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Electroosmotic flow reversal for the determination of inorganic anions by capillary electrophoresis with methanol–water buffers

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Abstract

Manipulation of the electroosmotic flow (EOF) is essential for achieving optimized separations of small anions by capillary electrophoresis (CE). In this work, efficient suppression or reversal of EOF is achieved upon addition of small amounts of the cationic surfactants, cetyltrimethylammonium bromide (CTAB) or didodecyldimethylammonium bromide (DDAB) to the electrophoretic buffer. Highly stable and reversed EOF are achieved using the surfactants in the presence of up to 50% MeOH. In aqueous and low methanol containing solutions (up to 30%, v/v) surface aggregation of the surfactants at the capillary wall occurs at a concentration below the critical micelle concentration (CMC). The impact of MeOH on reversed EOF is predominantly a function of the diminished zeta potential of the silica, and to a lesser extent on the CMC in the bulk solution of the surfactant. Fast baseline separation and selectivity changes for small inorganic anions are observed when mixed aqueous–organic buffers are employed. Changes in EOF, micellar properties of the surfactant and selectivity for inorganic anions upon addition of various percent of methanol are also discussed.

Keywords: Electroosmotic flow; Dynamic coating; Selectivity; Surfactants; Inorganic anions

1. Introduction

Capillary electrophoresis (CE) is often described as a complementary technique to ion chromatography for the determination of small organic and inorganic ions [1,2]. In particular, the separation selectivity of CE is distinctly different than that of ion chromatography. In capillary zone electrophoresis (CZE) using bare silica capillary, the mobilities of many small inorganic anions are comparable in magnitude or even greater than the electroosmotic flow (EOF) mobility. Using counter-EOF flow would result in long times, and possibly fail to detect the faster migrating anions. Jones and Jandik used the cationic surfactant tetradecyltrimethylammonium bromide (TTAB) as a buffer additive to reverse the EOF [3]. In this manner, the reversed EOF augments the migration of the anions. Using this co-EOF approach they separated 30 anions in just 3.1 min [3] and Melanson and Lucy separated NO_3^- and NO_2^- in 12 s [4].

However, while co-EOF separations are rapid, achieving full resolution can be challenging. The EOF rapidly sweeps the analytes towards the detector. Thus, there must be a significant difference in the electrophoretic mobilities between two species to achieve separation. For cations such as alkaline earth and transition metals, the mobility can be altered by complexation [5,6]. For anions, complexation has seen only limited use [7,8]. More commonly anion selectivity is optimized by adjusting buffer conditions in the background electrolyte (BGE) [9,10]. However, this approach has limited use for small inorganic species such as chloride, bromide, iodide and fluoride that have unattainable pK_a values [11].

Another important approach to modifying CE selectivity for inorganic anions is the addition of organic solvents to the buffer. The use of organic solvents in CE as either organic modifiers or as pure nonaqueous media offers many advantages compared to purely aqueous media. These include increased solubility of hydrophobic analytes, lower Joule heating and suitability for detection by mass spectrometry (MS). However, most important with respect to inorganic anions is that dramatic alterations in selectivity can be achieved by varying the type and content of the organic solvent in the buffer. Buchberger and Haddad investigated the effect of up to 30% methanol, acetonitrile, tetrahydrofuran, acetone and ethylene glycol on anion mobilities in a chromate electrolyte containing TTAB [12]. These selectivity changes result from

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changes in the intrinsic mobility of the fully charged ion or in the effective charge of the ion due to solvent-induced changes in the pK_a. The contribution of both of these effects on the mobility of organic anions [13,14] and cations [15] in MeOH/water buffers have been extensively studied.

Thus, addition of organic solvents can alter separation selectivity. However, reversal of the EOF is essential to achieving rapid separations. A number of techniques have also been employed to modify the charge on the capillary and thereby control the EOF. These include: application of an external radial voltage [16,17]; permanently modifying the capillary surface using polymeric materials [18,19]; and dynamically coating the capillary with ionic or non-ionic surfactants [20–22]. In particular, the use of surfactants as dynamic coatings has been commonly employed for EOF control in the separation of small anions. Such surfactant-based coatings offer a number of advantages. They are easy to apply, yield high separation efficiencies are inexpensive and are applicable over a wide range of buffer conditions. Surfactants such TTAB or cetyltrimethylammonium bromide (CTAB) adsorb onto the capillary surface through electrostatic and/or hydrophobic interactions and thus alter the surface charge [21,23].

For inorganic anion separations, it would be desirable to combine the selectivity alterations offered by organic solvents, with the quick analysis times offered by reversal of the EOF. Here, we present systematic studies of the EOF in a capillary dynamically coated with CTAB and DDAB in mixed water–methanol electrolytes. Methanol was chosen because it is a common solvent miscible with water and it is capable of solvating surfactants to a greater extent than other organic solvents [23].

2. Experimental

2.1. Instrumentation

All experiments were performed on a HP^{3D} CE (Hewlett-Packard, Palo Alto, CA, USA) instrument equipped with an on-column diode array UV absorbance detector. Data acquisition and control were preformed using ChemStation software (HP^{3D}, Hewlett-Packard) for Windows 95 on a Pentium II personal computer. Untreated fused silica capillaries (Polymicro Technologies, Phoenix, AZ, USA) with an inner diameter of 50 μm, an outer diameter of 365 μm, and a total length of 37 cm (28.5 cm to the detector) were used unless otherwise specified. In all experiments, the capillary was thermostatted at 25.0 °C.

2.2. Chemicals and sample solutions

All solutions were prepared with Nanopure 18 MΩ water (Barnstead, Chicago, IL, USA). All of the chemicals used were reagent grade or better, and were used without further purification. Buffers were prepared from hydrated sodium salts of dihydrogenorthophosphate and hydrogenorthophosphate (BDH, Toronto, Canada), HPLC-grade methanol (MeOH; Fisher, Fair Lawn, NJ, USA), and a surfactant: either cetyltrimethylammonium bromide (CTAB; Sigma, St. Louis, MO, USA), or didodecyldimethylammonium bromide (DDAB; Aldrich, Milwaukee, WI, USA). The pH was measured using a Model 445 digital pH meter (Corning, Acton, USA) calibrated with aqueous standards immediately prior to use. The pH was adjusted using 0.1 M NaOH (BDH) before the required amount of methanol or surfactant (0.0–2.5 mM) was added. A 1 mM aqueous solution of mesityl oxide (Aldrich) was used as the neutral EOF marker. Previous studies have shown that mesityl oxide is an effective EOF marker in dilute micellar solutions [21,24]. Anion samples were prepared from reagent grade sodium nitrite (BDH), potassium nitrate (BDH), potassium bromide (Fisher), potassium iodide (BDH), and potassium thiocyanate (BDH).

2.3. EOF measurements

New capillaries were used for each new coating study or buffer condition. Each new capillary was pretreated at high pressure (93.8 kPa) with sequentially 1.0 M NaOH for 10 min, 0.1 M NaOH for 10 min and H_2O for 8 min. Prior to each run, the capillary was rinsed at high pressure with 0.1 M NaOH for 2 min, H_2O for 2 min, and running buffer for 3 min. A 0.5 s hydrodynamic injection (5.0 kPa) was used for aqueous buffers while a 2.0 s injection (5.0 kPa) was used for all methanol–water electrolytes. EOF measurements were performed under an applied voltage of −15 kV unless otherwise indicated. All voltages used herein were experimentally verified to be within the linear region of the Ohm's plots. Direct UV detection at 214 nm or 254 nm was used with a data acquisition rate of 8 Hz.

Two methods were used to measure the electroosmotic mobility (μ_{EOF}). When the μ_{EOF} was greater than $2 \times 10^{-4}\,cm^2\,V^{-1}\,s^{-1}$ (i.e., in pure aqueous and low methanolic buffers), the EOF was measured by conventional injection of mesityl oxide and application of a constant voltage of ±15 kV at 25 °C. The EOF was then calculated using:

$$\mu_{EOF} = \frac{L_t L_d}{t V_{app}} \quad (1)$$

where L_t is the total length of the capillary, L_d is the length to the detector, t is the migration time of the neutral marker, and V_{app} is the voltage applied across the capillary.

When the μ_{EOF} was less than $2 \times 10^{-4}\,cm^2\,V^{-1}\,s^{-1}$ (i.e., in buffers containing more than 20% v/v methanol), the EOF generated was so slow that conventional EOF measurements required inconveniently long migration times. To overcome this problem the three-peak injection method developed by Williams and Vigh [25] was used. This procedure was found to be effective particularly when the EOF is strongly suppressed [13,20]. Briefly, mesityl oxide (neutral marker) was injected into the capillary for 0.5 s at 5.0 kPa. This band

was then pushed a certain distance through the capillary using low pressure (5.0 kPa) for 1 min. A second mesityl oxide marker was introduced as before, and both markers were pushed along the capillary by applying low pressure (5.0 kPa) for 0.5 min. A constant voltage of +15 kV was applied for 1.5 min during which the position of the two bands was altered by the resultant EOF. Finally, a third mesityl oxide band was injected and all the three bands were pushed past the detector by applying 5.0 kPa pressure for 15 min. Direct detection was performed at 254 nm. The EOF was calculated using [25]:

$$\mu_{\mathrm{EOF}} = \frac{[(t_3 - t_2) - (t_2 - t_1)]L_{\mathrm{d}}L_{\mathrm{t}}}{[(t_3 + \frac{1}{2}t_{\mathrm{inj}})(t_{\mathrm{mig}} - \frac{1}{2}t_{\mathrm{ramp\text{-}up}} - \frac{1}{2}t_{\mathrm{ramp\text{-}down}})]V_{\mathrm{app}}} \quad (2)$$

where t_1, t_2, and t_3 are the migration times of the first, second and third EOF markers, respectively; t_{inj} is the injection time; t_{mig} is the time necessary for which the voltage was applied; and $t_{\mathrm{ramp\text{-}up}}$ (3 s) and $t_{\mathrm{ramp\text{-}down}}$ (1 s) are the times necessary for the applied voltage to change between 0 and V_{app}. All other terms are as defined in Eq. (1).

2.4. Critical micelle concentration

Critical micelle concentrations (CMCs) were determined from surface tension measurements using a Fisher surface tensiometer (Model 20, Fisher Scientific, Pittsburgh, PA, USA). The platinum–iridium ring (6.0 cm circumference) was cleaned in 2-butanone (Fisher) and then heated in the oxidizing part of a gas flame to ensure it was free of any residue. The glass sample beaker was also washed with the ketone and rinsed with water prior to measurements. The surface tension was then measured in 10 mM aqueous or methanolic phosphate buffer (pH 8.0) solutions containing increasing concentrations of CTAB (from 0.01 to 2.0 mM). All measurements were made at room temperature (~25 °C) and in duplicate. The CMC was determined based on the inflection point/break point of a plot of surface tension versus the log of the surfactant concentration. To validate the procedure, the CMC of CTAB was determined in distilled water at room temperature and a value of 0.87 mM was obtained. This is comparable with literature values [26].

2.5. Anion separations

The effect of methanol content on selectivity of small inorganic anions was studied over the range of 0–60% (v/v) MeOH in a 15 mM phosphate buffer at pH 8.0 containing various concentrations of CTAB or DDAB. Mixed anion samples were prepared in the running buffer and contained 0.2 mM of each ion (NO_2^-, NO_3^-, Br^-, I^- and SCN^-). Sample was injected hydrodynamically for 2.0 s at 5.0 kPa. The direction of the EOF was reversed when cationic surfactants are used (i.e., from the cathode to the anode) so that the anions were separated in the co-EOF mode and detected using direct UV detection at 214 nm with a data acquisition rate of 8 Hz. An applied potential of −15 kV was used in all anion separations unless otherwise specified. Standard addition of each anion in each buffer was performed to confirm the identity of the peaks. All mobility measurements were performed in triplicate. The effective mobilities of the anions were calculated from the migration times under constant voltage conditions [11].

3. Results and discussion

3.1. EOF reversal using CTAB in aqueous buffers

The effect of CTAB concentration on EOF in a 10 mM aqueous phosphate buffer at pH 8.0 is shown in Fig. 1 (closed circles). The normal EOF in a bare silica capillary is towards the negative electrode (cathode), and is denoted with a positive EOF mobility. Upon addition of CTAB to the aqueous buffer there is a rapid transition from normal (cathodic) to a reversed (anodic) EOF as the CTAB concentration is increased from 0 to 0.1 mM. Thereafter the reversed EOF is constant at about $-4.45 \times 10^{-4}\,\mathrm{cm^2\,V^{-1}\,s^{-1}}$, independent of the CTAB concentration.

Previously, Tavares et al. reported flow reversal at a CTAB concentration of about 10% of the standard (i.e. distilled water) CMC value [27]. More commonly, the surfactant concentration at which the EOF is reversed is referenced versus the conditional CMC (i.e., the CMC of the surfactant in the buffer solution). Lucy and Underhill reported that EOF reversal was complete by the conditional CMC for CTAB in their pH 9.0 phosphate buffer (50 mM ionic strength) [21]. In contrast, Martin-Jouet et al. [28] also observed full reversed EOF at 1/3 of the conditional CMC of CTAB in 20 mM creatinine/4 mM nicotinic acid (pH 5.5) while Baryla et al. observed fully reversed EOF at half the conditional CMC of CTAB in a 10 mM phosphate (pH 7.0) buffer [29]. These latter observations are consistent with the aqueous phase behavior in Fig. 1 (closed circles), where

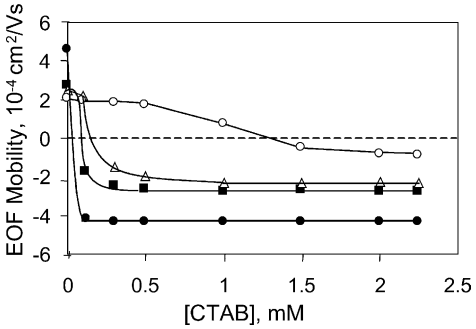

Fig. 1. Effect of concentration of CTAB and methanol on electroosmotic flow (EOF). Experimental conditions: $V = -15$ kV; $L = 37.0$ cm (28.5 cm to detector); 10 mM phosphate buffer, pH 8; direct UV detection at 254 nm using mesityl oxide as neutral marker. (A) 0% MeOH (●); (B) 30% MeOH (■); (C) 40% MeOH (△); (D) 60% MeOH (○).

Table 1
CMC values of CTAB in MeOH–water mixtures (buffer: 10 mM phosphate at pH 8.0)[a]

MeOH (%)	CMC (mM)	EOF reversal (mM)[b]
0	0.18	0.1
30	0.25	0.3
40	0.31	0.4
60	0.69	1.5

[a] CMC of CTAB in distilled water = 0.87 mM.
[b] [CTAB] at which full EOF reversal is observed.

the EOF is fully reversed by 0.1 mM, which is about half the conditional CMC for CTAB in 10 mM phosphate (pH 8) (0.18 mM, Table 1). Addition of electrolyte to the surfactant solution decreases the CMC of the surfactant by diminishing the electrostatic repulsion between the ionic headgroups of the surfactants. For instance, the CMC of CTAB decreases from 0.87 mM in distilled water to 0.18 mM in the aqueous 10 mM phosphate (pH 8.0) buffer used herein (Table 1). Thus, these observations suggest that EOF reversal in aqueous solution occurs at or just below the conditional CMC value of CTAB in the electrolyte buffers.

Recent atomic force microscopy (AFM) studies have shown that single chain cationic surfactants such as CTAB form spherical aggregates at silica surfaces [26,29]. These AFM studies also corroborate that surfactant aggregation occurs at the silica surface at one-third to one-half of the conditional CMC of the surfactant in the bulk solution. This sub-CMC aggregation is attributed to surfactant being concentrated in the wall region due to electrostatic attraction between the cationic headgroups of the surfactant and the negative charge on the capillary surface. The surface excess thus leads to micelle formation at the surface even when the surfactant concentration is below the bulk solution CMC. This concentration at which aggregation first occurs on the surface is referred to as the "critical surface aggregation concentration (CSAC)".

3.2. EOF reversal using CTAB in aqueous–methanol buffers

Fig. 1 shows the effect of increasing CTAB concentration on EOF in a 10 mM aqueous phosphate buffer at pH 8.0 containing 0–60% MeOH. A highly unstable baseline was observed for CTAB solutions containing more than 60% MeOH. The EOF reversal observed in the presence of methanol displays a number of changes in behavior relative to that in the pure aqueous buffer (Fig. 1). These are: a decrease in the magnitude of the normal EOF in the absence of surfactant; a decrease in the magnitude of the fully reversed EOF at high CTAB concentrations; and an increase in the CTAB concentration necessary to reverse the EOF.

The results in Fig. 1 shows that the magnitude of the EOF decreases as the amount of methanol increases in the absence of the surfactant. This is partly due to a decrease in the dielectric constant (ε) and an increase in the viscosity (η) of the buffer [30]. Schewer and Kenndler reported that the magnitude of ε/η in 60% MeOH solution is about 45% of that in pure aqueous solution [30]. Some additional decrease in EOF would be expected due decreases in the zeta potential in buffers containing the organic solvents. However, this latter effect is probably offset by variations in the ionic strength of our buffer upon dilution with the MeOH. Nonetheless, the potential effect of decreases in the zeta potential of the capillary wall on EOF reversal using CTAB will be discussed in Section 3.3.

The magnitude of the fully reversed EOF (>2 mM CTAB) in Fig. 1 decreases upon addition of MeOH. The EOF decrease from $-4.45 \times 10^{-4}\,\mathrm{cm^2\,V^{-1}\,s^{-1}}$ in pure aqueous buffer to $-0.78 \times 10^{-4}\,\mathrm{cm^2\,V^{-1}\,s^{-1}}$ in 60% MeOH. Between 0 and 40% MeOH the change in the reversed EOF is strongly correlated with the change in the normal EOF observed in the absence of CTAB ($R^2 = 0.985$). This suggests that the changes in the magnitude of the fully reversed EOF are directly related to solution conditions (ε/η), rather than a disruption of the adsorbed surfactant layer. However, the magnitude of the reversed EOF in 60% MeOH in Fig. 1 is much smaller than the corresponding normal EOF. This suggests that there is a disruption or alteration in the micellar layer under these conditions. This is similar to the observations of Colic and Fuerstenau who observed an order of magnitude decrease in the maximum surface coverage of sodium dodecylsulfate (SDS) on alumina (pH 3) upon switching from pure aqueous buffer to 50% ethanol [31].

As the concentration of MeOH in the buffer increases in Fig. 1, the transition from normal to reversed EOF becomes more gradual and the concentration of CTAB necessary to reverse the EOF also increases. As discussed above, EOF reversal in aqueous solution normally occurs below the conditional CMC of CTAB in the buffer. To determine if a similar relationship exists in MeOH–water electrolytes the CMC of CTAB in the methanol containing buffers was determined using a tensiometer, as discussed in Section 2.5. The CMC measured and CTAB concentrations at which a reversed and stable EOF is achieved under various buffer conditions are listed in Table 1.

In aqueous buffers reversal of EOF was complete by a CTAB concentration of about one-half the conditional CMC in solution. However, the behavior observed for buffers containing MeOH was different than the aqueous solutions as shown in Fig. 1. In 30% MeOH reversal of EOF occurred at approximately the conditional CMC and in 40% MeOH EOF reversal occurred at a concentration greater than the CMC. In 60% MeOH EOF reversal did not occur until a CTAB concentration much greater than the CMC was employed. Similar dramatic increases in the concentration of surfactant necessary to reverse the charge of a surface in mixed MeOH–water solutions have also been reported in the literature [32,33].

The CMC of CTAB increases only gradually with increasing percent methanol in the buffer. Short chain alcohols (methanol and ethanol) dissolve very little into micelles

and so only affect the CMC by altering the bulk properties and interactions of the solvent. For instance, the CMC of TTAB is 40% higher in 23% (w/w) ethanol than in pure water [34]. Long chain alcohols (more than four carbons) partition into the micelle and can screen the electrostatic repulsion between surfactant headgroups, thereby decreasing the surfactant CMC [35].

3.3. Effect of surface ionization of silica

The effect of zeta potential on surface aggregation of CTAB was conveniently explored by lowering the pH of the buffer. Fig. 2 shows the dependence of EOF versus CTAB concentration in 10 mM aqueous phosphate buffer at pH 3. The corresponding EOF behavior in aqueous pH 8 buffer is reproduced from Fig. 1 for reference. With no CTAB in the running buffer the EOF is much lower at pH 3 than 8, as is expected due to the reduced deprotonation of the silanols. At high CTAB concentration (>1 mM) the reversed EOF in Fig. 2A becomes independent of surfactant concentration and shows only a small dependence on pH.

The most striking difference between the EOF behaviors at pH 3 and 8 in Fig. 2A is the characteristics of the transition from normal to reversed EOF. The sharp transition in EOF at pH 8 has been discussed in Section 3.1. In contrast, at pH 3 the EOF undergoes a much more gradual transition (EOF is not fully reversed until 0.5 mM CTAB), as compared to the transition at pH 8 (EOF is reversed by 0.1 mM CTAB). This change in behavior is reminiscent of that observed in Fig. 1 as a result of the addition of ~40% MeOH to the buffer. Addition of 40% MeOH to the pH 3 buffer (Fig. 2B) shifts the point of EOF reversal to higher CTAB concentrations relative to the purely aqueous pH 3 buffer. Likewise the EOF transition in 40% MeOH is much more gradual at pH 3 than at pH 8.

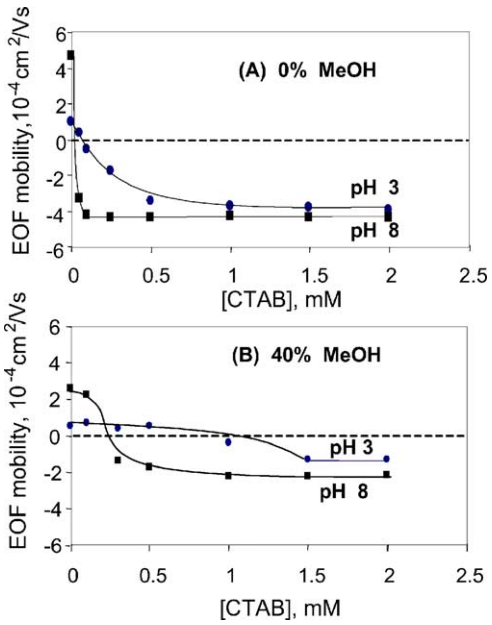

Fig. 2. Effect of pH on the magnitude of the EOF in a 10 mM phosphate buffer containing (A) no MeOH and (B) 40% (v/v) MeOH. All other experimental conditions as described in Fig. 1.

The results in Fig. 1 demonstrated that changes in the CMC of CTAB upon addition of methanol were not solely responsible for the changes in the EOF. A decrease in the zeta potential of the silica surface also could cause a decrease in surface aggregation of CTAB. Addition of organic solvents decreases the zeta potential of silica surfaces in two ways. First the intrinsic lower dielectric constant of the mixed aqueous–organic solvent results in a steeper drop of the potential. Secondly, the pK_a of the surface silanols shifts to a higher value in mixed aqueous–organic solvents [30] and hence the zeta potential is further diminished by incomplete ionization of the silanols. Thus, the impact of MeOH on CTAB concentration at which EOF reversal occurs is predominantly a function of the diminished zeta potential of the silica and to a lesser extent to changes in the CMC of the surfactant.

3.4. EOF reversal using DDAB in aqueous and mixed methanol–water solutions

We investigated the effect of double-chained surfactant, DDAB, on the EOF in aqueous and mixed aqueous–methanol buffers. This surfactant has previously been used for rapid separation of anions at low pH [4]. The results are shown in Fig. 3. The general trends in EOF observed are similar to those of CTAB (Fig. 1). A rapid transition from normal to reversed EOF is observed as the DDAB concentration increases from 0.05 to 0.1 mM. However, as the amount of MeOH increases from 0 to 20% the concentration of DDAB necessary to reverse the EOF rises only slightly. With the DDAB the EOF is reversed at much lower surfactant concentration than the CTAB both in aqueous and methanol containing buffers.

A stable reversed EOF is obtained with up to 20% MeOH in the running buffer. Above 20% MeOH the migration times become irreproducible and the baseline showed strong disturbances. Therefore, the use of DDAB in mixed aqueous–organic solvents was not explored further.

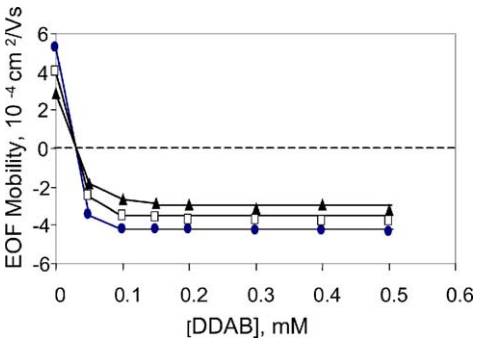

Fig. 3. Effect of concentration DDAB on EOF: (A) 0% MeOH (●); (B) 10% MeOH (□); (C) 20% MeOH (▲). Other conditions as in Fig. 1.

3.5. Anion separations

The effect of methanol content on the separation of small inorganic anions was studied over the range of 0–60% (v/v) MeOH in a 15 mM phosphate buffer at pH 8.0 containing cetyltrimethylammonium chloride (CTAC). Our attempts to use more than 60% MeOH were unsuccessful due to high background disturbances. The chloride form of the surfactant was used to reduce the UV background. The anions were separated in the co-EOF mode and detected using direct absorbance at 214 nm.

The electropherograms in Fig. 4 show the influence of increasing concentrations of MeOH on the separation of five anions. The mobility order in aqueous buffers is Br^- > NO_2^- ∼ NO_3^- > I^- > SCN^-. This is the same order as that observed in typical electrostatic ion chromatography [3,11]. In addition to reversing the EOF, cationic surfactants were reported to affect the migration times of anions through ion-pair interactions [3,36] or hydrophobic interaction [22]. The large but less hydrated anions such as iodide and thiocyanate undergo strong ion-exchange interactions with the surfactants. Thus, such polarizable anions are retained in the capillary longer. In addition, the peaks for these anions are broad and tailed. Interactions with the dynamically coated surfactant layers on the capillary wall would cause tailed peaks due to resistance to mass transfer of the anions to and from the surface to the bulk solution [20].

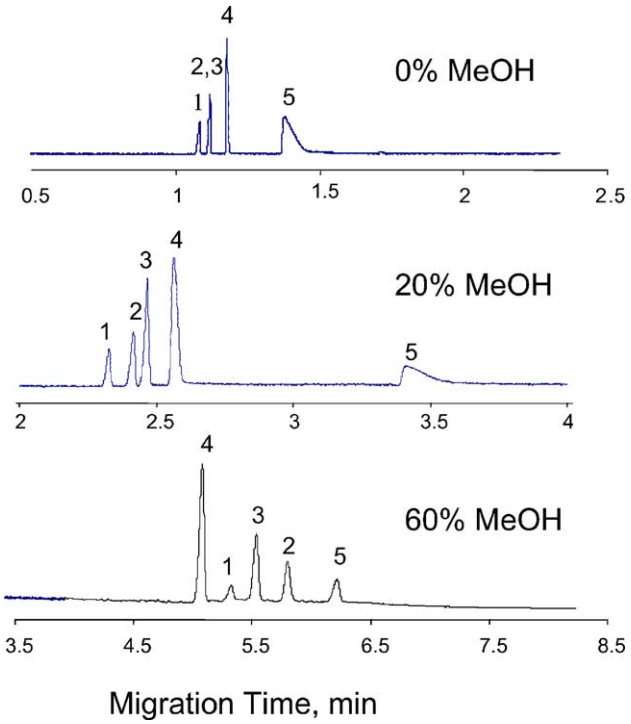

Fig. 4. Separation of five inorganic anions by co-EOF using mixed organic–aqueous systems. Experimental conditions: [CTAC] = 0.5 mM; $V = -15$ kV; $L = 37.0$ cm (28.5 cm to the detector); 15 mM phosphate buffer (pH 8.0); 0.2 mM sample concentration. Identification of peaks: (1) Br^-, (2) NO_3^-, (3) NO_2^-, (4) I^-, (5) SCN^-.

When methanol is used in the electrophoretic buffers significant changes in the separation order are observed relative to those in pure aqueous systems (Fig. 4). In all cases, the migration time of the anions increased with increasing MeOH concentration, as would be expected from the lower EOF velocity caused by the higher solvent viscosity and lower dielectric constant. NO_3^- and NO_2^- are not separated in aqueous solutions whereas these ions are baseline resolved in 20% MeOH. In 60% MeOH, the mobility of iodide and thiocyanate have been significantly altered, such that the mobility order is now: I^- > Br^- > NO_3^- > NO_2^- >SCN^-. I^- is the second slowest ion in 20% MeOH but the fastest in 60% MeOH. Changes in solvation of ions and ion-association interactions with the surfactant are likely the major factors determining the observed changes in selectivity [9,37].

We wished to investigate whether more drastic selectivity changes could be achieved by using a higher concentration of CTAC. Fig. 5 shows the effective mobilities (Section 2.5) of selected anions at increasing concentrations of CTAC and different % MeOH. In pure aqueous solutions, the mobility of the anions, particularly the more polarizable ones such as I^-, is retarded as the concentration of surfactant increases in the running buffer. This is due to ion-pair interactions between the analytes and the positively charged surfactants in solution. However, in methanolic buffers the effect is substantially reduced (Fig. 5). In 20% MeOH, there is a significant change in mobility of the anions but in buffers containing 60% MeOH, the mobility of I^- and SCN^- change

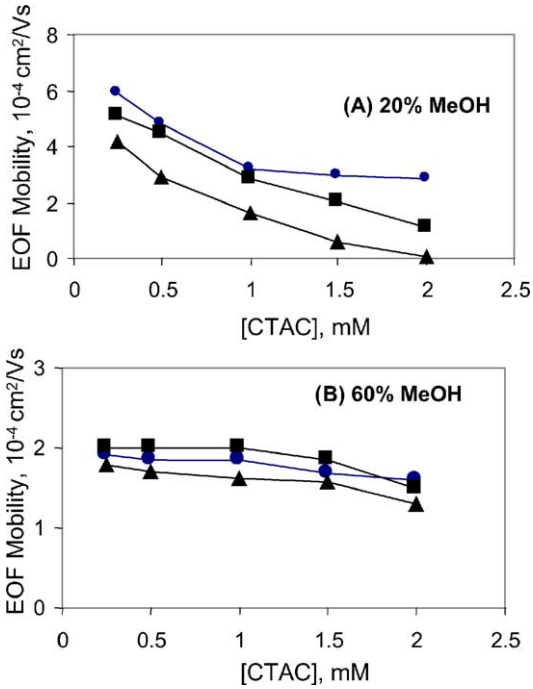

Fig. 5. Effect of CTAC concentration on the selectivity of inorganic anion separations: (A) 20% MeOH; (B) 60% MeOH. NO_3^- (●), I^- (■), SCN^- (▲). Other experimental conditions as in Fig. 4.

only by 4 and 6.5%, respectively when the concentration of the CTAC is increased from 0.5 to 2.5 mM. The presence of MeOH in the solution suppresses the interactions of analytes with the surfactant thereby increasing the effective mobility of the polarizable anion [10]. This reduction in ion-exchange interactions allows anions such as iodide to migrate faster than other anions and the peaks become more symmetrical, a big improvement over the aqueous buffers.

4. Conclusions

This work demonstrates that the reversed EOF generated by the cationic surfactants, CTAB and DDAB, can be systematically altered by the addition of methanol to the background electrolyte. For both aqueous and low methanol containing buffers surface aggregation of the surfactants at the capillary wall and EOF reversal occur at concentrations below the bulk solution CMC. In aqueous solutions, the resultant critical surface aggregation concentration (CSAC) is in the range of one-third to one-half of the CMC in the buffer solutions. Decreasing the zeta potential of the silica surface either by lowering the pH or adding MeOH results in an increase in the CSAC of the surfactant required to reverse the EOF. Also, dramatic selectivity changes for inorganic anions were observed upon increasing the % MeOH in the buffer. Changes in solvation of ions and ion-association interactions with the surfactant are likely the major factors responsible for the observed changes in selectivity. Hence, modifying the EOF using CTAC and methanol helps to obtain baseline separation of anions while retaining the rapid analysis times and high efficiencies associated with co-EOF separations.

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