

## Migration behaviour and optimization of selectivity of dichlorophenols in capillary zone electrophoresis

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### Abstract

The migration behaviour and selectivity of six isomeric dichlorophenols were systematically investigated using capillary zone electrophoresis. The pH, concentration of background electrolyte and applied voltage were optimized to give the best separation of these isomeric compounds. The migration behaviour and selectivity of each compound were affected strongly by pH, but much less by the composition and concentration of the electrolyte. The dependence on pH of differences in electrophoretic mobilities between two consecutively migrating solutes was used to determine the optimum buffer pH for the separation. The results confirmed that this optimum pH agrees satisfactorily with that predicted from the mean value of the solute  $pK_a$ . The  $pK_a$  values of these six dichlorophenols are reported.

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### 1. Introduction

Capillary electrophoresis (CE) is a popular and powerful separation technique that possesses many advantageous features, such as high resolution, high efficiency of separation and rapid analysis [1–9]. It has been successfully applied to diverse analytical samples. In CE, the migration times, resolution and separation efficiency are governed by the applied voltage, electrophoretic mobility ( $\mu_{ep}$ ) of a charged species of interest and the electroosmotic flow ( $\mu_{eo}$ ). In practice, among several separation parameters that affect mobility, the pH generally plays an important role in CE separation as it determines the extent of ionization of each individual analyte. Therefore, manipulation of the buffer pH becomes a key strategy in optimizing separation. However,

the combined effects of other separation parameters, such as composition and concentration of the background electrolyte and of the applied voltage, must also be considered.

Chlorinated phenols are of great environmental concern because of their high toxicity. The separation of chlorinated phenols by CE has been investigated in several laboratories [10–16]. Terabe et al. [10] successfully resolved a mixture of nineteen chlorinated phenols by means of micellar electrokinetic capillary chromatography (MECC) using a fused-silica capillary with sodium dodecyl sulfate (SDS) in phosphate–borate solution at pH 7.0. Ong et al. [13] separated eleven priority phenols in a similar manner at pH 6.6. The separation of eight chlorinated phenols in industrial waste water was achieved by Gaitonde and Pathak [14] using capillary zone electrophoresis (CZE) with a phosphate–borate electrolyte at pH 8.0. The pH for separating

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eight substituted phenols with  $pK_a$  in the range 7.04–10.28 was optimized by Smith and Khaledi [15]; they proposed a mathematical model to predict the mobility of each solute as a function of pH, and suggested that the optimum pH can be predicted with a window diagram. However, based on the resolution of the worst resolved peak pair, the optimum pH was about 10.5, which is even greater than the corresponding  $pK_a$ . The optimized CZE separation of thirteen chlorinated phenols as a function of pH, concentration of the background electrolyte and applied voltage was investigated by Gonnord and Collet [16]; they obtained the best resolution at pH 6.9. As the pH is varied for effective separation of diverse chlorinated or substituted phenols in an empirical manner, questions about the optimum pH for obtaining the best resolution of chlorinated phenols and about a baseline separation arise.

In order to address these issues, one has to achieve a profound understanding of not only the migration behaviour of these solutes but also the exact role of important parameters that have significant effects on the migration and selectivity of the solutes considered. We therefore investigated systematically the effect of pH and other parameters on migration behaviour and resolution in the CE of chlorinated phenols, even though the migration and selectivity depend strongly only on pH. we chose six isomeric dichlorophenols as test solutes and determined their  $pK_a$  values. We describe here an approach to determine the optimum buffer pH for the separation of these dichlorophenols.

## 2. Experimental

### 2.1. Chemicals and reagents

All six isomeric dichlorophenols (DCP) were purchased from Aldrich, sodium dihydrogenphosphate dihydrate from Showa Chemicals, anhydrous disodium tetraborate from Merck and methanol (HPLC grade) from Mallickrodt. All other chemicals were of analytical-reagent grade and used as received.

Sample solutions were prepared at a concentration about 20 ppm in methanolic solution. Phosphate–borate buffer solution was prepared by mixing disodium tetraborate and sodium dihydrogenphosphate solution in an appropriate ratio. The pH of the buffer solution was then adjusted with sodium hydroxide (0.1 M) or hydrochloric acid (0.1 M) to the desired value. All solutions were degassed ultrasonically and filtered through a membrane filter (0.22  $\mu\text{m}$ ) before use.

### 2.2. Apparatus

Capillary electrophoretic experiments were carried out on a Model 1000 capillary electrophoresis system (Spectra-Physics, Fremont, CA, USA), equipped with a UV detector, a thermostated fused-silica capillary cartridge (either 44 cm  $\times$  75  $\mu\text{m}$  I.D. or 44 cm  $\times$  50  $\mu\text{m}$  I.D.) and an autosampler. The length of capillary between injection and detection was 37 cm. The CE system was interfaced with a microcomputer and printer with CE 1000 1.05A software. A Model SP-701 pH meter (Suntex, Taipei, Taiwan) was used to measure pH with an accuracy of  $\pm 0.01$  unit.

### 2.3. Procedure

All experiments were performed with phosphate–borate buffer systems suitable for the desired pH at 25°C and the measurements were run at least in triplicate to ensure reproducibility. An applied voltage of 10 kV was selected to keep the total current less than 100  $\mu\text{A}$  in order to avoid experimental complications resulting for Joule heating when using a 75  $\mu\text{m}$  I.D. capillary. Sample injections were made in the hydrodynamic mode. The sample solution was typically injected for 1 s. All measurements were monitored at 215 nm.

When a new capillary was used, the capillary was washed for 10 min with sodium hydroxide solution (1.0 M) at 60°C, followed by 10 min with sodium hydroxide solution (0.1 M) at 60°C and 5 min with deionized and purified water at

60°C. The capillary was prewashed for 5 min with the running buffer before each injection.

#### 2.4. Calculations

Electrophoretic mobilities of solutes were calculated with the equation

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

where  $\mu_{ep}$  is the electrophoretic mobility of the solute tested,  $\mu$  is the apparent mobility,  $\mu_{eo}$  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 capillary,  $L_d$  is the length of capillary between injection and detection and  $V$  is the applied voltage.

### 3. Results and discussion

#### 3.1. Optimization of separation

Optimization of the separation of dichlorophenols in CZE is achieved by controlling either the difference in the electrophoretic mobilities of solutes ( $\mu_{cp}$ ) or the difference between the mobility and electroosmotic flow ( $\mu_{eo}$ ). In practice, both  $\mu_{cp}$  and  $\mu_{eo}$  can be modified by varying the separation parameters. We considered the combined effects of pH, composition and concentration of background electrolyte and applied voltage on the mobilities of dichlorophenols to optimize the separation, which involves a compromise among resolution, speed and sensitivity. We discuss each parameter in turn.

##### Applied voltage

The experiments were conducted below the maximum operating voltage for the conditions of pH, composition and concentration of the buffer electrolyte; otherwise the temperature of the buffer increases, resulting in peak broadening and irreproducible data. Fig. 1 shows the current generated as a function of applied voltage for a chosen concentration of as phosphate–borate

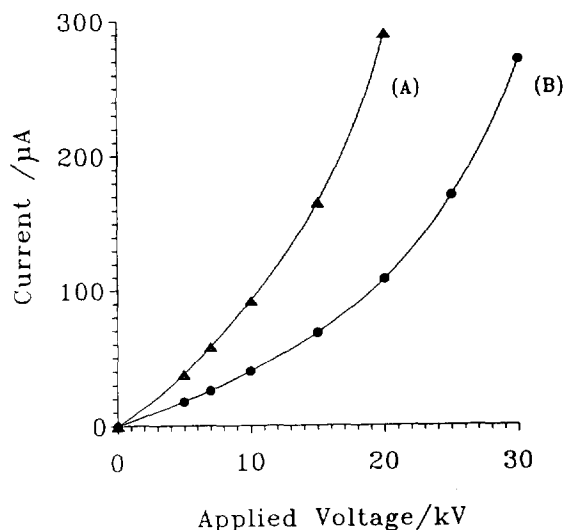


Fig. 1. Current generated as a function of applied voltage for 50 mM phosphate–100 mM borate buffer at pH 7.72 at 25°C. Fused-silica capillary: length, 44 cm; I.D. (A) 75 and (B) 50  $\mu\text{m}$ .

buffer system and a fused-silica capillary (length 44 cm, I.D. either 75 or 50  $\mu\text{m}$ ) to separate isomeric dichlorophenols at pH 7.72. In order to ensure the absence of complications due to Joule heating, we conducted all experiments at or below 10 kV when using a 75  $\mu\text{m}$  I.D. capillary or below 17 kV when using a 50  $\mu\text{m}$  I.D. capillary, so that the current generated was kept below 100  $\mu\text{A}$ . For this reason and because the number of theoretical plates obtained at 10 kV for a 75  $\mu\text{m}$  I.D. capillary has the largest value, we used an applied voltage 10 kV with a 75  $\mu\text{m}$  I.D. capillary.

##### Buffer composition

As the  $\text{p}K_a$  of dichlorophenols (DCPs) reported in the literature are in the range 6.5–8.5, a phosphate–borate buffer system was used as background electrolyte. Electropherograms of dichlorophenols obtained at various molar ratios of phosphate to borate are shown in Fig. 2. The migration order of the dichlorophenols is 3,4-DCP < 3, 5-DCP < 2, 4-DCP < 2, 3-DCP < 2, 5-DCP < 2,6-DCP. This order is consistent with that reported previously [16].

The effects of buffer composition on the

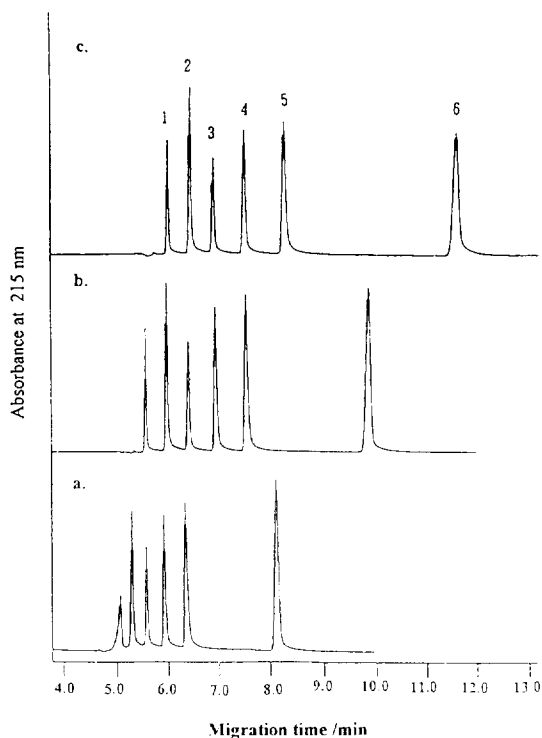


Fig. 2. Electropherograms of isomeric dichlorophenols obtained for various molar ratios of phosphate–borate of buffer composition: (a) 20:80; (b) 50:50; (c) 80:20. Total buffer concentration, 100 mM; buffer pH, 7.72 at 25°C; applied voltage 10 kV; fused-silica capillary, 44 cm  $\times$  75  $\mu$ m I.D. 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.

electroosmotic mobility and electrophoretic mobilities of each dichlorophenol are illustrated in Fig. 3. Both the electrophoretic and electroosmotic mobilities decrease when the ratio of phosphate to borate at a total buffer concentration of 100 mM varies from 20:80 to 80:20. As all dichlorophenols exhibit similar migration behaviour, variation of the electrolyte composition affects the relative selectivity only slightly. The difference between the electrophoretic mobility of each solute and the electroosmotic mobility decreased with increasing concentration of phosphate in the buffer solution. The conductivity increased linearly from  $5.10 \cdot 10^{-3}$  to  $6.64 \cdot 10^{-3}$  mho  $\text{cm}^{-1}$  before adjustment of the pH and from  $5.55 \cdot 10^{-3}$  to  $11.13 \cdot 10^{-3}$  mho  $\text{cm}^{-1}$  after adjustment of the pH to 7.72 when the molar ratio of

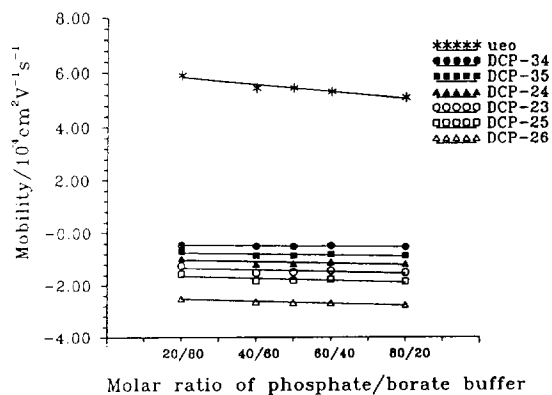


Fig. 3. Effect of buffer composition on electrophoretic mobility of dichlorophenols. Other conditions as in Fig. 2.

phosphate–borate buffer was varied from 20:80 to 80:20. Consequently, the migration times of dichlorophenols increased when the fraction of phosphate in the buffer solution was increased.

#### Buffer concentration

The variation of the electrophoretic mobility of dichlorophenols as a function of ionic strength of the buffer solution is shown in Fig. 4. The magnitudes of both the electroosmotic and electrophoretic mobilities of each isomeric dichloro-

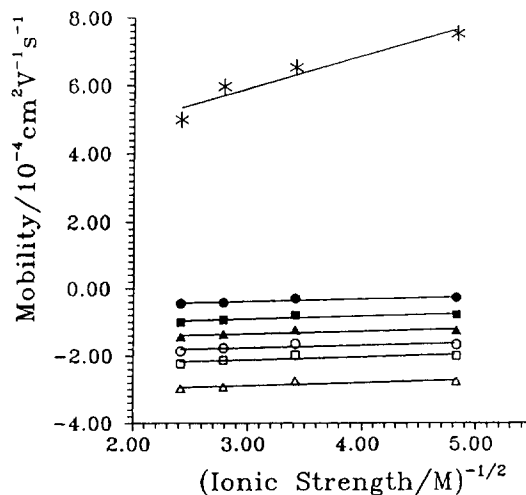


Fig. 4. Electrophoretic mobility as a function of ionic strength. Molar ratio of phosphate to borate, 1:2; buffer pH, 7.72; operating conditions, 10 kV, 25°C; fused-silica capillary, 44 cm  $\times$  50  $\mu$ m I.D. Symbols as in Fig. 3.

phenol decreased with increasing ionic strength. As the variation of mobility of each isomer was about the same, six straight lines nearly parallel to each other were observed. The migration times of dichlorophenols increased with increasing total concentration of phosphate and borate (Fig. 5).

#### Buffer pH

The electropherograms in Fig. 6 clearly illustrate that the selectivity of dichlorophenols was greatly affected by buffer pH in the range 6.29–9.63. Because at pH 6.29 we see a single feature for 3,4-DCP, 3,5-DCP and 2,4-DCP, these isomers co-migrate almost together, whereas 2,3-

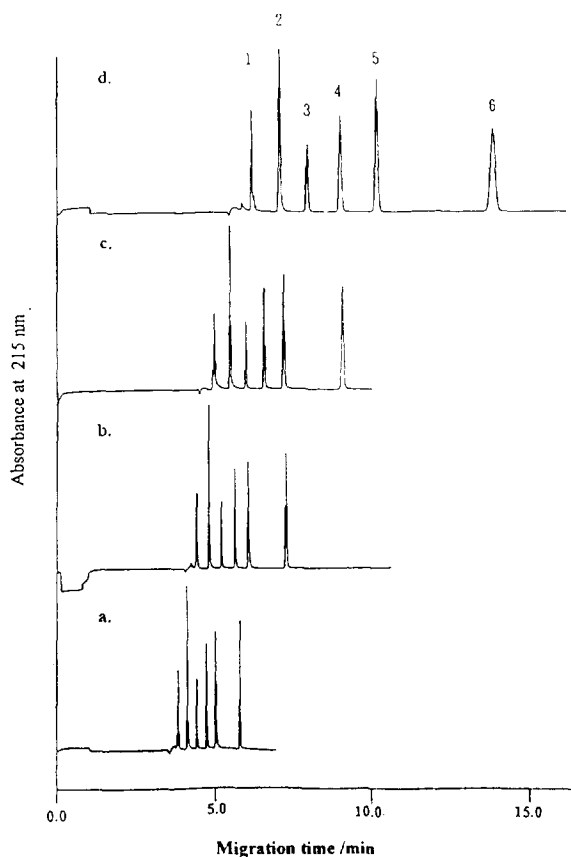


Fig. 5. Electropherograms of isomeric dichlorophenols obtained for various ionic strengths. Concentrations of phosphate/borate in phosphate–borate buffer (mM), (a) 12.5/25; (b) 25/50; (c) 37.5/75; (d) 50/100; other operating conditions as in Fig. 4. Peaks as in Fig. 2.

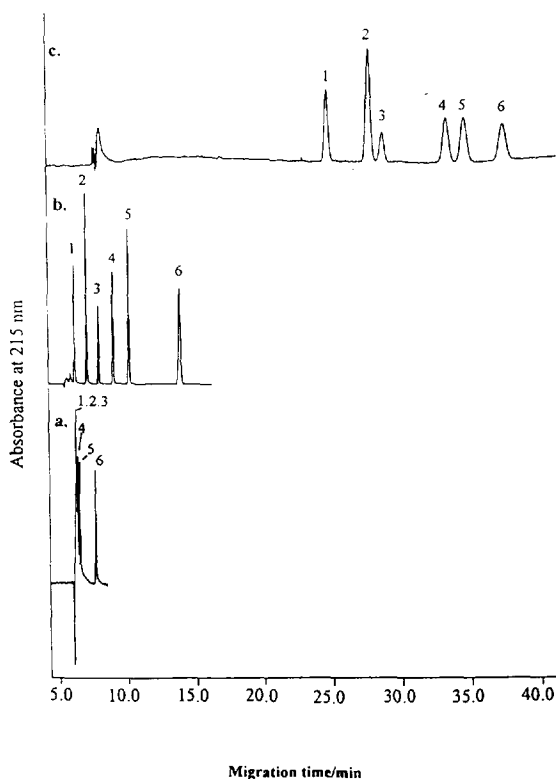


Fig. 6. Electropherograms of isomeric dichlorophenols obtained for various buffer pH values: (a) 6.29; (b) 7.72; (c) 9.63. Buffer composition, 50 mM phosphate–100 mM borate; fused-silica capillary, 44 cm  $\times$  50  $\mu$ m I.D.; other operating conditions and peak identification as in Fig. 2.

DCP, 2,5-DCP and 2,6-DCP isomers are barely separated. The resolution of the former three isomers improved with increasing buffer pH. Baseline separation of all six isomers was achieved when the buffer pH was in the range 6.7–9.6. At higher pH, the migration times of dichlorophenols were longer and electrophoretic peaks appeared broadened. This is mainly due to the longitudinal diffusion. Table 1 lists the mobility data for the six dichlorophenols; the relative standard deviation of the mobility was found to be less than 2%.

The migration behaviour of each dichlorophenol is described by the equation [17]

$$\mu_{ep} = \mu_{A^-} \left( \frac{K_a}{[H^+] + K_a} \right) \quad (2)$$

Table 1  
Mobilities ( $10^{-4} \text{ cm}^2 \text{ V}^{-1} \text{ s}^{-1}$ ) of dichlorophenols at various buffer pH values

pH	$\mu_{\text{eo}}$	$\mu_{\text{ep}}$					
		3,4-DCP	3,5-DCP	2,4-DCP	2,3-DCP	2,5-DCP	2,6-DCP
5.30	4.10	0.00	0.00	0.00	0.00	0.00	-0.14
5.80	4.31	0.00	0.00	0.00	0.00	-0.05	-0.34
6.14	4.32	0.00	0.00	0.00	-0.07	-0.13	-0.63
6.29	4.40	0.00	-0.02	-0.05	-0.11	-0.19	-0.81
6.70	4.49	-0.01	-0.08	-0.16	-0.29	-0.46	-1.39
6.99	4.56	-0.09	-0.22	-0.37	-0.56	-0.80	-1.82
7.35	4.60	-0.16	-0.39	-0.65	-0.95	-1.26	-2.20
7.72	4.52	-0.36	-0.77	-1.13	-1.15	-1.78	-2.47
8.14	4.34	-0.67	-1.22	-1.61	-1.94	-2.17	-2.58
8.53	4.24	-1.24	-1.83	-2.12	-2.37	-2.50	-2.71
9.11	4.05	-1.98	-2.24	-2.37	-2.54	-2.60	-2.70
9.63	3.69	-2.31	-2.45	-2.48	-2.62	-2.65	-2.71
10.10	3.37	-2.48	-2.53	-2.53	-2.64	-2.66	-2.70

where  $\mu_{\text{ep}}$  is the electrophoretic mobility of an isomeric dichlorophenol at a given pH,  $\mu_{\text{A}}$  is the mobility of the anionic form of the corresponding isomeric dichlorophenol and  $\text{p}K_{\text{a}}$  is the acid dissociation constant. Accordingly, a sigmoidal curved for the migration behaviour of each isomeric dichlorophenol is predictable when electrophoretic mobilities are plotted against buffer pH. Fig. 7 depicts the electrophoretic mobilities of six dichlorophenols as a function of buffer pH. At low pH ( $< \text{p}K_{\text{a}} - 2$ ), the electro-

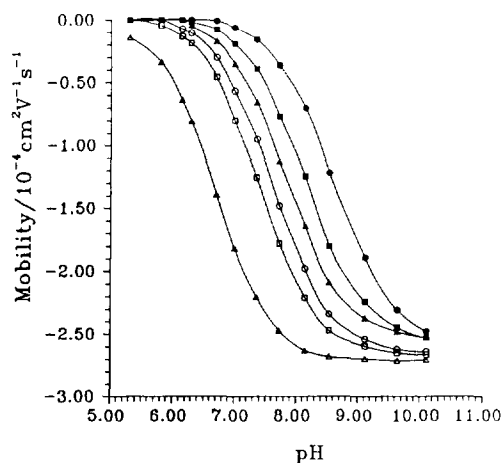


Fig. 7. Plots of electrophoretic mobility of dichlorophenols as a function of pH. Buffer, 50 mM phosphate–100 mM borate; fused-silica capillary, 44 cm  $\times$  50  $\mu\text{m}$  I.D.; other operating conditions as in Fig. 2. Symbols as in Fig. 3.

phoretic mobilities of dichlorophenols asymptotically approach zero, whereas at high pH ( $> \text{p}K_{\text{a}} + 2$ ), where the deprotonated form of the isomeric dichlorophenol becomes dominant, the mobilities approach their corresponding  $\mu_{\text{A}}$ -values. This electromigration behaviour is consistent with that observed for 2,5-DCP and 3,5-DCP by Smith and Khaledi [15].

Capillary electrophoresis is useful for determining the dissociation constants of acids [15] and bases [18]. With the aid of the plots in Fig. 7, one can easily determine the  $\text{p}K_{\text{a}}$  of each isomer of dichlorophenol, by estimating the best value of  $\mu_{\text{A}}$ - from measurement of the mobility of the anionic form of each isomeric dichlorophenol at high pH ( $> \text{p}K_{\text{a}} + 2$ ), and then measuring the pH corresponding to  $\mu_{\text{ep}} = \frac{1}{2} \mu_{\text{A}}$ - from the plot of  $\mu_{\text{ep}}$  versus pH. Hence, the  $\text{p}K_{\text{a}}$  of each isomeric dichlorophenol is equal to the buffer pH at  $\mu_{\text{ep}} = \frac{1}{2} \mu_{\text{A}}$ -. Table 2 gives the values of  $\text{p}K_{\text{a}}$  and  $\mu_{\text{A}}$ - determined for each isomeric species with values from the literature. The accuracy of the  $\text{p}K_{\text{a}}$  value is  $\pm 0.05$  unit.

Agreement between these two sets of data is satisfactory. We predicted the mobility of each isomeric dichlorophenol over the pH range 5.30–10.10 with Eq. 2 and calculated the  $\text{p}K_{\text{a}}$  and  $\mu_{\text{A}}$ -values. The predicted and actual mobilities are plotted in Fig. 8. The slope (1.0007) and the

Table 2  
 $pK_a$  and  $\mu_{A^-}$  values for dichlorophenol isomers

Solute	$pK_a$		$\mu_{A^-}$ ( $10^{-4} \text{ cm}^2 \text{ V}^{-1} \text{ s}^{-1}$ )	
	This work	Others	This work	Others <sup>b</sup>
3,4-DCP	8.50	8.62 <sup>a</sup>	-2.48	-
3,5-DCP	8.10	8.25 <sup>a</sup> , 7.98 <sup>b</sup>	-2.53	-2.83
2,4-DCP	7.90	7.90 <sup>b</sup>	-2.53	-
2,3-DCP	7.60	7.71 <sup>a</sup>	-2.64	-
2,5-DCP	7.30	7.51 <sup>a</sup> , 7.32 <sup>b</sup>	-2.66	-3.06
2,6-DCP	6.70	6.78 <sup>a</sup>	-2.70	-

<sup>a</sup> From Ref. [15].

<sup>b</sup> From Ref. [13].

correlation coefficient ( $r = 0.9995$ ) indicate an excellent correlation between predicted and actual mobilities. Hence the values of  $pK_a$  and  $\mu_{A^-}$  are demonstrated to be reliable.

### 3.2. Optimization of selectivity

Even if the six isomeric dichlorophenols are easily separated in a suitable range of buffer pH, it is still of interest to determine the optimum pH in an alternative way, based on optimizing the overall difference in the effective mobilities of these dichlorophenols.

An explicit equation for optimum pH of the

background electrolyte to separate two weak acids is [3]

$$\text{pH}_{\text{opt}} = \frac{1}{2} (pK_{a_1} + pK_{a_2}) - \log \frac{(\sqrt{\mu_{A_2^-}/\mu_{A_1^-}} - \sqrt{K_{a_2}/K_{a_1}})}{1 - \sqrt{(\mu_{A_2^-}K_{a_2})/(\mu_{A_1^-}K_{a_1})}} \quad (3)$$

where subscripts 1 and 2 refer to two solutes to be separated. Hence, if the  $pK_a$  and ionic mobilities are known, the optimum buffer pH can be calculated. For substances with equal ionic mobilities, such as positional isomers, the equation becomes simplified to

$$\text{pH}_{\text{opt}} = \frac{1}{2} (pK_{a_1} + pK_{a_2}) \quad (4)$$

Based on the  $pK_a$  values in Table 2, the optimum buffer pH values for the five pairs of consecutively migrating isomeric dichlorophenols are 8.35, 8.00, 7.74, 7.52 and 7.04, respectively, in the order of increasing migration time. For instance, the optimum pH for the pair 2,5-DCP and 2,6-DCP is predicted to be 7.04. Similarly, the optimum pH predicted for the six isomeric dichlorophenols is 7.61, which is the mean of the  $pK_a$  values of 3,4-DCP and 2,6-DCP.

Fig. 9 shows plots of differences in electrophoretic mobilities between each two consecutively migrating isomeric dichlorophenols ( $\Delta\mu_{ep}$ ) against buffer pH. The curves exhibit maxima at  $\text{pH} \approx 7.0, 7.5, 7.8, 8.0,$  and  $8.4$  for the five pairs of consecutively migrating isomers of dichloro-

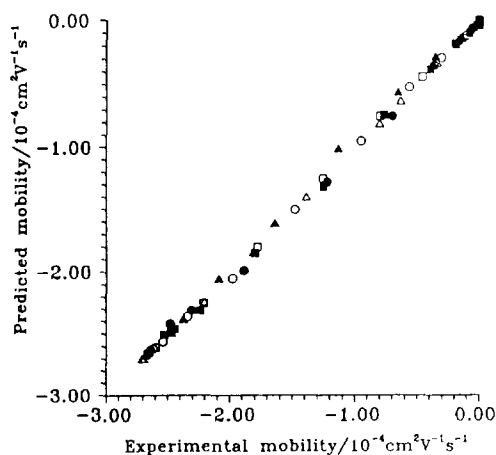


Fig. 8. Predicted and experimental mobilities for six isomeric dichlorophenols over the pH range 6.7–9.1. Symbols as in Fig. 3.

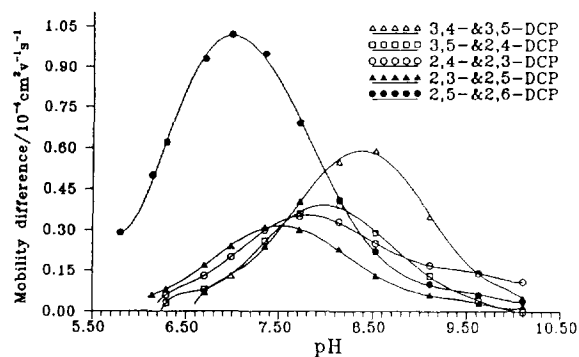


Fig. 9. Plots of differences in electrophoretic mobilities between two consecutively migrating isomeric dichlorophenols against buffer pH. Other conditions as in Fig. 7.

phenol. Thus, the optimum pH for resolving all six isomeric dichlorophenols is about 7.6–7.7, because the overall difference of effective mobilities of the six isomers is maximized. Table 3 summarizes the predicted and observed optimum pH values for each pair of consecutively migrating isomers and for all six isomeric dichlorophenols. The experimental results agree satisfactorily with the predicted values.

The resolution evaluated on the basis of the observed electropherograms at various buffer pH clearly indicates that, if the difference in electrophoretic mobilities between any two consecutively migrating isomers is not less than  $6 \cdot 10^{-6} \text{ cm}^2 \text{ V}^{-1} \text{ s}^{-1}$ , baseline separation of all isomeric dichlorophenols is achieved. This condition occurs at a buffer pH in the range 6.7–9.6. The minimum electrophoretic mobility difference for

Table 3  
Predicted and experimental optimum pH values of dichlorophenols

Migrating pair	Predicted value <sup>a</sup>	Experimental value <sup>b</sup>
3,4- and 3,5-DCP	8.35	8.40
3,5- and 2,4-DCP	8.00	8.00
2,4- and 2,3-DCP	7.74	7.80
2,3- and 2,5-DCP	7.52	7.50
2,5- and 2,6-DCP	7.04	7.00
3,4- and 2,6-DCP	7.61	7.6–7.7

<sup>a</sup> Based on the equation  $\text{p}K_{\text{a,opt}} = \frac{1}{2}(\text{p}K_{\text{a}_1} + \text{p}K_{\text{a}_2})$ .

<sup>b</sup> Determined from Fig. 9.

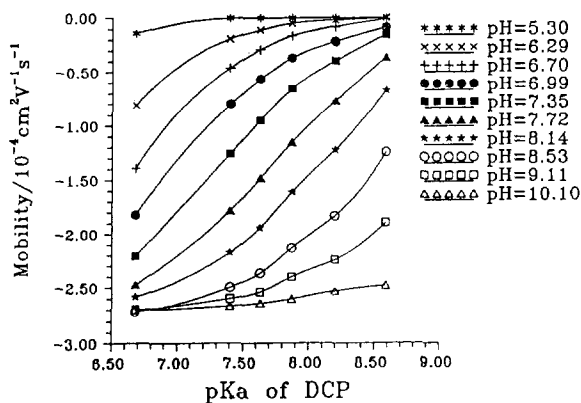


Fig. 10. Plots of electrophoretic mobility of dichlorophenols at a given buffer pH versus  $\text{p}K_{\text{a}}$ .

baseline resolution was evaluated on the basis of the equation  $R_s = 2\Delta w / (w_1 + w_2)$ , where  $R_s = 1.5$  is the resolution for baseline separation,  $w_1$  and  $w_2$  are the two peak widths at the base and  $\Delta w$  is the minimum difference in migration times for the pair of consecutively migrating isomers.  $\Delta w$  was then converted into  $\Delta\mu_{\text{ep}}$  to obtain the minimum difference in electrophoretic mobility for baseline separation.

To confirm that the present approach is suitable for determining the optimum buffer pH, the mobility data for six isomeric dichlorophenols at a given buffer pH were plotted against their corresponding  $\text{p}K_{\text{a}}$  values. Fig. 10 shows such plots obtained by treating the buffer pH as a parameter. The curves change from a concave to a convex shape as the pH is increased from 5.3 to 10.10. At pH 7.72, the curve is nearly a straight line when the overall difference in effective mobilities of the six isomers approaches its maximum value. As this pH falls in the predicted range of optimum buffer pH, this nearly straight line between the concave and convex conditions indicates the optimum condition.

#### 4. Conclusion

The electromigration behaviour and selectivity of six isomeric dichlorophenols were greatly affected by the buffer pH in capillary zone electrophoresis. In addition to the investigation



of the buffer pH, the effects of composition and concentration of the buffer electrolyte and of applied voltage on the electrophoretic mobility of these solutes were studied to optimize the separation. Baseline separation of these dichlorophenols was achieved at 10 kV with a phosphate–borate buffer with pH in the range 6.7–9.6. The optimum buffer pH determined for the best resolution of the dichlorophenols is about 7.7, in agreement with the prediction from the mean of  $pK_a$  values of 2,6-DCP and 3,4-DCP.

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