ELSEVIER

Journal of Chromatography A, 780 (1997) 165–178

JOURNAL OF CHROMATOGRAPHY A

Review

Control of separation selectivity in micellar electrokinetic chromatography by modification of the micellar phase with solubilized organic compounds

Shoichi Katsuta*, Koichi Saitoh[1]

Department of Chemistry, Graduate School of Science, Tohoku University, Sendai 980-77, Japan

Abstract

On the basis of the data on the distribution of various neutral solutes between sodium dodecyl sulfate (SDS) micelles and water, the control of separation selectivity in micellar electrokinetic chromatography (MEKC) by modification of the micellar phase with organic additives has been proposed and applied to the separation of simple model compounds. It was found that the distribution constants between the micelles and water ($K_{d,mc}$), which were determined by means of MEKC, of the solutes possessing hydrophilic functional groups are much larger than those between heptane and water ($K_{d,hep}$), whereas the $K_{d,mc}$ values of the solutes possessing no hydrophilic groups are comparable to their $K_{d,hep}$ values. This indicates that the former solutes are preferentially solubilized in the Stern layer of the micelles and that the latter are located in the hydrocarbon core. In MEKC separations of aromatic compounds and metal acetylacetonates, considerable changes in separation selectivity were caused by the addition of compounds possessing both hydrophobic and hydrophilic functional groups such as alcohols, phenol and ketones to the SDS micellar solution. The variations of the retention factors of the analytes could be explained in terms of saturation of the solubilization sites in the Stern layer with the modifiers, specific interaction of the modifiers with the analytes via hydrogen bonding in the micelles, and expansion of the core volume with the hydrocarbon parts of the modifiers. Such effects of the micellar modification could improve the resolution as well as the selectivity of MEKC separations. © 1997 Elsevier Science B.V.

Keywords: Selectivity; Distribution constants; Reviews; Buffer composition

Contents

*Corresponding author. Present address: Graduate School of Science and Technology, Chiba University, Yayoi-cho, Inage-ku, Chiba 263, Japan.

[1]Present address: Research Center for Higher Education, Tohoku University, Kawauchi, Aoba-ku, Sendai 980-77, Japan.

0021-9673/97/$17.00 © 1997 Elsevier Science B.V. All rights reserved.
PII S0021-9673(97)00344-0

1. Introduction

Micellar electrokinetic chromatography (MEKC) is a powerful analytical method which enables excellent separation of electrically neutral compounds utilizing capillary electrophoretic techniques [1]. The separation of neutral compounds by MEKC is usually performed on the basis of the differences in their distribution constants between the electroosmotically pumped aqueous phase and the ionic micellar phase which is slower moving by electrophoretic effects. Therefore, it is difficult to separate the compounds of which distribution constants (or partition coefficients) are close to each other. An approach for solving this problem is to use of some modifiers which can change the distribution of the analytes selectively. We can consider two types of the modifiers, i.e., modifiers of the aqueous phase and the micellar phase, respectively. To modify the aqueous phase, highly hydrophilic organic solvents (methanol [2], dimethylformamide [3], etc.) and solutes (urea [4], cyclodextrin [5], etc.) have been used; most of these additives are, however, originally used to enhance the solubility of hydrophobic analytes in the aqueous phase and usually decrease their distribution unselectively. On the other hand, the modification of the micellar phase has been so far made by the use of specially structured surfactants [6,7] and mixed micelles of plural surfactants [8,9]. The selection of the surfactants is surely an important factor affecting the separation selectivity in MEKC. However, the mechanisms of the effect of different surfactants on the solute–micelle interactions, to which attention was paid by a few workers [10,11], has not been well understood.

Recently, the authors proposed another simple and effective method to modifying the micellar phase, i.e., addition of hydrophobic organic compounds which are mostly solubilized in the micelles [12,13]. This method was invented on the basis of a fundamental study on the distribution properties of various neutral solutes between SDS micellar phase and bulk aqueous phase. In this paper, we compile our original studies on the distribution of solutes in the micellar system as well as the effect of modification of micelles with organic additives (alcohols, phenol and ketones) on MEKC separations; this paper is not intended to introduce the related literatures widely. Sodium dodecyl sulfate (SDS), which is the most usual surfactant in MEKC, was used throughout the studies. The analytes chosen were simple and well-characterized compounds, i.e., derivatives of benzene and naphthalene and, in addition, metal acetylacetonates considering increasing attention to the separation of metal complexes [14,15].

2. Distribution constants in SDS micelles–water system

The distribution constants of twenty-six aromatic compounds and four inert metal acetylacetonates between SDS micelles and water were determined by means of MEKC, and compared with the liquid–liquid distribution constants.

The migration time of an neutral solute (t_s) in MEKC is given by [16]

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

where t_0 and t_{mc} are the migration times of the electroosmotic flow and the micelles, respectively, and k is the retention factor defined as a mole ratio of the solute in the micellar phase to that in the aqueous phase. When the surfactant concentration is low, k is expressed as

$$k = K_{d,mc}V_{sf}(C_{sf} - CMC) \quad (2)$$

where $K_{d,mc}$ is the distribution constant defined as a molarity ratio of the solute in the micellar phase to that in the aqueous phase, V_{sf} the partial molar volume of the surfactant in the micelles, C_{sf} the total concentration of the surfactant, and CMC the critical micelle concentration. Therefore, the plot of k vs. C_{sf} should give a straight line with a slope of $K_{d,mc}V_{sf}$.

The MEKC experiments were performed with a fused-silica capillary column [70 cm (effective length 50 cm)×50 μm I.D.×375 μm O.D.] at 25°C. Carrier solutions used were phosphate–borate buffers (0.010 *M* NaH_2PO_4 and 0.0020 *M* $Na_2B_4O_7$, pH 6.8) containing 0.025–0.10 *M* SDS. Detection was made by on-capillary measurements of UV absorption at 260 nm. The retention factors of the solutes ($5\cdot10^{-5}$–$5\cdot10^{-3}$ *M* in sample solutions) were calculated according to Eq. (1), where t_0 and t_{mc} values

were assumed to be equal to the migration times of methanol as the solute insolubilized in the micellar phase and that of Sudan III or Oil Yellow OB as the solute completely solubilized in it, respectively. Sudan III and Oil Yellow OB, which differ considerably in molecular size and structure, always gave an identical migration time, and this result supports the contention that both dyes are completely solubilized in the micellar phase. The k values were independent of the applied voltage in the range +15.0–30.0 kV (current 14–32 μA), indicating that an increase in temperature in the capillary due to the Joule heat was negligibly small. The k values of phenols were confirmed to be independent of pH in the range 6.1–7.2, and therefore the acid-dissociation of the phenols is negligible under the present pH conditions.

As expected from Eq. (2), the plots of k vs. C_{sf} always gave straight lines with correlation coefficients of more than 0.99. The C_{sf} axis intercepts, $(6\pm1)\cdot10^{-3}$ M, were close to the CMC value of SDS, $4.6\cdot10^{-3}$ M, which was determined by conductometric titrations in the present buffer solution. The $K_{d,mc}$ values were determined from the slopes using the literature value of V_{sf} of SDS, 0.246 dm^3 mol^{-1} [17]. The $K_{d,mc}$ values obtained are summarized in Table 1.

The distribution constant in the micelles–water system has been often discussed by comparing with the liquid–liquid distribution constant in the 1-octanol–water system which is a well-known parameter for the hydrophobicity of chemicals [24,25]. However, since 1-octanol can specifically interact with hydrogen-bonding compounds (especially hydrogen-bond donors), the 1-octanol–water system is not always adequate as a reference to evaluate the distribution in the micelles–water system. Actually, the comparison with the 1-octanol–water system only gives rough correlations and little meaningful information. On the other hand, the comparison with the alkane–water system is significant to characterize the micelles–water system because the alkane phase can be regarded as a model of the hydrocarbon core of the micelles [26]. In this study, the distribution constant in the heptane–water system ($K_{d,hep}$) was used as a reference. Although dodecane may be strictly most adequate as a model of the core of SDS micelles, the distribution data in the dodecane–water system are little available. The $K_{d,hep}$ values were confirmed to be nearly equal to the distribution

Table 1
Distribution constants in SDS micelle–water system and in heptane–water system at 25°C

No.	Solute	Log $K_{d,mc}$	Log $K_{d,hep}$	No.	Solute	Log $K_{d,mc}$	Log $K_{d,hep}$
1	Benzene	2.02[a]	2.29[b]	16	2-Chlorophenol	2.19	0.79[d]
2	Toluene	2.50	2.85[c]	17	4-Chlorophenol	2.39	−0.11[c]
3	*o*-Xylene	2.86	3.39[c]	18	2,3-Dichlorophenol	2.89	1.33[d]
4	*m*-Xylene	2.91	3.54[c]	19	3,5-Dichlorophenol	3.03	0.42[d]
5	*p*-Xylene	2.91[a]	3.45[c]	20	1-Naphthol	2.85	0.55
6	Chlorobenzene	2.57	2.92[b]	21	2-Naphthol	2.83	0.30[c]
7	1,2-Dichlorobenzene	3.07	3.37[b]	22	Benzyl alcohol	1.76	−0.70
8	1,3-Dichlorobenzene	3.13	3.53[b]	23	Anisole	2.24	2.10[c]
9	1,4-Dichlorobenzene	3.00	3.53[b]	24	Acetophenone	2.29[a]	1.08[c]
10	Naphthalene	3.15	3.38[c]	25	Nitrobenzene	2.13	1.43[c]
11	Phenol	1.69[a]	−0.90	26	1,4-Dinitrobenzene	2.06	0.75
12	*o*-Cresol	2.08	−0.05[c]	27	$Cr(acac)_3$	2.09[a]	−0.43[d]
13	*p*-Cresol	2.12	−0.35[c]	28	$Co(acac)_3$	1.89[a]	−1.03[e]
14	2,3-Xylenol	2.48	0.49	29	$Rh(acac)_3$	2.05[a]	−0.62[f]
15	3,5-Xylenol	2.49	0.27	30	$Pd(acac)_2$	3.22[a]	1.43[f]

[a] Ref. [18].
[b] Ref. [19].
[c] Ref. [20].
[d] Ref. [21].
[e] Ref. [22].
[f] Ref. [23].

constants in the dodecane–water system ($K_{d,dod}$): for example, log $K_{d,hep}=2.92$ [19] and log $K_{d,dod}=3.04$ for chlorobenzene; log $K_{d,hep}=0.42$ and log $K_{d,dod}=0.52$ for 3,5-dichlorophenol; log $K_{d,hep}=-1.03$ and log $K_{d,dod}=-1.09$ [27] for $Co(acac)_3$. Then the distribution constant in the heptane–water system, where relatively many distribution data were available, was chosen, and the values are listed in Table 1. The $K_{d,hep}$ values without citation marks in Table 1 were determined by us in a similar manner as described previously [19,21,22,27].

The relation between log $K_{d,mc}$ and log $K_{d,hep}$ values are shown in Fig. 1, which indicates several interesting features of $K_{d,mc}$ as follows. A good correlation for methyl and chloro derivatives of benzene and naphthalene, which have no hydrophilic functional groups, is observed. For these compounds, the $K_{d,mc}$ value is comparable to the $K_{d,hep}$ value. This is also the case for the polar solutes such as 1,2-dichlorobenzene (dipole moment: 2.26 debye) as well as the nonpolar ones such as benzene and naphthalene. The study on the structure of SDS micelles by means of small-angle neutron scattering ($C_{sf}=0.4$ *M*, 30°C) [28] reveals that 99 SDS molecules form an spherical aggregate (radius 2.92 nm, volume 104.3 nm^3) composed of the dry hydrocarbon core (radius 1.82 nm, volume 25.3 nm^3) and the Stern layer (thickness 1.10 nm, volume 79.0 nm^3) including water, sodium ion and a part of hydrocarbon chains. Assuming that the above structure of SDS micelles can be applied to the present conditions and that most of the solutes in the micellar phase are located in the core, the distribution constant between the core and water ($K_{d,mc,core}$) can be estimated using the core volume per one mole of the surfactant in the micelles, 0.15 $dm^3\ mol^{-1}$, instead of V_{sf} in Eq. (2). The right-side ordinate in Fig. 1 denotes the $K_{d,mc,core}$ value, and the solid line indicates a relation of log $K_{d,hep}=$log $K_{d,mc,core}$. The line is close to the plot of the solutes possessing no hydrophilic functional groups. It follows from this that the SDS micelle behaves as a similar medium to heptane for these solutes.

On the other hand, in the case of 2-naphthol and the phenols possessing no *ortho*-substituents, the log $K_{d,mc}$ values, which also show a good correlation with the log $K_{d,hep}$ values, are 2.22 (3,5-xylenol)–2.61 (3,5-dichlorophenol) larger than the corresponding log $K_{d,hep}$ values. This suggests the stronger interaction of the solutes with the micelles than with heptane. The deviations, log $K_{d,mc}-$log $K_{d,hep}$, for *o*-cresol, 2,3-xylenol and 1-naphthol are 2.13, 1.99, and 2.30, respectively; those for 2-chlorophenol and 2,3-dichlorophenol are 1.40 and 1.56, respectively; these deviations are smaller than those for the phenols possessing no *ortho*-substituents. This indicates the weaker interaction of these solutes with the micelles, which would be due to the steric

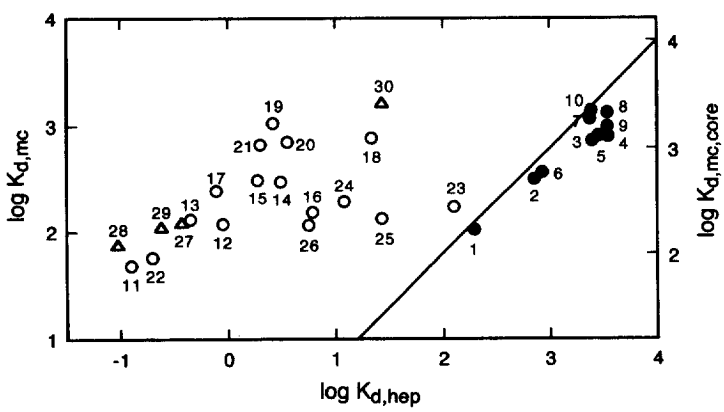

Fig. 1. Correlation between the distribution constant in the SDS micelle–water system ($K_{d,mc}$) and that in the heptane–water system ($K_{d,hep}$). The scale on the right-side ordinate indicates the distribution constant in the micellar system calculated based on the hydrocarbon-core volume ($K_{d,mc,core}$, see Section 2). The solid line displays a relation of log $K_{d,hep}=$log $K_{d,mc,core}$. Solutes: ● aromatic compounds possessing no hydrophilic groups; ○ aromatic compounds possessing hydrophilic groups; △ metal acetylacetones; the numbers correspond to those in Table 1.

hindrance of the *ortho*-substituent against the hydroxyl group. In each family of the phenols having the same steric structure around the hydroxyl group, the log $K_{d,mc} - \log K_{d,hep}$ value is generally greater for the phenol whose acidity constant is larger. The hydrogen-bond donor ability of the solute would be a factor affecting the solute–micelle interaction. However, even in the case of the other organic solutes which have hydrophilic groups but their hydrogen-bond donor ability is much weaker than phenols, the log $K_{d,mc}$ values are larger than the log $K_{d,hep}$ values: the log $K_{d,mc} - \log K_{d,hep}$ values are 2.46 for benzyl alcohol, 0.14 for anisole, 1.21 for acetophenone, 0.70 for nitrobenzene and 1.31 for 1,4-dinitrobenzene. Especially, the data of 1,4-dinitrobenzene, of which dipole moment is zero, clearly shows that one of the definitive factors governing the solute–micelle interaction is the existence of hydrophilic group rather than the polarity of the solute molecule. The $K_{d,mc}$ value of anisole is near to its $K_{d,hep}$ value, which would be due to the relatively low hydrophilicity of the methoxy group.

The metal acetylacetonates, which have symmetric structures and are not dipoles, also show much stronger interaction with the micelles than with heptane [18]: the log $K_{d,mc} - \log K_{d,hep}$ values are 2.92 for $Co(acac)_3$, 2.67 for $Rh(acac)_3$, 2.57 for $Cr(acac)_3$, and 1.79 for $Pd(acac)_2$. These complexes are known to behave as hydrogen-bond acceptors by their oxygen atoms [21,22,29]. The order of the log $K_{d,mc} - \log K_{d,hep}$ value is in accord with that of the association constant of these complexes with 3,5-dichlorophenol in heptane, $Co(acac)_3 > Rh(acac)_3 \approx Cr(acac)_3 > Pd(acac)_2$ [29], indicating that the complex with stronger hydrogen-bond acceptor ability has higher affinity for the SDS micelles.

The liquid–liquid distribution constant has been known to be governed by the volume of the solute molecule as well as the solute–solvent interaction, and that of the solutes possessing no hydrophilic groups such as halobenzenes is mainly determined by their molecular volume [19]. In Fig. 2A and B, the $K_{d,hep}$ and $K_{d,mc}$ values are shown as a function of the Van der Waals volume (V_w) of the solutes calculated from group contributions according to Bondi [30], respectively. In the heptane–water system, the distribution constants of the solutes possessing hydrophilic groups are remarkably smaller than those of the corresponding-sized solutes possessing no hydrophilic groups. Such deviations can be explained by the strong interaction through hydrogen bonding between the hydrophilic solutes and water in the aqueous phase. On the other hand, in the micelles–water system, the distribution constants of the solutes possessing hydrophilic groups remarkably increase and become close to the values of the

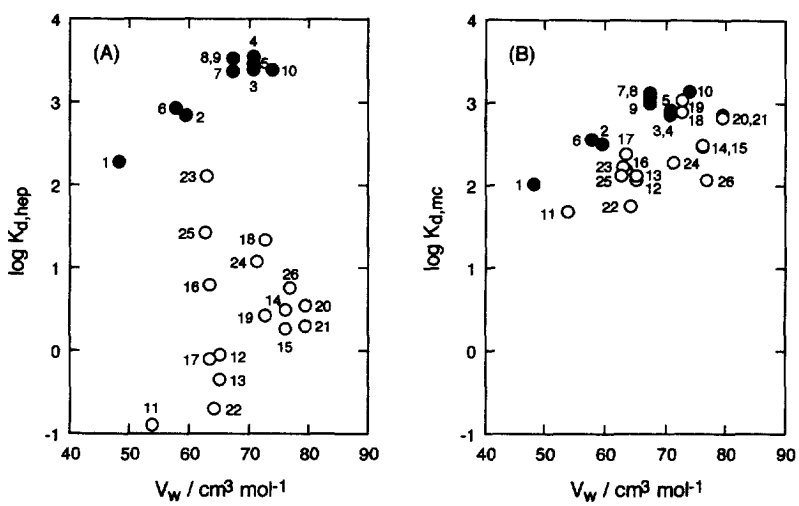

Fig. 2. Distribution constant in the heptane–water system (A) and that in the SDS micelle–water system (B) as a function of the Van der Waals volume of the solute (V_w). The symbols and numbers are the same as in Fig. 1.

solutes possessing no hydrophilic groups. These results indicate the existence of so strong interaction of the hydrophilic solutes with the micelles as to offset the interaction with water in the aqueous phase.

The specific interaction of the hydrophilic solutes with the micelles should occur in the Stern layer surrounding the hydrocarbon core. The negatively charged sulfate group of SDS may act as a hydrogen-bond acceptor and interact with the hydrogen-bond donors such as phenols [10,11]. However, this idea can not explain the strong interaction of the hydrogen-bond acceptors such as acetophenone, nitrobenzene and metal acetylacetonates with the micelles. A more probable explanation is the interaction of the solutes with water in the Stern layer. The transfer of the hydrophilic solutes from water to the micelles, where the solutes partially keep the solvating water molecules, should be energetically favorable compared with that from water to heptane where they are mostly dehydrated. This idea successfully explains that the solute whose hydrophilicity (hydrogen-bond donor ability or acceptor ability) is higher shows stronger interaction with the micelles and that the interaction of the solute with the micelles is comparable to that with water in the aqueous phase. It is also supported by the experimental results that the UV spectra of 3,5-dichlorophenol and $Co(acac)_3$ in the SDS micelles are almost the same as those in water. The solutes possessing no hydrophilic group such as benzene and *p*-xylene are known to be solubilized in the hydrocarbon part of the micelles [31,32], and hence it is reasonable that the distribution constants of these solutes in the micelles–water system are comparable to those in the heptane–water system. Consequently, the characteristics of distribution constants in the SDS micelles–water system could be clearly interpreted in terms of the solubilization sites of the solutes.

3. Modification of SDS micelles with organic additives

As mentioned in Section 2, in the micelles–water system, the solutes with and without hydrophilic functional groups have comparable values of the distribution constant, which is not favorable for the separation methods such as MEKC based on the differential distribution of analytes into the micelles. The characteristics of distribution in the micellar solution gave us useful hints for selective control of the solute distribution. Considering that the solutes possessing hydrophilic groups are generally solubilized in the Stern layer of the micelles and that the solutes possessing no hydrophilic groups are penetrated into the hydrocarbon core, the excess amount of additives possessing hydrophilic groups may saturate the solubilization sites in the Stern layer and cause a selective decrease in the distribution constant of the analytes possessing hydrophilic groups. On the other hand, if there are the specific attractive forces between additives and analytes in the micellar phase, the distribution constant of the analytes may be increased. In this study, in order to confirm the validity of such concepts of the modification of micelles, hydroxy compounds (alcohols and phenol) and ketones were examined as modifiers of the micellar phase in MEKC.

3.1. Effect of hydroxy compounds

As hydroxy modifiers, 1-hexanol, cyclohexanol, benzyl alcohol, and phenol were used for SDS micelles, and their effects on the MEKC separation of various neutral aromatic compounds and metal acetylacetonates have been investigated. These modifiers possess the similar number of carbon atoms (6 or 7), but the structure of their hydrocarbon parts and the hydrogen-bond donor ability of their hydroxyl groups are considerably different.

Fig. 3 shows examples of the chromatograms obtained with and without the addition of 0.10 *M* 1-hexanol to the carrier solution of 0.075 *M* SDS. In spite of the small amount of 1-hexanol added to the micellar solution [only 1.4% (v/v)], it makes a considerable effect on the migration order of the analytes: with the addition of 1-hexanol, the retention orders of phenol–$Co(acac)_3$; 1,4-dinitrobenzene–$Cr(acac)_3$; *p*-cresol–acetophenone, and naphthalene–$Pd(acac)_2$ are reversed, and unresolved peaks of nitrobenzene and *p*-cresol with SDS alone are well separated in the presence of 1-hexanol. Using the distribution constant of 1-hexanol at low concentration reported to be $2.25 \cdot 10^3$ on the mole fraction scale [33], it is estimated that ca. 70% of

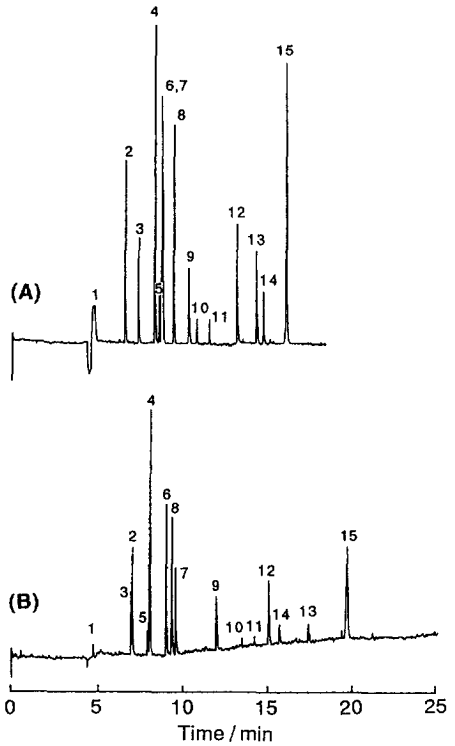

Fig. 3. Effect of addition of 1-hexanol on the micellar electrokinetic chromatogram of several neutral compounds. Micellar solution: (A) 0.075 *M* SDS in 0.01 *M* NaH_2PO_4–0.002 *M* $Na_2B_4O_7$ buffer (pH 6.8); (B) same solution as in (A) but containing 0.10 *M* 1-hexanol. Samples: (1) methanol; (2) phenol; (3) $Co(acac)_3$; (4) 1,4-dinitrobenzene; (5) $Cr(acac)_3$; (6) nitrobenzene; (7) *p*-cresol; (8) acetophenone; (9) 4-chlorophenol; (10) toluene; (11) chlorobenzene; (12) 2-napthol; (13) naphthalene; (14) $Pd(acac)_2$; (15) Oil Yellow OB. Separation tube: fused-silica capillary 70 cm (effective length 50 cm)×50 μm I.D.× 375 μm O.D. Detection wavelength: 260 nm. Applied voltage: +20.0 kV. Temperature: 25°C.

1-hexanol in the micellar solution is solubilized into the micellar phase at 0.075 *M* SDS. The number of moles of 1-hexanol in the micellar phase is comparable to that of the surfactant, and the properties of the micelles is expected to be modified with the solubilized hexanol. The migration time of the micelles, indicated by that of Oil Yellow OB, is considerably increased by the addition of 1-hexanol though that of the electroosmotic flow monitored by methanol is little changed. There are two possible explanations for an increase in electrophoretic mobility of the micelles, i.e., an increase of the negative charge and a decrease of the effective radius of the micelles. It was reported that the degree of ionization of SDS micelles increases in the presence of long-chain 1-alcohols (C_4–C_7) [34,35] and that the size of the micelles is enlarged by solubilization of 1-octanol [36]. Therefore, the increased mobility of the micelles is attributable to the increase of negative charge of the micelles.

In order to discuss the effect of modifiers on the distribution of analytes quantitatively, the variation of retention factor (k) was evaluated. The variations of the retention factors of several analytes with the addition of 0.10 *M* hydroxyl compounds are summarized in Table 2. Fig. 4 shows the dependence of the retention factors on the concentration of 1-hexanol, cyclohexanol, benzyl alcohol and phenol in the micellar solution. The addition of the alcohols remarkably decreases the retention factors of acetophenone, 1,4-dinitrobenzene, and metal acetylacetonates, slightly decreases those of 2-naphthol and nitrobenzene, has little effect on those of phenol and *p*-cresol, and increases those of benzene, toluene, chlorobenzene and naphthalene. The effects of these modifiers are similar to each other, and the differences among their hydrophobic parts, i.e., *n*-hexyl, cyclohexyl and benzyl groups, are not appreciably reflected. In contrast, the addition of phenol, of which hydroxyl group has higher hydrogen-bond donor ability than that of the alcohols, remarkably decreases the retention factors of *p*-cresol, changes little those of benzene, acetophenone and nitrobenzene, and increases those of the metal acetylacetonates. The hydrogen-bonding ability of the hydroxy modifiers is closely related to the selectivity of their effect.

The addition of cyclohexane as a modifier scarcely affected the retention factors of all the analytes investigated although those of the solutes possessing no hydrophilic groups (benzene, toluene and chlorobenzene) are slightly enhanced [13]. It is apparent that the existence of hydroxyl group in the modifier molecules is important.

Although it has been known that the solubilization of long-chain alcohols generally lowers the CMC [33,37], the selectivity will not be altered by an increase in the volume of the micellar phase induced by the decrease in CMC. In addition, the CMC value of SDS in the present buffer solution in the absence

Table 2
Variations of retention factors by the use of organic additives[a]

Solute	Log k without additive	Increment of log k with additive								
		1-Hexanol	c-Hexanol	Benzyl alcohol	Phenol	2-Butanone	2-Pentanone	2-Hexanone	2-Heptanone	c-Hexanone
Benzene	0.22	+0.14[b]	+0.06[b]		+0.01[b]					+0.03
Toluene	0.65	+0.15[b]	+0.06[b]	+0.16		−0.01	+0.04	+0.11	+0.23	+0.05
Chlorobenzene	0.76	+0.16[b]	+0.05[b]	+0.16		+0.01	+0.08	+0.15	+0.28	+0.10
Naphthalene	1.32	+0.09		+0.01						+0.02
Phenol	−0.08	+0.06		−0.03						+0.04
p-Cresol	0.34	+0.02[b]	−0.01[b]	−0.05	−0.16[b]	−0.02	+0.03	+0.06	+0.10	+0.03
p-Chlorophenol	0.58	+0.07		−0.01						+0.14
2-Naphthol	1.05	−0.03[b]	−0.05[b]	−0.07		0.00	+0.06	+0.10	+0.17	+0.07
Acetophenone	0.44	−0.11[b]	−0.14[b]	−0.10	−0.03[b]	−0.10	−0.11	−0.12	−0.12	−0.14
Nitrobenzene	0.33	−0.04[b]	−0.05[b]	−0.04	−0.04[b]	−0.05	−0.01	+0.02	+0.09	−0.01
1,4-Dinitrobenzene	0.26	−0.11		−0.05						−0.07
$Cr(acac)_3$	0.30	−0.18	−0.24	−0.09	+0.07	−0.17	−0.23	−0.23	−0.32	−0.28
$Co(acac)_3$	0.08	−0.21	−0.21	−0.07	+0.10	−0.17	−0.21	−0.25	−0.31	−0.30
$Pd(acac)_2$	1.45	−0.35		−0.29						−0.37

[a] SDS concentration: 0.075 M; additive concentration: 0.10 M; buffer: 0.01 M NaH_2PO_4–0.002 M $Na_2B_4O_7$ (pH 6.8); temperature: 25°C.
[b] Ref. [13].

of the modifiers was $4.6 \cdot 10^{-3}$ M, and therefore, in this study where the total concentration of SDS was 0.075 M, the amount of the surfactant forming micelles is little increased with the decrease in CMC (less than 6% increase).

The alcohols and phenol added as modifiers should be solubilized in the Stern layer of the micelles as described in Section 2. Therefore, it is probable that the modifiers, covering the micellar surface or saturating the Stern layer, inhibit the solubilization of the analytes possessing hydrophilic groups which are also solubilized in the Stern layer. On the other hand, in the hydrophobic field in the micelles, remarkable interaction is expected between hydrogen-bond donors (phenols) and acceptors (alcohols, ketones, nitrobenzenes and metal acetylacetonates); the hydrophobic association complexes of the hydrogen-bond donors with the acceptors could be

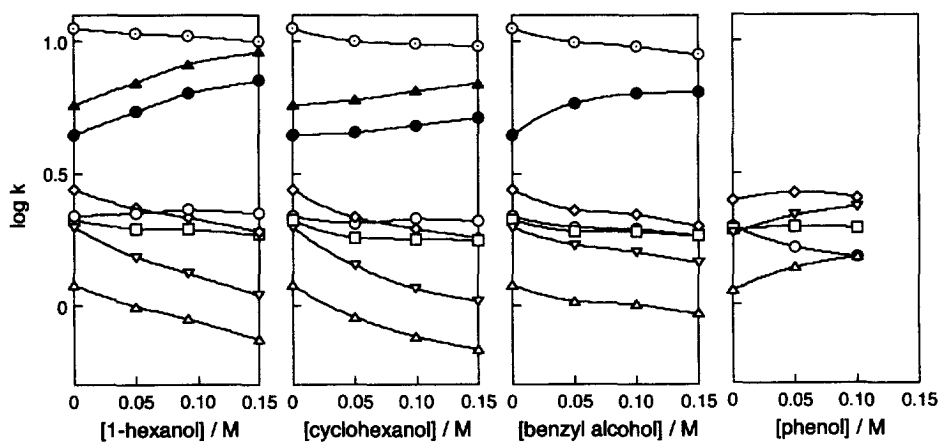

Fig. 4. Dependence of log k on the concentration of 1-hexanol, cyclohexanol, benzyl alcohol and phenol in the micellar solution (0.075 M SDS, pH 6.8, 25°C). Solutes: ○ 2-napthol; ▲ chlorobenzene; ● toluene; ◇ acetophenone; ○ p-cresol; □ nitrobenzene; ▽ $Cr(acac)_3$; △ $Co(acac)_3$.

present in the core of the micelles. When the modifiers can associate with the analytes in the micellar phase, the effect increasing the distribution of the analytes should occur along with the effect decreasing it due to the saturation of the Stern layer, and the apparent effect is governed by the balance between these opposite effects. Consequently, the contrastive effects of alcohols and phenol on the retention factors of the solutes possessing hydrophilic groups can be successfully explained in terms of the saturation of the Stern layer and the specific interaction with the analytes in the micelles. The reason for that the effect of the alcohols on nitrobenzene is smaller than that on acetophenone and metal acetylacetonates would be deeper permeation of nitrobenzene into the micellar core: the log $K_{d,mc} - \log K_{d,hep}$ value is smaller for nitrobenzene (0.70) than for acetophenone (1.21), 1,4-dinitrobenzene (1.31), and the metal acetylacetonates (1.79–2.92), suggesting that a larger portion of nitrobenzene in the micellar phase should be located in the core. A little larger effect on the metal acetylacetonates than that on acetophenone and 1,4-dinitrobenzene would be also explained in a similar manner.

On the other hand, the enhancement effect on the retention factors of the solutes possessing no hydrophilic groups is attributable to the expansion of the micellar core volume with the hydrocarbon part of the modifiers. The larger effect of 1-hexanol than cyclohexanol and phenol may be attributed to the deeper permeation of the straight hydrocarbon chain of 1-hexanol to the micellar core than the cyclic chain of cyclohexanol and phenol. However, as mentioned below, the magnitude of the effect of modifiers largely depends on the amounts of the modifiers solubilized in the micellar phase.

In the present MEKC experiments detecting absorbance at 250–260 nm, benzyl alcohol and phenol, which have large absorption in this region, remarkably enhanced the baseline noise. These aromatic modifiers would not be practical for the UV detection.

3.2. Effect of ketones

In this section, the contribution of hydrophobic parts of the modifier molecules is evaluated in detail using various aliphatic ketones, 2-butanone, 2-pentanone, 2-hexanone, 2-heptanone and cyclohexanone as modifiers. In Fig. 5, examples of the chromatograms with 0.075 *M* SDS solution obtained in the absence and presence of 0.10 *M* cyclohexanone are shown. The effect of cyclohexanone is similar to that of 1-hexanol shown in Fig. 3. The variations of the retention factors of several analytes with the addition of 0.10 *M* ketones are summarized in Table 2. In Fig. 6, the dependence of the retention factor on the concentration of ketones is shown. All the ketones decrease the retention factors of acetophenone and the metal acetylacetonates similarly to the alcohols, which is ascribed to saturation of the Stern layer with the modifiers; the magnitude of the effect in the

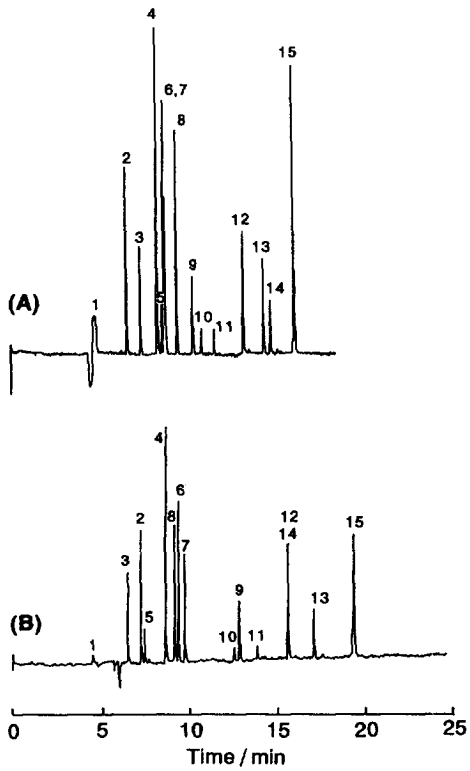

Fig. 5. Effect of the addition of cyclohexane on the micellar electrokinetic chromatogram of several neutral compounds. Micellar solution: (A) 0.075 *M* SDS in phosphate–borate buffer (pH 6.8); (B) same solution as (A) but containing 0.10 *M* cyclohexane. Samples: (1) methanol; (2) phenol; (3) $Co(acac)_3$; (4) 1,4-dinitrobenzene; (5) $Cr(acac)_3$; (6) nitrobenzene; (7) *p*-cresol; (8) acetophenone; (9) 4-chlorophenol; (10) toluene; (11) chlorobenzene; (12) 2-napthol; (13) naphthalene; (14) $Pd(acac)_2$; (15) Oil Yellow OB. Other conditions as in Fig. 3.

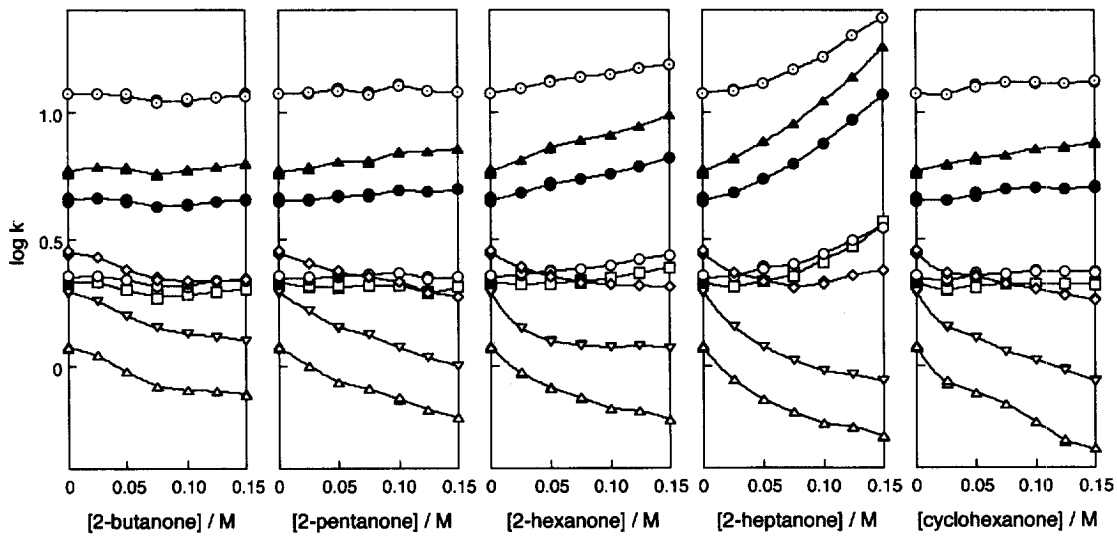

Fig. 6. Dependence of log k on the concentration of 2-butanone, 2-pentanone, 2-hexanone, 2-heptanone and cyclohexane in the micellar solution (0.075 M SDS, pH 6.8, 25°C). Solutes: ○ (with dot) 2-napthol; ▲ chlorobenzene; ● toluene; ◇ acetophenone; ○ p-cresol; □ nitrobenzene; ▽ $Cr(acac)_3$; △ $Co(acac)_3$.

lower concentration region of the ketones is increased in the order, 2-butanone<2-pentanone<2-hexanone<cyclohexanone<2-heptanone. However, in the higher concentration region, the effect on the retention factor of acetophenone is reduced. The distribution of acetophenone, which is deeper permeated into the micellar core than the metal acetylacetonates, might be more affected by the expansion of the core volume with the hydrocarbon part of these modifiers. The increased retention factor of nitrobenzene by the addition of 2-hexanone and 2-heptanone would be also explained in a similar manner. The ketone possessing a larger hydrocarbon component increases the retention factors of the solutes possessing no hydrophilic groups such as toluene and chlorobenzene more greatly, due to the expansion of the micellar core.

The addition of the ketones except for 2-butanone evidently increases the retention factors of the phenols, unlike the case of alcohols as modifiers. The increase of the retention factor can be explained by appreciable hydrogen-bond interaction between the carbonyl oxygen of the ketones and the hydroxyl hydrogen of the phenols in the micellar phase. In fact, the increment of the retention factor by the addition of 0.10 M cyclohexanone is larger for the phenol having stronger hydrogen-bond donor ability: p-cresol<phenol<2-naphthol<p-chlorophenol. In general, the bulkier ketone seems to have a larger effect on the retention factor of each solute.

In order to clarify the relationship between the magnitude of the effect and the hydrophobicity of the ketones, the amount of ketones in the micellar phase was evaluated. In the MEKC experiments using ketones as modifiers, a negative peak as observed in Fig. 5B always appeared when the concentration of ketones in the sample solution was lower than that in the running buffer solution. Since the ketones have weak absorption at the present detection wavelength (250–260 nm), the UV dip arises from the sample-solution band containing lower concentration of ketones, and its migration velocity should correspond to that of the ketones in the capillary [38]. Therefore, the retention factor of the ketones added as modifiers can be estimated from the migration time of the negative peak according to Eq. (1). In micellar systems, the distribution constant of a solute is generally a function of its concentration in the micellar phase [39,40] unless the molar ratio of the solubilized solute to the surfactant in the micellar phase is sufficiently small. Indeed, the retention factor of the ketones depends on its total concen-

tration (C_{ketone}) in the range 0.025–0.15 *M* at constant C_{sf}=0.075 *M* (further details will be reported elsewhere). Since the concentration of the surfactant forming micelles is approximately equal to its total concentration C_{sf}, the mole ratio of the ketone to the surfactant in the micellar phase ($R_{ketone/SDS}$) can be calculated as $R_{ketone/SDS} = C_{ketone}k(1+k)^{-1}C_{sf}^{-1}$, where *k* denotes the retention factor of the ketone determined from the migration time of the negative peak. In Fig. 7, the mole ratio is shown as a function of C_{ketone}. At a certain value of C_{ketone}, the amount of ketone solubilized in the micellar phase is generally greater for the bulkier ketone, i.e., 2-butanone (Van der Waals volume= 49.27 cm^3 mol^{-1})<2-pentanone (59.5 cm^3 mol^{-1})≈cyclohexanone (62.85 cm^3 mol^{-1})<2-hexanone (69.73 cm^3 mol^{-1})<2-heptanone (79.96 cm^3 mol^{-1}). Fig. 8 shows the variation of the retention factor of several analytes as a function of the mole ratio of the ketone to the surfactant in the micellar phase. Interestingly, the magnitudes of the effects of different ketones, except for the effects of cyclohexanone on acetophenone and $Co(acac)_3$, are nearly equal to each other at the same amounts of the ketones in the micellar phase. Therefore, the differences in magnitude of the effect among the homologous modifiers are primarily interpreted by their amounts in the micellar phase. On the other hand, the specifically large effect of cyclohexanone, compared with the other linear ketones, stands out for the retention factors of acetophenone and $Co(acac)_3$. It would be due to the relatively rigid structure of the cyclic hydrocarbon chain which may effectively occupy the sites in the Stern layer where the solutes possessing hydrophilic groups are solubilized.

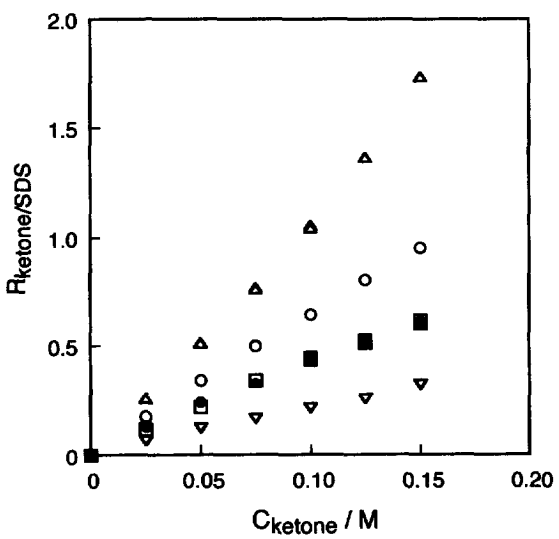

Fig. 7. A mole ration of the solubilized ketone to SDS in the micellar phase as a function of the total concentration of ketone in the micellar solution (0.075 *M* SDS, pH 6.8, 25°C). Ketones: ▽ 2-butanone; □ 2-pentanone; ○ 2-hexanone; △ 2-heptanone; ● cyclohexanone.

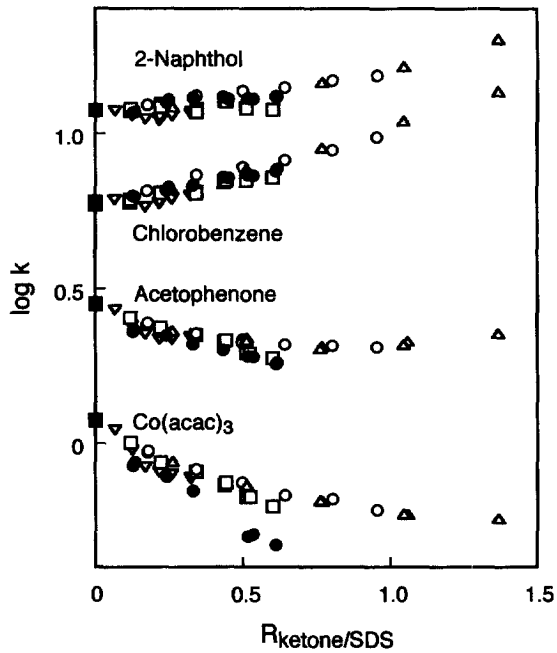

Fig. 8. Dependence of log *k* of 2-napthol, chlorobenzene, acetophenone and $Co(acac)_3$ on the mole ratio of the solubilized ketone to SDS in the micellar phase. The symbols are the same as those given in Fig. 7.

4. Conclusions

Comparing the micellar phase of SDS with the bulk solvent as a medium incorporating the solutes, the most striking characteristic of the micelle is the existence of two divided parts with different properties, i.e., the hydrocarbon core and the Stern layer. The solutes possessing no hydrophilic functional groups are mainly located in the hydrocarbon core of the micelles, and their distribution in the micelles–

water system is very similar to that in the alkane–water system. On the other hand, the solute possessing hydrophilic functional groups, of which transfer from water into alkane accompanying dehydration is thermodynamically unfavorable, can be favorably incorporated in the Stern layer of the micelles together with some water molecules. Consequently, in the SDS micelles–water system, the difference in the solute–water interaction is less reflected on the distribution constant than in the alkane–water system, and the main factor governing the distribution constant is the volume of the hydrophobic parts of the solutes. The solutes possessing analogous hydrophobic parts should have comparable values of the distribution constant regardless of the presence or difference of the hydrophilic parts in the solute molecules. Although MEKC itself has excellent separation efficiency and can recognize a small difference in the distribution constant, it must be important to consider how to control the solute distribution in order to further improve the separation. In this study, the selective control of the distribution in the micellar system using the modifiers possessing both hydrophilic and hydrophobic parts has been proposed and proved to be effective.

The probable mechanisms of the modification effect in the SDS micellar system are summarized in Fig. 9. When a considerable amount of the modifiers possessing both hydrophilic and hydrophobic parts dominantly occupies the solubilization sites in the Stern layer at the micellar surfaces, the distribution of the other solutes possessing hydrophilic functional groups should be competitively lowered (effect A, Fig. 9A). When the modifiers associate with the solutes in the micellar phase through the specific interaction such as hydrogen bonding, this interaction contributes to enhancement of the distribution of the solutes (effect B, Fig. 9B); the effect B is always attended by the effect A, and the net effect on the distribution of the solutes is the sum of these opposite effects. In the higher concentration region of the modifiers, the hydrocarbon core of the micelles would be enlarged by the contribution of hydrophobic part of the modifiers, which chiefly increases the distribution of the solutes possessing no hydrophilic groups (effect C, Fig. 9C). For the analytes possessing hydrophilic groups and not interacting with the modifiers, the effect A is mainly

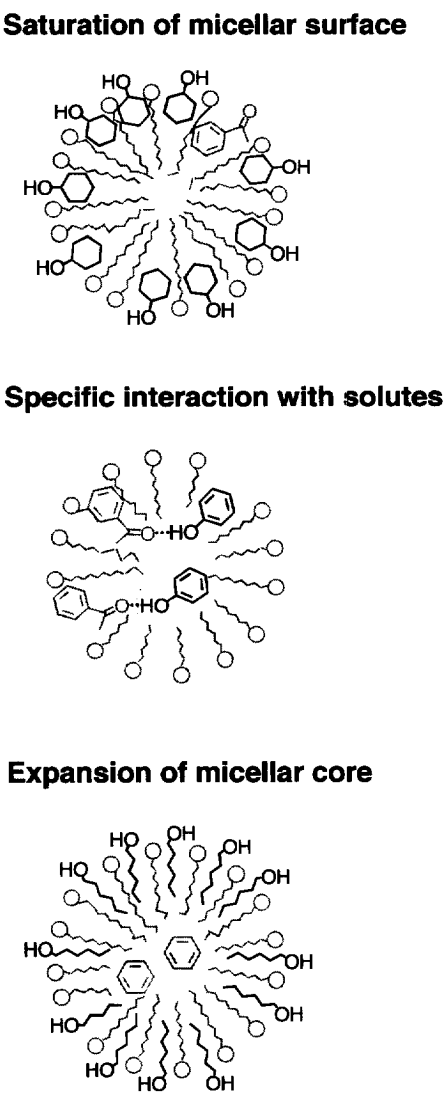

Fig. 9. Schematic illustrations explaining the typical effects of modifiers possessing both hydrophobic and hydrophilic parts on the solute distribution in the SDS micelle–water system: (A) saturation of the micelle surface by the modifiers decreases k of the solutes solubilized in the Stern layer; (B) specific interaction of the modifiers with the solutes increases k of the solutes; (C) expansion of the micellar core with the hydrocarbon parts of the modifiers increases k of the solutes solubilized in the core.

operated; for the solutes possessing hydrophilic groups and specifically interacting with the modifiers, the effects A and B are both operated; for the solutes possessing no hydrophilic groups, the effect C is operated. The hydrogen-bond donor or acceptor

ability of hydrophilic groups of the modifiers is very important for the effect B but not so important for the effects A and C. The magnitude of the effect is affected by the location of the solutes in the micellar phase for the effects A and C, by the strength of the intermolecular interaction for the effect B, and by the amount of the modifiers in the micellar phase for all the effects. The volume of the hydrophobic part of the modifiers is an important factor affecting the solubilization amount of the modifiers. In addition, the structure of the hydrophobic part somewhat influences the selectivity of the effect A.

Although we have examined the micelle-modification effect only for MEKC of simple model compounds, its applicability is sufficiently expected. In our previous paper [13], improved separation of *o*-cresol, *p*-cresol and nitrobenzene in MEKC with SDS by using 1-hexanol as a modifier was demonstrated. The distribution constants of these analytes between the micellar and aqueous phases are similar and the complete separation with the SDS micelle (0.075 *M*) is difficult. In the presence of 0.10 *M* 1-hexanol, the baseline separation was achieved accompanying the changes of retention order. This improvement of separation is mainly due to the selective decrease in the retention factor of nitrobenzene. In addition, the use of the modifiers was found to increase the resolution of separation in MEKC. Generally, the resolution in MEKC becomes higher when the value of t_{mc}/t_0 and the number of theoretical plates are greater [1]. For example, the use of 0.10 *M* cyclohexanol increased the t_{mc}/t_0 value from 3.63 to 4.47 by a little change in t_0 and a remarkable increase in t_{mc}, and it also raised the number of theoretical plates, e.g., from 170 000 to 220 000 for acetophenone and from 260 000 to 320 000 for 2-naphthol [13].

To improve the separation in MEKC, the use of another surfactants or mixed micelles may be alternative methods. However, the modification of micelles with organic additives must be a useful method because it is very easy, various compounds can be chosen as modifiers, and the results can be predicted. Additionally, this idea would be applicable to various separation methods utilizing micellar media such as micellar chromatography [41] and micellar enhanced ultrafiltration [42] as well as MEKC.

References

[1] R. Kuhn and S. Hoffstetter-Kuhn, Capillary Electrophoresis: Principle and Practice, Springer, Berlin, 1993, Ch. 5, p. 191.
[2] S. Takeda, S. Wakida, M. Yamane, A. Kawahara, K. Higashi, Anal. Chem. 65 (1993) 2489.
[3] C. Kiyohara, K. Saitoh, N. Suzuki, J. Chromatogr. 646 (1993) 397.
[4] S. Terabe, Y. Ishihama, H. Nishi, T. Fukuyama, K. Otsuka, J. Chromatogr. 545 (1991) 359.
[5] S. Terabe, Y. Miyashita, O. Shibata, E.R. Barnhart, L.R. Alexander, D.G. Patterson, B.L. Karger, K. Hosoya, N. Tanaka, J. Chromatogr. 516 (1990) 23.
[6] D.E. Burton, M.J. Sepaniak, M.P. Maskarinec, J. Chromatogr. Sci. 25 (1987) 514.
[7] S. Terabe, M. Shibata, Y. Miyashita, J. Chromatogr. 480 (1989) 403.
[8] E.L. Little, J.P. Foley, J. Microcol. Sep. 4 (1992) 145.
[9] E.S. Ahuja, B.P. Preston, J.P. Foley, J. Chromatogr. B 657 (1994) 271.
[10] S. Yang, M. Khaledi, Anal. Chem. 67 (1995) 499.
[11] S. Yang, M. Khaledi, J. Chromatogr. A 692 (1995) 301.
[12] S. Katsuta, K. Saitoh, Anal. Lett. 27 (1994) 743.
[13] S. Katsuta, T. Tsumura, K. Saitoh, N. Teramae, J. Chromatogr. A 705 (1995) 319.
[14] T. Saitoh, H. Hoshino, T. Yotsuyanagi, J. Chromatogr. 469 (1989) 175.
[15] K. Saitoh, C. Kiyohara, N. Suzuki, Anal. Sci. 7S (1991) 269.
[16] S. Terabe, K. Otsuka, K. Ichikawa, A. Tsuchiya, T. Ando, Anal. Chem. 56 (1984) 111.
[17] J.M. Corkill, J.F. Goodman, T. Walker, J. Chem. Soc., Faraday Trans. I 63 (1967) 768.
[18] S. Katsuta, K. Saitoh, Chem. Lett. (1994) 349.
[19] H. Watarai, M. Tanaka, N. Suzuki, Anal. Chem. 54 (1982) 702.
[20] C. Hansh and A. Leo, Substituent Constants for Correlation Analysis in Chemistry and Biology, Wiley, New York, 1979.
[21] S. Katsuta, N. Suzuki, Bull. Chem. Soc. Jpn. 64 (1991) 2470.
[22] H. Imura, S. Katsuta, N. Suzuki, Polyhedron 10 (1991) 1405.
[23] S. Katsuta, Ph.D. Thesis, Tohoku University, Sendai, 1992.
[24] E. Pramauro, C. Minero, G. Saini, R. Graglia, E. Pelizzetti, Anal. Chim. Acta 212 (1988) 171.
[25] B.K. Lavin, A.J. White, J.H. Han, J. Chromatogr. 542 (1991) 29.
[26] P. Mukerjee, J.S. Ko, J. Phys. Chem. 96 (1992) 6090.
[27] H. Watarai, H. Oshima, N. Suzuki, Quant. Struct.-Act. Relat. 3 (1984) 17.
[28] S.S. Berr, M.J. Coleman, R.R.M. Jones, J.S. Johnson Jr., J. Phys. Chem. 90 (1986) 6492.
[29] S. Katsuta, Bunseki Kagaku 44 (1995) 1001.
[30] A. Bondi, J. Phys. Chem. 68 (1964) 441.
[31] E.J. Fendler, C.L. Day, J.H. Fendler, J. Phys. Chem. 76 (1972) 1460.
[32] K.K. Fox, I.D. Robb, R. Smith, J. Chem. Soc., Faraday Trans. I 68 (1972) 445.
[33] K. Hayase, S. Hayano, Bull. Chem. Soc. Jpn. 50 (1977) 83.

[34] M. Manabe, M. Koda, K. Shirahama, J. Colloid Interface Sci. 77 (1980) 189.
[35] S. Backlund, K. Rundt, Acta Chem. Scand. A34 (1980) 433.
[36] M. Abe, Y. Tokuoka, H. Uchiyama, K. Ogino, J.F. Scamehorn, S.D. Christian, Colloids Surf. 67 (1992) 37.
[37] I.V. Rao, E. Ruckenstein, J. Colloid Interface Sci. 113 (1986) 256.
[38] S. Katsuta, H. Kato, K. Saitoh and N. Teramae, The 43rd Annual Meeting of the Japan Society for Analytical Chemistry, Fukuoka, October 1994, Abstr., p. 19.
[39] S.J. Dougherty, J.C. Berg, J. Colloid Interface Sci. 48 (1974) 110.
[40] M. Abe, K. Mizuguchi, Y. Kondo, K. Ogino, H. Uchiyama, J.F. Scamehorn, E.E. Tucker, S.D. Christian, J. Colloid Interface Sci. 160 (1993) 16.
[41] D.W. Armstrong, R.Q. Terrill, Anal. Chem. 51 (1979) 2150.
[42] R.O. Dunn, J.F. Scamehorn, S.D. Christian, Sep. Sci. Technol. 20 (1985) 257.