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Simultaneous separation of anions and cations by capillary electrophoresis with high magnitude, reversed electroosmotic flow

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Abstract

A method for simultaneous separation and indirect detection of anions and cations by capillary electrophoresis (CE) is reported. An anodic electroosmotic flow (EOF) in excess of $-100 \times 10^{-9} \, \mathrm{m^2 \, V^{-1} \, s^{-1}}$ was achieved by the addition of 0.1 mM didodecyldimethylammonium bromide (DDAB) to the background electrolyte (BGE). This high magnitude EOF enabled rapid co-EOF separation of inorganic anions and counter-EOF separation of inorganic cations having high electrophoretic mobilities. Single-end injection of the sample at the capillary inlet and detection near the capillary outlet was used. Indirect photometric detection of cations was accomplished using a cationic probe (imidazole), with counter-ionic (Kohlrausch) indirect detection being used for anions. A mixture consisting of chloride, bromide, nitrate, lithium, magnesium, sodium, calcium, potassium, and ammonium was separated in less than 3.5 min using a background electrolyte comprising 6 mM imidazole, 12 mM hydroxyisobutyric acid, 0.1 mM DDAB, 2 mM 18-crown-6 at pH 4.0, with indirect detection at 210 nm. The system was well suited to the separation and detection of inorganic cations, with limits of detection (LODs) in the range 0.92–4.65 μM and separation efficiencies from 44 400–208 5000 theoretical plates. By contrast, LODs for inorganic anions ranged from 6.66 to 11.43 μM, with separation efficiencies of 2500–26 300. Analytical potential of the method is discussed.
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1. Introduction

Simultaneous separation and detection of underivatized anions and cations (including high-mobility inorganic cations) by capillary electrophoresis (CE) requires that all analytes must be transported past the detection window in a fashion which will allow their separation. Two approaches to achieve this goal are possible. The first approach involves sample injection at both ends of the capillary with detection near the middle of the capillary. Electroosmotic flow (EOF) should be suppressed to a minimum to allow cations and anions to migrate past the detector

from opposite directions according to their electrophoretic mobilities. Sample injection can be realised either by hydrostatic or electrokinetic injection. This dual-end injection approach has been demonstrated successfully with both photometric [1–4] and contactless conductometric detection [5–8]. The second approach uses traditional single-end injection, with detection performed near the opposite end of the capillary. Under normal cathodic EOF, cations can be separated in the co-EOF mode and anions in the counter-EOF mode. Obviously, in this arrangement only those anions with electrophoretic mobilities of lower magnitudes than the EOF will be transported past the detection point. Therefore to separate a range of anions including high mobility inorganic anions within a reasonable time, a high magnitude EOF (in excess of approximately $90 \times 10^{-9} \, \mathrm{m^2 \, V^{-1} \, s^{-1}}$) or application of an additional external

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(hydrodynamic) pressure are necessary. The high magnitude EOF approach is preferred as hydrodynamic pressure will to some degree compromise the separation efficiency in a CE system.

The simplest way to increase the magnitude of cathodic EOF is to increase the pH of the background electrolyte (BGE). There are two limitations to this approach: alkaline earth metal ions may form hydroxide complexes and cationic probes used for indirect photometric detection may not be protonated at higher pH values. Examples of this approach include Jorgenson and Lukacs [9], Shamsi and Danielson [10] and Foret et al. [11]. The use of pressure to transport ions is generally avoided due to the associated band broadening. However, a successful application was demonstrated by Haumann et al. [12], in which potassium, sodium, lithium, formate, acetate, sulfate, chloride, bromide, nitrate, chlorate and propionate were separated in 13 min with the application of 200 mbar pressure to the inlet end of the capillary. This additional pressure generated a linear velocity of 0.66 cm/s in the BGE. Separation efficiencies were not provided.

The major limitation of high magnitude cathodic EOF to provide simultaneous separation of anions and cations is that it is difficult to generate an EOF of sufficient magnitude to separate high mobility inorganic anions, such as chloride and nitrate, in a counter-EOF mode. However, inorganic cations generally have mobilities of lower magnitude than anions, so this presents an opportunity for simultaneous separation of anions and cations using a reversed EOF and reversed polarity separation. Here, the anions are separated in the co-EOF mode and cations are separated in the counter-EOF mode (i.e., the reverse of the situation discussed above). To be successful for cations having the highest mobilities, this approach requires that a reversed EOF of high magnitude be used.

The double-chained cationic surfactant didodecyl-dimethylammonium bromide (DDAB) and its properties regarding EOF in CE have been studied extensively by Lucy and co-workers [13,14]. DDAB offers some properties which differ from those single-chain cationic surfactants [such as cetyltrimethylammonium bromide (CTAB) and tetradecyltrimethylammonium bromide (TTAB)] used commonly for EOF reversal. Melanson et al. [13] showed that DDAB formed a flat bilayer structure at the capillary wall. The coating was shown to be very stable, which allowed electrolytes not containing the surfactant to be used whilst retaining stable, reversed, anodic EOF. Baryla and Lucy [14] also used combinations of DDAB and the zwitterionic surfactant 1,2-dilauroyl-*sn*-phosphatidylcholine (DLPC) to vary EOF. Adjusting the ratio of DDAB and DLPC allowed the EOF magnitude to be tuned to optimise separations. In these works DDAB was not present in the electrolyte and the highest magnitude anodic EOF generated in pre-run coated capillaries was $-70 \times 10^{-9}\,\mathrm{m^2\,V^{-1}\,s^{-1}}$.

A further complication in the simultaneous determination of anions and cations is the identification of a suitable detection mechanism. Conductivity detection has been suc-

cessfully employed for detection, with examples including Kuban et al. [5,6]. Indirect photometric detection has also been used, based on well-established principles [15–18]. Indirect detection is based on the displacement of an absorbing co-ion (commonly referred to as the probe) in the BGE by an analyte ion, leading to a quantifiable decrease in absorbance. When indirect detection is used with simultaneous separations of anions and cations, two probes (one cationic and one anionic) are typically present in the electrolyte and are displaced by analytes of the same sign charge. The mechanisms of indirect detection stipulate that a number of principles should be followed to achieve optimal detection. The most important of these is that the presence of co-ions (ions of the same charge as the probe) in the electrolyte will lead to the formation of system peaks. Such peaks can diminish the detection of desired analytes through obscuring analyte peaks and decreasing the detection sensitivity. The presence of probes in the form of free acids and/or bases is a convenient means of avoiding such problems. Clearly, the BGE must be selected carefully in order to allow successful separation and detection of anions and cations in a single run. Some examples of the two probe, dual-end injection approach include (where the cationic probe is listed first and the anion probe second) imidazolium/nitrate [2], copper(II)-ethylenediamine/nitrate [2], copper(II)-ethylenediamine/chromate [1], 4-aminopyridinium/chromate [3], imidazolium/sulfosalicylate [19], benzylammonium/pyromellitate [20], 1,2-dimethylimidazolium/trimellitate [20], imidazolium/thiocyanate [12], and dimethyldiphenylphosphonium/trimesate [12].

Indirect detection of analytes can also be achieved through displacement of a counter-ion by the analyte, a process which is sometimes referred to as Kohlrausch detection. This indirect detection mode is based on the principle that the difference in mobility between the analyte ion and the co-ion will result in a change in the counter-ion concentration. This detection mode relies on a non-absorbing analyte being detected in a BGE consisting of a non-absorbing co-ion and an absorbing counter-ion, which acts as the probe. The detection sensitivity is dependent strongly upon the mobility difference between the analyte and the co-ion (with zero sensitivity occurring for matching mobilities) and this makes this detection mode less practical compared with the conventional co-ionic indirect detection. Foret et al. [11] detected cations (lithium and potassium) using an anionic probe (sorbate). Collet and Gareil [21] studied the indirect detection of alkali metal, quaternary ammonium and ammonium ions with an electrolyte containing a transparent co-ion and an absorbing counter-ionic probe. They demonstrated that the relative mobilities of the analyte (μ_i) and co-ion (μ_a) would determine whether the analyte is detected as a positive or negative peak. If the mobility of the analyte was greater than the mobility of the co-ion ($\mu_i > \mu_a$), then a positive peak was observed, and if the mobility of the analyte was less than the mobility of the co-ion ($\mu_i < \mu_a$) then a negative peak occurred. If the mobilities of the analyte ion and the

co-ion are identical then no peak will be observed. This approach was used to achieve simultaneous detection of anions and cations using 1,5-naphthalenedisulfonic acid to provide indirect photometric detection of anions and Kohlrausch detection of cations [22]. Single-end injection and a high magnitude cathodic EOF ($\sim +80 \times 10^{-9} \, \mathrm{m^2 \, V^{-1} \, s^{-1}}$) allowed co-EOF separation of potassium, ammonium, sodium, lithium and counter-EOF separation of ascorbate, sorbate, benzoate, lactate, acetate, hydrogen carbonate, phosphate, formate, fluoride in 8 min. However, highly mobility inorganic anions, such as chloride and nitrate, could not be separated as the cathodic EOF was not high enough to sweep them past the detection point. Macka et al. [23] used 4-aminopyridine as a co-ionic indirect detection probe for cations separated co-electroosmotically in a PTFE capillary modified with hexadecanesulfonate and the same probe was also acting as a counter-ionic indirect detection probe for anions separated counter-electroosmotically. However, the detection sensitivity for anions was significantly lower than for cations.

In this work we present the co-EOF separation of anions and counter-EOF separation of cations in a single run using a high magnitude anodic EOF generated through the use of DDAB as a dynamic EOF modifier added to the electrolyte. This allows anions and cations to be transported from the same injection point past the detection window without the use of external hydrodynamic pressure. An electrolyte containing only one probe is used, with the cationic probe imidazole being utilised for indirect detection of cations, and also as an absorbing counter-ion for the detection of anions. The advantages and limitations of this unique system are demonstrated and discussed.

2. Experimental

2.1. Instrumentation

The capillary electrophoresis instrument used during this work was an Agilent Technologies [3D]CE (Waldbron, Germany). This instrument was equipped with a deuterium lamp with a photodiode array detector. A voltage of −30 kV was applied during all separations, with temperature maintained at 25 °C. Injections were performed hydrodynamically with various pressures and injection times. Detection was performed at 210 nm. Fused silica capillaries (Polymicro Technologies, Phoenix, AZ, USA) of 75 μm inner diameter, 375 μm outer diameter of varying lengths were used.

2.2. Reagents

Calcium chloride, potassium chloride and sodium chloride were obtained from APS Chemicals (NSW, Australia). Lithium nitrate, ammonium chloride and magnesium nitrate were obtained from BDH (Vic., Australia). Sodium bromide was obtained from Sigma (NSW, Australia). Water treated with a Millipore (Bedford, MA, USA) Milli-Q water system was used to prepare standard solutions of the AR grade analytes. DDAB, imidazole, 18-crown-6 ether and 2-hydroxyisobutyric acid (HIBA) were all obtained from Aldrich (Milwaukee, WI, USA).

Imidazolium-HIBA electrolytes were prepared by titrating a given concentration of imidazole by HIBA to the desired pH, or for the final optimal electrolyte simply by mixing 6 mM imidazole and 12 mM HIBA, adding 18-crown-6 ether and DDAB, giving a final electrolyte pH of 4.0.

A test mixture of sodium chloride, calcium chloride, lithium nitrate, magnesium nitrate, potassium chloride, ammonium chloride and sodium bromide was prepared at a concentration of 50 μM each, corresponding to a total concentration of 50 μM calcium, lithium, magnesium, potassium, ammonium and bromide, 100 μM sodium, 150 μM nitrate, and 250 μM chloride.

2.3. Calculations

Separation efficiencies (plate numbers per capillary) were calculated using the peak width at half-height. Detection limits were calculated at a signal to noise ratio of two.

3. Results and discussion

3.1. Optimisation of BGE composition

In order to separate cations in a counter-EOF mode, it is necessary for a high magnitude anodic EOF to be generated. For example, with a 48 cm capillary, 40 cm to detector, an EOF of about $-80 \times 10^{-9} \, \mathrm{m^2 \, V^{-1} \, s^{-1}}$ would be required for cations with mobility up to $+65 \times 10^{-9} \, \mathrm{m^2 \, V^{-1} \, s^{-1}}$ to be detected in less than 8 min. Typical EOF values achieved with commonly used single-chain quaternary ammonium surfactants such as TTAB and CTAB are not sufficiently high to carry cations of medium to high mobility past the detection point. DDAB pre-coatings have been observed to provide an EOF of $-70 \times 10^{-9} \, \mathrm{m^2 \, V^{-1} \, s^{-1}}$ [14], and dynamic coatings of DDAB were therefore investigated as a means of obtaining higher anodic EOF values.

A BGE consisting of phosphoric acid titrated with sodium hydroxide to pH 2.5 used with a capillary preflushed with 0.1 mM DDAB produced an EOF of $-76 \times 10^{-9} \, \mathrm{m^2 \, V^{-1} \, s^{-1}}$, similar to the value reported previously [14]. The addition of DDAB to the BGE resulted in an increased EOF of $-88 \times 10^{-9} \, \mathrm{m^2 \, V^{-1} \, s^{-1}}$. A range of BGE compositions was studied (Table 1), all containing DDAB. Table 1 shows that the dynamic coating approach produced high anodic EOF values which significantly exceed those reported previously (e.g. [14,24],) and the magnitudes of these EOF values were suitable for the counter-EOF separation of high mobility inorganic cations. The values shown were the average of six replicate determinations showing high reproducibility (percentage relative standard deviation <3%).

Table 1
EOF values achieved with various background electrolytes (average of six determinations)

BGE composition	EOF ($\times 10^{-9}$ m^2 V^{-1} s^{-1})
Pre-flush with 0.1 mM DDAB for 3 min, electrolyte, 10 mM H$_3$PO$_4$, pH 2.5	−76
8.3 mM H$_3$PO$_4$, 0.17 mM DDAB, pH 2.5	−88
5 mM H$_3$PO$_4$, 0.17 mM DDAB, pH 2.5	−108
5 mM H$_3$PO$_4$, 0.5 mM DDAB, pH 2.5	−113
0.5 mM Ce(NO$_3$)$_3$, 4 mM HNO$_3$, 0.5 mM DDAB, pH 2.5	−86
1.8 mM 4-aminopyridine, 3.3 mM HNO$_3$, 0.5 mM DDAB, pH 2.5	−85
1.8 mM imidazole, 3.3 mM HNO$_3$, 0.5 mM DDAB, pH 2.5	−85
1.25 mM imidazole, 2.5 mM H$_3$PO$_4$, 0.5 mM DDAB, pH 2.5	−113
1.25 mM imidazole, 2.0 mM HIO$_3$, 0.5 mM DDAB, pH 2.5	−121
6.0 mM imidazole, 12.0 mM HIBA, 0.1 mM DDAB, pH 4.0	−105

For other conditions see Section 2.

Simultaneous indirect detection of anions and cations optimally requires a dual-probe BGE containing both an anionic probe for co-ionic indirect detection of anions and a cationic probe providing co-ionic indirect detection of cations. In the present case, this proved impossible due to precipitation reactions occurring when an anionic probe, such as chromate, was added. Combinations of cerium(III), aminopyridinium or imidazolium as cationic probes with nitrate as the anionic probe did not result in precipitates but produced EOF values which were too low for the desired application (Table 1). The combination of imidazolium with iodate gave a suitable EOF but detection sensitivity for anions was poor. For these reasons, further optimisation of the BGE composition was focused on a single cationic probe providing co-ionic indirect detection of cations and counter-ionic (Kohlrausch) detection of anions.

Imidazolium is a widely used probe for indirect detection of cations [25–30] and could be added to the electrolyte without a significant reduction in EOF. The commonly used combination of imidazolium as a probe for indirect detection of cations with the addition of HIBA as a weak complexing agent to provide selectivity was chosen as the template for all further work. The absorptivity of imidazolium at 210 nm is about 50 times that of HIBA, therefore HIBA can be regarded as a non-absorbing co-ion. It has been demonstrated previously that the concentration of the probe in the BGE should be maximised in order to minimise the electromigration dispersion and consequently to improve peak shapes, separation efficiencies and detection sensitivity [31,32]. Importantly, increasing the imidazolium concentration to 6 mM did not cause a large decrease in EOF. Buffering of the electrolyte at pH 4.0 was provided by the presence of 12 mM HIBA as a complexing agent. Increases in DDAB concentration in the electrolyte above 0.1 mM DDAB did not further increase the magnitude of the anodic EOF.

3.2. Interaction of analyte anions with DDAB

A test mixture consisting of chloride, bromide, sulfate, sodium, ammonium, potassium, magnesium, calcium and lithium was used to investigate separation selectivity and efficiency of the BGE. The high magnitude of the anodic EOF results in the availability of only a small time window for separation and detection of anions prior to the appearance of the EOF peak at ∼1.1 min. Injection of anionic analytes revealed the presence of interactions between the anions and the positively charged capillary surface, as evidenced by broadened peaks and electrophoretic mobilities reduced from their typical values. This interaction was particularly strong for sulfate and this species could not be separated from the EOF peak by using strategies such as a longer separation capillary, reduced concentrations of DDAB in the BGE, variation of the pH of the BGE, or addition of zwitterionic surfactants. The strong interaction of sulfate with DDAB both in the electrolyte and on the capillary wall is consistent with previous studies [14]. Sulfate was therefore omitted from samples used in further investigations of the analytical potential of this approach.

3.3. Simultaneous separation of anions and cations

A test mixture of sodium chloride, calcium chloride, lithium nitrate, magnesium nitrate, potassium chloride, ammonium chloride and sodium bromide was chosen to test the performance of an optimised electrolyte. 18-Crown-6 ether was added to the BGE in order to provide separation selectivity between potassium and ammonium [33–36]. A BGE consisting of 6 mM imidazole, 12 mM HIBA, 0.1 mM DDAB, 2 mM 18-crown-6 at pH 4.0 was found to provide a suitably high EOF, adequate separation selectivity, and suitable indirect detection of cations and counter-ionic detection of anions (via imidazolium). A 48 cm capillary was used as extending the length to 80 cm offered no appreciable benefits regarding separation selectivity or peak shapes for anions as the critical group of analytes. Using the 48 cm capillary also provided rapid run times of approximately 3.5 min, compared to around 8 min for the 80 cm capillary. It should be noted that the chosen BGE offered significant advantages over the earlier used single cationic probe 4-aminopyridine for simultaneous CE separation and detection of cations and anions [23] as HIBA in the newly developed BGE provided not only buffering but also improved separation selectivity for a number of cations.

A separation of the test analytes (50–250 μM each) with the optimised BGE is shown in Fig. 1. Effective electrophoretic mobilities, separation efficiencies and detection limits are provided in Table 2. The peak directions of the anions are in accordance with the guidelines provided by Collet and Gareil [21]. Here, the co-ion for anionic analytes was HIBA and since chloride, bromide and nitrate have higher electrophoretic mobilities than HIBA (-33.5×10^{-9} m^2 V^{-1} s^{-1} at pH 4) they were detected as positive peaks ($\mu_i > \mu_a$). Importantly, even though the effective mo-
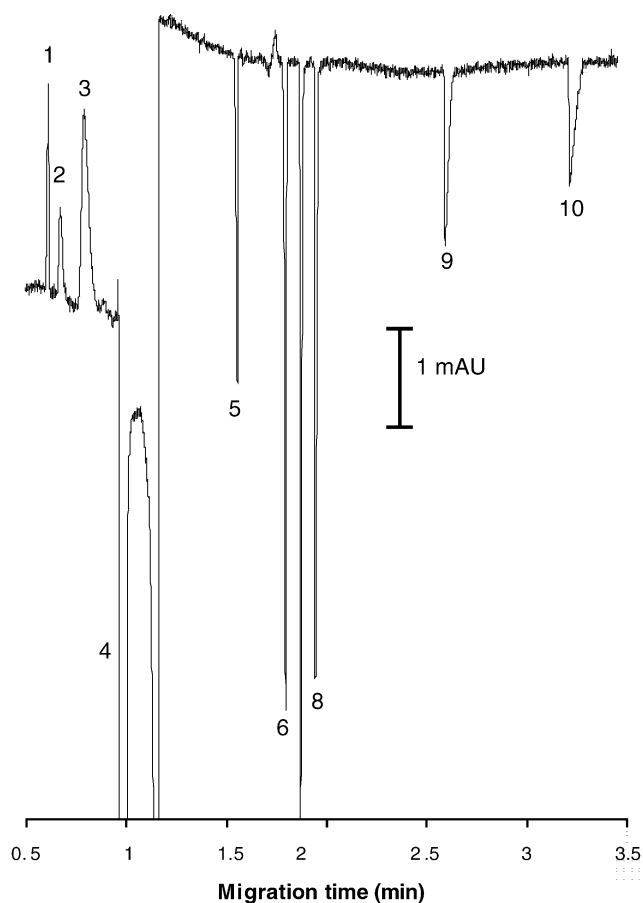
Fig. 1. Simultaneous separation of three inorganic anions and six inorganic cations in a single run. Key: 1: chloride, 2: bromide, 3: nitrate, 4: EOF, 5: lithium, 6: magnesium, 7: sodium, 8: calcium, 9: potassium, 10: ammonium. Conditions — sample: 50 μM each of sodium chloride, calcium chloride, lithium nitrate, magnesium nitrate, potassium chloride, ammonium chloride and sodium bromide; electrolyte: 6 mM imidazole, 12 mM HIBA, 0.1 mM DDAB, 2 mM 18-crown-6, pH 4.0; capillary: fused silica, 75 μm i.d., length 0.485 m, 0.40 m to detector; separation voltage: −30 kV; pressure injection: 3 s at 30 mbar (injection volume 16 nL, 0.75% capillary volume); temperature 25 °C; detection: indirect photometric at 210 nm.

Table 2
Limits of detection (LODs), electrophoretic mobilities and separation efficiencies obtained for simultaneous separation of anions and cations

Analyte	Electrophoretic mobility ($\times 10^{-9}\,m^2\,V^{-1}\,s^{-1}$)	Separation efficiency (theoretical plates)	LOD (μM)
Chloride	−67.2	26300	11.43
Bromide	−51.1	7600	6.66
Nitrate	−27.1	2500	9.05
Lithium	+39.6	182400	1.68
Magnesium	+48.8	205000	0.92
Sodium	+51.3	208500	1.54
Calcium	+53.4	185800	0.94
Potassium	+67.2	101800	2.99
Ammonium	+75.1	44400	4.65

Conditions — sample: 50 μM each of sodium chloride, calcium chloride, lithium nitrate, magnesium nitrate, potassium chloride, ammonium chloride and sodium bromide; electrolyte: 6 mM imidazole, 12 mM HIBA, 2 mM 18-crown-6 ether, 0.1 mM DDAB, pH 4.0; voltage: −30 kV; injection: 3 s at 30 mbar (injection volume 16 nL, 0.75% capillary volume); temperature: 25 °C; detection: indirect photometry at 210 nm.

bilities of the analyte anions were reduced due to interactions with DDAB, it is the absolute mobility rather than effective mobility which determines the peak direction [21]. It can be seen that the counter-EOF separation of cations provided good selectivity and well-shaped peaks. Detection limits were comparable to typical values using imidazolium as a probe. The interactions of the analytes with DDAB (both in the BGE and at the capillary wall) resulted in broadened peaks and separation efficiencies which were lowered by about an order of magnitude in comparison to typical CE values for these analytes. The detection limits for anions were relatively poor, but the proposed method still offered significant analytical potential for simultaneous separations of cations and a limited range of anions. Alternative detection methods, such as contactless conductivity [5–8], may be applicable and could lead to improved detection limits.

4. Conclusions

An electrolyte containing DDAB as an EOF modifier can achieve surprisingly high magnitudes of anodic EOF of up to $-121 \times 10^{-9}\,m^2\,V^{-1}\,s^{-1}$, which represent so far the highest magnitudes of anodic EOF reported in CE. This made possible to our best knowledge the first counter-EOF separation of the high mobility inorganic cations and also the first simultaneous separation of high mobility inorganic cations and anions in a single run.

Addition of a single cationic probe can provide co-ionic indirect photometric detection of cations and counter-ionic indirect detection of anions. Although the detection of anions via counter-ionic indirect detection offers only adequate performance in terms of sensitivity and peak shapes, this approach allows the separation and detection of three anions and six cations in one run in about 3 min. The disadvantage of the use of DDAB as an EOF modifier means that there is no simple means of adding an anionic probe for the indirect detection of anions. Addition of anionic probes leads to a decrease in EOF below a cut-off point for detection of cations. Also, pH restrictions (in order to protonate cationic probes) meant that solubility problems are encountered for some frequently used anionic probes such as chromate.

The key advantage of this approach is it allows high mobility cations to be separated in a counter-EOF mode within a reasonable time span. While UV-absorbing anions for counter-ionic detection of cations have been used previously, we believe that this approach using a cationic probe for the simultaneous separation of anions and cations with high magnitude anodic EOF is a first. It would be expected that the use of a cationic probe more strongly absorbing than imidazole, such as a dye, would lead to increased detection sensitivity. However, the use of DDAB may present a problem due to potential formation of insoluble complexes between the dye and DDAB. The magnitude of the reversed EOF may also be reduced, rendering it unsuitable for counter-EOF separation of cations. If an alternative means of generating a similarly

high magnitude reversed EOF could be found, however without the interaction of the anionic analytes with the cationic wall could be found, this may improve the analytical potential of this approach.

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