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Migration behavior of dichlorophenols for replicate separations without replenishment of buffer electrolyte in micellar electrokinetic capillary chromatography

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Abstract

The migration behavior of dichlorophenols in replicate separations, without replenishment of buffer electrolyte between runs, in micellar electrokinetic capillary chromatography (MECC) was investigated. The results indicated that the pH of the buffer electrolyte drifts as a result of electrolysis of the buffer solution during electrophoresis and that the pH drift, being independent of the micelle concentration, is responsible for the variation in electrophoretic mobilities of analytes in MECC when the buffer is not replenished. The influences of the buffer pH and micelle concentration on the migration behavior of analytes were demonstrated to be correlated at small micelle concentrations. The variation of electrophoretic mobility of an analyte as a function of buffer pH at a given micelle concentration is quantitatively described. 2,6-Dichlorophenol is selected for illustration. The variation of electrophoretic mobility agrees satisfactorily between predicted and observed data.

Keywords: Buffer composition; Dichlorophenols

1. Introduction

Micellar electrokinetic capillary chromatography (MECC) extends the powerful technique of capillary zone electrophoresis (CZE) to separate efficiently charged and uncharged species present in a small sample volume (<10 μl) [1–5]. The advantages of high efficiency, high resolution and rapid analysis in CE have attracted the attention of researchers in various fields [5–12].

In MECC, buffer pH and surfactant concentration are two important separation parameters that can greatly affect the migration behavior of analytes [13,14]. Thus the variation of these parameters can affect the migration selectivity of analytes or improve the resolution of capillary electrophoretic separation.

The pH of the buffer varies during electrophoresis because reactions occur at the electrodes, causing the buffer solution in the anodic buffer vial to become more acidic and that in the cathodic buffer vial to become more alkaline [15]. The buffer pH also varies as a result of electrolysis taking place at the electrodes [16]. The pH of the background electrolyte drifts at the electrodes and the extent of the pH variation

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depends on the buffer capacity of the solution employed [17]. Therefore, the decreased pH in the buffer reservoir is responsible for the decreased migration times of analytes when samples are introduced into a capillary at the anodic end.

Previously, we investigated the migration behavior of isomeric dichlorophenols (DCPs) in MECC using sodium dodecyl sulfate (SDS) as an anionic surfactant in a phosphate–borate buffer solution [14]. The influences of buffer pH and micelle concentration on the migration of analytes were correlated at low SDS concentrations when the solute is partially dissociated. In the present experiments, we observed that the electrophoretic mobilities of analytes drifted when consecutive injections were performed without buffer replenishment. We sought to explain the variation in the electrophoretic mobility of dichlorophenols in those circumstances and present our results here.

2. Theoretical considerations on mobility

The electrophoretic mobility of an acidic solute in a MECC system can be quantitatively described as [18]

$$\mu = F^{m}_{HA}\mu_{mc} + F^{aq}_{A^-}\mu^{aq}_{A^-} + F^{m}_{A^-}\mu_{mc} \quad (1)$$

where μ is the electrophoretic mobility of an acidic solute, μ_{mc} is the mobility of micelles and μ_{A^-} is the mobility of A^- in the aqueous solution; F values with the subscripts or superscripts HA, A^-, mc and aq represent the mole fraction of a solute in the protonated and dissociated forms in micelles and water, respectively. In the protonated form (HA), the mobility of a solute is its micellar mobility ($F^{m}_{HA}\mu_{mc}$), and in the dissociated form (A^-), the mobility of an anionic solute is a result of its aqueous mobility ($F^{aq}_{A^-}\mu_{A^-}$) and micellar mobility ($F^{aq}_{A^-}\mu_{mc}$). The mole fraction of protonated species is defined as

$$F^{m}_{HA} = \frac{[HA]_m}{[HA]_m + [HA]_{aq} + [A^-]_m + [A^-]_{aq}} \quad (2)$$

By substituting $K^{m}_{HA}[HA]_{aq}[M]$ for $[HA]_m$, $K^{m}_{A^-}[A^-]_{aq}[M]$ for $[A^-]_m$ and $[HA]_{aq}K_a/[H^+]$ for $[A^-]_{aq}$, where K^{m}_{HA} and $K^{m}_{A^-}$ are the binding constants of HA and A^- to the micelles, respectively, K_a is the acid dissociation constant and [M] is the micelle concentration, one can define F^{m}_{HA} in terms of K_a, K^{m}_{HA}, $K^{m}_{A^-}$, [M] and $[H^+]$ through the equation

$$F^{m}_{HA} = \frac{K^{m}_{HA}[M]}{(1 + K^{m}_{HA}[M]) + (K_a/[H^+])(1 + K^{m}_{A^-}[M])} \quad (3)$$

where [M] is [S] − CMC, [S] is the total concentration of surfactant and CMC is the critical micelle concentration. Similar equations can be derived for $F^{m}_{A^-}$ and $F^{aq}_{A^-}$:

$$F^{m}_{A^-} = \frac{(K^{m}_{A^-}[M])(K_a/[H^+])}{T} \quad (4)$$

$$F^{aq}_{A^-} = \frac{K_a/[H^+]}{T} \quad (5)$$

where T is the denominator of Eq. 3.

The apparent dissociation constant in micellar solutes ($K_{a,app}$) and the two limiting mobilities of the protonated and the dissociated anionic species (μ_{HA} and μ_{A^-}) are defined, respectively, by the following equations:

$$K_{a,app} = \frac{K^{m}_{A^-}[M] + 1}{K^{m}_{HA}[M] + 1} \cdot K_a \quad (6)$$

$$\mu_{HA} = \frac{K^{m}_{HA}[M]\mu_{mc}}{1 + K^{m}_{HA}[M]} \quad (7)$$

and

$$\mu_{A^-} = \frac{\mu^{0}_{A^-} + K^{m}_{A^-}[M]\mu_{mc}}{1 + K^{m}_{A^-}[M]} \quad (8)$$

where $\mu^{0}_{A^-}$ is the mobility of a fully dissociated anionic solute in the absence of micelles in the aqueous solution. Therefore, the electrophoretic mobility of an acidic solute in a MECC system can be expressed as

$$\mu = \frac{\mu_{HA} + \mu_{A^-}(K_{a,app}/[H^+])}{1 + (K_{a,app}/[H^+])} \quad (9)$$

As show in Eqs. 7–9, the pH and micelle

concentration of the buffer solution are two important experimental parameters that can greatly influence the electrophoretic mobility of acidic solutes. Therefore, for a selected solute at a given micelle concentration, it is expected that the variation of the electrophoretic mobility of this solute depends on the pH drift of the buffer in a series of replicate separations.

3. Experimental

3.1. Chemicals and reagents

Six isomeric DCPs (Aldrich), sodium dihydrogenphosphate dihydrate (Showa Chemicals), SDS and anhydrous disodium tetraborate (Merck) and methanol (HPLC grade; Mallinckrodt) were obtained from the indicated suppliers; all other chemicals were of analytical-reagent grade and used as received. Sample solutions were prepared at a concentration about 20 ppm im methanol. The phosphate–borate buffer solution containing SDS surfactant was prepared by dissolving 0.5031 g of disodium tetraborate in 100 ml of 50 m*M* sodium dihydrogenphosphate solution containing 10, 8.2 or 5 m*M* SDS and adjusting the pH with NaOH (0.1 *M*) or HCl (0.1 *M*) to a desired value. All solutions were degassed with ultrasonic equipment and filtered through a membrane (0.22 μm) before use.

3.2. Apparatus

Capillary electrophoretic experiments were carried out on a capillary electrophoresis system (Spectra PHORESIS 1000; Spectra-Physics, Fremont, CA, USA), equipped with a thermostated fused-silica capillary cartridge (44 cm × 75 μm I.D.) and an autosampler. The temperature of the oven was set at 25°C. We measured the UV absorption of the analytes at 215 nm. The length of capillary between injection and detection was 37 cm. A 0.3-cm segment of polyimide coating was burned off the tubing 7 cm from the cathodic end before installation in a capillary cartridge. The CE system was interfaced to a microcomputer and printer (with software CE 1000, 1.05A). For pH measurements, a Suntex (Taipei, Taiwan) SP-701 pH meter was employed with a precision of ±0.01 pH unit.

3.3. Electrophoretic procedures

A series of replicate separations were carried out using the same anodic buffer vial in each run without replenishment between injections. The capillary was prewashed with anodic buffer for 5 min before injection.

To avoid experimental complication resulting from Joule heating, we limited the applied voltage to 10 kV to keep the current below 100 μA. Injection was performed in the hydrodynamic mode for 1 s. When changing the buffer electrolyte, the capillary was washed for 10 min with NaOH (0.1 *M*) at 60°C, followed by deionized and purified water for 5 min.

3.4. Calculation

The electrophoretic mobility of analytes was calculated from the observed migration time, described as

$$\mu_{ep} = \mu - \mu_{eo} = \frac{L_t L_d}{V}\left(\frac{1}{t_m} - \frac{1}{t_{eo}}\right) \quad (10)$$

where μ_{ep} is the electrophoretic mobility of the solute, μ is the apparent mobility, μ_{eo} is the electroosmotic mobility, t_m is the migration time measured directly from the electropherogram, t_{eo} is the migration time for an uncharged solute (methanol as neutral marker), L_t is the total length of the capillary, L_d is the length of the capillary between injection and detection and V is the applied voltage.

4. Results and discussion

Several workers have emphasized the importance of buffer replenishment to obtain a reasonably reproducible migration time with a non-coated capillary, because the pH of the buffer solution varied with no buffer replenishment [15–17]. To minimize the variation of buffer pH, the buffer at both electrodes is generally re-

plenished before each injection. We found previously [14] that the migration selectivity of dichlorophenols altered from a CZE pattern to a MECC pattern, depending on the micelle concentration and the pH of the buffer electrolyte, when we varied the SDS concentration from 0 to 30 m*M* in a phosphate–borate buffer at pH 7.72. The buffer was replenished and migration times of isomeric DCPs were acceptably reproducible with R.S.D.s less than 2%. However, with no buffer replenishment between runs, the migration times of DCPs varied continuously in a series of replicate separations.

Fig. 1 presents plots of the electrophoretic mobilities of six isomeric DCPs obtained with 10 m*M* SDS added to a phosphate–borate buffer solution and with an initial pH of 7.72 versus run number for 25 replicate separations. The migration behavior varies with each isomeric DCP. The electrophoretic mobility of 2,6-DCP towards the anode decreases with increasing run number, varying by ca. 22% between the first and 25th runs. The electrophoretic mobilities of 2,5-DCP and 2,3-DCP also decrease but those of 3,4-DCP and 3,5-DCP increase with increasing run number to a much smaller extent than for 2,6-DCP, whereas the electrophoretic mobility of 2,4-DCP fluctuates slightly from run to run.

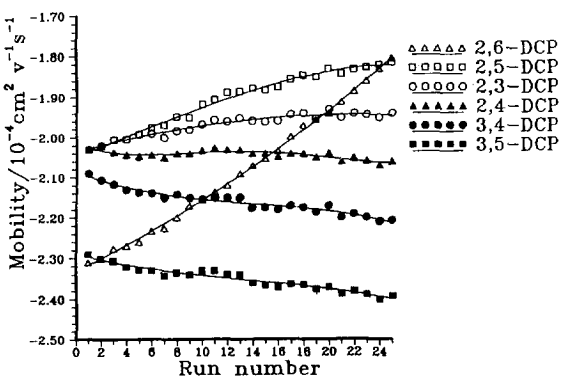

Fig. 1. Plots of electrophoretic mobilities of six isomeric DCPs obtained with SDS (10 m*M*) and initial pH 7.72 in a series of replicate separations without buffer replenishment between runs. Buffer: phosphate (50 m*M*)–borate (100 m*M*) with SDS (10 m*M*) and initial pH 7.72. Other operating conditions: 10 kV, 25°C. Capillary: 44 cm × 75 μm I.D. fused silica.

Fig. 2 shows typical electropherograms of DCPs obtained for runs 1, 9, 17 and 24 in a series of replicate separations. Migration patterns of DCPs similar to those for runs 9, 17 and 24 were observed when the pH was in the range 7.72–6.50 and when 10 m*M* SDS was added to the buffer solution with buffer replenishment between runs. Regardless of the magnitude of the migration mobilities of these solutes because of the presence of varied micelle concentrations, the migration patterns of DCPs obtained for runs 1, 9, 17 and 24 resemble those of DCPs obtained with buffer replenishment when SDS was added at concentrations of 10, 14, 16 and 18 m*M*, respectively, to the buffer at pH 7.72. These phenomena reveal that the influences of buffer pH and micelle concentration on the migration behavior of analytes are correlated in MECC.

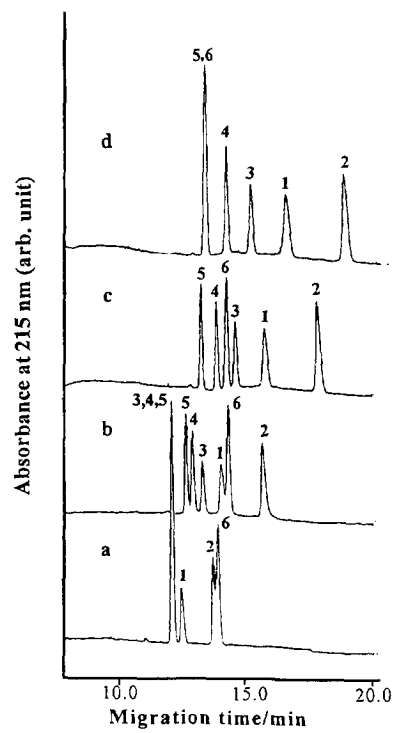

Fig. 2. Electropherograms of DCPs obtained for a series of replicate separations without buffer replenishment between runs: (a) run 1; (b) run 9; (c) run 17; (d) run 24. Peaks: 1 = 3,4-DCP; 2 = 3,5-DCP; 3 = 2,4-DCP; 4 = 2,3-DCP; 5 = 2,5-DCP; 6 = 2,6-DCP. Other operating conditions as in Fig. 1.

In order to discover whether the pH really drifted at the electrodes in the buffer vials, we measured the pH of the anodic buffer after each run. The pH of the cathodic buffer remains the same after each run because the Spectra PHORESIS 1000 is equipped with an automatic device to replenish the buffer in the cathodic vial. Fig. 3 shows the plot of buffer pH versus run number in a series of 25 replicate separations with 10 m*M* SDS added to a phosphate–borate buffer of initial pH 7.72. This experiment was performed in duplicate and the pH drifts were reproducible to within ±0.02 pH unit. The pH of the buffer in the anodic vial decreased continuously by about one pH unit during 500 min of electrophoretic separation at 10 kV. This result confirms previous findings [15–17] that the migration time of analytes depends upon the pH environment in the buffer reservoir and that the pH drifts in the phosphate–borate buffer solution during electrophoresis. The pH drifts of the buffer electrolyte containing SDS at 8.2 and 5.0 m*M* and initial pH 7.72 were also measured; plots of pH against run number are linear and almost coincide. Hence the pH drift of the buffer being independent of the micelle concentration is indicative. The pH drift of the buffer electrolyte as a result of electrolysis of the buffer solution during electrophoresis is responsible for the variation of electrophoretic mobilities of analytes in MECC when the buffer is not replenished.

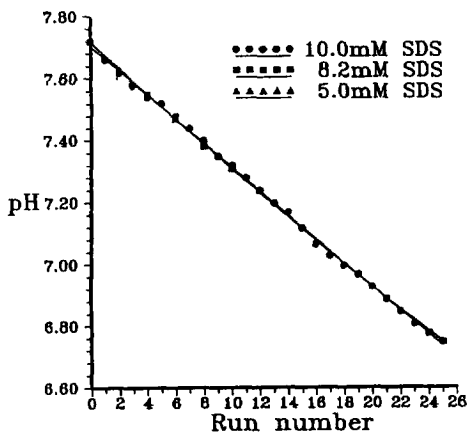

Fig. 3. Variation of buffer pH in an anodic vial for a series of replicate separations without replenishment of buffer between runs. Operating conditions as in Fig. 1.

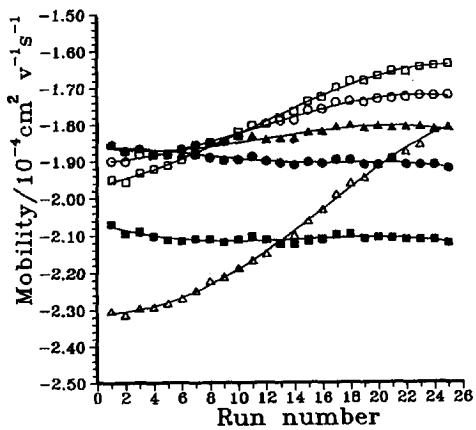

Fig. 4. Plots of the electrophoretic mobilities of six isomeric DCPs obtained with SDS (8.2 m*M*) and initial pH 7.72 in a series of replicate separations without replenishment of buffer between runs. Other operating conditions and symbols as in Fig. 1.

Fig. 4 shows plots of electrophoretic mobilities of DCPs versus run number for a series of 25 replicate separations without buffer replenishment under the same operating conditions as for Fig. 1, except that the SDS concentration was 8.2 m*M*. The plots of electrophoretic mobilities of DCPs versus run number after the eighth run in a series of replicate separations in Fig. 4 resemble those of DCPs in Fig. 1. Similar trends in the variation of electrophoretic mobilities of DCPs are observed in Figs. 1 and 4. Thus a decreased buffer pH at a given SDS concentration exhibits a similar effect on the migration behavior of DCPs as an increased SDS concentration at a given buffer pH. These results confirm that the buffer pH and micelle concentration are correlated separation parameters in MECC.

Fig. 5 shows plots of electrophoretic mobilities of DCPs versus run number for a series of 25 replicate separations without buffer replenishment under the same operating conditions as for Fig. 1, except that the SDS concentration was 5.0 m*M*. The electrophoretic mobilities of 2,6-, 2,5-, 2,3- and 2,4-DCP decreased with increasing run number, whereas those of 3,4- and 3,5-DCP remained almost invariant. The phenomena

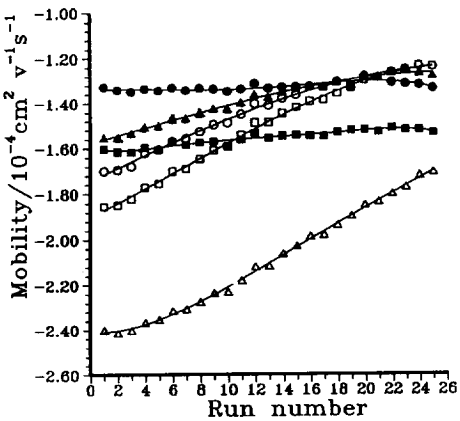

Fig. 5. Plots of the electrophoretic mobilities of six isomeric DCPs obtained with SDS (5 m*M*) and initial pH 7.72 in a series of replicate separations without replenishment of buffer between runs. Other operating conditions and symbols as in Fig. 1.

shown in Fig. 4 were essentially observable in Fig. 5. The migration patterns of these DCPs after the 14th run in a series of replicate separations resemble those of DCPs displayed in Fig. 4, whereas the migration patterns of DCPs after the 22nd run in a series of replicate separations resemble those of DCPs shown in Fig. 1. A similar conclusion is drawn for the correlated influences of decreased buffer pH at a given SDS concentration and increased SDS concentration at a given pH of the buffer on the migration behavior of DCPs.

In order to ascertain that the pH drift of the buffer is the origin of the variation of the electrophoretic mobility of DCPs for replicate separation with no buffer replenishment, electrophoretic mobilities of DCPs at various pH values with SDS at a given concentration should be evaluated with Eqs. 6–9. Table 1 presents all the parameters needed to predict the electrophoretic mobilities of six isomeric DCPs. According to Eq. 9, a sigmoidal curve for the migration behavior of each isomeric solute is predictable when electrophoretic mobilities are plotted against pH of the buffer at a given SDS concentration. It is also expected from Eqs. 7 and 8 that the difference between μ_{HA} and μ_{A^-} decreases as the SDS concentration increases from

Table 1
pK_a values and binding constants (K^m_{HA}) and ($K^m_{A^-}$) of dichlorophenols

Dichlorophenol	pK_a [a]	K^m_{HA} (M)[b]	$K^m_{A^-}$ (M)[b]
2,6-DCP	6.7	53	0
2,5-DCP	7.3	83	4
2,3-DCP	7.6	94	5
2,4-DCP	7.9	108	9
3,4-DCP	8.5	151	15
3,5-DCP	8.1	124	38

[a] From Ref. [19].
[b] From Ref. [14].

0 to 40 m*M* [14]. As an example, the plots of predicted and observed electrophoretic mobilities of 2,6-DCP as a function of buffer pH with an SDS concentration of 10 m*M* are shown in Fig. 6. The dashed line represents the variation of predicted values of the electrophoretic mobility, whereas the solid lines indicate the observed data obtained from the electropherograms of 2,6-DCP with and without buffer replenishment between runs. As illustrated in Fig. 6, the variation in electrophoretic mobility agrees satisfactorily between the predicted and observed values.

It should be noted that there is a small pH gradient along the capillary as a result of the

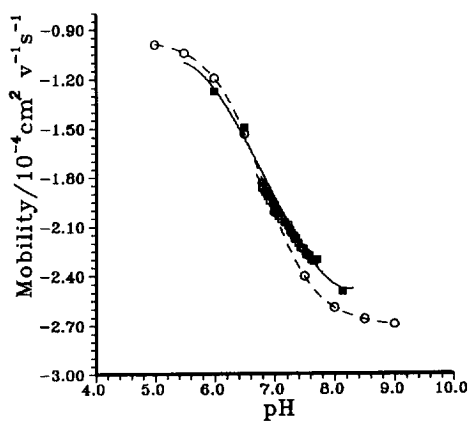

Fig. 6. Electrophoretic mobility of 2,6-DCP as a function of buffer pH with 10 m*M* SDS. (○) Predicted data; (■) observed values with buffer replenishment; □ = observed values without buffer replenishment. Operating conditions as in Fig. 1.

electrolytic reactions occurring at both electrodes during electrophoresis. However, the difference between the observed electrophoretic mobility with the pH gradient for 0.9 pH unit along the capillary and that without the pH gradient is less than $1.0 \cdot 10^{-6}$ $cm^2 V^{-1} s^{-1}$. Hence the discrepancy between them is insignificant.

5. Conclusion

In MECC, the migration behavior and selectivity of six isomeric DCPs varied continuously during a series of replicate separations with no replenishment of buffer electrolyte between runs. The migration behavior of DCPs varied with the pH of the buffer as a result of electrolysis of the buffer solution during electrophoresis. The influences of buffer pH and micelle concentration on the migration behavior and selectivity of DCPs are demonstrated to be correlated. The migration selectivity of DCPs varied from a pattern characteristic of CZE to a pattern characteristic of MECC in replicate separations with no replenishment of buffer electrolyte.

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