



FUNDAMENTAL STUDIES ON ELECTROKINETIC CHROMATOGRAPHY

KOJI OTSUKA

1986

DEPARTMENT OF INDUSTRIAL CHEMISTRY
FACULTY OF ENGINEERING
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CHAPTER 1

GENERAL INTRODUCTION

Since high-performance liquid chromatography (HPLC) was introduced as one of the most powerful analytical tools [1] in late 1960s, there have been remarkable developments and improvements in the field of this analytical technique. They include the use of chemically bonded stationary phases such as alkyl (C_2 , C_8 , C_{18} , etc.), phenyl or cyano group bonded silica supports, the developments of highly sensitive detectors, such as the electrochemical detector in addition to the improvement of conventional spectrophotometric and fluorometric detectors, and the use of micro-packed [2] or open-tubular micro-capillary columns [3]. Recently, extensive work has been done on the coupling HPLC with mass spectrometry (LC-MS), nuclear magnetic resonance spectrometry (LC-NMR), or Fourier transform infrared spectrophotometry (LC-FTIR) [4]. As a technique related with HPLC, a new chromatographic method using supercritical fluid has also been reported [5].

In all of above-mentioned chromatographic systems, there are two heterogeneous phases in a separation column, i.e., the mobile phase and the stationary phase. In conventional liquid chromatography, the stationary phase is immobilized in a column, while the mobile phase is percolated through the stationary bed. When a sample is added to the top of the column, components of the sample distribute themselves between mobile

and stationary phases in the ratio of the capacity factor while the mobile phase flows down continuously through the column. It is, however, not essential in chromatography that one phase is rigidly fixed in the column; the relative movement of two phases between which a solute is distributed is sufficient for the chromatographic separation. Thus, instead of the forced flow of the mobile phase through the stationary phase, any means of continuous displacement between two phases can be utilized to constitute a chromatographic system. The two phases need not always be heterogeneous if any distribution process of a solute can be held between them; they may be solvent and a pseudophase like micelle, or even a molecule. In this thesis, the author reports a new type of chromatographic technique based on electrokinetic migrations in an open-tubular capillary and the distribution of the solute between two phases through some specific interactions, i.e., micellar solubilization phenomenon and inclusion complex formation with cyclodextrin. This extends the scope of liquid chromatography to that of a homogeneous solution alone. For this technique, the term "electrokinetic chromatography" is used because this obviously belongs to a branch of chromatography.

Among various techniques available in electrophoresis, free zone electrophoresis in open-tubular capillaries [6,7] has been employed because of its high efficiency in separation and ease of instrumentation for continuous on-line detection. It has been reported [7] that in free zone electrophoresis with an open-tubular glass capillary a strong electroosmotic

flow of the electrolyte solution occurs in the tube to an extent that even small, triply charged anions are carried toward the negative electrode. This electroosmotic flow provides another advantage of shortening the analysis time.

The principle of electrokinetic chromatography using micellar solution and open-tubular capillary is schematically illustrated in Figure 1. An aqueous solution of an ionic surfactant, whose concentration is higher than the critical micelle concentration, is filled in an open-tubular capillary, and a high-voltage DC is applied between both ends of the tube. The whole solution is transported by the electroosmotic flow, while the charged micelle migrates with a different velocity from that of the solution because of an electrophoretic effect. When a solute is added to one end of the tube, the solute distributes itself between the micelle and the aqueous phase, while the two phases differentially move in the tube. The advantages of this technique over other chromatographic ones exist in high resolution in separation attained by the approximate flat flow of electrokinetic migrations and in the limited range of the retention time resulting from migrations of both phases to the same direction.

A schematic diagram of electrokinetic chromatograph is shown in Figure 2. The system consists of 4 parts, that is, an open-tubular capillary column, e.g., 50 μ m i.d. fused silica tube, a UV spectrophotometric detector, two beakers to stock a chromatographic solution, and a regulated high-voltage DC power supply. Details of the apparatus are dis-

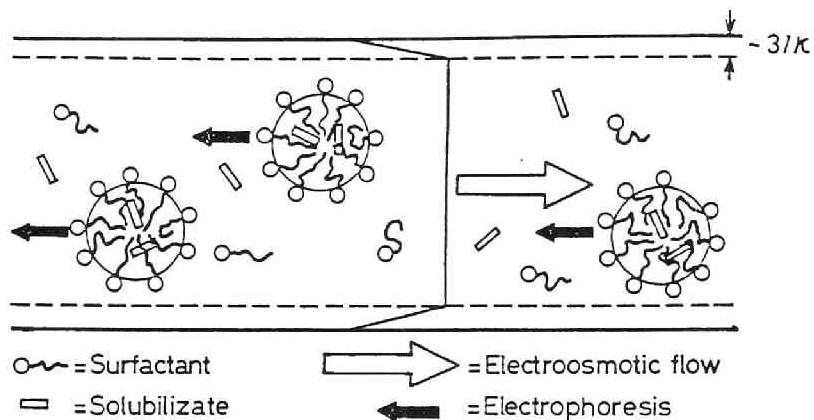


Figure 1. Schematic illustration of electrokinetic chromatography with micellar solution and open-tubular capillary.

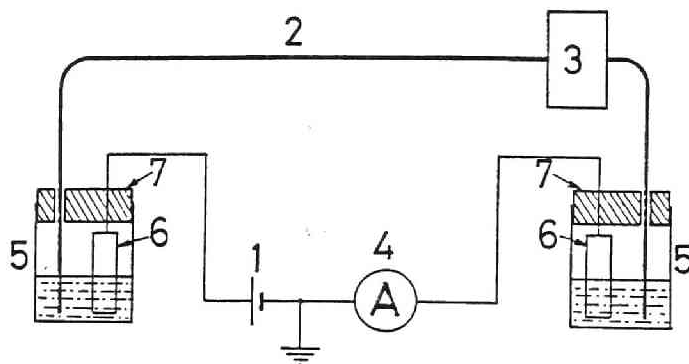


Figure 2. Schematic diagram of electrokinetic chromatograph: 1, regulated high-voltage DC power supply; 2, separation tube; 3, UV detector; 4, digital ampere meter; 5, glass beaker; 6, platinum electrode; 7, silicone rubber cap.

cussed in the following Chapters. Some chromatograms are shown in Figures 3 and 4. The former shows that this technique can completely resolve fourteen phenol derivatives injected as an aqueous solution within 19 min. The limited range of elution is shown in the latter. The method for calculation of the capacity factor is described in Chapter 2.

Fundamental characteristics of electrokinetic chromatography with micellar solution and open-tubular capillary are presented in Chapter 2. Electrokinetic migrations in this chromatographic system are treated by considering the dependence of the electroosmotic velocity, the electrophoretic velocity and the migration velocity of the micelle on the applied voltage and the electric current. Then, some chromatographic parameters of electrokinetic chromatography are described in comparison with conventional liquid chromatography. Distribution coefficients and thermodynamic parameters are also discussed.

In Chapter 3, band broadening in electrokinetic chromatography with micellar solution is discussed. Instrumental requirements to ensure the high efficiency in this chromatography are described in conjunction with effects of the lengths of the sample injection and the slit of the detector, the response times of the detector and the recorder on the plate height. Band broadening in column is theoretically treated by considering the observed dependence of the plate height on the electroosmotic velocity.

Applications of electrokinetic chromatography with micellar solutions are reported in Chapters 4 to 6 to show high-

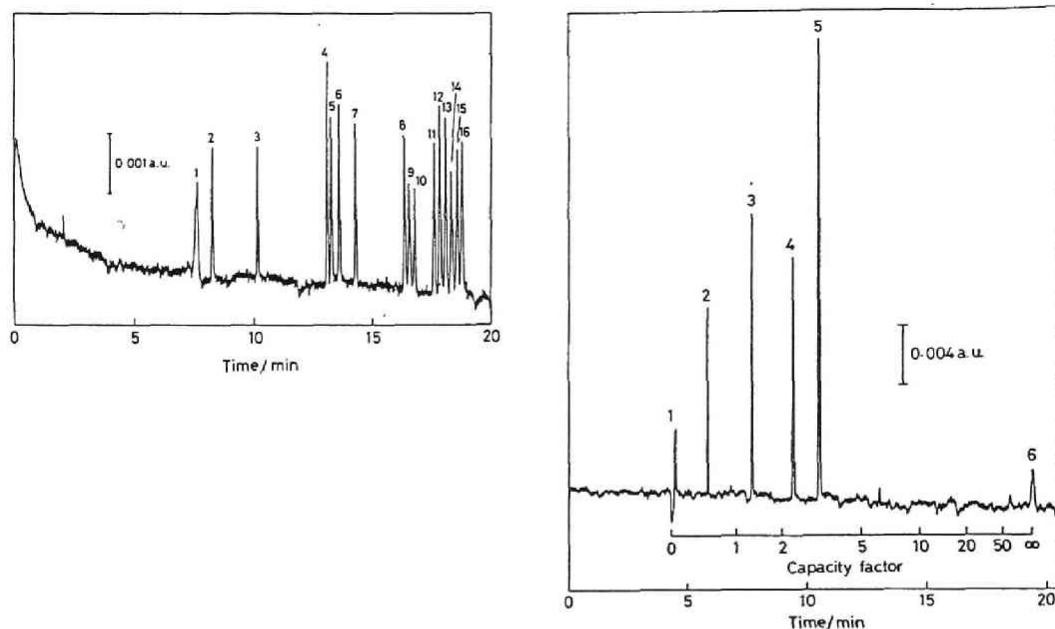


Figure 3. Electrokinetic chromatogram of phenols with an SDS solution: (1)water, (2)acetylacetone, (3)phenol, (4)*o*-cresol, (5)*m*-cresol, (6)*p*-cresol, (7)*o*-chlorophenol, (8)*m*-chlorophenol, (9)*p*-chlorophenol, (10)2,6-xyleneol, (11)2,3-xyleneol, (12)2,5-xyleneol, (13)3,4-xyleneol, (14)3,5-xyleneol, (15)2,4-xyleneol, (16)*p*-ethylphenol; micellar solution, 1 mmol of SDS in 20 mL of borate-phosphate buffer, pH 7.0; separation tube, 0.05 mm i.d. x 900 mm; length of the tube used for separation, 750 mm; total applied voltage, ca. 25 kV; current, 28 μ A; detection wavelength, 270 nm; temperature, ca. 25 °C.

Figure 4. Electrokinetic chromatogram indicating the total range of elution: (1)methanol, (2)phenol, (3)*p*-cresol, (4)2,6-xyleneol, (5)*p*-ethylphenol, (6)Sudan III; separation tube, 0.05 mm i.d. x 650 mm; length of the tube used for separation, 500 mm; total applied voltage, ca. 20 kV; current, 33 μ A; detection wavelength, 220 nm. Other conditions as in Figure 3.

resolving power and high-performance of this chromatography.

Separation of phenylthiohydantoin (PTH)-amino acids is described in Chapter 4. A mixture of 22 PTH-amino acids is successfully separated with a 0.05 M SDS solution and a 0.05 mm i.d. x 650 mm fused silica tube. As some solutes in the mixture are ionized under the conditions used, the electrophoretic effect of the ionized solute itself is discussed in addition to the micellar solubilization phenomenon and the electrophoretic migration of the micelle. Comparison of two surfactants, SDS and dodecyltrimethylammonium bromide, is also made.

Retention characteristics of chlorinated phenols, some of which are ionized, in electrokinetic chromatography with micellar solutions are presented in Chapter 5. By considering both effects of the electrophoretic migration of the ionized solute itself and the micellar solubilization, the capacity factors of the solutes are calculated. Complete separation of all the isomers of chlorinated phenols and phenol itself is achieved with a 0.07 M SDS solution and a 0.05 mm i.d. x 650 mm fused silica capillary.

In Chapter 6, separation of 11 aromatic sulfides by electrokinetic chromatography with micellar solutions is reported. The 10 sulfides of the 11 solutes are separated with a 0.02 M SDS solution. Distribution coefficients of the sulfides in micellar solubilization are calculated for SDS and sodium tetradecyl sulfate solutions at 35 °C. The use of a mixed solution of an SDS solution and methanol is also successful in separating the 10 sulfides of the 11.

The above-mentioned studies are all concerned with one type of electrokinetic chromatography which uses a micellar solution. In Chapter 7 are described preliminary results of the study on the other type of electrokinetic chromatography, which uses a solution of an ionizable β -cyclodextrin derivative, i.e., 2-*O*-carboxymethyl- β -cyclodextrin (β -CMCD), as a moving "stationary" phase. Some benzene derivatives are separated based on the formation of inclusion complexes by β -CMCD, and distribution coefficients of the solutes between aqueous phase and β -CMCD are calculated.

LITERATURE CITED

- [1] Snyder, L. R.; Kirkland, J. J. "Introduction to Modern Liquid Chromatography"; Wiley: New York, 1979; Chapter 1.
- [2] Ishii, D.; Asai, K.; Hibi, K.; Jonokuchi, T.; Nagaya, M. *J. Chromatogr.* 1977, *144*, 157-168.
- [3] Tsuda, T.; Novotny, M. *Anal. Chem.* 1978, *50*, 271-275.
- [4] Majors, R. E.; Barth, H. G.; Lochmuller, C. H. *Anal. Chem.* 1984, *56*, 300R-349R.
- [5] Peaden, P. A.; Lee, M. L. *J. Liq. Chromatogr.* 1982, *5*(Suppl. 2), 179-221.
- [6] Mikkers, F. E. P.; Everaerts, F. M.; Verheggen, Th. P. E. M. *J. Chromatogr.* 1979, *169*, 11-20.
- [7] Jorgenson, J. W.; Lucaks, K. D. *Anal. Chem.* 1981, *53*, 1298-1302.

CHAPTER 2

ELECTROKINETIC CHROMATOGRAPHY WITH MICELLAR SOLUTION AND OPEN-TUBULAR CAPILLARY

ABSTRACT

Fundamental characteristics of a new type of chromatography with micellar solutions of ionic surfactants were examined. Electrokinetic phenomena in open-tubular capillaries move two phases, aqueous and micellar, with different velocities and micellar solubilization operates as the distribution process of solutes. Sodium dodecyl sulfate (SDS) solutions and a 0.05 mm i.d. x 650 mm fused silica tube were employed and high DC voltages up to 25 kV were applied. Linear relationships were observed between current and migration velocities of water, micelle, and any solute, but not between applied voltages and the velocities. This discrepancy can be reasonably interpreted in terms of the temperature rise of the solution in the tube resulting from Joule heating. Optimum resolutions can be obtained when the capacity factor is about 2, because retention times are limited between those of an insolubilized solute or water and a totally solubilized solute or micelle. Observed dependence of capacity factors on current can also be explained by the temperature rise. Thermodynamic parameters in micellar solubilization were presented.

INTRODUCTION

The applicability of the micellar solubilization phenomenon to chromatography as a distribution process in combination with electrophoresis of an ionic micelle has briefly been discussed by Nakagawa [1], and he called this technique "solubilization chromatography" after the partition mechanism. The point of his discussion may be summarized as follows: Micelles of an ionic surfactant can migrate in an aqueous solution by electrophoresis. When a solubilize is added into a micellar solution, some portion of the solubilize may be solubilized into the micelle. Thus the solubilization by micelles can constitute a mechanism of retention in chromatography. The distribution ratio of a solubilize will increase with an increase of the micellar concentration but will be constant regardless of the concentration of the solubilize. If a solubilize is soluble in an aqueous solution to a certain extent, the distribution equilibrium is considered to be established very rapidly because, e.g., the stay time of benzene in the micelle of sodium dodecyl sulfate (SDS) has been estimated to be less than 10^{-4} s [2]. When electrophoresis is exclusively operating in the relative migration between the aqueous and micellar phases, the aqueous phase is considered to be stationary and the micellar phase mobile. Although micelles are understood as dynamic structures with a liquid core [3], it may be possible in the following discussion in this Chapter to depict micelles as a liquid phase distinct from the surrounding aqueous solution. It

should be noted that this technique is distinctly different from the reversed-phase liquid chromatography with micellar mobile phase [4]: In the latter, the separation is based on distribution processes among three phases, stationary bonded phase, micelle, and water, and micelles migrate with water as an aqueous pseudophase.

In this Chapter, some features of the electrokinetic migration will be first presented from the practical point of view, i.e., how electrokinetic velocities can be related to experimental variables. Then some fundamental characteristics of this technique as a chromatographic method will be described in comparison with conventional chromatography because the limited range of retention times requires different expressions of chromatographic parameters. Last, distribution coefficients and thermodynamic parameters in micellar solubilization will be discussed.

EXPERIMENTAL SECTION

Apparatus

Electrokinetic chromatography was performed in microbore vitreous silica tubing, 650 or 900 mm long, 0.05 mm i.d. (Scientific Glass Engineering Inc.), with a Model HSR-24P regulated high-voltage DC power supply (Matsusada Precision Devices, Otsu, Japan) delivering +3 to +25 kV. Each end of the capillary tube was dipped in a small glass beaker containing a surfactant solution covered with a silicone-rubber stop-

per having two small-bore holes, one for a platinum electrode and the other for the capillary tube. The electric current was monitored between the negative electrode and the negative terminal of the power supply with an ammeter throughout the operation. Detection was carried out by on-column measurement of UV absorption through a slit of 0.05 mm x 0.75 mm, the long axis of which was placed parallel to the column axis at a position 150 mm from the negative end of the tube. The polymer coating of the vitreous silica tubing was partly burned out at the detection point of the tube to make an on-column UV cell. A Jasco UVIDEK-100-II spectrophotometric detector (Tokyo, Japan) was used with minor modification to obtain a higher amplifier gain and a shorter response time than conventional one. The apparatus was placed in a thermostated oven. Although the oven was not equipped with a circulating fan, a fan incorporated in the detector to dissipate the heat from a UV lamp served as a substitute.

Reagent

Sodium dodecyl sulfate (SDS) of protein-research grade purchased from Nakarai Chemicals (Kyoto, Japan), sodium tetradecyl sulfate (STS) supplied by Nikko Chemicals (Tokyo, Japan) and sodium dodecanesulfonate purchased from Tokyo Kasei Kogyo (Tokyo, Japan) were used as they were received. Water was purified with a Milli-Q system. Other reagents were of analytical-reagent grade and were used without further purification. Borate-phosphate buffer solution, pH 7.0 was prepared by mixing a 0.025 M sodium tetraborate solution and a

0.05 M sodium dihydrogen phosphate solution in an appropriate ratio to indicate pH 7.0. A surfactant solution was prepared by dissolving an appropriate amount of the surfactant in the borate-phosphate buffer solution followed by filtration of the solution through a membrane filter of 0.45- μ m pore size. The solution was allowed to stand in the oven more than 1 night before use to equilibrate at the temperature. Concentrations of test samples were arbitrarily adjusted to give suitable peak heights, and estimated to be in the range from 0.1 to 1.0 mg mL⁻¹ except for methanol and Sudan III. About 1 % of methanol was added to each sample solution. Injection volume was about 2 nL throughout the experiment.

Procedure

A capillary tube was filled with a surfactant solution by use of a microsyringe and 1.5 mL each of the same surfactant solution was introduced in two beakers placed at the same level. For the sample injection, the positive end of the tube was moved into a vessel containing a sample solution and the level of the sample solution was raised about 4 cm higher than that of the surfactant solution to allow the sample solution to flow downward into the capillary tube. After 5 to 90 s depending on the desired amount of injection, the end of the tube was returned to the beaker and a high voltage was applied.

RESULTS AND DISCUSSION

Electrokinetic Migration

The electrokinetic chromatogram shown in Figure 1 was obtained with a 0.05 M SDS solution and a 0.05 mm i.d. x 500 mm tube at 35 °C. All the solutes injected at the positive end of the tube were detected with a UV photometer positioned at the negative side. Methanol is regarded as an insolubilized solute, i.e., as existing only in the aqueous phase, and Sudan III is assumed to be completely solubilized, i.e., to exist only in the micellar phase. The assumed behavior of methanol was justified by gel filtration chromatography with SDS solutions [5]: The distribution coefficient of methanol between SDS micelle and water was found to be 0.3 at 40 °C [6]. The validity of the use of a water-insoluble dye as a tracer of the micelle has been discussed by Stigter and Mysels [7]. Methanol is usually not detected by the absorption of UV light but absorbs here due to the slight change of the refractive index. The cylindrical cell employed in this work is susceptible to the influence of refraction when it is irradiated at right angles to the axis of the tube. Since other solutes are considered to be unionized under the conditions used, the retention times of these solutes fall between those of methanol and Sudan III as will be described later. The retention parameters in this method will also be discussed later.

Dependences of the migration velocities of these solutes on the electrical field strength and the current were examined under various applied voltages, and the results for methanol

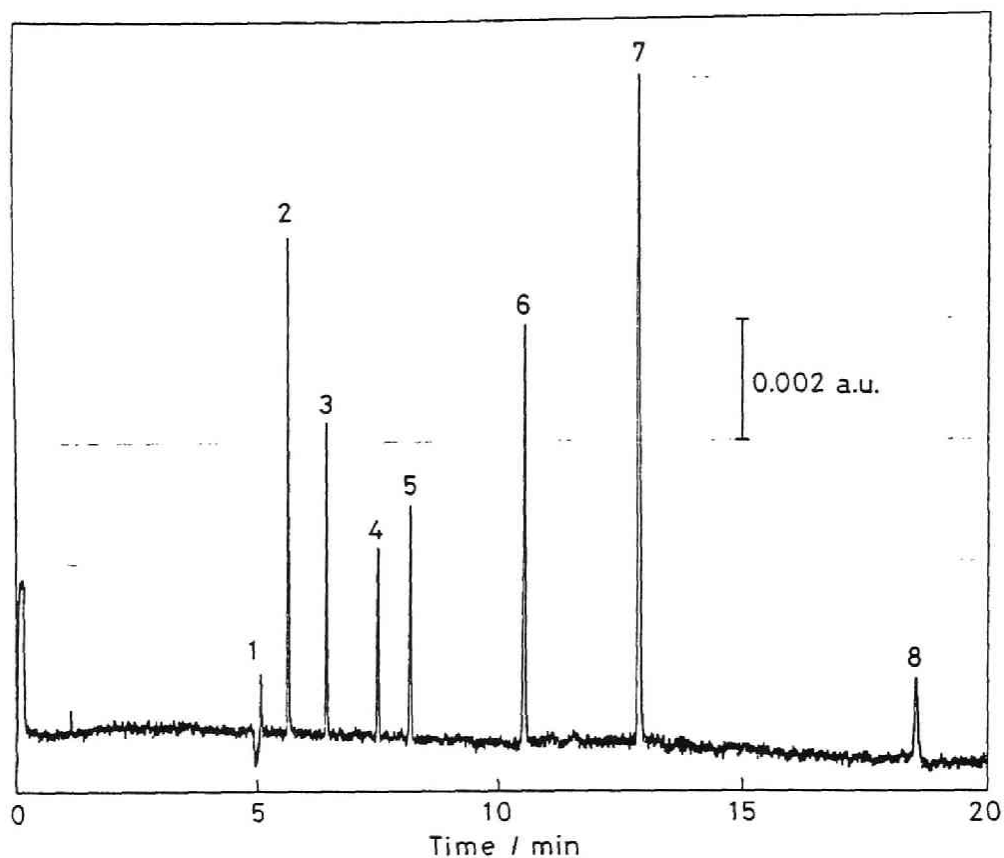


Figure 1. Electrokinetic chromatogram of some test samples: (1)methanol, (2)resorcinol, (3)phenol, (4)*p*-nitroaniline, (5)nitrobenzene, (6)toluene, (7)2-naphthol, (8)Sudan III; micellar solution, 0.05 M SDS in 0.1 M borate--0.05 M phosphate buffer, pH 7.0; separation tube, 0.05 mm i.d. x 650 mm; length of the tube used for separation, 500 mm; total applied voltage, ca. 15 kV; current, 26 μ A; detection wavelength, 210 nm; temperature of oven, 35 °C.

and Sudan III are given in Figure 2. Similar dependences as shown in Figure 2 were always recognized at different SDS concentrations. The migration of the aqueous phase which is indicated by the methanol band is interpreted to be brought about by electroosmosis and, therefore, the velocity of the methanol band should be essentially equal to the electroosmotic velocity v_{eo} . The Sudan III band is considered to represent the migration of the micelle as mentioned above. The velocity of the micelle should be equal to the difference between electroosmotic velocity of the aqueous phase and the electrophoretic one of the micelle, because the negatively charged SDS micelle is subject to both effects in opposite directions. The electroosmotic velocity is observed to be much larger than the electrophoretic one for the SDS micelle under the conditions employed.

Linear relationships were noticed between velocities of any solutes and the electrical current but not between velocities and applied voltages as shown in Figure 2 for methanol and Sudan III. A similar deviation from linearity has been reported by Jorgenson and Lukacs [8] and they suggested that Joule heating is responsible for this deviation.

The theoretical work of Rice and Whitehead [9] on electroosmotic flow in narrow cylindrical capillaries indicates that when the reciprocal of the Debye-Huckel parameter, $1/\chi$, is much smaller than the radius of a capillary r , v_{eo} is described by the classical formula [10]

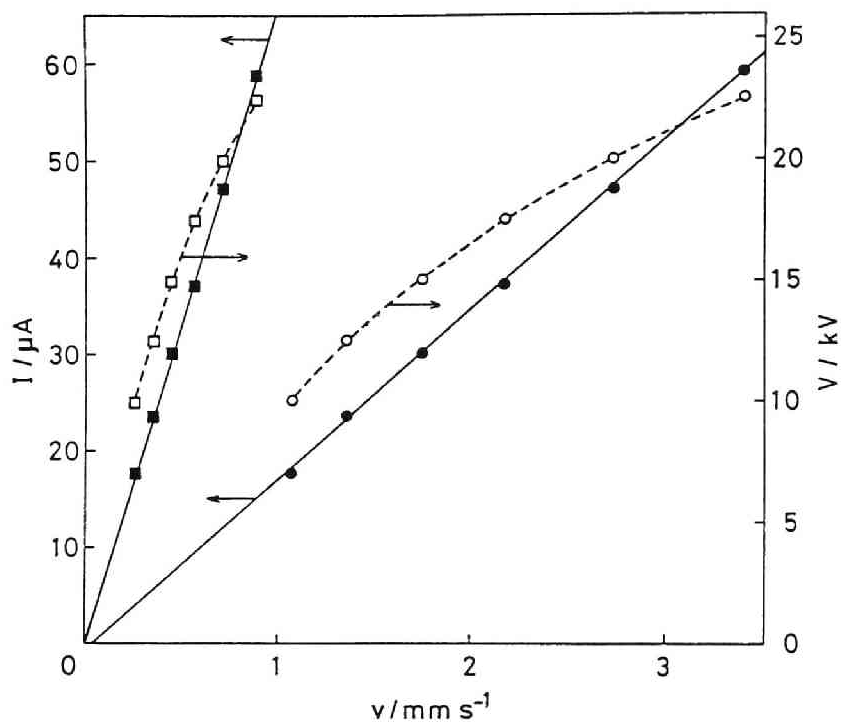


Figure 2. Dependence of migration velocities v of methanol ($\circ, \bullet$) and Sudan III ($\square, \blacksquare$) on applied voltage (dashed line) and current (solid line). Conditions are the same as those given in Figure 1 except for applied voltage and current.

$$v_{e0} = - \frac{\epsilon \xi}{\eta} E \quad (1)$$

where ϵ is the permittivity of the liquid, ξ is the zeta potential, η is the viscosity of the liquid, and E is the electrical field strength. The negative sign means that when ξ is negative, the liquid flow is toward the negative electrode [10]. In the case of the buffer solution employed in this study, 0.1 M borate--0.05 M phosphate, pH 7.0, the value of $1/\chi$ is estimated from electrolyte concentrations to be 0.8 nm, much smaller than the radius of the tube, 50 μ m. Since the surface conductivity may be neglected in this case, Ohm's law,

$$E = \frac{l}{\pi r^2 \sigma} I \quad (2)$$

where l is the length of the tube, σ is the specific conductivity of the liquid, and I is the current due to transport of charge by the fluid, can be used to rewrite eq 1 into the expression

$$v_{e0} = - \frac{l}{\pi r^2} \frac{\epsilon \xi}{\sigma \eta} I \quad (3)$$

The specific conductivity σ is given by the Kohlrausch's law of independent migration of ions

$$\sigma = \sum_j c_j z_j \lambda_j \quad (4)$$

where c_j , z_j , and λ_j are the number of moles per unit volume, charge number, and equivalent conductivity of the j th ion, respectively. Here, λ_j can be related to the viscosity by the combination of the Stokes--Einstein relation and the Nernst--Einstein equation [11] as

$$\lambda_j = \frac{z_j e F}{6 \pi a_j \eta} \quad (5)$$

where e is the elementary charge, F is the Faraday constant, and a_j is the radius of the solute ion j . Combination of eq 3 with eqs 4 and 5 gives

$$v_{e0} = - \frac{6 \epsilon \xi l}{r^2 e F} \sum_j \frac{a_j}{c_j z_j^2} I \quad (6)$$

One should note that η is not included in eq 6.

The dependence of v_{e0} on current and the applied voltage V shown in Figure 2 may be explained in terms of the decrease of η with increase of V or I . That is, if c_j , z_j , and a_j are assumed to be constant, v_{e0} increases linearly with increase of I as predicted from eq 6, but the deviation from linearity may be predicted between v_{e0} and V as a result of the change of η with V in eq 1. The experimental results strongly support this view and, in addition, indicate that the product of permittivity and the zeta potential remains virtually constant

regardless of E and I . The rise in temperature of the liquid in the tube by Joule heating must be the reason for the decrease of η as the applied voltage increases. In order to confirm this interpretation, the surface temperature of the tube was measured with a thermocouple under the same conditions as employed for obtaining Figure 2. The rise in temperature observed reached 45 °C higher than the ambient temperature at 25 kV/610 mm as shown in Figure 3, although the exact temperature was uncertain because of the relatively large contact point (0.2 mm o.d., of 0.1 mm o.d. alumel--chromel wire) compared with the diameter of the tube, 0.2 mm o.d. The temperature rise was proportional to the power dissipated per unit length of the tube and amounted to about 2.8 °C mW⁻¹ cm for 0.05 M SDS solution. Rather high temperature at the surface reveals that heat dissipation is not efficient because of a low film coefficient of heat transfer from the surface of the tube into the air.

The variation of current with the applied voltage is also illustrated in Figure 3. By considering the rise in temperature mentioned above, the nonlinear relationship between V and I can also be understood from a viewpoint of the alteration in conductivity as a result of the viscosity change as predicted from eq 5. It follows, therefore, that low viscosity at a high voltage causes positive deviation in the plot of I vs. V as shown in Figure 3.

By taking into account the sign of the migrating direction, we can describe the velocity of the micelle v_{mc} , as

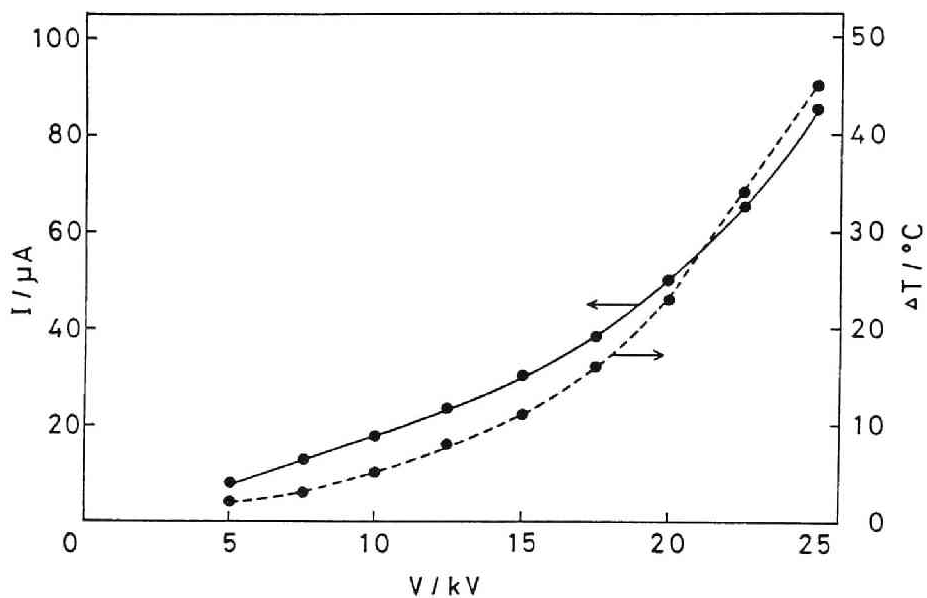


Figure 3. Dependence of current (solid line) and rise in surface temperature, ΔT , of the tube (dashed line) on applied voltage; length of the tube 610 mm. Other conditions are given in Figure 2.

$$v_{mc} = v_{eo} + v_{ep} \quad (7)$$

where v_{ep} is the electrophoretic velocity of the micelle. The velocity v_{ep} is given by

$$v_{ep} = \frac{2\epsilon\xi}{3\eta} f(\kappa a) E \quad (8)$$

where function $f(\kappa a)$ depends on the particle shape and, for sphere of $\kappa a = \infty$, is 1.50 and a is the radius of the particle [10]. It should be noted that the zeta potential in eq 8 is for the micelle, while that in eq 1 is for fused silica. Substitution of eq 1 and eq 8 into eq 7 gives

$$v_{mc} = - \frac{\epsilon}{\eta} \left\{ \xi_1 - \frac{2}{3} \xi_2 f(\kappa a) \right\} \quad (9)$$

where subscripts 1 and 2 denote fused silica and micelle, respectively. The observed movement of the methanol band from the positive end to the negative means that ξ_1 is negative. The fact that the Sudan III band moved in the same direction as the methanol but with lower velocity implies that ξ_2 is lower than ξ_1 in absolute value, because ξ_2 should also be negative for the negatively charged SDS micelle. The linear relationship of v_{mc} vs. I and the nonlinear one of v_{mc} vs. E as shown in Figure 2 agree with the probable change of viscosity as discussed above. Although the micellar size of an ionic surfactant is reported to decrease with an increase of

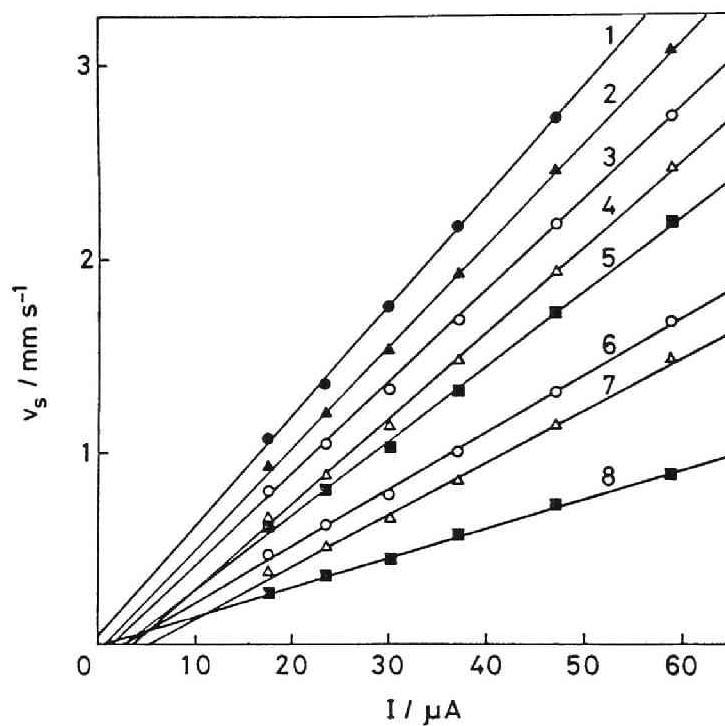


Figure 4. Velocities of solutes as a function of current. Solutes are indicated by the same numbers as given in Figure 1. Conditions are the same as those given in Figure 2.

temperature [12], the observed linearity between v_{mc} and I or v_{ep} and I indicates that $f(\chi a)$ may be regarded as unaltered in the temperature range of 35-80 °C.

Velocities of the solutes v_s are shown in Figure 4 as a function of the current. All the solutes have linear dependence but extrapolated values of v_s at $I = 0$ do not coincide with zero. These deviations seem to exceed the experimental error and may be ascribed to the small variation of the zeta potential for the micelle with temperature when the micelle contains solutes. The number of solutes solubilized into the micelle is roughly estimated to be less than 5 to 10 molecules per micelle under the condition shown in Figure 1 except for Sudan III where less than one molecule is solubilized.

At a constant voltage applied, v_{eo} remained virtually constant irrespective of the SDS concentration c_{SDS} , while the reciprocal of v_{mc} increased with an increase of c_{SDS} as shown in Figure 5. In this case, current was observed to increase linearly with c_{SDS} from 22 μA at 0.03 M to 45 μA at 0.15 M as predicted from eqs 2 and 4. Independence of v_{eo} from c_{SDS} suggests that the viscosity η remains approximately unchanged as expected from eq 1: The effect of the temperature rise by increasing current on η is accidentally compensated by that of increasing c_{SDS} . Thus, the viscosity of the aqueous SDS solution has been reported to increase linearly with the concentration [13]. The constant v_{eo} regardless of c_{SDS} also suggests that SDS has no effect on the zeta potential of the glass/solution interface. The observed increase of v_{mc}^{-1} with c_{SDS} , i.e., the increase of the electrophoretic velocity, is

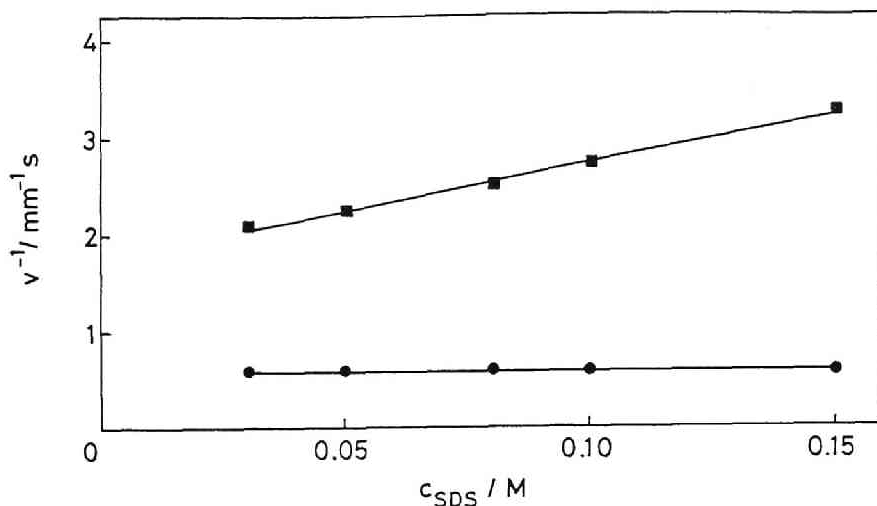


Figure 5. Reciprocal velocities of Sudan III (■) v_{mc}^{-1} and methanol (●) v_{eo}^{-1} as a function of SDS concentration; applied voltage was 15 kV. Other conditions are given in Figure 1.

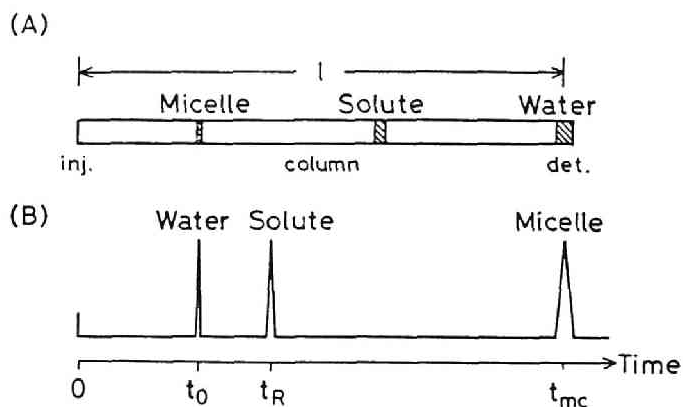


Figure 6. Schematic illustration of zones separated in the tube (A) and chromatogram (B) of the hypothetical mixture of water, solute, and micelle. Plate number, capacity factor of solute, and $v_0/v_{\text{mc}} = t_{\text{mc}}/t_0$ are arbitrarily assigned as 14000, 1, and 4, respectively.

in contrast to the result of Stigter et al. [7] that the electrophoretic mobility of the SDS micelle decreases linearly with the increase of c_{SDS} . This discrepancy may be interpreted in terms of differences in temperature rise by Joule heating, i.e., $2.8\text{ }^{\circ}\text{C mW}^{-1}\text{ cm}$ in this work and $0.2\text{ }^{\circ}\text{C mW}^{-1}\text{ cm}$ in the case of Stigter et al. [7, 14]. Thus, in this work η happened to be held nearly constant as described above but in the case of Stigter et al. [7, 14] the increase of η with c_{SDS} caused decrease of v_{ep} as anticipated from eq 8.

Retention Parameters

Figure 6A schematically illustrates migrating zones of water, a solute, and the micelle at the time t_0 , elution time of water, after a hypothetical mixture of the three is injected at the left end of the tube. Here, it is assumed that the zeta potential for fused silica has the same sign as that for the micelle, and the absolute value of the former is higher than the latter. The velocity v_{e0} , which is equal to l/t_0 , is presumed to be four times as large as the v_{mc} in Figure 6A. Figure 6B displays the schematic chromatogram of the three zones observed with an imaginary detector situated at the length l from the injection point. The number of theoretical plates N is arbitrarily assigned as 14000. Experimentally, the retention time of water t_0 is measured with methanol and that of the micelle t_{mc} with Sudan III as described above. The most significant difference in this method from conventional chromatography lies in the fact that the range of retention times of electrically neutral solutes

are limited between t_0 and t_{mc} . The chromatogram given in Figure 1 shows an example of the total range of retention.

A solute which is not solubilized by the micelle at all should migrate with the same velocity as the electroosmotic flow v_{eo} and be eluted first at the retention time t_0 . On the other hand, a solute which is completely solubilized with the micelle should migrate with the same velocity as that of a micelle v_{mc} and be eluted last at the retention time t_{mc} . The velocity v_{mc} is the difference between v_{eo} and the electrophoretic velocity of the micelle v_{ep} , or $v_{mc} = v_{eo} + v_{ep}$ as mentioned above. The retention time of an ordinary sample should depend on the capacity factor K' , which is given by the ratio of the total moles of the solute in the micelle n_{mc} to those in the aqueous phase n_{aq} , or $K' = n_{mc} / n_{aq}$. The retention time of the solute t_R should appear in the range $t_0 \leq t_R \leq t_{mc}$. The R value, the fraction of the solute in the aqueous phase, is given by

$$R = \frac{v_s - v_{mc}}{v_{eo} - v_{mc}} \quad (10)$$

where v_s is the migration velocity of the solute. Now, the R value can be related to K' by [15]

$$R = \frac{1}{1 + K'} \quad (11)$$

Inserting the relationship, $v_{eo} = l/t_0$, $v_{mc} = l/t_{mc}$, and $v_s =$

l/t_R , where l is the tube length from the injection end to the detector cell, into eq 10, followed by combination with eq 11 gives

$$K' = \frac{t_R - t_0}{t_0 (1 - t_R / t_{mc})} \quad (12)$$

Equation 12 can be written in another form as

$$t_R = \frac{1 + K'}{1 + (t_0 / t_{mc}) K'} t_0 \quad (13)$$

In this thesis, the capacity factor is given the symbol K' instead of widely accepted k' in order to emphasize the difference in the relationship of the capacity factor to t_R from the well-known equation

$$t_R = (1 + k') t_0 \quad (14)$$

The value of t_{mc}/t_0 can be regarded as a parameter to indicate the width of the total range of elution for electrically neutral solutes. When t_{mc} becomes infinite, eq 13 is equivalent to eq 14. It should be stressed that the capacity factor of infinity in this method implies that the solute stays in the micellar phase at all times.

It is interesting to compare the retention behavior observed in this method whose total range of retention is limited between t_0 and t_{mc} with that obtained under the

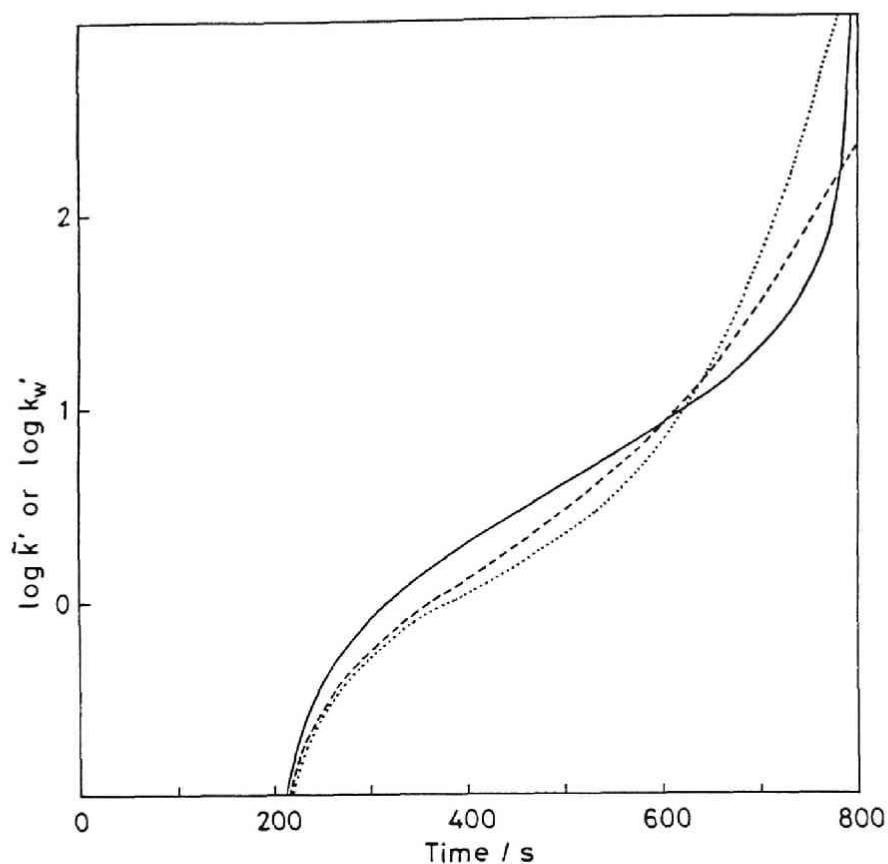


Figure 7. Logarithmic capacity factor as a function of retention time for electrokinetic chromatography (solid line) and for concave gradient elution in liquid chromatography from water to methanol (dashed line) and from water to tetrahydrofuran (dotted line).

gradient elution in conventional liquid chromatography. The relationship between the retention time and the capacity factor in this method can be calculated from eq 13, and an example is shown on a solid line in Figure 7, where the following conditions are assumed: $t_0 = 200$ s, $t_{mc} = 800$ s, and tube length $l = 500$ mm. Similar relationship between the retention time for a concave gradient elution and the capacity factor k_w' in water under reversed-phase chromatography is calculated for two cases according to the procedure described in the Appendix. The result for a concave gradient from water to methanol ($S = 3.0$ [16]) is shown on the dashed line, and that for the case from water to tetrahydrofuran ($S = 4.4$ [16]) is on the dotted line. In both cases, column length and t_0 , the retention time of an unretained solute, are taken as the same value as the above example, and t_c as 600 s, that is, pure methanol or tetrahydrofuran reaches the exit of the column at 800 s. The gradient shapes are chosen to give a curve close to that for the above example: $m = 2.1$ in eq A1 for water--methanol and $m = 3.0$ for water--tetrahydrofuran. Although the curve for electrokinetic chromatography is not in good agreement with those for the concave gradient, it can be said that the relationship between the capacity factor and the retention time in electrokinetic chromatography is roughly approximated to that in reversed-phase chromatography with the concave gradient elution technique from water to methanol except for solutes having larger capacity factors than 150 in water.

Resolution Equation, Effective Plates, and Peak Capacity

The following resolution equation can be derived by considering eq 12 in the same manner [15] as in usual elution chromatography:

$$R_s = \frac{\sqrt{N}}{4} \left(\frac{\alpha - 1}{\alpha} \right) \left(\frac{k_2'}{1 + k_2'} \right) \left(\frac{1 - t_0/t_{mc}}{1 + (t_0/t_{mc}) k_1'} \right) \quad (15)$$

Here, R_s is the resolution, N is the plate number, and α is the separation factor which is equal to k_2'/k_1' . Owing to the last term, the selectivity term in this chromatography is not independent of the capacity factor term in contrast to conventional chromatography [15].

$$R_s = \frac{\sqrt{N}}{4} \left(\frac{\alpha - 1}{\alpha} \right) \left(\frac{k'}{1 + k'} \right) \quad (16)$$

The last term in eq 15 reveals again the characteristic of this method. The infinite value of t_{mc} reduces the last term of eq 15 to unity, and then eq 15 becomes equal to eq 16. To evaluate the effect of k' on resolution, the approximation, $k_1' = k_2' = k'$, is made for closely eluting peaks. The function $f(k')$, the product of the last two terms in eq 15, is written as

$$f(k') = \left(\frac{k'}{1 + k'} \right) \left(\frac{1 - t_0/t_{mc}}{1 + (t_0/t_{mc}) k'} \right) \quad (17)$$

The dependence of the function $f(k')$ on k' is shown in Figure

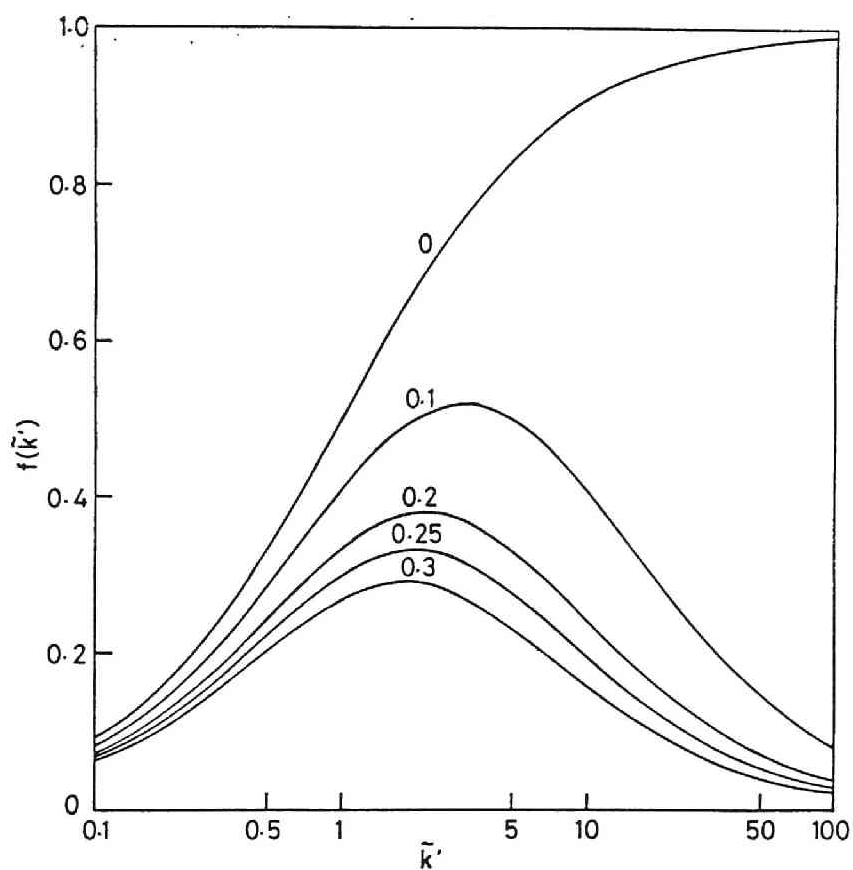


Figure 8. Dependence of $f(K')$ on capacity factor K' . The values of t_0/t_{mc} are given on each line.

8 for some different values of t_0/t_{mc} . The value of zero for t_0/t_{mc} corresponds to conventional chromatography, where the larger capacity factor leads to the better resolution as generally known. Large K' values, however, are unfavorable to obtain good resolutions in this method because the last term in eq 15 approaches zero. The optimum value of K' depends on the ratio t_0/t_{mc} as shown in Figure 8. The ratio t_0/t_{mc} remains constant for an SDS concentration irrespective of V or I as found from Figure 2 but decreases from 0.28 for 0.03 M SDS to 0.18 for 0.15 M SDS as found from Figure 5. For example, since t_0/t_{mc} is about 0.22 for 0.1 M SDS solution, the optimum range of K' lies between 0.84 and 5.4 which gives $f(K')$ values between 0.30 and 0.36. The maximum value 0.36 is found at $K' = 2.13$. Although Figure 8 is calculated based on the assumption $K_1' = K_2'$, if we set 1.1 for K_2'/K_1' in the above example, the range of K' which gives the value higher than 0.30 for the product of the last two terms in eq 15 becomes 0.74 to 5.6 and the maximum value of the product increases to 0.37 at $K_1' = 2.0$. That is, the better resolution will be attained for actual cases than predicted from eq 17 only from the viewpoint of the last two terms in eq 15 in addition to the selectivity term.

The smaller maximum value of $f(K')$ than that of the term $k'/(1 + k')$ in eq 16, which may be close to unity, implies a disadvantage of this method in view of maximum resolution. However, the smaller value of this can be well compensated by the large plate numbers, e.g., 250000 obtained for a 500 mm tube as will be discussed in Chapter 3. The smallest value of

α to obtain $R_s = 1$ calculated by eq 15 is 1.023 at $K' = 2.13$ for $N = 250000$ and $t_0/t_{mc} = 0.22$.

Since the capacity factor K' is proportional to the volume ratio of a micelle to an aqueous phase, the K' value can be easily adjusted by changing the concentration of a micelle to yield an appropriate value for optimum resolution. The dependence of K' on the concentration of a surfactant and temperature will be discussed later in this Chapter. Any of the three parameters, K' , α , and t_0/t_{mc} , may be altered by the structure of a surfactant molecule. Examples of variations of these parameters are given in Table I for three surfactant solutions at 0.05 M along with current, v_{eo} , v_{mc} , and v_{ep} . It is obvious from Table I that changes in ratio t_0/t_{mc} among three solutions are mainly attributable to the changes in electrophoretic velocities which depend on both surface charges and micellar sizes as predicted from eq 8. The alteration of elution order is not observed for listed solutes but values of K' are significantly different among three solutions. Since critical micelle concentrations (CMC) of these surfactants are different, the effect of the structural change of a surfactant molecule on the capacity factor should be examined at the same micellar concentration or with the distribution coefficient as discussed below.

In regard to the resolution equation, the number of effective plates N_{eff} is given for this method as

$$N_{eff} = N \left(\frac{K'}{1 + K'} \right)^2 \left(1 - \frac{t_0}{t_{mc}} \right)^2 \quad (18)$$

Table I

Comparison of Current, Velocities, and Capacity Factors
among Three Anionic Surfactant Solutions at Constant Applied
Voltage^a

	surfactant		
	R ₁₂ SO ₄ Na ^b	R ₁₄ SO ₄ Na ^c	R ₁₂ SO ₃ Na ^d
$I/\mu\text{A}$	44.6	41.4	41.0
$v_{eo}/\text{mm s}^{-1}$	2.67	2.62	2.50
$v_{mc}/\text{mm s}^{-1}$	0.685	0.769	0.674
$v_{ep}/\text{mm s}^{-1}$	1.985	1.851	1.826
t_o/t_{mc}	0.257	0.294	0.270
K^o (resorcinol)	0.158	0.207	0.155
K^o (phenol)	0.378	0.509	0.326
K^o (<i>p</i> -nitroaniline)	0.657	0.860	0.454
K^o (nitrobenzene)	0.986	1.37	0.693
K^o (toluene)	2.27	3.56	1.80
K^o (2-naphthol)	3.79	5.74	3.35

^a Electrical field strength, 308 V cm⁻¹; concentration of surfactant, 0.05 M; oven temperature, 35 °C. ^b Sodium dodecyl sulfate (SDS). ^c Sodium tetradecyl sulfate (STS). ^d Sodium dodecanesulfonate.

To see the performance of this method, the maximum effective plates per second is calculated by setting $N = 250000$, $l = 500$ mm, $v_{e0} = 2 \text{ mm s}^{-1}$, and $t_0/t_{mc} = 0.22$ to give 148 s^{-1} at $K' = 5.9$. This suggests that the efficiency of this method is comparable to that of gas chromatography with open-tubular glass capillaries.

Peak capacity is defined as the maximum number of peaks that can be resolved within a specified range of retention time. For cases in which peaks are to be separated with 4σ resolution, where σ is the standard deviation of the peak, under a constant plate number N , peak capacity n is given according to Giddings [17] as

$$n = 1 + \frac{\sqrt{N}}{4} \ln \frac{t_{mc}}{t_0} \quad (19)$$

If we take the parameters again as $N = 250000$ and $t_0/t_{mc} = 0.22$, n becomes 190.

Capacity Factor K' and Distribution Coefficient K

The dependence of the capacity factor K' on electroosmotic velocity v_{e0} was observed to be remarkable as shown in Figure 9. The velocity v_{e0} can be related to current as shown in Figure 2 and, therefore, a similar relationship obviously applies between K' and I . The capacity factor K' linearly decreased with an increase of v_{e0} , but slopes of these plots were different from one another. The similar plots were obtained with other concentrations of SDS and with other surfac-

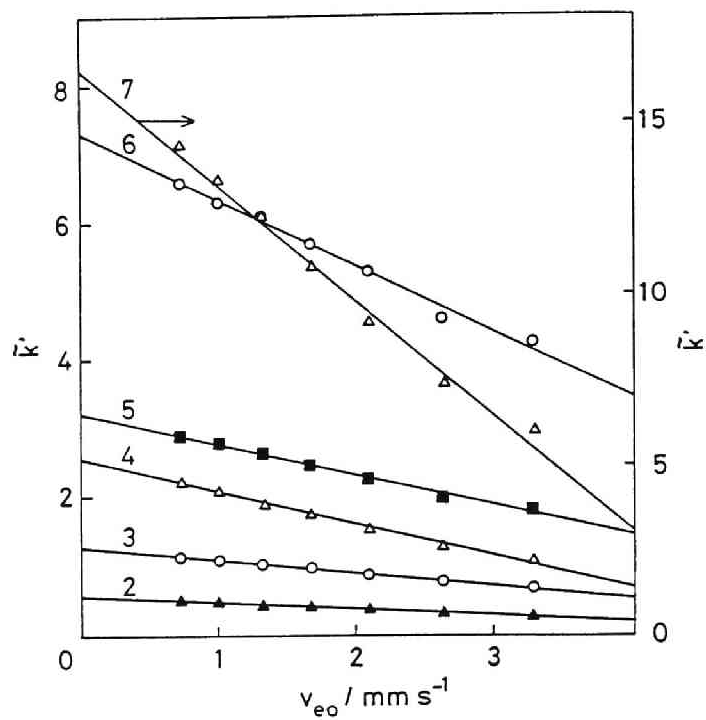


Figure 9. Dependence of capacity factor K' on velocity v_{eo} . The number on each plot corresponds to the solute shown in Figure 1; concentration of SDS was 0.10 M. Other conditions are the same as those in Figure 1 except for applied voltages.

tant solutions. In order to eliminate the effect of v_{e0} or I on K' , each line of the plot was extrapolated to the intercept at $v_{e0} = 0$, and the value of K' at $v_{e0} = 0$ was designated K'_0 . The capacity factor K'_0 may be taken as K' at the ambient temperature in the oven thermostated at a constant temperature, because the temperature of liquid in the tube should be higher than the ambient unless v_{e0} is equal to zero as described above.

The capacity factor K' can be related to the distribution coefficient K of a solute between micellar and aqueous phases through

$$K' = K \frac{V_{mc}}{V_{aq}} \quad (20)$$

where V_{mc} and V_{aq} are volumes of micellar and aqueous phases. Here, V_{mc} can be determined from a micellar concentration c_{mc} , which is equal to the concentration of a surfactant c_{sf} minus CMC, and partial specific volume $\bar{v}$ of micelle, and V_{aq} can be regarded as the difference between the total internal volume of the tube and V_{mc} . Thus, the distribution coefficient can be easily calculated from the capacity factor in contrast to conventional chromatography.

Equation 20 means that K' is proportional to the phase ratio V_{mc}/V_{aq} which is described as

$$\frac{V_{mc}}{V_{aq}} = \frac{\bar{v}(c_{sf} - \text{CMC})}{1 - \bar{v}(c_{sf} - \text{CMC})} \quad (21)$$

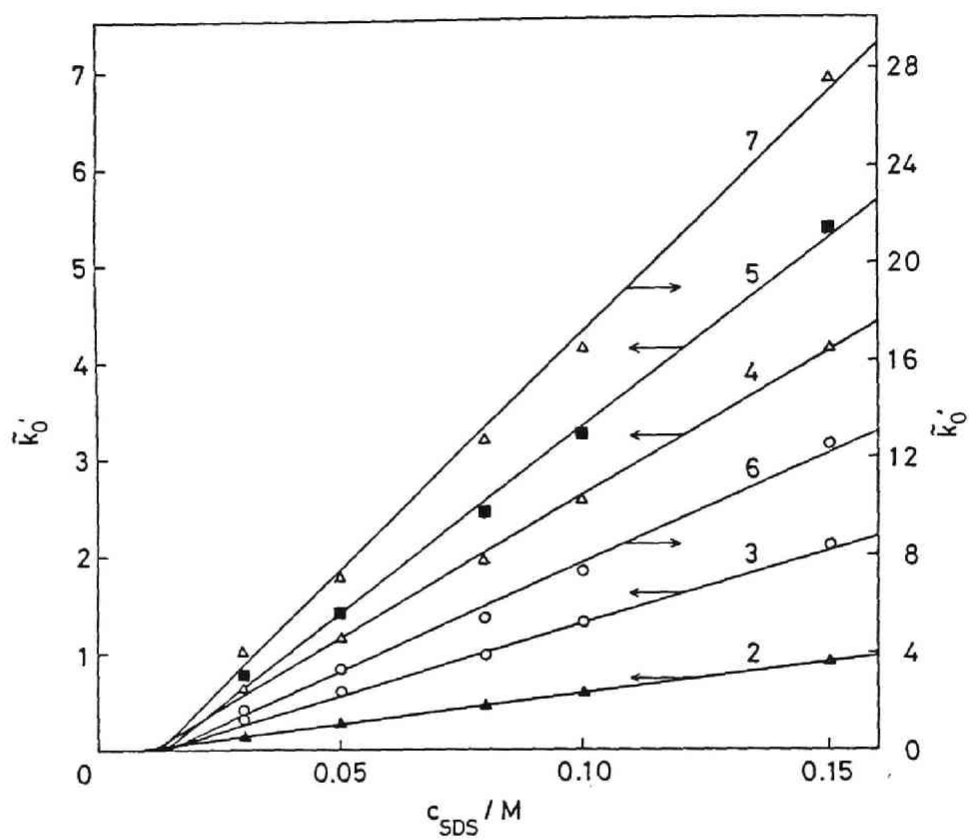


Figure 10. Dependence of capacity factor k'_0 on the concentrations of SDS. Solutes are indicated by the same numbers as those in Figure 1.

When micellar concentrations are low, the denominator at the right side of eq 21 may be approximated to be equal to unity, i.e.,

$$K' \cong K\bar{v}(C_{sf} - CMC) \quad (22)$$

The plots of K_0' vs. concentration of SDS, C_{SDS} , are given in Figure 10. The linear relationship observed in these plots implies that the distribution coefficients remain constant at least at the SDS concentrations below 0.15 M. Similar linear plots were recognized for STS and sodium dodecanesulfonate solutions.

Distribution coefficients at 35 °C calculated from slopes of these plots are listed in Table II for three surfactants. It is interesting to note that distribution coefficients are almost the same between SDS and STS for less solubilized solutes from resorcinol to nitrobenzene in Table II but significantly different for toluene and 2-naphthol. On the other hand, the situation is reversed between SDS and sodium dodecanesulfonate. These data indicate that distribution coefficients of solutes partially soluble in water are more sensitive to the change of the polar moiety of a surfactant molecule than to that of the nonpolar moiety and that distribution coefficients of solutes almost insoluble in water are sensitive to the length of the alkyl chain. These facts suggest that solutes partially miscible with water are solubilized by adsorption on the micellar surface with orienting polar groups toward the aqueous phase and nonpolar groups

Table II

Distribution Coefficients at 35 °C Calculated from Slopes of
Plots K_0' vs. c_{SDS} in Figure 10

solute	distribution coefficient ^a		
	$R_{12}SO_4Na^b$	$R_{14}SO_4Na^b$	$R_{12}SO_3Na^b$
resorcinol	21.6	20.8	27.7
phenol	52.1	52.3	56.1
<i>p</i> -nitroaniline	103	100	84.3
nitrobenzene	135	138	111
toluene	318	345	288
2-naphthol	656	789	698

^a Partial specific volumes ($\bar{v}$) at 35 °C used to calculate distribution coefficients are taken from Shinoda et al. [18]; for SDS, $\bar{v} = 0.8721 \text{ mL g}^{-1}$; for STS, $\bar{v} = 0.9019 \text{ mL g}^{-1}$; for sodium dodecanesulfonate, $\bar{v} = 0.8809 \text{ mL g}^{-1}$. ^b See Table I for the abbreviations.

inside the micelle, and that less water-soluble solutes are uptaken into the interior of the micelle in agreement with the postulated modes of incorporation [19]. The differences in capacity factors of solutes from resorcinol to nitrobenzene listed in Table I between SDS and STS are exclusively ascribed to the difference in micellar volume, that is, differences in CMC and the partial specific volume, because distribution coefficients are almost the same between the two solutions.

Intercepts of plots in Figure 10 extrapolated to $K_0' = 0$ are virtually concentrated at around 10 mM (averaged value 10.4 mM) and this concentration can be interpreted to be CMC under these conditions. This value of CMC seems too high in comparison with the reported value of 8.6 mM in water at 35 °C [20], since CMC is generally reduced with additions of electrolytes [21]. The values of CMC for STS and sodium dodecanesulfonate at 35 °C found from similar plots as in Figure 10 are 2.2 mM and 12.1 mM. Reported values for STS in water at 35 °C is 2.12 mM [20] and for sodium dodecanesulfonate in water at 25 °C 8.1 mM [22]. Thus, this method seems not to be reliable to obtain CMC.

Temperature dependence of the distribution coefficient can be described as

$$\ln K = - \frac{\Delta H^0}{RT} + \frac{\Delta S^0}{R} \quad (23)$$

where ΔH^0 is the enthalpy change associated with micellar solubilization, ΔS^0 is the corresponding entropy change, and

Table III

Enthalpy, Entropy, and Gibbs Free Energy Changes in Micellar Solubilization with SDS Solution^a

solute	$\Delta H^\circ /$ kJ mol ⁻¹	$\Delta S^\circ /$ J mol ⁻¹ K ⁻¹	corr. coeff.	$\Delta G^\circ (35^\circ \text{C}) /$ kJ mol ⁻¹
resorcinol	-17	-30	0.990	-7.8
phenol	-13	-7.8	0.991	-11
<i>p</i> -nitroaniline	-19	-22	0.982	-12
nitrobenzene	-10	8.5	0.953	-13
toluene	-17	-5.7	0.792	-15
2-naphthol	-20	-9.8	0.995	-17

^a 0.1 M, pH 7.0. Calculated from distribution coefficients measured at four different temperatures: 31, 35, 40, and 45 °C.

R is the gas constant. Logarithms of distribution coefficients determined from K_0' through eq 20 at different temperatures were plotted against the reciprocal temperature according to eq 23 to give straight lines. The values of ΔH^0 and ΔS^0 calculated from the above van't Hoff plots are listed in Table III for six solutes along with correlation coefficients of the plots and Gibbs free energies ΔG^0 for micellar solubilization at 35 °C. Since the range of the temperature change is only 14 °C, accuracies of these thermodynamic data may be low. There were no available thermodynamic data in the literature concerning micellar solubilization of these solutes with SDS. These data will be helpful in understanding this phenomenon and the present method may provide a useful technique to determine these thermodynamic quantities as well as a high resolution separation method.

Since the capacity factor should be constant irrespective of the velocity v_{e0} , we may assume that the decrease of K' with an increase of v_{e0} or current is ascribed to the rise in temperature resulting from Joule heating. On the basis of this assumption, it is possible to estimate the temperature which gives the K' value observed at any v_{e0} or current by using eqs 20 and 23 from values of ΔH^0 and ΔS^0 listed in Table III. Thus, calculated temperatures of the solution corresponding to the observed K' values at the oven temperature of 35 °C under the applied field of 308 V cm⁻¹ are listed in Table IV. Although the calculated temperatures are scattered between 59 and 77 °C, these discrepancies may be accounted for by taking into account the fact that ΔH^0 and ΔS^0 are deter-

Table IV

Estimated Temperature of the Solution from Capacity Factor
with Enthalpy and Entropy Changes Listed in Table III^a

solute	K'	$T_{\text{calcd}} / ^\circ\text{C}$
resorcinol	0.29	59
phenol	0.72	68
<i>p</i> -nitroaniline	1.18	65
nitrobenzene	1.86	77
toluene	4.40	59
2-naphthol	6.78	68

^a Solution, 0.1 M SDS, pH 7.0; electrical field strength, 308 V cm⁻¹; current, 61.4 μ A; oven temperature, 35 $^\circ$ C.

Values of CMC and partial specific volume of micelle are taken as those at 35 $^\circ$ C to estimate the phase ratio.

mined over a narrow range of temperatures. The average value of 66 °C is close to the observed surface temperature of 65 °C. The temperature gradient in the liquid and the wall of the tube is estimated to be negligibly small from coefficients of heat transfer in the solution and the wall of fused silica tubing. The agreement between the calculated and actual temperatures strongly suggests that the observed dependence of K' on the v_{eo} or current is attributable to the temperature rise by Joule heating.

In conclusion, the heat dissipation in open-tubular capillaries is not efficient and, therefore, the effect of the temperature rise of the solution by Joule heating should be taken into account in the interpretation of experimental results. Thus, since the capacity factor or distribution coefficient is dependent on both current and applied voltage, it is desirable to keep the energy dissipated per unit time constant throughout a series of runs in order to eliminate the temperature effects. The technique described here is the first example of chromatography with a homogeneous solution alone as the phases or entities between which the solute is distributed. This concept of chromatography with a homogeneous solution and electrokinetic migrations can be readily extended to the incorporation of the solute through some specific interactions, e.g., complexation or clathration, by a molecule differentially migrating from the surrounding solvent. The greatest advantage of this technique as a new separation method lies in its high efficiency because of the low axial dispersion in open-tubular capillaries with the

electroosmotic flow and the electrophoretic migration of micelles and the rapid establishment of the distribution equilibrium in micellar solubilization. Band broadening in this method will be discussed in Chapter 3.

APPENDIX

The relationship between the retention time and the capacity factor in water under the concave gradient elution in reversed-phase liquid chromatography was calculated with a personal computer as follows: The concave gradient shape can be written as

$$\phi_b = (t/t_G)^m \quad (A1)$$

where ϕ_b is the volume fraction of organic solvent B in the mobile phase entering the column at time t and t_G is the time from the beginning to the end of gradient at the inlet of column. The dependence of logarithm of the capacity factor k' on ϕ_b is assumed as [15]

$$\log k' = \log k_w' - S\phi_b \quad (A2)$$

where k_w' is the capacity factor obtained in water. The parameter S is the value of the negative slope of plots of $\log k'$ vs. ϕ_b .

In order to make numerical calculation possible, the

gradient shape was approximated by the stepwise elution with 1000 steps of equal change of ϕ , i.e., $\phi(i+1) - \phi(i) = 1/1000$ for $i = 1$ to 1000. After setting an arbitrary value for m in eq A1, time t_i when the i th solvent enters the inlet of the column is determined for $\phi(i) = i/1000$ with eq A1. The moving length of the mobile phase during the i th step, $x_m(i)$, is given as

$$x_m(i) = u(t_{i+1} - t_i) \quad (A3)$$

where u is the linear velocity of the mobile phase. The capacity factor k_i' of a given solute in the i th solvent is calculated from eq A2. Since the velocity $u_s(i)$ of the solute in the i th solvent is given by

$$u_s(i) = u/(1 + k_i') \quad (A4)$$

the differential velocity $v_m(i)$ of the mobile phase relative to $u_s(i)$ can be expressed as

$$v_m(i) = u - u_s(i) = k_i' u / (1 + k_i') \quad (A5)$$

The period $\tau_s(i)$ that the solute spends in the i th solvent is described as

$$\tau_s(i) = x_m(i) / v_m(i) \quad (A6)$$

Then, the distance $x_s(i)$ that the solute travels in the i th

solvent can be calculated from eq A6 in combination with eqs A4 and A5 as

$$x_s(i) = u_s(i) \tau_s(i) = x_m(i)/k_i' \quad (A7)$$

When the condition

$$\sum_1^i x_s(i) = 1 \quad (A8)$$

where l is the column length, is established, the solute should be eluted from the exit of the column. The retention time t_R can be written as

$$t_R = \sum_1^i \tau_s(i) \quad (A9)$$

When t in eq A1 is equal to t_G , pure solvent B will begin to enter the column, and the column will be entirely filled with solvent B after l/u from t_G . The concave gradient is considered to be completed at this time. A solute whose retention time is equal to $(t_G + l/u)$ is eluted at last while the gradient elution is running, and k_w' of the last eluting solute is regarded as the maximum value. Thus, the relationship between t_R and k_w' is calculated for k_w' from 0.1 to the maximum.

LITERATURE CITED

- [1] Nakagawa, T. *Newsl., Div. Colloid Surf. Chem., Chem. Soc. Jpn.* 1981, 6(3), 1.
- [2] Nakagawa, T.; Tori, K. *Kolloid Z. Z. Polym.* 1964, 194, 143-147.
- [3] Attwood, D.; Florence, A. T. "Surfactant Systems: Their Chemistry, Pharmacy and Biology"; Chapman and Hall: London, 1983; Chapter 3.
- [4] Armstrong, D. W.; Nome, F. *Anal. Chem.* 1981, 53, 1662-1666.
- [5] Herries, D. G.; Bishop, W.; Richards, F. M. *J. Phys. Chem.* 1964, 68, 1842-1852.
- [6] Terabe, S.; Tanaka, H.; Otsuka, K.; Ando, T. unpublished work, Kyoto University, 1984.
- [7] Stigter, D.; Mysels, K. D. *J. Phys. Chem.* 1955, 59, 45-51.
- [8] Jorgenson, J. W.; Lucaks, K. D. *Anal. Chem.* 1981, 53, 1298-1302.
- [9] Rice, C. L.; Whitehead, R. *J. Phys. Chem.* 1965, 69, 4017-4024.
- [10] Hunter, R. J. "Zeta Potential in Colloid Science"; Academic Press: London, 1981; Chapter 3.
- [11] Bockris, J. O' M.; Reddy, A. K. N. "Modern Electrochemistry", Vol. 1; Plenum: New York, 1970; Chapter 4.
- [12] Kuriyama, K.; Inoue, H.; Nakagawa, T. *Kolloid Z. Z. Polym.* 1962, 183, 68-71.

- [13] Hess, K.; Philippoff, W.; Kiessig, H. *Kolloid Z.*
1939, *88*, 40-51.
- [14] Hoyer, H. W.; Mysels, K. J.; Stigter, D. *J. Phys. Chem.*
1954, *58*, 385-388.
- [15] Karger, B. L.; Snyder, L. R.; Horvath, C.
"An Introduction to Separation Science"; Wiley: New York,
1973; Chapter 5.
- [16] Snyder, L. R.; Dolan, J. W.; Grant, J. R. *J. Chromatogr.*
1979, *165*, 3-30.
- [17] Giddings, J. C. *Anal. Chem.* 1967, *39*, 1027-1028.
- [18] Shinoda, K.; Soda, T. *J. Phys. Chem.* 1963, *67*, 2072-2074.
- [19] Shinoda, K.; Nakagawa, T.; Tamamushi, B.; Isemura, T.
"Colloidal Surfactants: Some Physicochemical Properties",
Academic Press: New York, 1963; p.140.
- [20] Flockhart, B. D. *J. Colloid Sci.* 1961, *16*, 484-492.
- [21] Corrin, M. L.; Harkins, W. D. *J. Am. Chem. Soc.*
1947, *69*, 683-688.
- [22] Williams, R. J.; Phillips, J. N.; Mysels, K. J.
Trans. Faraday Soc. 1955, *51*, 728-737.

CHAPTER 3

BAND BROADENING IN ELECTROKINETIC CHROMATOGRAPHY WITH MICELLAR SOLUTION AND OPEN-TUBULAR CAPILLARY

ABSTRACT

The minimum plate height observed was $2\ \mu\text{m}$ in electrokinetic chromatography with a $50\ \mu\text{m}$ i.d. open-tubular capillary. Instrumental conditions required not to impair the efficiency were studied based on theoretical considerations and some experimental data. Since both on-column injection and on-column detection were employed, no connecting tube was used. The injection length which was the length of the tube occupied by a sample solution and the slit length of the detector cell was desired to be less than $0.8\ \text{mm}$. The response time of the detector was required to be less than $0.16\ \text{s}$. Band broadening in column was theoretically investigated on the basis of the observed dependence of the plate height on the electroosmotic velocity. Longitudinal dispersion must have seriously contributed to band broadening for solutes having small capacity factors when the electroosmotic velocity was less than $1\ \text{mm s}^{-1}$. Mass transfer dispersion may not have been significant unless the desorption rate of the solute from the micelle is extremely slow. Although electroosmotic dispersion may be negligible, electrophoretic dispersion remains to be evaluated.

INTRODUCTION

Electrokinetic chromatography with micellar solutions is a newly developed chromatographic method which utilizes the technique of free zone electrophoresis in open-tubular capillaries [1,2]. The micelle of an ionic surfactant participates in the distribution mechanism of a solute as a moving "stationary" phase in this method and another phase is an aqueous solution. The micelle and the aqueous phase migrate with different velocities by the electrokinetic force, i.e., electrophoretic and electroosmotic effects, which permits the chromatographic elution in a time-window determined by the velocities of the aqueous phase and the micelle. That is, the two phases between which the solute is distributed consist of a homogeneous solution but the phases move differentially. Although this method obviously belongs to a chromatographic technique, it can be considered that this approach has widened the electrophoretic separation technique to neutral molecules by an indirect process.

High efficiencies attainable by free zone electrophoresis in open-tubular capillaries have been discussed by Mikkers et al. [1,3] and Jorgenson and Lukacs [2]. The migrational dispersion can be ascribed to differences in electrophoretic mobilities between carrier constituents and sample constituents [3], and it has been shown that the asymmetric concentration distribution of the band frequently observed in free zone electrophoresis can be suppressed only by the application of very small amounts of samples [1]. The

electroosmotic flow has been observed to be much stronger than electrophoretic migrations even for multiply charged solutes in free zone electrophoresis in glass open-tubular capillaries [2] and this electroosmotic flow provides advantages: Both anions and cations can be analyzed in a single run and the separation time can be reduced. Jorgenson and Lukacs [2] have discussed that as the electroosmotic flow can approximate to plug flow, longitudinal molecular diffusion should contribute to the major band broadening effect in free zone electrophoresis in open-tubular glass capillaries, while that contribution is generally negligible in conventional liquid chromatography.

In Chapter 2, it is reported that plate heights of 1.9 to 3.6 μm were obtained for alkylphenols in electrokinetic chromatography. Similar efficiencies were observed for phenylthiohydantoin-amino acids (Chapter 4) and chlorinated phenols (Chapter 5). In order to attain the maximum performance band broadening in this chromatographic method has been systematically studied. In this Chapter, instrumental considerations will be first presented, which are necessary to record the chromatogram without any loss of performance characteristic of electrokinetic chromatography. Second, extra-column effects such as injection volumes, the cell volume of the detection part of the tube, the time constant of the detector, and the response of the recorder pen will be considered. Then, band broadening in the tube while solutes are migrating will be discussed in terms of molecular diffusion, the kinetic process of the solute distribution be-

tween the micelle and the aqueous phase, and electrokinetic migrations including both electroosmotic and electrophoretic dispersion. It should be noted that the discussion below is limited to the separation of uncharged solutes by electrokinetic chromatography.

EXPERIMENTAL SECTION

Apparatus, Reagents, and Procedure

An apparatus similar to that employed previously was used. A Shimadzu Chromatopac C-R3A data processor was used to measure the retention time t_R , the peak height h , and the peak area A , and the plate number N was calculated by the following equation,

$$N = 2\pi \left(\frac{t_R h}{A} \right)^2 \quad (1)$$

The sampling frequency of the data processor was 10 Hz. Time constants of the detectors employed in this study were 0.05 s for a Jasco UVIDEC-100-V and about 0.1 s for a Jasco UVIDEC-100-II. The latter detector was slightly modified in the electronic circuit to reduce the original time constant.

The amount of the injection volume of the sample was estimated as follows: A solution of a known concentration of phenol (ca. 1 mg/mL) was filled in the whole inside of the capillary tube with a microsyringe and the absorbance of the

solution at 220 nm was measured against a blank solution in order to determine the response of the detector. Phenol solution of a known concentration was injected into the positive end of the tube by holding up the solution for a given period of time at a constant level higher than that of the sodium dodecyl sulfate (SDS) solution at the negative electrode as described previously, and a chromatogram was recorded. By assuming Gaussian peak shape, the total amount of the injected phenol was calculated. It was straightforward to determine the amount of injection volume or the corresponding length of the tube from these procedures. Other procedures and reagents are given in the preceding Chapter.

RESULTS AND DISCUSSION

Observed Plate Heights

The dependence of the plate height H on the electroosmotic velocity v_{eo} is shown in Figure 1 which was obtained with a 0.05 M SDS solution and a 0.05 mm i.d. x 500 mm fused silica tube at ambient temperature. The solutes were the same as those described in Chapter 2 and an example of the chromatogram was also shown in Figure 1 in Chapter 2. Since peaks were very narrow and the trace of the recorder pen was not always in a constant thickness, it was difficult to obtain reproducible values for plate heights if they were calculated from retention times and peak widths at half heights measured on recorded chromatograms. On the contrary, the utilization

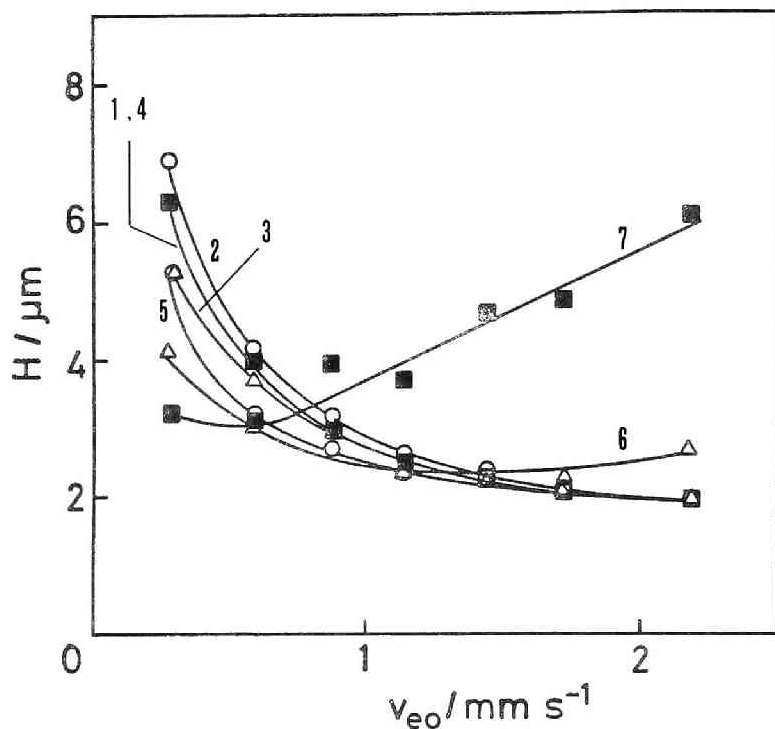


Figure 1. Dependence of plate height on electroosmotic velocity: Solute, (1)resorcinol, (2)phenol, (3)*p*-nitroaniline, (4)nitrobenzene, (5)toluene, (6)2-naphthol, (7)Sudan III; micellar solution, 0.05 M SDS in 0.1 M borate--0.05 M phosphate buffer, pH 7.0; separation tube, 0.05 mm i.d. x 650 mm; length of the tube used for separation, 500 mm; detector, Jasco UVIDEC-100-V at 210 nm; temperature, ambient (ca. 25 °C); data processor, Shimadzu C-R3A.

of a data processor gave satisfactorily reproducible values for every peak although the sampling interval (0.1 s) of the data processor was not very short compared with the peak width as it will be discussed later.

The minimum plate height observed was 2 μ m for most of the solutes, 2.5 μ m for 2-naphthol, and 3 μ m for Sudan III as shown in Figure 1. In addition, the electroosmotic velocity which gave the minimum plate height was different among three kinds of solutes mentioned above. These values of plate heights correspond to plate numbers of 250000, 200000, and 167000 for a 500 mm tube. It should be mentioned that velocity of every solute including electroosmotic and electrophoretic velocity was linearly dependent on electric current, although the electroosmotic velocity is adopted in Figure 1.

Instrumental Considerations

In free zone electrophoresis in open-tubular capillaries employed in this study, no connecting part is necessary between the injection port and the separation tube (column) and between the column and the detector cell, because a sample solution is displaced at the inlet end of the tube and the solute band is detected while the solute is migrating in the tube. That is, the on-column injection and the on-column detection are employed as described previously.

Because of the additivity of variances, the observed total peak variance σ_{ot}^2 can be expressed as

$$\sigma_{\text{tot}}^2 = \sigma_{\text{col}}^2 + \sigma_{\text{inj}}^2 + \sigma_{\text{det}}^2 \quad (2)$$

where σ_{col}^2 is the variance generated while the solute stays in the column and σ_{inj}^2 and σ_{det}^2 are extra-column variances originating in the injection system and detection system, respectively. It is generally accepted that the efficiency and resolution of the column is not seriously impaired by the 5 % increase of the band width of a peak [4]. Thus,

$$\sigma_{\text{tot}}^2 \leq (1.05 \sigma_{\text{col}})^2 \quad (3)$$

From the data shown in Figure 1, we can take the minimum value of the plate height to be 2 μm and, then, the distance variance σ_{col}^2 becomes equal to 1 mm^2 for a 500 mm column. If we assume the condition described in eq 3, the sum of extra-column variances should not exceed 10.3 % of the column variance, i.e., 0.103 mm^2 in this case. In the following discussion the author will mainly describe the variance and the standard deviation in the distance unit, because a portion of the length of an open-tubular capillary is utilized as an injection port, a column, or a detection cell in this study. The distance unit is easily converted to the volume unit by multiplying a cross section, e.g., $1.96 \times 10^{-3} \text{ mm}^2$ for 50 μm i.d. The contribution of each of extra-column variances will be first evaluated and the column variance will be discussed later.

As the sample solution is very slowly siphoned into the injection end of the tube, e.g., 0.03 mm s^{-1} , in this

technique, it is reasonable to assume that a certain length of the inside of the tube is completely displaced by a plug of sample solution. Under these circumstances, the distance variance σ_{inj}^2 is written as

$$\sigma_{inj}^2 = \frac{1}{12} \cdot l_{inj}^2 \quad (4)$$

where l_{inj} is the length of the sample solution in the tube. If it is assumed that the contribution of σ_{inj}^2 is equal to a half of the total extra-column variance, the sample length l_{inj} should be less than 0.79 mm, which corresponds to 1.55 nL in volume. Although this volume seemed too small to inject a desired amount, the procedure employed gave fairly reproducible results in injected volumes.

The dependence of the observed plate height H on the injection length l_{inj} is shown in Figure 2. Different volumes of the phenol solution of a known concentration were introduced into the tube by changing the siphoning time. It may be accepted that the total variance σ_{ot}^2 is expressed as the product of the observed plate height H and the tube length l including the injection port and the detector cell portion. The combination of eqs 2 and 4 gives

$$H = \frac{1}{12l} \cdot l_{inj}^2 + \frac{1}{l} (\sigma_{col}^2 + \sigma_{det}^2) \quad (5)$$

The second term of the right-hand side of eq 5 can be con-

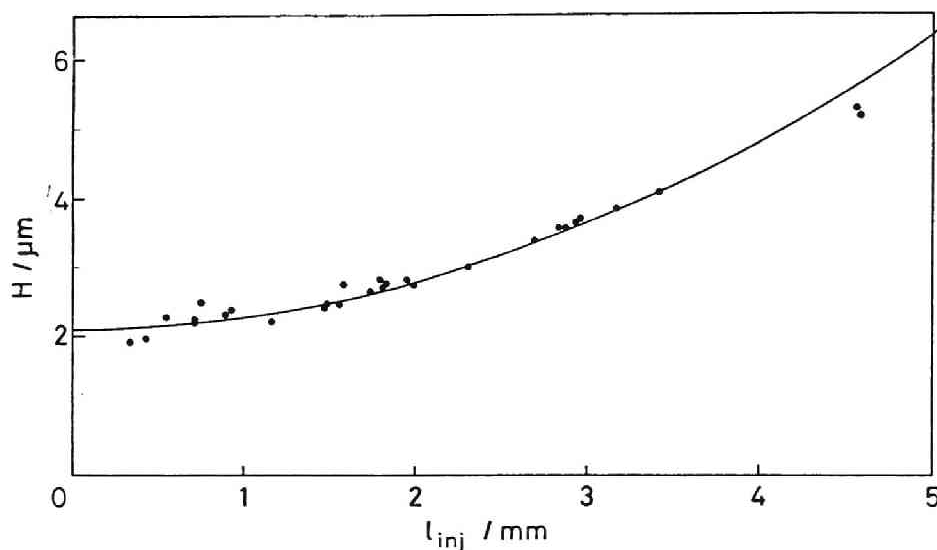


Figure 2. Dependence of plate height on injection length of solution: sample solution, 12 mM phenol in 0.1 M borate--0.05 M phosphate buffer, pH 7.0; detector, Jasco UVIDEK-100-II at 220 nm; temperature, 35 °C; applied voltage, 20 kV; current, 37 μ A. Other conditions are the same as those given in Figure 1. Solid line is calculated according to $H = 0.167 l_{inj}^2 + 2.1$.

sidered to remain constant under the conditions in Figure 2. The solid line in Figure 2 is calculated with eq 5 where l and the second term are assumed to be 500 mm and $2.1 \mu\text{m}$, respectively. The good agreement between the calculated and found plate heights supports the above view. When phenol as a sample was dissolved in pure water instead of the buffer solution, no concentrating effect [3] was recognized. This result seems reasonable by taking into account that phenol is neutral under the experimental conditions.

The above discussion on injection can be employed for the role of the detector cell in the same way, because of the additivity of the variances. Therefore, the cell length should be less than 0.79 mm and the cell volume 1.55 nL. The actual length employed was 0.75 mm. The detection limit of phenol was found to be about 50 pg in an injected amount at 220 nm.

Extra-column band broadening in the on-column detection technique has been discussed by Yang [5-8]. He has described the percentage loss of resolution $\% \Delta R$ as

$$\% \Delta R = [1 - (1 + \frac{H_{\text{det}} l_{\text{det}}}{H_{\text{col}} l_{\text{col}}})^{-1/2}] \times 100 \% \quad (6)$$

where H_{det} and H_{col} are the plate heights for the flow cell and the column, respectively, and l_{det} and l_{col} are the length of the flow cell along the tube axis and the column length, respectively. The author wishes to point out that eq 6 is not applicable to the evaluation of band broadening attributable to the detector cell. In the derivation of eq 6, the follow-

ing relationship was used:

$$\sigma_{\text{det}}^2 = H_{\text{det}} l_{\text{det}} \quad (7)$$

where σ_{det}^2 is the distance variance in the detector. The variance given in eq 7 expresses band broadening resulting from the migration of an impulse band from the entrance to the exit of the cell and it does not apply to the variance necessary to evaluate band broadening ascribed to the slit length. If H_{det} is assumed to be equal to H_{col} , which is a reasonable assumption for the open-tubular column, the erroneous results obtained from eq 6 are evident: e.g., eq 6 predicts only 29.3 % loss of resolution for equal length of the column and detector, in which case resolution will be lost almost completely.

By assuming the Gaussian profile at the entrance of the cell, the concentration profile observed through the whole cell can be approximated as (see Figure 3)

$$\frac{C(t)}{C_0} = \frac{1}{T} \cdot \frac{1}{2\pi} \int_{t - T/(2\sigma_t)}^{t + T/(2\sigma_t)} \exp\left[-\frac{1}{2} \left(\frac{\tau}{\sigma_t}\right)^2\right] d\tau \quad (8)$$

where $C(t)$ is the concentration measured at the moment t , C_0 and σ_t are the maximum concentration and the standard deviation of the original Gaussian profile, respectively, τ is a dummy time variable, and T is the residence time of the solute in the cell, i.e., l_{det} divided by linear velocity of the

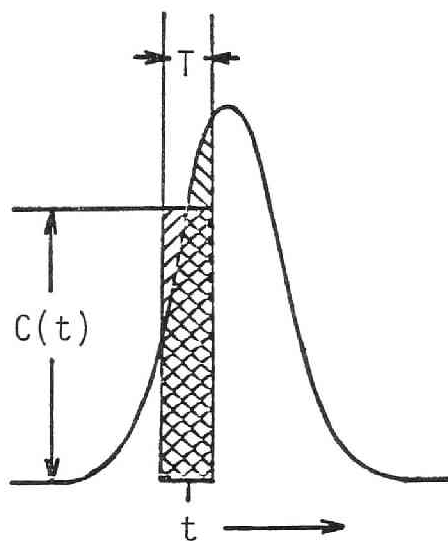


Figure 3. Illustration of relationship between the concentration observed through the slit and the original Gaussian profile in the on-column detection.

solute. Peak profiles calculated according to eq 8 are shown in Figure 4 for $T = \sigma_t$, $2\sigma_t$, and $3\sigma_t$. Peak widths at half heights are also shown in Figure 4. The peak width for $T = 0.7\sigma_t$ is 2.4 % broader than the original width, or the time variance for the calculated peak is 4.9 % larger than the original σ_t^2 . Thus, l_{det} should be less than about 0.7 mm which is obtained from the relationship that T/σ_t is equal to l_{det}/σ_{col} . This result agrees well with the value obtained from the calculation based only on the variance.

The situation noted above is equivalent to the one encountered in treating the problem of the wide initial band [9,10] when the sample solution is injected as a plug at the entrance of the column.

The time variance and the time standard deviation depend not only on the band width in the column but also on the migration velocity of the solute. In order to evaluate time dependent terms, a condition will be taken from the data shown in Figure 1 that the plate height is 2 μ m when the migration velocity of the solute is equal to 2 mm s⁻¹. Thus, the total time variance $\sigma_{t,tot}^2$ becomes 0.25 s² for the tube length of 500 mm. In this case, the peak width at the half height is 1.18 s. Since effects of the time constant of the detector amplifier and the balancing time of the recorder on band broadening have been described in detail by Scott [4], required values of these times for the measurement without any significant loss of column efficiencies are briefly discussed.

The total time variance can be expressed based on the summation of variance with similar equation to eq 2:

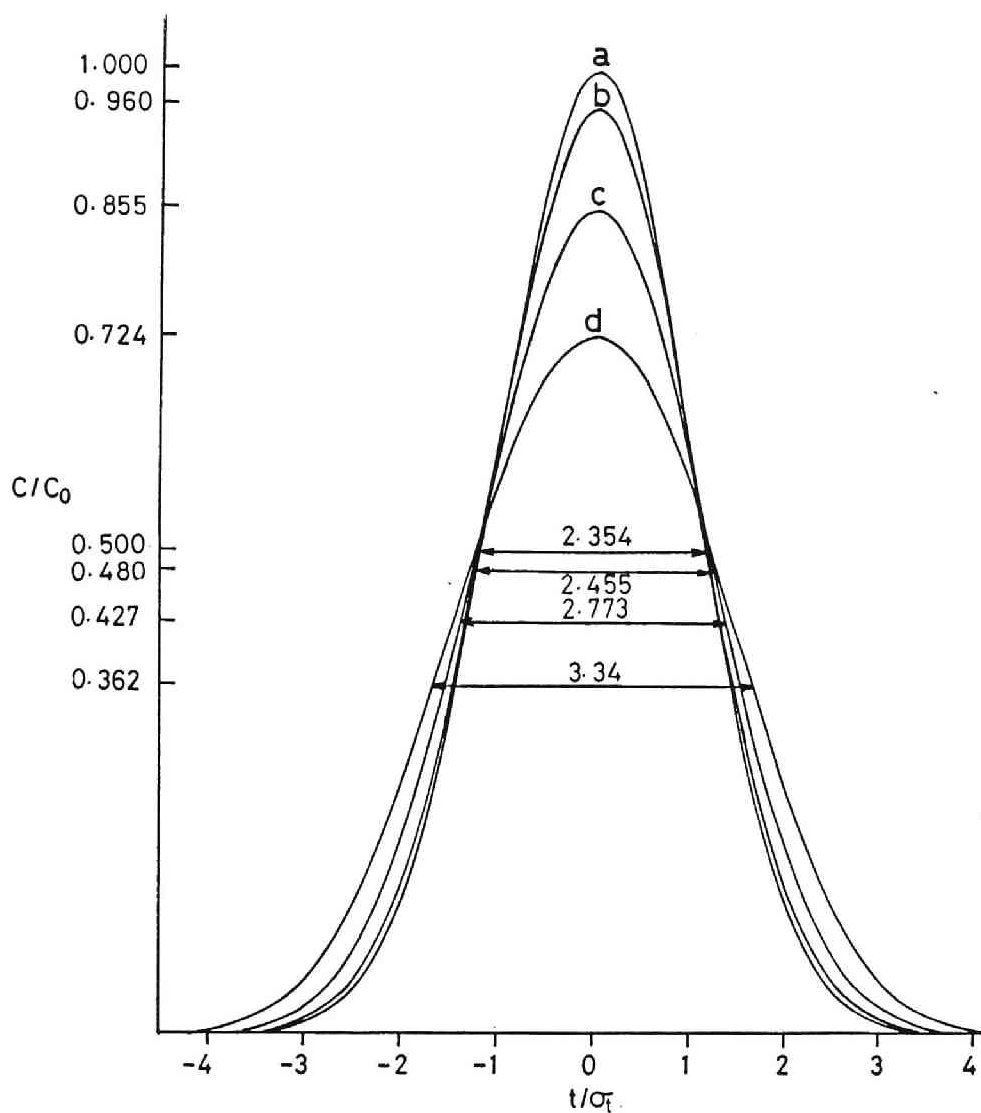


Figure 4. Peak profiles calculated according to eq 8, where t is taken as zero when the center of the band coincides with the center of the cell: The original Gaussian profile (a) and profiles when T is equal to σ_t (b), $2\sigma_t$ (c), or $3\sigma_t$ (d). Peak widths at half-heights are also shown in t/σ_t unit.

$$\sigma_{t, \text{tot}}^2 = \sigma_{t, \text{col}}^2 + \sigma_{t, \text{det}}^2 + \sigma_{t, \text{rec}}^2 \quad (9)$$

where each term is self-explanatory. Here, it should be noted that eq 2 and eq 9 are not independent of each other. Although these two equations are to be combined, the separate equations are described in different dimensions for convenience. According to the above discussion on the contribution of extra-column effects, the equivalent relationship to eq 3 is also to be applicable here. Then, the sum of time variances of the detector and the recorder must be suppressed below 10.3 % of the time variance of the column. In this case, the sum of the second and third terms should be less than 0.0258 s^2 . The time constant of the detector employed to obtain data shown in Figure 1 was 0.05 s , i.e., $\sigma_{t, \text{det}}^2 = 0.0025 \text{ s}^2$. The balancing time of the recorder employed in this study was about 0.5 s for the full scale deflection, and, therefore, the time variance of the recorder was equal to $(0.5 \text{ s})^2/18$, i.e., 0.0139 s^2 . The data processor employed in this study to measure plate numbers was not specified with the time constant or the balancing time, but the time variance of the data processor may be well below the 0.0258 s^2 , because its sampling interval was 0.1 s .

Band Broadening in Column

It is convenient to discuss band broadening in the column in terms of the plate height H_{col} , which is related to the distance variance σ_{col}^2 by

$$\sigma_{c o l}^2 = H_{c o l} I_{c o l} \quad (10)$$

Thus, eq 10 indicates that the variance and the plate height are replaceable each other if the column length remains constant. The overall plate height of the column can be described as

$$H_{c o l} = H_l + H_m + H_e \quad (11)$$

where H_l , H_m , and H_e are plate height contribution of longitudinal dispersion, mass transfer dispersion, and electrokinetic migrational dispersion, respectively.

Although longitudinal dispersion is negligibly small in conventional liquid chromatography while the solute is sorbed on the stationary phase, it should be mentioned that the solute is subject to the diffusional effect even when it is incorporated into the micelle as well as when it stays in the aqueous phase, because the micelle itself is subject to molecular diffusion. According to the Einstein equation, the distance variance by longitudinal dispersion σ_l^2 is given as

$$\sigma_l^2 = 2 \cdot D \cdot t_R \quad (12)$$

where D is the overall molecular diffusion coefficient of the solute in a surfactant solution and t_R is the retention time. The variance in eq 12 can be divided into two components:

$$\sigma_{l, aq}^2 = \frac{2 D_{aq}}{1 + K'} \cdot t_R \quad (13)$$

$$\sigma_{l, mc}^2 = \frac{2 K' D_{mc}}{1 + K'} \cdot t_R \quad (14)$$

where D_{aq} and D_{mc} are diffusion coefficient of the solute in the aqueous phase and the apparent diffusion coefficient of the solute in the micelle, respectively, K' is the capacity factor defined by the ratio of total moles of the solute in the micelle to those in the aqueous phase [1], and $\sigma_{l, aq}^2$ and $\sigma_{l, mc}^2$ are the distance variance when the solute is in the aqueous phase and in the micelle, respectively. Here, D_{mc} may be regarded to be equal to the diffusion coefficient of the micelle itself in the aqueous phase. The retention time is expressed with the capacity factor as follows (Chapter 2):

$$t_R = \frac{1 + K'}{1 + (t_0/t_{mc}) K'} t_0 \quad (15)$$

where t_0 and t_{mc} are the retention times of an insolubilized solute and the micelle. As mentioned previously, experimentally the former was measured with the methanol peak and the latter was with the tracer of the micelle such as Sudan III. The plate height H_l is described by combining eq 10 and eqs 12-15 and substituting v_{e0} in L_{col}/t_R as

$$H_l = \frac{2(D_{aq} + K' D_{mc})}{1 + (t_0/t_{mc}) K'} \cdot \frac{1}{v_{e0}} \quad (16)$$

If it is assumed that $D_{aq} = 1 \times 10^{-3} \text{ mm}^2 \text{ s}^{-1}$, $D_{mc} = 1 \times 10^{-4} \text{ mm}^2 \text{ s}^{-1}$ [11], and $t_0/t_{mc} = 0.22$ (Chapter 2), it is seen from eq 16 that the plate height by longitudinal diffusion becomes higher than $2 \mu\text{m}$ when the electroosmotic velocity is lower than 0.4 mm s^{-1} for $K' = \infty$, 0.62 mm s^{-1} for $K' = 10$, 0.83 mm s^{-1} for $K' = 2$, or 1.0 mm s^{-1} for $K' = 0$. The increase in plate heights except for that for Sudan III with decrease in the electroosmotic velocity as shown in Figure 1 can be explained in terms of longitudinal diffusion based on the above discussion. The observed minimum plate height at the low v_{eo} region for Sudan III is reasonable because Sudan III can be regarded as a tracer of the micelle whose diffusion coefficient is about one tenth [11] of those of other small solutes. The lower plate height for 2-naphthol than the other solutes except for Sudan III when v_{eo} is lower than 1 mm s^{-1} is also explainable as a result of the large capacity factor for 2-naphthol (Chapter 2). Thus, longitudinal diffusion can be a dominant factor determining the efficiency of this method.

The contribution of mass transfer dispersion to the plate height in chromatography has been discussed by Giddings [12]. According to his treatment, the plate height ascribed to the nonequilibrium process in this method is given as

$$H_m = \frac{2K'}{(1 + K')^2} \cdot \frac{(v_{eo} - v_{mc})}{k_d} \quad (17)$$

where v_{mc} is the migration velocity of the micelle and k_d is the desorption rate constant of the solute from the micelle.

Equation 17 predicts that the plate height caused by the kinetic process increases with an increase in the differential velocity $v_{e0} - v_{mc}$ and with a decrease in the rate constant k_d . The capacity factor term of eq 17, $2K'/(1+K')^2$, stays between 0.5 at $K' = 1$ and zero at $K' = 0$ or $K' = \infty$, the differential velocity $v_{e0} - v_{mc}$ being from 0.21 to 1.71 mm s⁻¹ in data shown in Figure 1.

The desorption rates of some aromatic hydrocarbons from the SDS micelle have been reported by Almgren et al. [13] and the values of rate constants have ranged from 4.1×10^2 s⁻¹ for perylene to 4.4×10^6 s⁻¹ for benzene [13]. In addition, Almgren et al. [13] have found that the exit rates approximately parallel the solubilities in water, i.e., the greater the solubility in water, the larger the exit rate from the particular micelle. This means that the solute having the greater capacity factor has the slower desorption rate. This relationship makes it difficult to evaluate the plate height H_m with eq 17, because the great capacity factor leads to a small value for the capacity factor term $2K'/(1+K')^2$ but it simultaneously results a great value for $1/k_d$.

The data shown in Figure 1 indicate that mass transfer dispersion is not significant for the solutes employed except for Sudan III and 2-naphthol, because observed plate heights did not increase with an increase in the electroosmotic velocity. It may be probable for Sudan III that the contribution of H_m is dominant to the observed plate height. If it is presumed that the capacity factor of Sudan III be 100 for convenience although it was previously regarded as infinity

(Chapter 2) and that the rate constant k_d be equal to 5 s^{-1} , eq 17 gives the H_m value of $6.7 \text{ } \mu\text{m}$ for $v_{eo} - v_{mc} = 1.71 \text{ mm s}^{-1}$. Thus, this calculation suggests the possibility of band broadening by the nonequilibrium process for Sudan III, but there is no strong reasoning for the assumed values given above. Although the dependence of the plate height for 2-naphthol on the electrokinetic velocity shown in Figure 1 also suggests the considerable contribution of H_m , the calculation with eq 17 gives a negative answer to this view: $H_m = 4.1 \times 10^{-2} \text{ } \mu\text{m}$ was obtained for reasonable values of parameters in eq 17: $K' = 10$ (Chapter 2), $k_d = 2.5 \times 10^5 \text{ s}^{-1}$ which is the value for naphthalene [13], and $v_{eo} - v_{mc} = 1.71 \text{ mm s}^{-1}$.

The velocity profile of the electroosmotic flow in open-tubular capillary has theoretically been studied by Rice and Whitehead [14] and they have described that the velocity profile is practically flat when the reciprocal of the Debye-Huckel parameter $1/\kappa$ is much smaller than the radius of a capillary. Under the experimental conditions employed in this study, the thickness of the electrical double layer should be negligible compared with the radius of the tube (Chapter 2). Therefore, the velocity profile of electroosmotic flow must be almost completely flat if the effect of the radial temperature gradient can be ignored.

As described in Chapter 2, the good agreement between the surface temperature at the outside wall of the tube measured with a thermocouple and the average liquid temperature inside the tube estimated from the thermodynamic data strongly sug-

gests that the temperature differences between the axis and the inner wall of the tube should be less than a few degrees. Another fact supporting the view that the temperature gradient effect on the plate height is not significant is found in the observed dependences of plate heights on the electroosmotic velocity or current shown in Figure 1: Since the heat generated by joule heating is greater at higher current, the plate height must have increased with an increase in the electroosmotic velocity if the velocity profile of the electroosmotic flow substantially contributed to the total plate height; moreover, the plot of H vs v_{eo} for every solute must have exhibited the similar dependence, because dispersion by the electroosmotic flow should have affected every solute to the same extent.

It should be noted that the above discussion on band broadening by the electrophoretic flow is different in situations from the recent discussions by Martin et al. [15,16] on peak broadening in open-tubular liquid chromatography with the electroosmotic flow: There is no immobilized stationary phase on the inner wall of the tube in this method and, therefore, this method corresponds to the case where the capacity factor is equal to zero in the discussions by Martin et al. [15,16].

Electrophoretic dispersion has been discussed in detail by Boyak and Giddings [17] and Wieme [18], and the concentration distribution in free zone electrophoresis has been evaluated by Mikkers et al. [3]. It should be remembered first that the micelle which can be displaced by electrophoresis is filled in the whole tube in this method, i.e., the

concentration of the micelle is always kept constant anywhere in the tube even while the voltage is applied. The solute itself is not directly subject to the electrophoretic force but it is indirectly transferred by the electrophoretic movement of the micelle. Therefore, electrokinetic dispersion of the micelle containing the solute should be taken into consideration in this method. According to the discussion of Wieme [18], the following factors are responsible for band broadening: Linear thermal diffusion, microheterogeneity, electrodiffusion, eddy diffusion, and electrosorptive spreading. The last two factors are not involved in this method, and linear thermal diffusion and electrodiffusion are already discussed above as longitudinal dispersion and mass transfer dispersion, respectively.

Microheterogeneity is related to randomness in size, shape and/or charge of the migrating species [18] and the variance σ_h^2 resulting from electroheterogeneity is given from random walk theory as [18]

$$\sigma_h^2 = (h_e E t'_{mc})^2 \quad (18)$$

where h_e is the standard deviation of the mobility distribution curve normalized to unity, E is the electrical field strength, and t'_{mc} is the time that the solute is incorporated into the micelle. Since t'_{mc} is equal to $K' t_R / (1 + K')$, the plate height contribution of microheterogeneity H_h is written by combining eq 10, eq 15 and eq 18 as

$$H_h = \left\{ \frac{K'}{1 + (t_0/t_{mc}) K'} \right\}^2 \left\{ \frac{(h_e E t_0)^2}{I_{c01}} \right\} \quad (19)$$

In the right side of eq 19, the value of the first term containing K' ranges from zero to $(t_{mc}/t_0)^2$, e.g., from zero to 21 for $t_0/t_{mc} = 0.22$, and the product of $E t_0$ is considered to be practically constant because it can be presumed that the viscosity of the solution does not vary significantly with the change of E , as discussed in Chapter 2. In other words, H_h is independent of the field strength or the electroosmotic velocity. If it is assumed that the values of parameters in eq 19 as follows: $K' = \infty$, $t_0/t_{mc} = 0.22$, $E = 31 \text{ V mm}^{-1}$, and $t_0 = 240 \text{ s}$, the value of h_e should be less than $9.3 \times 10^{-4} \text{ mm}^2 \text{ V}^{-1} \text{ s}^{-1}$ for $H_h < 0.1 H_{c01}$. Under conditions assumed above, the electroosmotic mobility is $5.2 \times 10^{-2} \text{ mm}^2 \text{ V}^{-1} \text{ s}^{-1}$. Therefore, the standard deviation of the mobility distribution must be less than 1.8 % of the average mobility of the micelle.

The polydispersity of the micelle size seems important as an origin of microheterogeneity. Although the polydispersity is a controversial problem [19], the aggregation number and the standard deviation of the distribution width for SDS micelle have been reported to be 64 and 13 at 25 °C, respectively [20]. This value of the standard deviation appears appreciable, but, unfortunately, the dependence of the electrophoretic mobility on the micelle size is not available. Thus, the problem of microheterogeneity remains to be evaluated.

The effect of concentration distribution on the peak width discussed by Mikkers et al. [3] is not considered to be significant in this method, because most peaks have practically symmetrical shapes irrespective of the injection amount of the solute. This observation for neutral solutes implies that the electrophoretic mobility of the micelle incorporating the solute is almost the same as that of the micelle free from the solute.

In conclusion, band broadening in electrokinetic chromatography with micellar solutions and open-tubular capillaries is mainly due to longitudinal dispersion when the electroosmotic velocity is less than 1 mm s^{-1} . However, the fact that the minimum plate height ($3 \mu\text{m}$) for Sudan III which was used as a tracer of the micelle was higher than those ($2 \mu\text{m}$) for the other solutes in spite of about one-order smaller diffusion coefficient of the micelle than those of the other solutes strongly suggests that another mechanism contributing to appreciable band broadening should be operating. The polydispersity of the micelle size is a probable reason for electrokinetic migrational dispersion. In electrokinetic chromatography with a cyclodextrin derivative having a ionizing group which was employed as a moving "stationary" phase instead of the micelle (Chapter 7), the minimum plate height observed ($4 \mu\text{m}$) was higher than those obtained with micellar solutions. These observations indicate that more extensive investigation on the mechanism of band broadening are required in order to attain the maximum efficiency in electrokinetic chromatography.

LITERATURE CITED

- [1] Mikkers, F. E. P.; Everaerts, F. M.; Verheggen, Th. P. E. M. *J. Chromatogr.* 1979, 169, 11-20.
- [2] Jorgenson, J. W.; Lukacs, K. D. *Anal Chem.* 1981, 53, 1298-1302.
- [3] Mikkers, F. E. P.; Everaerts, F. M.; Verheggen, Th. P. E. M. *J. Chromatogr.* 1979, 169, 1-10.
- [4] Scott, R. P. W. "Liquid Chromatography Detector"; Elsevier: Amsterdam, 1977; Chapter 3.
- [5] Yang, F. J. *HRC CC, High Resolut. Chromatogr. Chromatogr. Commun.* 1980, 3, 589-590.
- [6] Yang, F. J. *HRC CC, High Resolut. Chromatogr. Chromatogr. Commun.* 1981, 4, 83-85.
- [7] Yang, F. J. *J. Chromatogr.* 1982, 236, 265-277.
- [8] Yang, F. J. *J. Chromatogr. Sci.* 1982, 20, 241-251.
- [9] Said, A. S. *HRC CC, High Resolut. Chromatogr. Chromatogr. Commun.* 1979, 2, 45-47.
- [10] Said, A. S. *HRC CC, High Resolut. Chromatogr. Chromatogr. Commun.* 1979, 2, 93-94.
- [11] Corti, M.; Degiorgio, V. *J. Phys. Chem.* 1981, 85, 711-717.
- [12] Giddings, J. C. "Dynamics of Chromatography: Part 1"; Dekker: New York, 1965; Chapter 4.
- [13] Almgren, M.; Griesen, F.; Thomas, J. K. *J. Am. Chem. Soc.* 1979, 101, 279-291.
- [14] Rice, C. L.; Whitehead, R. *J. Phys. Chem.* 1965, 69, 4017-4024.

- [15] Martin, M.; Guiochon, G. *Anal Chem.* 1984, 56, 614-620.
- [16] Martin, M.; Guiochon, G.; Walbroehl, Y.; Jorgenson, J. W. *Anal. Chem.* 1985, 57, 559-561.
- [17] Boyak, J. R.; Giddings, J. C. *J. Biol. Chem.* 1960, 235, 1970-1972.
- [18] Wieme, R. J. In "Chromatography: A Laboratory Handbook of Chromatographic and Electrophoretic Methods", 3 rd ed., Heftmann, E., Ed.; Van Nostrand Reinhold: New York, 1975; pp 267-273.
- [19] Attwood, D.; Florence, A. T. "Surfactant Systems: Their Chemistry, Pharmacy and Biology"; Chapman and Hall: London, 1983; pp 85-87.
- [20] Aniasson, E. A. G.; Wall, S. N.; Almgren, M.; Hoffmann, H.; Kielmann, I.; Ulbricht, W.; Zana, R.; Lang, J.; Tondre, C. *J. Phys. Chem.* 1976, 80, 905-922.

CHAPTER 4

ELECTROKINETIC CHROMATOGRAPHY WITH MICELLAR SOLUTIONS

SEPARATION OF PHENYLTHIOHYDANTOIN-AMINO ACIDS

ABSTRACT

A mixture of 22 phenylthiohydantoin (PTH)-amino acids was separated by electrokinetic chromatography with a sodium dodecyl sulfate (SDS) solution and a 0.05 mm i.d. x 650 mm fused silica tube. This chromatography is based on micellar solubilization and electrokinetic migration in an open-tubular capillary. The retention times of the solutes could be controlled by changing the SDS concentration, the applied voltage or the length of the separation tube. Dodecyltrimethylammonium bromide (DTAB) solutions were also employed. The separation characteristics with SDS solutions were completely different from those with DTAB solutions. The resolutions were better with the former than with the latter solution, but for some solutes the latter gave better results.

INTRODUCTION

Micellar solubilization chromatography, or more generally electrokinetic chromatography, is a new type of chromatography based on micellar solubilization and electrokinetic migration. In this method, micellar solubilization operates as the partition mechanism, that is, a solute is distributed between the micelle and the aqueous phase, and the electroosmotic flow of a solution filled in an open-tubular capillary and the electrophoretic migration of the ionic micelle make possible differential displacement between the micelle and the aqueous phase. Although micelles are understood to be dynamic structures and are called a pseudo-phase, this technique can be regarded as belonging to a branch of liquid-liquid partition chromatography having no solid support. The fundamental characteristics of this method have been described in previous Chapters from the viewpoints of electrokinetic migration, chromatographic parameters and the micellar solubilization phenomenon.

In previous work, the author employed solutes that were electrically neutral under the experimental conditions used. If a solute can be ionized, the chromatographic behavior of the ionized solute should be different from that of the neutral solute, because the ionized solute is subject to electrophoretic attraction in addition to the electroosmotic migration of the whole solution when it exists in the aqueous phase. The electrophoretic attraction for the ionized solute should also be effective when the solute is incorporated into

the micelle as well as that for the ionic micelle. This kind of electrophoretic effect on the ionized solute should be taken into account in order to achieve a good separation of a complex mixture containing ionized species.

Phenylthiohydantoin (PTH)-amino acids, which are amino acid derivatives resulting from the Edman degradation of peptides and proteins, are important materials for determining amino acid sequences. Since Zimmerman et al. [1] first introduced high-performance liquid chromatography (HPLC) for separating PTH-amino acids, several studies have been published in which various kinds of techniques were used for HPLC. Isocratic [2-7] and binary gradient [8-13] elution were performed with acidic eluents, in some instances at high temperatures. In any event, however, some disadvantages were noted, such as long analysis times, complexity of the operation or failure of complete separation. Recently it has been shown that the use of micro HPLC under the conditions of isocratic elution and room temperature operation can shorten the analysis time [14], but the satisfactory separation was not obtained.

In this Chapter, the separation of PTH-amino acids is described to demonstrate the applicability of electrokinetic chromatography with micellar solutions. Some strategies for improving the resolution with this technique are also considered.

EXPERIMENTAL SECTION

The same apparatus as described in Chapter 2 was used. As a chromatographic column, a 0.05 mm i.d. x 650 mm or a 0.05 mm i.d. x 1150 mm fused silica tube was employed. The temperature of the thermostated oven was maintained at 35 °C.

All the reagents and samples were used as received. Sodium dodecyl sulfate (SDS) solutions were prepared by dissolving SDS in a 0.05 M sodium dihydrogen phosphate--0.0125 M sodium tetraborate buffer solution (pH 7.0). Dodecyltrimethylammonium bromide (DTAB), supplied by Tokyo Kasei Kogyo (Tokyo, Japan), was dissolved in a 0.1 M tris(hydroxymethyl)aminomethane--0.1 M hydrogen chloride buffer (Tris--HCl buffer) (pH 7.0). PTH-amino acids, obtained from Wako Pure Chemical Industries (Osaka, Japan), were dissolved in acetonitrile--water (1:1) or in acetonitrile and stored at -20 °C. Sudan III or Yellow OB (Tokyo Kasei Kogyo) was used after dissolution in surfactant solution or methanol.

The experimental procedure was the same as that described in Chapter 2.

RESULTS AND DISCUSSION

Figure 1 shows an electrokinetic chromatogram of a mixture of 22 PTH-amino acids with a 0.05 M SDS solution. In this chromatogram, the peaks of all the solutes in the mixture were separately recognized. However, four pairs of peaks were

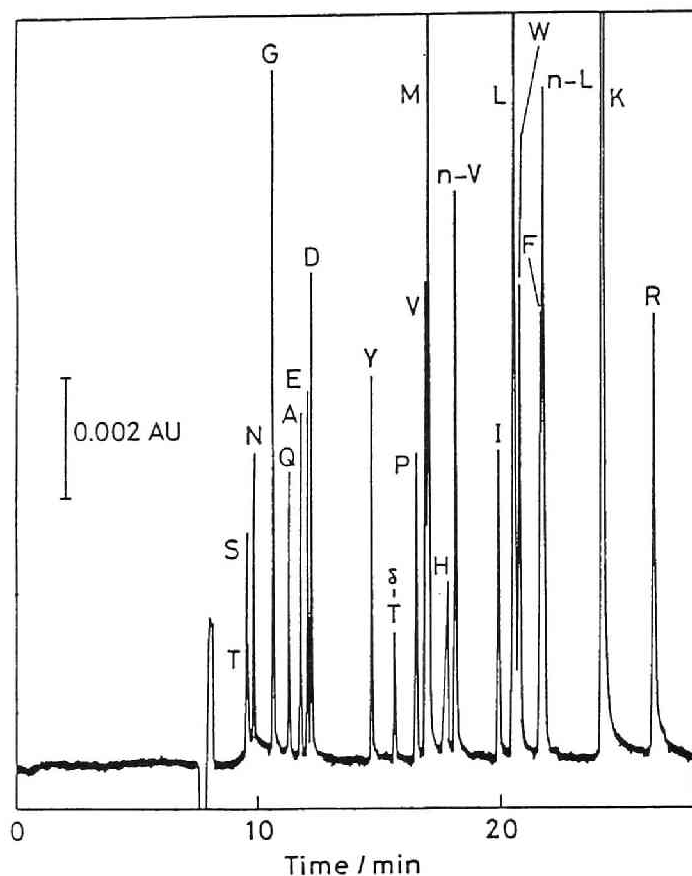


Figure 1. Electrokinetic chromatogram of a mixture of 22 PTH-amino acids. Micellar solution, 0.05 M SDS in phosphate--borate buffer, pH 7.0; separation tube, 0.05 mm i.d. x 650 mm; length of the tube used for separation, 500 mm; total applied voltage, ca. 10 kV; current, 14 μ A; detection wavelength, 260 nm; temperature of oven, 35 $^{\circ}$ C. The peaks are labelled with one-letter abbreviations for the amino acid.

poorly resolved: Thr(T)-Ser(S), Val(V)-Met(M), Leu(L)-Trp(W) and Phe(F)-Nle(n-L). Here, three- or one-letter abbreviations for the amino acid are used to indicate the corresponding PTH-amino acid. As noted in Chapter 2, the capacity factor, K' , of an electrically neutral solute in this chromatography is given by

$$K' = \frac{t_R - t_0}{t_0 (1 - t_R/t_{mc})} \quad (1)$$

where t_R , t_0 and t_{mc} are the elution times of the solute, the insolubilised solute and the micelle, respectively. In the experiments, t_0 was regarded as the retention time of methanol and t_{mc} that of Sudan III or Yellow OB (Chapter 2). The resolution, R_s , of two adjacent peaks whose capacity factors are K_1' and K_2' is expressed as follows:

$$R_s = \frac{\sqrt{N}}{4} \cdot \frac{\alpha - 1}{\alpha} \cdot \frac{K'}{1 + K'} \cdot \frac{1 - t_0/t_{mc}}{1 + (t_0/t_{mc}) K'} \quad (2)$$

where N is the plate number, α is the separation factor and K' is approximately given by $K' = K_1' = K_2'$ (Chapter 2). The term t_0/t_{mc} is a specific parameter for electrokinetic chromatography which shows the width of the total range of elution, and in Figure 1 its value was about 0.25: $t_0 = 470$ s and $t_{mc} = 1860$ s. As already mentioned, R_s should be maximal at $K' = 2.0$ for the value of $t_0/t_{mc} = 0.25$. In the chromatogram shown in Figure 1, the retention time correspond-

ing to $K' = 2.0$ is about 16 min. The value of K' of a solute changes linearly with the concentration of surfactant and with the applied voltage (Chapter 2): An increase in the concentration of surfactant or a decrease in the applied voltage causes an increase in the value of K' . Therefore, for pairs of peaks that are eluted at a time far from the optimum, e.g., pairs such as Ser-Thr, Leu-Trp or Phe-Nle, we can adjust the K' to more appropriate values by changing the SDS concentration and/or the applied voltage, and expect to obtain better resolutions than those given under the conditions used in Figure 1.

For the pair Ser-Thr, whose K' value is small (ca. 0.33) in Figure 1, an increase in the SDS concentration gave a better separation, as shown in Figure 2, where the Ser-Thr pair is completely resolved. It should be noted in Figure 2 that Glu(E) and Asp(D) were eluted faster than Gly(G) and Gln(Q), in contrast to the elution order in Figure 1. The result can be interpreted by considering two effects on the retention of PTH-amino acids in this chromatography: One is the micellar solubilization phenomenon in combination with electrophoresis of micelles and the other is electrophoresis of an ionized solute. Both PTH-Glu and -Asp have a free carboxyl group but PTH-Gly and -Gln do not. That is, PTH-Gly and -Gln are transported in the tube with a velocity $v_s(n)$, which is given by (Chapter 2)

$$v_s(n) = \frac{1 + (t_0/t_{mc})K'_n}{1 + K'_n} v_{eo} \quad (3)$$

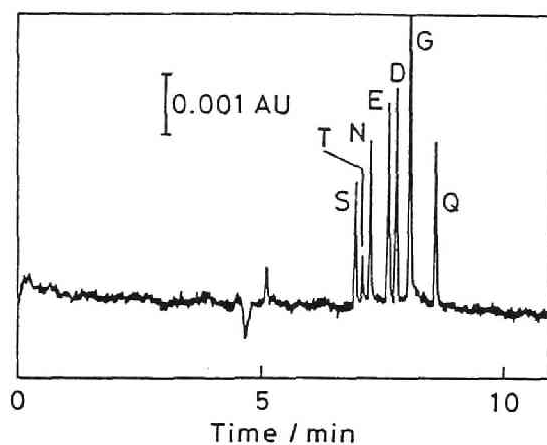


Figure 2. Electrokinetic chromatogram of a mixture of some PTH-amino acids, showing shorter retention times in Figure 1. Micellar solution, 0.10 M SDS; total applied voltage, ca. 15 kV; current, 31 μ A. Other conditions as in Figure 1.

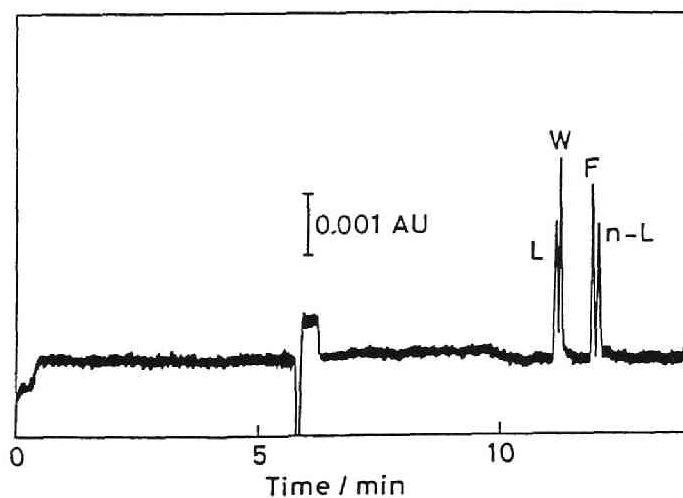


Figure 3. Electrokinetic chromatogram of a mixture of PTH-Leu, -Trp, -Phe and -Nle. Micellar solution, 0.02 M SDS; total applied voltage, ca. 12.5 kV; current, 13 μ A. Other conditions as in Figure 1.

where K_n' is the capacity factor for the electrically neutral solute and v_{e0} is the electroosmotic velocity, which is equal to the migration velocity of the unretained solute. On the other hand, PTH-Glu and -Asp can be considered to be ionized under the conditions given in Figure 2, so that these solutes are subject to the electrophoretic attraction to the positive electrode in addition to the electrokinetic chromatographic migration expressed by eq 3. By assuming that the electrophoretic velocity of the micelle, $v_{ep}(mc)$, remains constant, even when the micelle solubilizes the ionized solute, the migration velocity of the ionized solute, $v_s(i)$, is represented by

$$v_s(i) = v_{e0} + \frac{1}{1 + K_i'} v_{ep}(i) + \frac{K_i'}{1 + K_i'} v_{ep}(mc) \quad (4)$$

where K_i' is the capacity factor for the ionized solute and $v_{ep}(i)$ is the electrophoretic velocity of the ionized solute itself when it exists in the aqueous phase. Here, it should be noted that the sign of the migrating direction is taken into account. As the migration velocity of the micelle, v_{mc} , is given by (Chapter 2)

$$v_{mc} = v_{e0} + v_{ep}(mc) \quad (5)$$

eq 4 can easily be rewritten as

$$v_s(i) = \frac{1 + (t_0/t_{mc})K_i'}{1 + K_i'} \cdot v_{eo} + \frac{1}{1 + K_i'} \cdot v_{ep}(i) \quad (6)$$

where the direction of $v_{ep}(i)$ is the reverse of that of v_{eo} or $v_s(i)$. Comparison of eq 3 with eq 6 reveals that an increase in capacity factor, which is caused by an increase in the SDS concentration, should decrease the velocity of the non-ionized solute, $v_s(n)$, to a greater extent than that of the ionized solute, $v_s(i)$, because the second term in eq 6 becomes smaller in absolute value with an increase in K_i' . As had been expected, the retention times of PTH-Gly and -Gln could be made longer than those of PTH-Glu and -Asp by increasing the SDS concentration, as shown in Figures 1 and 2.

For other poorly resolved pairs in Figure 1, i.e., Leu-Trp and Phe-Nle, which were eluted with larger K' (4.9 and 5.8) than the optimum values, a decrease in SDS concentration was required in order to attain better separations. The chromatogram in Figure 3 shows that a decrease in SDS concentration from 0.05 to 0.02 M brought about better resolutions of both pairs than those observed in Figure 1.

The pair Val-Met was not completely separated in Figure 1, although it was eluted with the nearly optimum value of $K' = 2.6$. The author tried to improve the resolution of this pair by doubling the length of the initially used column to 100 cm. Under these conditions, the number of theoretical plates should be twice and the resolution about 1.4 times as large as those in Figure 1. The two peaks were successfully separated, as shown in Figure 4.

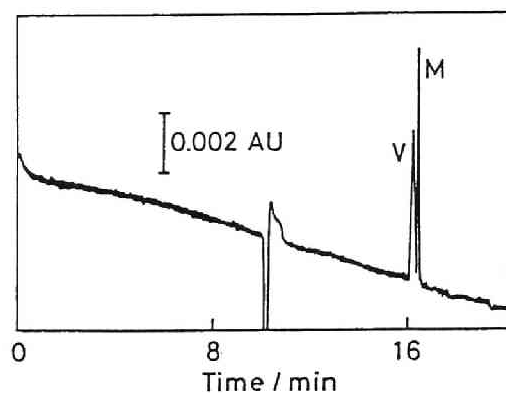


Figure 4. Electrokinetic chromatogram of a mixture of PTH-Val and -Met. Separation tube, 0.05 mm i.d. x 1150 mm; length of the tube used for separation, 1000 mm; total applied voltage, ca. 25 kV; current, 17 μ A. Other conditions as in Figure 3.

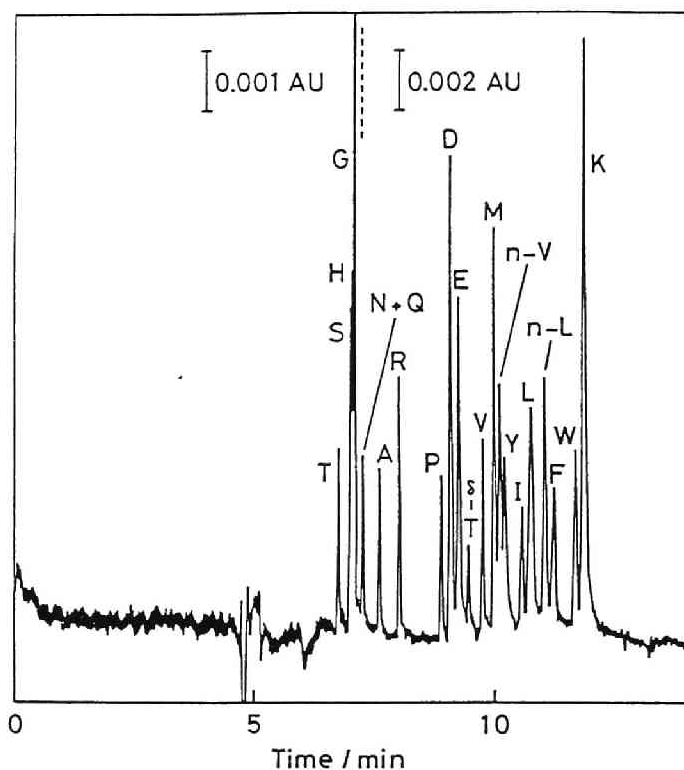


Figure 5. Electrokinetic chromatogram of a mixture of 22 PTH-amino acids. Micellar solution, 0.05 M DTAB in Tris--HCl buffer, pH 7.0; total applied voltage, ca. 15 kV; current, 37 μ A. Other conditions as in Figure 1.

Dodecyltrimethylammonium bromide (DTAB), which is a cationic surfactant, was also used to examine the effect of surfactant molecules on the separation of PTH-amino acids. The directions of both electroosmotic flow and electrophoretic migration of the DTAB micelle were the opposite of those in SDS solutions: The direction of the electroosmotic flow was from the negative to the positive electrode and that of the electrophoretic migration of the DTAB micelle was from positive to negative. These observations can be explained in terms of the reversal of the signs of the zeta potentials at the fused silica wall and the micelle when DTAB is substituted for SDS. In fact, the zeta potential of the quartz/DTAB solution has been reported [15] to be positive if the concentration of DTAB is higher than 10^{-4} M, and the micelle of DTAB apparently has positive charges. The chromatogram shown in Figure 5 is an example of the separation of PTH-amino acids with a 0.05 M DTAB solution. It is worth noting that any pair of solutes that were eluted as partially overlapping peaks under the conditions employed in Figure 1 was completely resolved in the chromatogram shown in Figure 5, although some other solutes were not separated in Figure 5 even with concentrations of DTAB different from 0.05 M.

In Table I, some migration variables in electrokinetic chromatography for the SDS and DTAB solutions are presented. The current in each surfactant solution increased with increase in the concentration of the surfactant (Chapter 2). A higher current is observed in the DTAB solution than in the SDS solution at the same concentration of the surfactants. The

Table I

Comparison of Variables^a of Electrokinetic Migration between SDS and DTAB Solutions^b

Surfactant	<i>c</i> /M	<i>I</i> /μ A mm s ⁻¹	<i>v</i> _{eo} / mm s ⁻¹	<i>v</i> _{mc} / mm s ⁻¹	<i>t</i> _o / <i>t</i> _{mc}
SDS	0.03	19.4	1.76	0.522	0.298
	0.05	22.1	1.71	0.494	0.288
	0.10	30.4	1.68	0.363	0.217
DTAB	0.03	32.2	1.73	0.733	0.425
	0.05	37.4	1.70	0.698	0.412
	0.10	45.5	1.71	0.651	0.380

^a *c*, concentration of the surfactant; *I*, current; *v*_{eo}, electroosmotic velocity; *v*_{mc}, migration velocity of the micelle. ^b Separation tube, 0.05 mm i.d. x 650 mm; total applied voltage, ca. 15 kV; temperature of oven, 35 °C.

differences in these values seem to be due to the differences in the buffer solutions used, borate--phosphate and Tris--HCl, rather than to the difference in the surfactants themselves. The electroosmotic velocities, v_{eo} , were almost constant, regardless of the surfactant concentration, but the migration velocities of micelles, v_{mc} , decreased with increase in the micellar concentration in both SDS and DTAB solutions. These facts can be explained in terms of the change in the viscosity of the solutions and the temperature rise due to Joule heat (Chapter 2). As a specific parameter in electrokinetic chromatography, we obtained smaller values for t_0/t_{mc} in the SDS solutions than in DTAB solutions at any concentration. From the viewpoint of the maximum resolution, a smaller value of t_0/t_{mc} is advantageous, and this is probably the main reason why the SDS solution gave a better resolution as a whole than the DTAB solution for the 22 PTH-amino acids.

In conclusion, a satisfactory separation of 22 PTH-amino acids was achieved by electrokinetic chromatography with a micellar solution and an open-tubular capillary, and this indicates the high resolving power of this chromatographic technique.

LITERATURE CITED

- [1] Zimmerman, C. L.; Appella, E.; Pisano, J. J.
Anal. Biochem. 1977, 75, 569-573.
- [2] Abrahamsson, M.; Groningsson, K.; Castensson, S.
J. Chromatogr. 1978, 154, 313-317.
- [3] Rose, S. M.; Schwartz, B. D. *Anal. Biochem.*
1980, 107, 206-213.
- [4] Tarr, G. E. *Anal. Biochem.* 1981, 111, 27-32.
- [5] Kim, S. M. *J. Chromatogr.* 1982, 247, 103-110.
- [6] Pucci, P.; Sannia, G.; Marino, G. *J. Chromatogr.*
1983, 270, 371-377.
- [7] Silver, M. R.; Trosper, T. D.; Gould, M.R.;
Dickson, J. E.; Desotelle, G. A. *J. Liq. Chromatogr.*
1984, 7, 559-568.
- [8] Bhowan, A. S.; Mole, J. E.; Weissinger, A.; Bennett, J. C.
J. Chromatogr. 1978, 148, 532-535.
- [9] Moser, P. W.; Rickli, E. E. *J. Chromatogr.*
1979, 176, 451-455.
- [10] Johnson, N. D.; Hunkapiller, M. W.; Hood, L. E.
Anal. Biochem. 1979, 100, 335-338.
- [11] Henderson, L. E.; Copeland, T. D.; Oroszlan, S.
Anal. Biochem. 1980, 102, 1-7.
- [12] Somack, R. *Anal. Biochem.* 1980, 104, 464-468.
- [13] Hawke, D.; Yuan, P.-M.; Shively, J. E. *Anal. Biochem.*
1982, 120, 302-311.

- [14] Takeuchi, T.; Yamazaki, M.; Ishii, D.
HRC CC, J. High Resolut. Chromatogr. Chromatogr. Commun.
1984, 7, 101-102.
- [15] Somasundaran, P.; Healy, T. W.; Fuerstenau, D. W.
J. Phys. Chem. 1964, 68, 3562-3566.

CHAPTER 5

ELECTROKINETIC CHROMATOGRAPHY WITH MICELLAR SOLUTIONS

RETENTION BEHAVIOR AND SEPARATION OF CHLORINATED PHENOLS

ABSTRACT

The retention behavior of chlorinated phenols in electrokinetic chromatography with micellar solutions and open-tubular capillary was investigated. The capacity factor for each compound of a test mixture was calculated by considering both the effects of micellar solubilization and electrophoresis of the ionized solute, even when a solute was ionized. Under conditions where a solute, a chlorinated phenol, was estimated to be partially ionized, the capacity factor of the solute decreased with an increase of pH, whereas that of an electrically neutral solute was almost constant regardless of the change in pH. All the isomers of chlorinated phenols were completely separated within 18 min by using a 0.05 mm i.d. x 650 mm fused silica tube with a 0.07 M sodium dodecyl sulfate solution, pH 7.0.

INTRODUCTION

The fundamental characteristics of electrokinetic chromatography with micellar solution have been described in previous Chapters, and it has been mentioned that electrokinetic chromatography has great ability to give high resolution within a shorter time than conventional high-performance liquid chromatography (HPLC). As an example that demonstrates the high resolving power of this chromatography, the separation of a mixture of 22 phenylthiohydantoin-amino acids has been described in Chapter 4. In Chapter 4, the retention characteristics of an ionized solute were briefly discussed, by considering the electrophoretic effect on the solute in addition to the phenomenon of micellar solubilization.

Separations of phenolic compounds have been widely studied, and some reports of the HPLC separation of those materials have been published [1-4] recently. Among such compounds, chlorinated phenols are one of the most popular and important compounds to be analyzed because they are serious environmental pollutants and toxic or hazardous materials, and were formerly widely used for various purposes, such as pesticides and sterilants. A number of studies to separate chlorinated phenols with HPLC have also been reported [5-9]. One of them [6] succeeded in separating a mixture of phenol and all the isomers of chlorinated phenols except 2,3,5-trichlorophenol.

In this Chapter, some retention characteristics of chlorinated phenols in electrokinetic chromatography with

micellar solutions will be given for both an electrically neutral and an ionized solute, in comparison with free zone electrophoresis in an open-tubular capillary [10,11], and then separations of all the isomers of chlorinated phenols, including phenol will be shown.

EXPERIMENTAL SECTION

Apparatus and Procedure

A 0.05 mm i.d. x 650 mm fused silica tube was employed as a separation capillary. At 150 mm from the negative end of the tube, the on-column measurement of UV absorption was carried out with a Jasco UVIDEC-100-II spectrophotometric detector (Tokyo, Japan). For data processing a Shimadzu Chromatopac C-R3A (Kyoto, Japan) was used. All experiments were performed in a thermostated oven at 35 °C. Other apparatus and experimental procedures were the same as those described previously.

Reagents

Chlorinated phenols were purchased from Wako Pure Chemical Industries (Tokyo, Japan), Nakarai Chemicals (Kyoto, Japan), Tokyo Kasei Kogyo (Tokyo, Japan) or Aldrich Chemical Company (Milwaukee, U.S.A.), and Yellow OB from Tokyo Kasei Kogyo. All the reagents were used as they were received and dissolved in methanol or a methanol-water (1:1) mixture. Sodium dodecyl sulfate (SDS) was dissolved in a 0.05 M sodium

Table I

List of Compounds Included in a Test Mixture^a

No.	solute	p <i>K</i> _a ^b
1	phenol	9.92
2	2-chlorophenol	8.52
7	2,5-dichlorophenol	7.51
14	2,4,5-trichlorophenol	6.72
17	2,3,4,5-tetrachlorophenol	5.64
20	pentachlorophenol	4.74
21	Yellow OB	--

^a The mixture was dissolved in methanol.

^b Logarithm of the reciprocal of the acid dissociation constant taken from the literature [6].

dihydrogen phosphate--0.025 M sodium tetraborate buffer solution, which was adjusted to an appropriate pH.

RESULTS AND DISCUSSION

Retention Characteristics of Chlorinated Phenols

A test mixture whose components are listed in Table I was used to investigate the retention behavior of chlorinated phenols in electrokinetic chromatography with SDS solutions. Yellow OB was added to the mixture as a tracer of the micelle, and the retention time of Yellow OB was regarded as that of the micelle, t_{mc} (Chapter 2). Methanol can be considered to be insolubilized by the micelle as described in Chapter 2, so the retention time of methanol corresponds to that of the unretained solute, t_0 . Five SDS solutions, 0.10 M SDS dissolved in borate-phosphate buffers whose pH values were 5.0, 6.0, 7.0, 8.0 and 9.0, were employed. A sample electrokinetic chromatogram of the test mixture, which was obtained with a 0.10 M SDS solution of pH 7.0, is shown in Figure 1. Here, a small peak followed by pentachlorophenol (peak No. 20) seems to be due to an impurity contained in the sample. With the solution of pH 5.0 no successful separation was obtained because of low solubilities of the samples in such an acidic solution, so in the following experiments the solution of pH 5.0 was omitted.

In electrokinetic chromatography, the capacity factor k' of an electrically neutral solute is given by (Chapter 2)

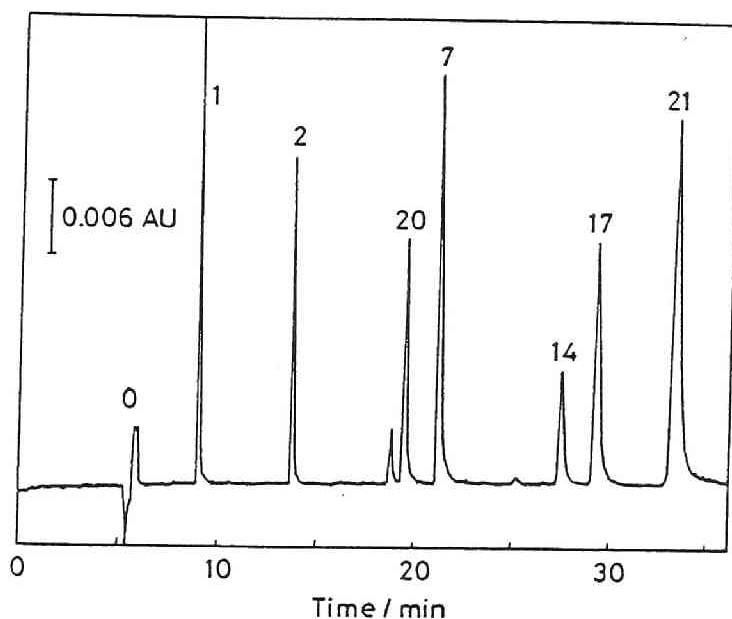


Figure 1. Electrokinetic chromatogram of a test mixture of chlorinated phenols: (0)methanol, for other solutes, see Table I; micellar solution, 0.10 M SDS, pH 7.0; separation tube, 0.05 mm i.d. x 650 mm; length of the tube used for separation, 500 mm; total applied voltage, ca. 15 kV; current, 33 μ A; detection wavelength, 220 nm; temperature, 35 $^{\circ}$ C.

$$K' = \frac{t_R - t_0}{t_0 (1 - t_R / t_{mc})} \quad (1)$$

where t_R is the retention time of the solute. Although some solutes in the test mixture might be ionized under the conditions used, the apparent capacity factor K_{app}' for each component was calculated by eq 1. The dependence of K_{app}' on the pH of the SDS solution is shown in Figure 2 for each compound. It can be seen from Figure 2 that the retention times of 2,5-dichlorophenol (peak No. 7), 2,4,5-trichlorophenol (No. 14), 2,3,4,5-tetrachlorophenol (No. 17) and pentachlorophenol tended to decrease with an increase of pH, whereas those of phenol (No. 1) and 2-chlorophenol (No. 2) remained almost constant regardless of the change in pH. The former fact, however, does not always mean that the ratio of solubilized to insolubilized solutes decreased with an increase of pH, because the negatively charged solute could be subject to an electrophoretic effect and electrostatic repulsion toward the negatively charged SDS micelle in solubilization. That is, the retention time of the ionizable phenol increased with an increase of the degree of ionization or the pH value of the solution, since the direction of electrophoretic migration of an anion is the reverse of that of electroosmotic flow as described in Chapter 2. On the other hand, the electrostatic repulsion between the ionized solute and the SDS micelle will suppress micellar solubilization.

To investigate the effect of the change in pH on micellar solubilization, the electrophoretic velocity of the negatively

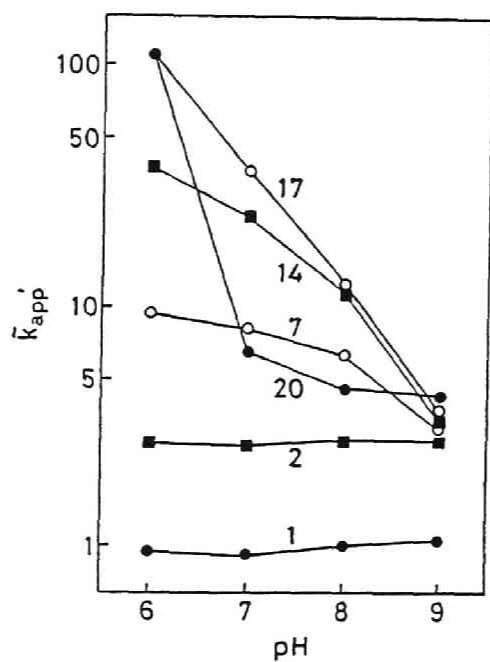


Figure 2. Dependence of apparent capacity factor K'_{app} on pH. For solute numbers see Table I. Conditions as in Figure 1, except for the value of the pH.

charged solute must be evaluated. Thus, free zone capillary electrophoresis was carried out with borate--phosphate buffer solutions of the same pH as mentioned above, either without SDS or with 5 mM SDS. The SDS micelle could not be formed under the latter conditions. A separation of the mixture by high-voltage capillary electrophoresis with the buffer solution of pH 7.0 is shown in Figure 3. Then, the electrophoretic velocity of a solute in the buffer solution, $v_{ep}(s)$, was calculated by (Chapter 2)

$$v_{ep}(s) = v_{eo} - v_b(s) \quad (2)$$

where $v_b(s)$ is the observed migration velocity of the solute and can be calculated from the column length l and the retention time t_R , i.e., $v_b(s) = l/t_R$. As described in Chapter 2, the electroosmotic velocity is defined as

$$v_{eo} = -(\epsilon \xi / \eta) \cdot E \quad (3)$$

where ϵ , ξ and η are the permittivity, the zeta potential and the viscosity of the solution, respectively. Among these three solutions, 0 mM, 5 mM and 0.10 M SDS, the values of v_{eo} could be regarded as constant at each pH (Table II). Therefore, the term $\epsilon \xi / \eta$ should be held constant regardless of the SDS concentration. Although it is difficult to measure the electrophoretic velocity of the ionized solute in the micellar solution, it is reasonable to assume that the velocity is the same as that in the buffer solution containing

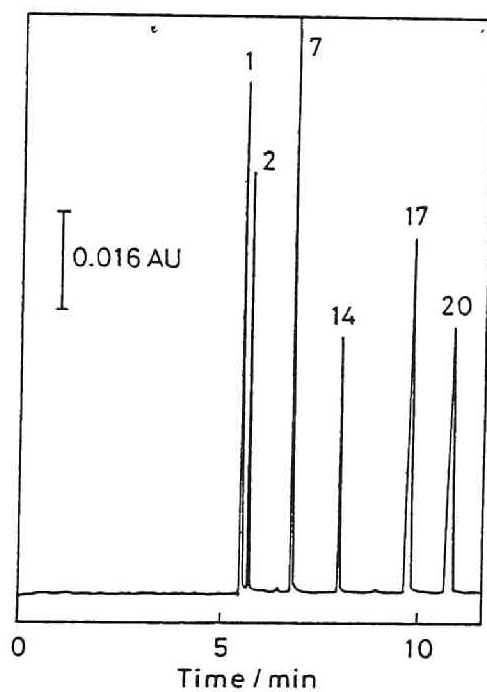


Figure 3. Separation of the test mixture by high-voltage capillary electrophoresis: Solution for separation, phosphate--borate buffer, pH 7.0; current, 17 μ A. For solute numbers see Table I. Other conditions as in Figure 1.

Table II

Electroosmotic Velocity, v_{eo} , in Three Solutions
of Different SDS concentrations at Four Different
pH Values ^a

pH	v_{eo} (mm s ⁻¹)		
	0 ^b	5 ^b	100 ^b
6.0	1.57	1.45	1.62
7.0	1.57	1.57	1.52
8.0	1.60	1.54	1.60
9.0	1.59	1.50	1.50

^a Separation tube, 0.05 mm i.d. x 650 mm; total applied voltage, ca. 15 kV; temperature of oven, 35 °C. ^b Concentration of SDS (mM).

5 mM SDS. On the other hand, the difference between the electroosmotic velocity v_{eo} and the migration velocity of the ionized solute in the micellar solution, $v_m(s)$, can be considered as the apparent electrophoretic velocity of the solute in the micellar solution, $v_{ep}^*(s)$:

$$v_{ep}^*(s) = v_{eo} - v_m(s) \quad (4)$$

Assuming that the electrophoretic velocity of the micelle, $v_{ep}(mc)$, is constant even when the micelle incorporates solutes (Chapter 4), we can write

$$v_{ep}^*(s) = \frac{n_{aq}}{n_{mc} + n_{aq}} \cdot v_{ep}(s) + \frac{n_{mc}}{n_{mc} + n_{aq}} \cdot v_{ep}(mc) \quad (5)$$

where n_{mc} and n_{aq} are the numbers of the solutes in micellar and aqueous phases. As the capacity factor K' is defined by (Chapter 2)

$$K' = n_{mc} / n_{aq} \quad (6)$$

by substituting eq 6 into eq 5, K' can be expressed as

$$K' = \frac{v_{ep}^*(s) - v_{ep}(s)}{v_{ep}(mc) - v_{ep}^*(s)} \quad (7)$$

Now we can calculate K' even for the ionized solute by eq 7. The dependence of K' on pH for each compound of the

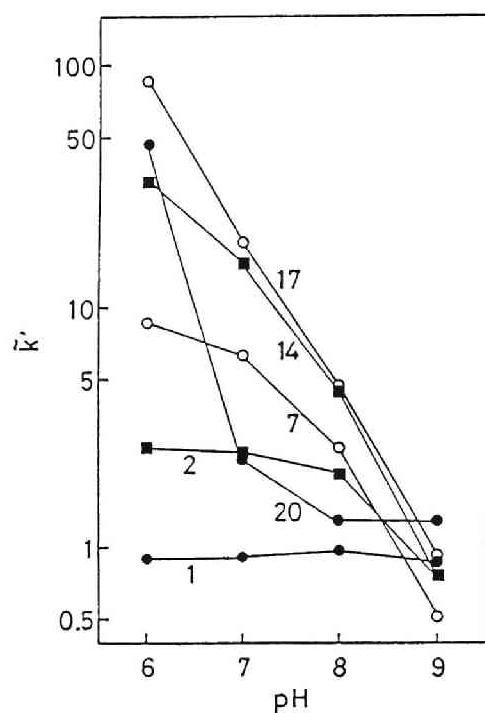


Figure 4. Dependence of capacity factor K' on pH. The number on each plot corresponds to the solute shown in Table I. Conditions as in Figure 1, except for the value of the pH.

test mixture is shown in Figure 4. The plots are similar to those shown in Figure 2. But the value of k' was smaller than k_{app}' at a pH where the solute was estimated to be ionized, and that was especially obvious for the case of 2-chlorophenol. In the pH range 6.0-9.0, k_{app}' was almost constant whereas k' began to decrease at pH 8.0 and became much smaller at pH 9.0. This fact means that the degree of micellar solubilization of the phenol by the SDS micelle decreases with an increase of the ionization of the solute.

On the other hand, the fact also suggests that even the negatively charged solute can be slightly solubilized by the SDS micelle which has a negative charge, because the value of k' for the solute was not zero.

Separation of Chlorinated Phenols

Separations of a mixture of all the isomers of chlorinated phenols, including phenol, were investigated under various conditions of SDS concentration and pH. Complete separation of all the solutes was accomplished with a 0.07 M SDS solution of pH 7.0, and the electrokinetic chromatograms shown in Figures 5 and 6 were obtained. The applied voltages were ca. 15 kV and 10 kV in Figures 5 and 6, respectively. In Figure 5, it can be seen that the peak of 2,6-dichlorophenol (peak No. 8) is seriously broadened. Although the reason has not been clarified, this may be due to an impurity in the sample since the same broadened peak was also observed even when only 2,6-dichlorophenol solution in methanol was chromatographed. On the other hand, the broadening was not

observed in Figure 6, probably owing to the lower resolution than in Figure 5. As mentioned in Chapter 2, the resolution R_s between two adjacent peaks whose capacity factors are k_1' and k_2' in electrokinetic chromatography is given by

$$R_s = \frac{\sqrt{N}}{4} \cdot \frac{\alpha - 1}{\alpha} \cdot \frac{K'}{1 + K'} \cdot \frac{1 - t_0/t_{mc}}{1 + (t_0/t_{mc})K'} \quad (8)$$

where N is the number of theoretical plates, α is the separation factor, and K' is given approximately by $K' = k_1' = k_2'$. Since the value of the parameter t_0/t_{mc} is ca. 0.25 in both Figures 5 and 6, the maximum resolution should be obtained under a constant N value when the value of K' is ca. 2 (Chapter 2). A method for adjusting K' to the optimum value by changing the concentration of a surfactant has been discussed in Chapter 4. But in Figures 5 and 6, the values of N for either chromatogram seemed to be different owing to an effect of molecular diffusion in the capillary tube. The plate height responsible for the molecular diffusion generally increases as the retention time increases. Thus, N in Figure 5 was larger than that in Figure 6, so a higher resolution was obtained in the former than in the latter.

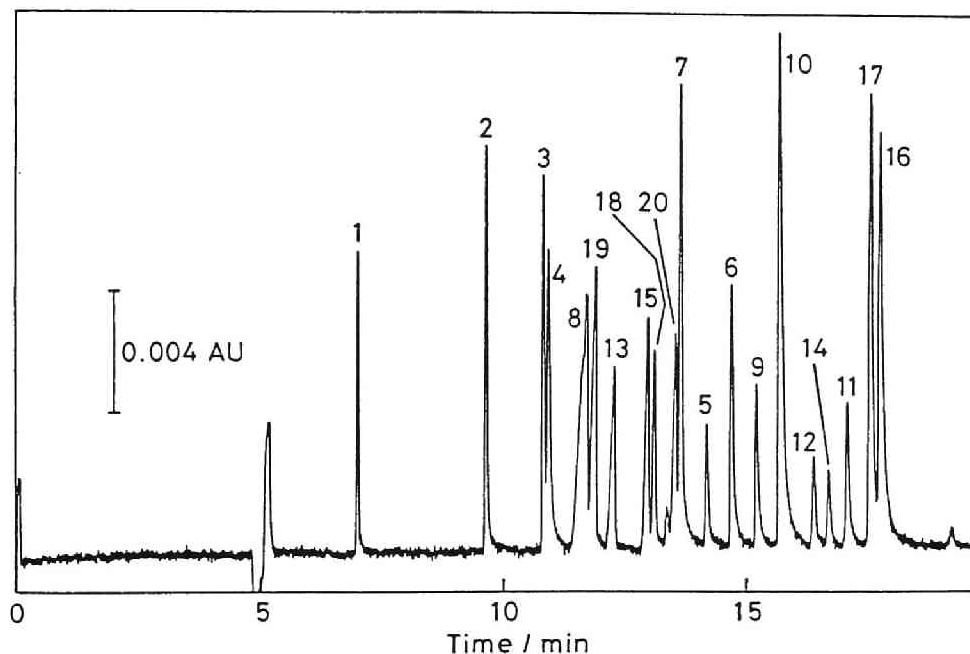


Figure 5. Electrokinetic chromatogram of a mixture of all the isomers of chlorinated phenols, including phenol: (1)phenol, (2)2-chloro, (3)3-chloro, (4)4-chloro, (5)2,3-dichloro, (6)2,4-dichloro, (7)2,5-dichloro, (8)2,6-dichloro, (9)3,4-dichloro, (10)3,5-dichloro, (11)2,3,4-trichloro, (12)2,3,5-trichloro, (13)2,3,6-trichloro, (14)2,4,5-trichloro, (15)2,4,6-trichloro, (16)3,4,5-trichloro, (17)2,3,4,5-tetrachloro, (18)2,3,4,6-tetrachloro, (19)2,3,5,6-tetrachloro, (20)pentachloro; micellar solution, 0.07 M SDS, pH 7.0; total applied voltage, 15 kV; current, 28 μ A. Other conditions as in Figure 1.

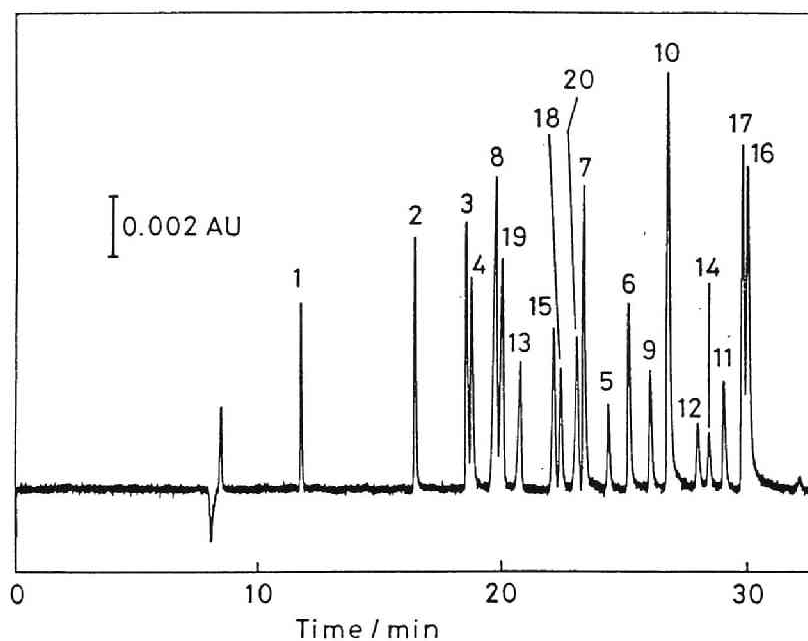


Figure 6 The same chromatogram as in Figure 5, except for the total applied voltage, ca. 10 kV, and the current, 17 μ A.

LITERATURE CITED

- [1] Chao, G. K.-J.; Suatoni, J. C. *J. Chromatogr. Sci.* 1982, 20, 436-440.
- [2] Burtscher, E.; Binder, H.; Concin, R.; Bobleter, O. *J. Chromatogr.* 1982, 252, 167-176.
- [3] Casteelle, K. V.; Geiger, H.; van Sumere, C. F. *J. Chromatogr.* 1983, 258, 111-124.
- [4] Buckman, N. G.; Hill, J. O.; Magee, R. J.; McCormick, M. J. *J. Chromatogr.* 1984, 284, 441-446.
- [5] Ivanov, Z.; Magee, R. J. *Microchem. J.* 1980, 25, 543-547.
- [6] Ugland, K.; Lundanes, E.; Greibrokk, T.; Bjørseth, A. *J. Chromatogr.* 1981, 213, 83-90.
- [7] Lores, E. M.; Edgerton, T. R.; Moseman, R. F. *J. Chromatogr. Sci.* 1981, 19, 466-469.
- [8] McLeod, H. A.; Laver, G. *J. Chromatogr.* 1982, 244, 385-390.
- [9] Nair, J.; Munir, K. M.; Bhide, S. V. *J. Liq. Chromatogr.* 1983, 6, 2829-2837.
- [10] Mikkers, F. E. P.; Everaerts, F. M.; Verheggen, Th. P. E. M. *J. Chromatogr.* 1979, 169, 11-20.
- [11] Jorgenson, J. W.; Lukacs, K. D. *Anal. Chem.* 1981, 53, 1298-1302.

CHAPTER 6

ELECTROKINETIC CHROMATOGRAPHY WITH MICELLAR SOLUTIONS

SEPARATION OF AROMATIC SULFIDES

ABSTRACT

Separation of a mixture of 11 aromatic sulfides, 7 alkyl phenyl sulfides and 4 alkyl benzyl sulfides, was investigated. The 10 sulfides of the 11 were separated with a 0.02 M sodium dodecyl sulfate (SDS) solution, pH 7.0 and a 0.05 mm i.d. x 900 mm fused silica tube at ambient temperature. Distribution coefficients of the sulfides in micellar solubilization were calculated for SDS and sodium tetradecyl sulfate solutions at 35 °C. The use of a mixed solution of 80 % of a 0.03 M SDS solution, pH 7.0 and 20 % of methanol was also successful in separating the 10 aromatic sulfides of the 11 with a 0.05 mm i.d. x 650 mm fused silica tube at ambient temperature. The unresolved pair was different from that observed with a 0.02 M SDS solution.

INTRODUCTION

Since organic sulfur compounds, including aliphatic and aromatic sulfides, are one of the most popular and important compounds to be analyzed as environmental pollutants or biological substances, chromatographic methods which are capable of separating sulfides are strongly required. Although many efforts were made to separate these compounds by gas chromatography (GC) or high-performance liquid chromatography (HPLC), very few gave successful results [1-3].

Takayanagi et al. have recently reported, as an application of ligand-exchange (LE) chromatography, the separation of dialkyl sulfides by LE-HPLC [4] and LE-GC [5], and of aromatic sulfides by LE-GC [6]. But there is no report of separation of aromatic sulfides by HPLC.

In this Chapter, the separation of 11 aromatic sulfides by electrokinetic chromatography with aqueous micellar solution is first reported. Then, distribution coefficients of the sulfides between the micelle and the aqueous phase will be calculated for both a sodium dodecyl sulfate (SDS) and a sodium tetradecyl sulfate (STS) solutions. The use of a mixed solution of an SDS solution and methanol to separate the same sulfides will also be shown.

EXPERIMENTAL SECTION

All the aromatic sulfides used were synthesized in this laboratory, except for methyl phenyl sulfide which was purchased from Wako Pure Chemical Industries (Tokyo, Japan), and used as methanol solutions. As separation tubes, a 0.05 mm i.d. x 650 mm and a 0.05 mm i.d. x 900 mm fused silica capillaries were used. A Glassman LG40R3.5 (Whitehouse Station, NJ, U.S.A.) and the current supply of a Shimadzu IP-2A isotachophoretic analyzer (Kyoto, Japan) were used to deliver a high voltage DC. Experiments were performed at ambient temperature (ca. 25 °C) or in a thermostated oven adjusted at 35 °C. Other apparatus, reagents and procedures were the same as those previously described.

RESULTS AND DISCUSSION

Use of Aqueous Micellar Solution

First, separations of 4 alkyl benzyl sulfides, whose alkyl groups were methyl, ethyl, propyl and isopropyl, and 7 alkyl phenyl sulfides, whose alkyl groups were methyl, ethyl, propyl, isopropyl, butyl, isobutyl and *s*-butyl, were tested by using a 0.05 mm i.d. x 650 mm fused silica tube with various concentrations of SDS solutions, pH 7.0 and STS solutions, pH 7.0 at ambient temperature.

For the mixture of 4 alkyl benzyl sulfides, the complete separation was obtained with a 0.05 M SDS solution as shown in

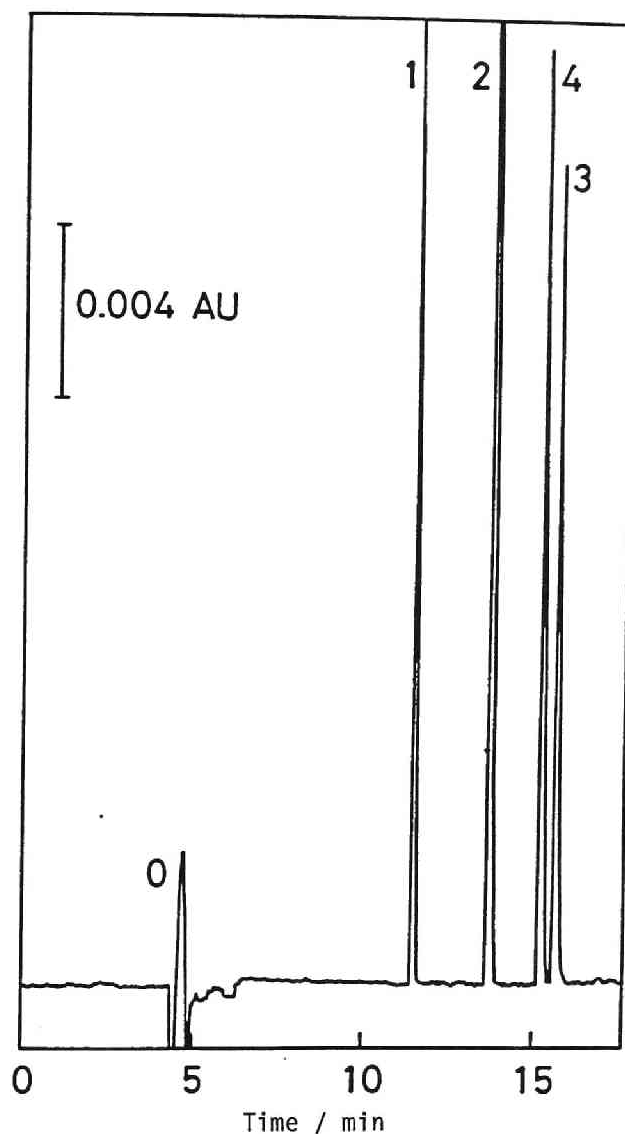


Figure 1. Electrokinetic chromatogram of alkyl benzyl sulfides: (0)methanol, (1)benzyl methyl sulfide, (2)benzyl ethyl sulfide, (3)benzyl propyl sulfide, (4)benzyl isopropyl sulfide; micellar solution, 0.05 M SDS, pH 7.0; separation tube, 0.05 mm i.d. x 650 mm; length of the tube used for separation, 500 mm; total applied voltage, ca. 18 kV; current, 30 μ A; detection wavelength, 210 nm; temperature, ambient.

Figure 1. The order of elution was the same as that in LE-GC [6]. The use of a 0.05 M STS solution gave the same resolutions and the order of elution.

On the other hand, for the mixture of 7 alkyl phenyl sulfides no successful separation was achieved with a 0.05 M SDS solution as shown in Figure 2: The peaks of butyl, isobutyl, and *s*-butyl phenyl sulfides were overlapped. In this case, the value of t_0/t_{mc} was ca. 0.26, where t_0 is the retention time of a unretained solute measured with methanol and t_{mc} is of the micelle measured with Sudan III. Since values of capacity factors K' for the unresolved components (ca. 70) were larger than the optimum value (ca. 2) as described in Chapter 2, a lower concentration of SDS was chosen to improve the resolution of the components. The complete separation of 7 alkyl phenyl sulfides was obtained with a 0.02 M SDS solution as shown in Figure 3. Here, the order of elution was again the same as that in LE-GC [6]. No successful separation, however, was achieved with any concentration of STS: The pair of butyl and isobutyl phenyl sulfides was not separated even when the value of K' was close to the optimum, i.e., $K' = 1.5$ for a 0.02 M STS solution, the value of t_0/t_{mc} being ca. 0.3.

Separations of a mixture of 4 alkyl benzyl sulfides and 7 alkyl phenyl sulfides mentioned above were not obtained with a 0.02 M SDS solution: The peaks of phenyl propyl and benzyl propyl sulfides were overlapped, while other peaks were successfully resolved each other. In this case, the values of K' for these unresolved two sulfides were almost optimum. So

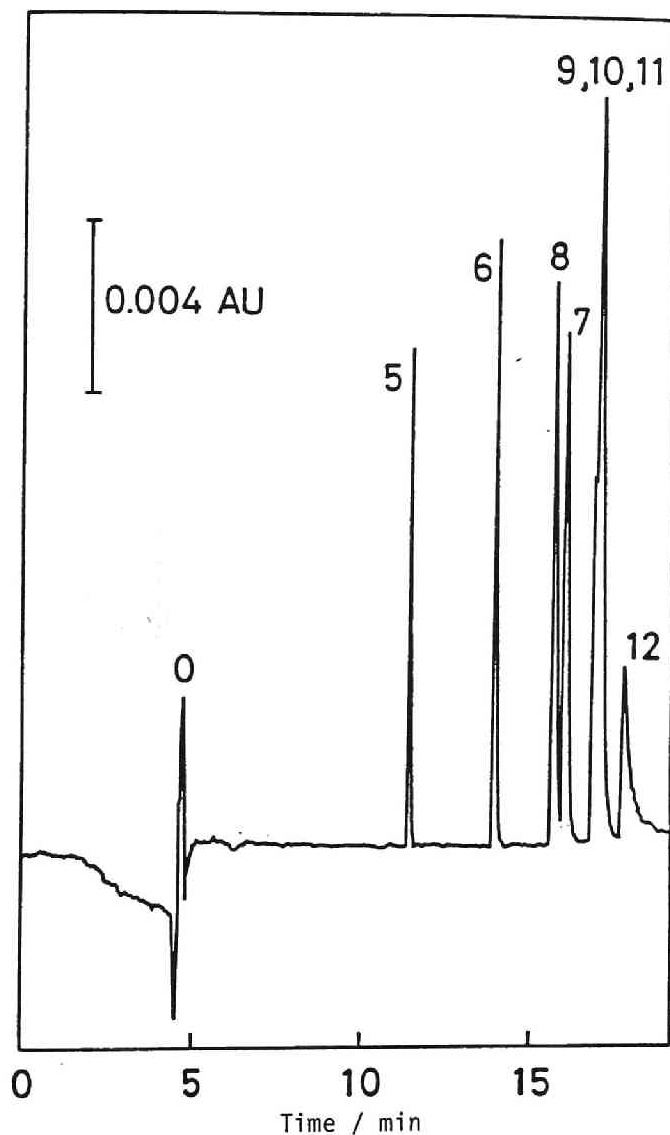


Figure 2. Electrokinetic chromatogram of alkyl phenyl sulfides: (5)methyl phenyl sulfide, (6)ethyl phenyl sulfide, (7)phenyl propyl sulfide, (8)isopropyl phenyl sulfide, (9)butyl phenyl sulfide, (10)isobutyl phenyl sulfide, (11)*s*-butyl phenyl sulfide, (12)Sudan III. Other conditions are the same as those given in Figure 1.

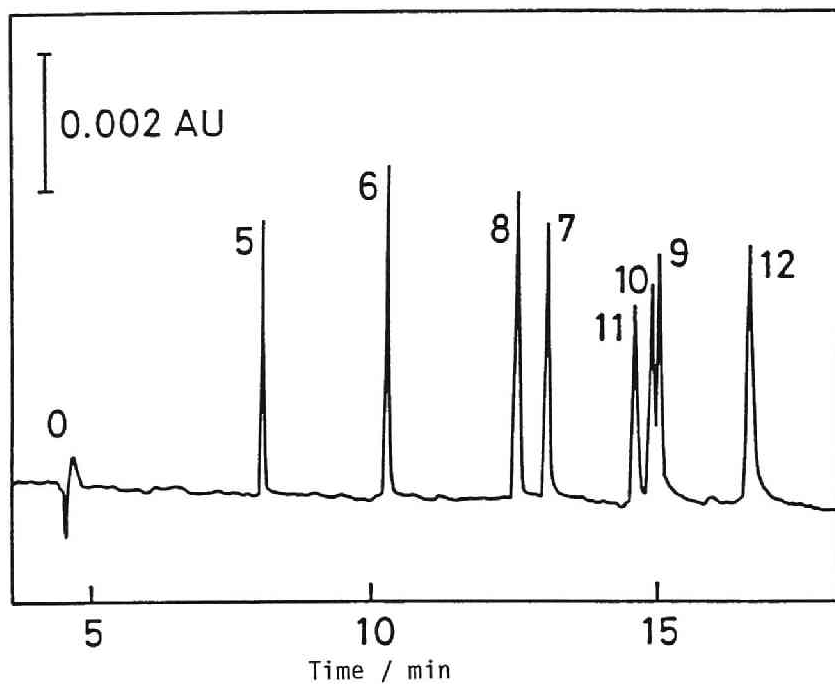


Figure 3. Electrokinetic chromatogram of 7 alkyl phenyl sulfides. Micellar solution, 0.02 M SDS, pH 7.0; total applied voltage, ca. 18 kV; current, 25 μ A. Other conditions as in Figure 2.

a longer separation column (750 mm) was employed. Although that pair could not be separated at all, the other 9 sulfides were separated as shown in Figure 4.

Calculation of Distribution Coefficients

As discussed in Chapter 2, the distribution coefficient in micellar solubilization between the micelle and the aqueous phase is related to the capacity factor K' as

$$K' = K \frac{V_{mc}}{V_{aq}} \quad (1)$$

where V_{mc} and V_{aq} are the volumes of the micelle and the aqueous phase. The value of K is considered to be constant regardless of the concentration of the surfactant as reported in Chapter 2. Distribution coefficients of the 11 aromatic sulfides were calculated for both SDS and STS solutions at 35 °C using eq 1. The concentration of each surfactant used was 0.02 M. The capacity factor K' was linearly decreased with an increase in the electroosmotic velocity v_{eo} as mentioned in Chapter 2. Figure 5 shows an example of dependence of K' of the 7 alkyl phenyl sulfides on v_{eo} in the SDS solution. The same plots were obtained for the other sulfides and in the STS solution. Each line of the plot was extrapolated to the intercept at $v_{eo} = 0$, and the value of K' at $v_{eo} = 0$ was designated K'_0 . Then, K'_0 may be taken as K' at the ambient temperature in the thermostated oven or 35 °C. On the other hand, the value of the phase ratio V_{mc}/V_{aq} was calculated by

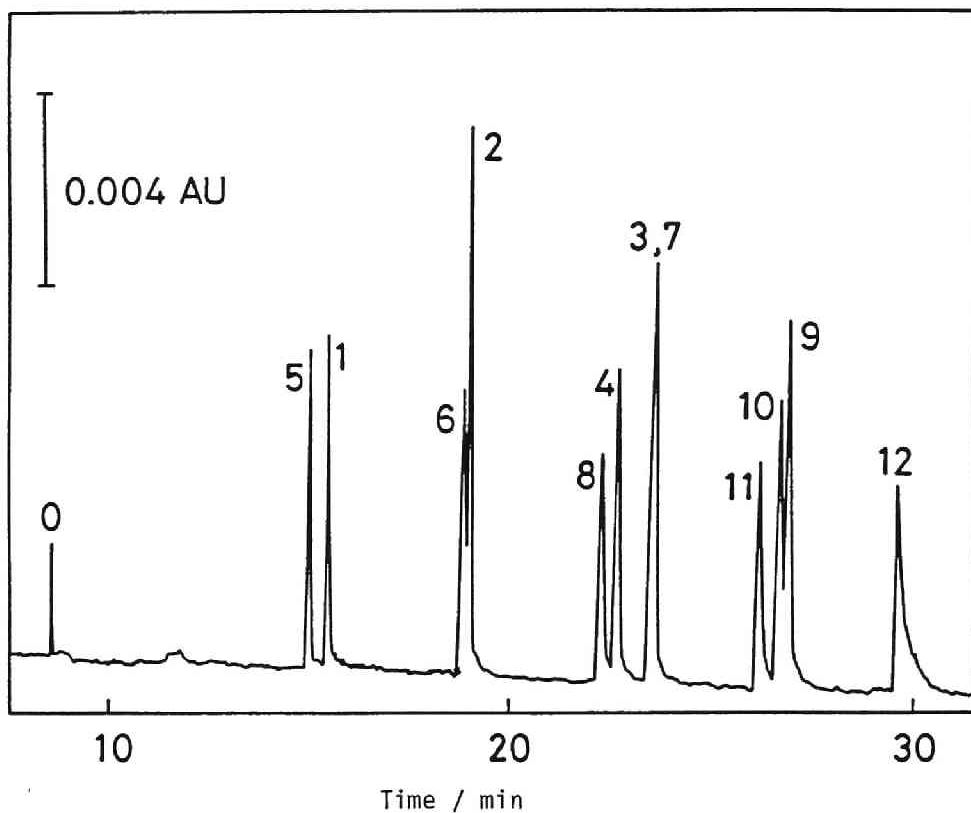


Figure 4. Electrokinetic chromatogram of 11 aromatic sulfides. Separation tube, 0.05 mm i.d. x 900 mm; length of the tube used for separation, 750 mm; total applied voltage, ca. 21 kV; current, 20 μ A. Peaks are labelled by the same numbers as in Figures 1 and 2. Other conditions as in Figure 3.

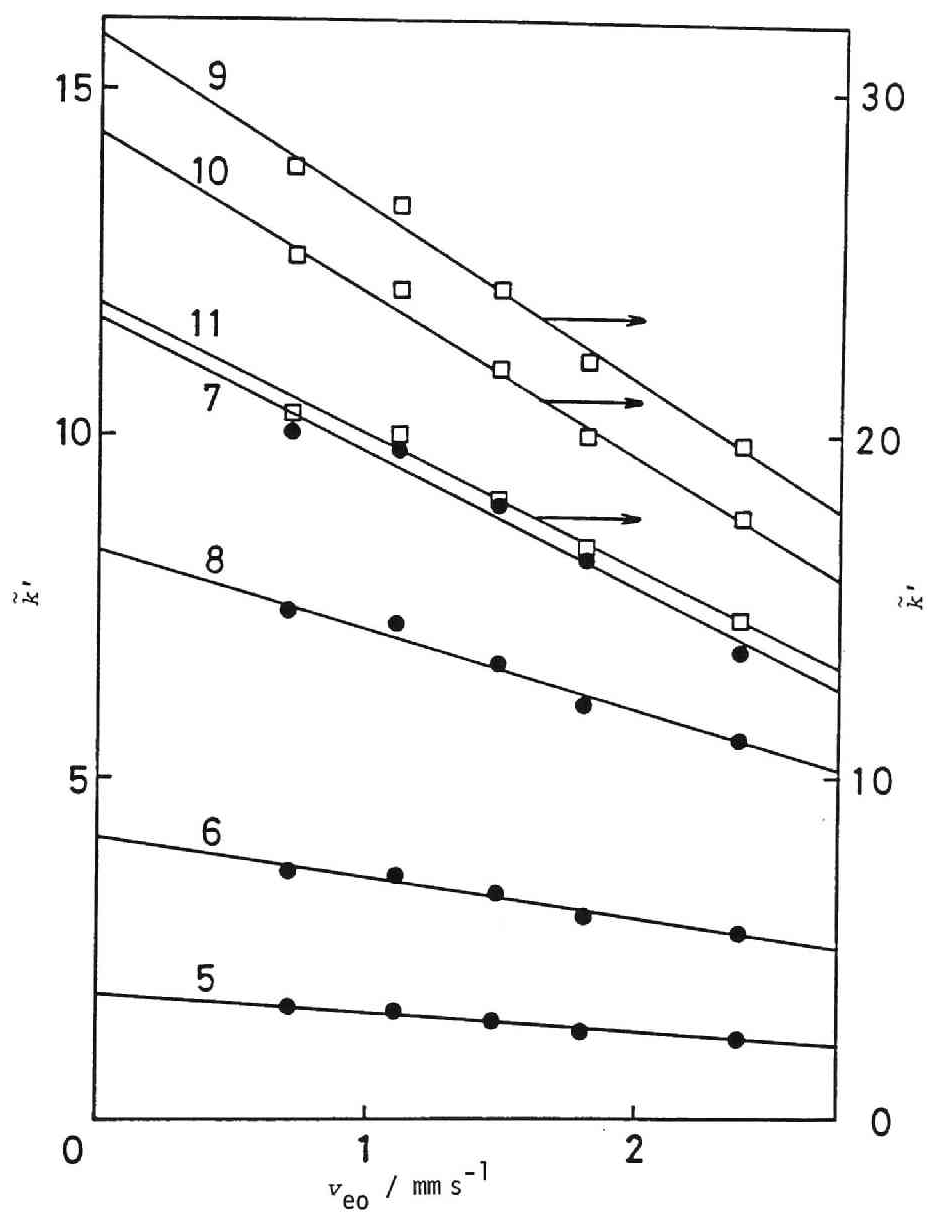


Figure 5. Dependence of capacity factors (K') of 7 alkyl phenyl sulfides on electroosmotic velocity (v_{eo}). Number on each plot corresponds to that in Figure 2. Conditions are the same as those in Figure 4 except for total applied voltage, current, and temperature. Temperature was adjusted at 35 °C.

(Chapter 2)

$$\frac{V_{mc}}{V_{aq}} = \frac{(C_{sf} - CMC) \bar{v}}{1 - (C_{sf} - CMC) \bar{v}} \quad (2)$$

where C_{sf} , CMC, and $\bar{v}$ are the concentration of the surfactant, the critical micellar concentration, and the partial specific volume of the micelle, respectively. Here, the values of V_{mc}/V_{aq} for a 0.02 M SDS and a 0.02 M STS solutions are 2.9×10^{-3} and 5.0×10^{-3} , respectively. The calculated distribution coefficients of the sulfides are shown in Table I. The values for STS tend to be slightly smaller than those for SDS, but there must be no difference between these values by considering experimental errors.

Use of Mixture of Aqueous Micellar Solution and Methanol

In all the experiments described in previous Chapters, only aqueous micellar solutions without any organic modifiers were employed. Here, a mixture of an aqueous SDS solution and methanol was used to explore possibilities of improving the separation of the sulfides. Separation of 11 aromatic sulfides with a mixture of 80 % of a 0.03 M SDS solution and 20 % of methanol and a 0.05 mm i.d. x 650 mm fused silica tube is shown in Figure 6. Changes in selectivities were recognized in marked contrast to the case in Figure 4: The peaks of phenyl propyl and benzyl propyl sulfides were now completely resolved, while the peaks of methyl phenyl and benzyl methyl sulfides were not separated at all. Moreover, two pairs,

Table I

Distribution Coefficients of Aromatic Sulfides at 35 °C

solute	distribution coefficient ^a / 10 ²	
	R ₁₂ SO ₄ Na ^b	R ₁₄ SO ₄ Na ^c
methyl phenyl sulfide	6.4	4.8
ethyl phenyl sulfide	14	12
phenyl propyl sulfide	45	35
isopropyl phenyl sulfide	29	25
butyl phenyl sulfide	110	110
isobutyl phenyl sulfide	100	110
<i>s</i> -butyl phenyl sulfide	82	77
benzyl methyl sulfide	7.1	5.4
benzyl ethyl sulfide	15	12
benzyl propyl sulfide	45	35
benzyl isopropyl sulfide	32	27

^a Concentration of surfactant, 0.02 M; partial specific volumes ($\bar{v}$) at 35 °C used to calculate distribution coefficients are taken from Shinoda et al. [7]: for sodium dodecyl sulfate (SDS), $\bar{v} = 0.8721$ mL g⁻¹; for sodium tetradecyl sulfate (STS), $\bar{v} = 0.9019$ mL g⁻¹. ^b SDS. ^c STS.

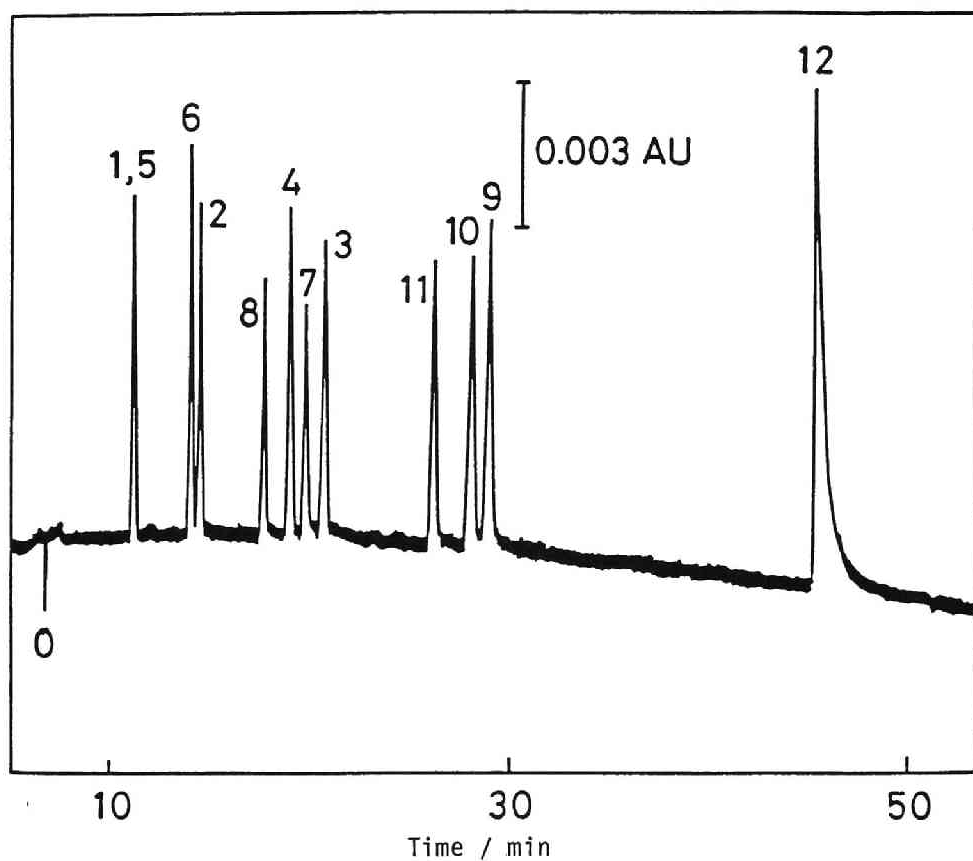


Figure 6. Electrokinetic chromatogram of 11 aromatic sulfides. Micellar solution, mixed solution of 80 % of 0.03 M SDS, pH 7.0 and 20 % of methanol; total applied voltage, ca. 22 kV; current, 20 μ A. Other conditions as in Figure 4.

which were not completely separated with a 0.02 M SDS solution, such as ethyl phenyl and benzyl ethyl sulfides and isobutyl phenyl and butyl phenyl sulfides were also separated.

Further experiments on the use of an aqueous organic solution in electrokinetic chromatography with micellar solution are in progress.

In conclusion, the successful separation of aromatic sulfides have been made by electrokinetic chromatography with a mixture of an SDS solution and an organic modifier, methanol, which shows a high resolving power of this chromatography.

LITERATURE CITED

- [1] Vose, C. W.; Stevens, P. M.; Dickens, J. P. *J. Chromatogr.* 1981, 225, 488-492.
- [2] Steudel, R.; Strauss, E.-M. *Z. Naturforsch* 1983, 38B, 719-725.
- [3] Appler, B.; Christmann, K. *J. Chromatogr.* 1983, 264, 445-452.
- [4] Takayanagi, H.; Hatano, O.; Fujimura, K.; Ando, T. *Anal. Chem.* 1985, 57, 1840-1846.
- [5] Takayanagi, H.; Hashizume, M.; Fujimura, K.; Ando, T. *J. Chromatogr.* 1985, 350, 63-73.
- [6] Takayanagi, H.; Akita, M.; Fujimura, K.; Ando, T. *J. Chromatogr.* 1985, 350, 75-85.
- [7] Shinoda, K.; Soda, T. *J. Phys. Chem.* 1963, 67, 2072-2074.

CHAPTER 7

ELECTROKINETIC CHROMATOGRAPHY WITH 2-*O*-CARBOXYMETHYL- β -CYCLODEXTRIN AS A MOVING STATIONARY PHASE

ABSTRACT

Preliminary results are presented on a new type of liquid chromatography, based on inclusion complex formation in a solution of a β -cyclodextrin derivative having an ionized group, 2-*O*-carboxymethyl- β -cyclodextrin (β -CMCD), combined with electrokinetic migrations. Only the host-guest interaction between β -CMCD and the solute operates as the distribution process, and electroosmosis and electrophoresis in an open-tubular capillary filled with a β -CMCD solution permit differential migrations between the host and guest molecules. Separations of some aromatic isomers are shown, and retention parameters and the distribution coefficient are discussed.

INTRODUCTION

In electrokinetic chromatography where a solution of an ionic surfactant is filled in an open-tubular glass capillary and is subject to free zone electrophoresis, the whole solution is transported by the electroosmotic flow with an approximately flat velocity profile in a direction determined by the sign of the zeta potential at the solution-glass interface. On the other hand, the ionic micelle is concurrently moved by the electrophoretic attraction generally in the direction opposite to the electroosmotic flow. As the electroosmotic flow is usually much stronger than the electrophoretic migration of the micelle (Chapter 2), the micelle is also transported in the same direction as the whole solution but with a slower velocity than the latter. A mixture of solutes added to the micellar solution at one end of the tube can be separated while it moves towards the other end according to the extent to which each component is incorporated into the micelle by the micellar solubilization phenomenon.

This concept of chromatography can be readily extended to the utilization of some specific interactions, e.g., complexation or clathration. This Chapter presents preliminary results on electrokinetic chromatography with an ionic cyclodextrin derivative that can be expected to form inclusion complexes with a variety of organic molecules; 2-*O*-carboxymethyl- β -cyclodextrin (β -CMCD) was employed as a substitute for the chromatographic phase corresponding to the micelle in the above-mentioned chromatographic system.

Recently, cyclodextrins have attracted wide attention in high-performance liquid chromatography (HPLC), e.g., cyclodextrins chemically bonded to silica gel as stationary phases have revealed interesting features for the separation of aromatic isomers [1,2] and optical isomers [3]. In these chemically bonded cyclodextrin phases, adsorption by spacer groups employed to link the cyclodextrin to the silica gel could be involved in the distribution process in addition to the formation of inclusion complexes of cyclodextrins with the solutes [1,2]. In electrokinetic chromatography, however, the cyclodextrin derivative is free in the solution and, therefore, only inclusion complex formation or host-guest interaction should be operating as the distribution process, although inclusion by ionic cyclodextrins may be slightly different from that by electrically neutral cyclodextrins.

EXPERIMENTAL SECTION

Reagents and Materials

β -Cyclodextrin was purchased from Nakarai Chemical (Kyoto, Japan). All compounds employed as samples and reagents used for the preparation of the cyclodextrin derivative and for the preparation of the chromatographic solution were of analytical-reagent grade and used without further purification.

Preparation of 2-*O*-Carboxymethyl- β -cyclodextrin (β -CMCD)

β -CMCD was synthesized by the reaction of sodium iodoacetate with an excess of β -cyclodextrin anion in dimethyl sulfoxide (DMSO) according to the method described by Kitaura and Bender[4] for the preparation of carboxymethyl- α -cyclodextrin (α -CMCD), and isolated by the method described by Gruhn and Bender [5] with some modifications, viz., DEAE Toyopearl 650M (Toyo Soda, Tokyo, Japan) was employed instead of DEAE-Sephadex A-25 and a 0.02 M phosphate buffer solution (pH 4.6) was delivered into the column as an isocratic eluent. Each eluate fraction was subject to HPLC analysis with a DEAE-5500PW column, 6 mm i.d. x 15 cm (Toyo Soda), and a 0.02 M phosphate buffer solution (pH 3.85). Fractions containing β -CMCD were collected, concentrated by evaporation and desalted with a Sephadex G-10 (Pharmacia, Uppsala, Sweden) column to give an aqueous solution of the sodium salt of β -CMCD. β -CMCD was isolated by lyophilization and its molecular structure was confirmed by NMR spectroscopy as described below.

About 40 mg of the sodium salt of β -CMCD was dissolved in ca. 0.5 mL of D₂O and proton NMR spectra at 90 MHz and carbon-13 NMR spectra at 22.49 MHz were measured at room temperature by using tetramethylsilane as an external standard. The proton NMR spectrum of β -CMCD was similar to that reported for α -CMCD [5]. Two peaks at δ 5.34 (1H, doublet, J = 4 Hz) and δ 4.21 (2H, singlet) were apparently observed, distinct from the spectrum of β -cyclodextrin recorded under the same conditions. These peaks can be assigned to the C₁

anomeric proton on the carboxymethylated glucose unit and methylene protons of the carboxymethyl group in the order cited above.

Only two weak peaks were recognized in the carbon-13 NMR spectrum of β -CMCD other than those observed for β -cyclo-dextrin [6], i.e., δ 100.0 and δ 70.4, which may be assigned to C₁ and C₃, respectively, of the glucose unit having the substituent. Major peaks of C₁ and C₃ were observed at δ 101.9 and δ 72.2, respectively. The absorption peak due to the saturated carbon of the carboxymethyl group seemed to overlap with the peak due to C₅ (δ 71.9), because only the C₅ peak was stronger than the others. Whereas the major peak of C₂ appeared at δ 73.1, the absorption by the C₂ of the glucose unit having the substituent could not be detected. Probably it accidentally coincides with that due to C₄ (δ 81.1).

Apparatus

Electrokinetic chromatography was performed at room temperature with an apparatus similar to that described in previous Chapters. The current supply of a Shimadzu IP-2A isotachophoretic analyzer was used, which could deliver a constant current up to 500 μ A at below 25 kV. The on-column detection technique was employed with a Jasco UVIDEC-100-V spectrophotometric detector with a modified slit, as described in Chapter 2. Proton NMR spectra were recorded on a Varian EM-390 and carbon-13 NMR spectra on a Jeolco FX-90Q NMR spectrometer.

Procedure

The chromatographic solution was prepared by dissolving β -CMCD in a 0.1 M phosphate buffer solution (pH 7.0) consisting of sodium salts alone. The two solutions at the two electrodes were interchanged at an appropriate interval to avoid changes in pH owing to the electrolysis of water. The apparatus was operated at the constant current of 50 μ A. All the solutes were injected as methanol solutions. Other chromatographic procedures were the same as those described in Chapter 2.

RESULTS AND DISCUSSION

Retention data obtained in this work are listed in Table I. The retention time of unretained solutes, t_0 , was regarded as being equal to that of methanol used as the solvent for the solutes. On the assumption that methanol cannot be included at all by β -CMCD, the velocity of methanol band should be identical with that of the aqueous phase that is moved by electroosmosis (Chapter 2). The observed variations in t_0 shown in Table I for identical solutions are considered to be due to temperature fluctuations and/or slight changes in the pH of the solution as a result of the electrolysis of water. As the tube is filled with β -CMCD solution of a constant concentration, it is necessary for the measurement of the velocity of β -CMCD to label β -CMCD with a UV-absorbing molecule. Iodine was employed as a candidate for the tracer

Table I

Retention Times, Capacity Factors^a and Distribution
Coefficients^b of Substituted Benzenes^c

No.	Solute	t_0 /min	t_R /min	K'	K
1	Acetophenone	15.6	18.5	1.00	49
	Anisole		18.8	1.19	58
	Methyl benzoate		19.6	1.88	92
	Ethyl benzoate		19.9	2.23	109
	Propyl benzoate		20.4	3.04	149
	Butyl benzoate		20.7	3.71	182
2	<i>o</i> -Cresol	15.6	18.1	0.78	38
	<i>m</i> -Cresol		18.6	1.04	51
	<i>p</i> -Cresol		19.3	1.55	76
3	<i>m</i> -Nitroaniline	16.1	18.6	0.75	37
	<i>o</i> -Nitroaniline		18.9	0.89	44
	<i>p</i> -Nitroaniline		20.8	2.54	124
4	<i>o</i> -Chloroaniline	15.8	18.7	0.96	47
	<i>m</i> -Chloroaniline		18.9	1.08	53
	<i>p</i> -Chloroaniline		19.7	1.68	82
5	<i>m</i> -Dinitrobenzene	14.5	15.5	0.29	14
	<i>p</i> -Dinitrobenzene		15.7	0.36	18
	<i>o</i> -Dinitrobenzene		16.6	0.78	38
6	<i>m</i> -Nitrophenol	15.3	18.9	— ^d	—
	<i>o</i> -Nitrophenol		20.3	— ^d	—
	<i>p</i> -Nitrophenol		22.5	— ^d	—

Table I (continued)

No.	Solute	t_0 /min	t_R /min	K'	K
7 ^e	<i>m</i> -Nitrophenol	9.8	10.4	—	—
	<i>p</i> -Nitrophenol		12.3	—	—
	<i>o</i> -Nitrophenol		14.2	—	—
8 ^f	<i>m</i> -Nitrophenol	8.0	8.6	—	—
	<i>o</i> -Nitrophenol		12.2	—	—
	<i>p</i> -Nitrophenol		13.0	—	—
9	3,5-Xylidine	15.6	16.4	0.18	9
	2,6-Xylidine		17.0	0.35	17
	2,3-Xylidine		17.4	0.48	24
	2,5-Xylidine		18.1	0.77	38
	2,4-Xylidine		18.3	0.86	42
	3,4-Xylidine		18.6	1.02	50
10	3,5-Xylenol	15.4	17.6	0.68	33
	2,6-Xylenol		17.6	0.68	33
	2,5-Xylenol		18.5	1.18	58
	2,4-Xylenol		18.8	1.41	69
	2,3-Xylenol		19.1	1.67	82
	3,4-Xylenol		19.4	2.00	98

^a An electrophoretic mobility of $9.03 \times 10^{-3} \text{ mm}^2 \text{ s}^{-1} \text{ V}^{-1}$ for β -CMCD was adopted in calculating t_{CD} . ^b As specific volume of 0.671 mL g^{-1} for β -CMCD was assumed in calculating V_{CD} . ^c Conditions as in Figure 1. Three to six solutes listed under the same run No. were injected as a mixture. ^d See text. ^e α -cyclodextrin (0.032 M) was employed instead of β -CMCD. ^f Phosphate buffer (0.1 M, pH 7.0) containing no cyclodextrin was used.

of β -CMCD, but the iodine peak could not be recognized when it was injected in the same way as the other samples. The chromatogram for the separation of cresol isomers is shown in Figure 1 and that for the separation of xylidine isomers in Figure 2.

The solutes listed in Table I can be assumed to be electrically neutral under the experimental conditions used, except for nitrophenols. These neutral solutes were not separated by electrophoresis alone when 0.032 M α -cyclodextrin was used in place of β -CMCD. The lower solubility of β -cyclodextrin than α -cyclodextrin in water is the reason why β -cyclodextrin was not used in the blank experiment. The fact that the separation of these isomers was effected when β -CMCD was present in the solution clearly implies that the inclusion of solutes by β -CMCD takes part in the distribution process. The order of elution of isomers of cresol, nitroaniline and dinitrobenzene is the same as that reported [1,2] in HPLC with β -cyclodextrin-bonded stationary phases. It should be noted that isomers of xylidine or xylenol were successfully separated even if 3,5- and 2,6-xylenols were not resolved. These results suggest that this method is promising for separating isomers of substituted benzenes.

The isomers of nitrophenol were separated in every instance shown in Table I (Nos. 6, 7 and 8), and these results can be explained as follows. In the absence of cyclodextrins (Table I, No. 8), the isomers were separated only by electrophoresis of free isomers in the phosphate buffer, that is, the isomers were eluted in decreasing order of their pK_a

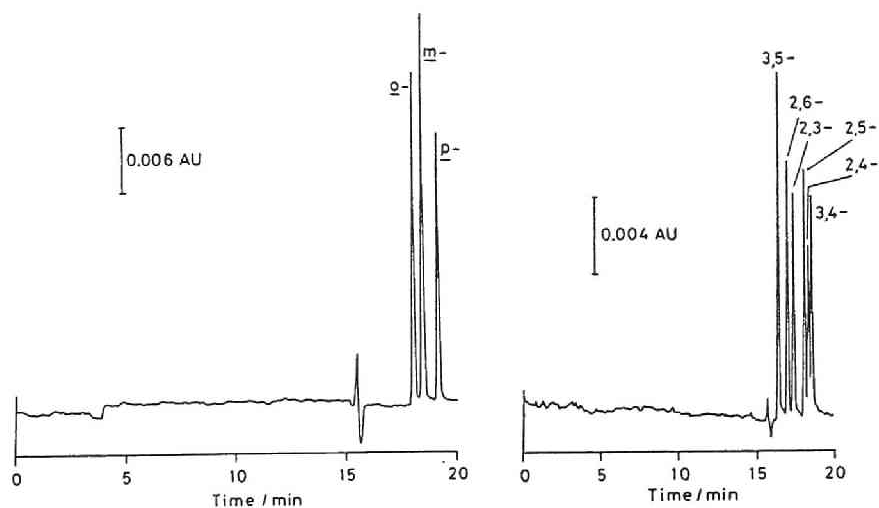


Figure 1. Electrokinetic chromatogram of cresol isomers. Chromatographic solution, 0.025 M β -CMCD in 0.1 M phosphate buffer (pH 7.0); separation tube, 0.05 mm i.d. $\times$ 650 mm; length of the tube used for separation, 500 mm; current, 50 μ A; total applied voltage, ca. 12 kV; detection wavelength, 210 nm.

Figure 2. Electrokinetic chromatogram of xylidine isomers. Conditions as in Figure 1.

values. When α -cyclodextrin was added to the buffer solution (Table I, No.7), the *p*-isomer was included by α -cyclodextrin to a much greater extent than the other isomers and the electrophoretic mobility was reduced, causing reversal of the order of elution between the *o*- and *p*-isomers. Comparison of the results with α -cyclodextrin (Table I, No.7) and β -CMCD (Table I, No.6) reveals that the *p*-isomer was also included by β -CMCD more strongly than the *o*-isomer.

The capacity factor, K' , which is defined as the ratio of the total number of moles of the solute included by β -CMCD, n_{CD} , to those in the aqueous phase, n_{aq} , i.e., $K' = n_{CD}/n_{aq}$, is related to the retention time, t_R , for electrically neutral solutes by the following equation, which is similar to that derived for electrokinetic chromatography with micellar solutions as described in Chapter 2:

$$K' = \frac{t_R - t_0}{t_0(1 - t_R/t_{CD})} \quad (1)$$

where t_{CD} is the elution time of β -CMCD and indicates a longer limit of retention times for electrically neutral solutes. As shown in eq 1, the values of both t_{CD} and t_0 are required for calculation of the capacity factor. This means that the possible range of the retention time should be known in order to determine the capacity factor in this method.

In order to measure the elution time of β -CMCD, an experimental approach was used in addition to the tracer method mentioned above. A ca. 0.015 M aqueous solution of β -CMCD

was injected and detected by UV absorption at 200 nm under the same conditions as those given in Table I, except that a 0.1 M phosphate buffer solution (pH 7.0) containing no β -CMCD was used instead of that containing β -CMCD. The current was kept at 50 μ A, but the voltage increased to 15 kV from the 12 kV observed with the 0.025 M β -CMCD solution. The electroosmotic velocity was measured with methanol added to the aqueous β -CMCD solution. The electrophoretic mobility of β -CMCD in the 0.1 M phosphate buffer solution (pH 7.0) was $9.03 \times 10^{-3} \text{ mm}^2 \text{ s}^{-1} \text{ V}^{-1}$. The estimated value of t_{CD}/t_0 from the above value of the electrophoretic mobility was about 1.4 under the conditions given in Table I.

Capacity factors calculated by eq 1 from the retention time and t_0 listed in Table I and the values of t_{CD}/t_0 estimated from the electrophoretic mobility of β -CMCD described above are also given in Table I, together with the distribution coefficient, K , which was obtained according to

$$K' = K \cdot (V_{\text{CD}} / V_{\text{aq}}) \quad (2)$$

where V_{aq} and V_{CD} are the volumes of the aqueous phase and β -CMCD, respectively, in the tube. The specific volume of β -CMCD required in order to know V_{CD} was assumed to be equal to that of α -cyclodextrin (0.671 mL g^{-1}) [7]. It should be stressed that the capacity factors and hence the distribution coefficients listed in Table I are approximate values because the estimated value of t_{CD} is adopted in the calculation.

The resolution equation in electrokinetic chromatography

is given by considering eq 1 (Chapter 2):

$$R_s = \frac{\sqrt{N}}{4} \cdot \frac{\alpha - 1}{\alpha} \cdot \frac{K'}{1 + K'} \cdot \frac{1 - t_0/t_{CD}}{1 + (t_0/t_{CD})K'} \quad (3)$$

where R_s is the resolution, N is the plate number and α is the separation factor. In eq 3, capacity factors for two closely eluted peaks are assumed to be approximately equal. The resolution expressed by eq 3 is apparently a function not only of N , α , and K' , but also of t_0/t_{CD} . As discussed in Chapter 2, the capacity factor that gives the maximum resolution at constant values of the other parameters is dependent on the ratio t_0/t_{CD} . As the value of t_0/t_{CD} is estimated to be about 0.7, as discussed above, the maximum value at $K' = 1.1$ can be predicted to be about 0.07 for the product of the last two terms of the right-hand side of eq 3, which depend only on the capacity factor among four terms. This large value of 0.7 for t_0/t_{CD} in this method results in a much poorer resolution in comparison with electrokinetic chromatography with micellar solutions where t_0/t_{mc} lies in the range (Chapter 2) 0.2-0.3, where t_{mc} is the elution time of the micelle and corresponds to t_{CD} . The low electrophoretic mobility of β -CMCD, which results from the small ratio of the charge number to the molecular weight, must be the reason for the large value of t_0/t_{CD} and it is therefore desirable to introduce more charges into a molecule in order to improve the resolution in this method.

Plate number calculated from peak area, peak height and

retention time are 120000-130000 in the chromatogram shown in Figure 1. These numbers correspond to plate heights of about 4 μ m. These plate heights are considerably higher than the 2-3 μ m observed for chromatograms with micellar solutions (Chapter 2), the reason for which remains to be clarified.

In conclusion, the method described is an example of chromatography with a homogeneous solution and electrokinetic migrations, supplementing that with micellar solutions reported previously. It demonstrates the possibility of widening the scope of electrokinetic chromatography. Only the formation of inclusion complexes or the host-guest interaction between the cyclodextrin derivative and the solute, both of which are free in the solution, operates as the distribution process. This method seems very promising, especially for separating aromatic isomers, although much remains to be done before practical application.

LITERATURE CITED

- [1] Fujimura, K.; Ueda, T.; Ando, T. *Anal. Chem.* 1983, 55, 446-450.
- [2] Kawaguchi, Y.; Tanaka, M.; Nakae, M.; Funazo, K.; Shono, T. *Anal. Chem.* 1983, 55, 1852-1857.
- [3] Hinze, W. L.; Riehl, T. E.; Armstrong, D. W.; DeMond, W.; Alak, A.; Ward, T. *Anal. Chem.* 1985, 57, 237-242.
- [4] Kitaura, Y.; Bender, M. L. *Bioorg. Chem.* 1975, 4, 237-249.
- [5] Gruhn, W. B.; Bender, M. L. *Bioorg. Chem.* 1974, 3, 324-342.
- [6] Takeo, K.; Hirose, K.; Kuge, T. *Chem. Lett.* 1973, 1233-1236.
- [7] Manor, P. C.; Saenger, W. *J. Am. Chem. Soc.* 1974, 96, 3630-3639.

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