PROTOCOL

Micellar Electrokinetic Chromatography

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1. Introduction

Recently, capillary electrophoresis (CE) or high performance capillary electrophoresis (HPCE) has become popular as a high-resolution separation method. The technique was first introduced by Mikkers et al. *(1)*, Jorgenson and Lukacs *(2)*, and Hjertén *(3)*, as an instrumental version of electrophoresis. Although CE can generally achieve a higher resolution within a shorter time compared with conventional high performance liquid chromatography (HPLC), only ionic or charged solutes can be analyzed by CE, in principle. This was a serious limitation of CE. However, the development of electrokinetic chromatography (EKC) *(4)* has been able to solve such problems. Electrokinetic chromatography is based upon chromatographic principles using homogeneous solutions containing an ionic "pseudo-stationary phase" and the same apparatus as CE. The unique characteristic of EKC is that both neutral and charged analytes can be separated electrophoretically. Among various modes of EKC, micellar EKC (MEKC) *(5–7)*, which uses micellar solutions of ionic surfactants, has become the most popular technique for the separation of small neutral molecules.

Many papers on fundamental characteristics and applications of MEKC have been published *(8)*, and some reviews on overview of MEKC have been also available *(9–15a)*. In this article, the separation principle and chromatographic consideration of MEKC will be described first as basic properties of MEKC. Then, strategies for selectivity manipulation will be discussed briefly, followed by the description of some applications.

2. Separation Principle of MEKC

The separation principle of MEKC is schematically shown in **Fig. 1** *(11)*. A fused silica capillary is filled with an ionic surfactant solution, in which the concentration of the surfactant is higher than its critical micelle concentration (CMC) so that micelles are formed. When an anionic surfactant, such as sodium dodecyl sulfate (SDS), is employed, the micelle is forced toward the anode by electrophoresis. The electro-osmotic flow (EOF) migrates toward the cathode owing to the negative charge of the capillary surface. The EOF is larger than the electrophoretic migration of the micelle under neutral or basic conditions and therefore, the anionic SDS micelle also migrates toward the cathode at a retarded velocity.

When a neutral analyte is injected into the micellar solution at the anodic end of the capillary, it will be distributed between the micelle and surrounding aqueous solution. The analyte will migrate at the same velocity of the micelle when it is incorporated into the micelle, whereas at the electro-osmotic velocity when it is free from the micelle or exists in the bulk solution. Thus, the migration velocity of the analyte depends on the distribution coefficient of the micellar solubilization. As long as the analyte is electrically neutral, it must migrate at a velocity between the two extremes, i.e., the electro-osmotic velocity, v_{eo}, and the velocity of the micelle, v_{mc} as shown in **Fig. 2A** *(6)*. In other words, the migration time of the analyte, t_R, is limited between the migration time of the bulk solution, t_0, and of the micelle, t_{mc} **(Fig. 2B)** *(6)*.

When an acidic solution or pH below 5.0 is employed, the EOF becomes smaller than the electrophoretic velocity of the SDS micelle and then the micelle migrates toward the anode *(16)*. When a cationic surfactant, e.g., dodecyltrimethylammonium bromide, is employed instead of SDS, the direction of the EOF will be reversed

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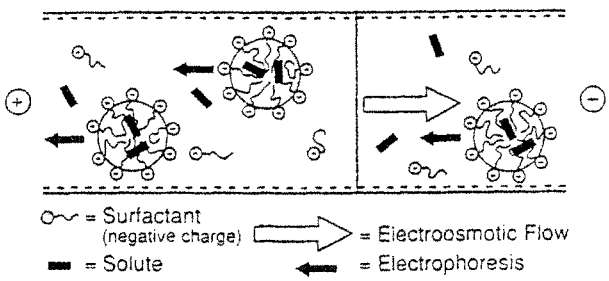

Fig. 1. Schematic illustration of the separation principle of MEKC. (Reprinted with permission from **ref. *11*.**)

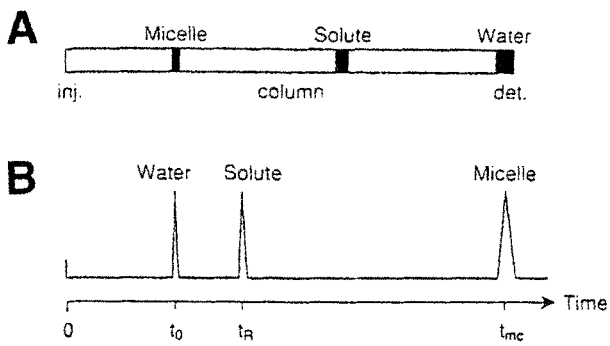

Fig. 2. Schematic of the zone separation in MEKC (**A**) and chromatogram (**B**). (Reprinted with permission from **ref. *6*.**)

or toward the anode through the adsorption of the surfactant molecule to the inside wall of the capillary ***(17)***.

3. Chromotographic Properties

3.1. Chromatographic Parameters

3.1.1. Retention Factor

Retention factor, k, can be defined as the case of the conventional chromatography ***(6)***:

$$k = (n_{mc} / n_{aq}) \quad (1)$$

where n_{mc} and n_{aq} are the amount of the analyte incorporated into the micelle and in the surrounding aqueous solution, respectively. Then we can obtain the relationship between the retention factor and the migration time as:

$$k = (t_R - t_0) / [t_0 (1 - t_R / t_{mc})] \quad (2)$$

It can be rewritten as:

$$t_R = [(1 + k) / 1 + (t_0 / t_{mc})k] \quad (3)$$

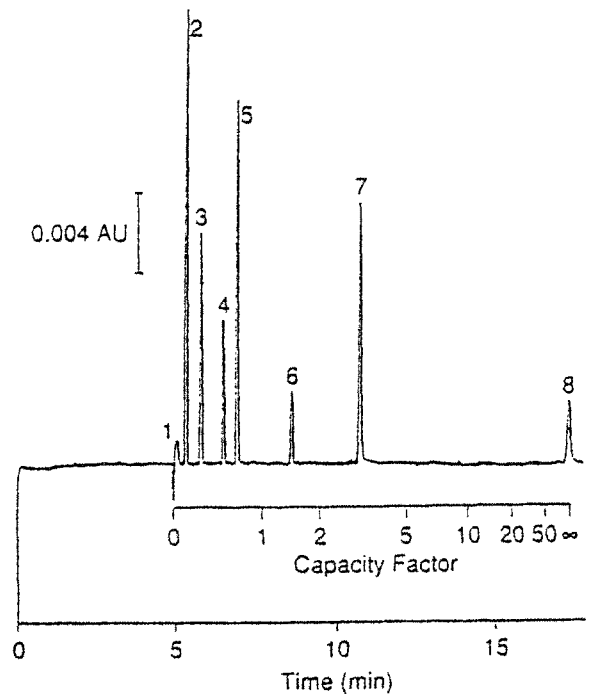

Fig. 3. An example of MEKC separation of the test solutes: (1) methanol, (2) resorcinol, (3) phenol, (4) *p*-nitroaniline, (5) nitrobenzene, (6) toluene, (7) 2-naphthol, (8) Sudan III. Micellar solution, 50 m*M* SDS in 100 m*M* borate–50 m*M* phosphate buffer, pH 7.0; capillary, 50 μm id × 650 mm (effective length, 500 mm); applied voltage, 15 kV; current, 33 μA; detection wavelength, 210 nm; temperature, 35°C. (Reprinted with permission from **ref. *6*.**)

Here, the reciprocal of t_0/t_{mc} or t_{mc}/t_0 is a parameter representing the migration time window.

If the analyte is not incorporated into the micelle or does not interact with the micelle at all, the migration time of such the solute is equal to t_0 and hence $k = 0$. On the other hand, when the analyte is totally incorporated into the micelle, the migration time becomes t_R and k becomes infinity. Thus, the migration time window is limited between t_0 and t_{mc}.

A typical example of MEKC separation of neutral compounds is shown in **Fig. 3** ***(6)***. In the figure the scale of the retention factor is inserted to see the relationship between the migration time and retention factor.

When t_{mc} is infinity or the micelle never comes out from the capillary, that condition is attained

only when the absolute value of the electro-osmotic velocity is identical to that of the electrophoretic velocity of the micelle in opposite directions, **Eq. 3** becomes:

$$t_R = (1 + k)t_0 \quad (4)$$

This is the same situation as the conventional chromatography, that is, the parameter of the migration time window is equal to infinity.

When t_0 is infinity or the electro-osmotic flow is completely suppressed, **Eq. 3** becomes:

$$t_R = (1 + 1 / k)t_{mc} \quad (5)$$

In this case, the aqueous phase never comes out from the capillary and only the micelle migrates toward the anode through the aqueous phase. Thus, the electro-osmotic flow is not essential in MEKC.

According to **Eq. 2** t_0, t_R, and t_{mc} are required to obtain the retention factor. As a marker of the electro-osmotic flow or t_0, methanol is usually used because the distribution coefficient of methanol between the micelle and aqueous phase is negligibly small. Although methanol is transparent to ultraviolet (UV), it can be detected as a baseline deflection with a UV detector owing to a refractive index change. Sudan III or IV is often employed as a tracer of the SDS micelle *(6)*, which is completely incorporated into the micelle. Timepidium bromide is also useful as a tracer for an anionic micelle *(18)*. It should be noted, however, that to find good tracers for t_0 and t_{mc} applicable to every conditions is difficult.

3.1.2. Resolution

Resolution, R_s, in MEKC is given as:

$$R_s = (N^{1/2} / 4) \times [(\alpha - 1) / \alpha)] \times [k_2 / (1 + k_2)] \times \{[1 - (t_0 / t_{mc})] / [1 + (t_0 / t_{mc})k_1]\} \quad (6)$$

Here, N is the theoretical plate number, α the separation factor equal to k_2/k_1, and k_1 and k_2 are the retention factors of analytes 1 and 2, respectively. Effects of these parameters on resolution are briefly discussed below.

3.1.2.1. Plate Number

Resolution increases proportionally with an increase in square root of the plate number. Usually average plate numbers for most analytes are 10,0000–20,0000. Normally, the higher the voltage is applied, the higher the plate number can be attained, unless much Joule heating in the higher applied voltage is generated. Because the diffusion coefficient of the micelle is small, solutes having larger retention factors can yield higher plate numbers.

3.1.2.2. Separation Factor

The separation factor reflects the relative difference of the distribution coefficients between two analytes, and is a unique variable to a given separation condition. Thus, we can manipulate the value by changing the type of micelles or the bulk solution using modifiers.

3.1.2.3. Retention Factor

The optimum value of the retention factor is represented as $(t_{mc}/t_0)^{1/2}$. Under neutral conditions, the optimum value is close to 2 for most long alkyl chain surfactants.

The retention factor can be related to the distribution coefficient, K, between the micelle and aqueous phase as:

$$k = K(V_{mc} / V_{aq}) \quad (7)$$

Here, V_{mc} and V_{aq} are the volume of the micelle and aqueous phase, respectively. The phase ratio, V_{mc}/V_{aq}, can be written by using the concentration of the surfactant, C_{sf}, and specific volume of the micelle, v as:

$$(V_{mc} / V_{aq}) = \bar{v}\,(C_{sf} - CMC) / [1 - \bar{v}\,(C_{sf} - CMC)] \quad (8)$$

Then, at low micellar concentrations, **Eq. 7** can be rewritten as:

$$k = K\bar{v}\,(C_{sf} - CMC) \quad (9)$$

This reveals that the retention factor increases linearly with an increase in the surfactant concentration. The retention factor can be easily adjusted by manipulating the surfactant concentration through **Eq. 9** if CMC is known.

3.1.2.4. Electro-osmotic Flow

The effect of the EOF can be discussed in terms of the migration time ratio, t_0/t_{mc}, or the migration time window, t_{mc}/t_0. The velocity of the micelle is given as:

$$v_{mc} = [\mu_{eo} + \mu_{ep}(mc)]\,E \quad (10)$$

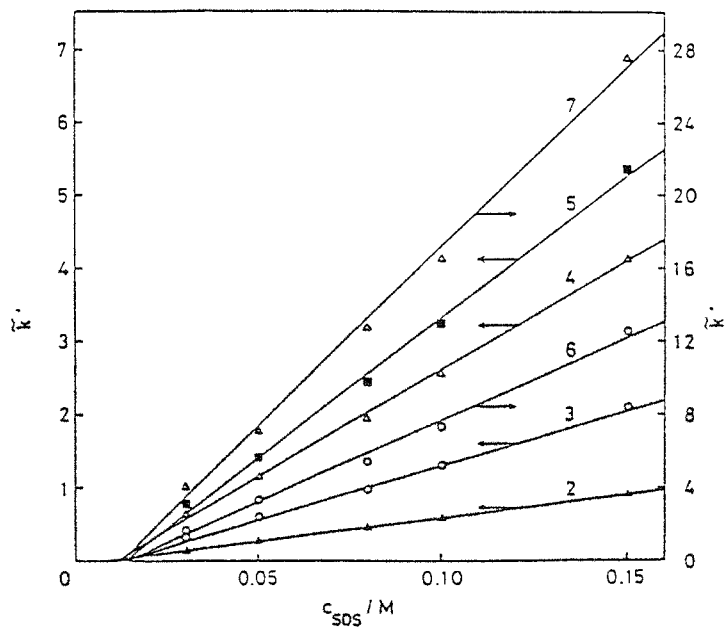

Fig. 4. Dependence of the retention factor (k) on the SDS concentration (c_{SDS}). (Reprinted with permission from **ref. 6**.)

where E is the electrical field strength. Then:

$$t_0 / t_{mc} = (1 + \mu_{ep}(mc) / \mu_{eo})\, E \qquad (11)$$

The mobilities μ_{eo} and $\mu_{ep}(mc)$ usually have different signs and the ratio $\mu_{ep}(mc)/\mu_{eo}$ is smaller than 0 and larger than −1. Therefore, t_0/t_{mc} is smaller than 1. The smaller the value of t_0/t_{mc} becomes, the larger resolution will be attained. Assumed a negative sign for t_0/t_{mc} when $\mu_{ep}(mc)/\mu_{eo}$ is smaller than −1, an extremely high resolution is expected for an analyte having the retention factor close to $-(t_{mc}/t_0)$ according to **Eq. 6** although a quite long migration time is required *(16)*.

3.2. Thermodynamic Parameters

As mentioned above, the retention factor increases linearly with an increase in the concentration of the surfactant. The dependence of the retention factor on the SDS concentration is shown in **Fig. 4** *(6)*. This reveals that the distribution coefficient is almost constant regardless of the SDS concentration.

The distribution coefficient measured at different temperatures should follow the vant Hoff equation:

$$\ln K = (\Delta H^0 / RT) + (\Delta S^0 / R) \qquad (12)$$

where H^0 is the enthalpy change associated with micellar solubilization or the transfer of the solute from the aqueous phase to the micelle, S^0 the corresponding entropy change, R the gas constant, and T the absolute temperature. Thus, **Eq. 12** allows the calculation of the enthalpy and entropy changes in micellar solubilization from the temperature dependence of K.

In various buffer system, such as borate-phosphate (B-P), piperazine-*N*,*N*'-bis(2-ethane-sulfonic acid) mono sodium salt (PIPES) - sodium hydroxide, *N*,*N*'- bis(2-hydroxyethyl)-2-aminoethanesulfonic acid (BES) - sodium hydroxide, and B-P with urea, CMC and the partial specific volume were measured for the SDS micelle *(19)* as shown in **Table 1**. **Table 2** lists distribution coefficients of test solutes at different temperatures between the SDS micelle and a B-P buffer. Corresponding enthalpy, entropy and Gibbs free energy changes were calculated as shown in **Table 3**. Obviously, the effect of the buffer solution is not significant on these thermodynamic quantities. The entropy changes for resorcinol, phenol, *p*-nitro-aniline, and 2-naphthol were negative, and the contribution of ΔS^0 to ΔG^0 was significant except for phenol. These solutes favored the aqueous phase from the view point of entropy.

Table 1
CMC of SDS and the Partial Specific Volume ($\bar{v}$) of the SDS Micelle

	Buffer[a]					
	CMC/m*M*				$\bar{v}$/mL/g	
Temp./°C	B-P	PIPES	BES	Urea	B-P	Urea
20	—	—	—	—	0.8562	—
22	2.8	—	—	—	—	—
25	2.9	3.8	3.1	4.4	0.8610	0.8126
30	2.5	4.2	3.3	4.5	0.8686	0.8160
35	2.6	4.3	3.3	5.3	0.8710	0.8242
40	3.0	4.2	3.5	5.9	0.8758	0.8248
45	—	4.4	3.6	5.9	—	0.8290
50	—	4.8	3.8	6.4	—	—

[a]B-P, 100 m*M* borate–50 m*M* phosphate buffer, pH 7.0; PIPES, 20 m*M* PIPES–20 m*M* NaOH, pH 7.0; BES, 100 m*M* BES–100 m*M* NaOH, pH 7.0; urea, 5*M* urea in 100 m*M* borate–50 m*M* phosphate buffer, pH 7.0.

Table 2
Distribution Coefficients of Alkylphenols Between the SDS Micelle and a 100 m*M* Borate–50 m*M* Phosphate Buffer, pH 7.0[a]

	Temp. / °C				
Solute	30	35	40	45	50
o-Cresol	100	93.0	86.4	81.1	76.3
m-Cresol	104	96.5	89.9	84.5	79.5
p-Cresol	112	104	96.8	90.7	85.5
2,6-Xylenol	203	187	173	161	150
2,3-Xylenol	233	214	197	183	170
3,4-Xylenol	250	230	212	197	183
2,4-Xylenol	269	248	229	213	198
p-Propylphenol	788	729	668	622	571
p-Butylphenol	2320	2140	1940	1800	1620
p-Amylphenol	7120	6660	5870	5580	4900

[a]Separation solution, 50 m*M* SDS in 100 m*M* borate–50 m*M* phosphate buffer, pH 7.0.

4. Selectively Manipulation

Selectivity in chromatography can be discussed by using the separation factor, α. The separation factor can be manipulated with chemical considerations. The micelle in MEKC corresponds to the stationary phase in reversed phase high-performance liquid chromatography (RP-HPLC), whereas the bulk solution or the surrounding aqueous phase to the mobile phase, from the view point of selectivity manipulation. Mainly, following four factors can be controlled to manipulate selectivity:

1. The micellar structure;
2. Temperature;
3. pH; and
4. Additives to the aqueous phase.

4.1. Effect of the Micellar Structure

4.1.1. Surfactants

A surfactant molecule has a hydrophobic and hydrophilic group and both groups affect selectivity in MEKC. Since most analytes interact with the micelle on its surface, the hydrophilic or ionic group is generally more important than the hydrophobic one in determining selectivity. For example, SDS and cetyltrimethylammonium bromide (CTAB) show considerably different

Table 3
Enthalpy, Entropy, and Gibbs Free Energy Changes in Micellar Solubilization of Alkylphenols by the SDS Micelle[a]

Solute	ΔH^0/kJ/mol	ΔS^0/J/mol/K	ΔG^0/kJ/mol, 35°C
o-Cresol	−11.1	1.6	−11.6
m-Cresol	−10.8	2.9	−11.7
p-Cresol	−10.9	3.3	−11.9
2,6-Xylenol	−12.4	3.2	−13.4
2,3-Xylenol	−12.7	3.3	−13.7
3,4-Xylenol	−12.7	3.9	−13.9
2,4-Xylenol	−12.4	5.5	−14.1
p-Propylphenol	−13.1	12.3	−16.9
p-Butylphenol	−14.4	16.9	−19.6
p-Amylphenol	−15.0	24.3	−22.5

[a]The separation solution was the same as in **Table 2**.

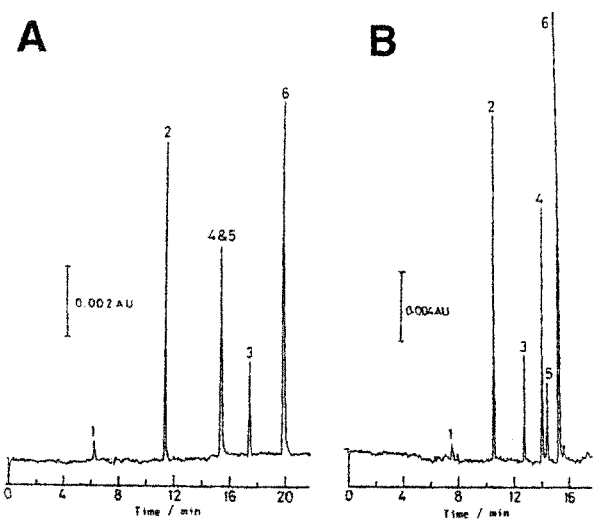

Fig. 5. Effect of the surfactant structures on selectivity: (1) water, (2) aniline, (3) nitrobenzene, (4) *m*-nitroaniline, (5) *p*-nitroaniline, (6) *o*-nitroaniline. Micellar solution, (**A**) 100 m*M* SDS in 100 m*M* borate–50 m*M* phosphate buffer, pH 7.0, (**B**) 50 m*M* CTAB in 100 m*M* Tris-HCl, pH 7.0; capillary, 50 μm id × 650 mm (effective length, 500 mm); applied voltage, 15 kV; detection wavelength, 230 nm; temperature, ambient. (Reprinted with permission from **ref. *20***.)

selectivity as shown in **Fig. 5** ***(20)***. The structural difference between SDS and CTAB is mainly ionic groups: sulfate in SDS and quaternary ammonium in CTAB. The hydrophobic groups are similar, only different in the length of alkyl chains. Similar results have also been published ***(21)***.

In **Table 4**, differences in distribution coefficients of the test solutes among three surfactants, such as SDS, sodium tetradecyl sulfate (STS), and sodium dodecanesulfonate (SDDS), are shown ***(6)***. The distribution coefficients for resorcinol, phenol, *p*-nitroaniline, and nitrobenzene are virtually identical between SDS and STS, which have the identical ionic groups but different alkyl chain lengths. On the other hand, significantly different distribution coefficients are observed between SDS and SDDS, which have the identical alkyl chains but different ionic groups. These results suggest that many compounds are adsorbed on or at least strongly interact with the surface of the micelle.

Bile salts, such as sodium cholate, sodium deoxycholate, sodium taurodeoxycholate, which form helical micelles ***(22)*** can make the significantly different selectivity compared with the long alkyl-chain surfactants ***(23)***. Bile salts are natural-occurring chiral surfactants and hence, they can also be used for enantiomeric separation ***(22,24–26)***.

4.1.2. Mixed Micelles

In MEKC, ionic micelles are usually used and they can be easily modified by adding ionic or nonionic surfactants to form mixed micelles. The mixed micelle consisted of ionic and nonionic surfactants is usually larger than the original ionic micelle and has a lower charge density,

Table 4
Distribution Coefficients at 35°C

Solute	Distribution coefficient		
	SDS[a]	STS[b]	SDDS[c]
Resorcinol	21.6	20.8	27.7
Phenol	52.1	52.3	56.1
p-Nitroaniline	103	100	84.3
Nitrobenzene	135	138	111
Toluene	318	345	288
2-Naphthol	656	789	698

[a]Sodium dodecyl sulfate.
[b]Sodium tetradecyl sulfate.
[c]Sodium dodecanesulfonate.

and hence, a lower electrophoretic mobility. Consequently, the narrower migration time window is obtained *(27)* and also different selectivity is expected *(27)* because the surface of the mixed micelle is different from that of the original one.

4.1.3. High-Molecular Mass Surfactants

As foregoing descriptions, an ionic micelle or mixed micelle, which is in principle a molecular aggregate of the surfactant molecule, is employed as a pseudo-stationary phase. Here, as can be seen from **Eq. 8**, the micellar concentration, C(mc), is represented as C_{sf} CMC. Because the analyte is distributed between the micelle and surrounding aqueous phase, the migration time of the analyte depends on C(mc). CMC varies with temperature, salt concentration, and additive to the surfactant solution and therefore, C(mc) is dependent on these parameters. To keep C(mc) constant throughout the MEKC run is essential to obtain reproducible migration times.

On the other hand, the high-molecular-mass surfactant (HMMS) forms a molecular micelle, which consists of a single molecule and hence, CMC of HMMS is zero or C(mc) is equal to C_{sf}, so that we can expect better reproducibility of the migration time with HMMS. Recently, some research on the use of HMMS, such as butyl acrylate-butyl methacrylate-methacrylic acid copolymer (BBMA) *(28–30)*, sodium 10-undecenyl sulfate (SUS) polymer *(31)*, and sodium undecylenate oligomer *(31a)*; in MEKC have been reported. These reports showed that the HMMSs could be utilized as pseudo-stationary phases in MEKC with almost the same separation performance as conventional low-molecular-mass surfactants. In the BBMA-MEKC system, reproducibility of the migration time was better than that in the SDS-MEKC, whereas reproducibility of the peak area was not comparable with the SDS-MEKC *(32)*. However, the use of HMMSs in MEKC is useful for the alteration of the selectivity and theoretical treatments on separation characteristics.

4.2. Effect of Temperature

The distribution coefficient is dependent on temperature: the distribution coefficient decreases with an increase in temperature, which causes a reduced migration time. The increase in temperature also causes increases in v_{eo} and v_{ep}(mc) by the same extent because of a reduced viscosity. Dependences of the distribution coefficients on temperature are different among solutes, therefore, temperature affects selectivity as shown in **Fig. 6** *(19)*.

It should be noted that temperature seriously affects the migration time although its effect on selectivity is not remarkable, and hence, it is important to maintain temperature precisely to obtain reproducible results.

4.3. Effect of pH

Effect of the constituents of the buffer is not significant while the pH is critical factor to ionizable analytes. If the ionized form of the solute has the same charge as the micelle, it will be incorpo-

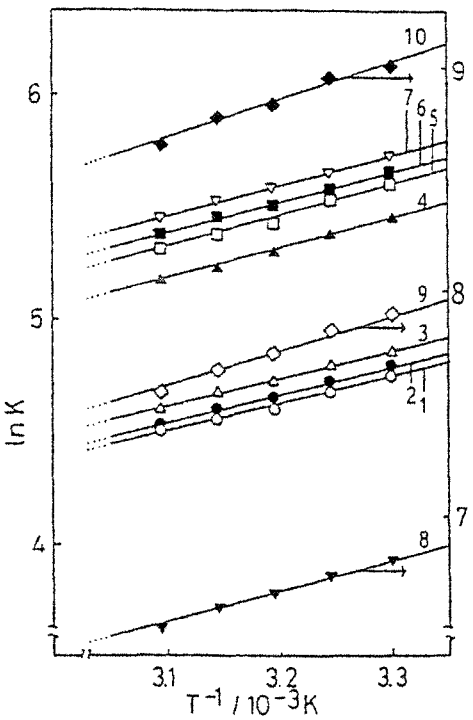

Fig. 6. Van't Hoff plots of alkylphenols: (1) *o*-cresol, (2) *m*-cresol, (3) *p*-cresol, (4) 2,6-xylenol, (5) 2,3-xylenol, (6) 3,4-xylenol, (7) 2,4-xylenol, (8) *p*-propylphenol, (9) *p*-butylphenol, (10) *p*-amylphenol. Micellar solution, 50 m*M* SDS in 100 m*M* borate–50 m*M* phosphate buffer, pH 7.0; applied voltage, 10 kV. (Reprinted with permission from **ref. *19***.)

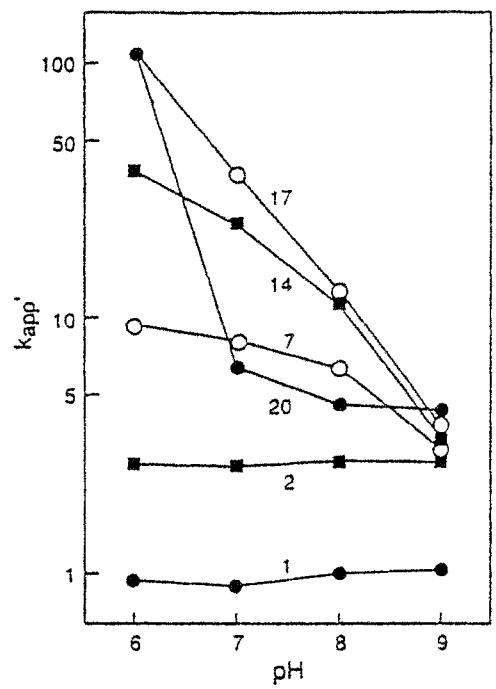

Fig. 7. Dependence of apparent retention factors (k_{app}) of chlorinated phenols on pH: (1) phenol, (2) 2-chlorophenol, (7) 2,5-dichlorophenol, (14) 2,4,5-trichlorophenol, (17) 2,3,4,5-tetrachlorophenol, (20) pentachlorophenol. Micellar solution, 100 m*M* SDS in 50 m*M* phosphate–borate buffer; capillary, 50 μm id × 650 mm (effective length, 500 mm); applied voltage, 15 kV; detection wavelength, 220 nm; temperature, 35°C. (Reprinted with permission from **ref. *33***.)

rated into the micelle less than its neutral form. **Figure 7** demonstrates the dependence of the apparent retention factor on the buffer pH for some chlorinated phenols *(33)*. Here, the apparent retention factor was calculated by **Eq. 2** regardless of whether the solutes were ionized or not. For acids, the increase in pH will promote ionization, then the distribution coefficient to the anionic micelle or SDS will be reduced. It should be noted that the change of the buffer pH, especially in the lower pH region, causes a significant change in the electro-osmotic velocity as mentioned the previous section *(16)*.

4.4. Effect of Additives to the Aqueous Phase

The most versatile and effective methods to manipulate selectivity in MEKC are the use of additives to the aqueous phase as well as the choice of surfactants. In conventional HPLC, modifications with additives to the mobile phase have been well established, and the know-how can be applied in similar manner to MEKC. Of course, we must understand the difference between the micellar phase in MEKC and the stationary phase in HPLC to use such additives. Mainly, there are four categories of additives to be useful in MEKC:

1. Cyclodextrins (CDs);
2. Ion-pair reagents;
3. Urea; and
4. Organic modifiers.

4.4.1. Cyclodextrins

Recently, CD has become a popular additive or compound in chromatography. In most cases,

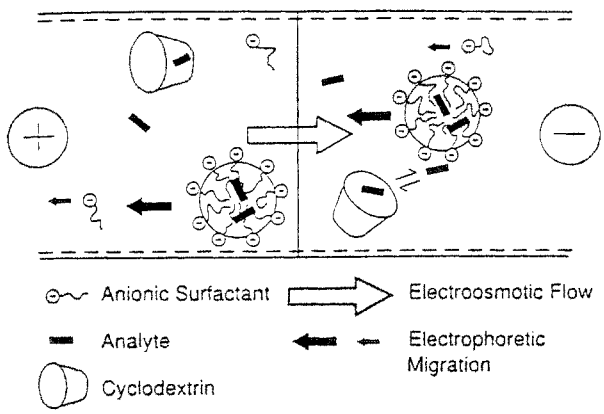

Fig. 8. Schematic of the separation principle of CD-MEKC. (Reprinted with permission from **ref. *34***.)

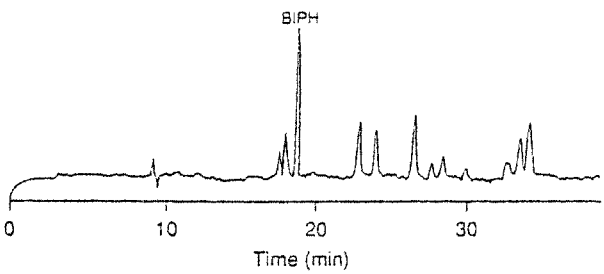

Fig. 9. Separation of 11 trichlorobiphenyl isomers by CD-MEKC: BIPH = biphenyl. Separation solution, 60 m*M* γ-CD, 100 m*M* SDS, and 2*M* urea in 100 m*M* borate–50 m*M* phosphate buffer, pH 8.0; capillary, 50 μm id × 650 mm (effective length, 500 mm); applied voltage, 15.4 kV; current 50 μA. (Reprinted with permission from **ref. *34***.)

CD's capability of recognizing specific molecules which fit its hydrophobic cavity is used for chromatographic separations. The use of CDs is especially effective for the separation of aromatic isomers and aromatic enantiomers which have the chiral center close to the aromatic ring.

Originally, CD is electrically neutral and not affected by the electrophoresis. It means that CD itself cannot be used as a carrier in EKC, unless an ionic group is introduced into CD. The surface of CD is hydrophilic and hence, we can assume that CD is not incorporated in to the micelle. A surfactant molecule, however, may be included into the CD cavity.

The separation principle of CD modified MEKC (CD-MEKC) is schematically shown in **Fig. 8** *(34)*. In this system, CD migrates at the same velocity as the electro-osmotic flow. The analyte molecule, which is assumed to be neutral, both included by CD and in the aqueous phase migrates at the same velocity as the electro-osmotic flow. On the other hand, the analyte migrates at the different velocity from the electro-osmotic flow when it is incorporated into the ionic micelle. In the case of highly hydrophobic analytes, they seem to be totally incorporated into the micelle in the absence of CD. It means that the addition of CD reduces the apparent distribution coefficient of the analytes between the bulk phase and the micelle and makes possible to separate such solutes. The higher the concentration of CD becomes, the smaller the distribution coefficient will be observed. In CD-MEKC, therefore, the retention factor can be manipulated by varying both the concentrations of CD and the micelle. An example of CD-MEKC separation of hydrophobic compounds is shown in **Fig. 9** *(34)*. CD-MEKC is also effective for enantiomeric separation, which will be discussed later.

It should be noted that CD-MEKC is a different technique from CD-EKC, although these two terms are somewhat confusing. In CD-MEKC, CD is added to micellar solutions, while in CD-EKC, an ionic CD derivative is used as a pseudo-stationary phase of EKC in a solution without micelles. Recently, the technique CD-EKC is sometimes referred as a branch of CD modified CZE (CD-CZE).

4.4.2. Urea

Urea is usually used to increase the solubility of hydrophobic compounds in water. In MEKC, a successful separation of highly lipophilic compounds was achieved with an SDS solution containing high concentration of urea, as shown in **Fig. 10** *(35)*. By adding urea to the micellar solution, the electro-osmotic velocity is slightly reduced, while the migration velocity of the micelle is considerably reduced, which causes the reduced retention factors. Urea is also effective to improve peak shapes especially in the separation of amino acids *(36)*.

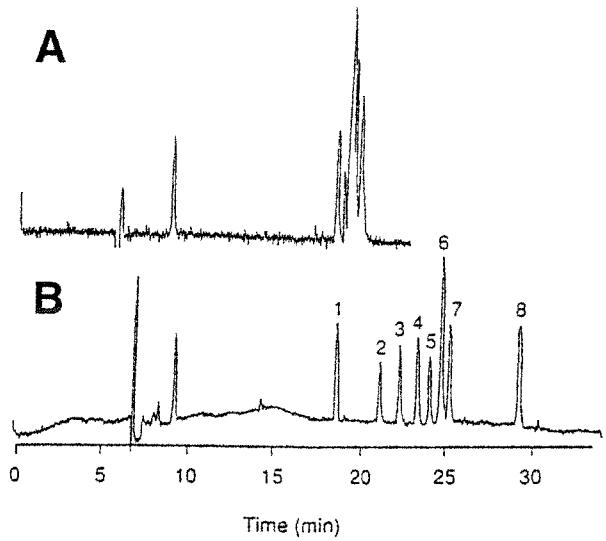

Fig. 10. The effect of urea addition to the SDS solution: (1) hydrocortisone, (2) hydrocortisone acetate, (3) betamethasone, (4) cortisone acetate, (5) triamcinolone acetonide, (6) fluocinolone, (7) dexamethasone acetate, (8) fluocinonide. Separation solution, 50 m*M* SDS in 20 m*M* borate-phosphate buffer, pH 9.0. (**A**) Without urea and (**B**) with 6*M* urea; capillary, 50 μm id × 650 mm (effective length, 500 mm); applied voltage, 20 kV; detection wavelength, 210 nm. (Reprinted with permission from **ref. *35***.)

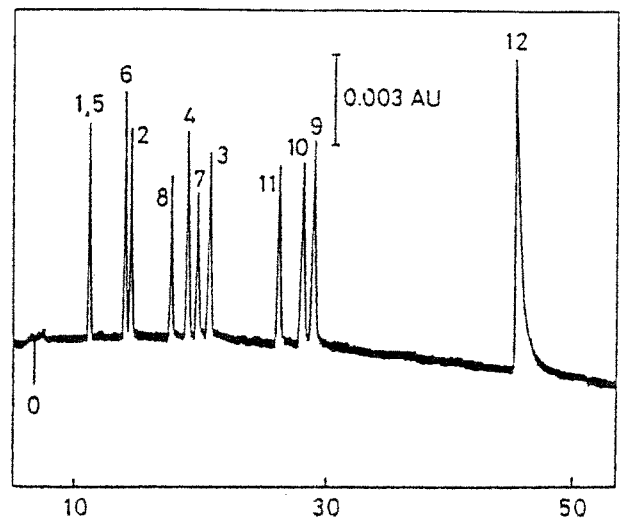

Fig. 11. Separation of 11 aromatic sulfides: (1) benzyl methyl sulfide, (2) benzyl ethyl sulfide, (3) benzyl propyl sulfide, (4) benzyl isopropyl sulfide, (5) methyl phenyl sulfide, (6) ethyl phenyl sulfide, (7) phenyl propyl sulfide, (8) isopropyl phenyl sulfide, (9) butyl phenyl sulfide, (10) isobutyl phenyl sulfide, (11) *s*-butyl phenyl sulfide, (12) Sudan III. Separation solution, 30 m*M* SDS, pH 7.0, containing 20% (v/v) methanol; capillary, 50 μm id × 900 mm (effective length, 750 mm); applied voltage, 22 kV; current, 20 μA; detection wavelength, 210 nm; temperature, ambient. (Reprinted with permission from **ref. *37***.)

Although a remarkable change of the selectivity is not attained by the urea addition, a slight change of the selectivity can be recognized, especially for the separation of closely related compounds.

4.4.3. Organic Modifiers

Similar to the case in HPLC, an organic solvent miscible with water can be used as an additive to the micellar solution to manipulate the retention factors or selectivity. In HPLC, highly hydrophobic compounds can be analyzed by using a high concentration of the organic solvent, whereas in MEKC, a high concentration of the organic solvent cannot be employed because of the break down of the micellar structure. In general, the usable maximum content of the organic solvent is 20% or so. The use of the organic solvent usually provides an improved resolution and/or a change in the selectivity. In MEKC, methanol *(37–40)*, 2-propanol *(41)*, and acetonitrile *(38)* are used as the organic modifiers, and they contribute to reduce the electro-osmotic velocity and expand the migration time window. An example of the use of methanol for the separation of aromatic sulfides is shown in **Fig. 11** *(37)*.

Recently, Tanaka *(42)* has reported that the use of a quite high concentration of methanol in an SDS solution is effective for the MEKC separation of some hydrophobic compounds. In such circumstances, it is not clear whether the SDS micelle still exists in the solution or not, but some interactions between the solutes and the SDS molecule or micelle might occur.

Imasaka and coworkers *(43)* has reported that the addition of *N,N*-dimethylformamide (DMF) to a bile salt micellar solution is effective for the separation of polycyclic aromatic hydrocarbons (PAHs). Similarly, the use of a high concentration of dimethyl sulfoxide (DMSO) added in the

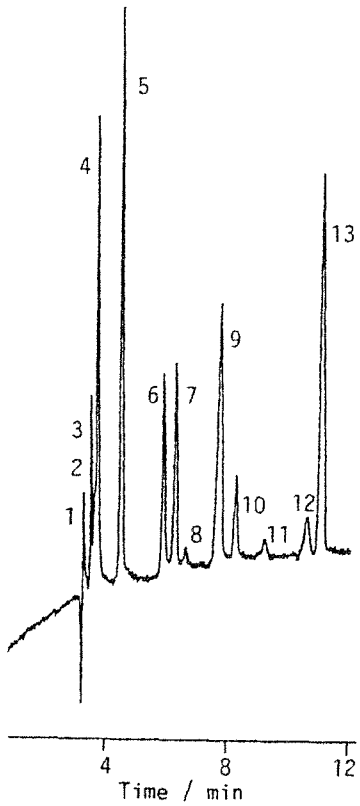

Fig. 12. Separation of PAHs by MEKC with acetone: (1) *p*-quinone, (2) quinoline, (3) benzene, (4) benzoin, (5) naphthalene, (6) benzanthrone, (7) phenanthrene, (8) anthracene, (9) pyrene, (10) 1,2-benzanthraquinone, (11) 2,3-benzofluorene, (12) benz(a)anthracene, (13) fluoresceine. Separation solution, 25 m*M* SDS in borate-phosphate, pH 7.0, containing 30% acetone; capillary, 52 µm i.d. × 370 mm (effective length, 300 mm); detection wavelength, 200 nm; applied voltage, 20 kV (541 V cm^{-1}); temperature, 30 °C. (Reprinted with permission from **ref. *44***.)

SDS-MEKC system is found to be useful for the analysis of PAHs *(44)*. Acetone is also a useful additive to the SDS solution for the separation of PAHs *(44)*, as shown in **Fig. 12**.

4.4.4. Ion-Pair Reagents

In MEKC, the use of an ion-pair reagent causes a remarkable change in separation characteristics, which is mainly owing to the charge of the micelle. When a tetraalkylammonium salt is added to the SDS micellar solution, anionic analytes form paired ions with the ammonium ion and hence, the electrostatic repulsion between the anionic SDS micelle and the anionic analyte is reduced. That formation of the paired ion is promoted with an increase of the concentration of the ammonium salt; that is, the higher the concentration of the ammonium salt, the larger the retention factor of the anionic analyte. On the other hand, a cationic analyte competes with the ammonium ion in pairing to the anionic micelle, so the migration time of the cation decreases with an increase of the concentration of the ammonium salt.

The effect of the addition of tetraalkylammonium salts to SDS micellar solutions on the selectivity is shown in **Fig. 13** *(18)*. The normal CZE separation, without SDS, using the buffer containing the salt is also shown. The effect strongly depends on the structure of the ion-paring reagent, e.g., the length of the alkyl chain.

4.4.5. Metal Salts

Cohen et al. *(45)* reported the effect of the addition of metal salts to the SDS micellar solution. By adding magnesium, zinc, or copper(II) to the SDS micellar solution, the separation of oligonucleotides was successfully achieved and good selectivity could be obtained, as shown in **Fig. 14**.

5. Applications

5.1. General Scope

Almost 10 years have passed since the first paper on MEKC *(5)* has been published, and a number of applications of MEKC have appeared. Although separation characteristics of MEKC are similar to those of reversed phase HPLC (RP-HPLC), the range of analytes applied to MEKC is limited compared with RP-HPLC: MEKC is mainly employed for the separation of small molecules, because the size of the micelle is relatively small and not able to incorporate big molecules such as proteins, whereas RP-HPLC can treat such molecules as analytes. Regardless of such a limitation, MEKC has recognized as a useful and powerful technique in various analytical fields, due to its advantages over RP-HPLC. The main advantage of MEKC is the higher separation efficiency. Nishi and Terabe *(46,47)* have shown some such examples, especially in the pharmaceutical analyses. Other advantages of MEKC over RP-HPLC are as follows:

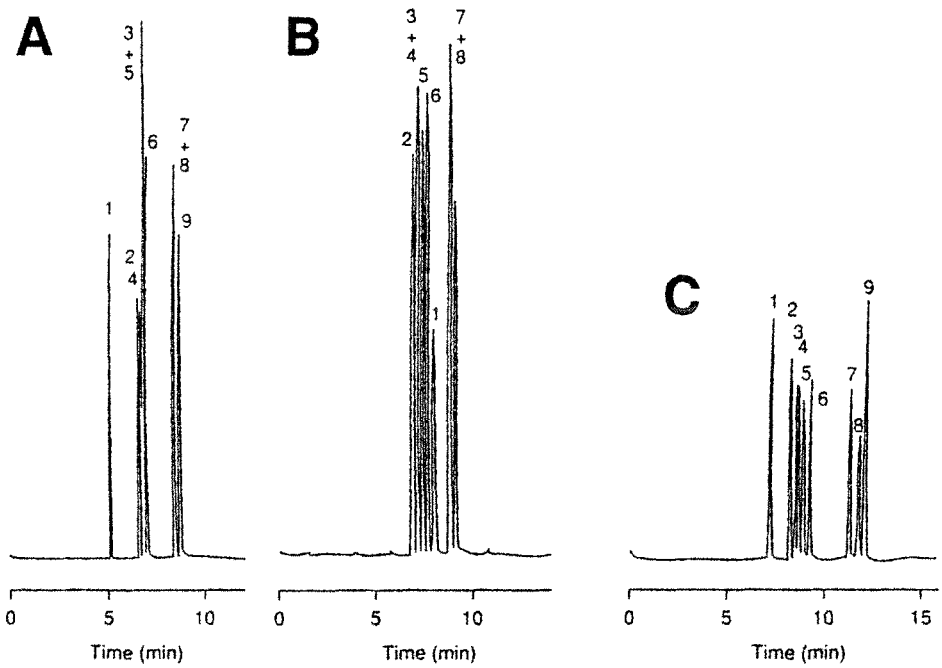

Fig. 13. Separation of cephalosporin antibiotics by (**A**) CZE, (**B**) MEKC with SDS, and (**C**) MEKC with SDS and tetramethylammonium salt: (1) C-TA, (2) ceftazidime, (3) cefotaxime, (4) cefmenoxime, (5) cefoperazone, (6) cefpiramide, (7) cefpimizole, (8) cefminox, (9) ceftriaxone. Separation solution, (A) 20 m*M* borate–phosphate buffer, pH 9.0, (B) 50 m*M* SDS added to (A), (C) 40 m*M* tetramethylammonium bromide added to (B); capillary, 50 μm id × 650 mm (effective length, 500 mm); applied voltage, 20 kV; detection wavelength, 210 nm. (Reprinted with permission from **ref. *18***.)

1. MEKC analysis can be carried out with smaller amounts of sample and separation solutions.
2. Separation can be usually completed within a shorter time.
3. Maintenance of the separation capillary, e.g., cleaning or replacing, can be easily operated.

Typical applications of MEKC is the separation of closely related compounds. A mixture of phenylthiohydantoin amino acids (PTH-AAs) was successfully separated by using an SDS, as shown in **Fig. 15A**, and a dodecyltrimethylammonium bromide (DTAB) solutions *(17)*. By adding urea to the SDS micellar solution, better resolution and selectivity could be obtained, as shown in **Fig. 15B** *(35)*. Separation of all isomers of chlorinated phenols including phenol could also be achieved with an SDS solution as shown in **Fig. 16** *(33)*. These separations cannot be carried out by a simple isocratic HPLC, i.e., a gradient method is required. The overall discussions on the MEKC applications are available in the review by Janini and Issaq *(9)*.

5.2. Enantiomer Separation

Recently, enantiomer separation has become one of major objectives in chromatographic separation and many papers on optical resolution by HPLC have appeared. A number of reports on chiral separations by MEKC has been also published at the present stage. In MEKC, following two methods are usually employed to achieve enantiomer separation: MEKC with chiral surfactants and cyclo-dextrin modified MEKC (CD-MEKC).

Brief reviews on chiral separations by MEKC and also by CE have been published previously *(48–50)*.

5.2.1. MEKC with Chiral Surfactants

Various amino acid derivatives, which form chiral micelles, have been used as pseudo-stationary phases in MEKC for chiral separations.

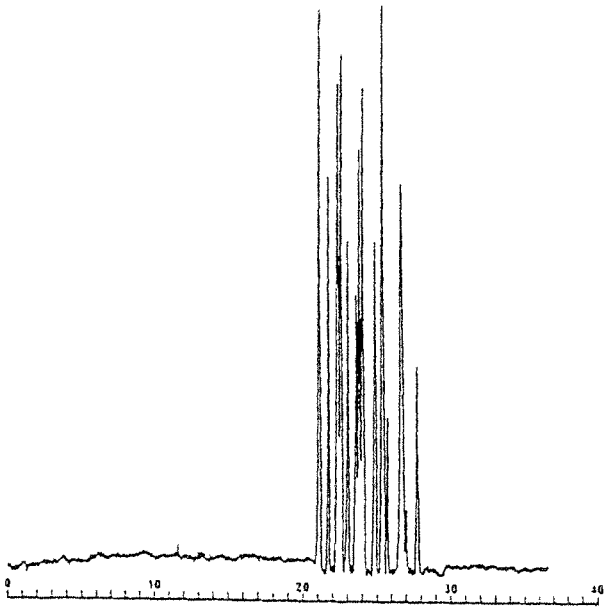

Fig. 14. Separation of 18 oligonucleotides, each with 18 bases, by MEKC with a metal ion. Separation solution, 50 m*M* SDS, 3 m*M* Zn(II), and 7*M* urea in 20 m*M* Tris-5 m*M* sodium phosphate buffer; capillary, 50 μm id × 850 mm; applied voltage, 22 kV; current, 10 μA; detection wavelength, 260 nm; temperature, 25°C. (Reprinted with permission from **ref. *45***.)

Sodium *N*-dodecanoyl-L-valinate (SDVal) ***(36,51–54)*** and related *N*-alkanoyl-L-amino acids ***(55–57)*** were effective to resolution of PTH-DL-AAs, as shown in **Fig. 17 (54)**. Similarly, Swartz et al. have reported on enantiomer separations by MEKC with novel chiral surfactants of amino acid derivatives ***(58–61)***.

Digitonin, which is a glycoside of digitogenin, could achieve the optical resolution of some dansylated DL-amino acids (Dns-DL-AAs), used as the mixed micelle with SDS or bile salts ***(53,55)***.

Bile salts are useful to chiral separations as mentioned previously. By using sodium taurocholate (STC) and sodium taurodeoxycholate (STDC), some Dns-DL-AAs were optically resolved ***(24)***. Some chiral drugs, e.g., diltiazem hydrochloride and trimetoquinol hydrochloride, have also been resolved ***(25,26,62)***. Enantiomeric separation of bina-phthyl analogues by MEKC with bile salts was reported by Cole et al. ***(63)***.

As other chiral surfactants, saponins such as glycyrrhizic acid and -escin have been used for optical resolution of Dns- or PTH-DL-AAs ***(64)***. Camilleri and coworkers ***(65,66)*** demonstrated the use of synthetic chiral compounds, such as alkyl glycopyranosides, as pseudo-stationary phases in MEKC for enantiomeric separations.

5.2.2. Cyclodextrin Modified MEKC (CD-MEKC)

As mentioned previously, CD-MEKC is capable of optical resolution, especially of aromatic and related enantiomers. Some Dns-DL-AAs were optically resolved by CD-MEKC using SDS solutions containing β- or γ-CD ***(67)***. Not only the underivatized CDs but also some CD derivatives, e.g., 2,6-di-*O*-methyl- -CD, in SDS solutions can be used for the resolution of the optical isomers ***(68)***.

Recently, the CD-MEKC system becomes one of popular techniques for chiral separations in HPCE: Optical resolution of some labelled amino acid enantiomers ***(69)*** and RS-chlorpheniramine ***(70)*** has been reported. It should be noted that the CD modified capillary zone electrophoresis (CD-CZE) system without micelles is usually more effective than CD-MEKC for chiral separation of ionic compounds, especially for the analyte having a high electrophoretic mobility, and CD-MEKC and CD-CZE are complementary techniques to each other.

5.3. Separation of Hydrophobic Compounds by MEKC

As mentioned in **Subheading 4.4.3.**, separation of hydrophobic compounds, such as polycyclic aromatic hydrocarbons (PAHs) has been recognized as one of important objectives in MEKC. Some PAHs were successfully separated with a CD-MEKC mode, i.e., using and -CD-SDS solution ***(71)***, and also by MEKC with an SDS-acetone solution ***(44)***. As new pseudostationary phases in EKC other than micelles, starburst dendrimers were introduced first by Tanaka et al. ***(42,72,73)***. They showed remarkably different selectivity from that in the SDS-MEKC system and successful separations of various PAHs. The use of dendrimers in EKC has also been reported by several groups ***(74–76)***. Other techniques for

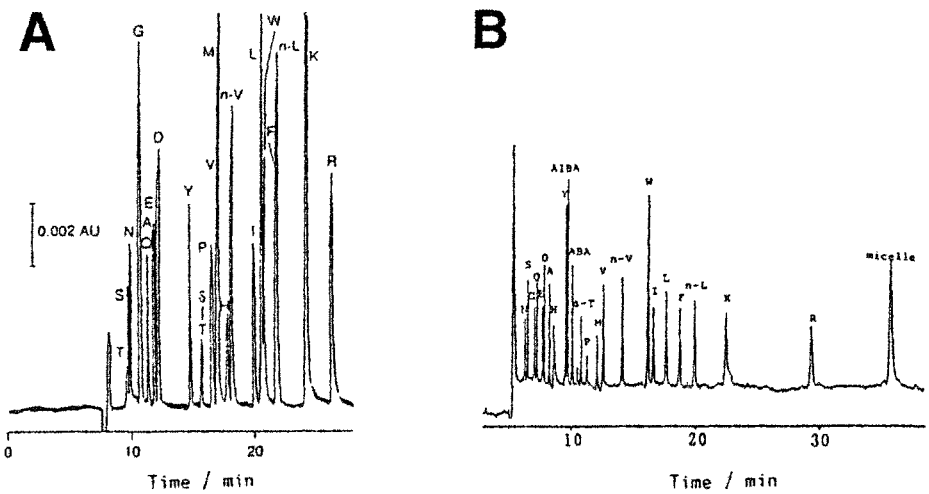

Fig. 15. Separation of PTH-amino acids by SDS-MEKC (**A**) without urea and (**B**) with urea: The peaks are labeled with one-letter abbreviations for amino acid. Separation solution, (A) 50 m*M* SDS, pH 7.0, (B) 100 m*M* SDS containing 4.3*M* urea; capillary, (A) 50 μm id × 650 mm (effective length, 500 mm), (B) 52 μm id × 500 mm (effective length, 300 mm); applied voltage, (A) 10 kV, (B) 10.5 kV; detection wavelength, (A) 260 nm, (B) 220 nm; temperature, (A) 35°C, (B) ambient. (Reprinted with permissions from **refs.** *17* and *35*.)

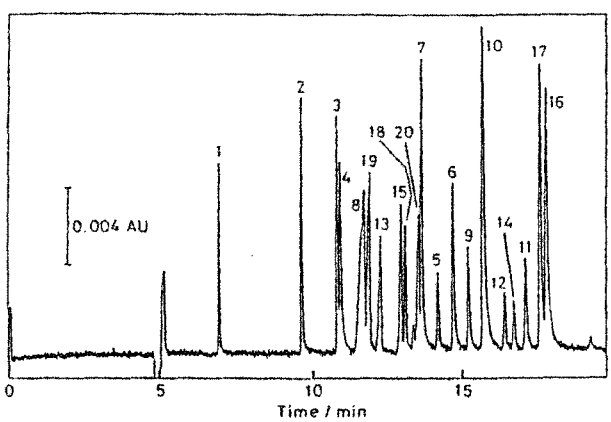

Fig. 16. Separation of chlorinated phenols by MEKC: Phenols, (1) phenol, (2) 2-chloro, (3) 3- chloro-, (4) 4-chloro-, (5) 2,3-dichloro-, (6) 2,4-dichloro-, (7) 2,5 -dichloro-, (8) 2,6-dichloro- (9) 3,4- dichloro-, (10) 3,5 -dichloro-, (11) 2,3,4-trichloro-, (12) 2,3,5-trichloro-, (13) 2,3,6-trichloro-, (14) 2,4,5 -trichloro-,(15) 2,4,6-tri-chloro-, (16) 3,4,5-trichloro-, (17) 2,3,4,5-tetrachloro-, (18) 2,3,4,6-tetrachloro-, (19) 2,3, 5,6-tetrachloro-, (20) pentachloro-; separation solution, 70 m*M* SDS, pH 7.0; capillary, 50 μm id × 650 mm (effective length, 500 mm); applied voltage, 10 kV, current, 17 μA; detection wavelength, 220 nm; temperature, 35°C. (Reprinted with permission from **ref.** *33*.)

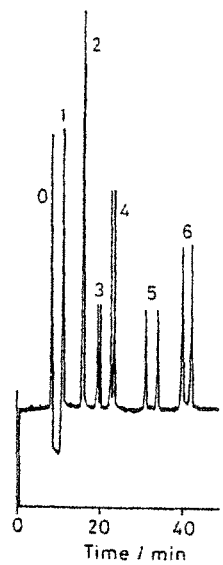

Fig. 17. Chiral separation of six PTH-DL-amino acids by MEKC with SDVal: Corresponding AAs: (1) Ser, (2) Aba, (3) Nva, (4) Val, (5) Trp, (6) Nle. (0) Acetonitrile. Micellar solution, 50 m*M* SD-Val—30 m*M* SDS—0.5*M* urea, pH 9.0, containing 10% (v/v) methanol; capillary, 50 μm id × 650 mm (effective length, 500 mm); applied voltage, 20 kV; current, 17 μA; detection wavelength, 260 nm; temperature, ambient. (Reprinted with permission from **ref.** *54*.)

the separation of hydrophobic compounds, nonaqueous CE *(77)* and hydrophobic interaction electrokinetic chromatography (HI-EKC) *(78)* were demonstrated.

6. MEKC-MS

Recently, mass spectrometry (MS) has become one of powerful detection schemes in CE. The development of MEKC-MS system, however, have not successfully progressed in last couple of years, because most surfactants normally used in MEKC often deteriorate ionization efficiency and cause high background signals in an electrospray ionization-MS (ESI-MS). One of solutions of these limitations is to use HMMSs instead of a conventional one, such as SDS, as pseudo-stationary phases in MEKC, as mentioned previously. Terabe and coworkers investigated on the use of BBMA for an MEKC-MS system. In an ESI-MS system, BBMA was successfully used for the separation and detection of some quaternary ammonium salts, alkaloids, and sulfamids *(79)*. The other technique to make an MEKC-ESI-MS possible is the partial filling (PF) method *(80)*, and PF-MEKC has been successfully coupled with an ESI-MS *(81,82, 82a)*.

As an alternate ionization method, the atmospheric pressure chemical ionization (APCI) has been developed *(83,84)*. In an MEKC-APCI-MS system, micellar solutions, even containing SDS, can be introduced directly into the ionization area of MS without severe decrease in sensitivity.

7. Conclusion

At the present stage, many papers on MEKC, which include fundamental characteristics and applications, have been available. Because only brief discussion on some aspects of MEKC was described in this article, it is necessary to refer some of those literature when the detailed information is required. Especially, for optimization strategies of MEKC, which was not discussed in this article, theoretical discussions by Foley *(85)*, Vindevogel and Sandra *(86)*, and Khaledi and coworkers *(87,88)* should be cited, along with the review article *(11)*.

Recently, a concept of retention index was extended to MEKC *(89,90)*. By using the retention index concept, we can discuss more easily on theoretical treatments of separation characteristics in MEKC.

There are some EKC techniques other than MEKC: Cyclodextrin EKC (CD-EKC) *(91)*, ion-exchange EKC (IX-EKC) *(92)*, and micro emulsion EKC (ME-EKC) *(93,94)*. In CDEKC, a cyclodextrin derivative having an ionic function is used instead of the micelles in MEKC. Similarly, polymer ions and microemulsions are used in IX-EKC and ME-EKC, respectively.

Electrokinetic chromatography, which is indeed a branch of CE, has become the normal technique for high-resolution separation of neutral species by CE, and will be used in much wider fields in the future.

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