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Selectivity enhancement for free zone capillary electrophoresis using conventional ion-pairing agents as complexing additives

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Abstract

Analyte selectivity in capillary zone electrophoresis may be limited due to similarities in the charge/size ratio for related compounds. Conventional cationic and anionic ion-pair agents including alkylsulfonic acids and tetraalklyammonium salts have been investigated as additives to the electrophoretic run buffer to improve both the selectivity and resolution for organosolutes, peptides and proteins. Several different factors are responsible for changes in selectivity and resolution, including ionic interaction and hydrophobic association between solutes and additives as well as the modulation of electroosmotic flow. Selectivity improvements due to ionic interaction can be systematically optimized by varying size and concentration of the ion-pairing agent(s) in the buffer system. Butanesulfonic acid in particular appears to very useful for a diversity of applications including improved neurotransmitter separations, peptide mapping and separations of protein mixtures.

Keywords: Ion-pairing reagents; Selectivity; Complexation; Buffer composition; Peptides; Proteins; Organic acids; Neurotransmitters

1. Introduction

Since the early development of capillary electrophoresis (CE) almost two decades ago [1–3], one of the most attractive features of the free zone format (CZE) has been its inherent simplicity. For the straightforward analysis of conventional organic acids, bases, amino acids and peptides, CZE combines unparalleled efficiency and high throughput, requires minimal sample and buffer volumes and is conducive to rapid method development. It is well established that the intrinsic separation mechanism of CZE invokes differences electrophoretic mobility (μ_e) as a function of the charge-to-size ratio. Although the favorable operating efficiencies associated with zone electrophoresis frequently result in exceptionally sharp peaks, as a practical issue insufficient selectivity between analytes of similar charge-to-size ratios often precludes their resolution. To increase electrophoretic selectivities for ions or molecules undergoing separation, a number of approaches have historically been presented that take advantage of complexation equilibria. Several well-known examples include the use of chelating agents [4], alkylammonium salts [5,6], tetraborate [7,8] and cyclodextrins [9,10] to differentially complex the analytes of interest, thus promoting the desired enhancement in resolution.

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Walbroehl and Jorgenson demonstrated early in the development of CZE that *n*-alkylammonium salts at concentrations below the critical micelle concentration (CMC) could be used for the separation of neutral polyaromatic hydrocarbons based on hydrophobic association in predominantly organic solvents [5]. More recently, Chen and Pietrzyk used the principle of ion-pairing for improving resolution for anionic surfactants by adding Mg^{2+} to the CZE run buffer [11]. In bioanalytical applications, Nashabeh and El Rassi found that the addition of tetrabutylammonium bromide to the run buffer improved resolution for glycoprotein digests [6], and McGlaughlin et al. noted that the addition of hexanesulfonic acid to the CZE run buffer significantly increased the number of resolved components for peptide digests [12]. While the concept of adding complexing agents to the CZE buffer system is not new, to our knowledge a systematic study that considers both ionic and hydrophobic effects of conventional HPLC ion-pair additives as manifested by changes in selectivity and resolution has not been published.

In the present paper we investigate the use of a broad range of conventional HPLC ion-pairing agents for altering selectivity and improving resolution for a diverse series of solute types including conventional organic acids and bases, peptides and proteins. In principle, by taking advantage of ionic and hydrophobic interactions between individual analytes and a common additive, selectivity can be altered so long as the individual association constants are different. Since the kinetics of such processes are rapid and reversible, complexation is observed as a shift in migration time for each component as in affinity capillary electrophoresis [13].

It is expected that the concentration, size and charge of the selected additive will affect migration as a result of capillary wall (electroosmotic flow, EOF) effects in combination with complexing effects with the solute. If ionic interactions are significant (e.g., ion-pair formation), the buffer pH and resulting solute charge should greatly influence the equilibrium established between additive and solute, thereby creating changes in selectivity. Hydrophobic association may also play a role in the separation, particularly for non-polar solute types as previously demonstrated [5]. The inclusion of ionic additives in the CZE run buffer will produce additional changes in the overall solution ionic strength relative to the buffer by itself, and this will result in an alteration of the zeta potential at the capillary wall and possibly prevent solute–wall effects that lead to increased dispersion. In the following discussion we address these issues individually by examining several simple mixtures in which these effects are evaluated. The practical benefits of adding small concentrations of such inexpensive and readily available compounds to the background run buffer is realized in the vastly improved separations achieved for both small molecule and macromolecule mixtures.

2. Experimental

2.1. Chemicals

Tetramethylammonium chloride (TMAC), tetraethylammonium chloride (TEAC), tetrabutylammonium perchlorate (TBAP), tetrahexylammonium chloride (THAC), butanesulfonic acid (BSA), heptanesulfonic acid (HSA), decanesulfonic acid (DSA), Brij-35, benzaldehyde and ultrapure buffer salts were obtained from Aldrich (Milwaukee, WI, USA). Dopamine, norepinephrine, epinephrine, serotonin, caffeic acid, *p*-hydroxycinnamic acid, vanillic acid, ferulic acid, bovine cytochrome *c*, ribonuclease A, bovine serum albumin, bovine trypsin (EC 3.4.21.4) and human hemoglobin A0 stabilized to ferrohemoglobin were obtained from Sigma (St. Louis, MO, USA). Water deionized to 18.3 MΩ·cm was obtained from a Barnstead Nanopure deionization system (Fisher Scientific, Atlanta, GA, USA).

2.2. Equipment

All CZE separations were performed using Spectraphoresis 500 or Spectraphoresis 1000 CE systems (Thermo Separation Products, Fremont, CA, USA). Uncoated fused-silica capillary (75 μm I.D.×363 μm O.D. or 50 μm I.D.×363 μm O.D.) was obtained from PolyMicro Technologies (Phoenix, AZ, USA). Protein digestion was carried out in a Forma-Temp Jr. Model 2095 constant temperature bath (Forma Scientific, Marietta, OH, USA).

2.3. Sample/buffer conditions

Sodium phosphate buffers (pH 2.5 and 7.0) were

prepared by dissolution of monobasic or dibasic sodium phosphate in deionized water followed by the addition of phosphoric acid to achieve the desired pH, and dilution with DI water to reach the reported Na^+ concentration. Sodium tetraborate (pH 9.0) buffers were prepared using similar methods. All buffers and samples were filtered through Nylon Acrodisk 0.22-μm membranes (Gelman Sciences, Ann Arbor, MI, USA) prior to use. HPCE capillaries were initially activated for 10 min using 1.0 *M* NaOH followed by a 5-min water rinse. Between successive injections, the capillary was rinsed with 0.10 *M* NaOH (2 min) and electrolytic run buffer (3 min), and the cathode electrolyte was replaced. All runs were carried out at a temperature of 25°C. All injections were carried out hydrodynamically at the anode at a relative pressure of 0.10 bar.

Neurotransmitter separations were carried using 20 m*M* sodium phosphate at pH 2.5 as the run buffer. All analytes were prepared directly in run buffer (without ion-pairing agent) at 100 m*M* concentration, and were injected into the capillary for 3 s. The separation voltage was 15 kV applied across a capillary 75 cm×50 μm, and detection was carried out 67 cm from the inlet end by UV absorbance at 210 nm. Phenolic acid separations were carried out similarly using 20 m*M* sodium phosphate at pH 7.0 as the run buffer. All analytes were prepared directly in the run buffer at 100 μ*M* concentration, and were injected for 5 s. The separation capillary was 42 cm×50 μm, and detection was carried out 34 cm from the inlet end by UV absorbance at 288 nm.

For all peptide digests, 25 mg of cytochrome *c* were dissolved in 5 ml of 1.0% (w/v) ammonium carbonate. The resulting 5 mg/ml cytochrome *c* solution was added to 0.20 ml of 0.01 *M* HCl containing 1 mg of trypsin. This mixture was incubated for 24 h in a thermostated water bath at 37°C. Following enzymatic cleavage, the digest was diluted 1:1 (v/v) with 0.50 *M* phosphoric acid and stored frozen until use. Final samples were prepared as a 5:1 (v/v) mixture of 2.5 m*M* sodium phosphate buffer–acidified digest to permit stacking conditions for injection. Injection was carried out for 5 s into a separation capillary 75 cm×75 μm, and detection was carried out 67 cm from the inlet end using UV absorbance at 210 nm. In all digest separations the applied voltage was 15 kV.

Cytochrome *c*, ribonuclease and bovine serum albumin were prepared in the 20 m*M* phosphate run buffer, pH 2.5, at 1.0 mg/ml concentrations. Samples were injected for 5 s into a 75 cm×50 μm capillary, and detection was carried out 67 cm from the inlet end using UV absorbance at 210 nm. Separation voltage was constant at 15 kV for each run. Hemoglobin samples were prepared at 0.10 mg/ml concentration in 25 m*M* phosphate buffer, pH 2.5, and injected at the anode hydrodynamically for 5 s at 0.10 bar into a 42 cm×75 μm capillary. Detection was carried out 34 cm from the inlet end using UV absorbance at 200 nm.

2.4. Calculation of selectivity values

Selectivity values were measured for critical pairs of the neurotransmitters and phenolic acids based on $\Delta\mu/\mu_{avg}$, where $\Delta\mu$ represents the difference in the calculated values of the electrophoretic mobilities of the critical pair and μ_{avg} represents the average mobility of the critical pair. To account for changes in EOF as a result of changing the background electrolyte, μ_e for each analyte was calculated using $v_T = \mu_e E + v_{Eo}$ where the velocity of EOF (v_{Eo}) was determined based on the migration of a neutral benzaldehyde marker.

To determine if any differences in separation are observed by maintaining the ion-pair agents in bulk solution with the analytes prior to injection, comparative runs were carried out using additives in the run buffer, with and without additives included in the sample. No significant change in migration behavior was observed in any of these studies. Further, when ion pairing agents were added *only* to the sample solution and not contained in the run buffer, no significant changes in selectivity were observed relative to injecting the sample with no additives present. This supports our assumption that rapid/reversible kinetics are associated with the complexation that occurs on-column, and facilitates the simple use of these compounds since they need only be added to run buffer.

3. Results

3.1. Effects with small molecules

As an initial simple organosolute system for

examining the selectivity effects of these additives, the well-studied separation of four common neurotransmitters was considered. Catecholamines have been previously used by Wallingford and Ewing [7,8] and Yoshida et al. [14] in their demonstration of the effect of borate ligand complexation. Three of the neurotransmitters investigated in this study (i.e., dopamine, epinephrine and norepinephrine) are structurally related catechols metabolically derived from tyrosine, and the fourth, serotonin, is an indole derived from tryptophane. In earlier work, the 3,4-dihydroxyphenol functionality of the catechols was shown to selectively complex with borate such that baseline resolution between serotonin and dopamine (which have similar electrophoretic mobilities, μ_e) was facilitated [8]. As shown in Fig. 1, resolution between serotonin and dopamine is not usually achieved under typical CZE conditions in an acidic buffer. However, the use of tetraborate in the run buffer at pH 9.0 permits baseline resolution of all four species. Presumably, this is due to the formation of a borate–catechol complex which migrates significantly slower than serotonin. In practice, an important drawback of using borate buffers for catecholamine analysis is the rapid oxidation of these compounds in basic media.

An alternative approach to improving the selectivity between dopamine and serotonin is to add *n*-alkylsulfonic acids to the run buffer at levels below their respective critical micelle concentrations. Fig. 2 shows the separation obtained following the addition of 100 m*M* BSA to the phosphate run buffer. Under these conditions, serotonin is observed to migrate more slowly than dopamine, permitting baseline resolution between these two compounds. As demonstrated in Fig. 3A, a significant increase in selectivity is observed between dopamine and serotonin as the BSA concentration is increased from 0 to 100 m*M*, ultimately providing for complete resolution of these two species. Since the selectivity between the second critical pair, norepinephrine and serotonin, decreases as BSA concentration is elevated (Fig. 3B), it may

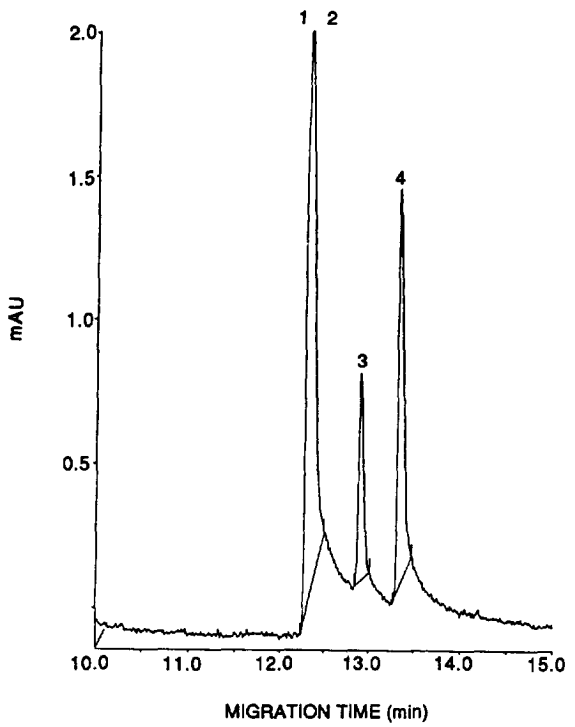

Fig. 1. Separation of neurotransmitters in 20 m*M* sodium phosphate, pH 2.5. Peaks: 1 = dopamine, 2 = serotonin, 3 = norepinephrine, 4 = epinephrine. Other conditions as in Section 2.3.

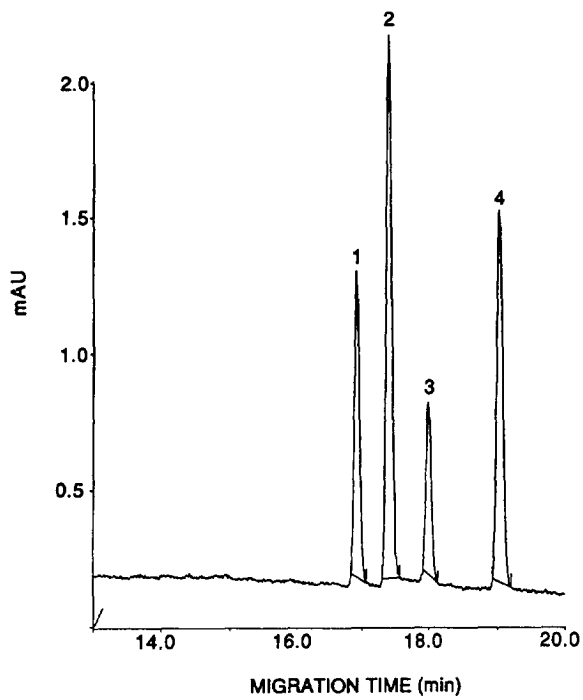

Fig. 2. Separation of neurotransmitters in 20 m*M* sodium phosphate, pH 2.5 with 100 m*M* added BSA. Peaks: 1 = dopamine, 2 = serotonin, 3 = norepinephrine, 4 = epinephrine.

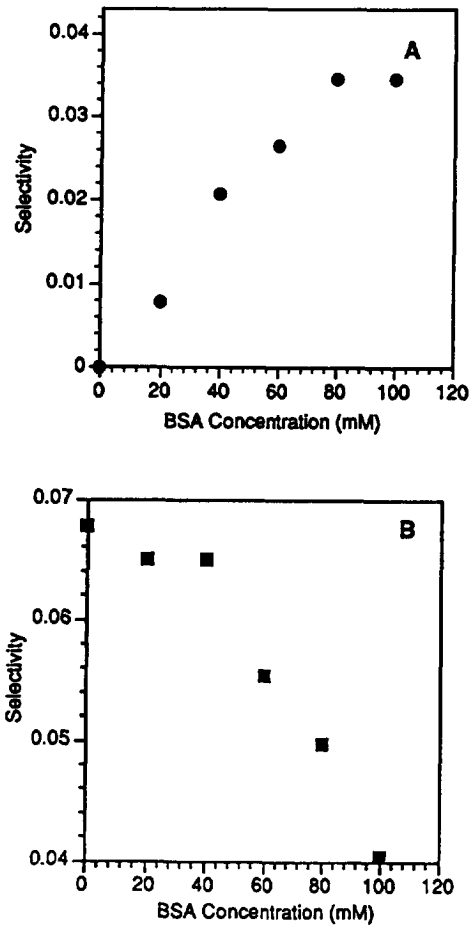

Fig. 3. Selectivity plots as a function of BSA concentration for (A) dopamine vs. serotonin, (B) serotonin vs. norepinephrine.

be inferred that reversible complexation between serotonin and BSA occurs to a greater extent than with the catechols used in this study. Improved resolution due to reduced capillary wall effects is frequently observed at higher ionic strengths of background electrolyte, but in this work increased concentrations of phosphate buffer ($\geq$100 m*M*) without added BSA failed to produce significant changes in the selectivity of the dopamine/serotonin critical pair. A plateau in the selectivity change is observed at higher BSA concentrations for the dopamine/serotonin critical pair, consistent with total saturation of the analytes at a molar ratio of approximately 1000:1 BSA/analyte. Although the optimized separation shown in Fig. 2 requires relatively high concentrations of BSA, similar improvements in selectivity are observed using much lower concentrations of longer chain alkylsulfonic acids. Partial resolution of serotonin and dopamine occurs with only 1.0 m*M* DSA (1:10 molar ratio DSA/analyte) added to the run buffer, and optimal separation is achieved at 20 m*M* DSA. Although the selectivity between dopamine and serotonin continues to increase at higher DSA concentrations, selectivity between the second critical pair (norepinephrine/serotonin) deteriorates to the extent that comigration occurs at DSA concentrations of 25 m*M* or higher. One possible drawback to the use of the longer chain acid is the longer analysis times encountered at equivalent field strength due to the reduction in EOF (i.e., 19 min at 20 m*M* DSA concentration).

In examining the specific mechanism(s) responsible for the observed change in migration, several factors may be considered. If ionic interaction is indeed a significant feature in the observed changes in migration behavior for these solutes, it is anticipated that larger alkyl chains of similar net charge (i.e., DSA) would produce a correspondingly greater change in migration than the their smaller counterparts (i.e., BSA) at any given concentration. Serotonin exhibits a greater effective positive charge than dopamine in aqueous solution as evidenced by its identical mobility but considerably larger size, and this is consistent with the change in selectivity that is observed assuming that serotonin interacts more strongly with BSA. Alternatively, purely hydrophobic considerations would result in similar effects with the extended chain sulfonic acids, since serotonin is more non-polar than any of the catechols used in this study. We have found in related studies (conducted in both aqueous and totally non-aqueous media (publication forthcoming) that neutral phenols cannot be separated in completely aqueous systems simply as a result of hydrophobic interaction, so it would appear that the ionic interactions are of major significance.

To further evaluate the nature of the complexes formed, similar concentration studies were performed using cationic agents TMAC, TEAC and TBAC. Under these conditions, selectivity improvements due to ionic interaction are not anticipated since the presence of like charge between the

additive and the analytes will not facilitate pairing; however, hydrophobic interaction would still be expected. In both phosphate and borate buffer systems, no change in selectivity was observed, supporting the assumption that charge effects rather than purely hydrophobic effects are largely responsible for the observed differences in electrophoretic behavior when the sulfonic acids are used. As a further indication of the importance of the charge interaction, Brij-35 was added to the run buffer at concentrations up to its critical micelle concentration, and produced no apparent change in analyte selectivity. It is useful to point out that EOF was observed to moderately decrease in the presence of Brij, and was reduced by a factor of two or more for the bulky, cationic alkylammonium agents.

To demonstrate that cationic alkylammonium salts could in fact be used for improving selectivity through complexation with anionic species, the separation of several phenolic acids (which are of particular importance as flavor constituents in natural beverages [15]) was investigated in phosphate buffer at pH 7.0. As shown in Fig. 4, peak resolution is significantly improved by the addition of the TEAC ion-pair agent. As demonstrated by Jorgenson and Walbroehl [5], and Yoshida et al. [14] and indicated by the neurotransmitter studies, EOF is greatly reduced in these quartenary amine systems; nonetheless, selectivity values for the 2nd and 3rd critical pairs are increased by the incorporation of TEAC in the run buffer between 5 and 50 m*M*, and virtual baseline resolution of all species is accomplished in under 13 min at a concentration of 40 m*M*. From a practical standpoint, the EOF effects can be overcome while maintaining the improvements in selectivity. In Fig. 4B, the run voltage is increased from 10 V to 15 V, yielding similar total run times to the control in Fig. 4A, but with the improved selectivity associated with the presence of TEAC at 10 kV. At concentrations of TEAC up to 100 m*M* in 20 m*M* phosphate buffer (pH 7.00) and field strengths of 400 V/cm, peak broadening or peak distortion were not observed in this system.

In considering differences in the degree of com-

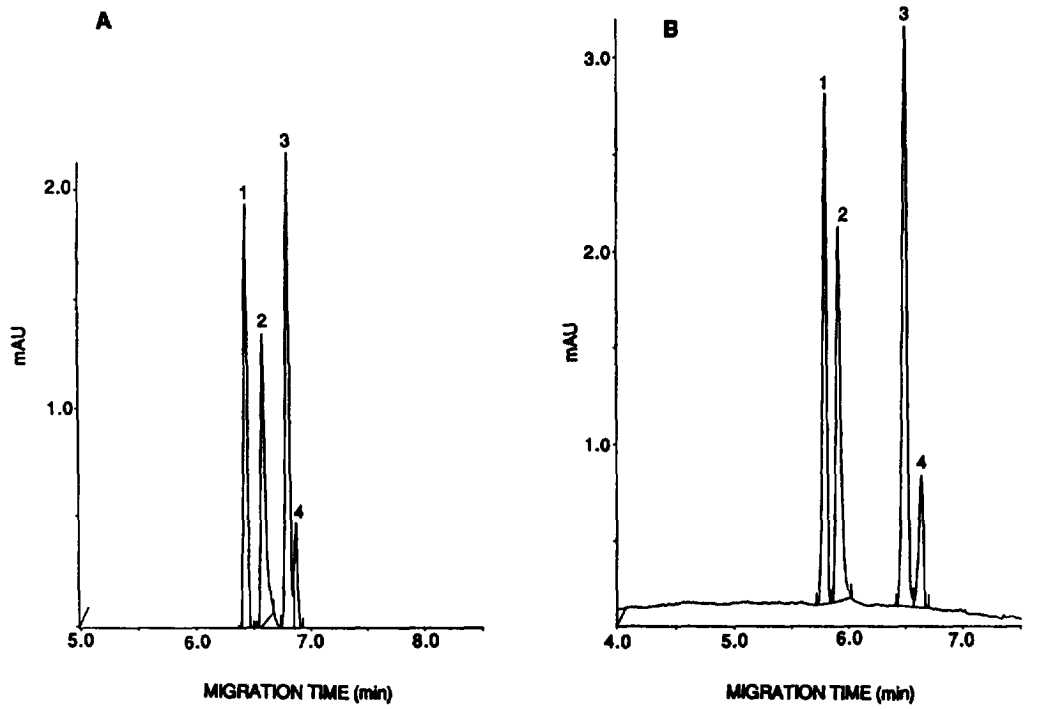

Fig. 4. Separation of phenolic acids in 20 m*M* sodium phosphate, pH 7.0. (A) Buffer only at 10 kV, (B) buffer+40 m*M* TEAC at 15 kV. Peaks: 1=ferulic acid, 2=caffeic, 3=*p*-hydroxycinnamic acid, 4=vanillic acid. Other conditions as in Section 2.3.

plexation as a function of solute structure, we first point out that any interaction with alkylammonium cations must increase the rate of migration of these anionic phenols as a result of lowering their effective charge and increasing their effective size. From the noticeable changes in the electropherogram that occur in the presence of TEAC, the first three solutes, all of which are hydroxyphenylpropenoic acid derivatives, apparently interact with TEAC to a greater extent than vanillic acid, a hydroxybenzoic acid. This difference in complexation equilibria as a function of the polarity and availability of the charged site for interacting with the additive seems consistent with the behavior observed with the neurotransmitters.

3.2. Peptide digest separations

McGlaughlin et al. have previously pointed out that the addition of hexanesulfonic acid to the electrophoretic run buffer can produce improvements in the separation of peptide digests [12]. To further investigate these effects as a function of ion-pair agent charge, size, concentration and buffer pH, we investigated an extensive group of ion-pair additives using tryptic digests of cytochrome *c* as a model analyte system. Studies were performed over a broad range of concentrations for each of the agents listed in Section 2.3, and the results for optimized concentrations of each agent are given in Table 1.

Fig. 5 shows a comparison of the electropherograms obtained using a 25 m*M* phosphate buffer at pH 2.5 with and without the addition of 75 m*M* HSA. As in the neurotransmitter separations, the addition of the anionic alkylsulfonic acids under pH conditions where the peptide fragments are predominantly positively charged produces a marked improvement in separation. In this case, this results in an approximate doubling of the number of individual peptide fragments that are resolved. Studies in which the buffer concentration was increased without the addition of ion-pair agent failed to show any significant improvement in separation, providing credibility to the argument that complexation events result in the improvement in separation selectivity, and not simply changes in solution ionic strength (efficiency).

Similarly positive results were obtained using 75 m*M* HSA (or any of the other alkylsulfonic acids) in a neutral pH phosphate buffer, where once again the number of resolved components in the ion-pair sample was approximately two-fold that observed in the absence of the ion-pair agent (see Table 1). Under these buffer pH conditions, it is anticipated that ionic interaction with anionic buffer additives remains favorable due to the presence of positive charges on the zwitterionic peptide fragments. Even so, the possibility exists for selectivity to be decreased as well as increased for any discrete critical pair based on the individual equilibria between each fragment and the additive. Based on peak shapes for identifiable peptide fragments in both buffer systems, the improvements that are observed at neutral pH are a combination of selectivity enhancement and a reduction in analyte/wall interactions. In experiments in which the pH was adjusted to 9.0 using

Table 1
Results of peptide digest separations using CZE with ion-pairing

Buffer system	pH	Ion-pair agent	Number of components[a]
25 m*M* sodium phosphate	2.5	none	17
25 m*M* sodium phosphate	2.5	HSA (75 m*M*)	30
25 m*M* sodium phosphate	7.0	none	15
25 m*M* sodium phosphate	7.0	BSA (100 m*M*)	34
25 m*M* sodium phosphate	7.0	HSA (75 m*M*)	33
25 m*M* sodium phosphate	7.0	DSA (5 m*M*)	26
25 m*M* sodium phosphate	7.0	TEAC (75 m*M*)	35
25 m*M* sodium phosphate	7.0	THAC (5 m*M*)	27
25 m*M* sodium tetraborate	9.0	none	21
25 m*M* sodium tetraborate	9.0	HSA (75 m*M*)	26

[a] The number of components was determined as the number of peaks that could be independently integrated with SpectraPhoresis software using identical threshold/peak width parameters for each system.

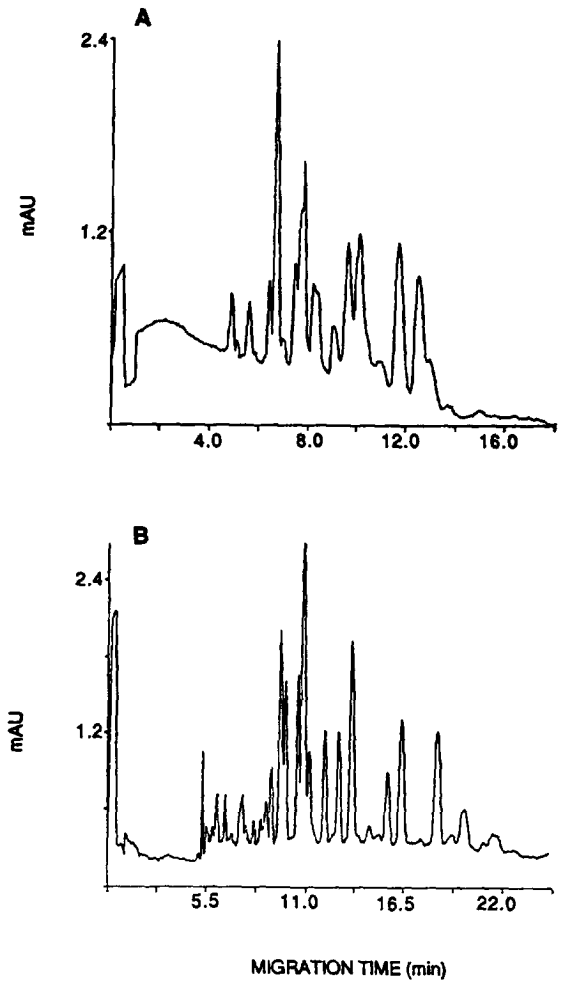

Fig. 5. Separation of peptide digest of cytochrome *c* in 25 m*M* sodium phosphate, pH 2.5. (A) Buffer only, (B) buffer + 75 m*M* HSA. Other conditions as in Section 2.3.

borate buffers, only slight improvement was observed in peptide resolution when the anionic ion-pairing agents were included (i.e., number of resolved components increased by five). The fact that resolution is increased at all suggests, however, that both the decrease in EOF and hydrophobic association between the additives and solutes play a minor role in the separation, since most peptide fragments exhibit greatly reduced positive charge in this pH region.

Changes in selectivity were also observed when 75 m*M* TEAC was added to the pH 7.00 phosphate run buffer. Although the signal-to-noise ratio was somewhat compromised at 210 nm due to residual absorption by the TEAC, significant improvements in resolution were nonetheless realized through the addition of these cationic agents to the peptide zwitterions, and the selectivity of the separation was differentially altered versus that achieved with the anionic ion-pair agents. It is useful to once again point out that much lower molar concentrations of the bulkier surfactants were required to produce significant improvements in peptide separations versus shorter chain additives as a result of the greater decrease in EOF and greater effective change in peptide electrophoretic mobilities.

3.3. Protein separations

Final studies conducted with ion-pair agents involved the addition of these additives to protein mixtures. In one example, anionic agents including BSA, HSA and DSA were added to a protein mixture containing cytochrome *c*, albumin and ribonuclease. As shown in Fig. 6A, 50 m*M* sodium phosphate buffer at pH 2.5 fails to produce baseline separation between the cytochrome *c* and albumin proteins. Fig. 6B demonstrates the significant improvement in resolution associated with the addition of 40 m*M* BSA to phosphate buffer. It is likely that the addition of the comparatively small ion-pair agent results in ionic interaction at multiple sites on the protein surface, and this further contributes to the magnitude of the selectivity change that is observed. The HSA and DSA agents also provided some enhancement in selectivity, but resulted in a greater loss of electrophoretic efficiency, presumably due to effects of denaturation and the reduction of EOF.

Free-zone electrophoresis of human hemoglobin A0 is shown in Fig. 7 with and without BSA contained in the phosphate run buffer at an optimized concentration. In aqueous solution at neutral pH, heme iron is normally dominated by the Fe^{3+} oxidation state, forming ferrihemoglobin. In our samples, a stabilizing antioxidant is provided with the lyophilized protein by the supplier to prevent stoichiometric conversion to Fe^{3+} and thus maintain an appreciable concentration of ferrohemoglobin in solution. As observed in Fig. 7B, BSA is apparently able to interact more strongly with the Fe^{3+} than Fe^{2+} heme, resulting in separation of the two

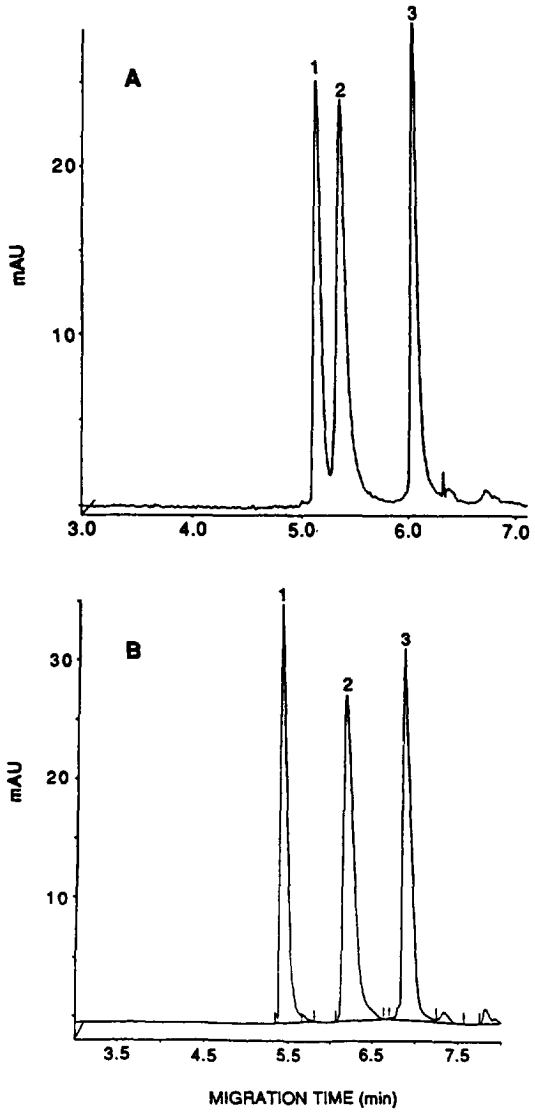

Fig. 6. Separation of proteins in (A) 50 m*M* sodium phosphate, pH 2.5, (B) 20 m*M* sodium phosphate, pH 2.5 + 40 m*M* BSA. Peaks: 1 = cytochrome *c*, 2 = albumin, 3 = ribonuclease. Other conditions as in Section 2.3.

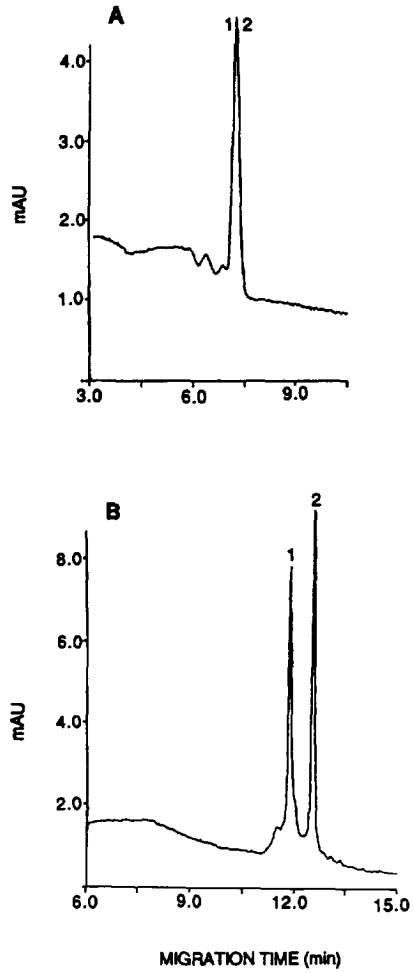

Fig. 7. Separation of (1) ferri and (2) ferrohemoglobin in 20 m*M* sodium phosphate, pH 2.5. (A) Buffer only, (B) buffer + 100 m*M* BSA. Other conditions as in Section 2.3.

hemoglobin forms. These results were independently confirmed by comparing the relative ferri/ferro peak areas using capillary isoelectric focusing (cIEF), since cIEF can be used to achieve baseline separation of the heme oxidation states based on an approximate 0.15 p*I* difference in their respective isoelectric points [16]. Unlike the small molecule separations, longer chain length additives were not successful in these studies due to peak broadening and the appearance of new peaks suggestive of surfactant-mediated denaturation of the native protein structure.

4. Conclusions

In this work we have demonstrated that modification of selectivity can be accomplished for many analytes through the straightforward addition of conventional ion-pairing agents to the electrophoretic run buffer, and that the changes in migration which occur are predominantly the result of fast, reversible

ionic interaction and the modulation of EOF. Conventional organic cationic and anionic surfactants have shown particular promise for selectivity enhancement due to their availability, high solubility and UV transparency. In general, we have found that larger ion-pair agent constituents result in greater changes in analyte migration at reduced molar concentrations. Peptides and protein systems have also been shown to be excellent candidates for these applications. Better separations result not only from changes in selectivity but also as a result of improved efficiencies associated with reduced wall adsorption. Longer alkyl-chain agents in particular were found to significantly disrupt the native protein structure as a result of hydrophobic association.

Acknowledgments

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