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Migration behavior and separation of benzenediamines, aminophenols and benzenediols by capillary zone electrophoresis

Ching-Erh Lin*, Yu-Tai Chen

Department of Chemistry, National Taiwan University, Taipei 106, Taiwan

Abstract

The migration behavior and separation of five benzendiamines, five aminophenols and three benzenediols were investigated in capillary zone electrophoresis. The results indicate that benzendiamines and aminophenols are optimally separated with a phosphate buffer at pH 5, whereas benzenediol isomers are best separated at pH about 12. The addition of surfactant monomers of tetradecyltrimethylammonium bromide to a phosphate buffer at pH 5 under the conditions of reversed electroosmotic flow is effective for separating these dye intermediates, except for the separation of 1,2-benzenediol from 1,3-benzenediol. The addition of sodium tetraborate as an electrolyte modifier is effective in the separation of 1,2-benzenediol from 1,3-benzenediol, but the latter comigrates with the 1,4-benzenediol isomer at pH 5.0. The electrophoretic mobility of ionized analytes can be described with Offord's equation, and the migration order depends on their ratios of charge to mass. In addition, the pK_a values of these analytes in 50 m*M* phosphate buffer are reported.

Keywords: Buffer composition; Benzenediamines; Aminophenols; Benzenediols; Amines; Phenols; Dyes

1. Introduction

Oxidative hair dyes usually contain a mixture of dye intermediates which include benzendiamines, aminophenols and benzenediols. As the color of hair is greatly affected by the content and composition of dye intermediates in a hair dye, a simple and rapid analytical method is needed for quality control and performance evaluation of commercial products. Moreover, dye intermediates used in a hair dye are of great health concern because allergic dermatitis due to 1,4-benzenediamine and nefrotoxic effect due to aminophenols have been reported [1]. As most of the dye intermediates are toxic compounds [2] and some dye intermediates are even carcinogenic [3,4], the development of new analytical methods to separate and analyze dye intermediates is certainly desirable.

Various chromatographic methods, including GC [5,6], HPLC [7–11], and capillary electrophoresis (CE) [12,13], have been used for the separation and determination of oxidative dye intermediates in permanent hair colorants. Among them, CE is a very attractive and powerful separation technique owing to its many advantageous features, such as extremely high efficiency, high resolution, rapid analysis, small consumption of sample and solvent and little matrix interferences, in comparison with HPLC. However, reports on the capillary electrophoretic separation of these dye intermediates are few [12–14]. Separation of three benzenediamines and three aminophenols was studied with isotachophoresis [12]. More recently, a mixture of fourteen dye intermediates was separated by micellar electrokinetic chromatography

*Corresponding author.

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(MEKC) using relatively high concentrations of sodium dodecyle sulfate and cetyltrimethylammonium chloride as surfactants at alkaline pH [13]. In our previous paper [15], the simultaneous separation of five benzenediamines, three benzenediols and five aminophenols in oxidative hair dyes by MEKC using cationic surfactants at acidic pH was reported. Complete separation of thirteen dye intermediates was satisfactorily achieved with the addition of tetradecyltrimethylammonium bromide (TTAB) and hexadecyltrimethylammonium bromide (CTAB) at a concentration of 18 m*M* and 13 m*M*, respectively, in a phosphate buffer at pH 5.0. To the best of our knowledge, the separation of these dye intermediates by capillary zone electrophoresis (CZE) has not been reported. We present here the results of our investigation by CZE in a phosphate buffer at pH 5 and 12 with an applied voltage of 20 kV, and with the addition of TTAB at a concentration below the critical micelle concentration at pH 5 under the conditions of reversed electroosmotic flow (EOF) at −20 kV. In addition, the pK_a values of these analytes are reported.

2. Experimental

2.1. Chemicals and reagents

Thirteen dye intermediates shown in Fig. 1, and TTAB were purchased from TCI (Tokyo, Japan). Sudan III were obtained from Aldrich (Milwaukee, WI, USA). Highly sulfated β-cyclodextrin (HS-β-CD) was obtained from Beckman (Fullerton, CA, USA) as a gift. All other chemicals were of analytical-reagent grade from various suppliers. Deionized water was prepared with a Milli-Q system (Millipore, Bedford, MA, USA).

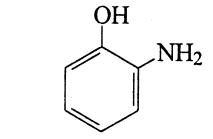
Standard stock solutions of dye intermediates at a concentration of 1000 μg/ml were prepared in a 50% (v/v) methanolic solution containing sodium sulfite (0.2%) in brown bottles and were stored in a refrigerator at 4°C. When needed, various concentrations of sample solution ranging from 10 to 50 μg/ml were prepared by dilution from the stock solution. The pH of the phosphate buffer was adjusted by mixing various proportions of a certain concentration of sodium dihydrogenphosphate solution with the same concentration of disodium hydrogenphosphate or phosphoric acid solution in order to adjust the pH to a desired value in the range 3.5–7.0. All solutions were filtered through a membrane filter (0.22-μm) before use.

2.2. Apparatus

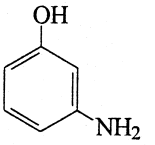
Separation were made with a capillary electrophoretic system (Spectra-Physics model 1000, Fremont, CA, USA), equipped with a programmable and high-speed scanning multiple-wavelength UV–visible detector, a fused-silica capillary cartridge thermostated with a Peltier thermoelectric device, and an automatic injection system. The capillary dimensions were 43 cm×50 μm I.D., purchased from Polymicro Technologies (Tucson, AZ, USA), or 67 cm×50 μm I.D. as indicated. The UV detection position is 7.0 cm from the cathodic end. Sample injection was done in a hydrodynamic mode during 1 s. The CE system was interfaced with a microcomputer and printer with software CE 1000 1.05A. For pH measurements, a pH meter (Suntex Model SP-701, Taipei, Taiwan) was employed with a precision of ±0.01 pH unit.

2.3. Electrophoretic procedure

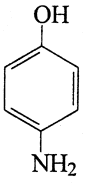
When a new capillary was used, the capillary was washed using a standard sequence described previously [16]. The procedure was 10 min with deionized water at 60°C, 60 min with 1.0 *M* NaOH at 60°C and then 10 min with deionized water at 25°C. To ensure reproducibility, all experiments were performed at 25°C and measurements were run at least in triplicate. The capillary was prewashed with deionized water at 25°C for 5 min, followed subsequently with sodium hydroxide solution (1.0 and 0.1 *M*) at 60°C for 10 min, and then with deionized water at 25°C for 10 min to maintain proper reproducibility for run-to-run injections everyday, and the capillary was washed with running buffer for 5 min before each injection. The detection wavelength was set at 200 nm or at 220 nm under the conditions of reversed EOF. Mesityl oxide (MO) was used as neutral marker.



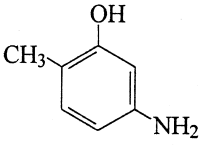
2-aminophenol (8)



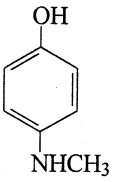
3-aminophenol (10)



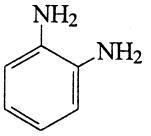
4-aminophenol (4)



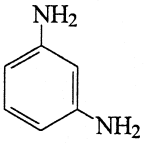
6-methyl-3-aminophenol (7)



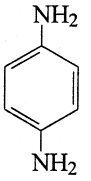
4-(methylamino)phenol (3)



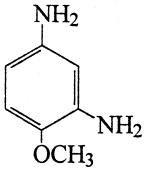
1,2-benzenediamine (9)



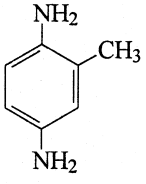
1,3-benzenediamine (6)



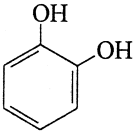
1,4-benzenediamine (1)



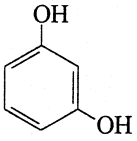
4-methoxy-1,3-benzenediamine (5)



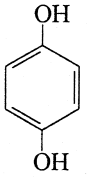
2-methyl-1,4-benzenediamine (2)



1,2-benzenediol (11)



1,3-benzenediol (12)



1,4-benzenediol (13)

Fig. 1. Structures of thirteen dye intermediates studied.

2.4. Calculation of electrophoretic mobility

The electrophoretic mobility of analytes was calculated from the observed migration times with the equation:

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

where μ_{ep} is the electrophoretic mobility of the analyte 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 unchanged solute, 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. Prediction of migration behavior of selected solutes

Structures of the selected benzenediamines, benzenediols and aminophenols are shown in Fig. 1. Benzenediamines possess two amino groups with pK_{a_2} and pK_{a_1} values in the range of 4.6–6.3 and 1.8–3.0, respectively [2,17]. Benzenediols possess two hydroxy groups with pK_{a_1} and pK_{a_2} values in the range 9.2–9.9 and 11.0–13.0, respectively [2]. Aminophenols have one amino group with pK_a values in the range 4.3–6.5 and one hydroxy group with pK_a values in the range 9.8–11.8 [2,17]. Hence, benzenediamines exist as cationic species at pH below about 7 (or as neutral species at pH above about 7); benzenediols exist as neutral species at pH above about 7.5 (or as anionic species at pH below about 7.5). Aminophenols exist as cationic, neutral or anionic species, depending on whether buffer pH is below 7, in between 7 and 8, or above 8, respectively. As the pK_a values of benzenediamines are greatly different from those of benzenediols, it is impossible to separate simultaneously these two structural types of analytes at a single buffer pH by CZE without the addition of charged complexing agents or electrolyte modifiers.

In CZE, the effective electrophoretic mobility (μ_{eff}) of a protonated basic solute (BH^+) as a function of buffer pH can be described by the following relationship [18,19]:

$$\mu_{eff} = \alpha_{BH^+}\mu_{BH^+} = \frac{[H_3O^+]}{[H_3O^+] + K_a} \cdot \mu_{BH^+} \quad (2)$$

where μ^+_{BH} and α^+_{BH} are the electrophoretic mobility and mole fraction of the protonated form of a basic solute (BH^+), and K_a is the acid dissociation constant of BH^+. Likewise, for a diprotonated basic solute (BH_2^{2+}), such as benzenediamines, which involves the following two dissociation equilibria:

$$BH_2^{2+} \overset{K_{a_1}}{\rightleftharpoons} BH^+ \overset{K_{a_2}}{\rightleftharpoons} B$$

the effective electrophoretic mobility of such a basic solute is then given by:

$$\mu_{eff} = \alpha_{BH_2^{2+}}\mu_{BH_2^{2+}} + \alpha_{BH^+}\mu_{BH^+} = \frac{[H_3O^+]^2\mu_{BH_2^{2+}} + K_{a_1}[H_3O^+]\mu_{BH^+}}{[H_3O^+]^2 + K_{a_1}[H_3O^+] + K_{a_1}K_{a_2}} \quad (3)$$

where $\mu_{BH_2^{2+}}$ and μ_{BH^+} are the electrophoretic mobility of BH_2^{2+} and BH^+, respectively.

Similarly, for a diprotic acidic solute (H_2A), such as benzenediols, which involves the following two dissociation equilibria:

$$H_2A \overset{K_{a_1}}{\rightleftharpoons} HA^- \overset{K_{a_2}}{\rightleftharpoons} A^{2-}$$

the effective electrophoretic mobility of such an acidic solute can be expressed as:

$$\mu_{eff} = \alpha_{HA^-}\mu_{HA^-} + \alpha_{A^{2-}}\mu_{A^{2-}} = \frac{K_{a_1}[H_3O^+]\mu_{HA^-} + K_{a_1}K_{a_2}\mu_{A^{2-}}}{[H_3O^+]^2 + K_{a_1}[H_3O^+] + K_{a_1}K_{a_2}} \quad (4)$$

where μ_{HA^-} and $\mu_{A^{2-}}$ are the electrophoretic mobily of HA^- and A^{2-}, respectively.

On the other hand, for an amphoteric solute ($HABH^+$) possessing both acidic and basic characters, such as aminophenols, which involves the following two dissociation equilibria:

$$HABH^+ \overset{K_{a_1}}{\rightleftharpoons} HAB \overset{K_{a_2}}{\rightleftharpoons} BA^-$$

the effective electrophoretic mobility of such an amphoteric solute is given by:

$$\mu_{eff} = \alpha_{HABH^+}\mu_{HABH^+} + \alpha_{BA^-}\mu_{BA^-} = \frac{[H_3O^+]^2\mu_{HABH^+} + K_{a_1}K_{a_2}\mu_{BA^-}}{[H_3O^+]^2 + K_{a_1}[H_3O^+] + K_{a_1}K_{a_2}} \quad (5)$$

where μ_{HABH^+} and μ_{BA^-} are the electrophoretic mobility of $HABH^+$ and BA^-, respectively. It should be noted that μ_{HABH^+} is a positive value and μ_{BA^-} is a negative value. At $pH \leq pK_{a_1} - 2$, $HABH^+$ being the only existing species that is ionized, the electrophoretic mobility of this analyte can be approximately described with the equation:

$$\mu_{ep} = \frac{[H_3O^+]\,\mu_{HABH^+}}{[H_3O^+] + K_{a_1}} \quad (6)$$

At $pH \geq pK_{a_2} + 2$, BA^- being the only species that ionized, the electrophoretic mobility of the analyte is approximately given by:

$$\mu_{ep} = \frac{K_{a_2}\mu_{BA^-}}{[H_3O^+] + K_{a_2}} \quad (7)$$

Accordingly, the migration behavior of each individual analytes can be predicted using Eqs. (3), (4) or (5), provided that the pK_a values and the necessary mobility data are available.

3.2. Optimization of separation

3.2.1. Effect of buffer pH

In CZE, manipulation of buffer pH becomes a key strategy to optimize a separation of ionizable analytes because buffer pH determines the extent of the ionization of each individual analyte [20,21]. It is well known that the charge of the capillary wall surface and the zeta potential are influenced by buffer pH [22–24]. Thus, the negative charge is built up at the surface with increasing buffer pH. In fact, the magnitude of electroosmotic mobility (μ_{eo}) changes from $6.5\cdot10^{-5}$ at pH 3.0 to $5.6\cdot10^{-4}$ $cm^2\,V^{-1}\,s^{-1}$ at pH 8.0, and then leveling off in the pH range 10.0–12.0 with μ_{eo} equal to $6.0\cdot10^{-4}$ $cm^2\,V^{-1}\,s^{-1}$.

The variations of electrophoretic mobility of benzenediamines, benzenediols and aminophenols as a function of buffer pH in the range 2.0–12.0 are separately illustrated in Figs. 2A–2C. Fig. 2D shows the variation of electrophoretic mobility of all of the analytes selected using a phosphate buffer (50 m*M*) at varied pH in the range 2.0–12.0. Sigmoidal behavior for the variation of the mobility was observed. The trends in the variation of the electrophoretic mobility of three different structural types of analytes as a function of buffer pH are in accordance with the prediction of migration behavior based on Eqs. (3)–(5). As illustrated, the optimum pH for the separation of benzenediamines and aminophenols and for the separation of benzenediols are 5 and 12, respectively.

Fig. 3 shows the electropherograms of the thirteen analytes obtained at pH 5.0 and pH 12.0 using a 43 cm×50 μm I.D. capillary. As illustrated, three isomers of benzenediol were well separated at pH 12.0. As shown in Fig. 4, the resolution of peaks among analytes (7), (8), and (9) was markedly improved using a 67 cm×50 μm I.D. capillary, and complete separation of five benzenediamines and five aminophenols was achieved in CZE with the addition of 1.2 m*M* TTAB to the phosphate buffer under the conditions of reversed EOF at −20 kV using a 67 cm×50 μm I.D. capillary.

3.2.2. Addition of complexing agents or electrolyte modifiers

Simultaneous separation of benzenediamines aminophenols and benzenediol isomers at acidic pH was attempted by adding TTAB monomers (1.2–1.6 m*M*), highly sulfated-β-CD (HS-β-CD) (0.2–1.0%), or sodium tetraborate (10–50 m*M*) into the phosphate buffer solution.

By adding TTAB at a concentration below the CMC to the buffer solution, 1,2- and 1,3-benzenediols are separated from 1,4-benzenediol under the conditions of reversed EOF. The separability increases with increasing TTAB concentration and a baseline separation of all three isomers of benzenediol is achievable with the addition of 5 m*M* TTAB or 2 m*M* of cetyltrimethylammonium bromide [14]. However, within the framework of CZE separation, 1,2-benzenediol and 1,3-benzenediol are barely separated with the addition of TTAB at a concentration of 1.6 m*M* which is very close to the CMC value of TTAB at pH 5.0.

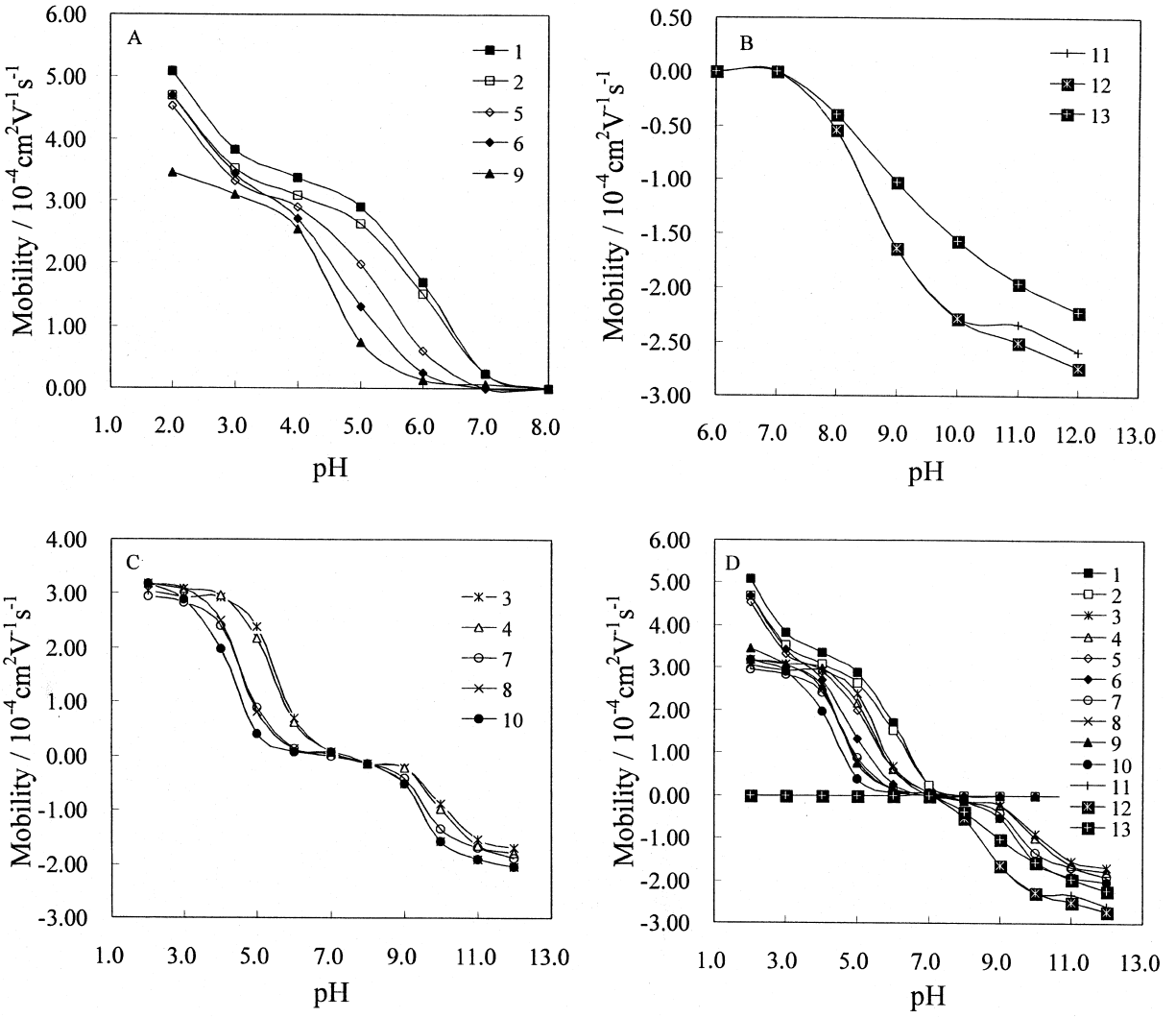


Fig. 2. Variation of the electrophoretic mobility of dye intermediates as a function of buffer pH in a phosphate buffer (50 m*M*) at pH 5.0. (A) benzenediamines, (B) benzenediols, (C) aminophenols, (D) all analytes. Capillary: 43 cm×50 μm I.D. Other operating conditions: 20 kV, 25°C. Curve identification: the numbers denote the analytes shown in Fig. 1.

The addition of charged β-CD such as HS-β-CD into the buffer solution would enhance the separation of 1,4-benzenediol from 1,2- and 1,3-benzenediol. Unfortunately, the resolution is deteriorated by peak broadening when adding more than 0.5% (w/v) of HS-β-CD into the buffer solution.

The use of phosphate-borate buffer was considered for separating 1,2-benzenediol from 1,3- and 1,4-benzenediols because 1,2-benzenediol can form anionic complexes with borate. Indeed, the addition of borate (30 m*M*) to phosphate buffer (50 m*M*) is effective in the separation of 1,2-benzenediol from 1,3- and 1,4-benzenediol isomers, but 1,3- and 1,4-benzenediol isomers are not resolvable.

3.3. Determination of pK_a values

Capillary electrophoresis is applied as a convenient method for precise pK_a determination [19–21,25–28]. In order to determine properly the pK_a

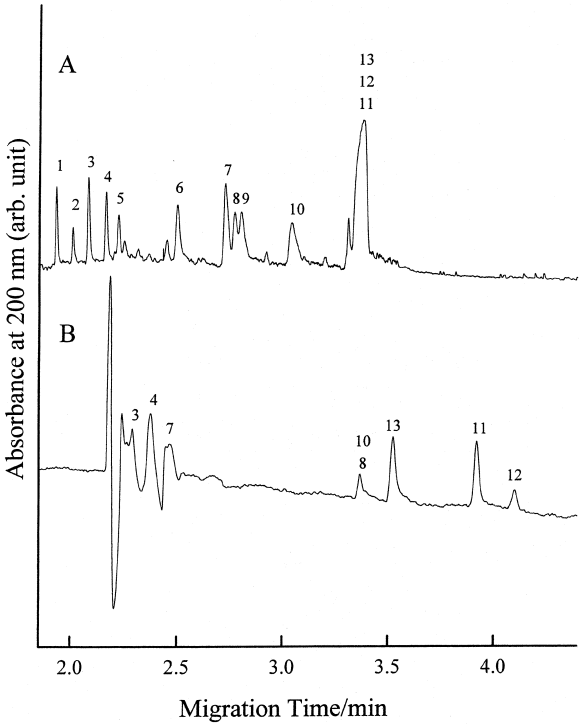


Fig. 3. Electropherograms of dye intermediates obtained with phosphate buffer (50 m*M*) at optimum buffer pH: (A) pH 5.0 (for the separation of benzenediamines and aminophenols), (B) pH 12.0 (for the separation of benzenediols). Other operating conditions and peak identification are the same as for Fig. 2.

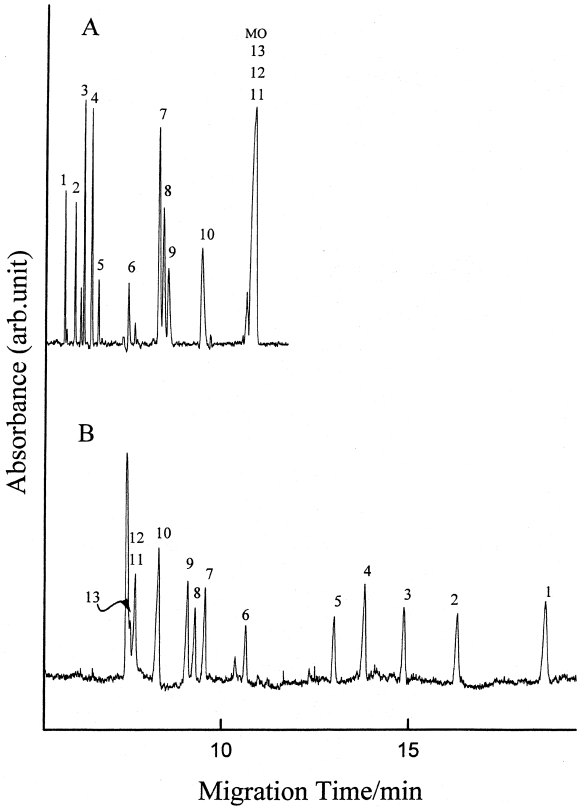


Fig. 4. Electropherograms of thirteen dye intermediates obtained with phosphate buffer (50 m*M*) at pH 5.0 using a 67 cm×50 μm I.D. capillary: (A) at 20 kV, (B) with the addition of 1.2 m*M* TTAB in the buffer solution under the conditions of reversed EOF at −20 kV.

values of benzenediamines, benzenediols and aminophenols, we measured electrophoretic mobility of these analytes over a wide range of pH. From the plots of electrophoretic mobility versus buffer pH, the values of four parameters (two pK_a values and two limiting mobilities) involved in Eqs. (3)–(5) for each individual analyte were estimated. The two pK_a values and the two limiting mobilities of each individual analyte are then determined by varying the trial values of these four parameters until the predicted mobility curve is best fitted to the experimental mobility curve through the utilization of EXCEL software. Fig. 5 shows the agreement of the mobility curves obtained for these thirteen analytes. As can be seen, the agreement between the predicted mobility curves (represented by solid lines) and the observed mobility curves (shown by data points) is very good, except at the two pH extremes because the experimental data are difficult to obtain. Table 1 lists the pK_a values and the mobility data evaluated, together with the literature pK_a values reported [2,17]. With the exception of 4-methylaminophenol, the evaluated and literature pK_a values of these analytes are in good agreement.

To evaluate the thermodynamic pK_a value (p$'K_a$) from the conditional pK_a value, the activity coefficients of ionized species and ionic strength of the buffer solution should be taken into consideration. The relationships between p$'K_a$ and pK_a for anionic and cationic species are given in Eqs. (8) and (9), respectively [29]

$$p'K_a = pK_a + \frac{0.51z^2\sqrt{I}}{1 + 1.5\sqrt{I}} \quad \text{(for anionic species)} \quad (8)$$

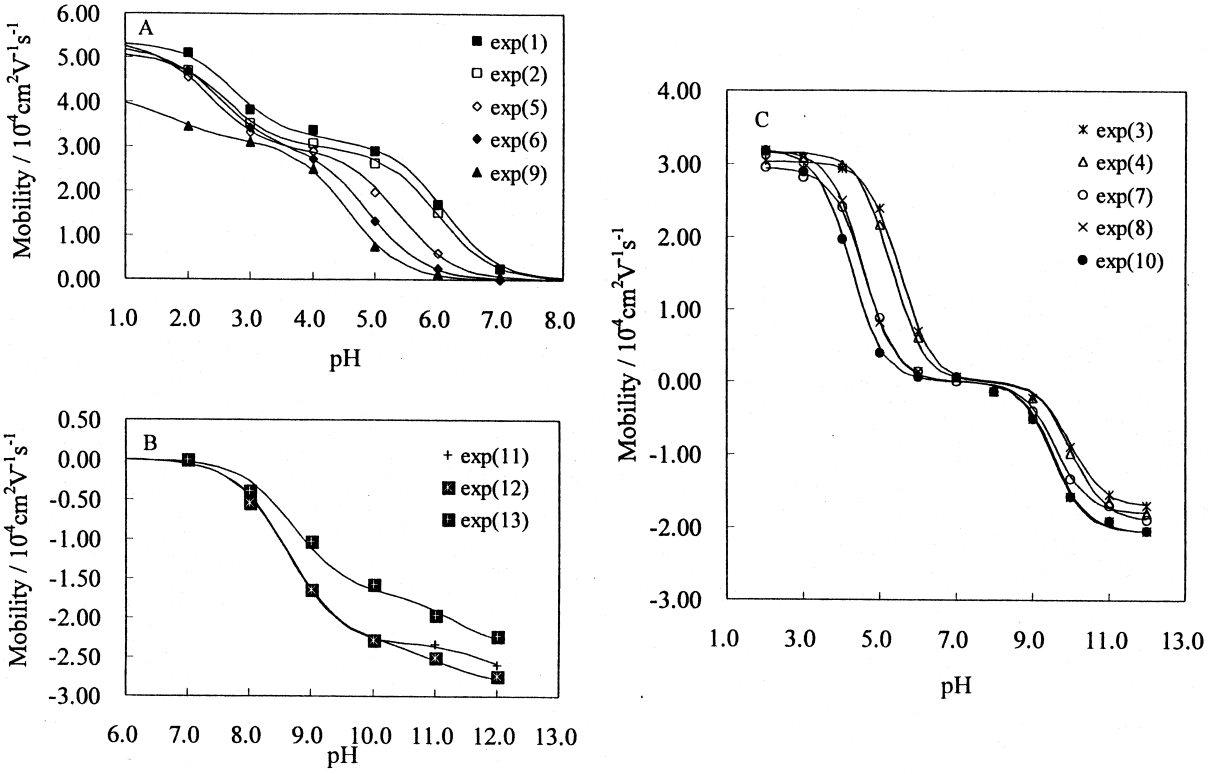


Fig. 5. The agreement between the predicted mobility curves (represented by solid lines) and observed mobility curves (shown by data points) for three different structural types of analytes: (A) benzenediamines, (B) benzenediols, and (C) aminophenols.

$$p'K_a = pK_a - \frac{0.51z^2\sqrt{I}}{1+1.5\sqrt{I}} \quad \text{(for cationic species)} \quad (9)$$

where I is the ionic strength of the buffer electrolyte and z is the charge of the ionized species. The $p'K_a$ values estimated for these analytes are also listed in Table 1 (the numbers in parentheses).

3.4. Migration order

The net charge (q) of ionized species of each analytes at pH 5.0 was calculated as the sum of the products of mole fraction (α) and charge for the singly-charged and doubly-charged species, according to the equation.: $q = \alpha_1(\pm 1) + \alpha_2(\pm 2)$, where α_1 and α_2 are the mole fractions of the singly-charged and doubly-charged species, respectively. Offord's parameter ($q/M^{2/3}$), where M is the molar mass of each analyte, was then evaluated [27,28]. As shown in Fig. 6, a linear relationship with correlation coefficient (R^2) equal to 0.9909 was obtained by plotting electrophoretic mobility against $q/M^{2/3}$ value for benzenediamines and aminophenols. The results clearly demonstrate that the migration order of these analytes is determined by their ratios of charge to mass ($q/M^{2/3}$).

4. Conclusion

Benzenediamines and aminophenols are effectively separated using a phosphate buffer at acidic pH by CZE. The optimum buffer pH for the separation is about 5, whereas that for benzenediol isomers is at pH 12. Thus, the determination of dye intermediates in oxidative hair dyes by CZE is feasible, although simultaneous separation of three different kinds of analytes is difficult at a single buffer pH. The

Table 1
The pK_a values and mobility data of aminophenols, benzenediamines and benzenediols

Peak	Analytes	pK_a						Limiting mobility[a]	
		Ref. [2]		Ref. [17]		This work[b]		This work	
Aminophenols									
4	2-Aminophenol	4.78	9.97			4.55 (4.63)	9.54 (9.42)	3.19	−2.06
2	3-Aminophenol	4.37	9.82			5.30 (5.38)	9.57 (9.45)	3.19	−2.06
10	4-Aminophenol	5.29	10.46			5.30 (5.38)	10.02 (9.90)	3.16	−1.90
8	6-Methyl-3-aminophenol			4.61	10.21	4.60 (4.68)	9.60 (9.48)	2.95	−1.08
11	4-Methylaminophenol			6.50	11.79	5.52 (5.60)	10.00 (9.88)	3.63	−1.70
Benzenediamines									
3	1,2-Benzenediamine	1.86	4.65			1.70 (1.57)	4.55 (4.47)	4.30	3.14
5	1,3-Benzenediamine	2.65	4.88			2.48 (2.35)	4.92 (4.84)	5.25	2.98
13	1,4-Benzenediamine	2.97	6.31			2.74 (2.61)	6.10 (6.01)	5.35	3.15
9	4-Methoxy-1,3-benzenediamine			2.65	5.39	22.32 (2.19)	5.39 (5.37)	5.35	2.95
12	2-Methyl-1,4-benzenediamine			2.68	6.02	2.61 (2.48)	6.00 (5.91)	5.10	2.96
Benzenediols									
6	1,2-Benzenediol	9.23	13.00			8.62 (8.74)	2.31 (12.57)	−2.34	−2.90
7	1,3-Benzenediol	9.30	11.06			8.62 (8.74)	11.07 (11.33)	−2.30	−2.83
1	1,4-Benzenediol	9.91	11.56			9.91 (10.00)	11.56 (11.82)	−1.69	−2.40

[a] Unit of mobility in 10^{-4} cm^2 V^{-1} s^{-1}.
[b] The numbers in parentheses are p′K_a values (thermodynamic dissociation constants).

addition of TTAB to a phosphate buffer is very effective for separating benzenediamines and aminophenols and is also helpful in the separation of 1,4-benzenediol from 1,2- and 1,3-benzenediol isomers at pH 5.0. The pK_a values of these analytes are satisfactorily determined. The results further support that CE is a convenient method to be used for precise pK_a determination.

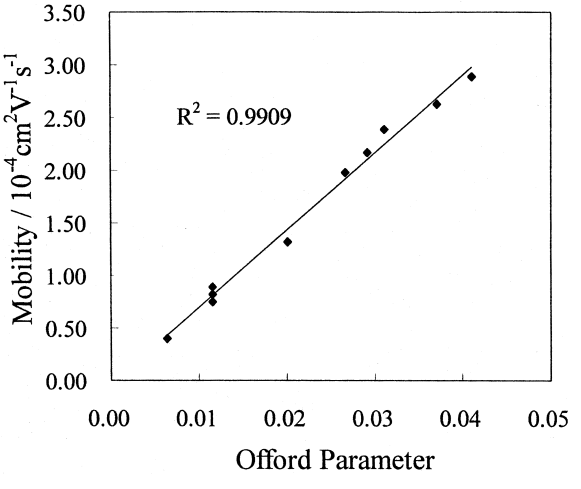


Fig. 6. Correlation of electrophoretic mobility of benzenediamines and aminophenols at pH 5.0 with Offord's parameter, $q/M^{2/3}$.

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References

[1] J.F. Corbett, in: F. Frost, S.N. Horwitz (Eds.), Principles of Cosmetics for the Dermatologist, C.V. Mosby, London, 1982, p. 160.
[2] A. Townshend et al. (Ed.), Dictionary of Analytical Reagents, Chapman & Hall, London, 1993.
[3] B.N. Ames, H.O. Kammen, E. Yamasaki, Proc. Nat. Acad. Sci. USA 72 (1975) 2423.
[4] Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Man, Vol. 16, International Agency for Research on Cancer, Lyon, 1977, p. 25.
[5] G. Choudhary, J. Chromatogr. 193 (1980) 277.
[6] H. Tokuda, Y. Kimura, S. Takano, J. Chromatogr. 367 (1986) 345.
[7] B. Schultz, J. Chromatogr. 299 (1984) 484.

[8] C.J. Dowle, A.P. Malyan, A.M. Matheson, Analyst 115 (1990) 105.
[9] M.C. Gennaro, P.L. Bertolo, E. Marengo, J. Chromatogr. 518 (1990) 149.
[10] V. Andrisano, R. Gotti, A.M. Di Pietra, V. Carrini, Chromatographia 39 (1994) 138.
[11] V. Andrisano, R. Gotti, A.M. DiPietra, V. Carrini, J. Liq. Chromatogr. 17 (1994) 2919.
[12] S. Fanali, J. Chromatogr. 470 (1989) 123.
[13] C. Sainthorant, Ph. Morin, M. Dreux, A. Baudry, N. Goetz, J. Chromatogr. A 717 (1995) 167.
[14] A. Cavallaro, V. Piangerelli, F. Nerini, S. Carvalli, C. Reschiotto, J. Chromatogr. A 709 (1995) 361.
[15] C.E. Lin, Y.T. Chen, T.Z. Wang, J. Chromatogr. A 837 (1999) 241.
[16] C.E. Lin, W.C. Lin, Y.C. Chen, S.W. Wang, J. Chromatogr. A 792 (1997) 37.
[17] D.D. Perrin, B. Dempsey, E.P. Serjeant, pK_a Prediction for Organic Acids and Bases, Chapman and Hall, London, 1981.
[18] J. Cai, J.T. Smith, Z.E. Rassi, J. High Resolut. Chromatogr. 15 (1992) 30.
[19] C.E. Lin, C.C. Chang, W.C. Lin, J. Chromatogr. A 768 (1997) 105.
[20] S.C. Smith, M.G. Khaledi, Anal. Chem. 65 (1993) 193.
[21] C.E. Lin, W.C. Lin, W.C. Chiou, J. Chromatogr. A 705 (1995) 325.
[22] K.D. Lukacs, J.W. Jorgenson, J. High Resolut. Chromatogr. 8 (1985) 407.
[23] R.M. McCormick, Anal. Chem. 60 (1988) 2322.
[24] S. Fujiwara, S. Iwase, S. Honda, J. Chromatogr. 447 (1988) 133.
[25] C.E. Lin, C.C. Chang, W.C. Lin, E.C. Lin, J. Chromatogr. A 753 (1996) 133.
[26] J.A. Cleveland Jr., M.H. Benko, S.J. Gluck, Y.M. Walbroehl, J. Chromatogr. A 652 (1993) 301.
[27] R.E. Offord, Nature 5049 (1966) 591.
[28] E.C. Rickard, M.M. Strohl, R.G. Nielsen, Anal. Biochem. 197 (1991) 197.
[29] J. Barbosa, D. Barron, E. Jimenez-Lozano, J. Chromatogr. A 839 (1999) 183.