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Ion association with alkylammonium cations for separation of anions by capillary electrophoresis

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Abstract

A background electrolyte (BGE) containing a 100 mM concentration of an alkylammonium cation with ethyl, propyl or butyl groups provides an excellent medium for separation of anions by capillary electrophoresis (CE). Two major effects were noted. Use of one of a series of alkylammonium cations in the BGE at a selected pH provides a simple and effective way to vary and control electroosmotic flow (EOF) over a broad range. It is believed that the alkylammonium cations are coated onto the capillary surface through a reversible dynamic equilibrium. Secondly, alkylammonium cations modify the electrophoretic migration of sample anions and the electroosmotic migration of neutral organic analytes by association interaction. This selective interaction results in improved anion separations and permits the simultaneous separation of neutral analytes. The degree of association interaction varies with the bulk and hydrophobicity of the alkylammonium cations. Incorporation of an aliphatic amine salt of moderate molecular weight in the running electrolyte provides a valuable new way to vary the migration times of sample anions and to optimize their resolution. The interactions between alkylammonium cations and sample anions or neutral organics appear to take place entirely within the liquid phase and do not require a polymeric or micellar pseudo phase.

Keywords: Capillary electrophoresis; BGE; Alkylammonium cations; Anions

1. Introduction

Although capillary electrophoresis (CE) is a powerful technique for the separation of ions, almost all of the work has been done with a background electrolyte (BGE) containing a relatively low ionic concentration. It is commonly thought that a BGE of high ionic strength would lead to excessive heating and peak distortion. However, Ding et al. obtained very satisfactory separations of both inorganic and organic anions in electrolyte solutions containing 0.1–0.2 M, and as high as 5 M sodium chloride using direct photometric detection [1]. Electroosmotic flow is reduced in electrolytes of higher salt concentrations, thus eliminating the need for an electroosmotic flow (EOF) modifier for the separation of anions. Keeping the ionic concentration of the BGE significantly higher than that of the analytical sample also gives sharper chromatographic peaks via an electrostacking mechanism.

One of the problems with CE is a lack of versatility regarding manipulation of the separation selectivity. The normal approach is to extend the residence time of the analytes in the capillary by adjusting both the direction and the magnitude of the EOF [2,3]. However, the electroosmotic migration is the same for all analytes, so other means must be sought to modify the electrophoretic migration of sample ions relative to one another. A cationic surfactant such as tetradecyltrimethyl ammoniumbromide (TTAB) can be added to the background electrolyte (BGE) to interact with sample anions and improve their resolution. However, TTAB strongly coats the surface of a fused-silica capillary, resulting in a rapid change in the magnitude and direction of electroosmotic flow (EOF) between 0 and 0.5 mM concentrations in the BGE [4]. At slightly higher concentrations, a positively charged micelle is formed that can also reverse the direction of electrophoretic of sample anions.

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The use of soluble polymers for modifying the electrophoretic has been reviewed [5]. In particular, a soluble cationic polymer known as poly(diallyl dimethylammonium chloride) (PDDAC) has proven to be very successful in modifying the relative migration of anions [6,7]. Modeling and optimization techniques have also been successfully applied [8].

An increasing number of observations in published papers indicate that ions of relatively small size can interact with sample ions to modify their electrophoretic migration in CE. An advantage of this approach is that semi-permanent coating of the capillary and problems with micelle formation encountered with larger modifiers are generally avoided.

Nashabeh and El Rassi [9,10] improved the selectivity and resolution of acid glycoprotein fragments by addition of tetrabutylammonium bromide to the running electrolyte. These effects were explained by ion-pair formation and/or hydrophobic interaction between the electrolyte cation and the analyte anions.

Chen and Pietrzyk [11,12] noted that Mg^{2+} as a buffer additive reduces the EOF in CE separations carried out at a neutral or alkaline pH. They suggested that the cation additive undergoes cation exchange at the capillary silanol sites, which alters the wall charge and consequently reduces the EOF. The Mg^{2+} in the BGE was found to have a significant effect on retention of organic sulfonates due to association between the cation and the sulfonate anions.

Yotsuyanagi and co-workers used 25 mM tetrabutylammonium bromide (TBAB) for the CE separation of anionic metal–organic complexes [13]. TBAB was also used in a study of ion association properties with aromatic divalent anions through mobility changes [14]. Ion association constants were calculated for a number of anions.

Conventional cation and anionic ion-pair agents, including alkylsulfonic acids and tetra alkylammonium salts, have been investigated as additives to the BGE to improve both the selectivity and resolution for organosolutes, peptides and proteins [15]. Ionic interaction and hydrophobic association between the solutes and additives, as well as modulation of EOF, were held to be responsible for the observed changes.

Moderately high (ca. 50 mM) concentrations of ethanesulfonic acid (ESA) or triethylamine (TEA) salts were found to give dramatically sharper peaks in the separation of protonated anilines [16]. Increasing concentrations of ESA reduced the electrophoretic migration of the aniline cations, while TEA had very little effect on the electrophoretic migration.

Although mostly large molecules such as surfactants and soluble polymers have been used to alter the EOF and hence the migration times of analyte ions in CE, it has been found that at concentrations of 100 mM or so, much smaller organic ions in the BGE can change the EOF in a controlled manner [17]. In acidic solution the migration times of protonated anilines became progressively slower in the series: methylamine, diethylamine, diethylaminoethanol and triethylamine. A major part of this effect was attributed to an opposing EOF resulting from a positively-charged coating of the capillary surface with the alkylammonium cations in the BGE via a dynamic equilibrium.

We now report the results of a more detailed study that includes a much larger variety of alkylammonium cations over a broad pH range of 3–9. Unlike conventional electrolytes that give a cathodic EOF of varying magnitude over the entire pH range, electrolytes containing alkylammonium salts give an anodic EOF in acidic solution. This changes gradually to a cathodic EOF at alkaline pH values. But most importantly, the migration velocities of sample anions are selectively reduced by associative interaction of alkylammonium cations with the sample anions.

2. Experimental

2.1. Reagents

All inorganic acids, bases and salts were obtained from existing laboratory stock or purchased from Fisher Scientific (Fairlawn, NJ, USA) and were of reagent grade quality or better. Organic amines and other organic reagents were purchased from Aldrich (Milwaukee, WI, USA) and were used as received. All solutions were prepared in purified 18.2 MΩ water from a Barnsted nanopure II water purification system (Barnsted Thermolyne, Dubuque, IA, USA). Analyte solutions were typically made up in acetonitrile and purified water in a concentration range of 5000 ppm and were diluted by a factor of 10 for the injected solutions.

2.2. Apparatus

A Waters Quantum 4000E capillary electrophoresis system was used with a positive power supply at +15 kV for some of the EOF measurements.

Polyimide coated fused-silica capillaries (Polymicro Technologies, Phoenix, AZ, USA) were 50 cm in length with effective length to the detector being 42.5 cm. The capillaries used were 50 μm inside diameter. A new capillary was prepared by rinsing the complete column volume three times each with 1 M hydrochloric acid, purified water and then followed by a third rinse solution, and finally the BGE. The third rinse solution in this preparation sequence is determined by whether the pH of the BGE is acidic, neutral or basic. For acidic BGE solutions no further rinse is necessary except for the final BGE rinse. A 1 M ammonium acetate solution is used for the third rinse solution for BGE solutions that have approximately neutral pH. Basic BGE solutions require 0.1 M NaOH as the third rinse solution. The final rinse solution is with the BGE, and on the final rinse the solution is allowed to flow through the capillary for 2 min with the potential applied. A single rinse in any of the solution steps consisted of filling the column volume with the rinse solution and purging the column of the rinse solution using air. This single rinse takes approximately 20 s and

brings the total column preparation time to approximately 5 min.

Direct UV detection at 214 nm and hydrodynamic injection for 2–5 s at 10 cm height were used for all separations. Electropherograms were sampled at a rate of 10–20 points/s by the Peak Simple chromatography data system software interfaced to a Gateway Solo 5300 laptop computer using the SRI Instruments model 202 interface (SRI Instruments, Torrance, CA, USA).

Aliphatic amines and quaternary ammonium hydroxides and chlorides used in the BGE were dissolved in water and carefully pH adjusted using either HCl or NaOH. The BGE contained 100 mM of an aliphatic amine, quaternary ammonium hydroxide or quaternary ammonium chloride salt and was buffered using a concentrated solution of a mixed buffer (PAB); which, upon dilution, provided a 5 mM concentration of each for phosphate, acetate and borate in the BGE. Hydrochloric acid or sodium hydroxide solution was then added to obtain the desired pH.

3. Results and discussion

In a previous study, a background electrolyte containing 100 mM concentrations of an aliphatic amine at an acidic pH was found to reverse the usual direction of electroosmotic migration in a fused-silica capillary [17]. For example, protonated diethylamine salts gave an anodic electroosmotic mobility ranging from approximately 1.5×10^{-4} cm^2 V^{-1} s^{-1} at pH 1.5 to 1.0×10^{-4} cm^2 V^{-1} s^{-1} at pH 4.0. This was attributed to a positively charged coating of the capillary surface with the amine cations in the BGE via a dynamic equilibrium.

This study has now been extended to include a much larger selection of alkylammonium cations and to cover a broader pH range. The electroosmotic mobilities of electrolytes containing 100 mM concentrations of sodium chloride and tetrapropylammonium chloride are compared at different pH values in Fig. 1. At pH 3 and 5, the quaternary ammonium salts reverse the direction of EOF from that normally encountered with a sodium chloride electrolyte. At pH 7 and 9, the EOF is cathodic but the magnitude is reduced significantly from that observed with sodium chloride. Additional experiments showed that the anodic EOF at pH 3 (due to a positive surface) increased in the order RNH_3^+, $R_2NH_2^+$ R_3NH^+, R_4N^+. A stronger anodic EOF was also observed as the size of the aliphatic R group was increased.

Previous investigators [9,10,13,14] used tetrabutylammonium salts to modify the migration of relatively large organic anions in CE. Our first experiments were designed to determine whether alkylammonium salts could be used effectively to modify the migration of inorganic anions. A relatively high concentration (100 mM) was used to improve peak sharpness through electrostacking and to enhance any cation–anion association effects that might be observed. A variety of alkylammonium salts was used to determine the effect of their chemical structure on analyte migration.

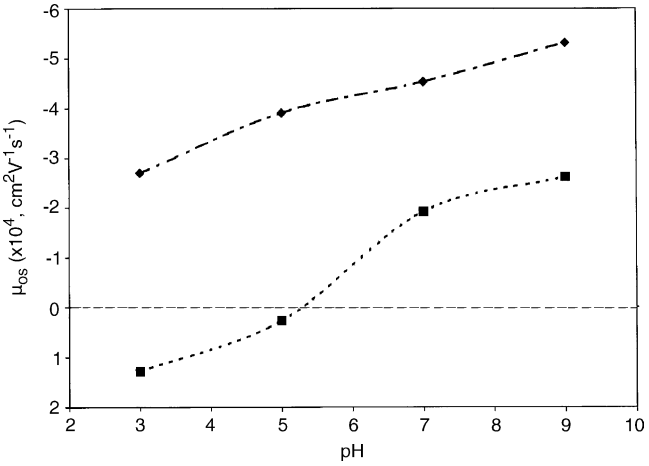

Fig. 1. Electroosmotic mobility (μ_{OS}, cm^2 V^{-1} s^{-1}) as a function of pH. Background electrolytes are 100 mM with 5 mM pH buffer. Upper curve: sodium chloride. Lower curve: tetrapropylammonium chloride.

The effects of BGE cation and pH on the migration times of several inorganic anions are compared in Table 1. Two general trends will be noted. The longer migration times at higher pH values are a consequence of an increasing cathodic EOF which slows migration of anions towards the detector near the anode. A second effect concerns the relative migration of iodide and bromide. These anions have almost identical equivalent conductivities and are therefore difficult to separate by CE. Electropherograms run in 100 mM sodium chloride failed to resolve bromide and iodide peaks at pH 3 and gave only 0.1 min difference between the two peaks at pH 9. By contrast, quaternary ammonium cations interact more strongly with iodide than with iodide than with bromide to

Table 1
Average migration times (min)

Electrolyte	Anion	pH			
		3	5	7	9
NaCl	Br^-	2.43	3.03	3.96	5.36
	I^-	2.43	3.03	4.02	5.48
	NO_2^-	5.55	3.33	4.52	6.48
	NO_3^-	2.68	3.48	4.84	7.20
Et_4N^+	Br^-	2.30	3.02	3.49	5.34
	I^-	2.38	3.18	3.70	5.88
	NO_2^-	4.34	3.26	3.76	5.95
	NO_3^-	2.51	3.36	3.94	6.41
Pr_4N^+	Br^-	2.40	3.37	4.79	5.79
	I^-	2.58	3.73	5.58	6.97
	NO_2^-	–	3.57	5.23	6.45
	NO_3^-	2.62	3.76	5.73	7.20
Bu_4N^+	Br^-	2.62	3.05	5.00	6.22
	I^-	2.95	3.455	5.99	7.96
	NO_2^-	5.03	3.22	5.33	7.05
	NO_3^-	2.89	3.92	5.83	8.03

All electrolytes 100 mM, −15 kV capilliary length 50 cm (42 cm to detector).

Table 2
Ratio of iodide:bromide migration times

Electrolyte				
PH	NaCl	Et_4N^+	Pr_4N^+	Bu_4N^+
3	1.00	1.03	1.08	1.12
5	1.00	1.05	1.11	1.13
7	1.015	1.06	1.16	1.20
9	1.02	1.09	1.21	1.28

All electrolytes 100 mM.

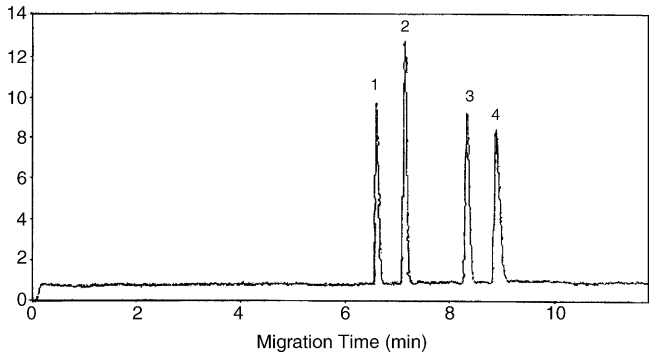

Fig. 2. Separation of inorganic anions at pH 9. BGE contains 200 mM tetrapropylammonium chloride, 50 mM tetrabutylammonium hydroxide, 5 mM PAB buffer and 10% v/v acetonitrile. Applied voltage, −015 kV. Peak identification: 1: bromide, 2: nitrite, 3: nitrate, 4: iodide.

cause a greater difference in their migration times. Table 2 shows that the ratio of iodide: bromide migration times increases with both the bulkiness of the tetrraalkyl ammonium cation and with increasingly higher pH values.

Even greater peak resolution can be obtained by using a higher concentration of a quaternary ammonium salt. In Fig. 2, bromide and iodide are separated by 2.25 min in 250 mM tetrabutylammonium chloride/hydroxide and 10% acetonitrile higher pH values. The relative migration times of nitrate and iodide are also strongly affected by the nature of the electrolyte cation. At pH 9 the ratio of nitrate: iodide is 1.31 in NaCl, 1.09 in Et_4N^+, 1.03 in Pr_4N^+ and 1.01 in Bu_4N^+.

The factors that affect migration of sample ions can be summarized in Fig. 3. The separations described were carried out with a negative power supply (−15 kV) so that electrophoretic migration of the sample anions is toward the anode where the detector is located. With alkylammonium cations in the BGE there is also an anodic electroosmotic migration in acidic solutions but this changes to the cathodic direction at alkaline pH values. A third effect occurs in which the alkylammonium cations interact with the sample anions to form a cation–anion association complex that reduces electrophoretic migration of free anions. This effect, which is indicated by a cathodic vector in the figure, explains the changes in the relative migration times of bromide, iodide and nitrate. Iodide associates more strongly with a quaternary ammonium cation than either bromide or nitrate and the magnitude of the cation:iodide association increases with greater bulk of the quaternary ammonium cation.

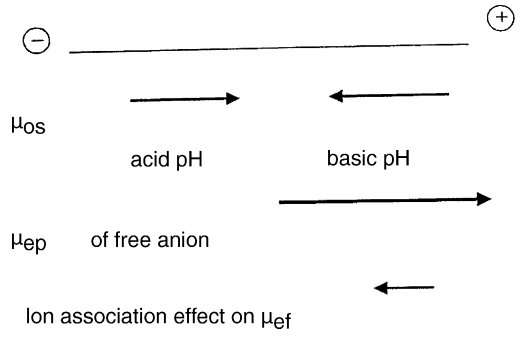

Fig. 3. Summary of migration effects with a negative power supply.

Retardation of sample anion migration velocity by formation of an association complex in solution would be greater with organic anions than with inorganic and would increase with the bulk of the R_4N^+ ions. An initial attempt to obtain a peak for *p*-toluenesulfonic acid (TSA) in 100 mM Bu_4N^+ at pH 9 failed. The opposing electroosmotic and ion association vectors were too large and the net migration was in the opposite direction. Shifting to an acidic pH where the μ_{OS} and μ_{ep} vectors were both anodic was much more successful. Fig. 4 shows excellent separations of several inorganic and organic anions with different quaternary ammonium cations in the BGE. As expected, retention times increased in the series: Et < Pr < Bu, especially for the organic anions. Resolution also improved in this series. In Pr_4N^+ solution, the 1-napthalene sulfonic acid was resolved into two peaks, the second presumably was 2-naphthalene sulfonic acid. Even greater resolution was evident in Bu_4N^+ solution. The anion peaks in these separations were also exceedingly sharp; actual theoretical plate numbers as high as 200,000–300,000 were obtained.

The observed mobilities (μ_{OB}) of the analyte anions were calculated from the migration times in Fig. 4. Then, the electrophoretic mobilities were calculated from the simple relationship:

$$\mu_{OB} = \mu_{ep} + \mu_{OS}$$

The μ_{ep} values listed in Table 3 decreases with increasing size and hydrophobicity of the anion, as would be expected. However, the ratios of μ_{ep} values of Pr_4N^+:Et_4N^+ and Bu_4N^+:Et_4N^+ also decrease in the same order. This means that the chain length of the alkyl groups on the quaternary ammonium cations has the greatest effect on the μ_{ep} of the larger anions.

3.1. Mechanism

It seems evident that the longer migration times of polarizable inorganic and organic anions in R_4N^+ solutions are due to some kind of interaction between sample anions and the amine cation. Since there is no micelle or other pseudo phase, interactions can only occur in solution or at the interface between the solution and the capillary wall. The latter could take place by ion-exchange between the sample anions and the

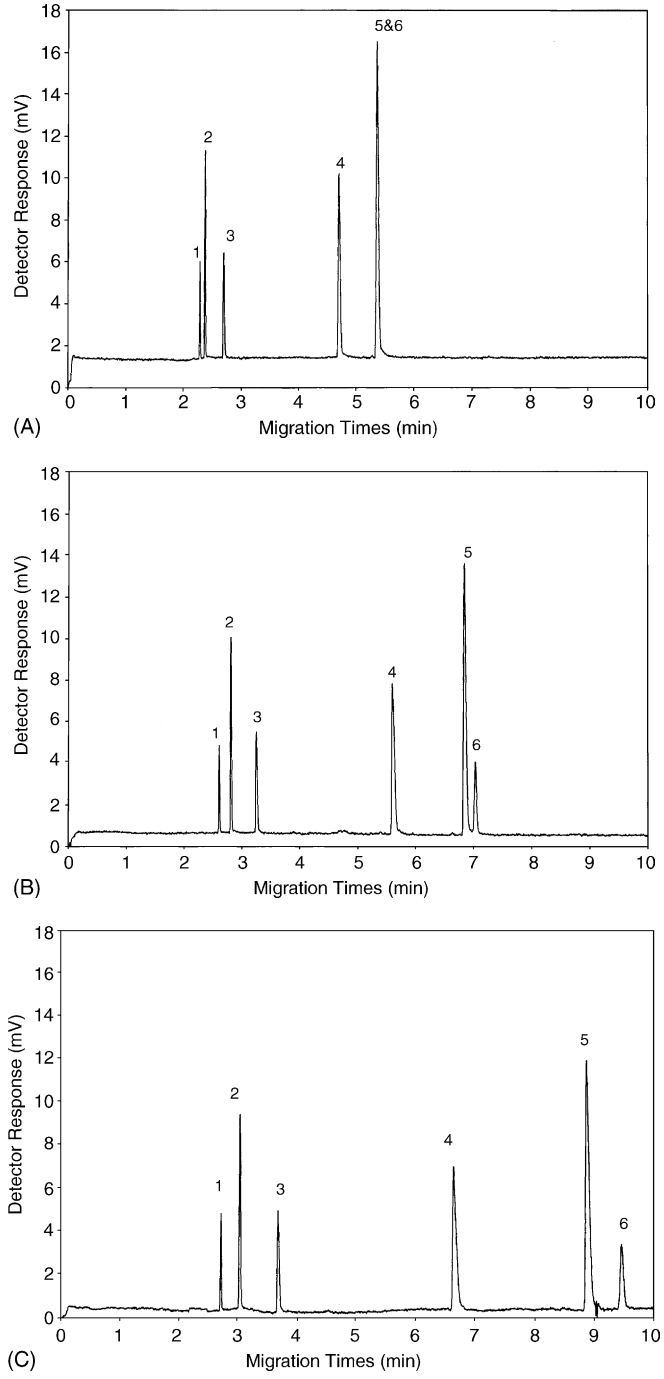

Fig. 4. Effect of the alkylammonium salt on the separation of anions at pH 3. BGE contains 100 mM R_4NCl + 5 mM buffer. Applied voltage, −15 kV. Column length, 50 cm, 42 cm to detector. Peak identification: 1: bromide, 2: iodide, 3: thiocyanate, 4: *p*-toluenesulfonate, 5: 1-napththalenesulfonate, 6: 2-naphthalenesulfonate. (A) Tetraethylammonium chloride; (B) tetrapropylammonium chloride; (C) tetrabutylammonium chloride.

positively-charged wall, resulting from adsorption of R_4N^+ at acidic pH values. However, the sample ion peaks are much sharper than would be expected if the primary mechanism involved ion exchange only at the solution–wall interface.

In CE separation of anions, a free sample anion in solution, A^-, migrates toward the anode and the detector at a certain

Table 3
Electrophoretic mobilities (μ_{ep}) of anions at pH 3.0 with 100 mM quaternary ammonium chloride

Anion	μ_{ep} ($\times 10^4$) ($cm^2\ V^{-1}\ s^{-1}$)			μ_{ep} ratio	
	Et_4N^+	Pr_4N^+	Bu_4N^+	Pr_4N^+:Et_4N^+	Bu_4N^+:Et_4N^+
Bromide	8.0	7.1	6.8	0.89	0.85
Iodide	7.7	6.5	5.9	0.84	0.77
Thiocyanate	6.5	5.4	4.6	0.83	0.71
TSA	2.9	2.3	1.7	0.80	0.59
1-NSA	2.2	1.6	0.8	0.73	0.36
2-NSA	2.2	1.5	0.7	0.68	0.32

The following values for the electroosmotic mobilities were used: Et_4N^+, $2.1 \times 10^{-4}\ cm^2\ V^{-1}\ s^{-1}$; Pr_4N^+, 1.8×10^{-4}; Bu_4N^+, $1.8 \times 10^{-4}\ cm^2\ V^{-1}\ s^{-1}$. TSA: toluenesulfonic acid; NSA: naphthalenesulfonic acid.

electrophoretic velocity. But if a portion of the anion is tied up as an association complex, RN^+A^-, which undergoes little if any electrophoretic migration, the result will be a slower net migration velocity and a longer retention time. This can be expressed as a simple equilibrium

$$RN^+ + A^- \rightleftharpoons RN^+A^- \quad (1)$$

$$K = \frac{[RN^+\ A^-]}{[RN^+][A^-]} \quad (2)$$

The ratio of $[RN^+A^-]$:$[A^-]$, and hence the degree with which the net migration of the sample anion is slowed, is seen to be a function of both K and the concentration of pairing cation, RN^+. The value of K will be different for each sample anion, thus providing an additional parameter for the resolution of anion mixtures by CE. Yotsuyangi and Motomizo [14] were able to calculate K values for association of several organic anions with tetrabutylammonium bromide.

If alkylammonium cations associate in solution with analyte anions, it seems likely that association would also take place between the BGE cations and neutral organic analytes. This association does indeed occur, as is demonstrated by the electropherogram in Fig. 5, which shows several peaks for various organic compounds in addition to the anion peaks.

Organic analytes apparently form cationic association complexes with the alkylammonium cation.

$$RN^+ + Org \rightleftharpoons Org : RN^+ \quad (3)$$

$$K = \frac{Org : RN^+}{[RN^+][Org]} \quad (4)$$

The ratio of complexed organic:free organic is proportional to $K\,[RN^+]$. The organic analytes have identical anodic EOF vectors toward the detector. During the time period each analyte is complexed, there is an opposing cathodic electrophoretic vector. Analytes that are more strongly complexed (higher K) have a greater electrophoretic vector and longer migration times. It should be noted that all of the neutral organic analytes have longer migration times than the EOF marker, which elutes at 15.5 min. Anionic analytes undergo both electrophoretic and electroosmotic migration toward the detector and thus elute before the EOF marker.

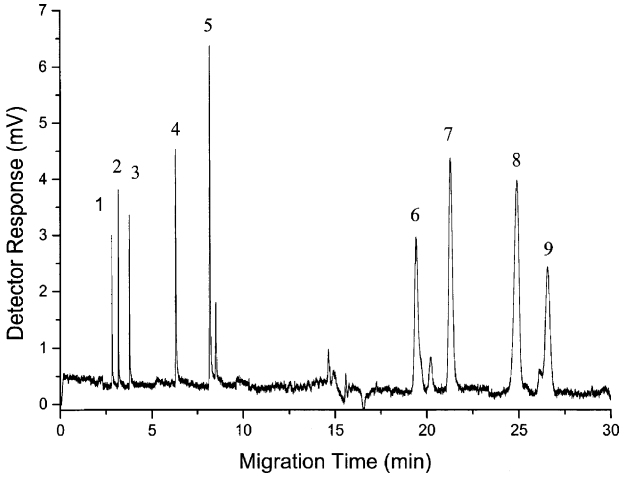

Fig. 5. Separation of anions and neutral compounds at pH 3. BGE contains 100 mM tetrapropylammonium chloride and 5 mM PAB pH buffer. Applied voltage, −15 kV. Peak identification: anion peaks 1–5 as in Fig. 4. Neutral analyte peaks: 6: benzaldehyde, 7: phenol, 8: benzophenone, 9: 4-methoxycinnamic acid.

The mechanism of ion-chromatographic method commonly known as "ion-interaction chromatography" or as "ion-pair chromatography" has been debated for some time [18,19]. The major thrust of the argument is that cation–anion pairing occurs in the stationary phase or at the interface between the phases but not in the liquid mobile phase. However, our experiments strongly suggest that both cation:anion association and cation:neutral association does occur in aqueous solution when both the BGE cation and the anionic or neutral analyte have sufficient hydrophobic character.

We may speculate that the amine cations are not arranged randomly in the aqueous solution but are concentrated in various domains in which the concentration of amine salts is higher than in other parts of the solution. Sample anions can then undergo ion association or ion exchange within these domains in much the same way that ions undergo conventional ion exchange between a liquid phase and a hydrophobic solid phase containing fixed cationic groups.

In a similar manner electroosmotic migration of neutral analytes is slowed by interaction within the hydrophobic domains. This is roughly analogous to the slowing of analyte velocities through an HPLC column due to selective partitioning between two distinct phases. Significantly greater chromatographic efficiency is obtainable when differences in analyte migration rates are achieved through interactions within a single phase.

4. Conclusions

A BGE containing a 100 mM concentration of an aliphatic alkylammonium cation alters the usual EOF behavior of a fused-silica capillary to give an anodic EOF, rather than a weak cathodic EOF, at pH 3. The direction of EOF is reversed at pH 7 or 9, but the cathodic flow is substantially lower than with conventional electrolytes. These effects are attributed to a dynamic equilibrium in which alkylammonium cations are attracted to the silica surface. "Conventional" flow modifiers of higher molecular size tend to give a semi-permanent surface coating, but the amine cations used in this work appear to operate by a reversible dynamic equilibrium.

The use of cationic alkylammonium salts in the BGE also has a major effect on the electrophoretic migration of anions to be separated by CE. The mobilities of polarizable inorganic anions and organic anions in particular are slowed by ion pairing or ion exchange with alkylammonium cations in the aqueous solution. This mechanism is different from micellar capillary electrophoresis (MEKC) in which neutral or ionic analytes interact with a micelle pseudo phase. Quaternaryammonium cations are shown to have a greater effect on the migration of organic anions than was observed in a technique called IC-CE in which the migration velocity of anions was modified by interactions with a soluble polymer such as PDDAC.

The approach used here, incorporation of an alkylammonium salt of moderate chemical size in the running electrolyte, is a simple and effective way to modify anion migration and improve resolution of complex mixtures. The extent of cation–analyte interaction can be varied over a broad range by choosing from a large number of available alkylammonium compounds. Adjusting the EOF by use of different alkylammonium cations at varying pH values offers additional separation parameters.

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