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Accounts

Micellar Electrokinetic Chromatography

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A review of the research on micellar electrokinetic chromatography (MEKC), carried out mainly in our laboratory, is described from the viewpoints of (1) fundamental characteristics of MEKC, such as the separation principle, chromatographic parameters, selectivity and resolution, thermodynamic parameters, retention index, and band broadening; (2) selectivity manipulation, including effects of surfactants, temperature, pH, and various additives; (3) on-line coupling of MEKC with mass spectrometry; (4) applications, such as the separation of closely related compounds and enantiomer separations; and (5) on-line neutral sample concentration techniques.

Chromatography is one of the most useful and important analytical separation methods and gas chromatography (GC) and high performance liquid chromatography (HPLC) are widely used as routine analytical techniques. The applicability of GC has been widely expanded due to the development of capillary GC, where quite high plate numbers can be attained. Similarly, a number of studies on the development of micro HPLC, in which a capillary tube was used as a separation column, has been carried out to achieve high theoretical plates. However, micro HPLC has not become popular as capillary GC. In around 1980, a few papers on capillary electrophoresis (CE) had been published[1—3] and many chromatographers were highly interested in the high separation efficiency in CE. However, only a few researchers began studies on CE at once, since handling capillaries was difficult because of the use of fragile glass tubing, special care was required for detection, and a high voltage power supply was needed. In about 1983, fused silica capillaries have become available commonly and the most difficult point in the experimental procedures was removed. Finally, it was in about 1989 that the studies on CE become active, when a commercially available CE apparatus came onto the market.

In CE, only ionic or charged solutes can be analyzed in principle, since its separation mechanism is based on the difference in electrophoretic mobilities of analytes. According to Nakagawa's suggestion,[4] we have developed micellar electrokinetic chromatography (MEKC)[5] where an ionic micelle is added to a CE solution. In MEKC, electrically neutral analytes can be separated without any modification of the CE instrument and the separation performance is almost comparable with CE, so that MEKC enabled to expand the application range of CE. On the other hand, MEKC is also a kind of chromatography and has both electrophoretic and chromatographic characteristics, that is why MEKC is an interesting technique in methodology. Some topics about the naming and the early stages of MEKC have been described in detail.[6]

In this paper, we summarize mainly our recent studies on MEKC, not over the entire broad range of MEKC. Basically important studies on MEKC, even not our results, will be included in this article. Some books and reviews on MEKC have been published;[7—10] some detailed descriptions on MEKC are also available in books on CE.[11—26]

1. Separation Principle and Separation Characteristics of MEKC

1.1. Principle of MEKC. In MEKC, an ionic surfactant micelle is used as a pseudostationary phase that corresponds to the stationary phase in liquid chromatography (LC). A micelle is a molecular aggregate of the surfactant and it cannot exist alone, but exists only dynamically in equilibrium between monomeric surfactant molecules and the micelle; the micellar solution is homogeneous. Under the electrophoretic condition, the ionic micelle migrates at a different velocity from the surrounding aqueous phase because of its electrophoretic mobility. A portion of the sample molecules introduced in the micellar solution is incorporated into the micelle, and a distribution equilibrium is established between the micelle and surrounding aqueous phase. In general, the distribution equilibrium completes within a much shorter time than the electrophoretic migration of the micelle so that the micelle can be considered as a stationary phase in chromatography. That is, the sample components are separated due to the difference in the strength of the interaction

with the micelle. A neutral analyte has no electrophoretic mobility, but it obtains an apparent electrophoretic mobility by binding to the micelle. Since the size of the micelle is larger than that of a low molecular mass compound, the electrophoretic velocity of the micelle is approximately constant even when the sample is incorporated into the micelle. When the degree of the interaction with the micelle is different among the analytes, the migration velocity of each analyte by electrophoresis becomes different, and hence the analytes will be separated.

Since sodium dodecyl sulfate (SDS) is widely used as an ionic surfactant in MEKC, the separation principle of MEKC with SDS micelles is shown in Fig. 1. A fused silica capillary is filled with an SDS solution, in which the concentration of SDS is higher than its critical micelle concentration (cmc) so that micelles are formed. When a DC high voltage is applied between both ends of the capillary, the entire solution migrates toward the cathode by the electroosmotic flow (EOF) owing to the negative charge on the capillary surface, while the micelle is forced toward the anode by electrophoresis since the SDS micelle is negatively charged. Under neutral or basic conditions, the EOF is stronger than the electrophoretic migration of the SDS micelle and hence, the micelle consequently migrates toward the cathode at a more retarded velocity than the aqueous phase.

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 the surrounding aqueous phase. The analyte that is not incorporated into the micelle at all migrates at the same velocity as the EOF toward the cathode, since it always exists in the surrounding aqueous phase. The analyte that is totally incorporated into the micelle migrates at the lowest velocity or the same velocity as the micelle toward the cathode. The more the analyte is incorporated into the micelle, the slower the analyte will migrate. As long as the analyte is electrically neutral, it migrates at a velocity between the two extremes or between the velocity of the EOF and that of the micelle. When a detector is settled at the cathodic side of the capillary, the analytes are detected in an increasing order of the distribution coefficients.

Assuming the injection of a hypothetical mixture of three components, such as water, micelle, and a solute that is equally distributed between the micelle and water, we can schematically illustrate the migrating zones (A) and obtain a schematic chromatogram of the three zones observed with an imaginary detector at the end of the capillary (B), as shown in Fig. 2.[27] Here, we assume the migration directions of the EOF and electrophoresis of the micelle are opposite and the absolute value of the former velocity is four-thirds of the latter velocity. The migration time of the electrically neutral analyte is limited between the two extremes: the migration time of a solute that is not incorporated into the micelle at all, t_0, and that of the micelle, t_{mc}.

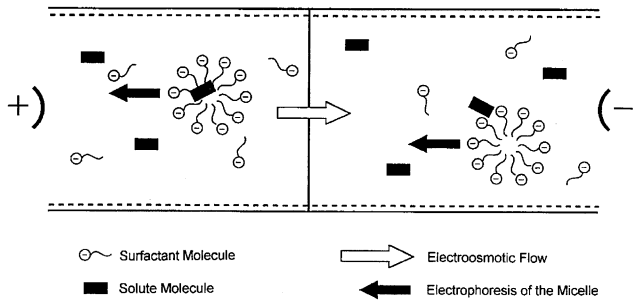

Fig. 1. Schematic illustration of the separation principle of MEKC.

When an acidic solution is employed, the absolute value of the electroosmotic velocity becomes lower than that of the electrophoretic velocity of the SDS micelle and then the micelle migrates toward the anode.[28] When a cationic surfactant, e.g., dodecyltrimethylammonium bromide (DTAB), is employed instead of SDS, the direction of the EOF will be reversed or toward the anode, through the adsorption of the surfactant molecule on the inside wall of the capillary and changing the surface charges.[29]

Comparing MEKC with conventional chromatography, the micelle corresponds to the stationary phase, although it is not immobilized in the capillary, and then the micelle will be referred to as a pseudostationary phase. On the other hand, the aqueous phase corresponds to the mobile phase, since it migrates at a different velocity from the micelle in the capillary. The use of pseudo-stationary phases other than the micelle is also possible, and, in that case, we will use the term electrokinetic chromatography (EKC) as a general name. In this article, other EKC techniques will also be described if necessary.

1.2. Resolution and Separation Parameters in MEKC.

1.2.1. Migration Time, Retention Factor, and Resolution.

(1) Retention Factor. For electrically neutral compounds, the retention factor, k, can be defined as n_{mc}/n_{aq}, where n_{mc} and n_{aq} are the number 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:

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

It can be rewritten as:

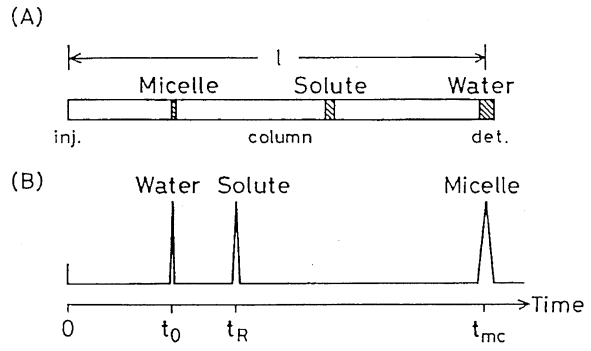

Fig. 2. Schematic of the (A) zone separation and (B) chromatogram in MEKC of the hypothetical mixture of water, solute, and micelle.[27]

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

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

When the migration time of the micelle is infinite or the micelle does not migrate in the capillary at all, t_0/t_{mc} will be zero; then Eqs. 1 and 2 become identical to those for conventional LC, Eqs. 3 and 4:

$$k = (t_R - t_0)/t_0, \quad (3)$$

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

In conventional LC, $k = \infty$ means that the solute is totally retained in the stationary phase and does not migrate at all or the retention time becomes infinite, whereas in MEKC $k = \infty$ means that the migration time of the solute, t_R, is equal to t_{mc}. In that case, such solute migrates at the same velocity as the micelle or at the lowest velocity and is then detected last.

On the other hand, when $t_0 = 0$ or the EOF is completely suppressed, Eq. 2 becomes

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

In this instance, the surrounding aqueous phase is completely suspended in the capillary and only the micelle migrates toward the anode. This also implies that the EOF is not essential in MEKC.

To calculate the retention factor by using Eq. 1, t_0, t_R, and t_{mc} are required. For the marker of the EOF or t_0, methanol is often used, since the distribution coefficient for methanol between the micelle and aqueous phase is negligibly small. Although methanol is UV transparent, it is detectable thorough the baseline fluctuation of the UV detector due to the change in refractive index. As for the tracer of the micelle, Sudan III or IV are widely used since they are highly lipophilic and totally incorporated into the micelle. Timepidium bromide is also useful as a tracer of an anionic micelle.[30]

Note that Eq. 1 was derived for the retention characteristics of neutral compounds. Hence, relationships between the migration time or mobility and retention factor will be more complicated when the solute has an electrophoretic mobility, that is, the migration of the ionic solute includes a portion generated by the micelle when the solute is incorporated into the micelle and also the other portion generated by the electrophoresis of the solute itself. For detailed discussions, refer to the literature.[31,32]

(2) Resolution. Resolution, R_s, in MEKC is given as:[27]

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

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. Equation 6 will be identical to the resolution equation for conventional chromatography when $t_0/t_{mc} = 0$. Effects of these parameters on resolution are briefly discussed below.

In MEKC, although the plate number is not proportional to the length of the capillary, the effective length of the capillary is preferably at least 30 cm. 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, or normally 15—20 kV is suitable. Since the diffusion coefficient of the micelle is small, solutes having larger retention factors can yield higher plate numbers.

The separation factor α is altered by the combination of the structure of the micelle as the pseudostationary phase and the aqueous phase as a solvent of the micelle. Since the retention factor is included in the last term of the right side of Eq. 6, the effect of the retention factor on resolution in MEKC is different from that in conventional chromatography. The optimum value of the retention factor, k_{opt}, for accomplishing the maximum R_s can be calculated by differentiating the following equation:[33]

$$f(k) = \{k_2/(1+k_2)\}\cdot[\{1-(t_0/t_{mc})\}/\{1+(t_0/t_{mc})k_1\}]. \quad (7)$$

Then,

$$k_{opt} = (t_{mc}/t_0)^{1/2}. \quad (8)$$

We can find that k_{opt} is a function of t_{mc}/t_0. Under neutral conditions, the optimum value is close to 2 for SDS micelles as the pseudostationary phase. For practical use, the range of k is recommended between 1 and 5, or at maximum between 0.5 and 10. Zhang et al. discussed the meaning of the last term of the right side of Eq. 6[34] which represents the column availability: Unlike conventional chromatography, in MEKC the micelle as the pseudostationary phase migrates by the flow of the whole liquid inside the capillary due to the EOF. This migration of the micelle by the EOF does not affect separation at all. However, the distance of the migration of the micelle cannot be used for the separation. Since the time available for the solute to interact with the moving micelle depends on its k, the column availability depends on k.

Although Eq. 6 was derived for the condition in which the EOF is larger than the electrophoretic velocity of the micelle, it is valid for other conditions. For example, under the acidic condition the electrophoretic velocity of the micelle will be higher than the EOF; then the migration directions of the micelle and aqueous phase will be opposite. In this case, the solutes having larger k or smaller k than $-(t_{mc}/t_0)$ migrate toward the same direction or the opposite direction as the micelle, respectively, whereas the solute of k equal to $-(t_{mc}/t_0)$ remains still in the capillary. Therefore, the closer to $-(t_{mc}/t_0)k$ becomes, the larger R_s becomes and the value of the last term in Eq. 6 becomes larger than unity.[28] Here, we assume that the migration directions of the EOF and the micelle are positive and negative, respectively, and t_{mc} is negative. Zhang et al.[34] reported detailed discussions on Eq. 6 by classifying the MEKC conditions based on the relative magnitude of t_{mc} and t_0 or on the ratio of the migration velocities of the micelle and EOF.

1.2.2. Retention Factor and Distribution Coefficient. In MEKC the retention factor can be related to the distribution

coefficient, K, between the micelle and aqueous phase as:

$$k = K(V_{mc}/V_{aq}). \quad (9)$$

Here, V_{mc} and V_{aq} are the volumes 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 the partial specific volume of the micelle $\bar{v}$ as:

$$V_{mc}/V_{aq} = \bar{v}(C_{sf} - \text{cmc})/\{1 - \bar{v}(C_{sf} - \text{cmc})\}. \quad (10)$$

At low micellar concentrations, we can rewrite Eq. 10 as:

$$k \cong K\bar{v}(C_{sf} - \text{cmc}) \quad (11)$$

This reveals that the retention factor can be easily adjusted by manipulating the surfactant concentration. Equation 11 shows that the retention factor increases linearly with the surfactant concentration. The linear relationship was observed between k and C_{sf} and we can calculate K from the slope of this relationship.[27] This also implies that K remains constant regardless of C_{sf}.[27] An example of the dependence of k on the surfactant concentration is shown in Fig. 3 for sodium 10-undecenyl sulfate (SUS) oligomer as a pseudostationary phase.[35] In this case, almost all lines pass through the origin, showing that cmc is essentially zero. In general, the intercept with the x axis corresponds to the cmc. The applicability of Eq. 11 under various conditions and for various surfactants has been explained by a number of reports.[27,31,32,36–41]

1.2.3. Thermodynamic Parameters. The distribution coefficient, K, can be related to the enthalpy change, ΔH°, and entropy change, ΔS°, associated with micellar solubilization as follows:

$$\ln K = -(\Delta H^\circ/RT) + (\Delta S^\circ/R), \quad (12)$$

where R and T are the gas constant and the absolute temperature, respectively. Thus, Eq. 12 allows the calculation of the enthalpy and entropy changes in micellar solubilization from the plot of K against the reciprocal of T or the van't Hoff plot.[27,42] This indicates that MEKC is a useful technique not only for analytical separation but also for calculating thermodynamic parameters.

To calculate K from Eq. 10, both cmc and the partial specific volume of the micelle must be known. In various buffer systems, such as borate–phosphate (B–P), piperazine-*N*,*N*′-bis(2-ethanesulfonic 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,[42] as shown in Table 1. Since cmc depends on both temperature and the composition of the buffer system, the cmc value under the separation condition must be estimated, whereas the difference in the partial specific volume among the above three buffer systems was not significant.

Table 2 lists distribution coefficients of some solutes at different temperatures between the SDS micelle and B–P buffer; corresponding enthalpy, entropy and Gibbs free energy changes were calculated, as shown in Table 3.[42] Obviously, the effect of the buffer composition is not significant

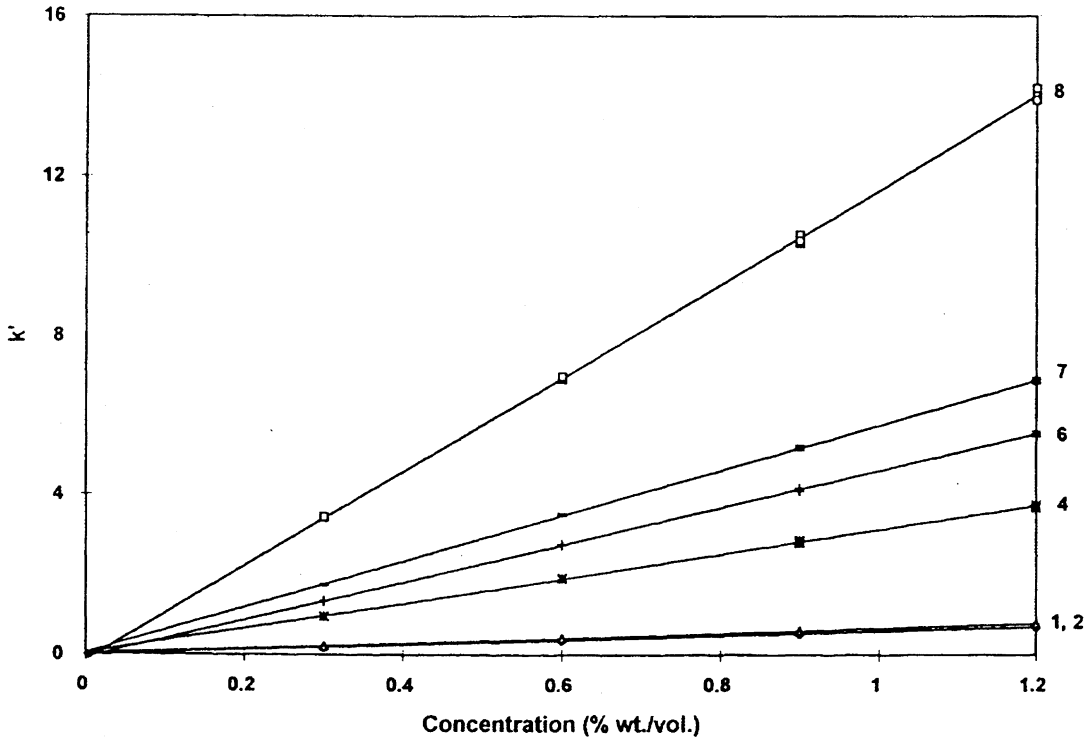

Fig. 3. Dependence of the retention factors (k') for substituted benzene and naphthalene compounds on the concentration of SUS oligomer:[35] (1) nitrobenzene, (2) *p*-nitroaniline, (4) naphthalene methanol, (6) naphthalene, (7) naphthalene ethanol, (8) diphenyl ether. Capillary, 50 μm i.d. × 570 mm, 500 mm effective; applied voltage, 14.1 kV.

Table 1. CMC of SDS and the Partial Specific Volume ($\bar{v}$) of the SDS Micelle[42]

Temp	Buffer[a] cmc/mM				$\bar{v}$/mL g^{-1}	
°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 mM borate-50 mM phosphate buffer (pH 7.0); PIPES, 20 mM PIPES-20 mM NaOH (pH 7.0); BES, 100 mM BES-100 mM NaOH (pH 7.0); urea, 5 M urea in 100 mM borate-50 mM phosphate buffer (pH 7.0) (1 M = 1 mol dm^{-3}).

Table 2. Distribution Coefficients of Alkylphenols between the SDS Micelle and a 100 mM Borate-50 mM Phosphate Buffer (pH 7.0)[a,42]

Solute	Temp/°C 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-Pentylphenol	7120	6660	5870	5580	4900

a) Separation solution, 50 mM SDS in 100 mM borate-50 mM phosphate buffer (pH 7.0).

on these thermodynamic quantities. The entropy changes for resorcinol, phenol, *p*-nitroaniline, and 2-naphthol were negative, and the contribution of $\Delta S°$ to $\Delta G°$ was significant except for phenol and nitrobenzene. Relatively a large positive entropy change for toluene is considered to be due to the disappearance of the highly structured hydrophobic cavity of water along with the solubilization. Measurements of thermodynamic quantities of micellar solubilization by MEKC have been reported elsewhere.[43—45]

1.2.4. Retention Index. The application of the retention index, which has been widely used in GC,[46] on MEKC to state retention behavior appeared recently.[47—49] The retention index was introduced to universally describe the retention behavior in chromatography based upon Martin's equation, which states that a linear relationship exists between the logarithm of the retention factor and number of carbons in a homolog series, that is,

$$\log k = az + b, \tag{13}$$

where k is the retention factor, z is the number of carbons, and a and b are constants: a reveals the degree of the change in retention factor through the difference in one carbon atom, and b depends on the functional group in the homolog. The retention index, I, is written as:

$$I = 100z + (\log k_s - \log k_z)/(\log k_{z+1} - \log k_z) \tag{14}$$

where k_s is the retention factor of the sample and k_z and k_{z+1} are the retention factors of the homologs of the number of carbon of z and z+1, respectively. From the limit of the detector response, a few series of homologs can be used for determining the retention index in MEKC. Alkylbenzenes and alkyl aryl ketones are usually employed for this purpose. The range of the number of carbons is narrow in MEKC since the range of the migration time is limited. However, the reproducibility of the retention index in MEKC is good, similar to values in GC and LC, since the index is hardly affected by experimental conditions and is independent of the change in the surfactant concentration.[47] Applications of the retention index include the qualitative analysis, classification of pseudostationary phases, studies on the interaction between the solute and pseudostationary phase, and correlation with the physiological activity. Figure 4 shows the comparison of retention indexes I_{SDS} and $I_{SDS/Brij35}$, for the SDS micelle and mixed micelle of SDS and Brij 35 as pseudostationary phases, respectively.[50,51] For *n*-alkylbenzenes a linear relationship was observed between I_{SDS} and $I_{SDS/Brij35}$, whereas for hydrogen bond-accepting compounds, such as aromatic compounds (A), xanthines (B), and corticosteroids

Table 3. Enthalpy, Entropy, and Gibbs Free Energy Changes in Micellar Solubilization of Alkylphenols by the SDS Micelle[a,42]

Solute	$\Delta H°$/kJ mol^{-1}	$\Delta S°$/J mol^{-1} K^{-1}	$\Delta G°$/kJ mol^{-1} (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-Pentylphenol	−15.0	24.3	−22.5

a) The separation solution was the same as in Table 2.

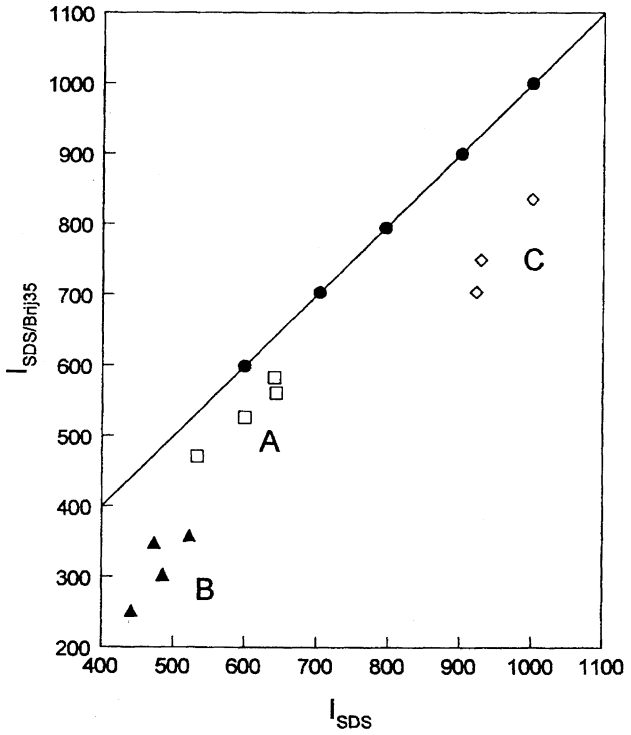

Fig. 4. Plot of $I_{SDS-Brij35}$ (50 mM SDS + 10 mM Brij 35) versus I_{SDS} for (line) *n*-alkylbenzenes, (A) hydrogen bond accepting aromatic compounds, (B) xanthines, and (C) corticosteroids.[51] Data from Ref. 50.

(C), no linear relationship was observed. So we can recognize that these compounds have stronger affinities to the SDS micelle than to mixed micelle. On the other hand, for hydrogen bond-donating compounds, such as phenol and nitrobenzene, points appeared above the straight line (data not shown) since these have stronger affinities to the mixed micelle than SDS micelle due to the interaction with the oxygen atom of the polyoxyethylene group in Brij 35. The retention index is also effective for ionic compounds if the retention factor can be obtained accurately.[49,52]

1.3. Band Broadening in MEKC. In capillary zone electrophoresis (CZE), the main cause of band broadening is the longitudinal diffusion, as long as both the length of the sample plug and the width of the detection window do not affect the band broadening.[2] In MEKC, the system contains the micelle and the distribution equilibrium of the solute between the micelle and aqueous phase exists, and therefore the effect of parameters on the plate height, H, is represented as:

$$H = H_l + H_{mc} + H_{aq} + H_t + H_{ep}, \quad (15)$$

where H_l, H_{mc}, H_{aq}, H_t, and H_{ep} are the contributions of the longitudinal molecular diffusion, the mass transfer between the micelle and aqueous phase, the inter micelle mass transfer of the solute in the aqueous phase, the distribution of the migration velocity due to joule heating, and the electrophoretic dispersion due to the heterogeneity of the micellar size, respectively. Here, only a brief description is given. Refer to reference[53] for detailed discussions.

Generally, H_l and H_{mc} are important among these five parameters; for the solute having a small retention factor only H_l is significant.[53] The description of H_l is as follows:

$$H_l = [\{2(D_{aq} + kD_{mc})\}/\{1 + (t_0/t_{mc})k\}][1/v_{eo}] \quad (16)$$

where D_{aq} and D_{mc} are the diffusion coefficients of the solute and micelle, respectively, and v_{eo} is the electroosmotic velocity. Generally, the magnitude of D_{mc} is one-tenth of that of D_{aq} and hence, Eq. 16 suggests that the solute having a larger retention factor attains a higher plate number and the contribution of the molecular diffusion in MEKC will always be smaller than that in CZE in terms of the longitudinal diffusion, except for polymers such as proteins.

The contribution of H_{mc} is represented as Eq. 17:

$$H_{mc} = \{2(1 - t_0/t_{mc})^2 k\}/[\{1 + (t_0/t_{mc})k\}(1 + k)^2]\cdot(v_{eo}/k_d). \quad (17)$$

Here, k_d is the desorption rate constant of the solute from the micelle. In general, k_d is smaller for a compound having larger k and hence, it contributes to the band broadening only for highly hydrophobic compounds. However, since high theoretical plate numbers are attained even for hydrophobic compounds, such as perylene and anthracene, the contribution of H_{mc} can be negligible.

It is an well-known fact that the size of the micelle, which is an aggregation of the surfactant molecules, is not uniform, but there is a distribution in the aggregation number. The difference in the micellar size seems to cause the difference in the electrophoretic velocity and hence to contribute the band broadening. However, we can imagine that the micelle is always in an equilibrium with a monomer of the surfactant molecule and a rapid equilibrium is always established in the micellar size, and consequently the contribution of the micellar size on the band broadening should be insignificant. On this point, there was an error in our previous report,[53] so we correct it by stating that the contribution of H_{ep} is three-orders smaller than that reported previously.

Therefore, the longitudinal molecular diffusion is the main cause of the band broadening even in MEKC, and a higher plate number is expected in MEKC than in CZE for small molecules. On the other hand, Davis et al.[54,55] examined the band broadening in MEKC in detail and supported our conclusion in the outline. However, for a solute having a large retention factor, a remarkable decrease in the plate number was observed when the concentration of the micelle or SDS was high and the temperature rise by joule heating was high. According to their conclusion, the reason was that the radial temperature gradient affected the electrophoretic velocity of the micelle.[56]

In many cases, the plate number observed in MEKC is not as high as that predicted from the theory. The main reason is considered to be a too large sample amount injected or some adsorption of the sample onto the inside wall of the capillary. Although the capillary rinsing is commonly performed by using an alkaline solution, this solution is not always suitable in MEKC and the use of the organic solvent should be attempted.

2. Improvement of Separation Selectivity in MEKC

Selectivity in chromatography can be discussed by using the separation factor, α. The micelle in MEKC corresponds to the stationary phase in reversed phase HPLC (RP-HPLC), while the bulk solution or the surrounding aqueous phase to the mobile phase, from the view point of selectivity manipulation. The following four factors should be mainly taken into account to manipulate the selectivity: (1) the surfactant molecule, (2) temperature, (3) pH, and (4) additives.

2.1. The Surfactant or Pseudostationary Phase.

2.1.1. Ionic Low-Molecular-Mass Surfactants (LMMSs). The hydrophobicity of the sample will greatly affect the selectivity according to the retention characteristics in MEKC reported to date. For example, a number of reports are available on the relationship between $\log k$ and $\log P_{ow}$ in MEKC.[43,44,57–63] Here, P_{ow} is the partition coefficient between octanol and water. Although relatively a good linear relation is recognized between these two, different slopes of the straight line and different correlation factors are observed among various kinds of surfactants and sample compounds.[63] This implies that the mechanism of the micellar solubilization is similar to that of the octanol-water distribution, but there is also a difference. Ishihama et al.[44,49] investigated the relationship between the retention characteristic and biological activity in EKC using a microemulsion as the pseudostationary phase instead of the micelle, and reported that better correlation with $\log P_{ow}$ was found by using the retention index rather than $\log k$. And they also described that the biological activity could be successfully related with the retention index more than with $\log P_{ow}$.

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.[27,29] For example, SDS and hexadecyltrimethylammonium bromide (CTAB) show considerably different selectivity, as shown in Fig. 5.[13] 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.

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.[27] 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.

A lot of reports have been published on the relation between the surfactant structure and separation selectivity. The most successful approach to relate physicochemical parameters of compounds to retention characteristics in MEKC is

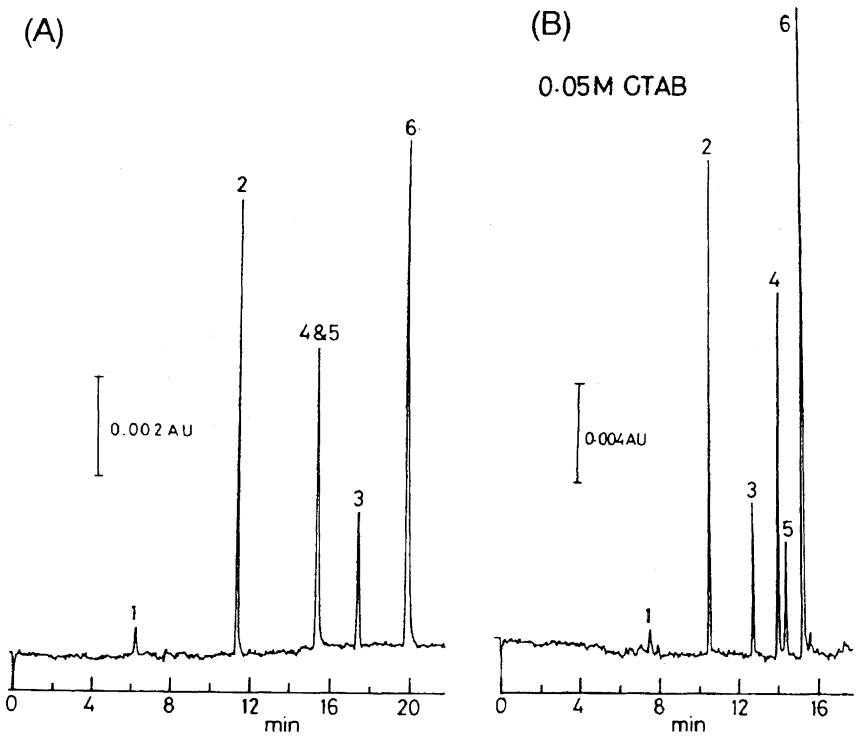

Fig. 5. Effect of the surfactant structures on selectivity:[13] (1) water, (2) aniline, (3) nitrobenzene, (4) *m*-nitroaniline, (5) *p*-nitroaniline, (6) *o*-nitroaniline. Separation solution, (A) 100 mM SDS in 100 mM borate-50 mM phosphate buffer (pH 7.0), (B) 50 mM CTAB in 100 mM Tris-HCl (pH 7.0); capillary, 50 μm i.d. × 650 mm, 500 mm effective; applied voltage, 15 kV; detection wavelength, 230 nm.

Table 4. Distribution Coefficients at 35 °C[27]

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.

the method using solvatochromic solvation parameters.[64—66]

Although cmc of SDS in pure water is reported as 8—9 mM, the value remarkably decreases in electrolyte solutions, as shown in Table 1. Properties of SDS, as a sodium salt, and potassium dodecyl sulfate are quite different. The Krafft point of the latter is too high to use in MEKC since it precipitates under ambient temperature. Therefore, potassium salts should not be used as buffer components for the SDS system.

Bile salts, such as sodium cholate, sodium deoxycholate, sodium taurodeoxycholate, which form helical micelles[67] can achieve the significantly different selectivity compared with the long alkyl-chain surfactants.[68]

2.1.2. 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. A mixed micelle consisted of ionic and nonionic surfactants is usually larger than the original ionic micelle and has a lower charge density, and consequently has a lower electrophoretic mobility. Thus, a narrower migration time window is obtained,[69] and also a different selectivity is expected[69,70] since the surface of the mixed micelle is different from that of the original one.

2.1.3. High-Molecular-Mass Surfactants. An ionic micelle or mixed micelle, which is in principle a molecular aggregate of the surfactant molecule and exits in a dynamic equilibrium state, is employed as the pseudostationary phase. As can be seen in Eq. 8, the net micellar concentration, C(mc), is represented as $C_{sf}-$cmc. Since the solute is distributed between the micelle and the surrounding aqueous phase, the migration time of the analyte depends on C(mc). cmc varies with temperature, salt concentration, and additives to the surfactant solution and therefore, C(mc) changes with these parameters. To keep C(mc) constant throughout the MEKC run is essential to obtain reproducible migration times.

Since the high-molecular-mass surfactant (HMMS) forms a molecular micelle, which consists of a single molecule, 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, several research on the use of HMMSs, such as butyl acrylate-butyl methacrylate-methacrylic acid copolymer sodium salt (BBMA),[71—73] SUS oligomer, and sodium undecenoate oligomer,[35,74] in MEKC have been reported, showing that the HMMSs are useful for pseudostationary phases in MEKC with almost the same separation performance as conventional LMMSs. Separation selectivity in an HMMS-MEKC is more or less different from that in an LMMS-MEKC system. In the BBMA-MEKC system, reproducibility of the migration time was better than that in SDS-MEKC, whereas reproducibility of the peak area was not comparable with that in SDS-MEKC, probably because of low purity of BBMA.[75]

HMMS-MEKC is effective for on-line coupling of MEKC with mass spectrometry (MS)[76,77] (see Section 3).

2.1.4. Optically Active Surfactants. Separation of optical isomers is one of major applications utilizing the high separation efficiency in CE, and a number of studies on enantioseparations by MEKC have also been carried out. For enantiomer separations by MEKC, two methods are used: one is the addition of a chiral agent to a micellar or separation solution, and the other is the use of a micelle that has an ability of enantiorecognition or a chiral surfactant (see Section 4.2.1).

The first report on the enantiomer separations by MEKC using chiral surfactants was about the use of bile salts as pseudostationary phases, in which dansyl-DL-amino acids (Dns-DL-AAs) were separated from each other and each enantiomer was optically resolved.[78]

MEKC with chiral surfactants is a promising technique, in which separation conditions can be easily manipulated, and has become an important method for the enantiomer separation in CE along with cyclodextrin modified CZE (CD-CZE).

2.2. Temperature. The distribution coefficient is dependent on temperature, that is, the distribution coefficient decreases with an increase in temperature. Thus, a reduced migration time of the solute is obtained with an increase in temperature. The increase in temperature also causes increases in v_{eo} and the electrophoretic velocity of the micelle, v_{ep}(mc), because of a reduced viscosity. Since dependences of the distribution coefficients on temperature are different among solutes, temperature affects selectivity, as shown in Fig. 6.[13]

Temperature seriously affects the migration time, whereas its effect on selectivity is not remarkable; hence, it is important to maintain temperature precisely to obtain reproducible results.

2.3. pH. Effect of the constituents of the buffer is not significant, while the pH is a quite critical factor to ionizable analytes. If the ionized form of the solute has the same charge as the micelle, it will be incorporated into the micelle less than its neutral form. Figure 7 demonstrates the dependence of the apparent retention factor on the buffer pH for chlorinated phenols.[79] Here, the apparent retention factor was calculated by Eq. 2 regardless whether the solutes were ionized or not. For acidic compounds, the increase in pH will promote ionization, then the distribution coefficient to the anionic micelle such as SDS will decrease. It should be noted that the change in the buffer pH, especially in the lower pH region, causes a significant change in the electroosmotic

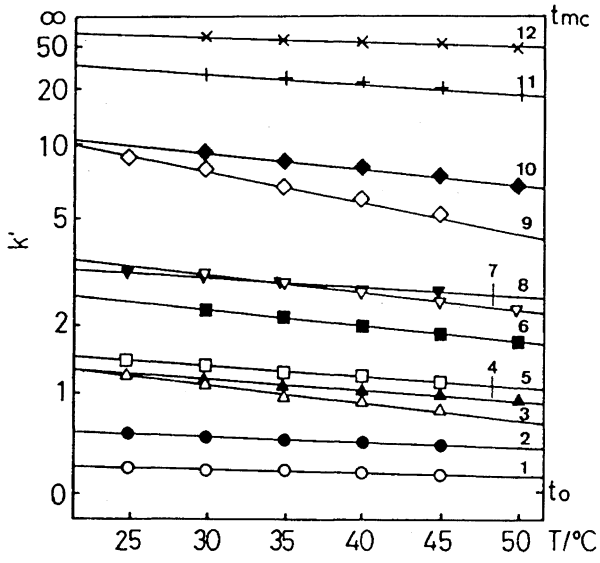

Fig. 6. Dependence of the retention factor (k') on temperature (T):[13] (1) resorcinol, (2) phenol, (3) *p*-nitroaniline, (4) *o*-cresol, (5) nitrobenzene, (6) 2,6-xylenol, (7) 2,4-xylenol, (8) toluene, (9) 2-naphthol, (10) *p*-propylphenol, (11) *p*-butylphenol, (12) *p*-pentylphenol. Micellar solution, 50 mM SDS in 100 mM borate-50 mM phosphate buffer (pH 7.0); capillary, 50 μm i.d. × 570 mm, 500 mm effective; detection wavelength, 214 nm; applied voltage, 15 kV.

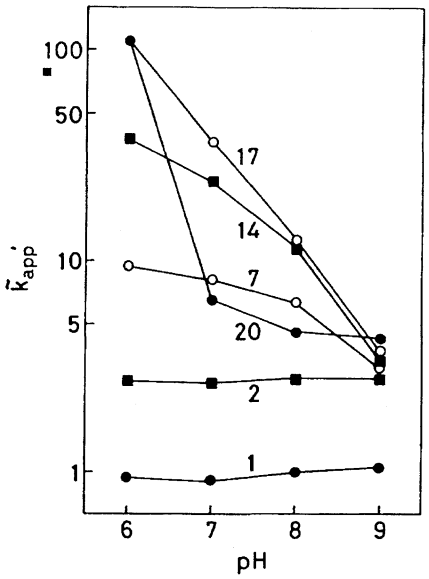

Fig. 7. Dependence of apparent retention factor (k'_{app}) of chlorinated phenols on pH:[79] (1) phenol, (2) 2-chlorophenol, (7) 2,5-dichlorophenol, (14) 2,4,5-trichlorophenol, (17) 2,3,4,5-tetrachlorophenol, (20) pentachlorophenol. Micellar solution, 100 mM SDS (pH 7.0); capillary, 50 μm i.d. × 650 mm, 500 mm effective; applied voltage, 15 kV; temperature, 35 °C.

velocity.[17]

2.4. Additives. The most versatile and effective methods to manipulate selectivity in MEKC are the use of additives to the aqueous phase and the selection of suitable surfactants. In HPLC, the methodology for the use of additives to the mobile phase is well established, and the procedures are useful in MEKC. The following categories of additives are mainly used in MEKC: (1) cyclodextrins (CDs), (2) organic modifiers, (3) ion-pair reagents, (4) others.

2.4.1. Cyclodextrins (CDs). Recently, CD has been widely used not only in LC but also in CE as a mobile phase modifier. In most cases, CD's capability of recognizing specific molecules which fit its hydrophobic cavity is used for chromatographic separations. The use of CD is especially effective for the separation of aromatic isomers and aromatic enantiomers which have the chiral center close to the aromatic ring.

Since CD is electrically neutral and is not affected by the electrophoretic force, CD itself cannot be used as a pseudo-stationary phase in EKC unless ionic groups are introduced into CD. However, by adding CD to the separation solution or the solution of an ionic surfactant, it has become possible to take advantage of the CD's specific capability of recognizing molecules.[80,81] This technique is designated as CD modified MEKC (CD-MEKC). The surface of the CD molecule is hydrophilic. Hence, it can be assumed that CD is not incorporated into the micelle, while a surfactant molecule may be included into the CD cavity.

The separation principle of CD-MEKC is schematically shown in Fig. 8.[80] In this system, CD migrates at the same velocity as the EOF. The neutral analyte molecule migrates at the same velocity as the EOF, whether included with CD or free from CD. On the other hand, the analyte migrates at the different velocity from the EOF when it is incorporated into the ionic micelle. In the absence of CD, highly hydrophobic analytes tend to be totally incorporated into the micelle. The addition of CD reduces the apparent distribution coefficients of the hydrophobic analytes between micelle and aqueous phase containing CD and makes possible the MEKC separation of such solutes. The higher the concentration of CD becomes, the smaller the distribution coefficient will be observed. Therefore, in CD-MEKC the retention factor can be manipulated by varying both the concentrations of the micelle and CD. An example of the separation of hydrophobic compounds by CD-MEKC is shown in Fig. 9.[80] CD-MEKC is also effective for enantiomeric separation (see Section 4.2.2).

It should be noted that CD-MEKC is a different technique from CDEKC, although these two terms are sometimes con-

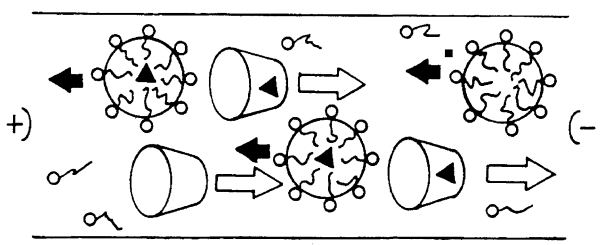

Fig. 8. Schematic illustration of the separation principle of CD-MEKC.[80] Filled arrows indicate the electrophoretic migration of the micelle and open arrows the electroosmotic migration of CD.

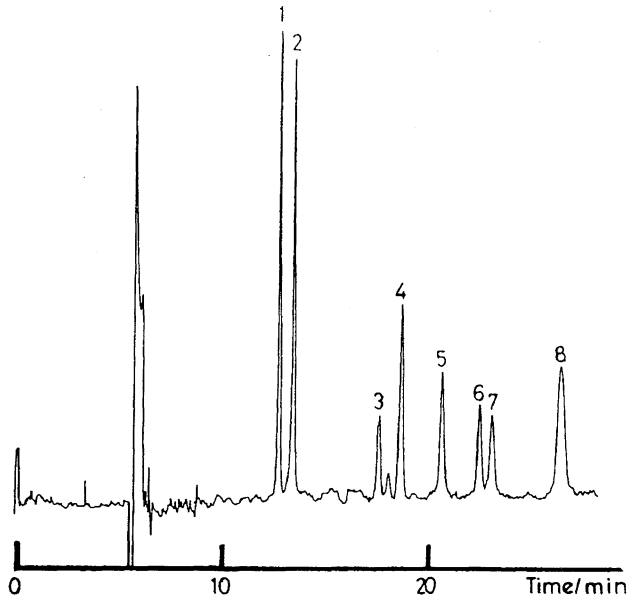

Fig. 9. Separation of a mixture of naphthalene and four tricyclic and three tetracyclic aromatic hydrocarbons by γ-CD-MEKC:[80] (1) naphthalene, (2) acenaphthene, (3) anthracene, (4) fluorene, (5) phenanthrene, (6) chrysene, (7) pyrene, (8) fluoranthene. Capillary, 50 μm i.d. × 700 mm, 500 mm effective; separation solution, 30 mM γ-CD, 100 mM SDS and 5 M urea in 100 mM borate buffer (pH 9.0); applied voltage, 20 kV; current, 41 μA; detection wavelength, 210 nm.

fused. In CD-MEKC, CD is added to micellar solutions, while in CDEKC, an ionic CD derivative is used as the pseudostationary phase of EKC in a solution, normally without micelles. Recently, the technique CDEKC is sometimes referred to as a modification of CD-CZE.

2.4.2. Organic Modifiers. Similar to HPLC, an organic solvent miscible with water can be used as an additive to the micellar solution to manipulate selectivity in MEKC. In HPLC, highly hydrophobic compounds can be analyzed by using a mobile phase containing a high concentration of the organic modifier, whereas in MEKC, the addition of an extremely high concentration of an organic modifier cannot be employed because of the breakdown of the micellar structure. In general, the maximum content of the organic modifier is 20—30% or so.

Organic modifiers usually used in MEKC are methanol,[82—86] 2-propanol,[87] acetonitrile,[83,86] and tetrahydrofuran,[86,88] which are the same as those used in HPLC. The use of the organic solvent usually provides a reduced EOF and hence an expanded migration time window.

The use of organic modifiers is also effective for the MEKC analysis of polycyclic aromatic hydrocarbons (PAHs). Separations of PAHs by MEKC using SDS as a micelle and methanol,[89] dimethyl sulfoxide,[90] and acetone[90] as organic solvents have appeared. In these cases, high concentrations of organic modifiers were used and the micellar structure might be different from that in the solutions without organic modifiers. However, any interaction between the solute and surfactant molecules occurred obviously. The separation of PAHs by MEKC using an SDS solution containing acetone is shown in Fig. 10.[90]

Separations of PAHs by MEKC using bile salts as surfactants and *N*,*N*-dimethylformamide as an organic modifier have also been reported.[91]

2.4.3. Ion-Pair Reagents. The use of the ion-pair reagent in MEKC causes a remarkable change in separation characteristics, which is mainly due to the charge of the micelle.

For example, when a tetraalkylammonium salt is added to an SDS micellar solution, anionic analytes form an ion pair with the ammonium ion; hence, the electrostatic repulsion between the anionic SDS micelle and the anionic analyte is reduced. This ion-pair formation is promoted with an increase in 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 interacting with the anionic micelle, so the migration time of the cation decreases with an increase in the ammonium salt

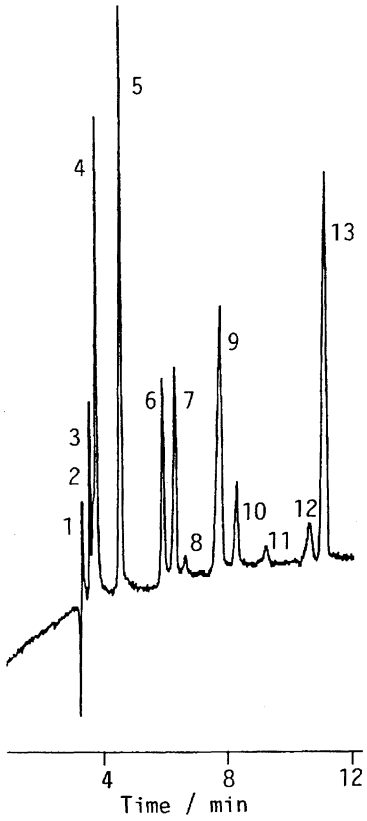

Fig. 10. Separation of 13 PAHs by SDS-MEKC in the presence of acetone:[90] (1) 1,4-benzoquinone, (2) quinoline, (3) benzene, (4) benzoin, (5) naphthalene, (6) benzanthrone, (7) phenanthrene, (8) anthracene, (9) pyrene, (10) 1,2-benzanthraquinone, (11) 2,3-benzofluorene, (12) benz(a)anthracene, (13) fluorescein. Separation solution, 25 mM SDS in borate-phosphate (pH 7.0) containing 30% (v/v) acetone; capillary, 52 μm i.d. × 370 mm, 300 mm effective; applied voltage, 20 kV (541 V cm^{-1}); detection wavelength, 200 nm; temperature, 35 °C.

concentration. It should be noted that the ammonium groups probably modify the surface of the SDS micelle by replacing a portion of sodium ions.

The effect of the addition of tetraalkylammonium salts to SDS micellar solutions on the selectivity is shown in Fig. 11.[30] The effect strongly depends on the structure of the ion-paring reagent, e.g., on the length of the alkyl chain.

2.4.4. Other Additives.

(1) 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 a high concentration of urea.[92] The addition of urea to the micellar solution causes the slightly reduced EOF, the considerably reduced migration velocity of the micelle, and the reduced retention factors. The addition of urea is also effective to improve peak shapes, especially in the separation of amino acid derivatives.[93]

Although a remarkable change of the selectivity is not

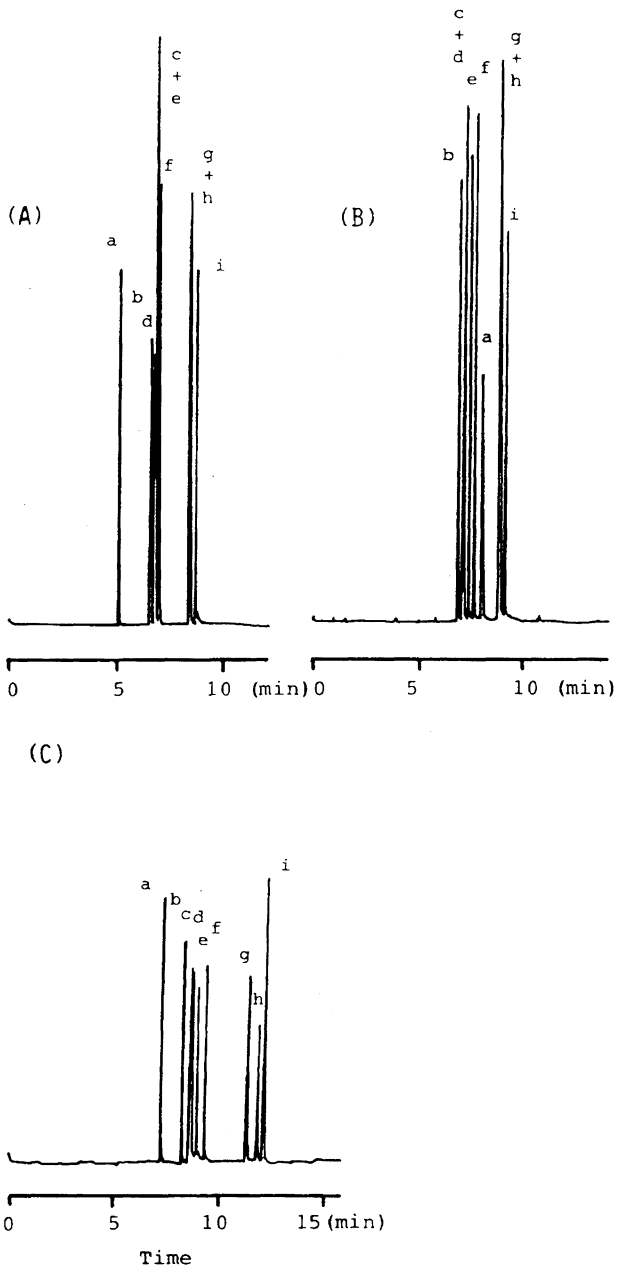

Fig. 11. Separations of cephalospolin antibiotics by (A) CZE, (B) SDS-MEKC, and (C) SDS-MEKC in the presence of tetraalkylammonium salt:[30] (a) C-TA, (b) ceftazidime, (c) cefotaxime, (d) cefmenoxime, (e) cefoperazone, (f) cefpiramide, (g) cefpimizole, (h) cefminox, (i) ceftriaxone. Separation solution, (A) 20 mM borate-phosphate buffer (pH 9.0), (B) 50 mM SDS in borate-phosphate buffer (pH 9.0), (C) 40 mM tetramethylammonium bromide added to the solution in (B); capillary, 50 μm i.d. × 650 mm, 500 mm effective; applied voltage, 20 kV; detection wavelength, 210 nm.

attained by the addition of urea, a slight change of the selectivity can be achieved, especially for the separation of closely related compounds.

(2) Metal Ions. Effects of the addition of metal ions to SDS solutions were reported.[94] In the separation of oligonucleotides, the addition of magnesium, zinc, or copper(II) ions to the SDS solution was effective to achieve separations and to improve the selectivity.

3. MEKC-MS

Recently, MS has become one of powerful detection schemes in CE, similar to schemes in GC and HPLC. The development of MEKC-MS system, however, has not remarkably progressed in the last couple of years, since most surfactants normally used in MEKC often deteriorate ionization efficiency and cause high background noise levels in electrospray ionization (ESI), which is typically used as an interface in CE-MS. However, several techniques have recently been developed to achieve MEKC-MS.[95]

One of the solutions to these problems is to use an HMMS instead of a conventional LMMS such as SDS as the pseudostationary phase in MEKC, as mentioned previously. We investigated the use of BBMA in MEKC-MS and demonstrated successful results.[76,77] In MEKC-ESI-MS with BBMA, successful separations and detections of some quaternary ammonium salts, alkaloids, and sulfaramides were achieved.[76]

As an alternate ionization method to ESI, the atmospheric pressure chemical ionization (APCI), which has already been widely used in LC-MS, has been applied to CE-MS.[96—98] In MEKC-APCI-MS, a micellar solution even containing SDS can be introduced directly into the interface without severe decrease in the MS sensitivity.

As one other technique to make MEKC-MS possible, the partial filling (PF) method was developed:[99] here the micellar solution or zone is filled only in a part of the capillary and the solute will interact with the micelle when passing through the micellar zone, as shown in Fig. 12. In PF-MEKC, the applied voltage for MEKC run will be cut off before the micellar zone reaches the end of the capillary or the interface to the mass spectrometer, so that the micelle is not introduced into the mass spectrometer. Therefore, by using the PF-MEKC technique, even SDS-MEKC can be coupled with ESI-MS.[100,101] Successful separations and detections of closely related peptides by PF-MEKC-ESI-MS were achieved.[101] The PF-MEKC technique is also applicable to APCI-MS. As for an example, PF-SDS-MEKC-APCI-MS analysis of several components of cold medicine have appeared,[102] as shown in Fig. 13.

The PF technique is also effective in CE-MS where an additive deteriorating the MS sensitivity is used in a run buffer: For enantiomer separations by CZE-ESI-MS using CDs and avidin as chiral selectors, the PF technique was applied to prevent introducing those chiral selectors into the ESI interface; good MS detectabilities were achieved.[103]

Recently, some attempts of EKC-ESI-MS without the PF technique have been also performed. A brief strategy for selecting separation conditions in EKC-ESI-MS, where SDS and a CD and a calix[6]arene derivatives were used as pseudostationary phases, was reported.[104] In this case, pseudostationary phases were sometimes introduced into the ESI interface. But good MS sensitivities were still attained.

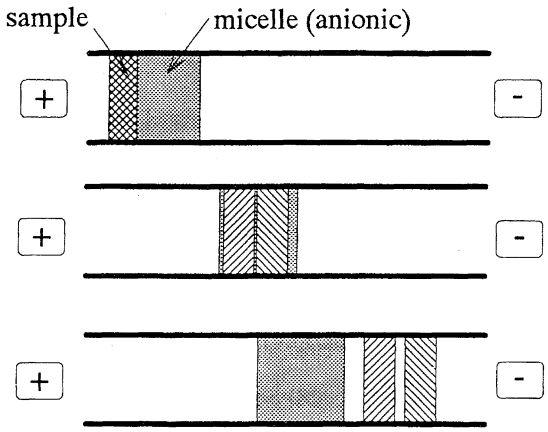

Fig. 12. Schematic illustration of the partial-filling MEKC (PF-MEKC) technique.[95] The sample solution contains two components.

4. Applications

4.1. Scope. MEKC has been recognized as a useful technique in various analytical fields owing to its advantages over RP-HPLC. The main advantage of MEKC is the higher separation efficiency than HPLC, typically shown in pharmaceutical applications.[105,106] Other advantages are: (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, and (4) running costs are low.

Typical applications of MEKC are separations of closely related compounds. A mixture of phenylthiohydantoin amino acids (PTH-AAs) was successfully separated by using an SDS solution, as shown in Fig. 14(a), and a DTAB solution.[29] With the addition of urea to the SDS solution, better resolution and selectivity could be obtained, as shown in Fig. 14(b).[92] Separation of all isomers of chlorinated phenols including phenol could also be achieved with an SDS solution.[79] These separations cannot be carried out by a simple isocratic HPLC, i.e., a gradient method is required.

4.2. Enantiomer Separations. As mentioned above, enantiomer separation has become one of the important applications in MEKC. For chiral separations by MEKC, the following two methods are usually employed: (1) MEKC with chiral surfactants and (2) CD-MEKC. Brief reviews on chiral separations by MEKC and also by CE have been available.[107—109]

4.2.1. MEKC with Chiral Surfactants. Various amino acid derivatives, which form chiral micelles, have been used as pseudostationary phases in MEKC for chiral separations. Sodium *N*-dodecanoyl-L-valinate (SDVal)[93,110—113] and re-

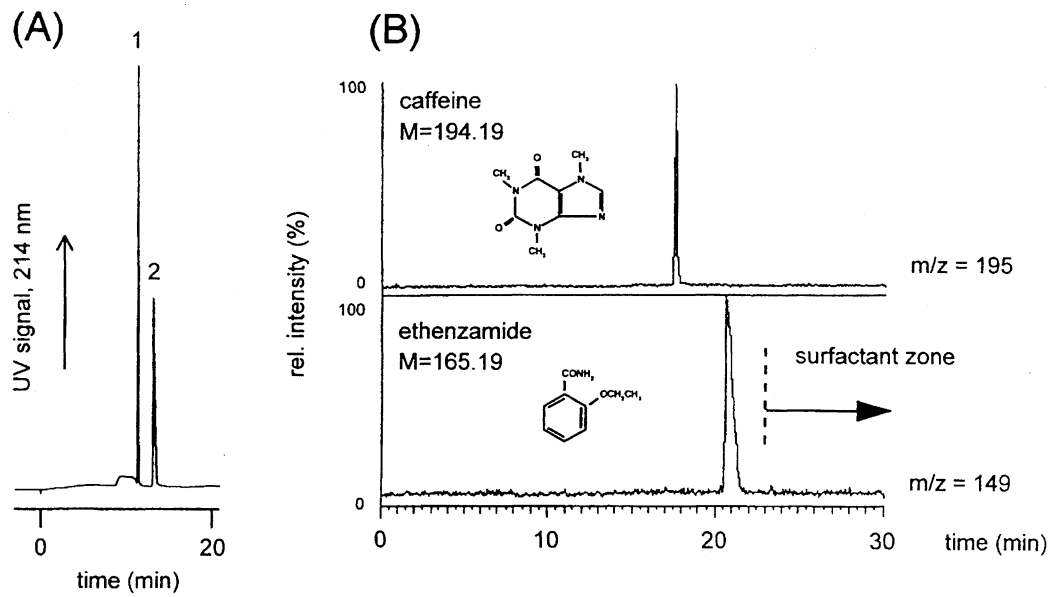

Fig. 13. PF-MEKC with (A) UV and (B) APCI-MS detections:[102] (1) caffeine (2) ethenzamide; (B) selected ion chromatograms. Run buffer, 20 mM ammonium acetate (pH 10); surfactant zone, 25 mM SDS in run buffer; zone injection, 200 mbar for 1 min; sample concentration, 0.5 mg mL^{-1}; sample injection, 50 mbar for 6 s; capillary, 50 μm i.d. × 100 cm, UV detection at 65 cm; inlet voltage, 18 kV; electrospray, 3 kV.

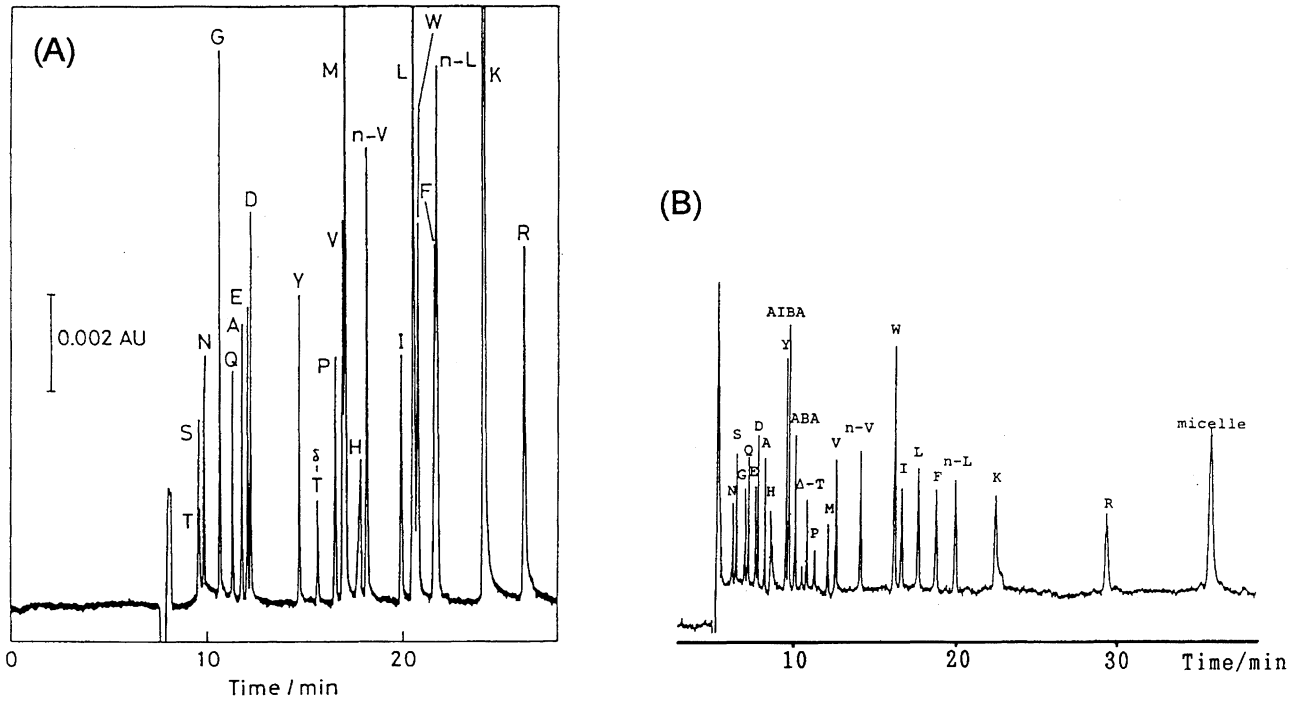

Fig. 14. Separations of PTH-AAs by SDS-MEKC (A) without urea[29] and (B) with urea:[92] The peaks are labeled with one-letter abbreviations for corresponding amino acid. Micellar solution, (A) 50 mM SDS (pH 7.0), (B) 100 mM SDS (pH 7.0) containing 4.3 M urea; capillary, (A) 50 μm i.d. × 650 mm, 500 mm effective, (B) 52 μm i.d. × 500 mm, 300 mm effective; applied voltage, (A) 10 kV, (B) 10.5 kV; detection wavelength, (A) 260 nm, (B) 220 nm; temperature, (A) 35 °C, (B) ambient.

lated *N*-alkanoyl-L-amino acids[114—116] were effective for resolution of PTH-DL-AAs. Similarly, enantiomer separations by MEKC with chiral surfactants of amino acid derivatives have also appeared.[117—120]

Digitonin, which is a glycoside of digitogenin, can be used for optical resolution of some Dns-DL-AAs as the mixed micelle with SDS or bile salts.[112,114]

As mentioned previously, by using a bile salt such as sodium deoxycholate or sodium taurodeoxycholate, enantioseparations of some Dns-DL-AAs,[78] chiral drugs, e.g., diltiazem hydrochloride and trimetoquinol hydrochloride,[121—123] and binaphthyl analogues[124] were achieved.

As other chiral surfactants, saponins such as glycyrrhizic acid and β-escin have been used for optical resolution of

Dns- or PTH-DL-AAs.[125] The use of synthetic chiral compounds, such as alkyl glycopyranosides, as pseudostationary phases in MEKC for enantiomeric separations have also been reported.[126—129]

4.2.2. 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.[130] Not only the non-derivatized CDs but also some CD derivatives, e.g., 2,6-di-*O*-methyl-β-CD, in SDS solutions can be used for the resolution of the optical isomers.[131]

Recently, the CD-MEKC system becomes one of popular techniques for chiral separations in CE: Optical resolution of some labeled amino acid enantiomers[132] and RS-chlorpheniramine[133] has been reported. It should be noted that the 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. On-Line Sample Concentrations

The low concentration sensitivity in CE is an inherent defect, which is caused by the limited volume of sample that can be injected into the capillary and the narrow optical pathlength for on-capillary detection. By using sample stacking,[134] this problem has been successfully reduced in CZE. Here, sample stacking is caused by the different mobilities of ions across a boundary, which separates regions of high and low electric fields. Sample stacking in MEKC, however, is not as straightforward as in CZE, since neutral analytes are unaffected by an enhanced electric field. Focusing is therefore dependent on the micelles in MEKC, which will provide neutral analytes with apparent electrophoretic mobilities. Few reports were published on neutral sample concentrations using stacking in MEKC with diluted micellar solutions as sample matrices,[135,136] in which micelles existed. Recently, we have achieved several methods for on-line neutral sample concentrations in MEKC using non-micellar solutions of low conductivity to prepare sample solutions, which are divided into two categories: One is the hydrodynamic sample injection mode, including normal stacking mode (NSM),[137] reversed electrode polarity stacking mode (REPSM),[138] stacking with reversed migrating micelles (SRMM),[139] and stacking using reverse migrating micelles and water plug (SRW),[140] and the other is the electrokinetic sample injection mode including field enhanced sample injection (FESI)[141] and field enhanced sample injection with reverse migrating micelles (FESI-RMM).[142]

We found that sample stacking of neutral analytes under acidic conditions, i.e., SRMM, SRW, and FESI-RMM, offers several advantages over those in neutral conditions, i.e., NSM, REPSM, and FESI. By using these concentration techniques, high stacking enhancement factors are easily obtained and optimization schemes are simply encountered. Together with a *z*-cell detection window, 1000-fold increases in detection sensitivity were realized.[143]

6. Conclusions

Micellar electrokinetic chromatography, which is actually a mode of CE, has become the most popular technique for high-resolution separation of neutral species by CE. In this work, we only summarize our previous and current studies on MEKC briefly. At the present stage, many papers on MEKC as well as CE are available, in which fundamental characteristics, theoretical treatments, and applications are described, so that it is necessary to refer to those items of literature when detailed information is required: For optimization strategies of MEKC, which was not discussed in this article, theoretical discussions[31—33,144] should be cited along with the review article.[10]

The above described work on MEKC has been performed by many co-workers whose names are on the references as coauthors mainly at Department of Industrial Chemistry, Faculty of Engineering, Kyoto University and Department of Material Science, Faculty of Science, Himeji Institute of Technology. Although individuals are not listed here, we are very grateful to all of them, in particular to Dr. Hiroyuki Nishi for his great contribution. We thank Yokogawa Electric Co. for the financial and technical supports at the early stage of the development of MEKC. Many financial supports including Grant-in-Aids for Scientific Research from Monbusho (the Ministry of Education, Science, Sports and Culture) are gratefully acknowledged.

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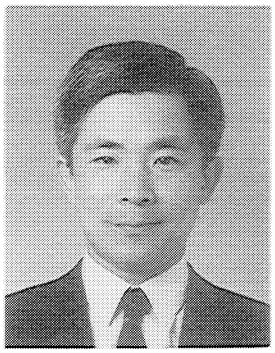

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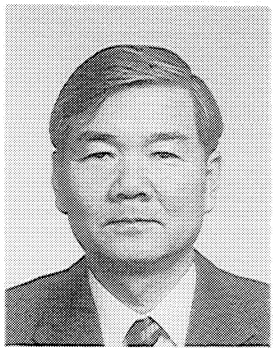

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