

Studies on the determination of inorganic anions by capillary electrophoresis

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Abstract

In order to separate anions effectively it is usually necessary to eliminate or reverse the direction of electroosmotic flow (EOF) resulting from attraction of cations to the negatively charged surface of a silica capillary. Previous workers have accomplished this by adding a quaternary ammonium salt (Q^+) as an EOF “modifier”. Under typical experimental conditions, a concentration of $Q^+ > 0.25 \text{ mM}$ is required to reverse the EOF direction. Addition of a low percentage of 1-butanol to the aqueous electrolyte was found to reduce the EOF. A combination of butanol and a very low concentration of Q^+ (typically 0.03 mM) was found to be particularly effective in controlling EOF and in giving effective separations of complex mixtures of anions. The probable mechanism of the butanol- Q^+ effect is discussed.

1. Introduction

Capillary electrophoresis (CE) has become an efficient technique for separating inorganic and organic anions. Several separations of inorganic and short-chain organic acid anions have been reported using UV detection [1–5]. Conductivity detection has also been used successfully for these separations [6,7].

In conventional CE using a fused-silica capillary, the electroosmotic flow (EOF) is toward the cathode where detection is performed. Anions, however, have electrophoretic mobilities toward the anode. Only anions having mobilities with magnitudes less than that of the EOF are detected with this configuration. Thus, it is necessary to eliminate or reverse the direction of the EOF for most anion separations.

There are several ways to control EOF. These

include altering the buffer pH [8–10] or the electrolyte composition by adding surfactants [6,11,12] or organic solvents [10,13,14]. Other methods include coating the inner capillary walls [15–17] or applying an external electric field [18–20]. Several studies of the effects of these parameters on anion separations have been published [21–23].

The most common method used to reverse the EOF for anion separations is to add a quaternary ammonium salt to the electrolyte solution. The positively charged compound is electrostatically attracted to the ionized capillary wall, thus creating a net positive charge on the wall. With the use of a negative power supply, all anions are detected at the anode.

In HPLC, Morris and Fritz [24] found that chromatographic behavior of polar compounds can be dramatically modified by use of a suitable mobile phase additive. A concentration of 4–5% 1-butanol in water was found to be particularly useful. A dynamic equilibrium of butanol exists

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between the mobile phase and the resin, thus coating the resin with a thin film of butanol.

It was thought that a similar effect was likely to occur in CE. Again, a dynamic equilibrium of butanol is expected between the aqueous electrolyte and the walls of the silica capillary. Several anion separations have been reported which use electrolyte solutions with organic solvent additives [2,22,23]. Most of these studies have been limited to low-molecular-mass solvents such as methanol, ethanol and acetonitrile.

In the present work, it is shown that a combination of low concentrations of a quaternary ammonium salt and 1-butanol added to the electrolyte solution affects anion separations. EOF can be controlled more easily when butanol is present and excellent anion separations are possible.

2. Experimental

The CE system used for all experiments was the Waters Quanta 4000 (Waters Chromatography Division of Millipore, Milford, MA, USA). Fused-silica capillaries (Polymicro Technology, Phoenix, AZ, USA) with lengths of 70 cm and inner diameters of 75 μm were used. At 62.5 cm from the injection end of the capillary, the polyimide coating was burned off to create a detection window.

New capillaries were conditioned by rinsing with 1 M NaOH for approximately 1 h, followed by a 15-min rinse with deionized water. A 5-min rinse with NaOH, followed by a 5-min rinse of deionized water was used to wash the capillaries at the start of each day and between runs with different electrolyte solutions.

Electrokinetic sampling was used with a sample voltage of 10 kV for 5 s (unless otherwise specified) or the hydrostatic sampling mode was used with a sampling time of 20 s and height of 10 cm. The capillary was purged with electrolyte solution for 2 min before each run. All separations were carried out at room temperature. The negative or positive power supply was used at 30 kV for each experiment.

On-column indirect UV detection at 254 nm

was used for all separations of inorganic and short-chain organic acid anions. The electrolyte solutions for these separations contained 5 mM sodium chromate as the visualization reagent. Direct UV detection at 254 nm was used for the separation of the aromatic organic acid anions.

All solutions were prepared using deionized water from a Barnstead Nanopure II system (Sybron Barnstead, Boston, MA, USA). All reagents and solvents used were reagent grade. Stock solutions of the inorganic anions were prepared from their corresponding sodium salts. Stock solutions of the organic acids were prepared from the organic acid and the pH raised to form the anion. The chromate electrolyte solutions were prepared from a stock solution of 100 mM sodium chromate (Fisher Scientific, Fair Lawn, NJ, USA). The borate buffer solutions were prepared from a stock solution of 20 mM sodium tetraborate decahydrate (Fisher Scientific). A 20 mM stock solution of OFM Anion-BT, a proprietary reagent from Waters Chromatography Division of Millipore, identified as $\text{R}(\text{CH}_3)_3\text{N}^+\text{Br}^-$, where R is a long-chain alkyl group [25], was used as the quaternary ammonium salt. All alcohols used were obtained from Aldrich (Milwaukee, WI, USA). Adjustments of pH were made with dilute solutions of reagent grade HCl or NaOH.

3. Effect of Q^+ on EOF

Separation of inorganic and short-chain organic acid anions by CE requires the reversal or elimination of EOF. A proprietary reagent developed by Waters, OFM Anion-BT (which will be referred to as Q^+), was used in our work as an EOF modifier. When there is no Q^+ present in a 5 mM chromate electrolyte solution and a negative power supply is used, the EOF is strong and toward the cathode. However, as increasing amounts of Q^+ are added to the electrolyte solution, the magnitude of the EOF to the cathode is decreased until its direction is eventually reversed to the anode. As the concentration of Q^+ in the electrolyte solution is increased, the charge on the capillary wall becomes less nega-

tive before obtaining a net positive charge. A dynamic equilibrium is likely to exist between the Q^+ on the surface of the capillary wall and the Q^+ in the electrolyte solution.

Fairly high concentrations of Q^+ (e.g. 0.5 mM) have been used successfully for several applications [2–5]. However, these concentrations of Q^+ in the electrolyte solution may result in a build-up of Q^+ on the capillary from run to run thus making it necessary to clean the capillary more often. Hydrophobic alkyl ammonium salts, such as Q^+ , also have limited solubility and may form insoluble pairs with some electrolyte components [23,26]. Therefore, a lower concentration of Q^+ in the electrolyte solution may be beneficial for the analysis of some samples.

4. Effect of organic solvents on EOF

Buchberger and Haddad [22] studied the effect of up to 30% methanol, acetonitrile, tetrahydrofuran, acetone and ethylene glycol on the migration order of ten different anions. They noted a general increase in migration times owing to: (1) a decrease in conductivity resulting in a lower current, and (2) a decrease in the amount of Q^+ adsorbed on the inner capillary wall, resulting in lower EOF to the anode.

If a low concentration (e.g. <0.05 mM) of Q^+ is used in the electrolyte solution with a negative power supply, the EOF is still in the opposite direction to the electrophoretic flow of the sample anions. This results in very long migration times.

Addition of alcohols such as methanol, ethanol, 1-propanol, 1-butanol and 1- and 2-pentanol decrease the EOF toward the cathode, thus resulting in faster migration times. However, even high concentrations (e.g. 20%) of methanol, ethanol and 1-propanol do not result in satisfactory decreases in migration times. Low concentrations of butanol (3–5%) and pentanol (1–2%) result in significant decreases in migration times. Anions that had migration times of ca. 30 min when a low concentration of Q^+ was used as the lone additive to the electrolyte solution showed migration times of ca. 3 min

when a low concentration of butanol or pentanol was added to the electrolyte solution. Since pentanol had limited solubility in the aqueous electrolyte solution and less controllable effects on the EOF, 1-butanol was chosen as the most promising alcohol to use as an EOF modifier.

5. Effect of 1-butanol on EOF

The effect of 1-butanol on the EOF was studied by measuring the EOF at increasing butanol concentrations. The EOF was measured using a positive power supply, a 5 mM chromate electrolyte solution at pH 8.0, and deionized water as the neutral marker. Electrolyte solutions containing greater than 8% butanol were not studied due to solubility limitations of butanol in the aqueous solution. Fig. 1 shows the effect of increasing butanol concentrations on the EOF. The EOF coefficient was calculated from the expression $\mu_{eo} = (LL_d)/(Vt)$, where L = capillary length (cm), L_d = capillary length from injection to detector (cm), V = applied voltage (Vs) and t = time (s). Although butanol does not reverse the EOF, there is a significant decrease in the EOF to the cathode as the concentration of butanol is increased. This effect is due in part to the butanol adsorbing to the capillary surface, thus cancelling the effect of the covered ionized groups on the EOF. It is likely that a dynamic equilibrium is established between the butanol in solution and the butanol on the capillary surface.

6. Effect of Q^+ and butanol on EOF

Since neither a low concentration of Q^+ or butanol alone reversed the EOF, a combination of the two modifiers was investigated. Fig. 2 shows the effect of increasing Q^+ concentrations on the EOF in electrolyte solutions containing 0, 3 or 5% butanol. The EOF direction is reversed at a much lower concentration of Q^+ when butanol is added to the electrolyte solution.

Three possible mechanisms were considered to explain the observed results. (1) Ion interaction

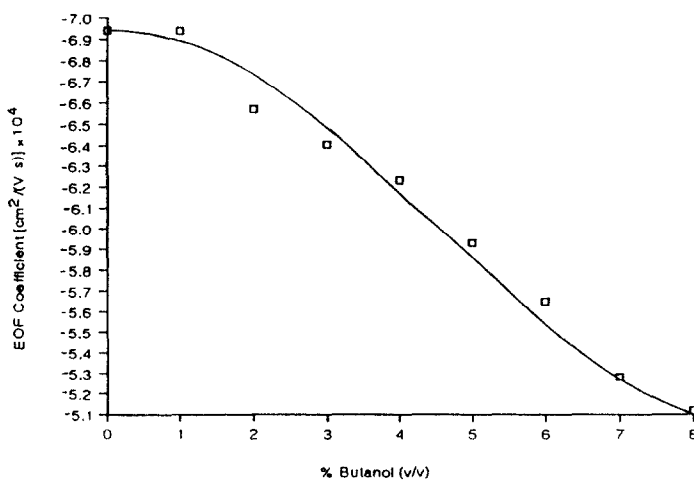


Fig. 1. Variation of EOF coefficient versus percentage 1-butanol added to an electrolyte solution of 5 mM chromate at pH 8.0. Negative coefficients indicate flow toward the cathode.

between the Q^+ and the analyte anions is unlikely to occur in the solution phase because of the very low concentrations of Q^+ (<0.1 mM) being

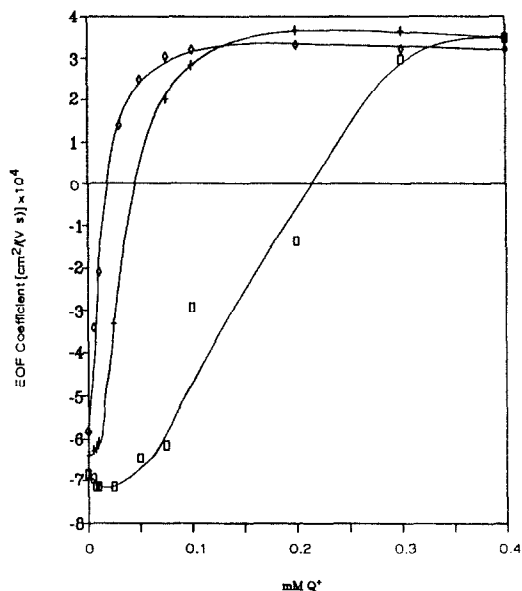


Fig. 2. EOF coefficient as a function of concentration of Q^+ added to electrolyte solutions of 5 mM chromate at pH 8.0 containing 0 (\square), 3 (+) or 5% (\diamond) 1-butanol. Negative coefficients indicate flow toward the cathode.

used. Furthermore, the relative migration times of several anions studied were unchanged when the concentration of Q^+ was varied from 0.03 to 0.1 mM. (2) Formation of a micelle was ruled out because the concentration of Q^+ in the electrolyte solution was far below the critical micelle concentration [27]. (3) The most plausible mechanism is one in which both butanol and Q^+ are adsorbed on the capillary surface by a dynamic equilibrium. The adsorbed butanol would shift the Q^+ equilibrium so that the surface achieves a net positive charge at a significantly lower Q^+ concentration. This mechanism is supported by the work of Scott and Simpson [28] on the distribution of several solvents including butanol between an aqueous phase and a reversed phase silica surface. They concluded that a dynamic equilibrium exists in which there is a monolayer coating of butanol on the surface.

The effect of using low concentrations of both Q^+ and butanol is more impressive when actual CE separations are viewed. Fig. 3A shows an attempted separation of an anion mixture using 0.075 mM Q^+ in an aqueous electrolyte solution containing 5 mM chromate at pH 8.0. Migration of the anions was so slow that only the first few anions had appeared after an hour. Using the

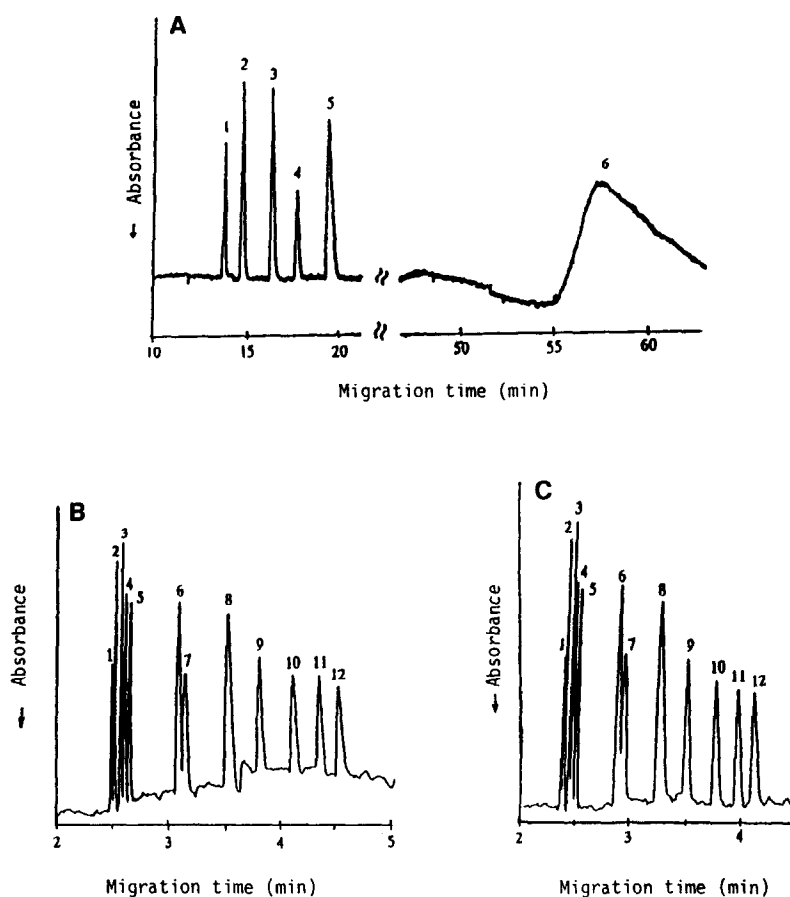


Fig. 3. Separation of inorganic and short-chain organic acid anions using varying concentrations of 1-butanol. Peaks: 1 = Br^- (5 ppm, w/w); 2 = Cl^- (5 ppm); 3 = SO_4^{2-} (6 ppm); 4 = NO_2^- (7 ppm); 5 = NO_3^- (8 ppm); 6 = F^- (8 ppm); 7 = HCOO^- (10 ppm); 8 = CO_3^{2-} (7 ppm); 9 = acetate (10 ppm); 10 = propionate (10 ppm); 11 = butyrate (10 ppm); 12 = valerate (10 ppm). (A) Electrolyte: 5 mM chromate, 0.075 mM Q^+ , pH 8.0; applied voltage: -30 kV, current: 23 μA , electromigration injection 6 s/10 kV. (B) Electrolyte: 5 mM chromate, 0.075 mM Q^+ , 3% 1-butanol, pH 8.0; applied voltage: -30 kV, current: 21 μA , electromigration injection 6 s/10 kV. (C) Electrolyte: 5 mM chromate, 0.075 mM Q^+ , 5% 1-butanol, pH 8.0; applied voltage: -30 kV, current: 21 μA , electromigration injection 10 s/10 kV.

same experimental conditions except for the addition of 3% butanol to the electrolyte solution, an excellent separation (Fig. 3B) was obtained in less than 5 min. The separation (Fig. 3C) observed when 5% butanol was added was even faster.

Several anions were separated using a combination of low concentrations of Q^+ and butanol. A typical separation of some of these anions using 0.03 mM Q^+ and 4% butanol is shown in Fig. 4. Without butanol, a much higher concen-

tration of Q^+ was required to obtain a comparable separation.

Several aromatic carboxylic acid anions were separated using conditions similar to those used previously (Fig. 5). Direct UV detection at 254 nm was used for these anions along with a borate buffer solution. Separation of this mixture was also attempted using the positive power supply and a borate buffer solution with no additives. Under these conditions, the EOF was toward the cathode with the electrophoretic mobilities of the

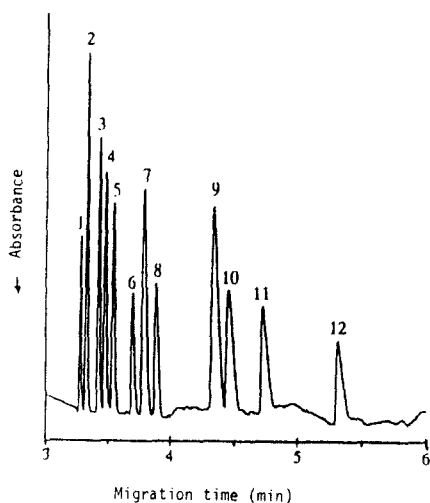


Fig. 4. Separation of inorganic anions. Electrolyte: 5 mM chromate, 0.03 mM Q^+ , 4% 1-butanol, pH 8.0; applied voltage: -30 kV, current: 23 μ A, electromigration injection 5 s/10 kV. Peaks: 1 = Br^- (5 ppm); 2 = Cl^- (5 ppm); 3 = SO_4^{2-} (6 ppm); 4 = NO_2^- (7 ppm); 5 = NO_3^- (7 ppm); 6 = MoO_4^{2-} (10 ppm); 7 = N_3^- (10 ppm); 8 = ClO_3^- (8 ppm); 9 = F^- (8 ppm); 10 = $HCOO^-$ (8 ppm); 11 = ClO_2^- (8 ppm); 12 = CO_3^{2-} (7 ppm).

anions in the opposite direction. The separation was unsuccessful, resulting in poor resolution of the less mobile anions and long migration times for the most mobile anions.

7. Conclusions

The combination of low concentrations of 1-butanol and a quaternary ammonium reagent in aqueous solutions is an excellent way to modify EOF in the CE separation of anions. Coating the capillary surface by a dynamic equilibrium involving butanol and Q^+ is an attractive alternative to other methods that have been proposed for adjusting EOF. Separations using this system are less noisy and more reproducible.

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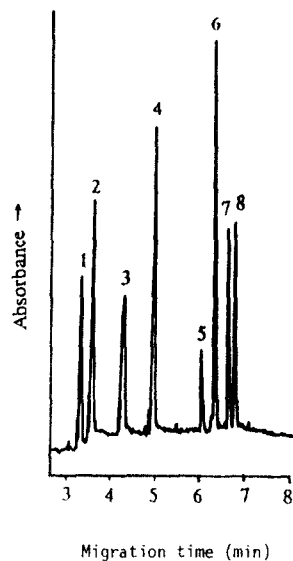


Fig. 5. Separation of aromatic organic acid anions. Electrolyte: 1 mM borate, 0.03 mM Q^+ , 4% 1-butanol, pH 8.5; applied voltage: -30 kV, current: 5.2 μ A, hydrostatic injection: 20 s. Peaks: 1 = 1,2,4,5-benzenetetracarboxylate ($5.9 \cdot 10^{-5}$ M); 2 = 1,2,4-benzenetricarboxylate ($7.2 \cdot 10^{-5}$ M); 3 = 3-nitrophthalate ($7.1 \cdot 10^{-5}$ M); 4 = 2,4-dihydroxybenzoate ($6.5 \cdot 10^{-5}$ M); 5 = benzoate ($1.2 \cdot 10^{-4}$ M); 6 = 4-nitrobenzoate ($4.8 \cdot 10^{-4}$ M); 7 = 3,5-diaminobenzoate ($6.6 \cdot 10^{-5}$ M); 8 = 1,2-naphthoquinone-4-sulfonic acid ($4.2 \cdot 10^{-5}$ M).

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