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Parameters influencing separation and detection of anions by capillary electrophoresis

Michael P. Harrold*, Mary Jo Wojtusik, John Riviello and Patricia Henson

Dionex Corporation, 1228 Titan Way, Sunnyvale, CA 94086 (USA)

ABSTRACT

Electrolyte composition is critical in optimizing separation and detection of ions by capillary electrophoresis. The parameters which must be considered when designing an electrolyte system for capillary electrophoresis include electrophoretic mobility of electrolyte constituents and analytes, detection mode, and compatibility of electrolyte constituents with one another. An electrolyte system based on pyromellitic acid is well suited for use with indirect photometric detection, and provides excellent separations of anions. The ability to modify the electrophoretic mobility of pyromellitic acid as a function of pH provides flexibility in matching electrophoretic mobilities of analytes. Additionally, the use of alkyl amines as electroosmotic flow modifiers allows the rapid separation of anions by reversing the direction of electroosmotic flow in a fused-silica capillary. The optimization of a capillary electrophoresis electrolyte for anion analysis is also discussed in terms of pH, ionic strength and applied voltage. The effect of organic solvent on separation selectivity is also discussed.

INTRODUCTION

The separation of ionic species by free zone electrophoresis in fused-silica capillaries has attracted considerable attention in recent years. Early work by Mikkers *et al.* [1,2] demonstrated the very high efficiency attainable for separations in inert capillaries and described the fundamental properties of capillary electrophoresis. Jorgenson and Lukacs demonstrated the utility of small diameter glass capillaries for the electrophoretic separation of fluorescent tagged alkyl amines [3] and dansyl amino acids [4]. These authors also described the characteristics of electroosmotic flow in narrow-diameter glass capillaries and derived expressions for resolution and efficiency as a function of many experimental parameters [3,4]. Tsuda *et al.* [5] characterized electroosmotic flow in glass, fused-silica, and polymeric capillaries and demonstrated high-efficiency separations of metal ions, pyridinium ions, and aromatic sulfonates using direct on-capillary UV detection.

The lack of a universal detection scheme for non-chromophoric analytes hampered early development of separations for inorganic anions. Some work has been done adapting on-capillary conductivity detection to capillary electrophoresis [6], but this detection mode has not yet found widespread commercial use. Indirect methods for detection of non-chromophoric ions are attractive because these methods are easily adapted for use with existing instrumentation and they are relatively universal in terms of response. The use of indirect fluorescence detection for HPLC separations has been described [7,8] and this same technique has been shown to be feasible in capillary electrophoresis for the detection of low-molecular-mass anions and nucleotides [9]. Indirect UV methods were described in chromatographic systems [10], as a detection scheme for ion chromatography [11], and their applicability to the capillary electrophoresis format has been documented in several papers [12,13]. Several reports have appeared recently describing the application of capillary electrophoresis for the analysis of organic and inorganic anions with indirect UV detection [14–17]. Jandik and Jones [18] discussed the

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* Corresponding author.

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optimization of indirect UV detection schemes and investigated selectivity for inorganic anion analysis using a chromate-based electrolyte system [19]. Applications of capillary electrophoresis with indirect detection for cation and metal analysis have also received attention [20]. Foret *et al.* [21] resolved a variety of alkali, alkaline-earth and lanthanide series metals using capillary electrophoresis with indirect UV detection [21]. The effect of solvent on separations has been explored using isotachophoresis [22–25] and capillary electrophoresis [26], although no systematic study has been performed for solvent effects on anion separations.

The goal of this work was to investigate the parameters that affect the separation of anions using capillary electrophoresis. Analyses were performed using a polycarboxylic acid as the primary electrolyte component. Modification of selectivity and peak shape as a function of pH was investigated. Selectivity changes as a result of organic solvent addition to electrolytes were also explored. As a result of this work, an electrolyte system suitable for the analysis of common inorganic anions was developed. This same electrolyte system, run at different pH values, was also optimized for the analysis of less mobile anions including haloacetates and alkyl sulfonates. Two difficult coelution problems were shown to be resolved by adding organic solvent to capillary electrophoresis electrolytes.

EXPERIMENTAL

Experiment

All electropherograms were generated using a Dionex CES-1 automated capillary electrophoresis system (Dionex, Sunnyvale, CA, USA). Fused-silica capillaries (Polymicro Technologies, Phoenix, AZ, USA) of 50 μm I.D., 375 μm O.D. and various lengths were used. The detection window was located 5 cm from the end of the capillary. Data were collected at five points/s using a Dionex AI-450 data acquisition system. Unless otherwise noted, all injections were hydrostatic and performed by raising the sample vial 100 mm above the level of the destination vial for 30 s. Molar absorptivities were determined using a Hitachi U-2000 UV–Vis spectrophotometer (Hitachi, Danbury, CT, USA) with 1 cm path length quartz cells.

Chemicals

Pyromellitic acid (PMA), 96% was obtained from Sigma (St. Louis, MO, USA). Hexamethonium bromide, monohydrate, 97% was obtained from Aldrich (Milwaukee, WI, USA) and triethanolamine, >99%, from Fluka (Ronkonkoma, NY, USA). Sodium hydroxide, 50% aqueous solution and methanol (Optima grade) were obtained from Fisher Scientific (Pittsburgh, PA, USA). Anion standards were prepared from sodium salts obtained from Fisher Scientific. Deionized water (18 MΩ/cm resistance) was used to prepare all reagents and standards. OnGuard A cartridges containing high-capacity anion-exchange resin for conversion of hexamethonium bromide to hexamethonium hydroxide were from Dionex.

Procedures

The standard electrolyte was 2.25 m*M* pyromellitic acid, 6.5 m*M* sodium hydroxide, 0.75 m*M* hexamethonium hydroxide, 1.6 m*M* triethanolamine at pH 7.7. The electrolyte was made from concentrated stock solutions.

A concentrate of 25 m*M* hexamethonium bromide was prepared and converted to the hydroxide form of the amine during the preparation of the working electrolyte. The conversion of hexamethonium from the bromide form to the hydroxide form was accomplished by first washing an OnGuard A sample preparation cartridge with 10 ml of 1.0 *M* sodium hydroxide followed by 10 ml deionized water.

The pH 3.5 electrolyte was prepared by dissolving 0.156 gram pyromellitic acid in 100 ml deionized water. Hexamethonium hydroxide was added as described above to give a final concentration of 0.75 m*M*. The working electrolyte was titrated to pH 3.5 with dropwise addition of 100 m*M* sodium hydroxide.

RESULTS AND DISCUSSION

Carrier ion

The anion present in the greatest quantity in the electrolyte is termed the carrier ion, and its properties effect the separation of anions by capillary electrophoresis. Perhaps the most important property of the carrier ion is the requirement that the electrophoretic mobility of the primary electrolyte component is closely matched to those of the analyte(s) of interest. This condition, the matching of electrophoretic mobility of carrier and analytes, governs the characteristic peak shapes observed in capillary electrophoresis. It has been demonstrated that the asymmetry of peak shapes in capillary electrophoresis is predictable and determined by the boundary anomalies that arise between the sample zone and the bulk carrier electrolyte when the sample contains ions of different ionic mobility than that of the carrier [1]. An electrolyte optimal for common inorganic anions must have a carrier ion with electrophoretic mobility closely matched to that of the anions of interest if a high-resolution separation is to occur.

A second important consideration when choosing a carrier ion for anion analysis is the mode of detection. Present commercial capillary electrophoresis instrumentation is restricted to the use of on-capillary UV–Vis or fluorescence detection. This presents a difficulty in the detection of inorganic anions many of which are UV transparent or absorb only at low UV wavelengths such as nitrite, nitrate and bromide. Using indirect UV detection, the decrease in absorbance observed when an analyte ion displaces a chromophoric ion in the electrolyte is measured. The technique results in a negative peak when a sample band passes through the detector window. Because indirect UV detection relies on displacement of a chromophore, the carrier (or visualizing) ion, should have a large molar absorptivity to maximize the decrease in signal resulting from its displacement. The correlation between peak response and carrier ion molar absorptivity in capillary electrophoresis has been documented by other workers [18]. Additionally, for use in indirect detection, the wavelength at which the chromophore absorbs should be well away from any wavelength at which analyte ions may absorb . This is to prevent an analyte's direct UV absorbance from counteracting the indirect absorbance mechanism.

These conditions, the match of electrophoretic mobility of carrier and analyte ions, and the restrictions arising from the use of indirect UV detection, limit the choice of potential electrolyte components for anion analysis by capillary electrophoresis. In our work, we have chosen pyromellitic acid as the carrier anion for the separation of common inorganic anions. Pyromellitic acid is well suited as an electrolyte because its electrophoretic mobility when fully ionized is closely matched to many common inorganic anions. In addition, pyromellitic acid has the required spectral characteristics, including high molar absorptivity ($7.8 \cdot 10^3$ l mol^{-1} cm^{-1}), and strong UV absorbance at 250 nm, well away from the low UV wavelengths at which several common inorganic anions absorb. The molar absorptivity for pyromellitic acid was measured at pH 7.5, almost 2 pH units above the highest pK_a for pyromellitic acid [27].

The noise in an indirect photometric system is proportional to the magnitude of the background absorbance [11]. This implies that using a very low concentration of carrier ion maximizes the signal-to-noise ratio in such a system. There are however, detection and separation considerations that limit the degree to which the concentration of carrier ion may be decreased. A low concentration of carrier ion results in a decrease of linear dynamic range. Additionally, as the concentration of the carrier ion is decreased, there is a subsequent decrease in loading capacity. A decreased loading capacity results from a decrease in efficiency as a function of sample ion to carrier ion concentration ratio. The optimum concentration of carrier ion is chosen such that desirable detection limits and adequate linear dynamic range are achieved.

Pyromellitic acid is a polycarboxylic acid whose net charge and resulting electrophoretic mobility is pH dependent. The highest pK_a for an acidic proton on pyromellitic acid is 5.6 and thus at pH 7.7, the pyromellitic acid is fully ionized [27]. Fig. 1 demonstrates the high efficiency separation of several inorganic anions using an optimized pyromellitic acid electrolyte at pH 7.7. At lower pH, the pyromellitic acid becomes increasingly protonated and a decrease in its electrophoretic mobility is observed. This pH dependence implies that by using a lower pH electrolyte, the peak symmetry and efficiency can be optimized for lower electrophoretic mobility anions that would display severe peak asymmetry (tailing) with pyromellitic acid electrolyte at higher pH.

Electroosmotic flow modifier

An important feature of capillary electrophoresis separations of anions is the potential for high peak capacity and short analysis time. Under normal

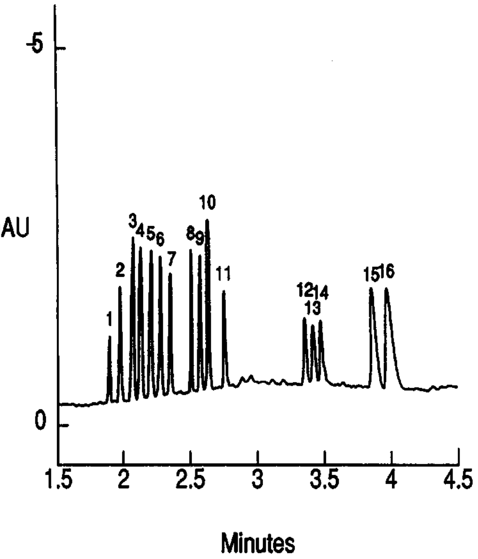

Fig. 1. Anion separation at pH 7.7. Capillary (50 μm), 50 cm length. Electrolyte composed of 2.5 m*M* pyromellitic acid, 6.5 m*M* NaOH, 0.75 m*M* hexamethonium hydroxide, 1.6 m*M* triethanolamine. 30 000 V applied for separation. Indirect UV detection at 250 nm, output polarity reversed to produce positive peaks. Gravity injection, 100 mm for 20 s. Peaks: 1 = dithionate (3 mg/l); 2 = thiosulfate (5 mg/l); 2 = bromide (8 mg/l); 4 = chloride (3 mg/l); 5 = sulfate (3 mg/l); 6 = nitrite (3 mg/l); 7 = nitrate (3 mg/l); 8 = molybdate (5 mg/l); 9 = azide (4 mg/l); 10 = thiocyanate (3 mg/l); 11 = chlorate (3 mg/l); 12 = fluoride (0.5 mg/l); 13 = bromate (3 mg/l); 14 = formate (2 mg/l); 15 = phosphate (3 mg/l); 16 = phthalate (10 mg/l).

electrophoretic conditions using uncoated silica capillaries, the bulk electroosmotic flow is from anode to cathode. This flow arises from the formation of a double layer of electrolyte cations along the surface of the ionized silanol groups of the capillary wall. When an electric field is applied, there is a migration of mobile cations toward the cathode and a resulting bulk solution flow in that direction. In most commercially available capillary electrophoresis instruments, the detector window is fixed near the destination end of the capillary. The natural direction of electroosmotic flow is toward the destination end of the capillary when the destination is cathodic. If the polarity of the applied voltage is reversed, the end of the capillary near the detector is anodic and the electroosmotic flow is away from the detector.

While a normal polarity (detector cathodic) configuration is useful for the determination of cations and neutral compounds, the direction of electroosmotic flow is opposite to the direction of anion migration, and thus detrimental in terms of analysis speed. If anions of very high mobility are being separated, their high intrinsic mobility may cause them to migrate out of the opposite end of the capillary (the anodic end) and not be detected. In order to minimize the time of analysis, it is desirable to produce an electroosmotic flow in the same direction as the migration of the analytes of interest. For the analysis of high mobility anions, the direction of electroosmotic flow should be from cathode to anode, reverse of the direction of a normal bare silica capillary.

Alkyl ammonium salts including cetyltrimethylammonium bromide (CTAB) [28], tetradecyltrimethylammonium bromide (TTAB) [6], and amines such as diethylenetriamine (DETA) [29] have been documented for several applications requiring suppression or reversal of electroosmotic flow. We have investigated a number of alkyl amines for electroosmotic flow reversal. We have found many different mono and poly amines at low to moderate concentrations are useful in controlling electroosmotic flow. Most of these electroosmotic flow modifiers have been found to be compatible with analytes and other electrolyte components. One disadvantage of hydrophobic alkyl ammonium salts, such as CTAB and TTAB is their limited solubility and tendency to form insoluble pairs with some electrolyte components [18,30]. For this reason, we chose 1,6-bis-(trimethylammonium)hexane, a C_6 alkyl diquaternary ammonium salt that does not interact with other electrolyte components. The common name for this compound is hexamethonium bromide. The bromide salt is converted to the hydroxide form to remove bromide ion that can compromise detection. The role of counterions is discussed in the next section. The hexamethonium ion is very effective in reversing electroosmotic flow, is highly soluble, and does not interact with other electrolyte components. In addition, because hexamethonium is a quaternary ammonium, its ability to modify the wall of the capillary is not changed as a function of pH, a phenomenon that could otherwise lead to unstable electroosmotic flow.

Counterion considerations

A phenomenon was observed which places restrictions on the types of counterions of electrolyte components. It is desirable to have only one carrier ion in the electrolyte, in this case, the pyromellitate anion. The presence of other anions can lead to baseline aberrations and a decrease in sensitivity. For example, the presence of bromide in the electrolyte causes a dip in the baseline at the migration time corresponding to bromide (a "dip" when using indirect UV is actually an increase in absorbance). This phenomenon has also been observed with sulfate and chloride when these anions are added to the electrolyte. This effect adversely affects accurate determination of an analyte anion that is also present in the electrolyte used for analysis. Additionally, the presence of non-chromophoric anions (at 250 nm) in the electrolyte results in a decrease in sensitivity when using indirect UV detection [11]. Many high purity, commercially available amines which are useful as electroosmotic flow modifiers are available only as the chloride or bromide salts. The addition of a flow modifier with either of these counterions causes disruptions in the baseline and affects quantitation. Chloride and bromide can still be accurately determined by exchanging the chloride or bromide counterion for hydroxide counterion with a sample pretreatment cartridge containing high capacity anion-exchange resin in the hydroxide form. Unlike bromide and chloride counterions, the addition of small amounts of hydroxide to the electrolyte solution has no adverse effect on separation or detection of inorganic anions.

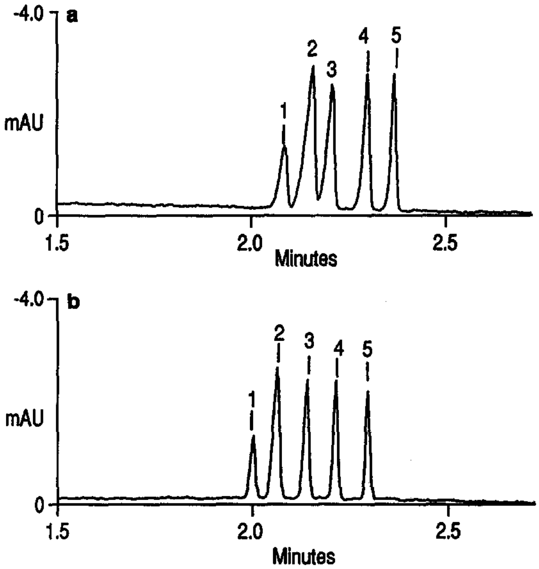

Fig. 2. Effect of ionic strength on peak symmetry. Electrolyte ionic strength: (a) 7.25 mM, (b) 11.10 mM. Capillary (50 μm), 50 cm length. 30 000 V applied for separaton. Indirect UV detection at 250 nm, output polarity reversed to produce positive peaks. Gravity injection, 100 mm for 30 s. (a) 1.5 mM pyromellitic acid, 3.5 mM NaOH, 0.75 mM hexamethonium hydroxide, 1.6 mM triethanolamine, pH 7.8. (b) 2.25 mM pyromellitic acid, 6.5 mM NaOH, 0.75 mM hexamethonium hydroxide, 1.6 mM triethanolamine, pH 7.8. Peaks (all 5.0 mg/l): 1 = bromide; 2 = chloride; 3 = sulfate; 4 = nitrite; 5 = nitrate.

Ionic strength

The ionic strength of the electrolyte is important for two distinct reasons. First, the current observed for a given applied voltage is proportional to the ionic strength of the electrolyte. Generally, effects due to heating increase at higher current resulting from the inability to dissipate heat. These effects, manifested as noise and baseline aberrations, are exacerbated by the use of a highly absorbing carrier ion for indirect UV detection. For this reason, the ionic strength must be minimized to prevent noise resulting from high current. Secondly, efficiency is proportional to electrolyte ionic strength. As ionic strength decreases, lower peak efficiency is observed. This effect is shown in Fig. 2. In both electropherograms, the applied voltage and pH of the electrolyte are identical, only the ionic strength was different. Clearly, there is an decrease in peak efficiency as the ionic strength is lowered. This effect can be explained in terms of the ratio of sample concentration to carrier electrolyte concentration.

In a system where other sources of dispersion have been minimized, the peak width in capillary electrophoresis has been shown to be proportional to the concentration ratio of sample ions to carrier ions [1]. This implies that in order to obtain a high-resolution separation of closely migrating peaks, the concentration of the injected sample must be kept low relative to the electrolyte. To attain higher efficiency with samples of high concentration, the concentration of the electrolyte must be increased in order to maintain a large ratio of electrolyte concentration to sample band concentration. The other possibility, the dilution of sample to minimize electromigrative dispersion, is generally not desirable due to the negative impact on peak response. The optimum ionic strength of an anion analysis electrolyte must be a balance which will produce an acceptably low current to minimize noise while maintaining good peak efficiency.

Applied voltage

The electroosmotic flow-rate as well as the velocity of a migrating ion is proportional to the applied voltage used for separation. Therefore, the analysis time can be shortened by increasing the applied voltage. For some separations described in this paper, short analysis times were attained by running the separation at 30 000 V. The relatively low ionic strength of the carrier electrolyte permits the use of such high voltages by producing only a modest current (8–12 μA) at 30 000 V.

pH

The pH of the electrolyte solution used for separation can have a large effect on the separation of anions using capillary electrophoresis. The pH of the electrolyte controls the mobility of anions of weak acids. For the pyromellitic acid based system, the mobility of the pyromellitic acid can be altered by decreasing the pH of the electrolyte. The analytes also display changes in electrophoretic mobility if the pH is changed in the vicinity of their p*K*. While most low-molecular-mass inorganic anions are unaffected by pH changes in the range 3 to 9, some anions such as phosphate, carbonate and acetate can display large changes in apparent mobility due to small changes in pH. The electrolyte system described here has been optimized at pH 7.7 which provides maximum resolution of phosphate from fluoride and carbonate. The pyromellitic acid–hexamethonium electrolyte system has been buffered at pH 7.7 with triethanolamine. Triethanolamine, with a p*K* of 7.7 is well suited for this electrolyte system not only because it has the proper p*K*, but also because it is available in the free base form and thus does not add a counter anion to the electrolyte. The use of a buffered system provides reproducible electrolyte pH as well as stable pH though the course of the run.

The pH of the electrolyte controls the ionization and thus the electrophoretic mobility of the carrier ion. The separation of lower mobility ions can be optimized by decreasing the pH. As the pH of a pyromellitic acid electrolyte is lowered, the pyromellitate ion becomes progressively protonated, decreasing its charge and its electrophoretic mobility. The predictable and controllable degree of ionization of pyromellitate ion is used to control the electrophoretic mobility of the carrier ion. Fig. 3 shows the optimization of the pyromellitic acid electrolyte system for the separation of haloacetates. Lowering the pH of the electrolyte lowers the electrophoretic mobility of pyromellitate so that it more closely matches the mobility of the analyte haloacetates.

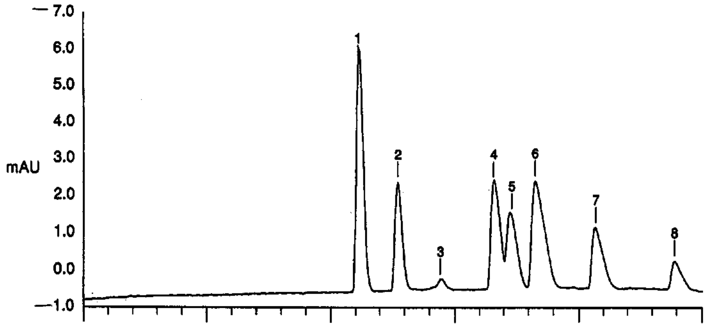

Fig. 3. Separation of haloacetic acids at pH 3.5. Capillary (50 μm), 50 cm length. Electrolyte composed of 2.5 m*M* pyromellitic acid, 0.75 m*M* hexamethonium hydroxide, adjusted to pH 3.5 with NaOH. 20 000 V applied for separation. Indirect UV detection at 250 nm, output polarity reversed to produce positive peaks. Gravity injection, 100 mm for 30 s. Peaks (all 5.0 mg/l): 1 = difluoroacetate; 2 = trifluoroacetate; 3 = unknown; = monochloroacetate; 5 = dichloroacetate; 6 = monobromoacetate; 7 = trichloroacetate; 8 = tribromoacetate.

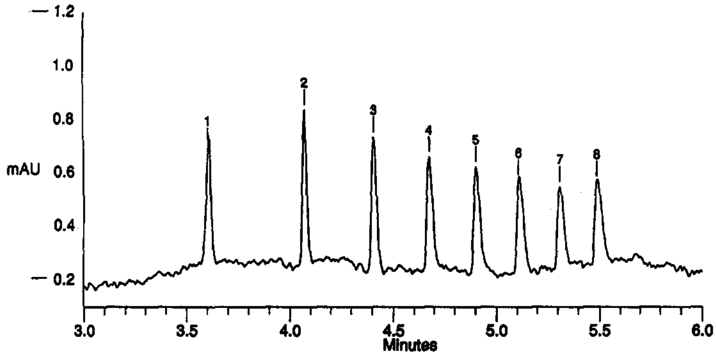

Fig. 4. Separation of alkylsulfonic acids at pH 3.5. Capillary (50 μm), 50 cm length. Electrolyte composed of 2.5 m*M* pyromellitic acid, 0.75 m*M* hexamethonium hydroxide, adjusted to pH 3.5 with NaOH. 20 000 V applied for separation. Indirect UV detection at 250 nm, output polarity reversed to produce positive peaks. Gravitiy injection, 100 mm for 20 s. Peaks (all 2.0 mg/l): 1 = methanesulfonate; 2 = ethanesulfonate; 3 = 1-propanesulfonate; 4 = 1-butanesulfonate; 5 = 1-pentanesulfonate; 6 = 1-hexanesulfonate; 7 = 1-heptanesulfonate; 8 = 1-octanesulfonate.

This results in good peak symmetry for these low mobility anions which have very poor peak shape in a higher pH pyromellitic acid electrolyte.

A low pH method also works well for alkylsulfonates which are shown separated in Fig. 4. In both cases, the charge on the pyromellitate carrier ion is estimated to be approximately −2. With an approximate charge of −2, the pyromellitate ion has a mobility well matched to that of the alkylsulfonates and haloacetates. The match of electrophoretic mobility of carrier and analyte anion results in symmetrical peak shape.

Solvation effects

The effect of solvent addition to a capillary electrophoresis electrolyte has been briefly investigated. Fig. 5 shows the effect of methanol addition to a pyromellitic acid electrolyte system. The conditions for the first electropherogram (standard conditions) are identical to those used for the electropherogram in Fig. 1. Under standard conditions, the migration times of iodide and chloride are identical. Perchlorate and azide also have identical electrophoretic mobility and thus co-migrate under standard conditions. The co-migration of these analytes is consistent with their limiting ionic conductances which are very similar [27]. The second electropherogram in Fig. 5 shows the separation of iodide, chloride, perchlorate, and azide in an electrolyte similar to that used for the first electropherogram, but with the inclusion of 15% (v/v) methanol.

This change in selectivity can be explained on the basis of the relative hydration enthalpies of the anions. The modification of selectivity with the ad-

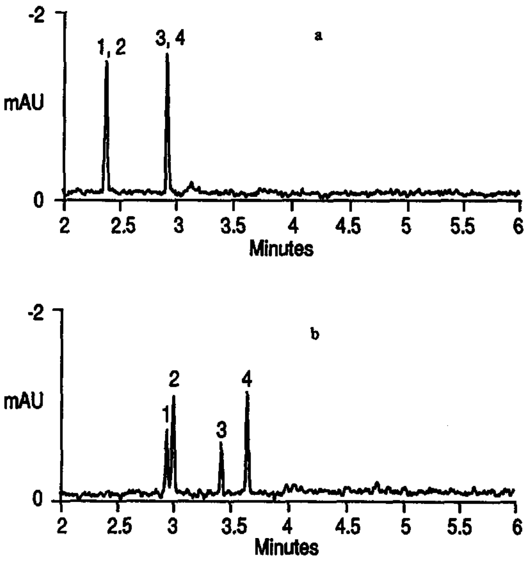

Fig. 5. Effect of solvent on separation of anions using pyromellitic acid electrolyte. Capillary (50 μm), 50 cm length. (a) Electrolyte composed of 2.5 m*M* pyromellitic acid, 6.5 m*M* NaOH, 0.75 m*M* hexamethonium hydroxide, 1.6 m*M* triethanolamine. (b) Electrolyte was same as electrolyte a, but with 15% methanol (v/v), pH = 7.7. 30 000 V applied for separation. Indirect UV detection at 250 nm, output polarity reversed to produce positive peaks. Gravity injection, 100 mm for 20 s. Peaks: 1 = iodide (5.0 mg/l); 2 = chloride (2.5 mg/l); 3 = perchlorate (2.5 mg/l); 4 = azide (2.5 mg/l).

TABLE I

ENTHALPIES OF HYDRATION FOR SELECTED ANIONS

From ref. 32.

Anion	$\Delta H_{\text{hydration}}$(kcal/mol)
Iodide	−331
Chloride	−348
Azide	−332
Perchlorate	−317

dition of organic solvent in ion chromatography of metal cations has been demonstrated by Strelow *et al.* [31]. They argued that the observed changes in selectivity are a result of the change in relative hydration of some metal ions in the presence of ethanol. We propose that the mechanism of separation of iodide and chloride as well as azide and perchlorate is similar and due to selective destruction of the hydration cloud which surrounds each ion in aqueous solution. Table 1 lists the standard enthalpies of hydration for several anions [32]. Because the enthalpy of hydration for iodide is less negative than that of chloride, it follows that the waters of hydration are more easily displaced from iodide than from chloride. When these two anions are placed in an electrolyte containing 15% methanol, it is expected that the solvation of iodide will be more pronounced than for chloride. This results in a lesser degree of hydration for iodide relative to chloride and thus an increase in effective mobility due to a decrease in effective mass. The separation shown in Fig. 5 is consistent with this hypothesis in that iodide migrated faster than chloride and the two peaks were fully resolved. The other co-migrating pair, perchlorate and azide, were fully resolved when 15% methanol was added to the electrolyte. The change in selectivity was even more pronounced with azide and perchlorate. Although the mechanism is thought to be the same, there may be an additional mechanism functioning in the case of perchlorate and azide. Perchloric acid is a strong acid whereas azide is a weak acid with pK = 4.7 [27]. The use of organic solvent in isotachophoresis has shown that an increase in pK of weak acids occurs in mixed aqueous–organic media and can be used to affect electrophoretic mobility [23]. The change in pK for azide in a 15% methanol solution is not known, however a pK increase of 2–3 units would be sufficient to slow the migration of azide relative to perchlorate. It is possible that resolution of perchlorate and azide in 15% methanol results from a combination of both hydration changes as well as pK changes for the two molecules.

It must also be noted that there is a decrease in electroosmotic flow when methanol is added to the electrolyte. The addition of organic solvent increases the viscosity of the electrolyte and changes the pK of the silanol groups on the wall of the capillary. The result is a decrease in electroosmotic flow when methanol is added to the electrolyte.

The beneficial effect of organic solvent on anion migration time has been demonstrated by Fig. 5. While a tentative mechanism has been proposed, the effect of methanol as well as other solvents must be explored in greater detail. The effect of solvation of anions has the potential to solve many co-migration problems that arise in capillary electrophoresis of ions.

CONCLUSIONS

The separation and detection of ions using capillary electrophoresis can be optimized by considering a number of parameters. To separate anions by capillary electrophoresis with high-efficiency peaks and therefore high resolution, a carrier electrolyte with an electrophoretic mobility matched to that of the analytes is required. If non-chromophoric analytes are to be determined by indirect UV detection, the carrier analyte must also fulfill certain spectral requirements. The speed of analysis is increased by dynamically modifying the surface of the silica capillary using an electroosmotic flow modifier, thereby reversing the direction of electroosmotic flow such that the analyte anions migrate in the same direction as the bulk electroosmotic flow.

An electrolyte for anion analysis using capillary electrophoresis has been systematically developed by modifying the various parameters described in this paper. The electrolyte system uses pyromellitic acid as a carrier ion and hexamethonium hydroxide as the electroosmotic flow modifier. The electrolyte system is buffered at pH 7.7 with triethanolamine so that the pyromellitic acid is in a fully ionized form.

The electrolyte system will separate a variety of common inorganic anions in less than 5 min with very high peak efficiency. The addition of solvent to the standard electrolyte was examined and found to be useful for the resolution of some co-migrating anions.

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