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Separation of benzenediamines, benzenediols and aminophenols in oxidative hair dyes by micellar electrokinetic chromatography using cationic surfactants

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Abstract

The separation of 13 dye intermediates, including benzenediamine, benzenediols and aminophenols, in oxidative hair dyes was investigated by micellar electrokinetic chromatography (MEKC) using tetradecyl- and hexadecyl-trimethylammonium bromides (TTAB and CTAB) as cationic surfactants in a phosphate buffer at acidic pH. The influences of separation parameters such as micelle concentration, buffer pH, buffer concentration and organic modifiers on the selectivity and migration order of these dye intermediates were examined. The results indicate that micelle concentration and buffer pH are the two most important factors that affect greatly the migration and the separation of these dye intermediates. The electrophoretic mobility of 6-methyl-3-aminophenol, 1,3-benzenediol and 1,2-benzenediol, owing to solute-micelle interactions, increases markedly with increasing micelle concentration, but decreases to a greater extent than that of the other analytes with increasing the proportion of organic modifiers. Thus the selectivity and the order of migration of the analytes which migrate consecutively with these three analytes may be altered on varying these separation parameters. Complete separation was achieved either with TTAB at a concentration of 18 m*M* or with CTAB at a concentration of 13 m*M* at pH 5.0. The analysis of a real sample of a commercial product of oxidative hair dyes was performed.

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

1. Introduction

A commercial oxidative hair dye usually contains a mixture of dye intermediates which include benzenediamines, benzenediols and aminophenols. In the process of hair dyeing, a dye precursor (e.g., 1,4-benzenediamine, 4-aminophenol) reacts with hydrogen peroxide to produce an imine, which in turn reacts with a coupler (e.g., 3-aminophenol, 1,3-benzenediol) to produce the dye [1,2]. Thus, the content and composition of dye intermediates in a hair dye affect greatly the color of hair. These dye intermediates are of great health concern because of allergic dermatitis due to 1,4-benzenediamine and nefrotoxic effects due to aminophenols [3]. Some dye intermediates are even carcinogenic [4,5]. For these reasons, interest in the development of new analytical methods to separate and analyze dye intermediates continues unabated.

Various methods, including GC [6,7], HPLC [8–12], mass spectrometry (MS) [13], and capillary electrophoresis (CE) [14,15], have been reported for

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the separation and determination of oxidative dye intermediates in permanent hair colorants. Among them, CE is one of the most attractive and promising methods to be applied. This is due to advantageous features of this technique, such as extremely high efficiency and rapid analysis.

Reports on the capillary electrophoretic separation of dye intermediates were very few. Complete separation of six dye intermediates, including three aminophenols and three benzenediamine isomers was achieved by capillary isotachophoresis [14]. More recently, the separation of a mixture of 14 dye intermediates was investigated by micellar electrokinetic chromatography (MEKC) using high concentrations of sodium dodecyl sulfate (SDS) and cetyltrimethylammonium chloride (CTAC) as surfactants at alkaline pH [15].

It was found that the reproducibility is rather low at alkaline pH due to increased dissolution of silica [15], and that dye intermediates such as benzenediamines and aminophenols are optimally separated at an acidic pH of about 5.0–5.5 in capillary zone electrophoresis (CZE) [16], Thus, it is expected that the separation of these analytes at acidic pH is better than at alkaline pH. In this paper, we report the results of the separation of 13 dye intermediates in a phosphate buffer at acidic pH using cationic surfactants at comparatively much lower concentrations. Separation parameters, such as micelle concentration, buffer pH, buffer concentration and the content of organic modifier, that affect the migration behavior and separation of these dye intermediates in MEKC were also investigated. A real sample of a commercial product of oxidative hair dyes was analyzed.

2. Experimental

2.1. Chemicals and reagents

Thirteen dye intermediates, as indicated in Table 1, and tetradecyl- and hexadecyl-trimethylammonium bromides (TTAB and CTAB) were purchased from TCI (Tokyo, Japan). Sudan III were obtained from Aldrich (Milwaukee, WI, USA). All other chemicals were of analytical-reagent grade. 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 0.2% sodium sulfite in brown bottles and were stored in a

Table 1
The pK_a values of aminophenols, benzenediamines and benzenediols

Peak no.	Dye intermediates	Abbreviations	pK_a					
			Ref. [21]		Ref. [22]		Ref. [16]	
	Aminophenols							
4	2-aminophenol	2-AP	4.78	9.97			4.55	9.54
2	3-aminophenol	3-AP	4.37	9.82			4.24	9.57
10	4-aminophenol	4-AP	5.29	10.46			5.30	10.02
8	6-methyl-3-aminophenol	6-M-3-AP			4.61	10.21	4.60	9.60
11	4-methylaminophenol	4-MAP			6.50	11.79	5.52	10.00
	Benzenediamines							
3	1,2-benzenediamine	1,2-BDA	1.86	4.65			1.70	4.55
5	1,3-benzenediamine	1,3-BDA	2.65	4.88			2.48	4.92
13	1,4-benzenediamine	1,4-BDA	2.97	6.31			2.74	6.10
9	4-methoxy-1,3-benzenediamine	4-MO-1,3-BDA			2.65	5.39	2.32	5.39
12	2-methyl-1,4-benzenediamine	2-M-1,4-BDA			2.68	6.02	2.61	6.00
	Benzenediols							
6	1,2-benzenediol	1,2-BDO	9.23	13.00			8.62	12.31
7	1,3-benzenediol	1,3-BDO	9.30	11.06			8.62	11.07
1	1,4-benzenediol	1,4-BDO	9.91	11.56			8.72	11.35

refrigerator at 4°C. When needed, various concentrations of sample solution ranging from 10–50 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

Separations were made with a capillary electrophoresis system described previously [17]. The capillary dimensions were 67 cm×50 μm I.D. With the use of cationic surfactants, the polarity of the electrodes was reversed under the conditions of reversed electroosmotic flow (EOF). Thus, the UV detection position is 7.0 cm from the anodic 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 [17]. To ensure reproducibility, all experiments were performed at 25°C and measurements were run at least in triplicate. The migration times were quite reproducible, with relative standard deviations varying by less than 0.5% ($n=6$). 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. The capillary was washed with running buffer for 5 min before each injection. The detection wavelength was set at 220 nm. Mesityl oxide was used as neutral marker.

2.4. Calculations

2.4.1. Electrophoretic mobility

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

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

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 a neutral 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.

2.4.2. Retention factor

The retention factor of analytes (k') was calculated from the observed migration times with the equation:

$$k' = \frac{t_m - t_o}{t_o \left(1 - \frac{t_m}{t_{mc}} \right)} \quad (2)$$

where t_o is the migration time in the absence of micelles, and t_{mc} is the migration time of micelles marker, or

$$k' = \frac{(\mu_{ep} - \mu_o)}{(\mu_{mc} - \mu_{ep})} \quad (3)$$

where μ_{ep}, $\mu_{o,}$ and μ_{mc} are the electrophoretic mobility of a solute, the electrophoretic mobility of a solute in the absence of micelles, and the electrophoretic mobility of micelle, respectively, calculated from the corresponding migration times. In this work, Sudan III was used as a micelle marker.

2.4.3. Partition coefficient

The retention factor in MEKC is directly proportional to the micelle concentration through the following equation [18,19]:

$$k' = P_{mw} \nu \{[S] - CMC\} \quad (4)$$

where P_{mw} is the partition coefficient of solutes between the aqueous and micellar phases, ν is the molar volume of the surfactant, [S] is the total

surfactant concentration and *CMC* is the critical micellar concentration. With TTAB as an cationic surfactant, ν is equal to 0.328 $Lmol^{-1}$ [20] and *CMC* is equal to 1.6±0.1 m*M* in a phosphate buffer (70 m*M*) at pH 6.0 [28].

3. Results and discussion

The 13 dye intermediates studied possess amino groups with two pK_a values in the range of 4.2–6.5 and 1.7–3.0, and/or hydroxy groups with the first and the second pK_a values in the range 8.6–11.8 and 11.0–13.0, respectively [16,21,22]. Table 1 lists the pK_a values of these dye intermediates. Benzenediamines and aminophenols which possess amino groups are partially protonated when buffer pH is in between their pK_a-2 and pK_a+2 values, whereas benzenediols with two hydroxy groups are neutral when the buffer pH is less than their p$K_{a_1}-2$ value. Therefore, benzenediamines and aminophenols, existing as cationic species at pH 5.0, migrate toward the cathodic end in the opposite direction of the reversed EOF. Benzenediols, existing as neutral species at pH 5.0, are incorporated with cationic micelles, thus migrating also toward the cathodic end. Since the reversed EOF is greater than the electrophoretic mobility of the analytes, all of the analytes are carried toward the anodic end by the reversed EOF.

3.1. Effect of micelle concentration

Fig. 1 shows the variation of electrophoretic mobility of 13 dye intermediates studied as a function of TTAB concentration and of CTAB concentration in the range 2–30 m*M* at pH 5.0. The electrophoretic mobility of 6-M-3-AP (8), 1,2-BDO (6) and 1,3-BDO (7) increases markedly with increasing surfactant concentration, whereas that of 2-AP (4), 3-AP (2) and 1,4-BDO (1) increases to a much less extent. The electrophoretic mobility of the rest of the solutes increases only slightly or remains almost unchanged.

The separation of ionic analytes depends mainly on their effective charges and molecular masses as in the case of CZE separation because of weak solute-micelle interactions. Thus 3-AP (2) and 1,4-BDA (13), having the smallest and the greatest electro-

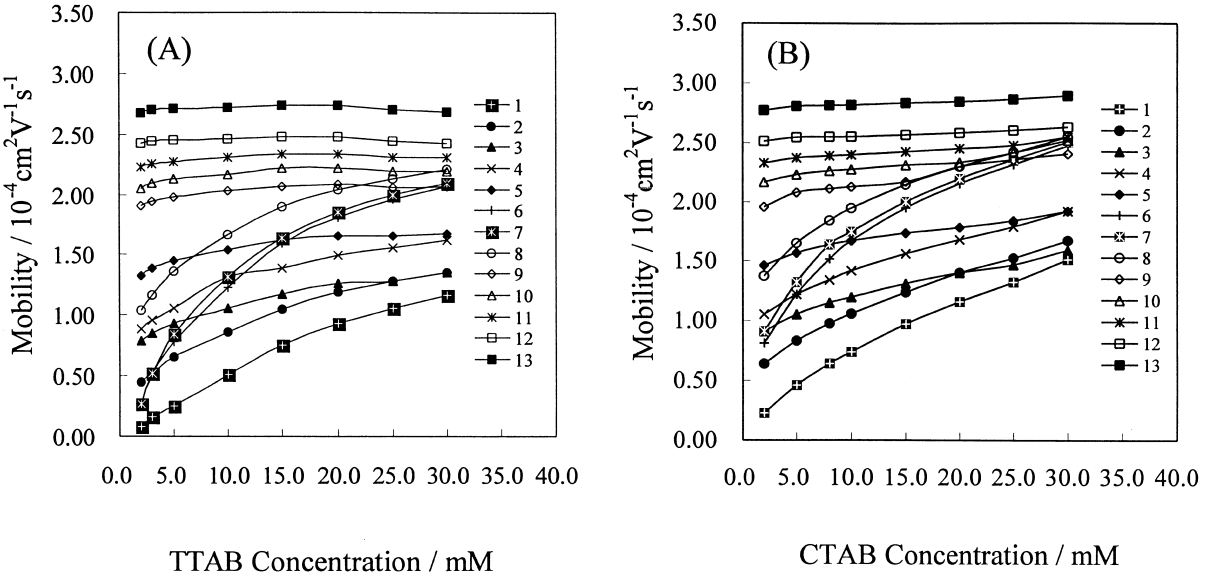

Fig. 1. Variation of the electrophoretic mobility of dye intermediates as a function of (A) TTAB concentration and (B) CTAB concentration in a phosphate buffer (50 m*M*) at pH 5.0. Other operating conditions: −20 kV, 25°C. Curve identification: the numbers denote the analytes indicated in Table 1.

phoretic mobility, are the first and the last eluted analytes, respectively, among ionic analytes. On the other hand, the separation of neutral species depends on both solute-micelle interactions and micelle concentration. Since the electrophoretic mobility of neutral analytes, depending on the solute-micelles interactions, increases with increasing micelle concentration, whereas that of ionic species are much less affected, the migration order and selectivity of the 13 dye intermediates may vary as the micelle concentration increases, especially when 6-M-3-AP (8), 1,2-BDO (6) and 1,3-BDO (7) are involved. This is because the interactions between these three dye intermediates and cationic micelles are stronger than those between the others and the micelles. For instances, as shown in Fig. 1A, 6-M-3-AP (8) migrates after 1,3-BDA (5) and 4-MO-1,3-BDA (9) when the concentration of TTAB is greater than 7 m*M* and 22 m*M*, respectively. Similar trends in the variation of electrophoretic mobility as for 6-M-3-AP (8) were also observed for 1,2-BDO (6) and 1,3-BDO (7), with 1,3-BDO (7) migrating slightly faster toward cathode than 1,2-BDO (6). 1,3-BDO (7) co-migrates subsequently with 3-aminophenol (2), 1,2-BDA (3), 2-AP (4), 1,3-BDA (5), and 4-M-1,3-BDA (9) at TTAB concentrations of 3, 6, 10, 15, and 30 m*M*, respectively. On the other hand, as shown in Fig. 1B, 6-M-3-AP (8) co-migrates subsequently with 1,2-BDA (3), 4-MO-1,3-BDA (9), and 4-AP (10) at CTAB concentrations of 3, 17, and 25 m*M*, respectively; 1,3-BDO (7) co-migrates subsequently with 2-AP (1), 1,3-BDA (5), 4-MO-1,3-BDA (9), and 4-aminophenol (10) at CTAB concentrations of 4, 8, 20, 25 m*M*, respectively.

The trends in the variation of electrophoretic mobility of these dye intermediates with CTAB are quite similar to those obtained with TTAB, except that the electrophoretic mobility and the extent of the variation in electrophoretic mobility of each individual solute are comparatively greater with CTAB in the same concentration range than with TTAB. This is attributed to differences in the binding constant of solutes to micelles and the molar volume of the micelles.

As demonstrated, effective separation of these dye intermediates was achieved with TTAB concentration at 18 m*M*. Fig. 2 shows the optimum electropherogram of these dye intermediates obtained. Effective separation of 1,2-BDO (6) and 1,3-BDO (7) were obtained, but not baseline resolved. However, complete separation of 1,2-BDO (6) and 1,3-BDO (7) was achieved with CTAB concentration at either 6 or 13 mM. As shown in Fig. 1A and B, the migration order of these dye intermediates with TTAB at a concentration of 18 m*M* happened to be the same as for CTAB at a concentration of 13 mM.

3.2. Effect of buffer pH

In the electrophoretic separation of ionizable solutes, buffer pH plays an important role as it determines the extent of ionization of each individual solute. Fig. 3 shows the variation of the electrophoretic mobility of three different structural types of dye intermediates as a function of buffer pH in the range 3.5–7.0 using TTAB as a cationic surfactant. Sigmoidal behavior for the variation of electrophoretic mobility of benzenediamines and aminophenols in this pH range is expected. The selectivity and the migration order of these two categories of analytes are primarily influenced by their pK_a values, molecular mass and the solute-micelle interactions. This is particularly true for 6-M-3-AP (8). The decrease in the electrophoretic mobility of this analyte with increasing buffer pH from 3.5 to 7.0 is comparatively more gradual than that of 3-AP (2). This is believed partly due to a much larger solute-micelle interactions and partly due to a slightly larger mole fraction of neutral species of solute (8). On the other hand, the migration order of benzenediols is solely determined by the solute-micelle interactions because benzenediols exist as neutral molecules in this pH range. This expectation is confirmed by the magnitudes of the partition coefficient of solutes to micelles evaluated for these three benzenediol isomers in Section 3.5. Sigmoidal behavior for the variation of electrophoretic mobility of benzenediols in the pH range 4.7–7.0 was observed. This is due to the decrease in the electrophoretic mobility of micelles (shown in Fig. 3) in a phosphate buffer using TTAB as a surfactant in this pH range [23].

The trends in the variation of the electrophoretic mobility of these dye intermediates using CTAB (13 m*M*) as a cationic surfactant in this pH range are more or less the same as in the case of TTAB shown

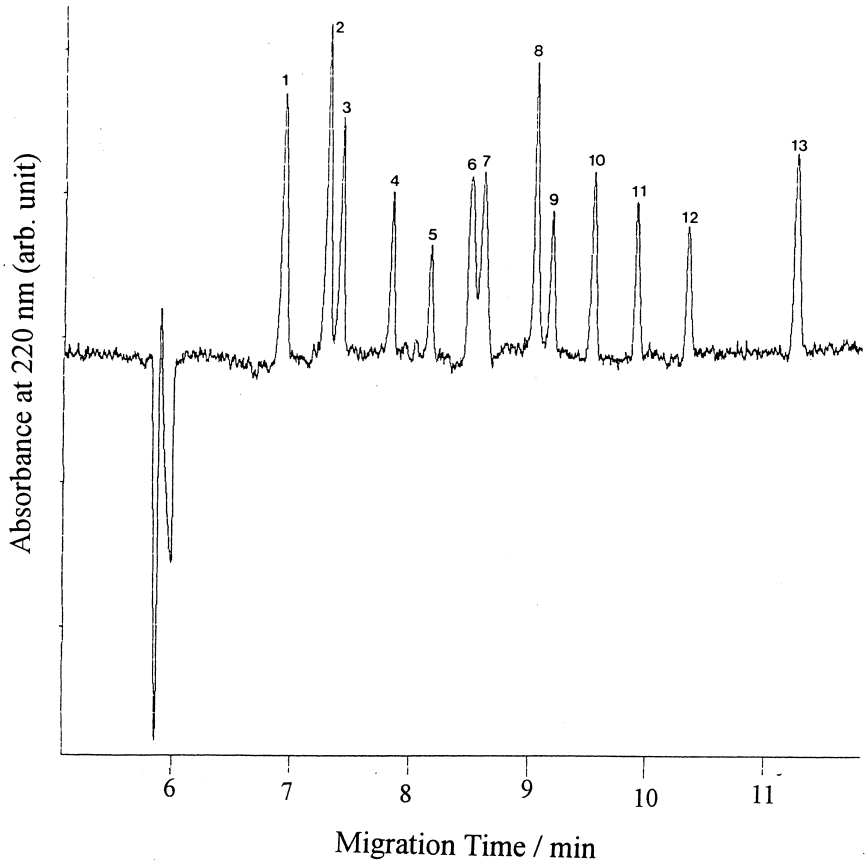

Fig. 2. Eletropherogram of dye intermediates obtained with phosphate buffer (50 m*M*) containing 18 m*M* TTAB at pH 5.0. Other operating conditions and peak identification are the same as for Fig. 1.

in Fig. 3. The optimum buffer pH for the separation of these 13 dye intermediates is about 5.0.

3.3. Influence of buffer concentration

The influence of the concentration of phosphate buffer in the range of 30–80 m*M* on the separation of 13 dye intermediates was examined with TTAB at a concentration of 20 m*M* at pH 5.0. The selectivity of most of the dye intermediates was little or insignificantly affected on varying buffer concentration, except that the separation of 6-M-3-AP (8) and 4-MO-1,3-BDA (9) became worse when buffer concentration exceeded 60 mM. Baseline separation of 1,3-BDO (7) and 1,2-BDO (6) was not achieved with buffer concentration less than 40 mM. Hence, the optimum concentration of phosphate buffer is about 50 mM.

3.4. Influences of organic modifiers

As the addition of organic modifiers to the buffer electrolyte may improve separation and resolution [24–27], the influence of organic modifiers on the separation of dye intermediates was examined. Fig. 4 shows the variation of electrophoretic mobility of these dye intermediates as a function of the proportion of organic modifiers. Methanol or acetonitrile as an organic modifier was added to the buffer electrolyte up to 20% (v/v). As can be seen, the electrophoretic mobility of 6-M-3-AP (8), 1,3-BDO (7) and 1,2-BDO (6) decrease to a greater extent than that of the other analytes with increasing the

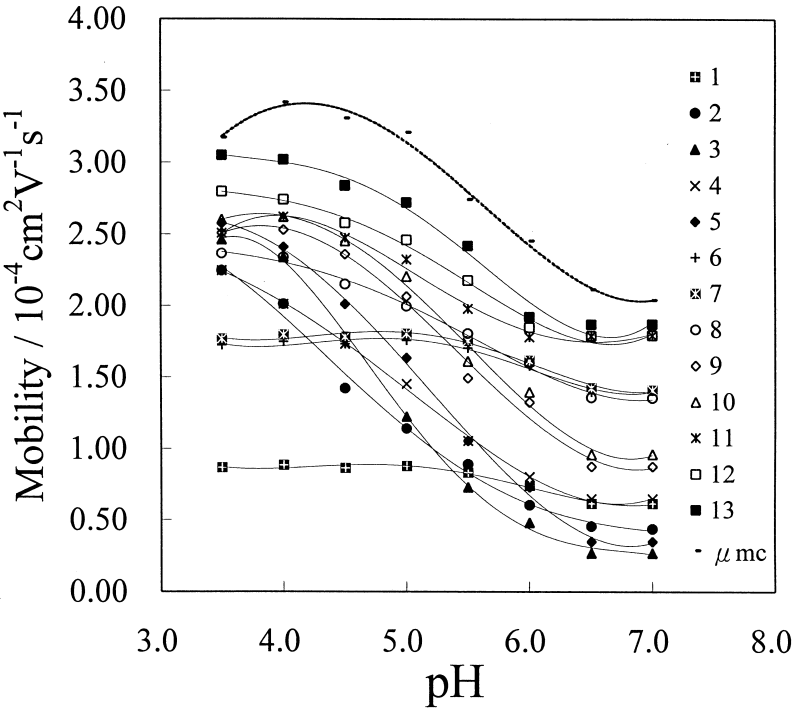

Fig. 3. Variation of the electrophoretic mobility for the 13 dye intermediates as a function of buffer pH with posphate buffer (50 m*M*) containing 18 m*M* TTAB. Other operating conditions and curve identification are the same as for Fig. 1. The variation of μ_{mc} is represented by the broken line.

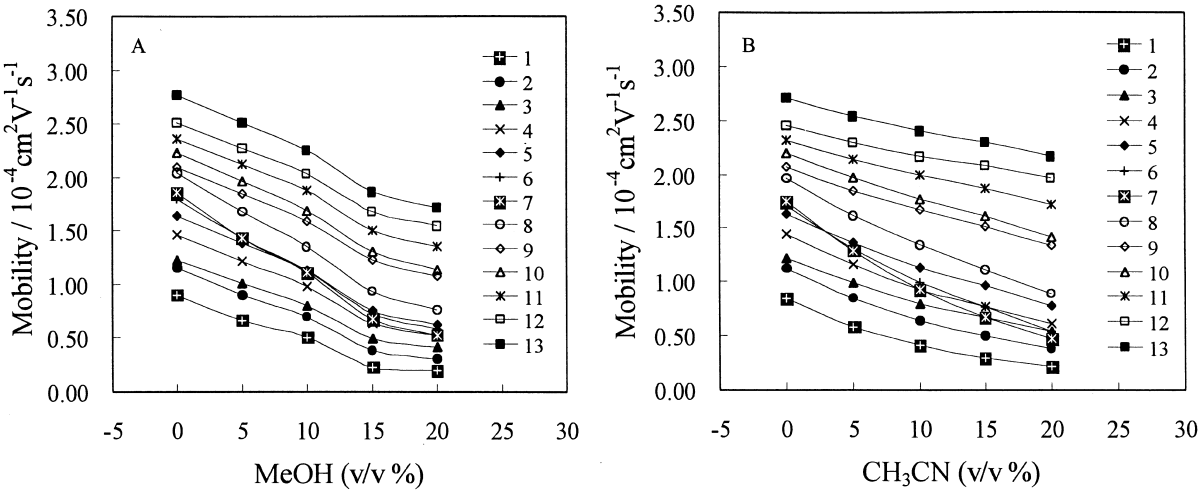

Fig. 4. Variation of the electrophoretic mobility of dye intermediates as a function of the content of organic modifiers in a phosphate buffer (50 m*M*) containing 18 m*M* TTAB at pH 5.0: (A) methanol, (B) acetonitrile. Other operating conditions and curve identification are the same as for Fig. 1.

proportion of organic modifiers. Thus, the selectivity and migration order of the dye intermediates which migrate consecutively with these three analytes may be altered on varying the proportion of organic modifiers. Fig. 5A and B show the electropherograms of 13 dye intermediates obtained in a phosphate buffer containing 18 m*M* TTAB at pH 5.0 with addition of methanol (15%, v/v) and acetonitrile (7%, v/v), respectively.

3.5. Partition coefficient vs. migration order

The principle of separation in MEKC is based on the differential partition of the solutes between the micellar and aqueous phases. In order to have better understanding on chemical interactions involved in partitioning process, partition coefficients of dye intermediates between the micellar and aqueous phases are evaluated.

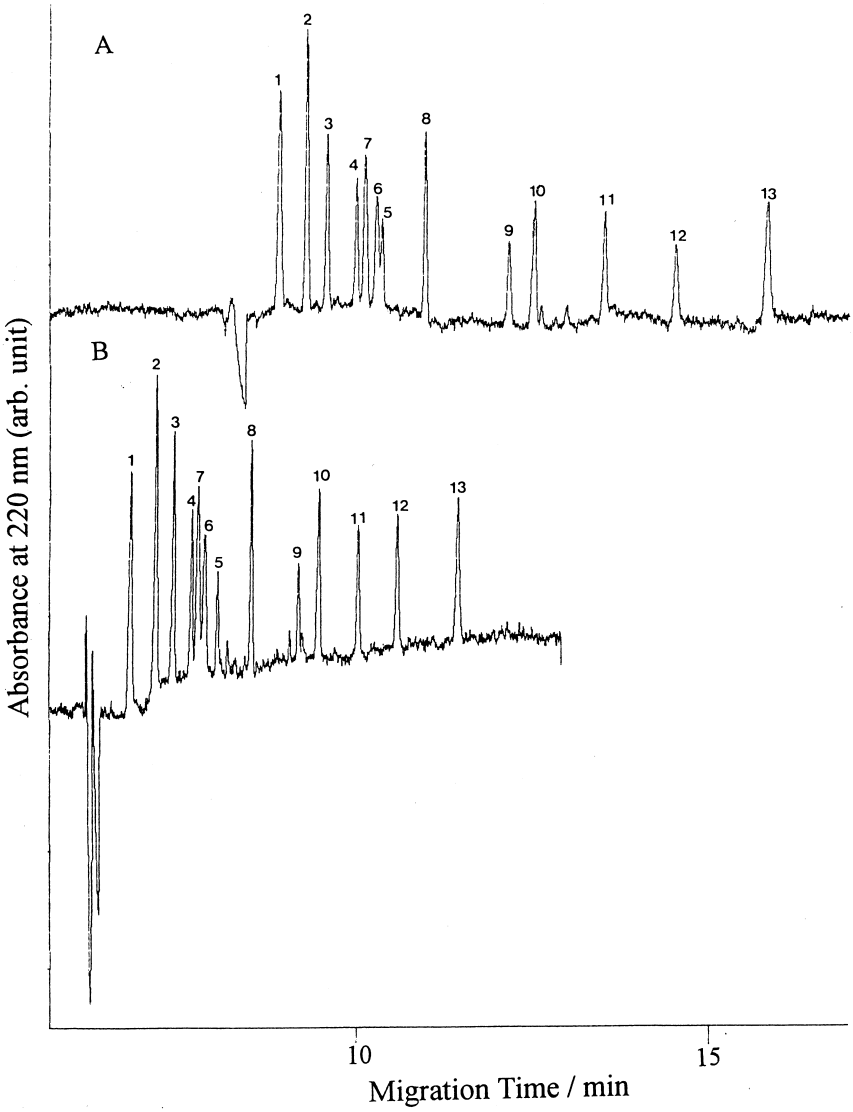

Fig. 5. Electropherograms of dye intermediates obtained in a phosphate buffer (50 m*M*) containing 18 m*M* TTAB at pH 5.0 with addition of organic modifiers: (A) methanol (15%, v/v); (B) acetonitrile (7%, v/v). Other operating conditions and peak identification are the same as for Fig. 1.

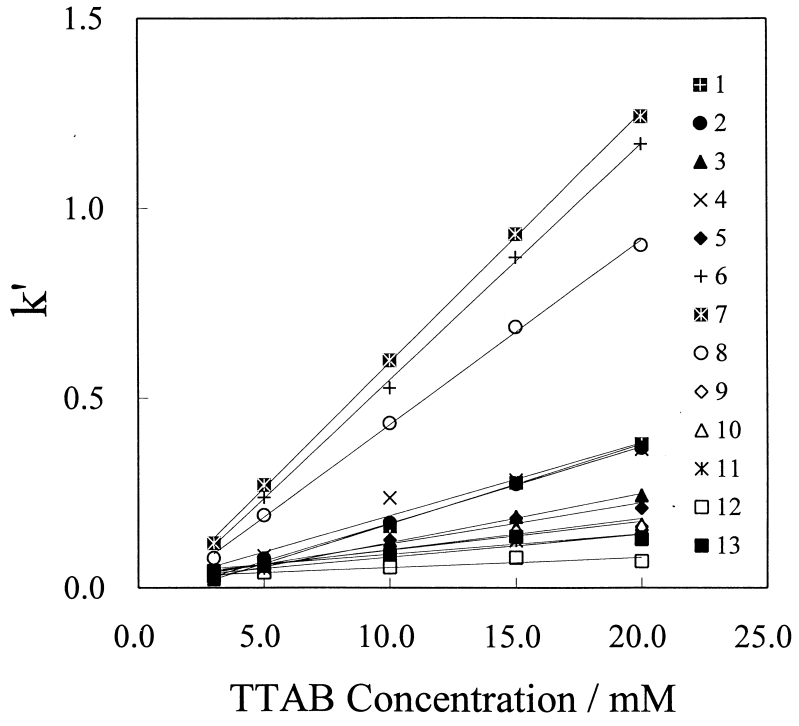

Fig. 6. Plots of retention factor (k') of dye intermediates as a function of TTAB concentration at pH 5.0.

The retention factors of benzenediamines and aminophenols at varied TTAB concentrations were calculated according to Eq. (3). Since these analytes are not electrically neutral at pH 5.0, the correction to the migration time in the absence of micelles must be taken into consideration. In this work, the mobility data with TTAB at 1.6 m*M*, which is the critical micelle concentration of TTAB at pH about 5.0–6.0

Table 2
Partition coefficients (P_{mw}), retention factor (k) and mobility data of dye intermediates measured with a phosphate-TTAB buffer at pH 5.0[a]

Peak no.	Dye intermediates	μ_{ep}[b]	μ_0[c]	k	P_{mw}	P_{ow}[d]
1	1,4-benzenediol	0.88	0.08	0.35	66	0.50
2	3-aminophenol	1.14	0.46	0.33	62	0.15
3	1,2-benzenediamine	1.22	0.79	0.22	39	0.15
4	2-aminophenol	1.45	0.88	0.33	59	0.52
5	1,3-benzenediamine	1.63	1.34	0.19	33	
6	1,2-benzenediol	1.76	0.20	1.09	198	1.01
7	1,3-benzenediol	1.80	0.20	1.15	210	0.77
8	6-methyl-3-aminophenol	1.99	1.01	0.82	152	0.77
9	4-methoxy-1,3-benzenediamine	2.06	1.91	0.14	23	0.50
10	4-aminophenol	2.20	2.06	0.15	28	0.04
11	4-methylaminophenol	2.32	2.23	0.11	22	
12	2-methyl-1,4-benzenediamine	2.46	2.43	0.05	10	
13	1,4-benzenediamine	2.72	2.68	0.08	24	

[a] Mobility in unit of 10^{-4} cm^2 V^{-1} s^{-1}; $\mu_{mc} = 3.19*10^{-4}$ cm^2 V^{-1} s^{-1}
[b] TTAB at concentration 18 m*M*.
[c] TTAB at concentration 1.6 m*M*.
[d] Ref. [29].

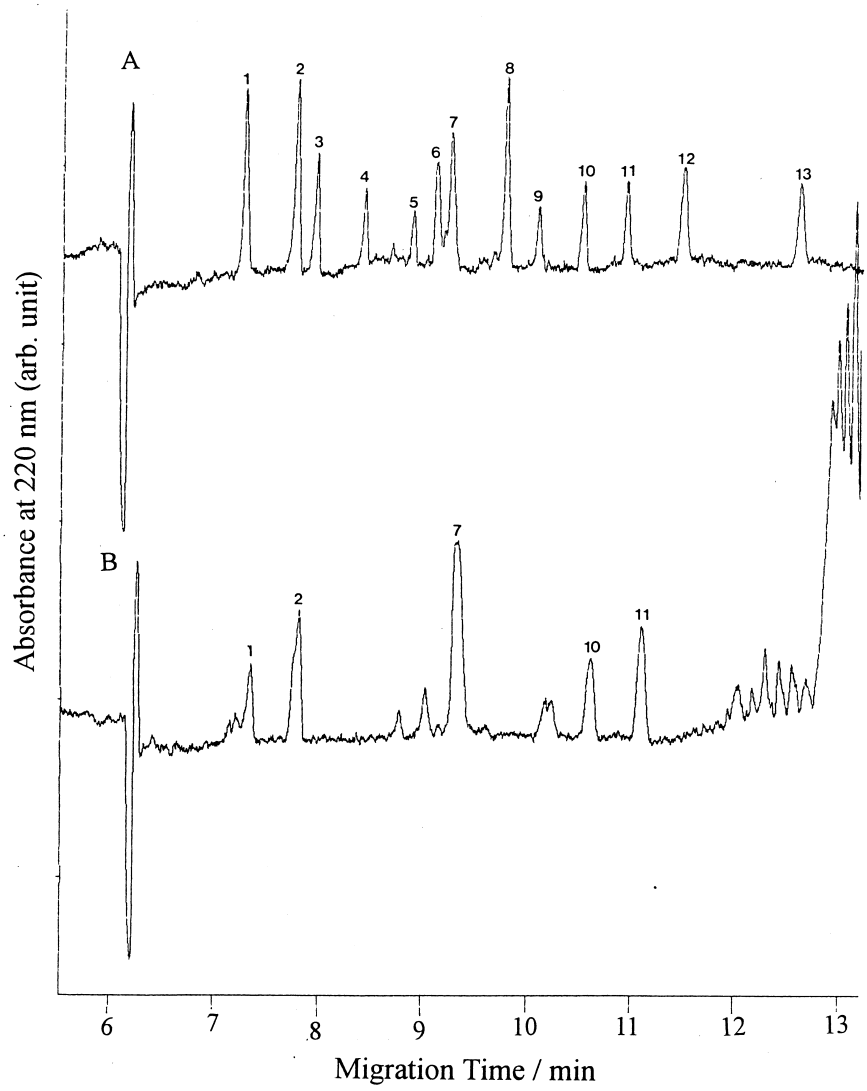

Fig. 7. Electrophrograms of dye intermediates obtained with phosphate buffer (50 m*M*) containing 13 m*M* CTAB at pH 5.0: (A) standards, (B) a real sample. Other operating conditions and peak identification are the same as for Fig. 1.

[28], were selected as μ_o. A minor correction to the migration time seem to be necessary for 1,3-BDO (7) and 1,2-BDO (6), even though they are neutral molecules at pH 5.0. This is due to the existence of interactions between neutral solutes and monomeric surfactant molecules. Fig. 6 shows the plots of retention factors of the 13 dye intermediates vs. total surfactant concentration of TTAB in the range 2–30 m*M* at pH 5.0. Good correlations with correlation coefficients (r^2) greater than 0.940 are obtained.

Since the slope of the line is directly proportional to P_{mw} as indicated in Eq. (4), the larger the slope, the greater the value of partition coefficient. The P_{mw} values were calculated according to Eq. (4), with the partial molal volume of TTAB being equal to 0.328 $lmol^{-1}$ [20]. Table 2 lists the P_{mw} and k' values evaluated and the mobility data obtained at pH 5.0, together with the P_{ow} values reported in the literature.

As shown in Table 2, the P_{mw} and P_{ow} values of 1,3-BDO (7), 1,2-BDO (6) and 6-M-3-AP (8) are comparatively much larger than those of the others.

As the P_{ow} value is an index of the hydrophobicity of a solute and the P_{mw} value is directly proportional to the binding constant of a solute to micelles, the results indicate that these three analytes interact hydrophobically to a much greater extent with cationic micelles. Therefore, the electrophoretic mobility of these three analytes increases markedly with increasing surfactant concentration, thus leading to the alteration of the migration order of the other analytes which migrate consecutively with these three analytes.

3.6. Analysis of a real sample

The analysis of a real sample of a commercial product of oxidative hair dyes (L'Oreal Excellence) was performed. Calibration curves for the quantitative analysis of 13 dye intermediate standards were obtained with CTAB at a concentration of 13 m*M* at pH 5.0. Excellent correlation with correlation coefficients (r^2) greater than 0.990 was obtained in the concentration range 20–70 μg/ml. The limits of detection, with signal-to-noise ratio equal to 3, for these dye intermediates are in the range from 3.37 μg/ml for 1,3-BDO (7) to 7.14 μg/ml for 3-AP (2).

Fig. 7 shows the electropherograms obtained for dye intermediate standards and a real sample of a commercial product. The real sample (forty-fold dilution with buffer electrolyte) was injected into the CE instrument. Evidently, this oxidative hair dye contains 1,4-BDO (1), 3-AP (2), 1,3-BDO (7), 4-AP (10) and 4-MAP (11), as indicated on the label. Although 1,4-BDA (13) is also indicated on the label, unfortunately, it is obscured by an intense unidentified peak (probably some unidentified polymer materials) and is unable to confirm the presence of this compound. The dye intermediates were quantified as follows: 1,4-BDO (2.40 μg/ml), 3-AP (1.27 μg/ml), 1,3 BDO (2.02 μg/ml), 4-AP (3.5 μg/ml) and 4-MAP (1.78 μg/ml). The results of the analysis agrees with the contents indicated by the manufacturer.

4. Conclusion

In separating oxidative dye intermediates with MEKC, micelle concentration and buffer pH are the two important separation parameters that affect greatly the selectivity and migration order of dye intermediates. Complete separations are satisfactorily achievable with cationic surfactants at low micelle concentrations at acidic pH. The analysis time is relatively short. The present method is applicable to the analysis of real samples.

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