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Note

Quantitation and reproducibility in electrokinetic chromatography with micellar solutions*

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Electrokinetic chromatography with micellar solutions[1,2] is a novel separation technique based on micellar solubilization and electrokinetic migration in open-tubular capillaries, which is related to capillary electrophoresis. Some applications of this chromatography have been reported[3–5].

As an efficient separation technique, free zone electrophoresis in open-tubular capillaries[6,7] has widely been employed in various analytical fields. Recently, Fujiwara and Honda[8] reported fundamental studies on the quantitation of cinnamic acid and its analogues by this method. The relationship between the injected amounts of sample and peak area or peak heights, and the reproducibility of the determination of the samples by the internal standard method, were described.

In this paper, we briefly discuss quantitation by the internal standard method and the reproducibility of retention times in electrokinetic chromatography with micellar solutions. The reproducibility of the peak area and peak heights for repeated injections of constant amounts of samples is also presented.

EXPERIMENTAL

Apparatus and reagents

Most of the apparatus and experimental conditions were as described previously[1,2]. The separation tube used was a 650 mm × 0.05 mm I.D. fused-silica capillary, the detection point being 500 mm from the point of injection. Data processing was performed by a Chromatopac C-R3A (Shimadzu, Kyoto, Japan). All experiments were carried out in a thermostatted oven at 35°C.

As a chromatographic solution, 0.10 *M* sodium dodecyl sulphate (SDS) (pH 7.0) was used. All other reagents were as described previously[1,2,4].

Procedure

In our system, the sample injection is carried out manually[1]: a sample solution was introduced into one end of the capillary tube by siphoning from sample solution

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at a higher level than the electrophoretic solution in which the other end of the tube was immersed. A sample mixture comprising phenol and five kinds of chlorinated phenols (Table I) was prepared by mixing appropriate volumes of methanol solutions of these compounds so that five different concentrations were obtained for each solute. Phenol was used as an internal standard, and the concentration of each chlorinated phenol relative to that of phenol was calculated.

TABLE I

CORRELATION COEFFICIENTS FOR THE PLOTS OF RELATIVE PEAK AREA OR RELATIVE PEAK HEIGHTS *vs.* RELATIVE CONCENTRATIONS

Micellar solution: 0.10 *M* SDS (pH 7.0). Separation tube: 650 mm × 0.05 mm I.D. Current: 50 μA. Temperature: 35°C. Phenol was used as the internal standard.

Solute	*Correltion coefficient*	
	Area	*Height*
2-Chlorophenol	0.9996	0.9956
2,5-Dichlorophenol	0.9983	0.9958
2,4,5-Trichlorophenol	0.9991	0.9982
2,3,4,5-Tetrachlorophenol	0.9992	0.9982
Pentachlorophenol	0.9995	0.9963

RESULTS AND DISCUSSION

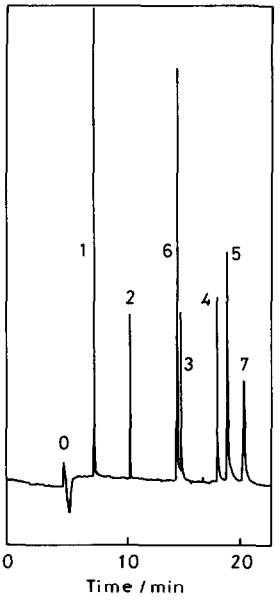
Quantitation

Since the sample volume at each injection seems to be imprecisely controlled in our system, quantitation by the internal standard method was examined with chlorinated phenols. Fig. 1 shows an example of an electrokinetic chromatogram of the test mixture. The dependence of the relative peak area, A_r, on the relative concentration, c_r, of each solute is shown in Fig. 2, where the concentration of phenol for $c_r = 1$ corresponds to 6.5 mg/ml, and each point is the average of three to five determinations. As is seen, good linear relationships between c_r and A_r were obtained over wide ranges of concentration. Similar relationships were observed between c_r and the relative peak height, H_r, but the linearity was slightly poorer for all the solutes. The correlation coefficients for these plots are shown in Table I. Thus, it may be better to use the relative peak area than the relative peak height for quantitative analysis by the internal standard method in electrokinetic chromatography.

A mixture of known amounts of 2,5-dichlorophenol and phenol was injected under the conditions given in Fig. 2, and the concentration of 2,5-dichlorophenol was calculated by using the plot shown in Fig. 2. The relative error was found to be 1.5%.

Reproducibility of retention time

In order to examine the reproducibility of retention times, the mean value, the standard deviation (S.D.) and the coefficient of variation (C.V.) of the retention time were calculated from chromatograms obtained by five repeated injections. The results



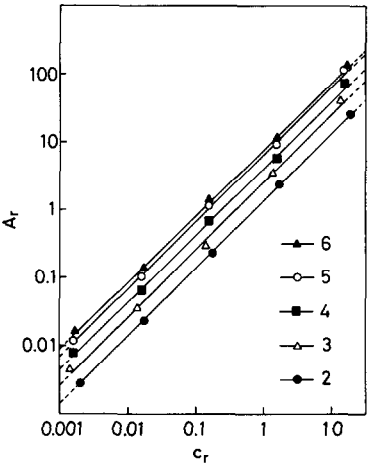


Fig. 1. Electrokinetic chromatogram of some chlorinated phenols: methanol (0); phenol (1); 2-chlorophenol (2); 2,4-dichlorophenol (3); 2,4,5-trichlorophenol (4); 2,3,4,5-tetrachlorophenol (5); pentachlorophenol (6); Yellow OB (7). Micellar solution: 0.10 *M* SDS (pH 7.0). Separation tube: 650 mm × 0.05 mm I.D. Length of tube used for separation: 500 mm. Current: 50 μA. Temperature: 35°C.

Fig. 2. Dependence of the relative peak area, A_r, on the relative concentration, c_r, of chlorinated phenols with respect to phenol. Conditions as in Fig. 1.

are shown in Table II. The retention time of methanol corresponds to that of an unretained solute, and that of Yellow OB which is a totally solubilized solute indicates the elution time of the micelle[2,4]. Although the S.D. tends to become large with increasing retention time, the C.V. is independent of the retention time. Values of the C.V. range from 0.3 to 1.2%, comparable to those obtained in conventional high-performance liquid chromatography.

Here, some polychlorinated phenols were considered to be partially ionized under the conditions used. The dependence of the retention times of these chlorinated phenols on pH has been discussed elsewhere[4]. Some neutral solutes were also examined to determine whether ionization of the solute affects the reproducibility of the retention time. A chromatogram of these solutes can be found in a previous paper[2]. The results are given in Table II. There was no significant difference in results between the samples of chlorinated phenols and neutral solutes.

It should be noted that all the data presented in this paper were obtained with an untreated fused-silica tube. We have already reported that electrokinetic phenomena, especially the electroosmotic velocity, can be controlled by treatment of the internal walls of a fused-silica capillary[9]. The reproducibility of retention times may also be affected by such treatment. Indeed, better results, *i.e.*, C.V. values of 0.2–0.25% for retention times, were reported for some phenols by using a methyl silicone-coated capillary[10].

TABLE II

REPRODUCIBILITY OF RETENTION TIMES OF CHLORINATED PHENOLS AND SOME NEUTRAL SOLUTES

Five repeated injections. Conditions as in Table I. The retention times of methanol and Yellow OB correspond to those of an unretained solute and the micelle, respectively[2,4].

Solute	*Mean (min)*	*S.D. (min)*	*C.V. (%)*
Phenol	7.44	0.080	1.10
2-Chlorophenol	10.7	0.101	0.95
2,5-Dichlorophenol	15.0	0.139	0.93
2,4,5-Trichlorophenol	18.1	0.171	0.95
2,3,4,5-Tetrachlorophenol	19.0	0.162	0.86
Pentachlorophenol	14.4	0.091	0.63
Methanol	4.84	0.015	0.31
Yellow OB	20.6	0.237	1.15
Resorcinol	6.57	0.025	0.38
p-Nitroaniline	10.4	0.062	0.60
Nitrobenzene	12.0	0.070	0.59
2-Naphthol	19.3	0.232	1.20

Reproducibility of peak area and peak height

As mentioned above, the sample injection was performed manually, using no mechanical or electrical device. To examine the precision of the sample size injected, the variances of the peak area and the peak height of each neutral solute mentioned above were calculated under a constant injection condition; that is, the difference in the siphoning levels between the sample solution and the SDS solution was held at 45 mm and the injection time at 50 s. The results are given in Table III as C.V. values. The reproducibility of this injection method was not good: C.V. = 1–8% for the peak area and 2–5% for the peak height.

Similar results for the reproducibility of peak heights in capillary electrophoresis were reported by Honda *et al.*[11] for manual sample introduction.

TABLE III

COEFFICIENTS OF VARIATION OF PEAK AREA AND PEAK HEIGHTS

Obtained by five repeated injections. Injection height: 45 mm. Injection time: 50 s. Other conditions as in Table I.

Solute	*C.V. (%)*	
	Area	*Height*
Resorcinol	4.4	1.7
Phenol	1.0	1.8
p-Nitroaniline	6.1	3.0
Nitrobenzene	3.4	1.5
2-Naphthol	7.6	5.2

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