Separation of Corticosteroids and Aromatic Hydrocarbons by Cyclodextrin-Modified Micellar Electrokinetic Chromatography

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SEPARATION OF CORTICOSTEROIDS AND AROMATIC HYDROCARBONS BY CYCLODEXTRIN-MODIFIED MICELLAR ELECTROKINETIC CHROMATOGRAPHY

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ABSTRACT

  Micellar electrokinetic chromatography (MEKC) permits the separation of uncharged or electrically neutral compounds by the electrophoretic technique, but highly lipophilic compounds, e.g., corticosteroids and aromatic hydrocarbons could not be resolved by MEKC with sodium dodecyl sulfate (SDS) solutions because such solutes migrated with almost the same velocity as that of the SDS micelle, owing to their large micellar solubilization. The addition of cyclodextrins (CDs) to the SDS solution, that is, cyclodextrin-modified micellar electrokinetic chromatography (CD/MEKC), remarkably improved the resolution of these lipophilic compounds. The effects of concentration and the type of CDs on the separation of corticosteroids and aromatic hydrocarbons were investigated.

INTRODUCTION

  Micellar electrokinetic chromatography (MEKC) is a relatively new type of analytical separation method, which belongs to a capillary zone electrophoretic (CZE) technique but whose separation principle is based on that of chromatography (1,2). The solute distributes itself between the micellar phase and the sourrounding aqueous phase. The separation is

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achieved by the differential solute distribution and the differential
migration of the micelle.

MEKC first permitted the separation of uncharged or electrically neutral
solutes by the electrophoretic technique (1-4) with high resolution and a
shorter analytical time than conventional high-performance liquid
chromatography (HPLC). In addition to neutral solutes, MEKC has brought
about many advantages for the separation of ionic compounds (5-7).

Selectivity in MEKC can be manipulated by changing the type of
surfactant (2,7), in addition to varing buffer constituents, ionic
strength, pH and temperature as well as in CZE. Changes in micellar
composition can produce micelles of different sizes, aggregation numbers and
geometries, leading to the different interaction or solubilization.
Selectivity can also be improved through the modification of the buffer
solution, using urea (8) or tetraalkylammonium (TAA) salts (9). The
addition of urea to the sodium dodecyl sulfate (SDS) solution caused a
decrease of the capacity factor of lipophilic compounds by increasing their
solubility in the aqueous phase. The addition of TAA salts to the SDS
solution caused a decrease of the migration times of cationic solutes, on
the contrary, an increase of those of anionic solutes just like the ion-pair
chromatographic method in HPLC.

Cyclodextrins (CDs) are widely used in analytical applications,
especially as a mobile phase additive or a chiral moiety of the stationary
phase in HPLC (10). It has been already reported that the use of CDs in
MEKC, it is called cyclodextrin-modified MEKC (CD/MEKC) (11), is effective
for the separation of lipophilic and closely related aromatic compounds (12).
Enantiomers of some drugs and compounds were also successfully separated by
CD/MEKC (13).

This paper describes the applicability of CD/MEKC to the separation of
highly lipophilic corticosteroids and some aromatic compounds. These
solutes could not be successfully separated by MEKC using an SDS solution
because of their large micellar solubilization. The effects of concentration
and species of CDs on the migration times of the solutes and the
selectivity are presented. The addition of CDs provided lipophilic solutes

with smaller migration times than those without CDs, hence improving the
separation of the compounds.

EXPERIMENTAL

Apparatus of MEKC

MEKC was performed in a fused-silica capillary that was 65 cm long
(effective length 500 mm) and had a 0.05 mm i.d. (Scientific Glass
Engineering Inc., Ringwood, Victoria, Australia). A high-voltage dc power
supply Model HJLL-25PO (Matsusada Precision Devices, Kusatsu, Shiga, Japan)
delivering from 0 to + 25 kV was used to drive MEKC separation. Detection
was carried out by the on-column measurement of UV adsorption at 220 nm with
a SPD-6A spectrophotometer (Shimadzu, Kyoto, Japan) at a time constant 0.05
s with a laboratory-made cell holder and a slit. A Chromatopac C-R5A
(Shimadzu, Kyoto, Japan) was used for data processing. Other apparatus and
experimental procedures were the same as those descreibed previously (5).

Reagents

Sodium dodecyl sulfate (SDS), urea, β-cyclodextrin (β-CD) and γ-
cyclodextrin (γ-CD) were obtained from Nacalai Tesque (Kyoto, Japan).
Water was purified by Milli-RO 60 water system (Millipore Japan, Tokyo,
Japan). Sudan III from Nacalai Tesque or 1,5-benzothiazepin derivatives
(13) were used as a tracer of the micelle (2). The micellar solution was
prepared by dissolving SDS in a 0.02 M phosphate-borate buffer solution and
these solutions were passed through a membrane filter of 0.45 μm pore
size (Gelman Science Japan, Tokyo) and degassed by sonication with an
ultrasonic cleaner prior to use.

Eight corticosteroids from Sigma Chemical Company (St. Louis, U.S.A.),
and nine aromatic compounds from Tokyo Kasei Kogyo (Tokyo, Japan) were used
as the test sample. These are summarized in Table I with the indication
symbols. All solutes were used as they were received and dissolved in
methanol at the concentration of 0.2 - 1 mg/ml to give adequate peak
heights. All other reagents and solvents used were of analytical-reagent
grade from Katayama Kagaku Kogyo.

Table I Test Samples

Aromatic hydrocarbons	Symbol	Corticosteroids	Symbol
toluene	1	hydrocortisone	a
naphthalene	2	hydrocortisone acetate	b
9-fluorenone	3	betamethasone	c
fluorene	4	cortisone acetate	d
xanthene	5	triamsinolone acetonide	e
dibenzyl	6	fluosinolone acetonide	f
phenanthlene	7	dexamethasone acetate	g
trans-stilbene	8	fluosinonide	h
fluoranthene	9		

RESULTS AND DISCUSSION

Effect of CDs addition to the SDS solution on the migration of eight corticosteroids and that of nine aromatic compounds was investigated employing two types of CDs. The result of 0.03 M β-CD addition to the 0.05 M SDS solution of pH 9.0 containing 4 M urea is shown in Figure 1. A typical chromatogram of nine aromatic compounds using a buffer solution of pH 9.0 containing 0.05 M SDS, 0.03 M β-CD and 4 M urea is shown in Figure 2. Urea was used to increase the solubility of the CDs in the aqueous phase (14). Migration times or capacity factors of all the solutes became shorter than those in the 0.05 M SDS solution of pH 9.0 without CD. Migration order was also altered by the addition of β-CD. These indicate that the solute was included into the CD and the interaction between the CD and the solute was different among the solutes tested. Trans-stilbene, fluorene and dibenzyl were effectively included by β-CD, resulting in larger change in the migration times.

In a buffer solution containing SDS and CD, a solute is distributed among three phases, that is, the aqueous phase, the micelle phase and the CD. Since CD has the hydrophilic outside surface due to the primary and secondary hydroxy groups in the CD molecule, it will not be solubilized by the micelle and will be transported with the same velocity as that of the electroosmotic flow. Therefore, the stable inclusion-complex formation of the solute with CD brings about faster migration of the solute under the
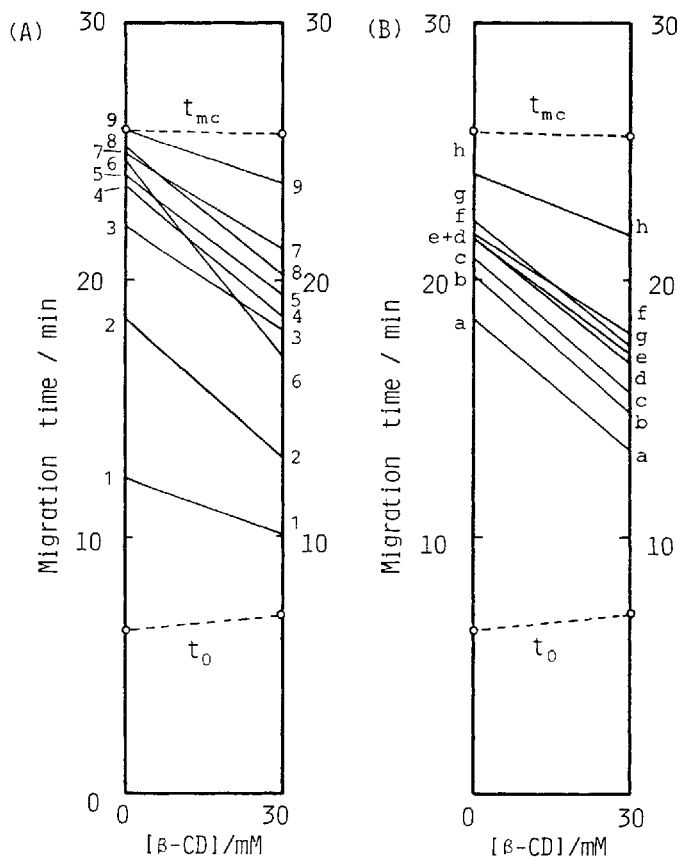
Figure 1. Effect of β-CD addition on the migration times of (A) nine aromatic hydrocarbons and (B) eight corticosteroids. Conditions: capillary, 500 mm effective length x 0.05 mm i.d.; applied voltage, 20 kV; buffer, a 0.02 M phosphate-borate buffer of pH 9.0 containing 0.05 M SDS and 4 M urea with or without CD; temperature, ambient; detection, 220 nm.

experimental conditions (pH 9.0), where the electroosmotic flow is strong compared with the micelle electrophoretic mobility. Stability of inclusion-complex formation is different among the solutes, hence migration order is altered. Accordingly, CD addition to the SDS solution in MEKC improves the selectivity of the solutes, which can be included by the CD.

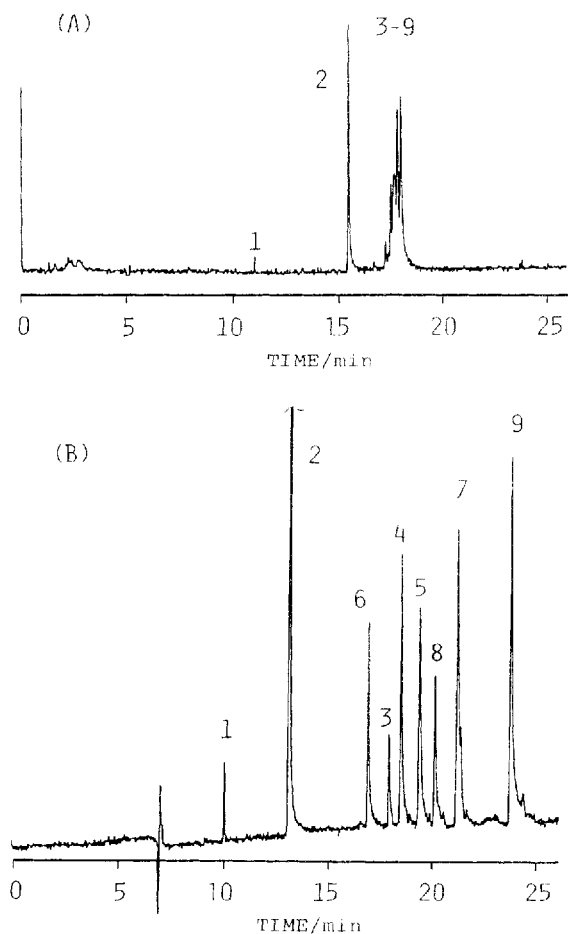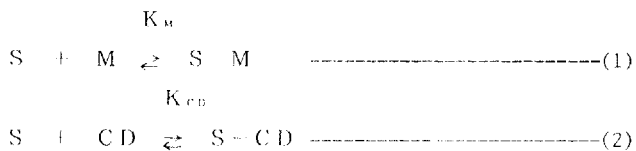The following equilibria occur in MEKC with CD:

Figure 2. Separation of nine aromatic hydrocarbons by the CD addition. Buffer, (A) a 0.05 M SDS solution of pH 9.0, (B) tha same buffer containing 4 M urea and 0.03 M β-CD. Other conditions are the same as given in Figure 1.

$$ S + M \underset{}{\overset{K_M}{\rightleftarrows}} S M \qquad \text{------------------------(1)} $$

$$ S + C D \underset{}{\overset{K_{CD}}{\rightleftarrows}} S - C D \qquad \text{------------------------(2)} $$

where S is the solute and M is the micelle. The formation constants of S–M and inclusion complex S–CD are given in equs. (3) and (4), respectively.

$$K_M = \frac{[S-M]}{[S]\ [M]} \qquad \text{----------------------(3)}$$

$$K_{CD} = \frac{[S-CD]}{[S]\ [CD]} \qquad \text{----------------------(4)}$$

The R value, the fraction of the solute in the aqueous phase, can be related to the capacity factor as follows (15):

$$R = \frac{1}{1 + \widetilde{k}'} = \frac{[S] + [S-CD]}{[S] + [S-CD] + [S-M]} \quad \text{---(5)}$$

From eqns. (3), (4) and (5), capacity factor k' is written as

$$1/\widetilde{k}' = \frac{1}{K_M\ [M]} \ (1 + K_{CD}\ [CD]) \text{ ----------(6)}$$

The capacity factor of the solute in MEKC is calculated according to the eqn. as follows (1,2):

$$\widetilde{k}' = \frac{t_R - t_0}{t_0\ (1 - t_R/t_{mc})} \qquad \text{----------------------------------(7)}$$

where t_R, t_0 and t_{mc} are the migration times of the solute, an unincorporated solute (methanol or mesityl oxide) and a totally solubilized solute (Sudan III or 1,5-benzothiazepin derivative), respectively. The dependence of capacity factors of the solutes on β-CD concentration was investigated over the concentration range of 0.03 M – 0.05 M. The results are shown in Figures 3 and 4. Migration times or capacity factors of the solute were reduced through the addition of CD as observed in the retention behavior of aromatic compounds in CD modified reversed-phase HPLC analysis (16,17). Graphs of $1/\widetilde{k}'$ vs. CD concentration gave fairly good linear relationships as expected in eqn. 6. Migration order was slightly altered through the CD concentration. Among aromatic compounds, the decrease in the migration times of fluoranthene and 9-fluorenone, which has a ketone structure outside the aromatic ring of the compound, through the CD addition
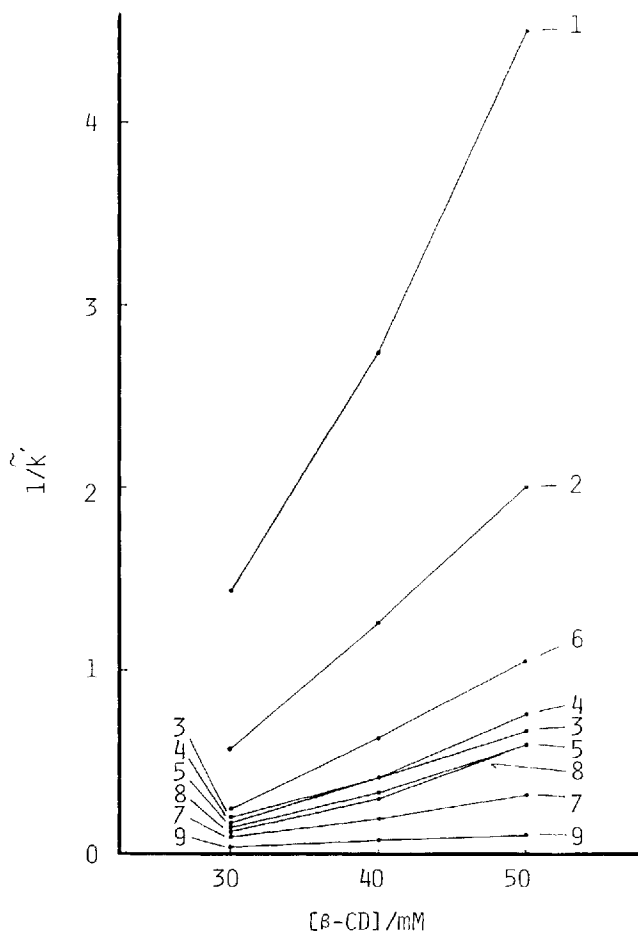
Figure 3. Plot of $1/\tilde{k}'$ vs. β-CD concentration for nine aromatic hydrocarbons with a 0.05 M SDS solution of pH 9.0 containing 4 M urea and β-CD. Other conditions are the same as given in Figure 1.

was relatively small. Steric hindrance of the solutes probably reduced their inclusion-complex formation with CD compared with other solutes.

Instead of β-CD, γ-CD was added to the SDS solution. The results of γ-CD addition on the migration of the solutes are shown in Figures 5 and 6. Migration times or capacity factors of the solutes were remarkably reduced by the γ-CD addition. The addition of 0.015 M γ-CD caused the almost the
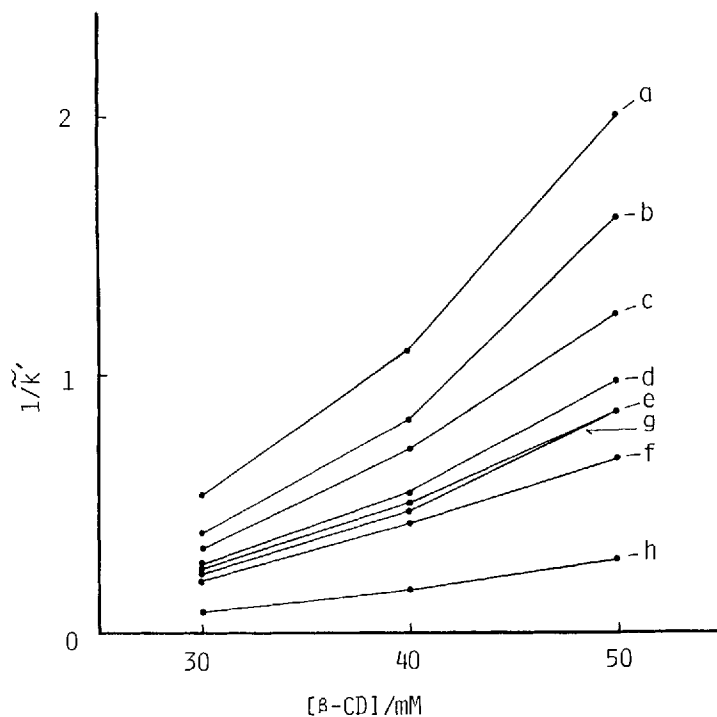
Figure 4. Plot of $1/\tilde{k}'$ vs. β-CD concentration for eight corticosteroids with a 0.05 M SDS solution of pH 9.0 containing 4 M urea and β-CD. Other conditions are the same as given in Figure 1.

same reduction of the migration times of the solutes as that in 0.03 M β-CD addition. This marked decrease in migration times was probably due to the stable or effective inclusion-complex formation between the solute and γ-CD. The important difference between γ-CD and β-CD is the diameter of the CD cavity. From the view points of cavity size, β-CD, which has a ca. 7.5 Å cavity diameter, will be much suitable for the inclusion of benzene or naphthalene analogues, especially, for the recognition of enantiomers as a mobile phase additive in HPLC separation (18,19). However, γ-CD having a ca. 9.5 Å cavity diameter was more effective for the inclusion-complex formation with such solutes in MEKC using an SDS solution, judged from the effect of the migration time reduction of the solute. This will be probably
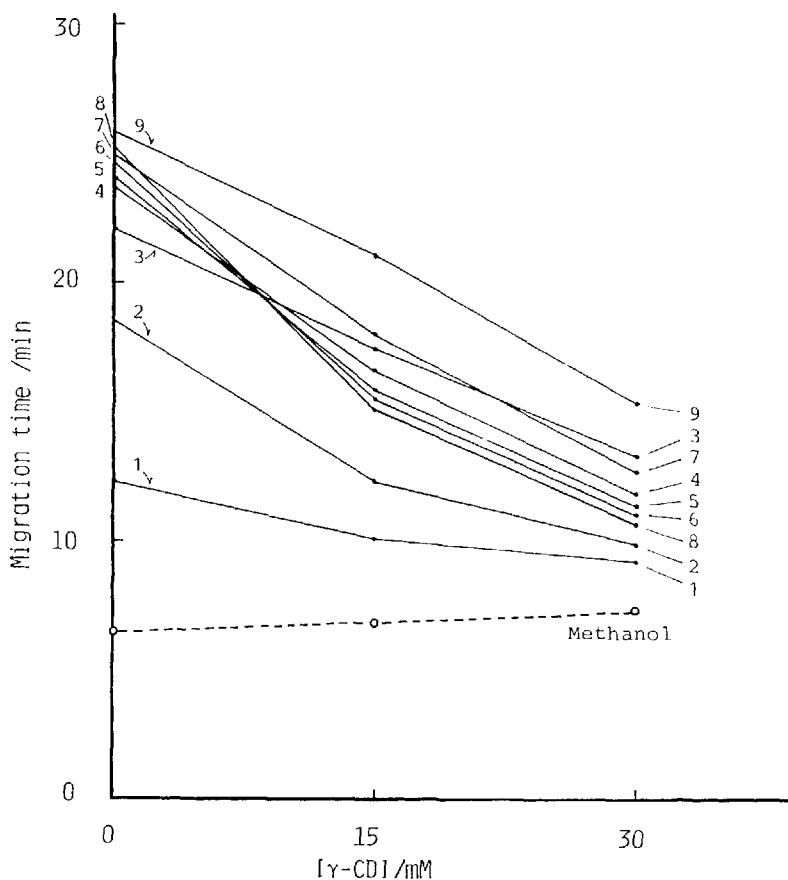
Figure 5. Effect of γ-CD concentration on the migration times of nine aromatic hydrocarbons. Buffer, a 0.05 M SDS solution of pH 9.0 containing 4 M urea and γ-CD. Other conditions are the same as given in Figure 1.

interpreted by the presence of a surfactant monomer, which has a lipophilic linear hydrocarbon chain. Some of the surfactant monomer, which is equilibrated to the micelle in a solution, will be probably included into the CD and the resulting inclusion-complex of the monomer with CD will prevent the solute inclusion, especially inβ-CD, which has a smaller cavity compared with γ-CD. γ-CD, which has a larger cavity, still had a capability of the solute inclusion after the inclusion of the surfactant
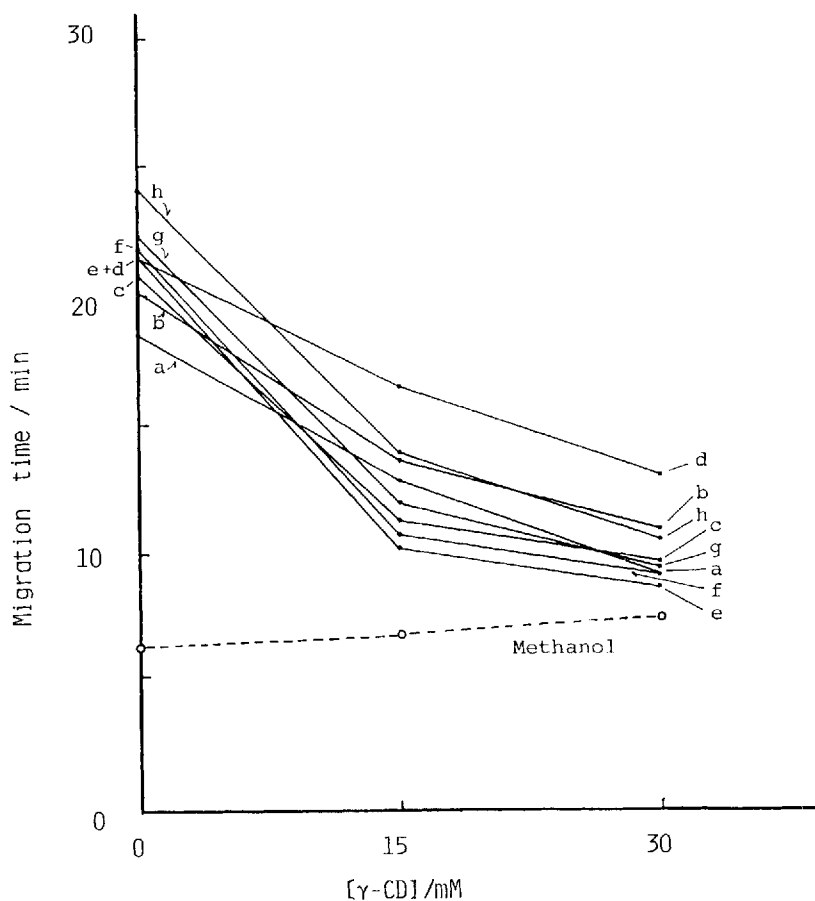
Figure 6. Effect of γ -CD concentration on the migration times of eight corticosteroids. Buffer, a 0.05 M SDS solution of pH 9.0 containing 4 M urea and γ -CD. Other conditions are the same as given in Figure 1.

monomer (20). Eight corticosteroids were successfully separated by the γ -CD addition as shown in Figure 7.

Migration order was remarkably changed by the γ -CD addition (see Figures 5 and 6). The formation of inclusion-complex between γ -CD and the solute effectively occured and the differential stability of the inclusion-complex brought about the differential solute distribution into the micelle
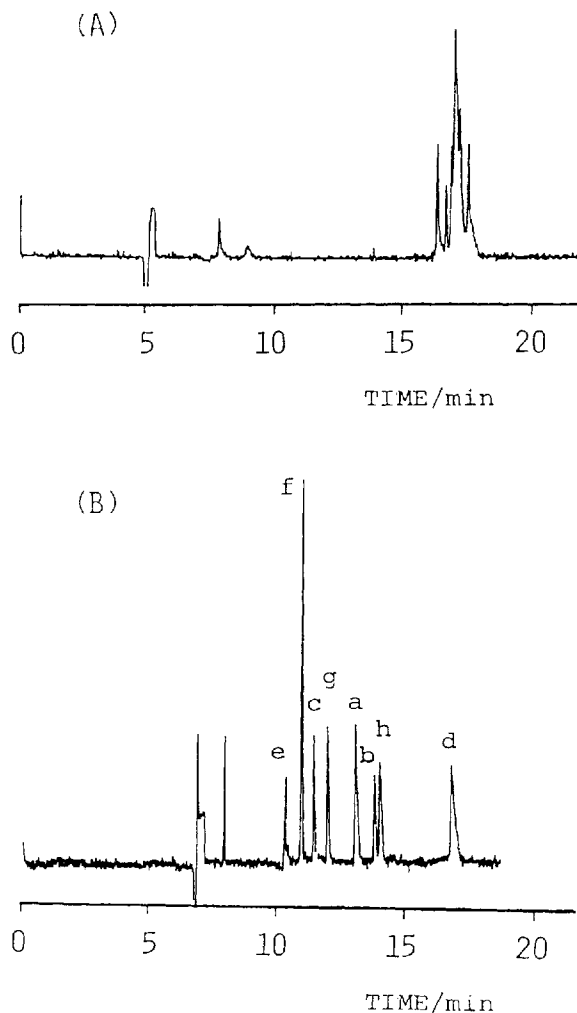
(A)

TIME/min

(B)

f

g
c   a
       h
e   b   d

0   5   10   15   20

TIME/min

Figure 7. Separation of eight corticosteroids by γ-CD addition. Buffer, (A) a 0.05 M SDS solution of pH 9.0, (B) tha same buffer containing 4 M urea and 0.015 M γ-CD. Other conditions are the same as given in Figure 1.

and hence their differential migration. Among nine aromatic compounds, smaller reduction of the migration times through the γ-CD addition was observed for 9-fluorene and fluoranthene. This will be ascribed to the steric hindrance as mentioned in β-CD addition. Lipophilic and rod-like compounds such as dibenzyl and trans-stilbene will be more easily incorporated into the CD, resulting their faster migration. Changes in the migration times of hydrocortisone, hydrocortisone acetate and cortisone acetate were relatively small in comparison with other corticosteroids. This may be ascribed to their hydrophilicity and their common geometry.

In conclusion, the CDs addition to the micellar solution decreased the migration times of lipophilic compounds and selectivity was manipulated by the type of CD and its concentration. Aside from the CDs mentioned in the work, we can use various kinds of additives to improve the separation in MEKC. Selectivity in MKEC will be successfully and easily manipulated through an additive to the micellar solution, in addition to varing the type of the surfactant.

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