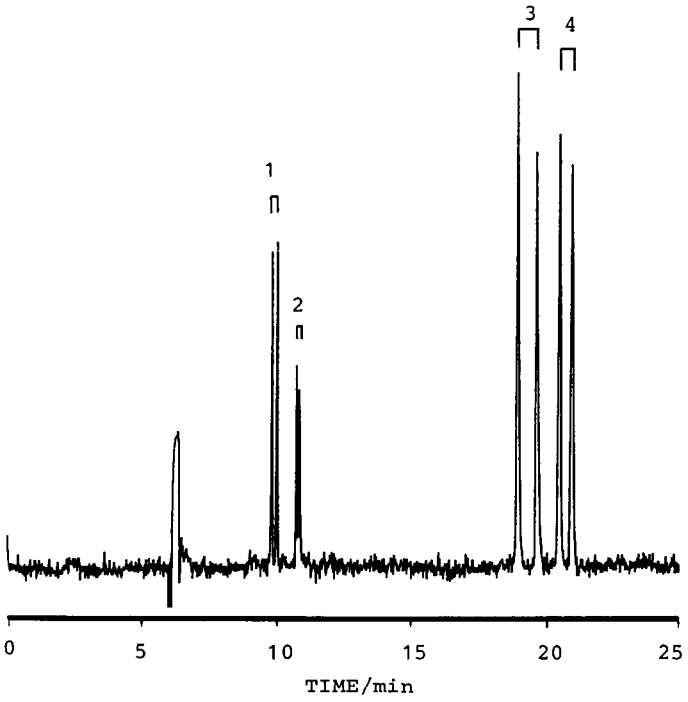


Fig. 7. Chiral separation by the addition of *l*-menthoxyacetic acid. Buffer, 0.06 *M* *l*-menthoxyacetic acid in the same buffer solution as in Fig. 2. Other conditions as in Fig. 2.

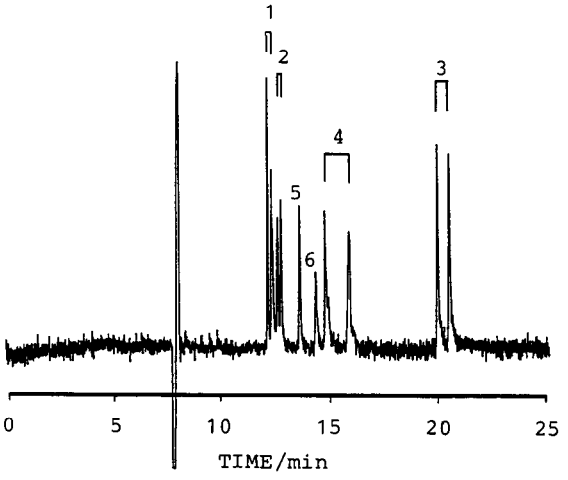


Fig. 8. Chromatogram of four enantiomeric solutes and two achiral solutes. Buffer, 0.05 *M* SDS solution of pH 9.0 containing 40 m*M* γ-CD and 20 m*M* sodium *d*-camphor-10-sulphonate. Other conditions as in Fig. 2.

togram obtained with addition of 60 m*M* *l*-menthoxyacetic acid is shown in Fig. 7. The migration times and capacity factors of the solutes increased with an increase in the concentration of these chiral additives, as shown in Tables IV and V.

This marked improvement in enantioselectivity may be ascribed to the inclusion of these chiral additives, which enhance the chirality of the CD cavity. The solute will be included in the γ-CD cavity together with the chiral additive. The cavity of γ-CD is large enough to include these two compounds in comparison with other CDs. It is well known that γ-CD often includes two molecules of the guest compound [19]. The interactions of the solute with both the CD and the chiral additive, through the hydrophobic or ionic portions, will probably give enhanced enantioselectivity.

Optimum chiral separation will be obtained by adjusting the concentrations of CDs and chiral additives. The addition of the former reduces the migration time of the solute; in contrast, the latter increases that of the solute. A chromatogram of four enantiomeric solutes and two achiral solutes is shown in Fig. 8, where a 0.05 *M* SDS solution of pH 9.0 containing 40 m*M* γ-CD and 20 m*M* sodium *d*-camphor-10-sulphonate was employed.

Chiral separation of aromatic compounds

The enantiomers of 2,2′-dihydroxy-1,1′-dinaphthyl were successfully resolved by using γ-CD and partially resolved by TM-β-CD. Chiral separation of 2,2,2-trifluoro-1-(9-anthryl)ethanol was only successfully achieved by using γ-CD. However, successful chiral separation of the enantiomers of 1,1′-binaphthyl-2,2′-diyl hydrogenphosphate was achieved by using DM-β-CD and TM-β-CD rather than γ-CD. This result also differs from that of the chiral recognition of two barbiturates, as shown in Table II. This indicates that the ionic group of the solute affects the chiral recognition in CD-MEKC. The selection of the pH of the buffer solution is important for the

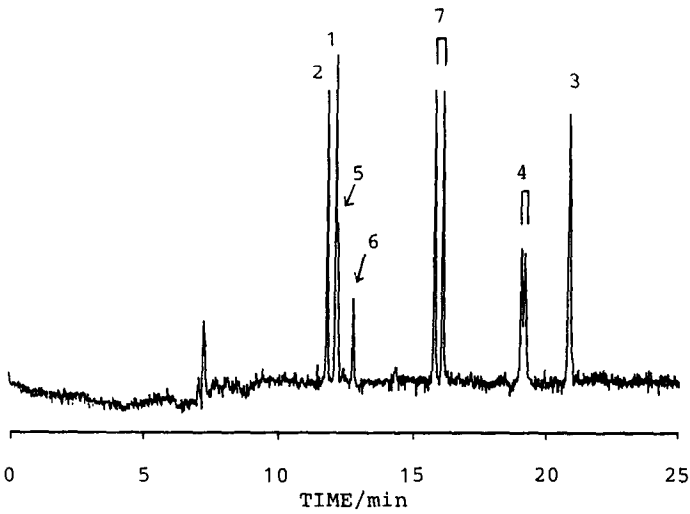


Fig. 9. Chiral separation using TM-β-CD. Buffer, 0.05 *M* SDS solution of pH 9.0 containing 15 m*M* TM-β-CD, 4 *M* urea and 40 m*M* sodium *d*-camphor-10-sulphonate. Other conditions as in Fig. 2.

successful chiral recognition of ionic enantiomeric solutes, in addition to the concentration and type of CDs. An example of the separation of 1,1'-binaphthyl-2,2'-diyl hydrogenphosphate is shown in Fig. 9, where the enantioselectivity is enhanced by adding sodium *d*-camphor-10-sulfonate.

Chiral separation of barbiturates

The separation of four barbiturates including two enantiomeric drugs was investigated by using several modes of capillary electrophoresis such as CZE and MEKC. As for the chiral separation of the enantiomers, addition of γ-CD to the SDS

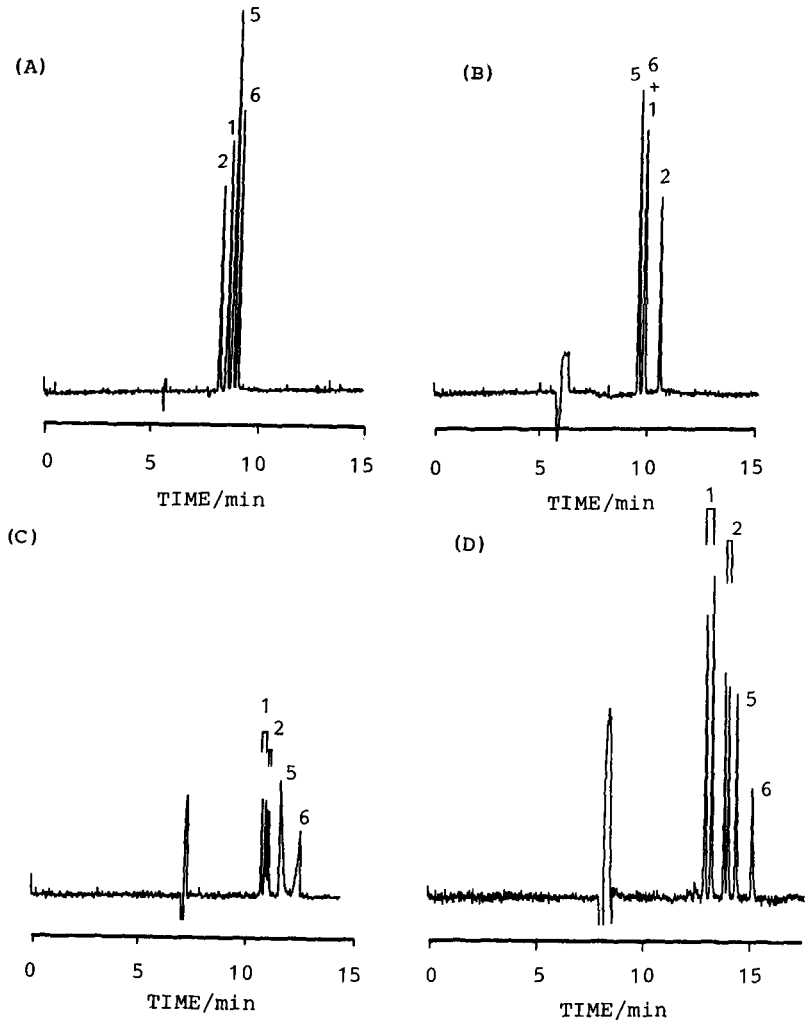


Fig. 10. Separation of four barbiturates including two enantiomeric drugs using different modes: (A) CZE using a 0.02 *M* phosphate–borate buffer solution of pH 9.0; (B) MEKC mode, 0.05 *M* SDS added to buffer in (A); (C) MEKC mode, 30 m*M* γ-CD added to buffer in (B); (D) CD-MEKC mode, 40 m*M* γ-CD and 40 m*M* sodium *d*-camphor-10-sulphonate added to buffer in (B). Other conditions as in Fig. 2.

solution was useful and a chiral additive in the SDS solution enhanced the enantioselectivity. These chromatograms are shown in Fig. 10. The migration times of thiopental and pentobarbital were reduced more than those of phenobarbital and sodium barbital by the γ-CD addition and therefore the migration order changed. The successful chiral separation of two enantiomeric drugs can be ascribed to the strong tendency of these drugs to interact with γ-CD. All the solutes including each enantiomer were separated by MEKC using γ-CD and sodium *d*-camphor-10-sulphonate as shown in Fig. 10D.

CONCLUSIONS

It was found that enantiomers of some drugs and chemicals can be successfully separated by CD-MEKC with SDS solutions. Chiral recognition was dependent on the type of CD and especially the cavity size. γ-CD was the most useful of five CDs studied and addition of some chiral additives such as sodium *d*-camphor-10-sulphonate or *l*-menthoxyacetic acid to the SDS solution containing CDs enhanced the enantioselectivity. Optimum capacity factors of the enantiomers for high resolution can be adjusted through the concentrations of CDs and chiral additives. Ionic interaction of the solute was one of the important factors for the chiral recognition of ionic enantiomers.

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