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Separation of phenylenediamine, phenol and aminophenol derivatives by micellar electrokinetic chromatography Comparison of the role of anionic and cationic surfactants

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Abstract

A mixture of phenylenediamine, aminophenol and phenol derivatives was studied with capillary zone electrophoresis (CZE) at different pHs. The use of acidic or alkaline pH did not give separations with a good resolution. Next, separation was performed using micellar electrokinetic capillary chromatography (MEKC), first with the anionic surfactant sodium dodecyl sulfate (SDS) and second with the cationic surfactant cetyl trimethylammoniumchloride (CTACl). In both cases, a study of pH, surfactant concentration and ionic strength of the electrolyte as function of the capacity factor allowed for the determination of the optimum conditions.

Since separation was obtained with both surfactants, we studied the distribution coefficient to determine the affinities of the solutes for the aqueous medium and the different surfactants.

1. Introduction

Capillary electrophoresis (CE) is a modern analytical technique in rapid development. The high efficiencies and high resolution achieved allow for separations similar to those obtained with high-performance liquid chromatography (HPLC). CE has a large field of applications, e.g. the separation of ionizable solutes by capillary zone electrophoresis (CZE) [1] and the separation of neutral compounds by micellar electrokinetic capillary chromatography (MEKC), developed by Terabe et al. [2]. Most reports on MEKC use sodium dodecylsulphate (SDS) at concentrations higher than its critical micellar concentration (CMC). Analytes are dissolved in the aqueous phase (moving at the electroosmotic flow velocity) and the pseudo-stationary phase (moving in the opposite direction as the electroosmotic flow of the negatively-charged micelles). Thus, the distribution of solutes over these two phases induces a difference in the migration velocities of the analytes which allows their resolution. One of the major advantages of capillary electrophoresis is the ability to resolve in the same run a mixture of anions, cations and neutral compounds.

Oxidative hair colorants are permanent hair colourers usually composed of a dye precursor and coupler in an alkaline medium and hydrogen peroxide. Hydrogen peroxide reacts with a primary intermediate (precursor, e.g. 1,4-diaminobenzene or 4-aminophenol) to produce an imine, which in its turn reacts rapidly with a

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second intermediate (coupler, e.g. 3-aminophenol or resorcinol) to produce the dyes [3–5]. The groups which are responsible for the attractive forces of the oxidative dyes are the amino and hydroxy groups, the attractive behaviour also being dependent on the positions of these groups. As precursors phenylenediamines, diaminophenols and aminophenols are used which have their amino or hydroxy groups in *ortho* or *para* positions. The *meta*-diamines, the *meta*-aminophenols and the polyphenols are used as couplers. For a good colour quality, the composition of the dye has to be carefully controlled. Several selective analytical methods have been proposed for the identification and determination of this class of compounds, e.g. thin-layer chromatography [6], gas chromatography [7], mass spectrometry [8], high-performance liquid chromatography [9,10] and more recently capillary electrophoresis (isotachophoresis) [11]. Nowadays, the analysis of oxidative dyes is achieved by HPLC.

Our aim was to determine the ability of capillary electrophoresis to resolve such aromatic solutes by CZE, and next by MEKC using anionic (SDS) and cationic [cetyl trimethylammoniumchloride (CTACl)] surfactants. To this end we selected the fourteen aromatic solutes (diamines, aminophenols, toluenediamines and phenols with different substituents) shown in Fig. 1.

2. Experimental

2.1. Apparatus

Part of the experiments were performed on a Spectraphoresis 2000 CE instrument (TSP, San José, CA, USA) equipped with a variable-wavelength UV–Vis absorbance detector, an automatic injector and an autosampler. It consists of a thermostated cartridge containing a silica capillary tube with an internal diameter of 50 μm, a total length of 70 cm (or 44 cm) and a injector–detector length of 63 cm (or 37 cm), respectively.

The other experiments were performed on a Beckman P/ACE 2100 (Beckman Instruments, Fullerton, CA, USA). The part of the capillary used for separation was kept at a constant temperature by immersion in a cooling circulation in the cartridge. The detection window was 100×800 μm. Fused-silica capillaries of 50 μm I.D. and 47 cm length (40 cm to the detector) were used. The UV detector was equipped with wavelength filters (190, 214, 254, 280 nm). In our experiments, the separation was carried out at 214 nm, using an applied voltage of +20 kV. A temperature of 25°C was selected to avoid dye oxidation. The pH of each electrolyte was checked using a Beckman pH meter (Model ϕ10, Beckman).

The capillaries were daily conditioned by rinsing first with 1 *M* sodium hydroxide (10 min) at 60°C, water (10 min) at 60°C, then 0.1 *M* sodium hydroxide (10 min) at 40°C, water (10 min) at 40°C, and finally the running electrolyte (30 min) at 25°C. The same washing procedure was used in MEKC, except for the last step where we used the electrolyte containing the surfactant (15 min) at 25°C. Between two analyses, the capillary tubes were flushed at 25°C with electrolyte (5 min) for CZE and with water (3 min) and electrolyte (5 min) at 25°C for MEKC, in order to improve reproducibility of the electroosmotic flow and migration times of the analytes.

2.2. Chemicals

All chemicals were of analytical reagent grade. Sodium dihydrogenphosphate (Fluka, Buchs, Switzerland), boric acid (Fluka), phosphoric acid (Carlo Erba, Milan, Italy), 1 *M* sodium hydroxide (Aldrich, Milwaukee, WI, USA), 0.1 *M* sodium hydroxide (Aldrich), sodium dodecylsulphate (SDS) (Fluka) and cetyl trimethylammoniumchloride (CTACl) (Aldrich) were used without further purification. Water used for dilutions or as electrolyte solution was of HPLC grade (Fison, Farmitalia, Milan, Italy).

Methanol and anthracene were used to determine the migration times of a neutral unretained solute and of micelle tracer, respectively.

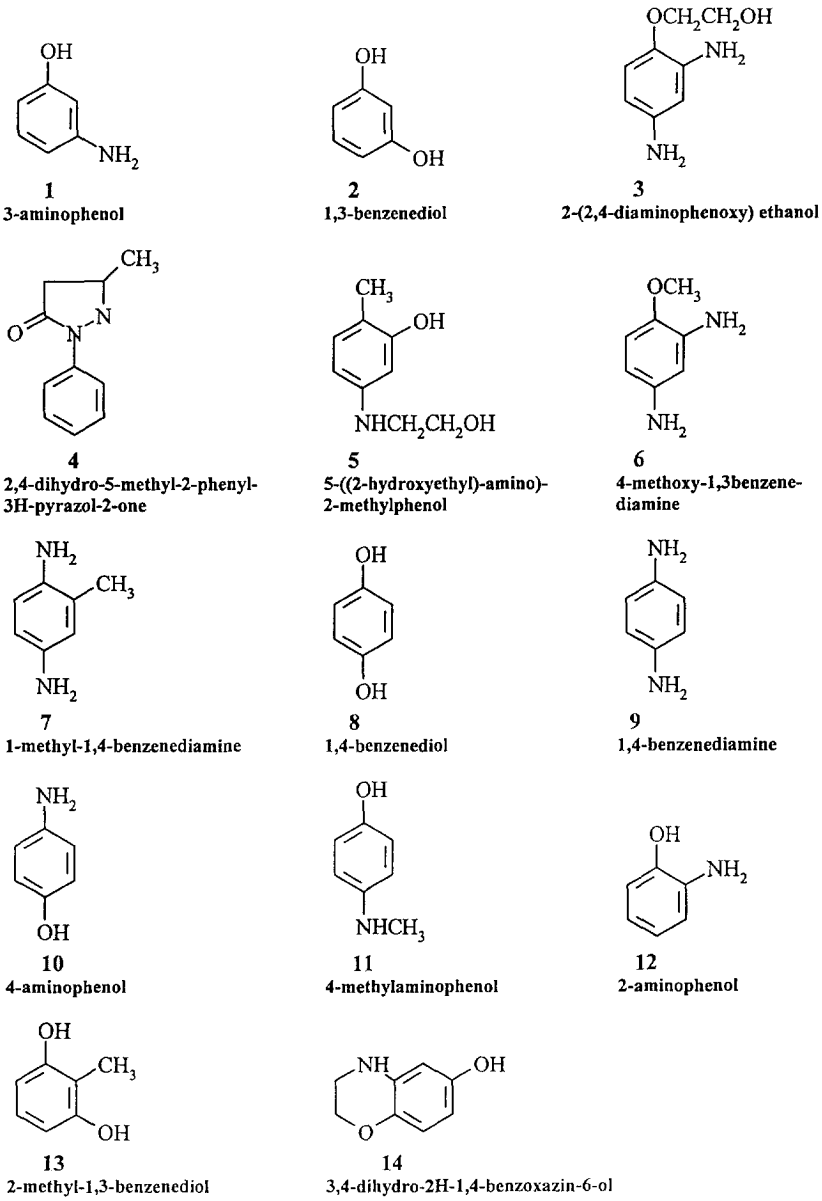


Fig. 1. Structures of the compounds analysed.

Authentic samples of oxidative dyes were a gift from Oreal (Aulnay-sous-Bois, France). The dye mixture was obtained by dissolving these solutes (50 mg/l) in the running buffer (pH 3)–methanol mixture (90:10, v/v). Finally, sodium sulphite (0.2%) was added to each sample to slow oxidation of the dyes.

3. Results and discussion

3.1. Capillary zone electrophoresis

Capillary zone electrophoresis (CZE) allows the separation of ionized molecules, which move according to their electrophoretic mobility [12]:

$$m_{ep} = \frac{L_d L_t}{V}\left(\frac{1}{t_m} - \frac{1}{t_0}\right) \quad (1)$$

where m_{ep} is the electrophoretic mobility of the solute, V the applied voltage, L_d the injector–detector capillary length, L_t the total capillary length, t_m the migration time of solute and t_0 the migration time of neutral marker.

Neutral molecules would migrate at the electroosmotic flow (EOF) velocity, and the electroosmotic flow mobility (m_{eo}) is given by

$$m_{eo} = \frac{L_d L_t}{V}\left(\frac{1}{t_0}\right) \quad (2)$$

The oxidative dyes contain amino groups (pK_a 3–5.5) or hydroxy groups (pK_a 9–11); the variation of their electrophoretic mobility with changes in running buffer pH is reported in Fig. 2.

The amino groups are ionized when the buffer pH is less than their pK_a values; in that case, the positive electrophoretic mobility of these organic cations is in the same direction as the electroosmotic flow. 3-Aminophenol (compound 1) has a positive m_{ep} at pH 4; when the buffer pH increases, its electrophoretic mobility decreases due to deprotonation of the amino group. At pH 6 the solute is neutral. When the pH is increased to the latter pK_a, the molecule moves with the electroosmotic flow. The hydroxy groups are ionized when the pH is higher than their pK_a values (pK_a 9–11), thus these anionic solutes have negative electrophoretic mobilities and migrate with the EOF. In contrast to the amino groups, the m_{ep} increases when the pH increases (because the solute gets more ionized hydroxy groups). For the aromatic solutes, the ionization constants of their amino and hydroxy groups may be determined by CZE. Figs. 3 and 4 show the results of the analysis of a mixture of the aromatic solutes using CZE. Under acidic pH the separation is better than at alkaline pH: pH

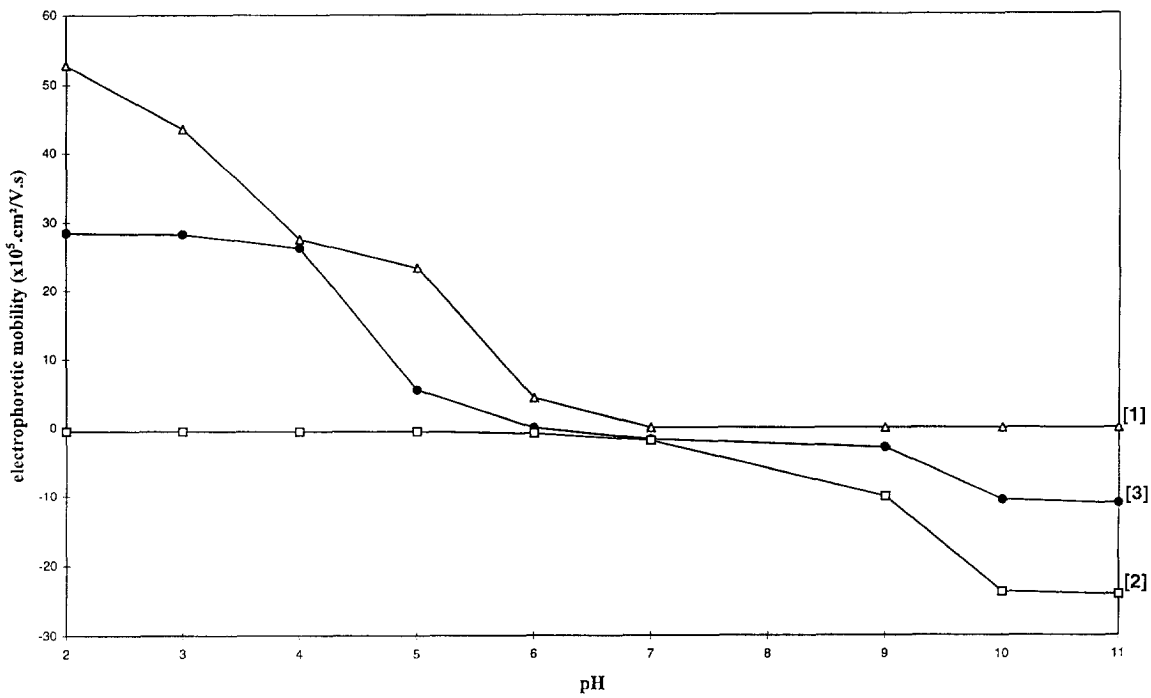


Fig. 2. Plot of electrophoretic mobility versus pH. Electrolyte, 25 mM phosphate–borate; capillary, 44 cm × 50 μm I.D.; UV detection, 214 nm; hydrodynamic injection, 2 s; voltage, 20 kV; temperature, 25°C. Compounds: [1] (△) 3-aminophenol (pK_a 4.3–9.4); [2] (□) 1,3-benzenediol (pK_a 9.1–11); [3] (●) 2-(2,4-diaminophenoxy)ethanol (pK_a 3.2–5.4).

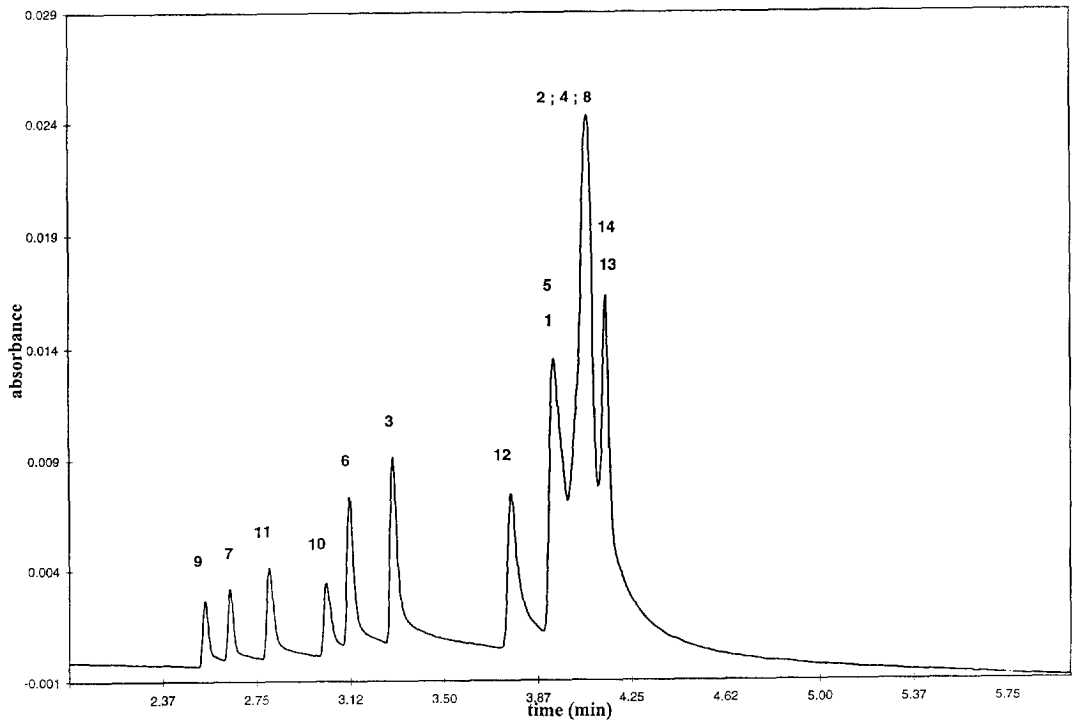


Fig. 3. Separation of oxidative dye mixture under acidic conditions (CZE). Electrolyte, 25 m*M* phosphate–borate, pH 5.5; capillary, 47 cm × 50 μm I.D.; UV detection, 214 nm; hydrodynamic injection 2 s; voltage, 20 kV; temperature, 25°C; concentration, 50 m*M*. For peak identification see Fig. 1.

increase causes deterioration of the resolution. The difficulties in realizing a separation with high resolution are caused by the pK_a of the molecules. Since these are small for the amino groups, the separation should be performed at low pH. In that case, the EOF is small and the neutral molecules (phenol) in the mixture will migrate only slowly. On the other hand, the pK_a of phenols are high and thus the pH must be increased to ionize those compounds (compounds 12, 13). However, reproducibility is rather low at alkaline pH due to increased dissolution of silica. At pH 5.5, the first peak corresponds with the aromatic diamines (di-cationic molecules). The diamine compounds (7, 9) are more ionized than the others, and thus their electrophoretic mobilities are higher. CZE separation depends on the ionic charge and the molecular mass of the analyte. Solute 7 migrates slower than solute 9 due to its higher molecular mass. This is also true for solutes 6 and 3. The compounds 1, 10 and 12 are isomers, the pK_a of their amino groups being 4.3, 5.5 and 4.6, respectively. When the separation is performed at pH 5.5, compound 10 is more ionized than compounds 1 and 12. Their order of migration is 10, 12, 1. The elution order follows the decreasing pK_a order. These solutes have the same molecular mass, so they migrate in order of their ionization. The benzenediols migrate slowest due to their neutral character at pH 5.5, which results in migration with the electroosmotic flow. At pH 9.5, the solutes having two amino groups migrate at a velocity equal to the electroosmotic flow and are not resolved (compounds 9, 7, 6 and 3). On the other hand, when the buffer pH is not alkaline enough to ionize all phenol groups, we will not see the normal migration order diamines, aminophenols, phenols. For example, compound 2 is more ionized than its isomer 8 (only one group ionized) and thus solute 8 comigrates with the aminophenols.

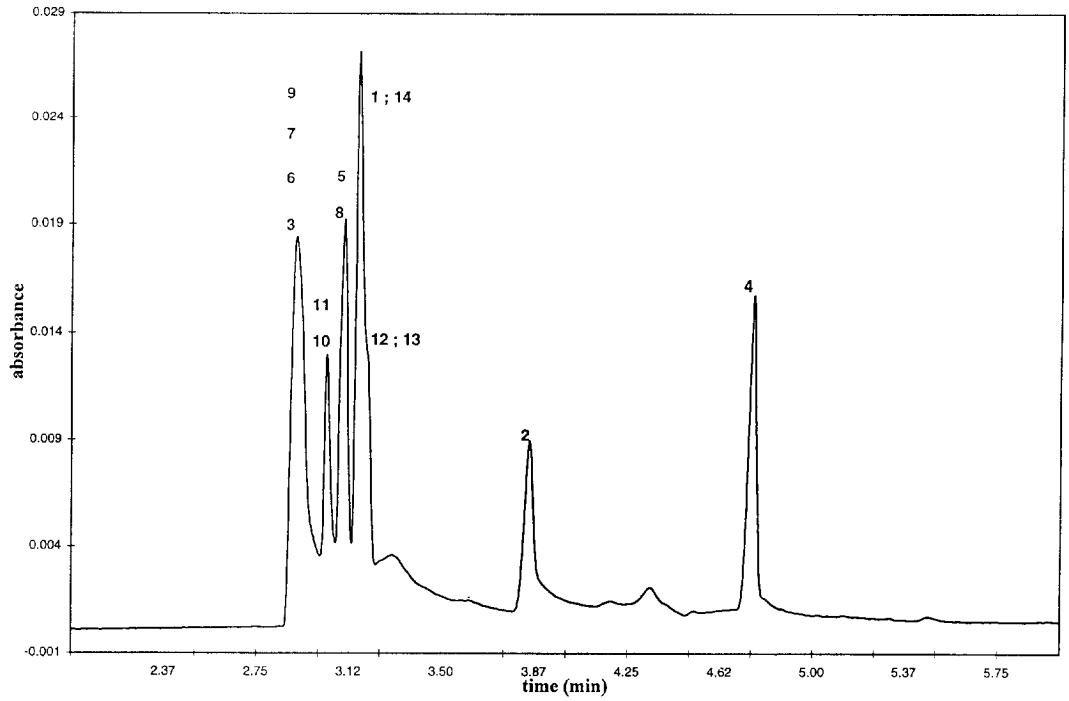


Fig. 4. Separation of oxidative dye mixture under alkaline conditions (CZE). Electrolyte, 25 m*M* phosphate–borate, pH 9.5; capillary, 47 cm × 50 μm I.D.; UV detection, 214 nm; hydrodynamic injection, 2 s; voltage, 20 kV; temperature, 25°C; concentration, 50 m*M*. For peak identification see Fig. 1.

3.2. Micellar electrokinetic chromatography

After the CZE experiments a more elaborate study was done using MEKC, first with SDS as surfactant, which is often used [13]. To determine the optimum conditions we do not calculate the m_{ep}, as in CZE, but instead we use the capacity factor, which takes the electrophoretic medium and the micelle interaction into consideration. The principle of separation in MEKC is based on the differential partition of the solute between the ionic micelles and the surrounding aqueous phase. In MEKC, the capacity factor of a neutral solute is given by [13]:

$$k' = \frac{t_r - t_0}{t_0(1 - t_r/t_{mc})} \tag{3}$$

where t_r, t_0 and t_{mc} are the migration times for the solute, the neutral marker (methanol) and the micelle tracer (anthracene). Thus, the capacity factor of a neutral compound in first approximation is linearly related to the micelle concentration through

$$k' = K\bar{\nu}(C_{sf} - CMC) \tag{4}$$

where K is the partition coefficient of the solute into the micelles, $\bar{\nu}$ the partial molar volume of the micelle, C_{sf} the total surfactant concentration and CMC the critical micellar concentration of the surfactant.

The migration behaviour of an anionic (or cationic) solute involves its own negative (or positive) electrophoretic mobility in the aqueous phase. Thus, in MEKC, the mobility of such an ionized solute is the weighted average of the mobility in the micellar phase and its mobility in the aqueous phase. Khaledi et al. [14] have derived the following equation:

$$k' = \frac{t_r - t_r'}{t_r'(1 - t_r/t_{mc})} \tag{5}$$

where t_r, $t_{r'}$ and t_{mc} are the migration times of the solute in MEKC, CZE and of the micelle tracer in MEKC. Several assumptions have been made in deriving this equation: firstly, the mobility of the micelles is assumed to be unchanged when analytes are dissolved in them, and secondly, the EOF is the same with or without micelles. The latter means that changes in the zeta potential and the buffer viscosity caused by adding micelles to the electrolyte are negligible.

Our study started at pH 7, because below this pH the electroosmotic flow decreases rapidly and the separation shows broadening of the peaks. A variation of the buffer pH from 7 to 10 (Fig. 5) allowed to determine the optimum resolution (pH 7). In contrast to the experiment performed at pH 8.5, the compounds 5, 6, 7 and 8 are well separated while the others comigrate. At pH 10, only compound 6 is resolved. The ionization of hydroxy groups increases in going towards alkaline pH values. The appearance of negative

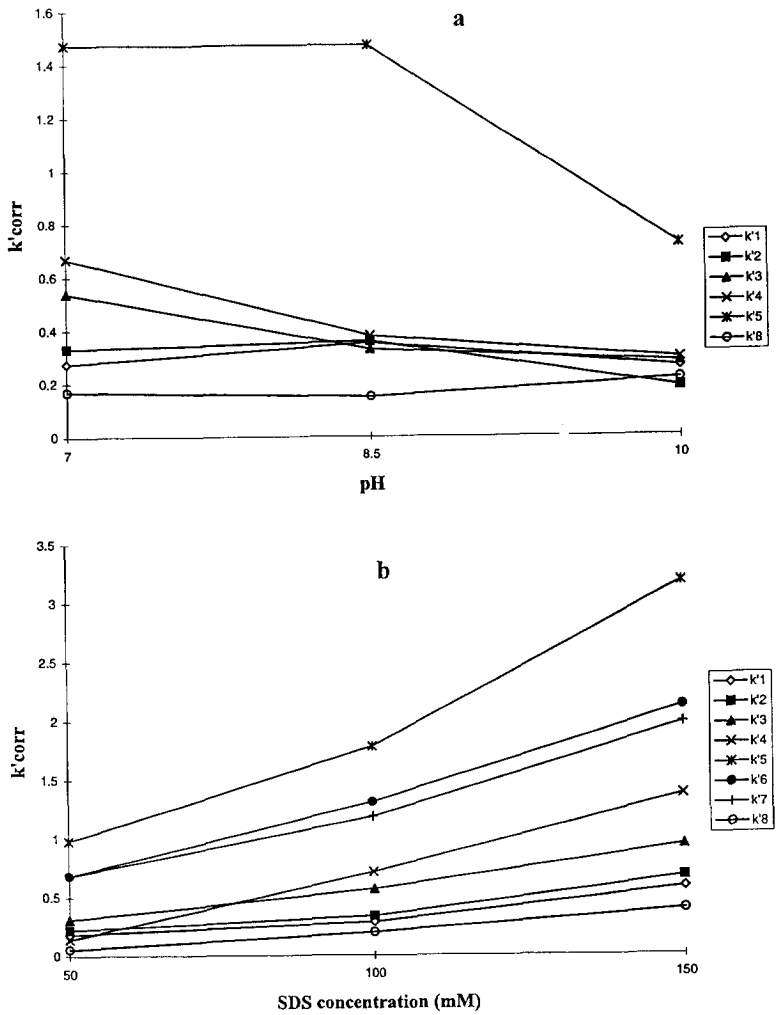


Fig. 5. (a) Influence of electrolyte pH in MEKC. Conditions: 75 m*M* SDS; capillary, 44 cm × 50 μm I.D. (b) Influence of SDS concentration in MEKC. Conditions: pH 7; capillary, 70 cm × 50 μm I.D. For (a) and (b): electrolyte, 25 m*M* phosphate–borate; UV detection, 214 nm; hydrodynamic injection, 2 s; voltage, 20 kV; temperature, 25°C.

charge on the compound decreases the micelle–compound interactions (as they are of similar sign), and thus resolution decreases. The same conclusion has been drawn by Takeda et al. [15] for the separation of aniline derivatives by MEKC. The capacity factor decreased significantly with increasing pH for *p*-anisidine and N-methylaniline due to a weaker protonation of amino groups; at alkaline pH the compounds interacted less with the micelles.

Next, the influence of the SDS concentration has been investigated. An increase of micelle volume improves the separation. However, it should be noted that the values of the capacity factors are rather low (under 3.5) even with an SDS concentration of 150 m*M*, in spite of a marked widening of the migration window. Comparing our results with those of Takeda et al. [15], we observed that the *k'*-values, which increase with the surfactant concentration, are always smaller than those reported for aniline derivatives. The aromatic solutes have pK_a values around 4–5 and consequently are partially ionized at pH 6.2. We cannot work below pH 7

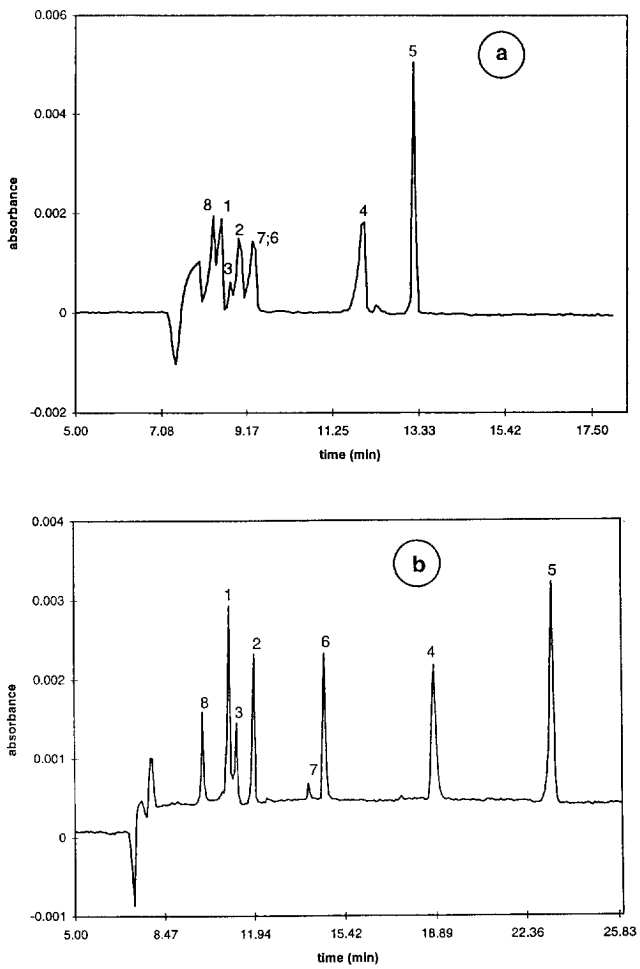


Fig. 6. Influence of ionic strength of electrolyte. (a) Electrolyte, 25 m*M* phosphate–borate, 150 m*M* SDS, pH 7. (b) Electrolyte, 75 m*M* phosphate–borate, 150 m*M* SDS, pH 7. Capillary, 70 cm × 50 μm I.D.; UV detection, 214 nm; hydrodynamic injection, 2 s; voltage, 20 kV; temperature, 25°C; concentration, 50 m*M*. For peak identification see Fig. 1.

with the oxidative dyes because the compounds have pK_a values smaller than those of the aniline derivatives. We can remark that diphenylamine and chloroaniline are more hydrophobic than aminophenol or phenylenediamine. Moreover, the mixture contains phenols which are neutral at this pH (compounds not present in the mixture of Takeda et al.).

For the mixture of oxidative dyes, improvement of the separation is obtained by increasing the electrolyte ionic strength. So the third parameter which has been changed, is the electrolyte ionic strength (Fig. 6). An increase of the buffer ionic strength induces a more hydrophilic aqueous medium, so a hydrophobic solute preferentially dissolves into the micelles. This fast mass transfer causes better peak efficiencies. The migration time has doubled, so the solutes are better separated: compounds 7 and 6 are resolved with 75 m*M* but not with 25 m*M* of electrolyte. Compound 2 is better resolved from compounds 3, 6 and 7, and has a better efficiency. However, compound 3 is not completely resolved, because the increase in ionic strength has incited a decrease of the electrophoretic mobility which is smaller than that for solute 1. Upon further increase of the ionic strength, compound 3 elutes before compound 1.

This study allowed us to determine the optimum conditions to realize the analysis of the fourteen oxidative dyes (Fig. 7). Among the fourteen solutes resolved in 27 min, two compounds (compounds 2 and 9) are not well resolved. If we compare the electropherogram with

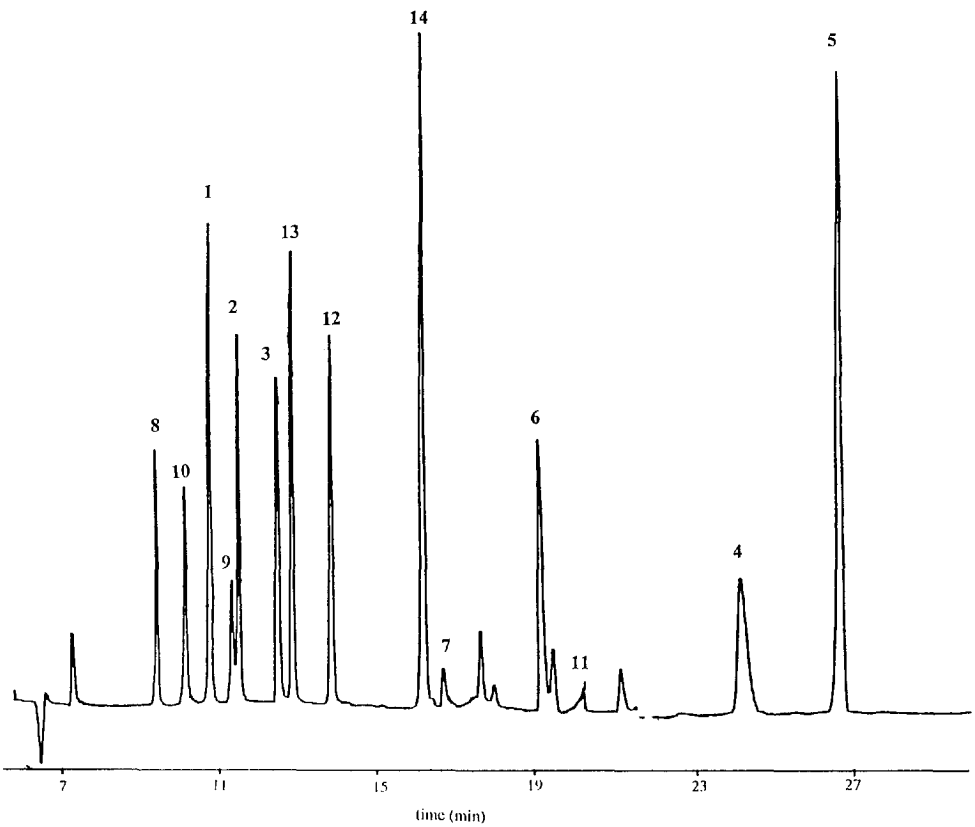


Fig. 7. MEKC separation with SDS surfactant under optimized conditions. Electrolyte, 75 m*M* phosphate–borate, 150 m*M* SDS, pH 7; capillary, 70 cm × 50 μm I.D.; UV detection, 214 nm; hydrodynamic injection, 2 s; voltage, 20 kV; temperature, 25°C; concentration, 50 m*M*. For peak identification see Fig. 1.

the HPLC chromatogram, the analysis time is similar but the MEKC resolution is better.

A similar study is realized using MEKC with the CTACl cationic surfactant. We selected CTACl because of its low Kraft temperature compared with CTABr. In the case of a cationic surfactant, ionic interactions exist between the ionized silanols and the CTA^+ cations. So, when the CTACl concentration increases, we note an evolution of the electroosmotic flow, which translates the modifications created at the silica capillary surface. At a given applied field strength and buffer system, the magnitude and the direction of the EOF is controlled solely by the capillary wall surface charge density. At pH 9.5, the ionization of Si–OH groups is very large at the wall surface. The addition of CTACl to the solution produces an EOF decrease, because the CTA^+ cations progressively cover the capillary surface due to attractive interactions with negative silanol groups. Then, a bilayer of CTACl molecules (hemimicelle) is formed at the capillary wall with a positive charge directed towards the centre of the capillary. So the direction of EOF is reversed [16–18].

In SDS-MEKC, few hydrophobic interactions exist between the aromatic molecules and the SDS micelles. So, we work at pH 9.5 to promote the ionization of hydroxy groups. Therefore, we will have hydrophobic interactions (same as SDS) and hydrophilic interactions [15].

Next, the variation in k' of each compound has been studied versus CTACl concentration. By increasing the CTACl concentration, the separation is improved (Fig. 8). To obtain a chromatogram of the mixture, we have chosen a CTACl concentration of 60 mM, since better separation will be obtained at higher concentrations. However, a high CTACl concentration produces a large current (100 μA) and results in

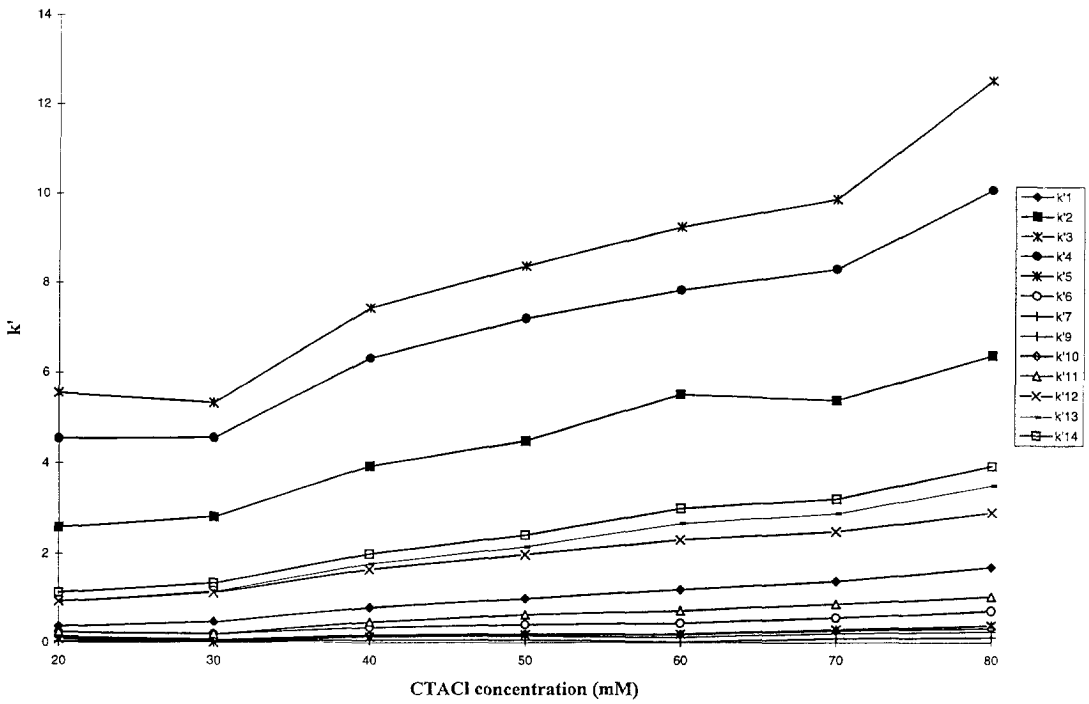


Fig. 8. Influence of CTACl concentration in MEKC. Electrolyte, 25 mM phosphate–borate, pH 9.5; capillary, 47 cm × 50 μm I.D.; UV detection, 214 nm; hydrodynamic injection, 2 s; voltage, 20 kV; temperature, 25°C.

a widening of peak 8 (and thus in loss of efficiency). A solution of phosphate–borate 30 m*M*, CTACl 60 m*M*, allows separation of the mixture with a good resolution; 14 peaks were obtained, which is better than the results obtained with HPLC, but less than obtained using MEKC with SDS. In MEKC, the analysis time using SDS is half of that found with CTACl, and thus separation could be improved. In the case of CTACl, the first migrating compounds are the phenylenediamines, which are neutral at pH 9.5 and have only hydrophobic interactions with the micelles. They are followed by the negative aminophenols (only one ionized group), having hydrophilic interactions with the positive Stern layer of the micelles. Lastly, we observe the migration of phenols (with two ionized groups), due to hydrophilic interactions with CTA^+ monomers (Fig. 9).

According to Kaneta et al. [19], we can calculate distribution coefficient of a compound between the aqueous medium and the micelles from Eq. 4. By plotting the variation of k'-values versus the surfactant concentration, we can graphically determine the CMC value (from the x-intercept) and K (from the slope $K\bar{v}$), as indicated in Fig. 10. The distribution coefficients for three solutes are reported in Table 1. The partial specific volumes given in the literature [20] are 0.977 ml/g for CTACl and 0.862 ml/g for SDS. The distribution coefficients are determined at pH 10, where hydroxy groups are ionized and amino groups are neutral. Finally, distribution coefficients are compared with the ionization degree of these three compounds. For the CTACl surfactant, decreasing order of the distribution coefficients is 1,3-benzenediol > 3-aminophenol > 2-(2,4-diaminophenoxy)ethanol,

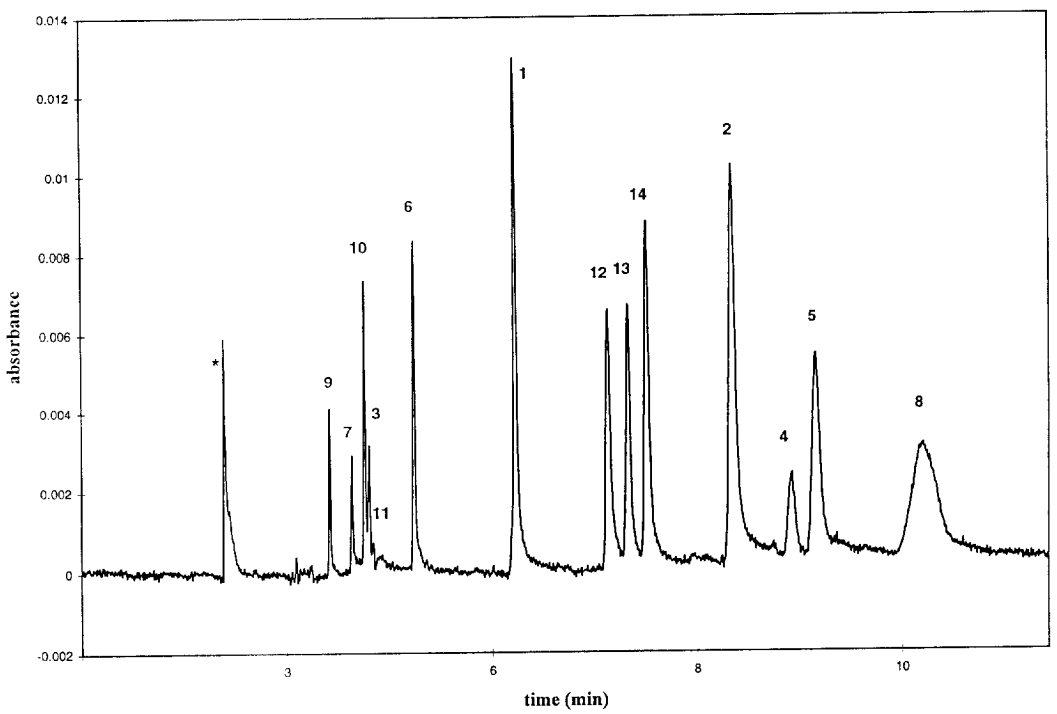


Fig. 9. MEKC separation with CTACl surfactant under optimized conditions. Electrolyte, 25 m*M* phosphate–borate, 60 m*M* CTACl, pH 9.5; capillary, 47 cm × 50 μm I.D.; UV detection, 214 nm; hydrodynamic injection, 2 s; voltage, 20 kV; temperature, 25°C; concentration, 50 m*M*. Asterisk indicates the peak of $Na_2S_2O_5$. For peak identification see Fig. 1.

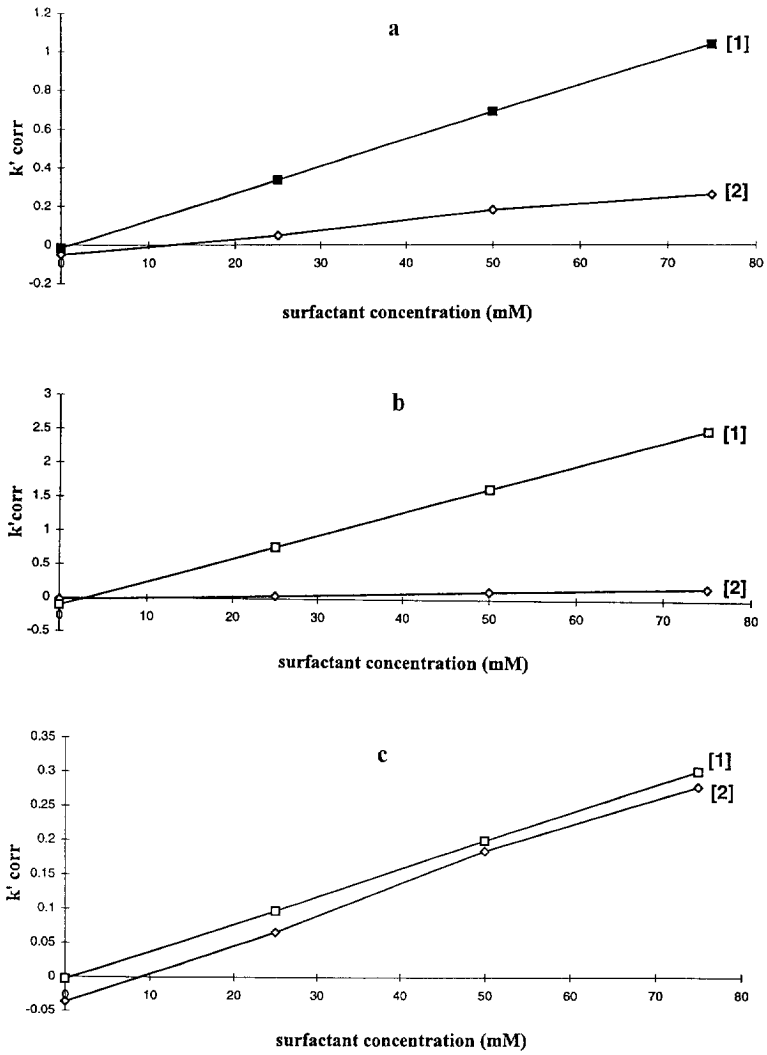


Fig. 10. Variation of corrected capacity factor versus CTACl surfactant concentration. Electrolyte, 25 m*M* phosphate–borate, pH 10; capillary, 47 cm × 50 μm I.D.; UV detection, 214 nm; hydrodynamic injection, 2 s; voltage, 20 kV; temperature, 25°C. Surfactant: [1] CTACl, [2] SDS. Compound: (a) 3-aminophenol, (b) 1,3-benzenediol, (c) 2-(2,4-diaminophenoxy)ethanol.

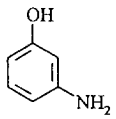
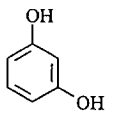
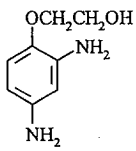
which is related with the decreasing interactions with the micelles. At pH 10, the neutral diamine group has a higher affinity for the aqueous phase than for the micellar phase. For hydroxy groups, the increase in *K*-value means an increase in the affinity with the micelles. Benzenediol has two groups ionized, and therefore it has a higher affinity for the micelle than aminophenol, which has only one hydroxy group.

For the SDS surfactant all *K*-values are small, because these compounds have less affinity for the micellar medium. The differences between *K*-values for anionic and neutral solutes are small.

4. Conclusion

The separation of oxidative dyes is realized by MEKC using two kinds of surfactant (SDS and

Table 1
Distribution coefficients of solutes for two surfactants

Solute		SDS surfactant			CTACl surfactant		
Structure	pK_a	K	CMC (mM)	r^2	K	CMC (mM)	r^2
	4.3 9.4	17.3	11.6	0.9812	45.1	1.13	0.9993
	9.1 11	10.2	9.1	0.9962	112.3	3.16	0.9880
	3.2 5.4	17.3	8.4	0.9954	13.1	1.39	0.9918

Electrolyte, 25 mM phosphate–borate, pH 10; capillary, 47 cm × 50 μm I.D.; UV detection, 214 nm; hydrodynamic injection, 2 s; voltage, 20 kV; temperature, 25°C.

CTACl). The separation of fourteen dyes has been satisfactorily achieved using 150 mM SDS at pH 7 or using 60 mM CTACl at pH 9.5. Thus with the method described here good resolution of the fourteen compounds was achieved. However, the analysis time differs from 30 min using SDS to 12 min using CTACl.

Acknowledgement

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