

Study of the selectivity of inorganic anions in hydro-organic solvents using indirect capillary electrophoresis

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Abstract

In capillary electrophoresis (CE) analysis of small inorganic anions, the ability to control the electroosmotic flow (EOF) and the ability to alter the electrophoretic mobility of the ions are essential to improve resolution and separation speed. In this work, a CE method for separation of small inorganic anions using indirect detection in mixed methanol/water buffers is presented. The suitability of different UV absorbing probes commonly used for indirect detection including chromate, iodide, phthalate, benzoate, trimellitate, and pyromellitate, in mixed methanol/water buffers is examined. The effect of the electrolyte buffer system, including the pH, buffer concentration and the organic solvent on the electrophoretic mobility of the probes and analytes are also investigated. The EOF was reversed using cationic surfactant, cetyltrimethylammonium bromide (CTAB) so ions were separated under co-EOF mode. The organic solvent alters the electrophoretic mobility of the probes and the analytes differently and hence choice of the appropriate probe is essential to achieve high degree of detection sensitivity. Separations of six anions in less than 2.5 min were accomplished in buffers containing up to 30% MeOH. Adjustment of the methanol content helps to improve the selectivity and resolution of inorganic anions. Limit of detection, reproducibility and application of the method for quantification of anions in water samples will also be discussed.

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1. Introduction

Ion chromatography (IC) is the most popular method for the analysis of small inorganic anions. However, capillary electrophoresis (CE) represents a valuable alternative to IC due to its complementary separation selectivity. CE also provides shorter analysis times, higher separation efficiency, and much lower sample and electrolyte volumes than IC [1–3].

Separation selectivity in CE depends on the difference in the effective electrophoretic mobilities of the analytes of interest. Selectivity can be achieved by changing the ionic strength, pH, and using additives. A number of studies have noted a change in selectivity in CE separations of anions due to the ionic strength [4,5]. In particular Li et al. [6] had demonstrated the influence of ionic strength on the mobility

of singly and multiply charged carboxylates and sulfonates using the Pitts equation. However, the extensive use of indirect detection in CE of inorganic anions limits the utility of ionic strength for controlling selectivity. Anion selectivity can also be achieved by adjusting the pH of the buffer to maximize the difference in electrophoretic mobility of the ions. Dramatic changes in selectivity have been observed particularly for borate, carbonate, and phosphate whose pK_a lie in the pH range 8–13 [7]. Melanson and Lucy have also used acidic buffer (pH 2.5) to separate nitrite and nitrate [8]. However, this approach has limited use for inorganic anions as many of these ions have no pK_a 's in the pH range that is attainable in most CE applications [3,9].

In recent years there have been significant interest in the use of organic solvents to modify selectivity in CE [10–12]. However, there has been a limited amount of work on CE separations of inorganic anions in the presence of organic solvents [4,13]. Salimi-Moosavi and Cassidy [13] investigated

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the effect of methanol and dimethylformamide for the separation of inorganic anions. They noted some significant changes in separation order relative to aqueous systems, with some instances in reversal of order of migration. Buchberger and Haddad [4] also studied the effect of up to 30% of methanol, acetonitrile, tetrahydrofuran, acetone or ethylene glycol on anion mobilities.

With respect to CE separations of inorganic anions, two unique issues that arise are detection and the control of the electroosmotic flow (EOF). In CE, detection is usually performed by directly monitoring absorption of UV light by the analytes. However, only a few inorganic anions absorb in the UV–vis range. As a result, it is necessary to use indirect detection [7,8,14]. The application of indirect UV detection in CE requires that a chromophore ion (a probe) be present in the separation buffer to provide a background absorbance. The analyte ion then displaces the probe as it migrates along the capillary, resulting in a decrease in the background absorbance. To maximize this displacement and the resultant relative change in the absorbance background, it is essential that the concentration of the probe be dilute and that the mobility of the probe be close to the mobility of the analytes [5]. If the mobility of the probe differs from that of the analyte, electrodispersion band broadening will occur [15]. Therefore, selection of a suitable UV absorbing probe must consider not only factors related to optimizing sensitivity, but also the separation efficiency [2]. Choosing a probe that has high molar absorptivity and whose mobility is comparable to the mobilities of the analytes optimizes both sensitivity and efficiency.

Control of the EOF is essential for optimizing the analysis time for inorganic anions. In bare silica fused capillaries, the electrophoretic mobilities of small inorganic anions are of the same magnitude but in the opposite direction to the electroosmotic flow. Such counter-EOF separations can enhance resolution, and have even been demonstrated to resolve the isotopes of chloride [16]. However, it also results in excessively long migration times. Hence, in the analysis of most small anions, an EOF modifier is added to the running buffer to slow or more commonly reverse the EOF. To establish co-EOF conditions for anions in fused-silica capillaries, dynamic coatings with positively charged additives or permanent coatings with positively charged function groups have been used [17–19]. Only limited studies have been performed on EOF reversal in the presence of organic solvents [11,20,21]. In our previous studies, we reported that cetyltrimethylammonium bromide (CTAB) can be used to manipulate the magnitude and direction of the EOF in buffers containing 0–60% methanol [20].

This paper develops methods for rapid separation of inorganic anions using indirect detection, wherein the selectivity is modified using methanol. Consideration is given to the choice of the absorbing probe used for indirect detection, based on the relative mobilities of the probe and the analytes and the desired limits of detection. A num-

ber of indirect probes are examined. The effect of the electrolyte buffer system, including the pH, buffer concentration and organic modifier on the probes and analytes are investigated. Adjustment of the methanol content helps to improve the selectivity and resolution of inorganic anions under the co-EOF mode. Separations two times faster than standard ion chromatography techniques are achieved. Limit of detection, reproducibility and application of the method for quantification of anions in water samples will also be discussed.

2. Experimental

2.1. Instrumentation

All experiments were performed on a HP^{3D} CE instrument (Agilent Technologies, Palo Alto, CA, USA) equipped with an on-column diode array UV absorbance detector. Data acquisition and control were performed using ChemStation software (HP^{3D}, Agilent Technologies) on an HP 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 the experiments, the capillary was thermostatted at 25.0 ± 0.1 °C.

2.2. Chemicals and sample solutions

All solutions were prepared with ultrapure (18 M Ω) water (Barnstead, Chicago, IL). The chemicals were all reagent grade or better, and were used without further purification. Buffers were prepared from sodium salts of orthophosphate (BDH), potassium chromate (BDH), and HPLC-grade methanol (MeOH; Fisher, Fair Lawn, USA). The surfactants; cetyltrimethylammonium bromide and chloride (CTA⁺; Sigma, St. Louis, MO, USA) and the carboxylic acids ((benzoic acid (BDH), phthalic acid (1,2-benzene-dicarboxylic acid; BDH), trimellitic acid (1,2,4-benzene-tricarboxylic acid; BDH), and pyromellitic acid (1,2,4,5-benzene-tetracarboxylic acid; BDH)) were used as received. 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 and/or surfactant (0.0–2.5 mM) was added.

Stock anion samples (5.0 mM) were prepared from reagent grade sodium nitrite (BDH), potassium nitrate (BDH), potassium bromide (Fisher Scientific, Fair Lawn, NJ, USA), potassium iodide (BDH), sodium chloride (BDH), sodium sulfate (Fisher), potassium thiocyanate (BDH), sodium fluoride (Fisher), potassium perchlorate (Sigma), and potassium iodate (BDH) without further purification.

2.3. EOF measurements

Each new capillary was pretreated at high pressure (93.8 kPa) with 1.0 M NaOH for 10 min, and H₂O for 8 min. Prior to each run, the capillary was conditioned by rinsing at high pressure (93.8 kPa) with 0.1 M NaOH for 2 min, H₂O for 2 min, and with the 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 buffers. 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 plot. Direct and indirect UV methods were applied at detection wavelengths of 214 and 254 nm, respectively with data acquisition rates of 10 Hz. A 1 mM solution of mesityl oxide (Aldrich, Milwaukee, WI, USA) or 1 mM solution of benzyl alcohol (Aldrich) was used as the neutral EOF marker for direct detection.

2.4. Separation and quantification of anions

Anion samples containing 0.2 mM of each of anions (NO₂[–], NO₃[–], Br[–], I[–], Cl[–], SO₄^{–2}, F[–], ClO₄[–], and SCN[–]) were prepared from the standard stock solutions. Sample was injected hydrodynamically for 2.0 s at 5.0 kPa. The direction of the EOF was reversed in the presence of the cationic surfactant, CTAB or CTAC (i.e., from the cathode to the anode), so the anions were separated in the co-EOF mode. An applied potential of –15 kV was used in all separations unless otherwise specified. Standard addition of each anion in each buffer was used to confirm the identity of each peak. All mobility measurements were performed in triplicate. The effective mobilities of the anions were calculated from the migration times under constant voltage conditions [3]. Efficiencies were computed using the peak width at half-maximum height method.

Limits of detection of the anions were determined using a procedure based on the US Environmental Protection Agency (EPA) methodology [22]. Using the experimental conditions described above, a calibration curve was generated over the concentration range 0.5–10 µg/mL by diluting the stock standard solution of the anions. Then, a 2.5 µg/mL sample of anions (about 5–10 times the estimated detection limit) was separated eight times using 0.25 mM CTAC, 30% MeOH and 5.0 mM chromate (pH 8.0). The standard deviation for these replicate injections was determined. The detection limit is the standard deviation multiplied by the Student *t*-value at the 99% confidence (one-sided) interval.

3. Results and discussion

The choice of the background electrolyte is an important factor in indirect detection in CE. The theoretical limit of

detection in indirect detection (C_{LOD}) is given by:

$$C_{\text{LOD}} = \frac{C_{\text{P}}}{T_{\text{R}} \times D_{\text{T}}} = \frac{N_{\text{BL}}}{T_{\text{R}} \times \epsilon \times l} \quad (1)$$

where C_{P} is the concentration of the visualization probe; T_{R} , the transfer ratio (the number of probe molecules displaced by one analyte molecule), and D_{T} is the dynamic reserve (ratio of the background absorbance to the noise). N_{BL} is the baseline noise, ϵ is the molar absorptivity of the probe, and l is path-length of the detection window. Thus, a low-concentration background electrolyte with a high molar absorptivity at the wavelength of detection is required to obtain the best possible detection sensitivity [15].

3.1. Mobility match in hydroorganic buffers

A key objective in developing an indirect CE method is that the probe used as the visualizing chromophore should have an electrophoretic mobility that closely matches the mobilities of the analytes. This is important for two reasons. Firstly, the transfer ratio T_{R} depends not only on the charges of the probe and analyte, but also on their electrophoretic mobility through Kohlrausch's regulating function [15]. The transfer ratio is greatest when the probe and analyte have very similar mobilities [15,23].

Secondly, with the dilute background electrolytes used in indirect detection, the analyte ions contribute significantly to the total solution conductance within the sample zone. Consequently, if the mobility of the analyte differs from that of the background electrolyte, there will be differences in the conductivity, and hence the electric field strength, across the sample zone. The resultant *electrodispersion* results in peak fronting if the mobility of the analyte is greater than that of the probe, and tailing if the analyte mobility is less than the probe [15,24]. Thus, electrodispersion can severely degrade peak shape, and consequently lower both the resolution and sensitivity of the method.

Addition of organic solvents to the background electrolyte will affect the mobility of both the analytes and probe. Thus, it is important to assess the mobility of probes relative to the mobility of the analytes in the mixed organic-aqueous solvent systems to ensure optimal efficiency and sensitivity. Five commonly used probes (Fig. 1) were selected for study on the basis of their relative mobilities and their high molar absorptivity [15,23]. Fig. 2 shows the effect of the addition of methanol on the electrophoretic mobility of these probes. The buffer concentrations are maintained the same in each buffer system.

As shown in Fig. 2, the electrophoretic mobility of chromate and all the aromatic carboxylic acids decrease substantially as the amount of methanol increases from 0 to 50%. This is not surprising as the viscosity of the electrolyte increases from 0.89 cP in pure water to a maximum of 1.60 cP in 45% methanol [25]. However, some more subtle relative changes in mobility are also evident in Fig. 2. The mobility of pyromellitate decreased by 55% in 40% MeOH relative

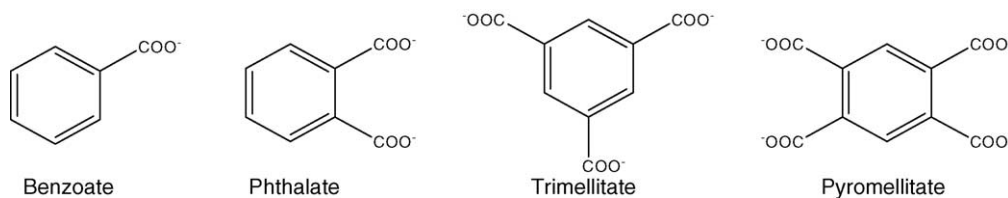


Fig. 1. Structure of commonly used visualization agents (probes) for indirect detection of small anions.

to pure aqueous buffer, while the mobility of benzoate decreased only by 42%. The mobility changes for all others probes are within this range. In 60% MeOH buffer, the mobilities of all except benzoate and phthalate are reduced by more than 50%.

In addition to viscosity, the mobilities of the probes could be affected by solvent induced pH or pK_a changes. Sarmini and Kenndler [11] have shown that the pK_a of benzoic acids change by one or two units upon switching from an aqueous solution to 80% MeOH buffer. Roses et al. also reported similar behavior of pK_a changes of weak acids in buffers containing a variety of organic solvents [26–28]. Herein the effect of the degree of dissociation of the probes shown in Fig. 1 was studied in MeOH/water buffer systems by measuring the effective mobility of the probes over pH values ranging from 6.5 to 9.0 (data not shown). The effective mobility of chromate increases from $1.0 \times 10^{-4} \text{ cm}^2/\text{Vs}$ to $3.4 \times 10^{-4} \text{ cm}^2/\text{Vs}$ as the pH rises from 6.5 to 7.2 and then remains unchanged at higher pH. All other acids show no significant changes over the entire pH range studied (6.5–9.0). In the presence of up to 60% MeOH and at pH 8.0 where the measurements are made, all the probes are completely dissociated/ionized. Thus, the reduction in the effective mobility of the probes upon methanol addition observed in Fig. 2 is not caused by protonation of the probes.

Overall trimellitate and pyromellitate show the largest decrease in mobility upon addition of methanol (Fig. 2). This is consistent with the expectation that these highly charged probes (-3 and -4 , respectively) would experience stronger dielectric friction and ionic strength effects than

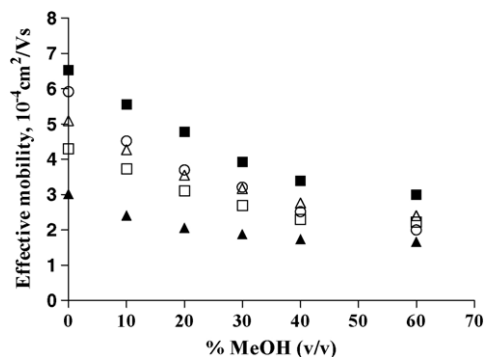


Fig. 2. Electrophoretic mobility of common indirect detection probes for anions detection in mixed methanol/water buffers. Chromate (■), pyromellitate (○), trimellitate (△), phthalate (□), benzoate (▲). Conditions: 15 mM phosphate, pH 8.0; detection at 214 nm.

the lower charged ions [10]. From these results, chromate is the best choice as an indirect detection probe in mixed organic-aqueous media, as it displays the highest mobility in all of the mixed methanol/water buffers systems studied. Chromate ion also has a high molar absorptivity at the wavelengths of detection ($\epsilon \sim 3900 \text{ L mol}^{-1} \text{ cm}^{-1}$ at 214 nm and $\epsilon \sim 2640 \text{ L mol}^{-1} \text{ cm}^{-1}$ at 254 nm) used in this work [24].

The next question is how good is chromate as a visualizing agent for small inorganic anions in mixed organic-water media? Fig. 3 shows the electrophoretic mobility of a few representative inorganic anions relative to that of chromate. In aqueous solution, the mobility of chromate is comparable to that of the anions. This is why it has been the preferred probe for indirect detection of these ions in CE [7,23]. Upon addition of methanol, the mobility of chromate is affected more dramatically than the mobility of the anions. Hence, the relative mobility of the anions increases as methanol is added. At 50% MeOH the mobilities of chloride and bromide are about 1.5 times that of chromate. Thus, even though chromate is the fastest migrating probe in methanol/water media (Fig. 2), its low mobility relative to the inorganic anions (and the resultant electrodispersion) may limit indirect CE to buffers containing less than 40% MeOH.

3.2. Selectivity changes in mixed organic/water buffers

The effect of methanol on mobility of small inorganic anions was examined under indirect detection conditions using a 5.0 mM chromate buffer. Separations were performed in the co-EOF mode by addition of 0.25 mM CTAC to the run buffer. The migration time of the water peak was used to cal-

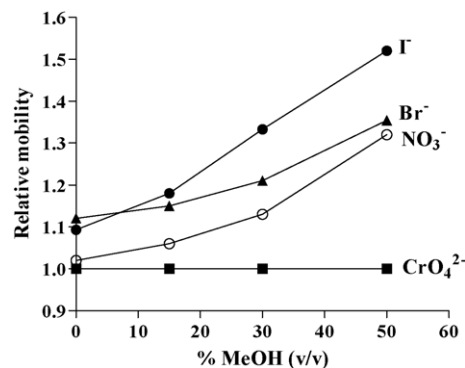


Fig. 3. Comparison of mobility of selected anions vs. that of chromate at different percent methanol. All other experimental conditions as in Fig. 2.

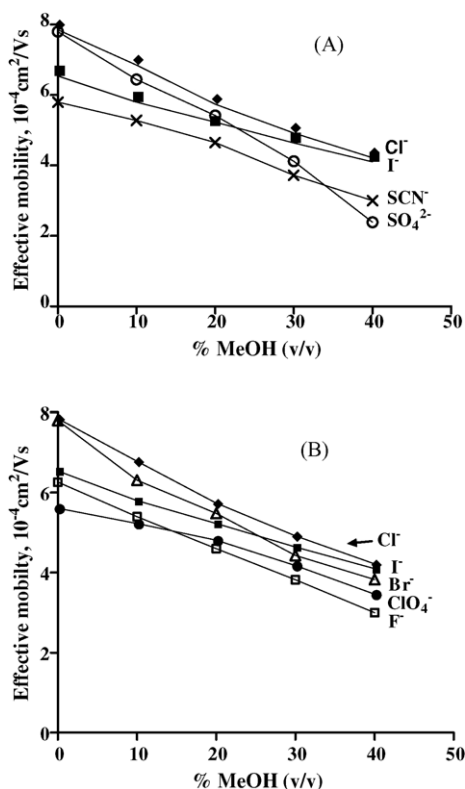


Fig. 4. Selectivity changes of anions with percent methanol. Conditions: 5 mM chromate, 0.25 mM CTAC; pH 8.0 and indirect detection at 254 nm. Cl⁻ (◆), SO₄²⁻ (○), I⁻ (■), SCN⁻ (×), Br⁻ (△), F⁻ (□), ClO₄⁻ (●).

culate the electrophoretic mobility of the anions. Fig. 4 shows the electrophoretic mobilities of the anions studied at different concentrations of methanol. In the presence of organic solvents, all ions exhibit lower mobility than in aqueous solutions. This is due to the increase in viscosity and the decrease in dielectric constant of the running buffer upon addition of methanol. The viscosity of methanol–water mixtures change from 0.89 cP in pure aqueous solution to 0.55 cP in 100% MeOH reaching a maximum value of 1.60 cP at about 45% MeOH [25]. The dielectric constant decreases almost linearly as the amount of organic solvent increases [25]. Hence, the ratio of the dielectric constant to the viscosity is an important parameter determining the mobility of an ion in different solvent systems. For methanol/water mixture this value first decreases and reaches a minimum at about 45% MeOH and there after increases slightly.

In Fig. 4A, sulphate shows significant decrease in mobility when the buffer is changed from pure aqueous to 40% MeOH buffer. Sulfate is the fastest migrating anion in aqueous solution but the slowest migrating in 40% MeOH. Chloride and sulphate have the highest mobility and co-migrate in aqueous solution but are well separated in 40% MeOH. Iodide and chloride are well resolved in aqueous solutions but their separation is compromised in 40% MeOH. An optimum condition for the separation of these four anions is 5 mM chromate with 30% MeOH and 0.25 mM CTAC. Fig. 4B shows the changes in mobility of a second set of anions versus changes

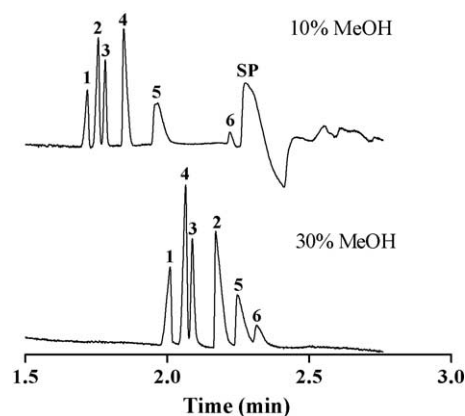


Fig. 5. Co-electroosmotic separation of six anions with buffers containing 0.25 mM CTAC, 5.0 mM chromate. Other conditions: $V = -15$ kV and indirect UV detection at 254 nm. Peaks: (1) Cl⁻; (2) SO₄²⁻; (3) NO₃⁻; (4) I⁻; (5) ClO₄⁻; (6) F⁻; SP: system peak.

in percent methanol. Here also significant selectivity changes are observed. The migration order in 10% MeOH buffer is Cl⁻ > Br⁻ > I⁻ > F⁻ > ClO₄⁻, while in 30% MeOH the order is Cl⁻ ~ I⁻ > Br⁻ > ClO₄⁻ > F⁻.

The electropherograms in Fig. 5 show the separation of six different anions using 5 mM chromate in the presence of 0.25 mM CTAC surfactant. Significant changes in mobilities among the anions are observed with small changes with the amount of methanol. Rapid separations with good resolution are also achieved under these conditions. Efficiencies ranging from 112,000 to 250,000 plates/m are achieved for the separations shown in Fig. 5. The occurrence and position of the system peaks (SPs) is of major concern in indirect detection because this peak will interfere with the detection of surrounding peaks. In 10% MeOH the system peak is close to the fluoride peak, and interferes with the detection of fluoride. However, the system peak is well removed from the analyte peaks in 30% MeOH, and thus does not affect detection of the analyte ions.

In CE the electrolyte is buffered to provide sufficient pH stability and separation reproducibility [29]. This can be accomplished by the addition of a buffer of the same charge as the probe. However, with indirect CE detection any additional co-ion present will compete with the probe and cause both a reduction in detection sensitivity and the occurrence of additional system peaks [30]. To avoid complications associated with system peaks, separations performed herein use only one co-ion in the electrolyte, namely the probe itself. However, this does leave the background electrolyte with limited buffering capacity. The buffer was observed to become cloudy after four runs. Therefore, in all further studies the inlet and outlet buffer reservoirs were changed after four runs.

As shown in Fig. 4, the inorganic anions show significantly different mobility behaviors in mixed methanol/water buffer than in aqueous solutions. In addition to the viscosity and dielectric constant of the solvent system, the solvated radius of ions will be affected in mixed organic/aqueous solvents due to changes in the solvation behavior of the medium. There are

two limiting theoretical views of the influence of solvation, the solvent-berg model and dielectric friction [31]. In the solvent-berg approach, addition of organic solvents is viewed to change the selectivity by altering the solvated radii of the ions. However, this parameter is difficult to measure and there are only few reports on the solvated radii of small inorganic anions in these solvent systems. Recently, Descroix et al. [32] used density functional theory (DFT) coupled with a polarizable continuum model to predict solvated radii of a few inorganic anions in water and methanol. In water, the predicted values agree with the experimental behavior but the model failed to predict the correct mobility order in pure methanol.

In a different but related model, the solvation phenomena have been explained in terms of changes in the frictional forces acting on the ions [33]. Roy and Lucy have shown that dielectric friction can be an important factor affecting the mobility of ions particularly in nonaqueous solvents in addition to the hydrodynamic friction [10,34]. They found that the Hubbard-Onsager (HO) dielectric friction model is successful at predicting solvent-induced selectivity changes in alcohol–water and acetonitrile–water media [35,36]. The HO model treats ion–solvent interactions as a dynamic perturbation of the solvent orientation caused by the ion's charge and size. Thus, dielectric friction can be considered as a charge-induced friction resulting from the finite relaxation time of the solvent dipoles surrounding the ion. The viscosity, dielectric constant, and relaxation time for methanol, respectively, are 0.55 cP, 32.7, and 53 ps [10,34]. For water these values are 0.89 cP, 78.4, and 10 ps, respectively. Hence, the dielectric friction in solvent systems with lower dielectric constant and higher relaxation time becomes more important as the charge density on the ion increases. This indicates that the contribution of the dielectric friction will be much stronger in methanol than in water.

As shown from the results herein the mobility of doubly charged sulphate decreased more significantly than the singly charged anions as the amount of MeOH increased from 0 to 40% (v/v). This is consistent with the predictions [33]. The contribution of dielectric friction for the halide ions would be smaller, as these ions possess only a single charge. For similarly charged ions, the HO model predicts a direct dependence of dielectric friction on ion size and solvent composition. Smaller ions will experience higher frictional forces. In methanol Ibuki and Nakaraha observed an increase in the residual frictional coefficient in the order $I^- < Br^- < Cl^-$ [37]. This is consistent with in Figs. 4 and 5, where the mobility of iodide increases relative to chloride and bromide as the methanol concentration is increased. However, the selectivity changes amongst the other singly charged ions are modest since these ions do not display large differences in size.

3.3. Effect of type of the background electrolyte

Based on the results presented in Fig. 3, we wondered whether iodide could be used as a probe rather than chro-

mate. Iodide's mobility remains comparable to that of the other inorganic anions in the presence of methanol, and has the strongest molar absorptivity at 214 nm [38,39]. Concentrations of KI ranging from 2 to 7 mM are investigated as carrier electrolyte for indirect detection of the other anions (Cl^- , NO_3^- , SO_4^{2-} , Br^- , ClO_4^-). At higher concentration (>7 mM) the iodide forms a precipitate with the EOF modifier (CTAC). Separation efficiencies of 176,000 and 280,000 plates/m are obtained for Br^- and Cl^- , respectively in 30% MeOH using 3 mM KI background electrolyte. These efficiencies are better than the 112,000–250,000 plates/m obtained using chromate buffer (Fig. 5) as expected from the better mobility match between the probe and analytes. Unfortunately, iodide yields poor detection sensitivity. This is mainly due to two reasons. First, some of the anions exhibit strong UV absorbances at 214 nm, which offsets the decrease in absorbance caused by displacement of the iodide probe. As a result, non-UV absorbing anions such as Cl^- and SO_4^{2-} display good detection sensitivities, whereas the UV absorbing anions such as Br^- and NO_3^- show poor sensitivities. Second, the poor detection sensitivity is also due to the high background absorbance at 214 nm from methanol.

3.4. Influence of concentration of background electrolyte

The effect of increasing the concentration of chromate on the separation selectivity of anions was also investigated. Chromate concentrations ranging from 2 to 12 mM were used as BGE. Effective mobilities were determined for each anion at each buffer concentration. Increasing the ionic strength of the BGE more than 12 mM, resulted in Joule heating, as evidenced by a noisier baseline and a reduction in separation efficiency.

Li et al. [6] have shown that Pitts equation can be used to describe the influence of ionic strength on mobility of singly and multiply charged organic anions. The assumptions used to derive the Pitts equation are analogous to those in the Debye-Huckel extended law for ionic activity [40]. Both equations predict that the impact of changes in the ionic strength of the buffer is directly related to the charge on the ion. As result anions of same charge are affected similarly by ionic strength and, the mobility of multiply charged ions will be more significantly affected by the ionic strength than singly charged ions [6]. The behavior of the univalent anions would be expected to follow the Pitts equation, and so changes in ionic strength would not be expected to significantly alter relative mobility of these anions [6]. Using literature ionic size parameters, a decrease in mobility of about 13% is expected for singly charged anions over the buffer concentration range 2–12 mM.

The influence of chromate concentration on selectivity in a BGE containing 0.25 mM CTAC at pH 8.0 is shown in Fig. 6. The results show that the mobilities of the anions decrease with increasing chromate concentration. However, the anions display different behavior. The mobilities of bromide and nitrate decrease by about 11%, in agreement with theory.

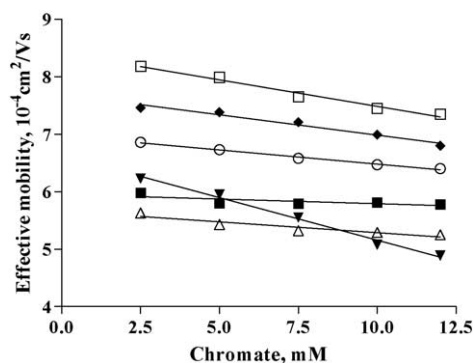


Fig. 6. Effect of concentration of chromate on mobility of inorganic anions in aqueous solutions. All other experimental conditions are as in Fig. 5. Br⁻ (□), NO₃⁻ (◆), I⁻ (○), SCN⁻ (■), ClO₄⁻ (△), F⁻ (▼).

The mobility of fluoride, however, decreases by 21% over the same buffer concentration range. This is greater than theoretically predicted. We have no explanation for the cause of this strong ionic strength effect on fluoride.

In contrast, thiocyanate, perchlorate and iodide exhibit only minor changes in mobility (much less than predicted) as function of chromate concentration. The changes in mobility observed for these anions are less than 7%. This suggests that there are factors other than ionic strength contributing to the observed mobility changes. Since the mobilities are determined in the presence of 0.25 mM CTAC, interaction of the anions with CTAC may be an additional factor for the observed mobilities. It has been shown that large and polarizable anions such as thiocyanate and iodide show greater tendency to interact with cationic surfactants in aqueous solution through ion pairing [20,21,41]. However, the chromate anion used as the background electrolyte also competes with analytes for ion association with the cationic surfactant. Introduction of more chromate ions would displace the iodide and thiocyanate from their ion-pair with CTAC. Disruption of the anion-CTAC ion-pairs would result in greater effective electrophoretic mobility for the anions, thereby offsetting some of the decrease in mobility due to the ionic strength effects. As a result, little change in mobility would be expected for strongly ion associating anions, as observed in Fig. 6. A more detailed investigation of the ion-association of selected anions with the surfactant is discussed in Section 3.5.

3.5. Ion association with the surfactant in MeOH/water buffers

In addition to reversing the EOF, the cationic surfactant might also influence the anion mobility through ion pairing or ion association [21,41,42]. This would lead to a decrease in the effective mobility of the anion. In this study, ion association of the anions with the surfactant molecules or micelles is examined by analyzing the change in the electrophoretic mobility of the anions as the surfactant concentration is varied. Assuming a 1:1 combination of the anion, A⁻, with the sur-

factant, CTA⁺, the association equilibrium can be described as:



where K_{ass} is the ion association or ion-pairing constant. The electrophoretic mobility of analyte anions can be derived from mass balance concentrations and the equilibrium expression (Eq. (2)) as [42]:

$$\mu_{\text{eff}} = \frac{\mu_{A^{-}}}{K_{\text{ass}}[CTA^{+}] + 1} \quad (3)$$

where $\mu_{A^{-}}$ is the electrophoretic mobility of the free anion, [CTA⁺] is the concentration of surfactant which is equal to total surfactant concentration in the buffer, and μ_{eff} is the effective mobility of the anion in the presence of the surfactant. Eq. (3) can be rewritten as:

$$\frac{\mu_{A^{-}}}{\mu_{\text{eff}}} - 1 = K_{\text{ass}}[CTA^{+}] \quad (4)$$

A plot of the term on the left side of Eq. (4) (measured experimentally) versus the total concentration of surfactant yields a straight line with slope equal to the ion-association constant (K_{ass}). With such technique, association constants for bromide and iodide were determined in pure aqueous and 40% MeOH solutions.

In aqueous solutions, both ions show strong binding, with iodide displaying slightly stronger interactions with the surfactant. Ion association constants calculated for bromide and iodide in aqueous solutions are 73 ± 3 and $301 \pm 4 \text{ L mol}^{-1}$, respectively. This behavior is consistent with the selectivity trends observed with ion-exchange chromatography [43]. Large polarizable ions such as iodide orient water molecules with difficulty because of their low charge density. The disruption of the local water structure results in tight binding of surrounding water molecules with one another and in a decrease in entropy; this unfavorable situation enhances the formation of ion pairs [44]. Because of the strong interaction with the CTA⁺, the iodide peak in the electropherogram is also tailed particularly when high concentrations of surfactant are used. Similar tailed peaks have been reported in the literature [41,45]. This is because 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. Hence, in aqueous solutions interaction of the anions with the surfactant could contribute for the reduction in the mobility of the anion and sometimes could lead to the loss of efficiency. Use of 0.25 mM CTAC in aqueous solutions causes the mobility of iodide to decrease by about 7% through ion association.

In 40% MeOH, the ion association constants for bromide and iodide are 196 ± 4 and $125 \pm 3 \text{ L mol}^{-1}$, respectively. Iodide shows weaker binding with the surfactant in MeOH/water buffer than in the aqueous solution. This suppressed ion-exchange interaction of iodide with the surfactant in 40% MeOH leads to faster relative mobility and the

peak is more symmetrical. The efficiency of iodide peak increases from 112,000 plates/m in aqueous solution to over 240,000 plates/m in 40% MeOH. This is consistent with expectation if the ion association is weakening.

In contrast with iodide, bromide shows stronger binding with the surfactant in MeOH/water buffer than the aqueous solution. This stronger ion interaction in methanolic solution is corroborated by a slight decrease in efficiency of the bromide peak from 187,000 to 160,000 plates/m upon going from pure aqueous to 40% MeOH/water buffer. This increase in association constant is consistent with the fact that electrostatic ion pairing requires small, highly charged ions and occurs more readily in solvents of low dielectric constant [44]. Similar enhanced ion-pairing of bromide in methanolic eluents have also been observed in ion chromatography [46]. Hadded and Croft [47] also reported increased retention times of hydrophilic inorganic and organic anions at higher concentrations of organic modifier (acetonitrile).

Thus, addition of methanol to the buffer is expected to increase peak widths due to increased electrodispersion (Section 3.1). However, methanol also affect ion interaction of the anions with CTAC. Thus, for strongly associating anions such as iodide, the addition of methanol actually results in improved efficiencies.

3.6. Quantification and application

Quantification of the anions was examined using the optimal conditions of 0.25 mM CTAC, 30% (v/v) MeOH and 5 mM chromate (pH 8.0). Limits of detection were determined using a procedure based on the US Environmental Protection Agency methodology [22]. This approach determines the minimum amount of sample that can be reported to be greater than the background noise (blank run) with 99% confidence. Using the experimental conditions described in Section 2.4, all the quantitative analyses were performed using calibration curves generated for each anion. Then, replicate separations of a 2.50 $\mu\text{g/mL}$ sample of each anion (about 5–10 times the estimated detection limit) were performed. The standard deviation for these replicate injections was determined and the detection limit is the standard deviation multiplied by the Student *t*-value for one-sided 99% confidence interval.

Calibration curves of the anions show linear dynamic ranges from 0.1 to 10 $\mu\text{g/mL}$ with correlation coefficients equal to or greater than 0.998. Detection limits (Table 1) range from 0.09 to 0.23 $\mu\text{g/mL}$, which are better or comparable to literature values in aqueous buffers [4,48,49]. To examine the run-to-run reproducibility, eight replicate measurements of a standard solution of 2.50 $\mu\text{g/mL}$ of each anion were performed. The percent relative standard deviations (% R.S.D.) for peak areas are presented in Table 1 and range from 1.3 to 2.0%. Good reproducibility of migration times is also obtained (0.2–0.6%). Another important advantage of this methanolic method is that it offers fast separation. The

Table 1

Limits of detection and reproducibility of migration time and peak areas

Anion	% R.S.D. (n = 8)		Linearity correlation (r^2)	LOD ($\mu\text{g/mL}$)
	Migration time	Peak area		
Cl^-	0.2	1.3	0.997	0.12
NO_3^-	0.5	1.5	0.984	0.20
SO_4^{2-}	0.5	1.8	0.993	0.14
ClO_4^-	0.6	2.1	0.996	0.23
F^-	0.3	2.0	0.998	0.09

Experimental conditions: 0.25 mM CTAC, 5.0 mM chromate, 30% MeOH, $V = -15$ kV and indirect UV detection at 254 nm.

method is at least two times faster than standard IC method and other reported CE methods [50,51].

The method was applied to the analysis of anions in tap and river waters (North Saskatchewan River, Edmonton, AB). The samples were diluted five times with de-mineralized water to bring the concentrations within the calibration range and filtered through 0.45 μm membrane before injection. The peaks are identified by standard addition. An efficient separation and good detection sensitivity of the anions (chloride, nitrate, sulphate) are obtained. Chloride and sulphate concentrations in river water were found to be 28 and 35 mg/L and 32 and 22 mg/L, respectively in tap water. The ability to move peaks selectively using different percent of methanol demonstrates the flexibility of this technique. This method could also offer the ability to determine directly small amounts of nitrite, bromide, nitrate, chlorate, or iodide in samples containing high levels of sulfate and/or chloride ions.

4. Conclusions

In CE analysis of small inorganic anions the ability to alter the electrophoretic mobility of the ions and control of the EOF are essential to improve resolution and separation speed. This work demonstrates that indirect detection of small anions can be achieved using CTAB as EOF modifier in the presence of organic solvent. Selection of the appropriate probe helps to improve resolution and sensitivity. Chromate shows better mobility match with small inorganic anions under mixed MeOH/water buffers. In addition, chromate also allows detection at wavelength much greater than the absorption by most anions and methanol. Significant changes in selectivity among anions are observed by using up to 40% methanol in the running buffer. Good resolution and separations times two times faster than standard ion chromatography techniques are achieved. The ability to move peaks selectively using different percent of methanol demonstrates the flexibility of this technique.

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