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Short communication

Migration behavior and separation of trichlorophenols by capillary zone electrophoresis

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Abstract

The influence of buffer pH on the migration behavior and separation of isomeric trichlorophenols (TCPs) was systematically investigated using capillary zone electrophoresis. Complete separation of isomeric trichlorophenols was achieved with a phosphate–borate buffer in the pH range 5.8–8.0 at 10 kV. The present results confirm that the optimum pH condition is indicated by the change in the shape of curves from concave to convex in the plots of electrophoretic mobilities of trichlorophenols at some given pH (roughly in the range $pK_a \pm 2$) versus the pK_a value of each corresponding isomer. At this optimum pH, electrophoretic mobility of isomeric trichlorophenols correlates linearly with their pK_a values. The similarity of such correlations in different pH ranges for trichlorophenols and dichlorophenols illustrates the dependence of the electrophoretic mobility of a chlorophenol isomer on both pK_a and the molecular size. The pK_a values of five isomeric trichlorophenols are reported.

Keywords: Trichlorophenols; Chlorophenols; Phenols

1. Introduction

Chlorophenols are of great environmental concern because of their high toxicity and tendency for bioaccumulation. Various modes of capillary electrophoresis, including capillary zone electrophoresis (CZE) [1–4], micellar electrokinetic chromatography (MEKC) [5,6], and capillary isotachophoresis (cITP) [7] have been applied to the separation and determination of chlorophenols. In our previous work, the migration behavior and selectivity of six isomeric dichlorophenols were systematically investigated using CZE [4]. Accurate pK_a values of these six isomers were reported. The optimum pH condition was indicated by the change in the shape of curves from concave to convex in the plots of electrophoretic mobilities of dichlorophenols at some given pH (in the range 5.30–10.10) versus the pK_a value of each corresponding isomer. Since the influence of buffer pH on the migration behavior and selectivity of trichlorophenols (TCPs) has never been systematically investigated, and the pK_a values of trichlorophenols reported in the literature do not

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satisfactorily agree, it would be worthwhile to report the results of our work on isomeric trichlorophenols.

2. Experimental

2.1. Chemicals and reagents

Trichlorophenols (2,3,4-TCP, 2,3,6-TCP and 2,4,5-TCP from TCI, 2,3,5-TCP from Aldrich and 2,4,6-TCP from Riedel-de Haen) were purchased. All other chemicals were of analytical-reagent grade. Deionized water was prepared with a Milli-Q system (Millipore, Bedford, MA, USA).

Standard solutions of trichlorophenols were prepared at a concentration of about 20 ppm in methanolic solution. The pH of the phosphate (60 m*M*)–borate (40 m*M*) buffer solution was adjusted with sodium hydroxide (0.1 *M*) or hydrochloric acid (0.1 *M*) to a desired value. All solutions were filtered through a membrane filter (0.22 μm) before use.

2.2. Apparatus

Separations were carried out on a Model 1000 capillary electrophoresis system (Spectra-Physics, Fremont, CA, USA), as described previously [4].

2.2.1. Electrophoretic procedure

All experiments were performed using a fused-silica capillary with phosphate–borate buffer systems suitable for the desired pH at 25 °C and measurements were run at least in triplicate to ensure reproducibility. An applied voltage of 10 kV was selected and the total current was kept below 100 μA in order to avoid Joule heating. Sample injections were made in the hydrodynamic mode. The sample solution was typically injected for 2 s. All measurements were monitored at 215 nm.

When a new capillary was used, the capillary was washed for 2 h with 1.0 *M* NaOH at 60 °C, followed by 0.5 h with deionized water at 25 °C. The capillary was prewashed for 3 min with running buffer before each injection and postwashed for 2 min with deionized water, 3 min with NaOH (0.1 *M*) and 2 min with deionized water to maintain proper reproducibility of run-to-run injections.

2.2.2. Calculations

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

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

where μ_{ep} is the electrophoretic mobility of the solute tested, 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 (mesityl oxide 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. Effect of buffer pH

The migration behavior of each isomeric trichlorophenol at various pH can be described by the equation

$$\mu_{ep} = \left(\frac{K_a}{[H^+] + K_a}\right)\mu_A^- \quad (1)$$

where μ_{ep} is the electrophoretic mobility of an isomeric trichlorophenol at a given pH, μ_A^- is the mobility of the anionic form of the corresponding isomeric trichlorophenol and pK_a is the acid dissociation constant. Accordingly, a sigmoidal curve for the migration behavior of each isomeric trichlorophenol is predictable when electrophoretic mobilities are plotted against buffer pH. Fig. 1 depicts the electrophoretic mobilities of five isomeric trichlorophenols as a function of buffer pH in the range 4.48–9.50.

Fig. 2 shows the electropherograms of trichlorophenols obtained at pH 5.80, 6.48 and 8.00 with a phosphate (60 m*M*)–borate (40 m*M*) buffer solution at 10 kV. As illustrated, effective separation of isomeric trichlorophenols was achieved in the pH range 5.80–8.00. The migration order follows 2,3,4-TCP <2,4,5-TCP <2,3,5-TCP <2,4,6-TCP <2,3,6-TCP. This is consistent with that observed by Gonnord and Collet [2].

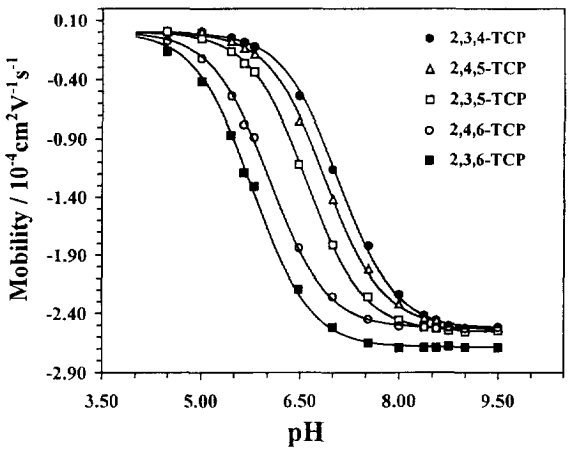

Fig. 1. Plots of the electrophoretic mobility of trichlorophenols as a function of buffer pH. Buffer, phosphate (60 m*M*)–borate (40 m*M*); fused-silica capillary, 44 cm×50 μm I.D.; applied voltage, 10 kV; 25 °C.

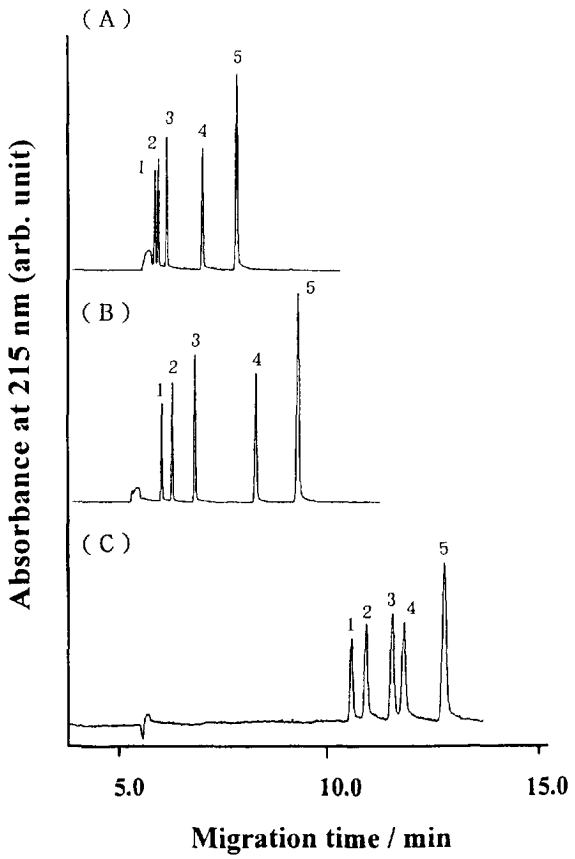

Fig. 2. Electropherograms of isomeric trichlorophenols obtained for various buffer pH values: (A) 5.80; (B) 6.48; (C) 8.00. Operation conditions as for Fig. 1. Peaks: 1=2,3,4-TCP; 2=2,4,5-TCP; 3=2,3,5-TCP; 4=2,4,6-TCP; 5=2,3,6-TCP.

As in the case of dichlorophenols [4], the resolution evaluated from the observed electropherograms at pH 8.00 and 5.80 in Fig. 2 clearly indicates that baseline separation of all isomeric trichlorophenols is achievable as long as the difference in electrophoretic mobilities between any two consecutively migrating isomers is not less than $6\cdot10^{-6}$ cm^2 V^{-1} s^{-1}. Accordingly, complete separation of isomeric trichlorophenols is achievable in the pH range 5.80–8.00. At pH >9.50, where isomeric trichlorophenol dissociates almost completely, the mobility approaches its corresponding μ_A^- value for each isomeric species. On the other hand, the electrophoretic mobility of each isomeric trichlorophenols, except 2,4,6- and 2,3,6-TCP, asymptotically reaches 0 at pH 4.48.

3.2. Determination of pK_a values

Capillary electrophoresis has been applied as a convenient method for precise pK_a determination [1,4,8,9]. With the aid of the plots in Fig. 1, the pK_a of each isomeric trichlorophenol can be easily determined either by curve-fitting the experimental mobility data as a function of buffer pH through the utilization of Sigmaplot software or by estimating the limiting values of μ_A^- and then measuring the pH corresponding to $\mu_{ep}=1/2\ \mu_A^-$ from plots of Fig. 1. Table 1 gives the values of pK_a, and μ_A^- determined by curve-fitting for each isomeric species, together with the pK_a values from the literatures. The precision of pK_a determined is within ±0.03 unit. The μ_A^- value of 2,3,6-TCP is 5–6% higher than those of other isomers. This is probably due to the slightly larger ratio of charge to mass.

The pK_a values of 2,4,6-TCP and 2,3,6-TCP determined in this work agree very well with those obtained by Ugland et al. [10], whereas the pK_a values of 2,3,4-TCP and 2,4,5-TCP fall in between those obtained by Ugland et al. [10] and those by Li et al. [11]. We predicted the mobility of each isomeric trichlorophenol over the range 4.48–9.50

Table 1
The pK_a and μ_A^- values for trichlorophenol isomers

Solute (TCPs)	pK_a				μ_A^{-a}
	This work	Others[b]			
		A	B	C	
3,4,5	–	7.55	7.57	7.74	–
2,3,4	7.09	6.97	7.34	7.59	−2.56
2,4,5	6.89	6.72	7.20	7.33	−2.53
2,3,5	6.60	–	6.92	–	−2.55
2,4,6	6.04	5.99	6.51	6.42	−2.52
2,3,6	5.78	5.80	6.10	6.12	−2.69

[a] μ_A^- in unit of 10^{-4} cm^2 V^{-1} s^{-1}.
[b] A from ref. [10]; B from ref. [11]; C from ref. [12].

with Eq. (1) and the calculated pK_a and $\mu_A -$ values. The correlation coefficient (r) between predicted and actual mobilities is 0.999. Hence, the values of pK_a and μ_A^- are demonstrated to be reliable.

3.3. Optimization of selectivity

As described previously [4], the optimum buffer pH for the separation of trichlorophenols can be estimated by plotting electrophoretic data for five isomeric trichlorophenols at some given buffer pH in a certain pH range against their corresponding pK_a values. Fig. 3 shows such plots obtained by treating buffer pH as a parameter in the pH range 4.48–8.38. The shape of the curves changes from concave to convex as the pH increased from 4.48 to 8.38; a

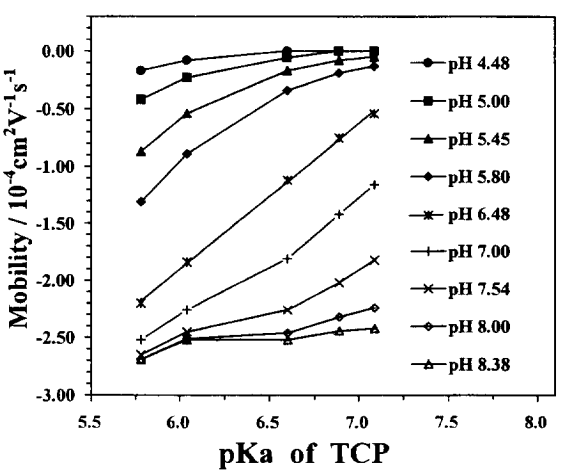

Fig. 3. Plots of electrophoretic mobility of trichlorophenols at some given buffer pH versus pK_a.

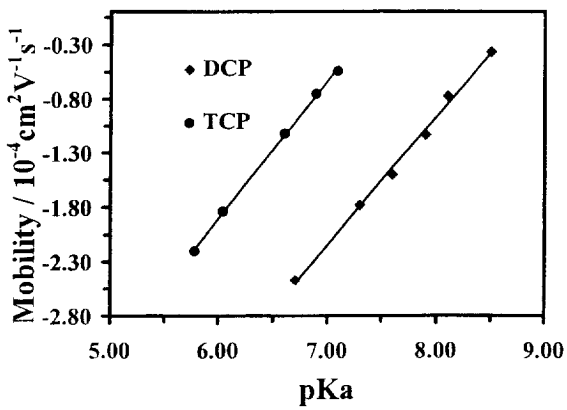

Fig. 4. Correlation plots of the electrophoretic mobility versus pK_a value for trichlorophenols (TCPs) and dichlorophenols (DCPs) at the optimum pH.

nearly straight line was obtained at pH around 6.48 and the overall difference in electrophoretic mobilities of isomeric trichlorophenols was found to approach the maximal value. As this pH falls in the predicted range of optimum buffer pH, this nearly straight line between the concave and convex curves indicates the approximation of the optimum pH condition. Thus, the present results provide further evidence to support previous findings that, at the optimum pH, the overall difference in the electrophoretic mobility of isomeric trichlorophenols in a fixed pH range reaches its maximum and that the electrophoretic mobility correlates linearly with the pK_a value of each corresponding isomeric species.

It is of interest to note that, as shown in Fig. 4, the correlation plots at optimum pH for trichlorophenols and dichlorophenols yield two straight lines which are nearly parallel to each other. The similarity of such correlations in two different pH ranges for trichlorophenols and dichlorophenols clearly indicates the dependence of the electrophoretic mobility of isomeric chlorophenols on both pK_a value and the size of molecule.

4. Conclusion

Baseline separation of five isomeric trichlorophenols is achievable using a phosphate–borate buffer in the pH range 5.80–8.00. The optimum pH is determined to be 6.48. At this optimum pH, the

electrophoretic mobility of each isomeric trichlorophenol was found to correlate linearly with its pK_a value. The present results demonstrate the dependence of the electrophoretic mobility of an isomeric chlorophenol on both pK_a value and its molecular size. The determination of pK_a values of trichlorophenols in this work is helpful in clarifying the inconsistency of literature values.

Acknowledgments

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