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Separation of anilines by capillary electrophoresis with small ionic compounds as buffer additives

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Abstract

Addition of either ethanesulfonic acid or protonated triethylamine to the background electrolyte was found to markedly improve the separation of protonated anilines by capillary electrophoresis. These additives appear to form a thin coating on the capillary surface via a dynamic equilibrium. This results in a change in electroosmotic flow and reduces interactions of the sample cations with the silica surface. A mixture of 10 substituted anilines could be separated, including several positional isomers. Migration times of the sample cations were reproducible with a RSD less than 1.0%. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: Background electrolyte composition; Anilines

1. Introduction

Organic bases can be separated by capillary electrophoresis (CE) as protonated cations by operating at an acidic pH. Lin et al. separated β-blockers using citrate buffer at a very acidic pH [1]. Basic proteins and peptides have been separated at acidic pH values with a positively-charged surfactant additive in the background electrolyte (BGE) [2,3]. The surfactant is adsorbed on the capillary walls, giving a positive surface that repels the protein cations and prevents their adsorption. Chiral bases have been resolved by CE using chiral additives such as native and derivatized β-cyclodextrins [4–6]. Organic bases may also be separated in their molecular form by micellar electrokinetic chromatography. Sodium dodecyl sulfate (SDS) [7], tetraalkylammonium salts [2–4,8], bile salts [9,10] and glycopyranoside-based surfactants [11,12] have been used for this purpose. In some cases, especially for protein separations, polymers were employed to coat the capillary surface covalently or adsorptively so that the interactions between the analytes and silanol groups on the capillary surface were decreased or eliminated. Poly(ethylene glycol) (PEG) [13–15], poly(vinyl alcohol) (PVA) [16] and poly(ethylene oxide) [17] are several examples of the polymers used.

Generally, the additives mentioned above are quite large. While they have usually worked well for the separation of basic compounds, there are some problems with their usage. Surfactants and other large additives may form thick coatings on the capillary surface. Wei and Fritz demonstrated that the coating could continue to build up from run to run, thus causing a gradual increase in migration time [2]. It may be difficult to remove completely when experimental conditions are changed.

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Published work suggests that the use of much smaller molecules as BGE additives may effectively inhibit unwanted interactions between sample cations and the silica surface of the capillary. Incorporation of ethanesulfonic acid in the BGE was found to markedly improve the CE separation of protonated amino acids [18] and basic drugs [19] at an acidic pH. Quang and Khaledi found that tetrabutylammonium salts improved the chiral separation of bases with β-cyclodextrin at pH 2.5 [4]. Triethylammonium salts have often been used in HPLC to block secondary interactions between analytes and the silica-based stationary phase [20], which indicates its potential as the BGE additive for CE separations.

This research has two main goals. One was to find conditions for a practical CE separation of substituted anilines of very similar chemical structure. Compounds of this type are potential environmental pollutants [21–23]. Several groups reported the separation of chloro- and nitro-substituted anilines by different techniques [24–26]; however, nothing about the separation of alkyl-substituted anilines could be found. The second, and perhaps more important, goal was to study the effect of some small organic ions as CE electrolyte additives on the separation of protonated organic bases. A low concentration of ethanesulfonic acid (ESA) or protonated triethylamine (TEA) in the BGE was found to greatly improve the separation of organic cations and to provide excellent reproducibility of the migration time. Experiments were performed to elucidate the mechanism of ESA and TEA with the silica surface of the capillary.

2. Experimental

A Waters Quanta 4000 capillary electrophoresis system (Millipore Waters, Milford, MA, USA), equipped with a positive power supply, was employed to separate anilines under acidic conditions and generate all electropherograms. Polyimide-coated fused-silica capillaries (Polymicro Technologies, Phoenix, AZ, USA) were 45 cm (37 cm effective length)×50 μm I.D. Direct UV detection was performed at 229 nm. A voltage of +15 kV was applied for all separations. All samples were injected electrokinetically, and electroosmotic flows (EOF) were measured with the 'accelerated method' introduced by Sandoval and Chen [27] with formamide and dimethyl sulfoxide (DMSO) as neutral markers. Electropherograms were collected at a speed of 15 points/s and plotted by the Chromperfect data acquisition system (Justice Innovations, Mountain View, CA, USA).

Standards of substituted anilines, ethanesulfonic acid (ESA), triethylamine and diethylenetriamine were purchased from Aldrich (Milwaukee, WI, USA). Organic solvents were obtained from Fisher Scientific (Fair Lawn, NJ, USA). All chemicals were the best grade available. All standards and buffer solutions were prepared with 18.2 MΩ deionized water by a Barnstead Nanopure II system (Sybron Barnstead, Boston, MA, USA). Buffers were made by mixing phosphoric acid and ethanesulfonic acid/amine additives, and adjusting the pH with 1 *M* sodium hydroxide or 1 *M* phosphoric acid before 2-propanol was added.

Each new capillary was conditioned with 1 *M* sodium hydroxide for 1 h, followed by 1-h rinsing with deionized water. Each day, prior to use, capillaries were rinsed with 0.1 *M* sodium hydroxide and deionized water for 10 min each. When different running buffers were used, capillaries were rinsed with the desired buffer for 10 min following the NaOH and deionized water rinsing. Before each sample injection, capillaries were rinsed with the running buffer for 3 min.

3. Results and discussion

3.1. Effect of ESA on the separation of anilines

3.1.1. Preliminary experiments

Preliminary experiments at an acidic pH gave very poor CE separations of various protonated anilines when no additive was used in the CE buffer solution. A similar situation had been observed for the CE separation of protonated amino acids [18]. Varying the pH or increasing the phosphate concentration in the buffer improved the resolution somewhat, but separation of the aniline mixture was still far from complete, and the peaks were broad and somewhat tailed. Addition of 30–50 m*M* ESA to the BGE was

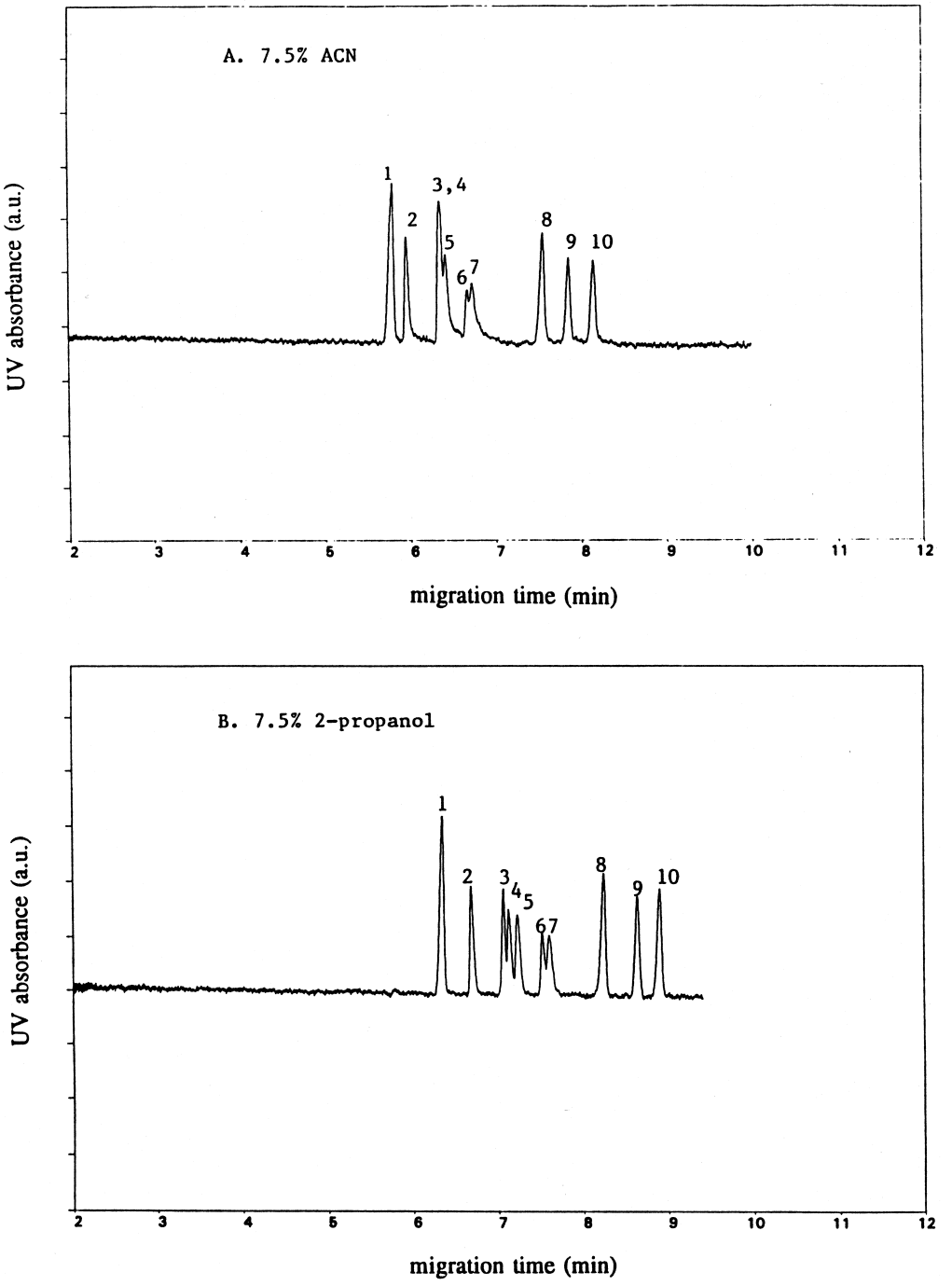

Fig. 1. Separation of substituted anilines. Electrolyte: 50 m*M* ESA, 10 m*M* phosphate, 7.5% organic solvent, pH 3.70; electrokinetic sampling, 8 s, 5 kV. Peaks: 1=aniline, 2=4-ethylaniline, 3=3-ethylaniline, 4=4-propylaniline, 5=4-isopropylaniline, 6=4-butylaniline, 7=4-*sec*.-butylaniline, 8=2-ethylaniline, 9=2-isopropylaniline, 10=2-propylaniline. Each analyte was 50 ppm in the sample solution.

found to greatly improve the separation of the substituted anilines.

Preliminary results on buffer pH established pH 3–4 as the optimum range, so the subsequent experiments were performed within this range.

3.1.2. Selection of an organic solvent

Experiments were performed in which the BGE contained 50 m*M* ESA in addition to a phosphate buffer, and part of the water in the buffer was replaced with an organic solvent. Three types of organic solvent, acetonitrile, 2-propanol and 1-butanol, were studied, and each solvent was tested with several concentrations in the buffer composition: 2.5, 5.0, 7.5 and 10% (2.5 and 5.0% only for 1-butanol). Electropherograms are given in Fig. 1 comparing acetonitrile and 2-propanol as organic modifiers. The results with 2-propanol showed better resolution and peak shape for the aniline separations, probably due to the better solvation of the analytes by 2-propanol, while the analysis time did not differ much for three solvents. So 2-propanol was chosen as the organic solvent. A concentration of 7.5% 2-propanol itself in the buffer gave slightly better separation of 10 substituted anilines than other concentrations. Therefore, 7.5% 2-propanol was added to the electrolytes for all the following studies.

3.1.3. pH effect

The pH study was confined to pH 3.0–4.0. Outside this range, the separation was not acceptable; the baseline was noisy and several analytes comigrated. Measured or predicted pK_a values are available for several of the substituted anilines and ranged approximately from 4.4 to 5.1 [28]. Between pH 3.0 and 4.0, the substituted anilines would be partially protonated to different degrees. This would give different mobilities and contribute to a better separation. Without this effect, the separation would be based only on small differences in analyte structure and would be much more difficult.

Fig. 2 shows the effect of buffer pH on the migration behavior of the substituted anilines. It is evident that all the analytes migrated slower at higher buffer pH, as would be expected. The pH for the best separation of all 10 of the anilines studied

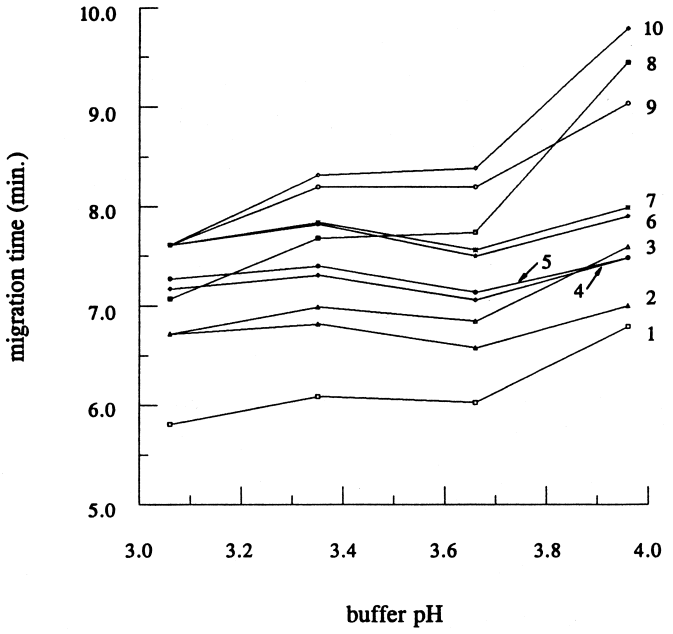

Fig. 2. Effect of buffer pH on the migration time of substituted anilines. Electrolyte: 10 m*M* phosphate, 50 m*M* ESA, 7.5% 2-propanol; electrokinetic sampling, 8 s, 5 kV. Sample identity: 1=aniline, 2=4-ethylaniline, 3=3-ethylaniline, 4=4-propylaniline, 5=4-isopropylaniline, 6=4-butylaniline, 7=4-*sec*.-butylaniline, 8=2-ethylaniline, 9=2-isopropylaniline, 10=2-propylaniline.

was determined from Fig. 2 to be 3.65 with ESA as the additive.

3.1.4. Effect of ESA on EOF and electrophoretic mobility

A more complete picture of the effect of the electrolyte additives can be obtained by measuring the electroosmotic flow (EOF) and the electrophoretic mobilities as a function of the additive concentration. Plots for ESA at pH 3.65 are given in Fig. 3. The EOF dropped rapidly and then decreased much more slowly as the ESA concentration was increased. This would indicate a decrease in surface negative charge. A thin layer of ESA is most likely

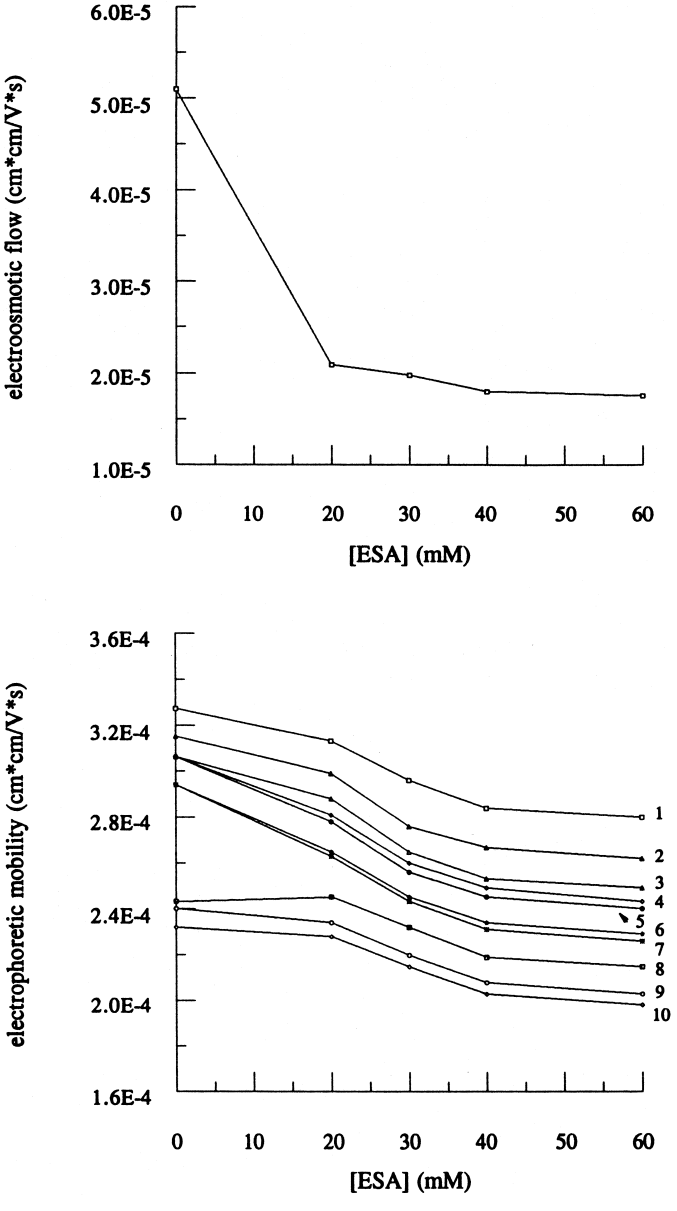

Fig. 3. Effect of ESA concentration on EOF and electrophoretic mobilities of substituted anilines. Electrolyte: 10 m*M* phosphate, 7.5% 2-propanol, pH 3.65; electrokinetic sampling, 8 s, 5 kV. Sample identity: same as Fig. 2.

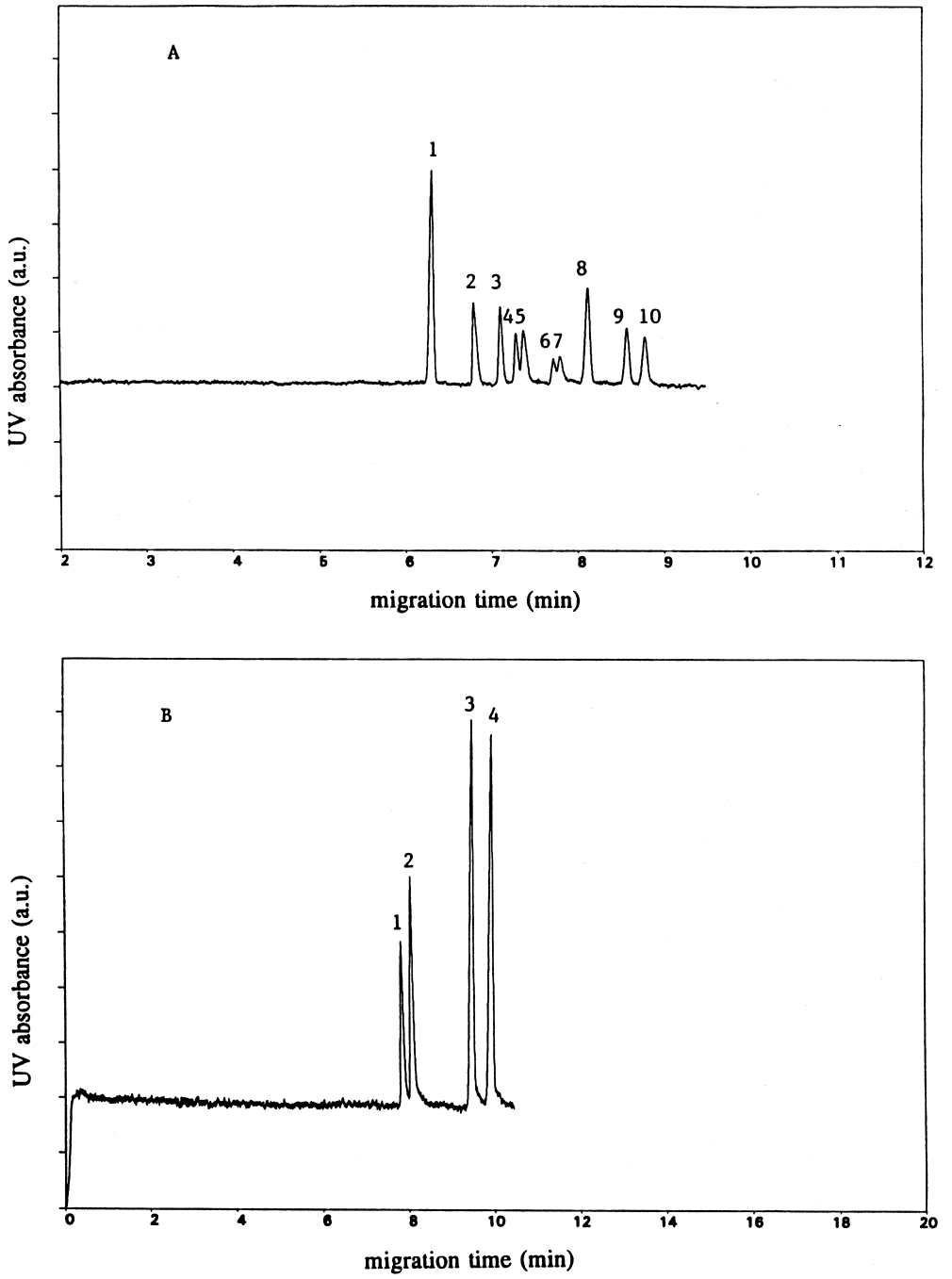

Fig. 4. Separation of substituted anilines. Electrolyte: 55 m*M* ESA, 15 m*M* phosphate, 7.5% 2-propanol, pH 3.65; electrokinetic sampling, 8 s, 5 kV. Peaks: (A) same as Fig. 1; (B) 1=4-butylaniline, 2=4-*tert*.-butylaniline, 3=2-*sec*.-butylaniline, 4=2-butylaniline. Each analyte was 50 ppm.

adsorbed on the silica surface via a dynamic equilibrium [18]. Adsorption of ESA may involve hydrogen bonding between the sulfonate groups and the surface silanol groups of the silica capillary, so that some of the surface negative charges are covered by ESA. Adsorption of a second layer of ESA, which would give a more negative surface, seems unlikely because the hydrocarbon chains of the ESA are too short to provide much hydrophobic attraction.

Fig. 3 shows that the electrophoretic mobilities also decrease somewhat with increasing ESA concentration. This is probably due to a certain amount of ion-pair formation between the protonated anilines and the negatively-charged ESA

$$BH^+ + ESA^- \rightleftharpoons \text{Ion pair} \quad K = \frac{[\text{ion pair}]}{[BH^+][ESA^-]}$$

A decrease in the fraction of analyte present as the free cation (BH^+) would decrease its electrophoretic mobility. Differences in the equilibrium constants (K) of the various analytes would lead to improved separations.

From Fig. 3 an ESA concentration within 40–60 m*M* seems to be optimal for the separation of substituted anilines. Included in Fig. 4 is the separation of some anilines with 55 m*M* ESA in the background electrolyte. Several butylaniline positional isomers were baseline resolved under these conditions.

3.1.5. Plate number

Ding found that addition of ethanesulfonic acid (ESA) to the BGE gave much sharper peaks for the separation of basic drugs at pH ~2.5 in aqueous electrolytes containing 10% acetonitrile [19]. In the present work, addition of ESA was shown to improve both peak sharpness and peak symmetry. Typical values for CE separation of anilines at pH 3.65 in aqueous solution containing 7.5% 2-propanol with or without ESA as BGE additive are shown in Table 1.

Table 2
Reproducibility test. For ESA, electrolyte contained 15 m*M* phosphate, 55 m*M* ESA and 7.5% 2-propanol at pH 3.65; for TEA, electrolyte contained 50 m*M* phosphate, 40 m*M* TEA and 7.5% 2-propanol at pH 3.45. Electrokinetic sampling, 8 s, 5 kV

Compound	RSD (%)	
	ESA	TEA
Aniline	0.8	0.4
4-Ethylaniline	0.8	0.5
3-Ethylaniline	0.9	0.5
2-Ethylaniline	1.1	0.7

3.1.6. Reproducibility

The run-to-run reproducibility of the aniline migration times was determined by replicate injections ($n=5$) on the same capillary. Four anilines were used as sample analytes. No treatment of the capillary was performed between runs except for a brief rinsing with fresh buffer. The data in Table 2 for buffers containing 55 m*M* ESA gave migration times with an average RSD of 0.9%. These results indicate that ESA in the buffer leads to excellent reproducibility and that no appreciable buildup of ESA occurs on the capillary surface.

3.2. Effect of amine additives

3.2.1. pH effect

Addition of 40 m*M* of protonated TEA to the BGE instead of ESA also improved the peak sharpness and resolution of the aniline analytes. The pH study in Fig. 5 shows that a pH of 3.45 gave the best resolution of the test mixture. Migration times were longer with TEA than with ESA as the additive.

Table 1
Comparison of plate number (N) and peak asymmetry factor (PAS) for separations with and without ESA as the BGE additive. Electrolyte: 10 m*M* phosphate, 7.5% 2-propanol, pH 3.65. Electrokinetic sampling, 8 s, 5 kV

Compound	*N*		PAS	
	0 m*M* ESA	50 m*M* ESA	0 m*M* ESA	50 m*M* ESA
Aniline	22,000	81,000	2.34	0.60
2-Propylaniline	54,000	127,000	0.32	0.88

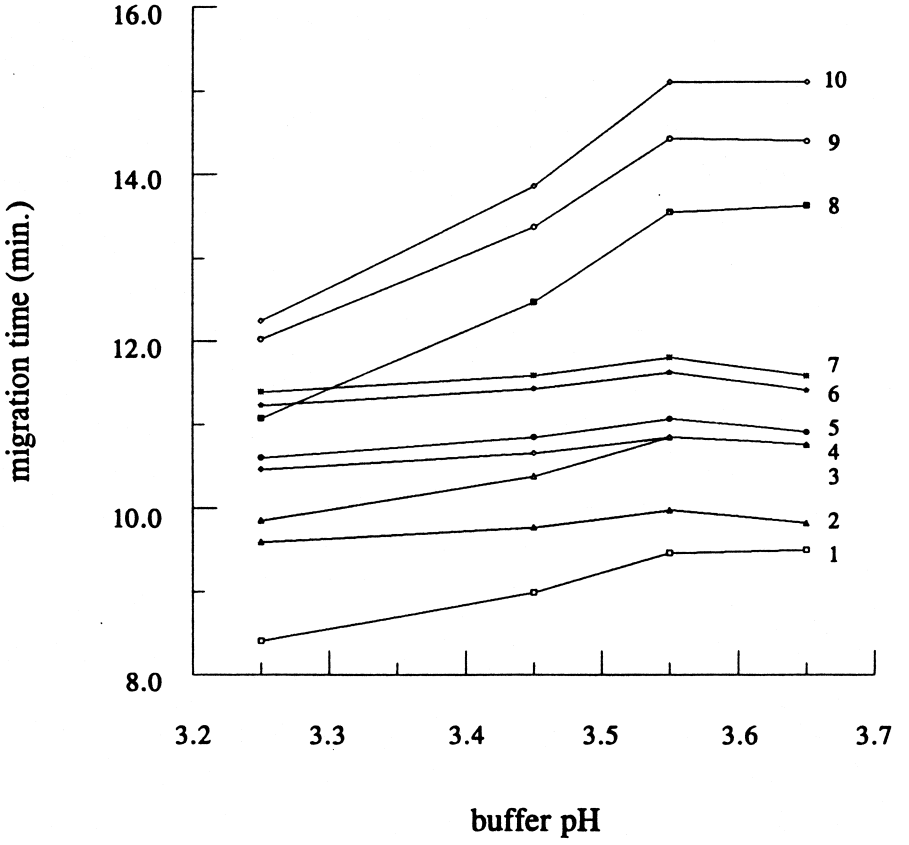

Fig. 5. Effect of buffer pH on the migration time of substituted anilines. Electrolyte: 40 m*M* triethylamine, 50 m*M* phosphate, 7.5% 2-propanol; electrokinetic sampling, 12 s, 3 kV. Sample identity: same as Fig. 2.

3.2.2. Effect of amine additives on EOF and electrophoretic mobility

The protonated forms of two amine additives were studied: triethylamine (TEA) and diethylenetriamine (DETA). Fig. 6 shows a much greater change in EOF for TEA than was observed for ESA. The EOF decreased steadily within the concentration range studied, and its direction was reversed from positive to negative as the amount of triethylamine in the buffer increased. This clearly pointed out the adsorption of triethylamine onto the capillary surface. This adsorption could probably involve several aspects: the electrostatic interactions between the negative capillary surface and the triethylamine cations, the hydrogen bonding between the silanols and the amino groups, and perhaps the hydrophobic interactions between the alkyl chains and the siloxane groups, which are known to exhibit hydrophobic character [29,30]. The electrophoretic mobilities of the analytes did not vary much within the same concentration range, which indicated the absence of interactions between the substituted anilines and triethylamine. A higher concentration of TEA gave a better separation of substituted anilines. This occurred because of a higher EOF counter to electrophoretic mobilities, which increased migration times and gave better peak resolution. Fig. 7 shows the separation of anilines with 40 m*M* TEA as the BGE additive.

Diethylenetriamine (DETA) was also briefly investigated as a buffer additive. Under the conditions for this study (pH 3.65), each DETA molecule possesses more than two positive charges, so it is more polar than both ESA and triethylamine. As the DETA concentration was varied from 0 to 30 m*M*, the EOF decreased and reached a certain value

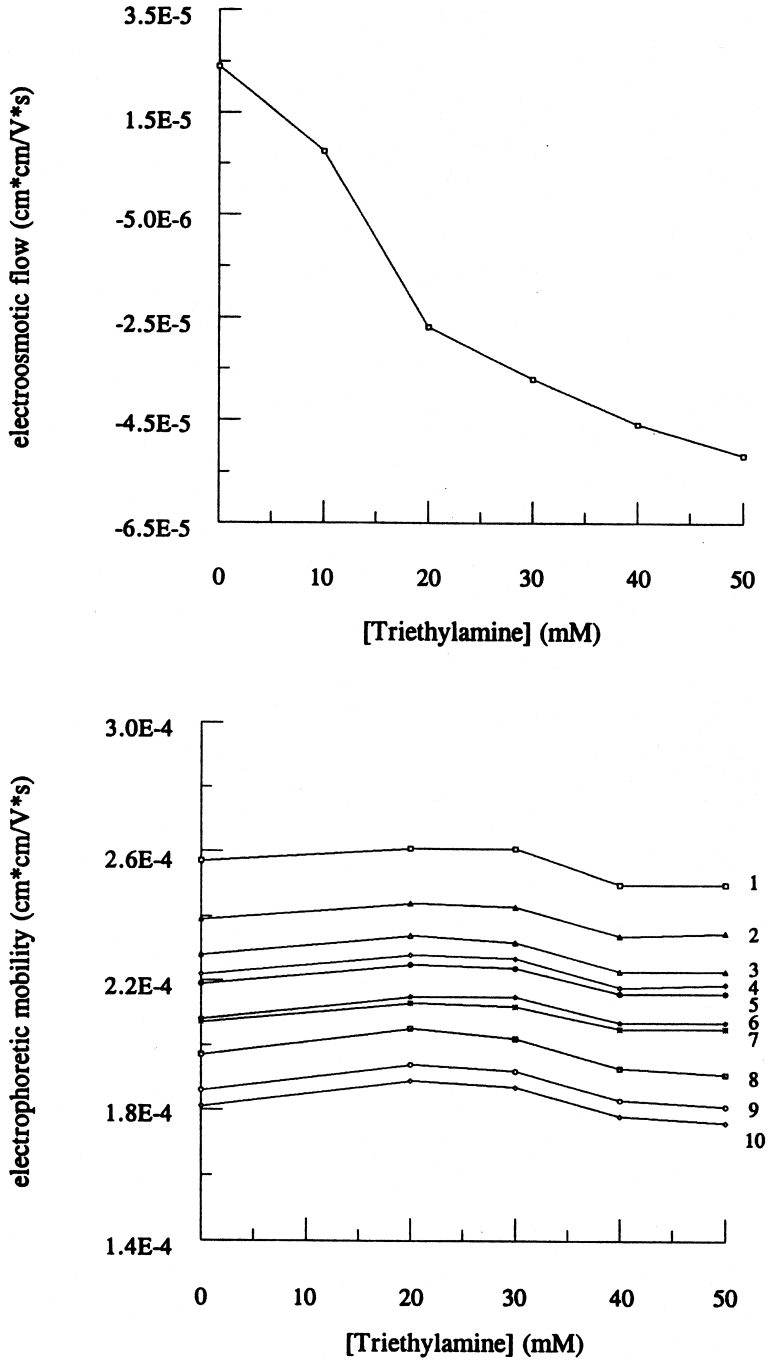

Fig. 6. Effect of triethylamine concentration on EOF and electrophoretic mobilities of substituted anilines. Electrolyte: 50 m*M* phosphate, 7.5% 2-propanol, pH 3.45; electrokinetic sampling, 12 s, 3 kV. Sample identity: same as Fig. 2.

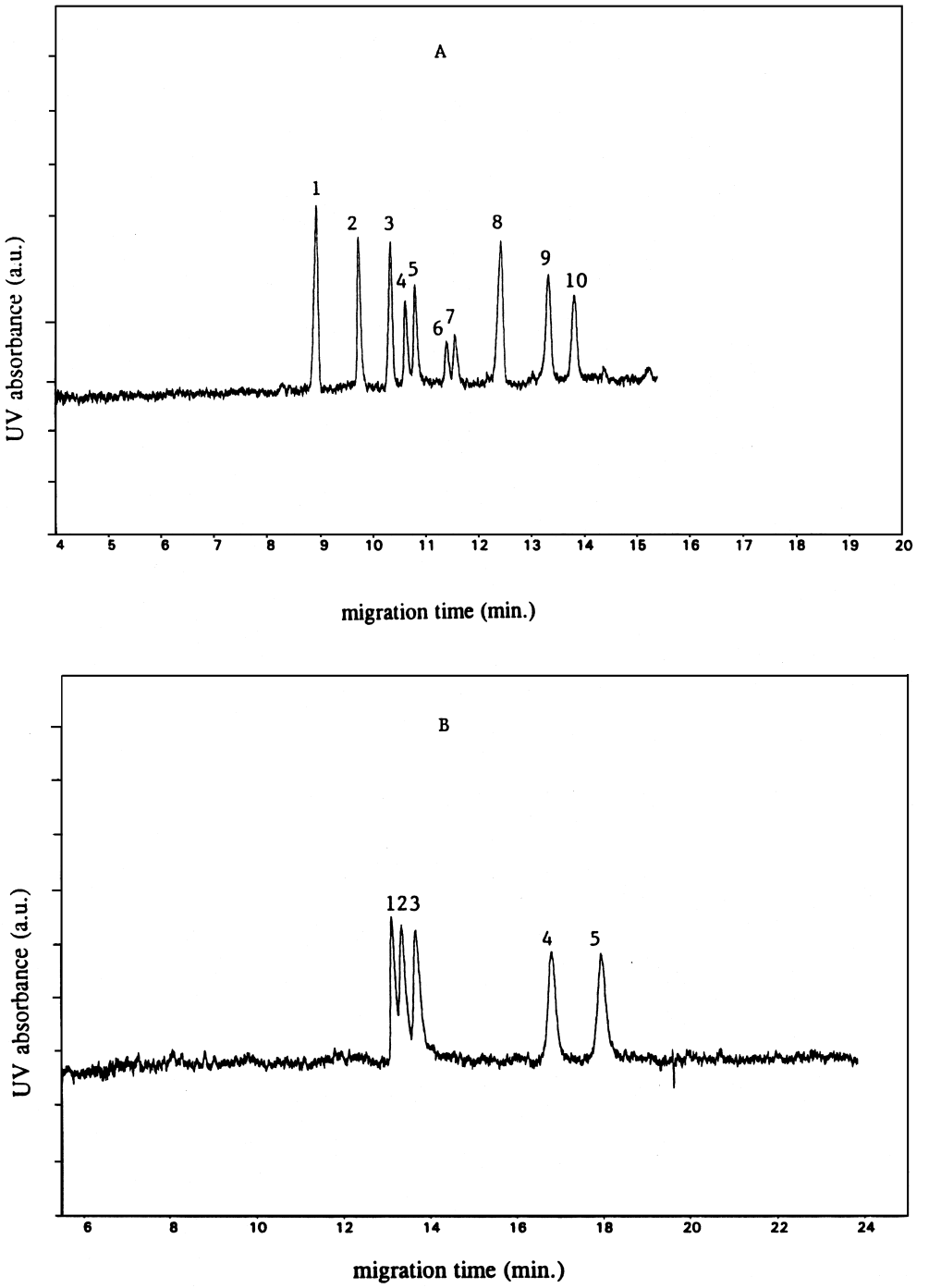

Fig. 7. Separation of substituted anilines. Electrolyte: 40 m*M* triethylamine, 50 m*M* phosphate, 7.5% 2-propanol, pH 3.45; electrokinetic sampling, 10 s, 4 kV. Peaks: (A) same as Fig. 1; (B) 1=4-butylaniline, 2=4-*sec*.-butylaniline, 3=4-*tert*.-butylaniline, 4=2-*sec*.-butylaniline, 5=2-butylaniline. Each analyte was 50 ppm.

instead of being reversed. Probably, DETA could interact with the surface only through hydrogen bonding. Unlike triethylamine, its high polarity prevented the hydrophobic interaction between DETA molecules and the siloxane groups, and its structure made it impossible to form a DETA bilayer on the surface and reverse the EOF. However, the electrophoretic mobilities did not change much, which is similar to the variation with triethylamine. Therefore, it is likely that there is no interaction between the analytes and these cationic additives.

3.2.3. Reproducibility

Run-to-run reproducibility ($n=5$) of migration times was determined at pH 3.45 in CE buffer solutions containing 7.5% 2-propanol and 40 m*M* protonated TEA. The average RSD (Table 2) was 0.5%.

4. Conclusions

Small anionic or cationic buffer additives, such as ESA and protonated TEA, were shown to improve the separation of protonated organic bases. These additives appear to form a thin coating on the capillary surface which modifies the electroosmotic and electrophoretic mobilities. Most likely, the additives reduce or prevent interaction of the sample cations with the capillary surface, thereby giving sharper sample peaks. Excellent reproducibility ($<1\%$ RSD) of migration times was obtained.

A mixture of 10 substituted anilines was separated with near baseline resolution. The compounds separated included several positional isomers as well as isomers with primary, secondary and tertiary butyl groups.

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