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ELECTROKINETIC CHROMATOGRAPHY WITH MICELLAR SOLUTIONS

RETENTION BEHAVIOUR AND SEPARATION OF CHLORINATED PHENOLS

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SUMMARY

The retention behaviour of chlorinated phenols during electrokinetic chromatography with micellar solutions and open-tube capillary columns 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 650 × 0.05 mm I.D. fused-silica tube with a 0.07 *M* sodium dodecyl sulphate solution (pH 7.0).

INTRODUCTION

Electrokinetic chromatography with a micellar solution and an open-tube capillary column[1–3] is a new type of liquid chromatography based on micellar solubilization phenomena and electrokinetic migrations, which has a pseudo-stationary phase, a micelle, and can be considered as a kind of liquid–liquid partition chromatography consisting of a homogeneous solution alone. We have already described the fundamental characteristics of electrokinetic chromatography with micellar solutions in previous papers[1,2], and have 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 reported[3]. In that paper we briefly discussed the retention characteristics of an ionized solute, 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 re-

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ports of the HPLC separation of those materials have been published[4–7] recently. Among such compounds, chlorinated phenols are one of the most popular and important compounds to be analysed 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[8–12]. One of them[9] succeeded in separating a mixture of phenol and all the isomers of chlorinated phenols except 2,3,5-trichlorophenol.

In this paper, we shall give some retention characteristics of chlorinated phenols in electrokinetic chromatography with micellar solutions for both an electrically neutral and an ionized solute, in comparison with free-zone electrophoresis in an open-tube capillary column[13,14], and then show separations of all the isomers of chlorinated phenols, including phenol.

EXPERIMENTAL

Apparatus and procedure

A 650 × 0.05 mm I.D. 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[1,2].

Reagents

Chlorinated phenols were purchased from Wako (Tokyo, Japan), Nakarai (Kyoto, Japan), Tokyo Kasei Kogyo (Tokyo, Japan) or Aldrich (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 sulphate (SDS) was dissolved in a 0.05 *M* sodium 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 behaviour 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}[2]. Methanol can be considered to be insolubilized by the micelle[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 Fig. 1. Here, a small peak followed by pentachlorophenol (peak No. 20) seems to be due to an

TABLE I

LIST OF COMPOUNDS INCLUDED IN A TEST MIXTURE*

No.	*Solute*	pK_a**
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	–

* The mixture was dissolved in methanol.

** Logarithm of the reciprocal of the acid dissociation constant taken from the literature[9].

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 $\tilde{k}'$ of an electrically neutral solute is given by[1,2]

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

where t_R is the retention time of the solute. The symbol $\tilde{k}'$ for the capacity factor is used instead of k' widely accepted in conventional HPLC[2]. Although some solutes in the test mixture might be ionized under the conditions used, the apparent capacity factor $\tilde{k}'_{app}$ for each component was calculated by eqn. 1. The dependence of $\tilde{k}'_{app}$ on

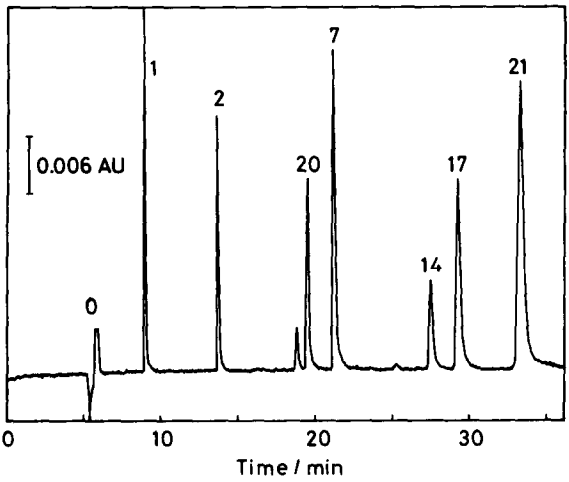

Fig. 1. Electrokinetic chromatogram of a test mixture of chlorinated phenols. Peak 0 = methanol; for other solutes, see Table I; micellar solution, 0.10 *M* SDS, pH 7.0; separation tube, 650 × 0.05 mm I.D.; length of the tube used for separation, 500 mm; total applied voltage, *ca.* 15 kV; current, 33 μA; detection wavelength, 220 nm; temperature, 35°C.

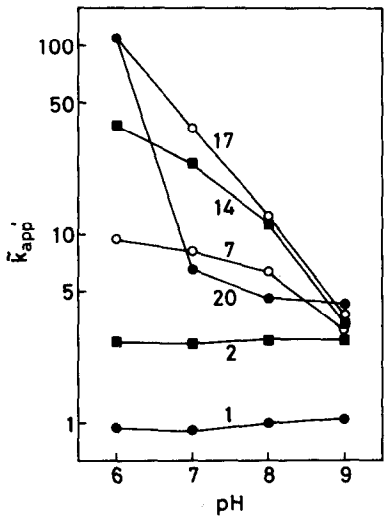

Fig. 2. Dependence of apparent capacity factor k'_{app} on pH. For solute numbers see Table I. Conditions as in Fig. 1, except for the value of the pH.

the pH of the SDS solution is shown in Fig. 2 for each compound. It can be seen from Fig. 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[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, we must evaluate the electrophoretic velocity of the negatively charged solute. 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 m*M* 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 Fig. 3. Then, the electrophoretic velocity of a solute in the buffer solution, v_{ep} (s), was calculated by[2]

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

where v_b (s) is the observed migration velocity of the solute and can be calculated

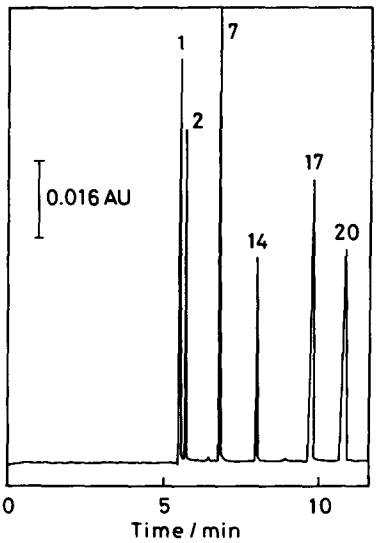

Fig. 3. Separation of the test mixture by high-voltage capillary electrophoresis: Solution for separation, phosphate–borate buffer, pH 7.0; current, 17 μA. For solute numbers see Table I. Other conditions as in Fig. 1.

from the column length l and the retention time t_R, *i.e.*, v_b (s) = l/t_R. The electroosmotic velocity is defined as[2]

$$v_{eo} = -(\varepsilon\zeta/\eta)E \tag{3}$$

where ε, ζ 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 $\varepsilon\zeta/\eta$ 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

TABLE II

ELECTROOSMOTIC VELOCITY IN THREE SOLUTIONS OF DIFFERENT SDS CONCENTRATIONS AT FOUR DIFFERENT pH VALUES

Separation tube, 650 × 0.05 mm I.D.; total applied voltage, *ca.* 15 kV; temperature of oven, 35°C.

pH	v_{eo} (*mm* s^{-1})		
	*0**	*5**	*100**
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

* Concentration of SDS (mM)

solution, it is reasonable to assume that the velocity is the same as that in the buffer solution containing 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):

$$v^*_{ep}\,(s) = v_{eo} - v_m\,(s) \tag{4}$$

Assuming that the electrophoretic velocity of the micelle, v_{ep} (mc), is constant even when the micelle incorporates solutes[3], we can write

$$v^*_{ep}\,(s) = \frac{n_{aq}}{n_{mc} + n_{aq}} v_{ep}\,(s) + \frac{n_{mc}}{n_{mc} + n_{aq}} v_{ep}\,(mc) \tag{5}$$

where n_{mc} and n_{aq} are the numbers of the solutes in micellar and aqueous phases, respectively. As the capacity factor $\tilde{k}'$ is defined by[2]

$$\tilde{k}' = n_{mc}/n_{aq} \tag{6}$$

by substituting eqn. 6 into eqn. 5, $\tilde{k}'$ can be expressed as

$$\tilde{k}' = \frac{v^*_{ep}\,(s) - v_{ep}\,(s)}{v_{ep}\,(mc) - v^*_{ep}\,(s)} \tag{7}$$

Now we can calculate $\tilde{k}'$ even for the ionized solute by eqn. 7. The dependence of $\tilde{k}'$ on pH for each compound of the test mixture is shown in Fig. 4. The plots are similar

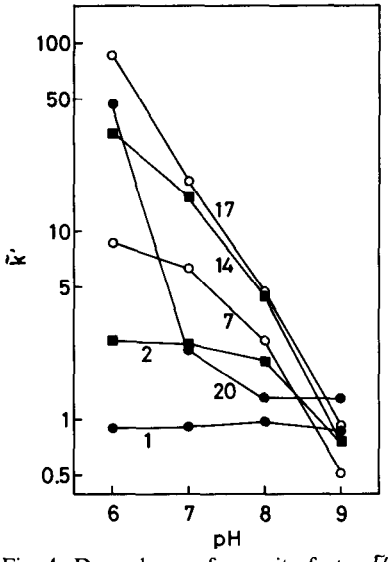

Fig. 4. Dependence of capacity factor $\tilde{k}'$ on pH. The number on each plot corresponds to the solute shown in Table I. Conditions as in Fig. 1, except for the value of the pH.

to those shown in Fig. 2. But the value of $\tilde{k}'$ was smaller than $\tilde{k}'_{app}$ 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, $\tilde{k}'_{app}$ was almost constant whereas $\tilde{k}'$ 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.

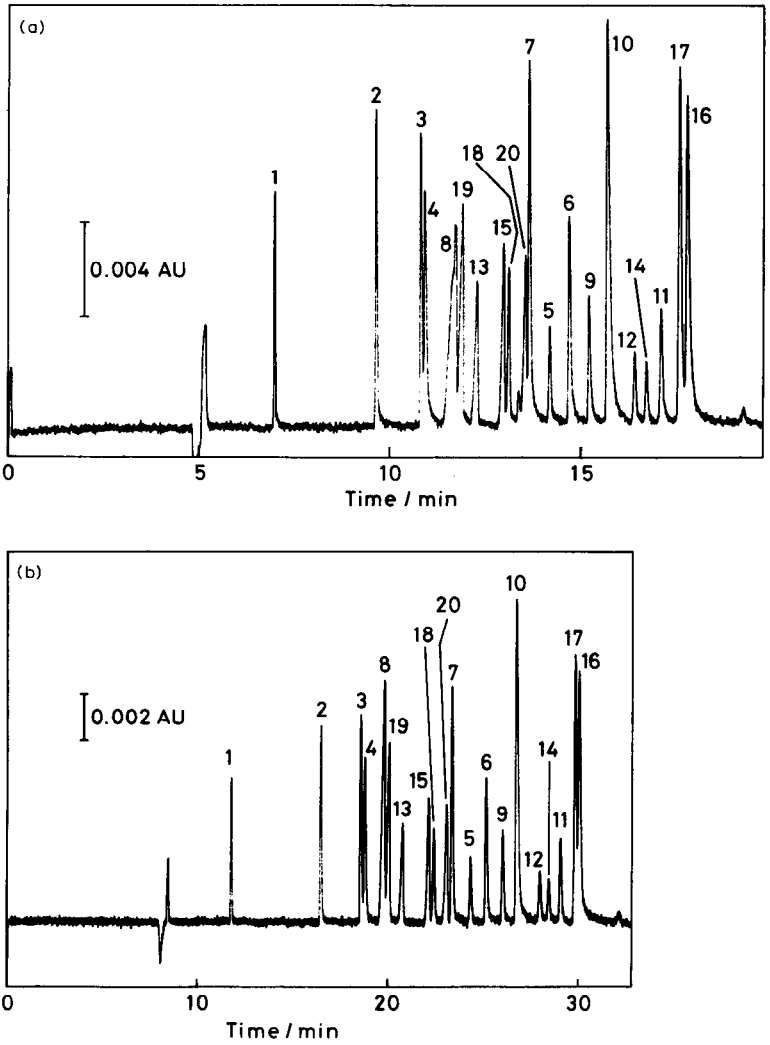

Fig. 5. Electrokinetic chromatogram of a mixture of all the isomeric chlorinated phenols, including phenol. Peaks: 1 = phenol; 2 = 2-chloro; 3 = 3-chloro; 4 = 4-chloro; 5 = 2,3-dichloro; 6 = 2,4-dichloro; 7 = 2,5-dichloro; 8 = 2,6-dichloro; 9 = 3,4-dichloro; 10 = 3,5-dichloro; 11 = 2,3,4-trichloro; 12 = 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, (a) *ca.* 15 kV, (b) *ca.* 10 kV; current, (a) 28 μA, (b) 17 μA. Other conditions as in Fig. 1.

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 $\tilde{k}'$ 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 Fig. 5 were obtained. The applied voltages were *ca.* 15 kV and 10 kV in (a) and (b), respectively. In Fig. 5a, 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 Fig. 5b, probably owing to the lower resolution than in Fig. 5a. As already mentioned[2], the resolution R_s between two adjacent peaks whose capacity factors are $\tilde{k}'_1$ and $\tilde{k}'_2$ in electrokinetic chromatography is given by

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

where N is the number of theoretical plates, α is the separation factor, and $\tilde{k}'$ is given approximately by $\tilde{k}' = \tilde{k}'_1 - \tilde{k}'_2$. Since the value of the parameter t_0/t_{mc} is *ca.* 0.25 in both Figs. 5a and b, the maximum resolution should be obtained under a constant N value when the value of $\tilde{k}'$ is *ca.* 2 (ref. 2). A method for adjusting $\tilde{k}'$ to the optimum value by changing the concentration of a surfactant has been discussed previously[3]. But in Fig. 5, 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 Fig. 5a was larger than that in Fig. 5b, so a higher resolution was obtained in the former than in the latter. Detailed discussions for parameters affecting the plate height will be discussed in a future paper.

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