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Optimization of inorganic capillary electrophoresis for the analysis of anionic solutes in real samples

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ABSTRACT

Inorganic capillary electrophoresis (ICE) is a separation technique which offers many advantages for the analysis of anionic solutes in real samples. Parameters which influence ICE separations such as system configuration, choice of electrolyte anion, electrolyte pH and the addition of electroosmotic flow modifier were investigated and a number of electrolytes of varying mobilities were studied. Optimized conditions were established for the separation of inorganic anions, organic acids and alkylsulfonates and the technique was applied to the analysis of a variety of anionic solutes in several complex sample matrices.

INTRODUCTION

Ion chromatography (IC) has seen remarkable growth since the introduction of the technique in 1975 [1]. Perhaps the greatest reason for this growth has been the ability to provide rapid and simple solutions to a large number of analytical problems, particularly the determination of inorganic anions and short-chained carboxylic acids. The technique of IC has expanded significantly to encompass a wide variety of separation and detection methods and is now extensively used in application areas such as water, environmental, industrial, food and clinical analysis [2].

Capillary zone electrophoresis (CZE) is a relatively new separation technique which has also been demonstrated to be applicable to the quantitation of inorganic anions and short-chained carboxylic acids. This technology utilizes narrow diameter capillaries (typically polyimide-coated, 50–100 μm fused-silica) and ionic species are separated according to their mobility under the influence of an applied potential (usually 10–30 kV). In CZE, the direction and magnitude of the bulk fluid flow, or electroosmotic flow (EOF), is dictated by the charge on the inner wall of the capillary [3]. In conventional CZE using a fused-silica capillary, the direction of the EOF is toward the negative electrode at most pH values, hence detection is carried out at this end. Under the influence of an applied potential, inorganic anions migrate rapidly toward the positive (non-detection) electrode and are typically not quantitated as they have excessive migration times or are not eluted at all. Only anions with a mobility less than that of the EOF can be determined using this approach. However, the addition of

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a cationic surfactant such as cetyltrimethylammonium bromide to the electrolyte has been demonstrated to significantly reduce migration times for small anionic species by the reducing or reversing the electroosmotic flow [3–5]. Detection of inorganic anions and short-chained carboxylic acids in CZE is carried out using similar strategies to those employed in ion chromatography for solutes which are typically non-UV absorbing. Conductivity [5,6] and both indirect fluorescence [7,8] and indirect UV absorbance [9,10] have all been used for the CZE determination of low-molecular-weight anionic species.

The combination of using a fused-silica capillary with an electrolyte containing a UV absorbing anion plus an electroosmotic flow modifier and indirect photometric detection has recently been termed "inorganic capillary electrophoresis" or ICE [11]. Determination of complex ionic mixtures with analysis times under five minutes and separation efficiences greater than 250 000 plates have been demonstrated using this analytical approach. There are a number of advantages of this approach for anion analysis. Ions are separated according to their mobilities resulting in different selectivities when compared to IC. Extremely efficient separations give more peak information from nanoliter sample volumes with ultra fast analysis times. Also, the instrumentation is very simple and allows rapid methods development.

This study is primarily concerned with parameters that influence the separation in ICE in order to optimize the analysis of anionic solutes in complex sample matrices. The differences (and similarities) between inorganic capillary electrophoresis and ion chromatography will be also discussed.

EXPERIMENTAL

Instrumentation

The capillary electrophoresis instrument used was a Quanta 4000 (Waters Chromatography Division of Millipore, Milford, MA, U.S.A.) with either a Waters 840 or 820 data station. It proved necessary to collect data at 20 points per second due to the very narrow peak widths resulting in ICE. The separations were carried out using a conventional fused-silica capillary (60 cm × 75 μm I.D.) obtained from Waters. While the Quanta 4000 is capable of both hydrostatic and electromigration injections, hydrostatic injection was used as the sample introduction mode for all this work. Typically the sample was elevated at 10 cm for 30 s. Electrolytes were prepared daily, filtered and degassed with a Waters solvent clarification kit. Detection was carried out using indirect photometry at 254 nm. Specific operating conditions are provided as captions to the figures.

Reagents

Purified (18 MΩ) water using a Millipore Milli-Q water purification system (Bedford, MA, U.S.A.) was used for all solutions. Sodium chromate tetrahydrate and the alkylsulphonate standards were obtained from Aldrich; potassium hydrogen phthalate and benzoic acid were obtained from Sigma and 1-naphthalenesulphonic acid (technical grade) was obtained from Kodak. Acetonitrile (HPLC grade) was obtained from J. T. Baker, as were the analytical-grade sodium salts used for the preparation of all the anion standards. C_{18} Sep-Pak cartridges were obtained from Waters and the electroosmotic flow modifier, Nice-Pak OFM Anion BT, is a propriety chemical obtained from Waters.

RESULTS AND DISCUSSION

Instrumental configuration for ICE

The system configuration for the analysis of anionic species by ICE differs from the approach taken in conventional CZE in that detection is carried out at the anodic (positive) electrode and injection is carried out at the cathodic (negative) electrode. The instrumentation for ICE has been described previously [11]. Sample introduction into the capillary differs from that used in IC or HPLC in that no injection valve is used. The most common mode is a hydrostatic (or gravity) injection as this mode, unlike electromigration, introduces a representative sample to the capillary. Electromigration injection involves applying a voltage to the sample in order to force the ions to migrate into the capillary. This injection mode is selectively biased toward faster migrating ions [12]. Hydrostatic injection was used as the sample introduction mode for all this work. As a matter of convention throughout this paper, the power supply used in the ICE configuration (*i.e.*, detection at the anodic electrode) will be referred to as negative. Unless otherwise indicated, the separations in this work were carried out with a cationic surfactant, Nice-Pak OFM Anion BT (patent applied for), added to the electrolyte in order to reverse the direction of the EOF [11]. The high mobility of inorganic anions in the same direction as the EOF (toward the negative electrode) enables very rapid, high efficiency separations to be obtained in ICE. As detection for all the separations was carried out by indirect photometry at 254 nm, electrolytes were chosen on the basis of both mobility and detection properties.

Anion selectivity by ICE

The selectivity for anion separations using ICE differs significantly from that obtained using conventional anion-exchange columns in IC. In an anion-exchange separation, cations and neutral species elute at the void volume of the column. Short-chained monocarboxylic acids and weakly retained anions, such as fluoride and methanesulphonate, are all eluted early and tend to be poorly resolved in many instances. Also, the presence of elevated carbonate and/or high levels of sample cations may further complicate the early portion of the chromatogram. The use of gradient [13] or coupled [14] IC can often overcome these resolution problems, however, both of these approaches are somewhat complex. A separation of inorganic anions and short-chained carboxylic acids obtained using ICE exhibits several significant differences from that obtained using an anion-exchange separation in IC. First, cations do not participate in the separation since they travel in the opposite direction to the anions. As the inorganic anions have high charge-to-ionic radii ratios, they tend to migrate much faster than weak acid anions and are well resolved from these species. Neutral solutes are carried along by the EOF and have appreciably longer migration times, however, these can be simply purged from the capillary once the desired separation is obtained. Fig. 1 shows an electropherogram of low ppm levels of the seven inorganic anions most commonly analyzed in IC [2] obtained using ICE with an electrolyte containing electroosmotic flow modifier (Nice-Pak OFM Anion BT) and the highly mobile chromate anion. All peaks are well resolved in under four minutes with this electrolyte. The retention order for these anions using an ion-exchange separation and an eluent at a pH of 8.5 would be fluoride, chloride, nitrite, bromide, nitrate, phosphate and sulphate. The "tailing" of the fluoride and phosphate peaks

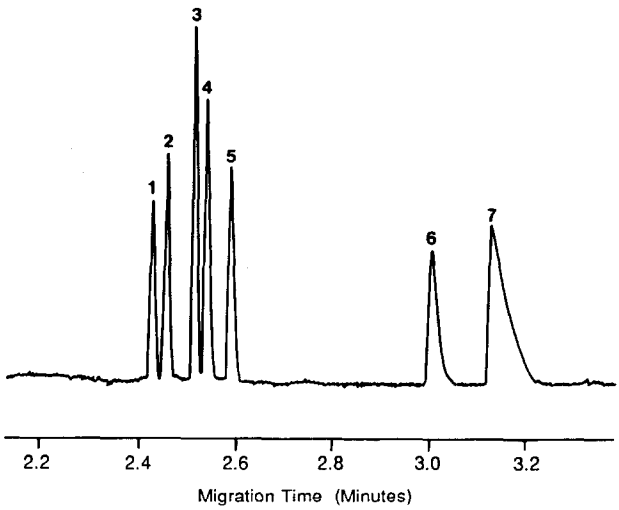

Fig. 1. Electropherogram of standard anions. Conditions, capillary: 60 cm × 75 μm I.D. fused-silica; power supply: negative; electrolyte: 5 m*M* chromate with Nice-Pak OFM Anion BT (patent applied for) at pH 8.0; injection: hydrostatic for 30 s; detection: indirect UV at 254 nm; solutes: 1 = bromide (4 ppm); 2 = chloride (2 ppm); 3 = sulphate (4 ppm); 4 = nitrite (4 ppm); 5 = nitrate (4 ppm); 6 = fluoride (1 ppm); 7 = phosphate (6 ppm).

occurs as a result of these peaks having a lower mobility than the electrolyte (chromate) anion. Solute anions with a higher mobility than the electrolyte anion exhibit "fronting" while solutes with a lower mobility than the electrolyte anion show tailing, as predicted by Mikkers *et al.* [9]. Optimal peak shape results if the electrolyte and solute anions have equivalent mobilities. The chromate anion has a mobility similar to that of bromide as indicated by peak shapes shown in Fig. 1 and is sufficiently absorbing at 254 nm to allow sensitive indirect photometric detection. These selectivity differences, along with rapid run times, high efficiencies and low sample and reagent consumption make ICE an ideal tool for practical analyses.

Practical applications using ICE

Fig. 2 shows a standard separation of chloride, sulphate, citrate, C_1–C_4 carboxylic acids and carbonate obtained using the same chromate electrolyte as above. This particular combination of solutes would be impossible to separate isocratically by IC as citrate is generally very strongly retained while the carboxylic acids are all weakly retained. Typically, a strong eluent species such as phthalate [15] is required to elute citrate in IC and such an eluent would result in poor resolution of the carboxylic acids.

Kraft black liquor is a complex sample from the pulp and paper industry which contains high concentrations of anions and organic acids in a high pH matrix. This sample is difficult to analyze by IC as the species of interest range from weakly retained organic acids such as formate and acetate to very strongly retained anions such as thiosulphate. Two isocratic separations (anion-exchange and ion exclusion) are typically used to quantitate the anions and organic acids in black liquor by IC [16]. Fig. 3 shows an ICE separation of a black liquor sample (diluted 1:1000 followed by

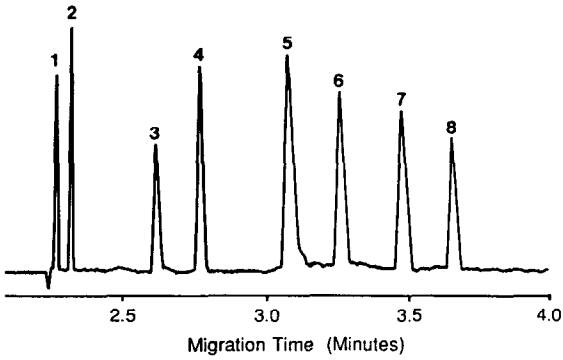

Fig. 2. Electropherogram of standard anions and organic acids. Conditions as for Fig. 1 except, solutes: 1 = chloride (2 ppm) and 4 ppm each of 2 = sulphate; 3 = citrate; 4 = formate; 5 = carbonate; 6 = acetate; 7 = propionate; 8 = butyrate.

C_{18} Sep-Pak clean-up) using the high mobility chromate electrolyte at pH 10.0. Thiosulphate, chloride, sulphate, oxalate, formate, carbonate, acetate, propionate and butyrate were present in the sample, along with several unidentified peaks. The less mobile species (formate, acetate, etc.) all show peak tailing as expected when using a chromate electrolyte. Sulphite, which also may be of interest in such samples, can be stabilized by the addition of mannitol and is well resolved from both oxalate and formate under these conditions.

The high mobility chromate electrolyte is of limited utility for the separation of lower mobility anions and organic acids. A lower mobility electrolyte anion, phthalate, was then employed in an attempt to better resolve a wider range of organic acids. An example of an electropherogram using a phthalate electrolyte at pH 5.6 is shown in Fig. 4. Formic, succinic, acetic, lactic, propionic and butyric acids (and phosphate) are well separated at this electrolyte pH. Variation of the electrolyte pH can be used to readily alter the migration times of weak acids, especially polyprotic acid anions such as phosphate. The higher the electrolyte pH, the more "ionized" is the solute, hence the greater its mobility. The determination of the organic acids shown in Fig. 4a in plaque is of interest in tooth decay research. Fig. 4b shows a electropherogram of a dental

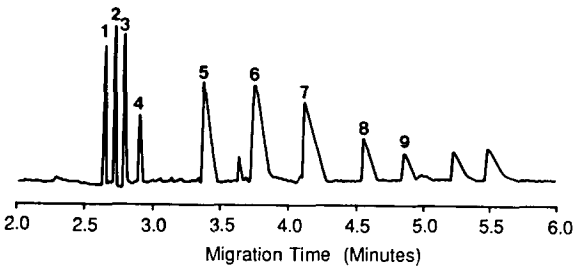

Fig. 3. Electropherogram of Kraft black liquor. Conditions as for Fig. 1 except, electrolyte: 5 m*M* chromate with Nice-Pak OFM Anion BT at pH 10.0; injection: hydrostatic for 20 s; sample preparation: 1000× dilution in deionized water, solutes: 1 = thiosulphate; 2 = chloride; 3 = sulphate; 4 = oxalate; 5 = formate; 6 = carbonate; 7 = acetate; 8 = propionate; 9 = butyrate.

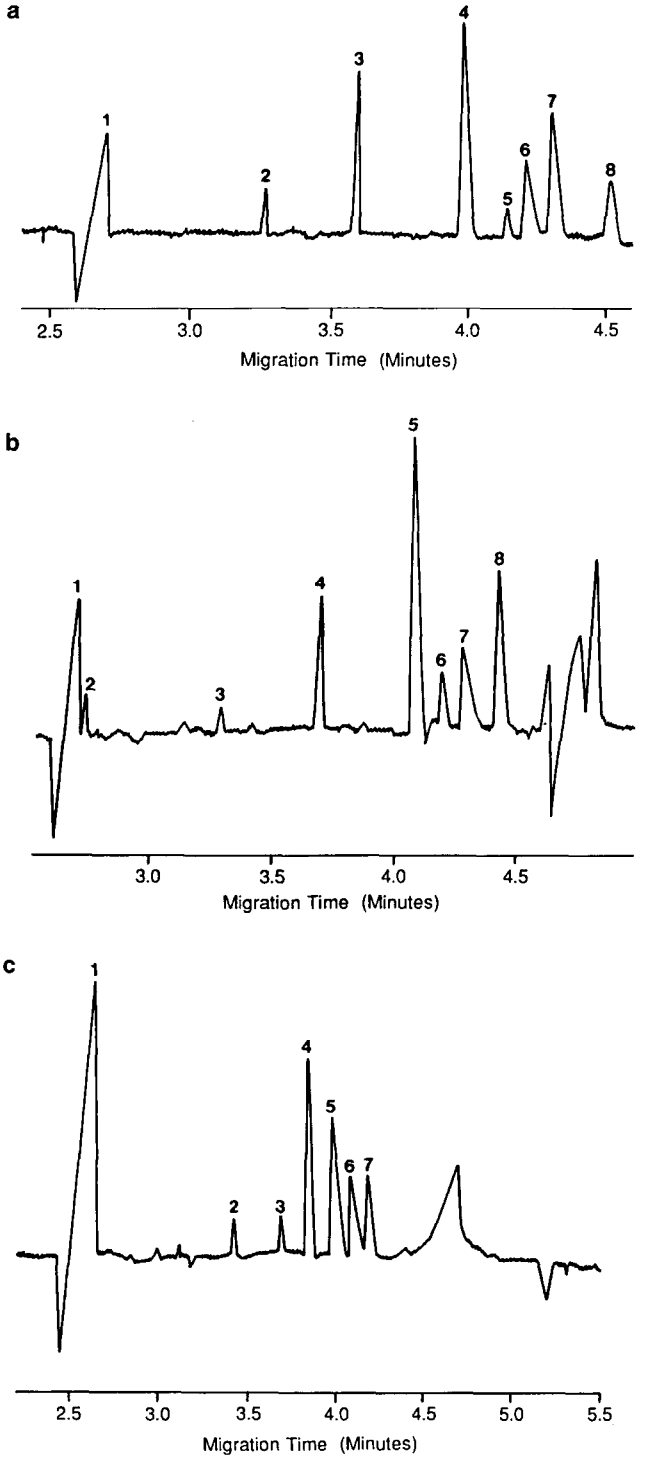
a
1
2
3
4
5
6
7
8
2.5
3.0
3.5
4.0
4.5
Migration Time (Minutes)
b
1
2
3
4
5
6
7
8
3.0
3.5
4.0
4.5
Migration Time (Minutes)
c
1
2
3
4
5
6
7
2.5
3.0
3.5
4.0
4.5
5.0
5.5
Migration Time (Minutes)

Fig. 4.

plaque extract run under the same conditions as the above standard. While similar separations can be achieved using ion-exclusion chromatography [17] or gradient IC [14], ICE is particularly appropriate for this analysis as the volume of sample obtained is limited to approximately 1 μl per patient. Injection volumes in ICE are usually in the order of 10–50 nl. A similar group of organic acids can also be found in saliva, Fig. 4c shows a electropherogram of a 1:10 dilution of a saliva sample using the phthalate electrolyte. The negative peaks in the sample electropherograms are anionic species (unidentified) which must absorb more than phthalate at 254 nm.

The peak shape of the later migrating solutes in Fig. 4a–c can be improved by selecting a still less mobile electrolyte anion. Fig. 5a shows a separation of a similar group of organic acids to those shown in Fig. 4a using a low mobility benzoate electrolyte. Acids such as butyric and caproic give Gaussian peak shapes with this electrolyte while the (relatively) more mobile acids such as formate exhibit fronting. The ICE separation of any group of solutes can be readily optimized by selecting an electrolyte anion with a mobility closely matched to the ions of interest. If a sample contains ions of widely varying mobilities, it can simply be analyzed using more than one electrolyte as the run times in ICE are so short and there is only a two-minute purge required between changing electrolytes. As the same concentration of the electro-osmotic flow modifier is used in the different mobility electrolytes, migration time stability is established within one run when changing between electrolytes. Fig. 5b shows an electropherogram of a diluted butyric acid extract of an air (filter) sample using the benzoate electrolyte; glycolate, acetate and valerate were present, along with two unidentified organic acids and a large butyric acid peak.

The same low mobility electrolyte (benzoate) can be used for the separation of short (C_1–C_7) chained linear alkylsulphonates as shown in the electropherogram in Fig. 6a. This analysis can be used for the determination of alkylsulphonates in a 0.1% dilution of an isopropyl alcohol process extract from a petroleum refinery as shown in Fig. 6b. The large peak at the start of the electropherogram is sulphate as the undiluted sample contained 60% sulphuric acid along with various crude petroleum fractions. A simple dilution and filtration was all the sample preparation necessary for this relatively complex matrix. ICE separations are less affected by sample pH than IC, however, resolution (and migration times) are somewhat dependent upon sample loading. High ionic strength samples will cause a shift in migration times, *e.g.*, compare the migration times of butanesulphonate in Fig. 6a (peak 4) and Fig. 6b (peak 2). Calibration curves in ICE are linear up to approximately 200 ppm [18] depending upon the analyte and the electrolyte being used.

Fig. 4. (a) Electropherogram of organic acid (and phosphate) standard with phthalate electrolyte. Conditions as for Fig. 1 except, electrolyte: 5 m*M* phthalate with Nice-Pak OFM Anion BT at pH 5.6; solutes: 1 = chloride; 2 = formate (0.167 μM); 3 = succinate (0.167 μM); 4 = acetate (0.178 μM); 5 = lactate (0.167 μM); 6 = phosphate (0.167 μM); 7 = propionate (0.175 μM); 8 = butyrate (0.175 μM). (b) Electropherogram of dental plaque extract. Conditions as above except, solutes: 1 = chloride; 2 = sulphate; 3 = formate (0.015 μM); 4 = succinate (0.042 μM); 5 = acetate (0.441 μM); 6 = lactate (0.021 μM); 7 = phosphate (0.081 μM); 8 = propionate (0.164 μM). (c) Electropherogram of human saliva. Conditions as above except, sample preparation: 10 × dilution in deionized water; solutes: 1 = chloride; 2 = formate (0.023 μM); 3 = succinate (0.013 μM); 4 = acetate (0.427 μM); 5 = lactate (0.167 μM); 6 = phosphate (0.101 μM); 7 = propionate (0.115 μM).

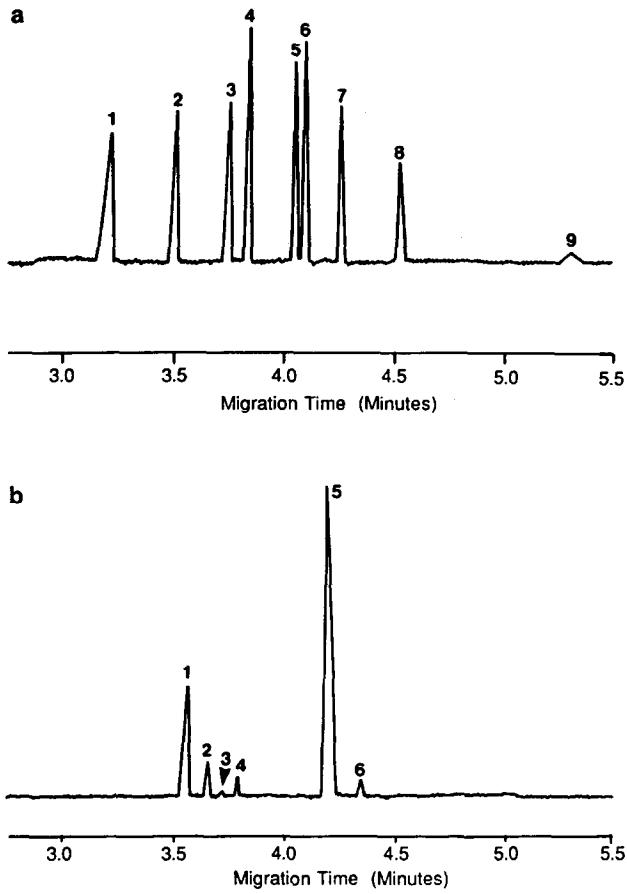

Fig. 5. (a) Electropherogram of organic acid (and phosphate) standard with benzoate electrolyte. Conditions as for Fig. 1 except, electrolyte: 10 m*M* benzoate with Nice-Pak OFM Anion BT at pH 6.0; solutes: 10 ppm each of 1 = formate; 2 = succinate; 3 = glycolate; 4 = acetate; 5 = phosphate; 6 = propionate; 7 = butyrate; 8 = caproate; 9 = caprylate. (b) Electropherogram of butyric acid extract of an air (filter) sample. Conditions as above except, sample preparation: 5000 × dilution in deionized water; solutes: 1 = unknown; 2 = unknown; 3 = glycolate (0.4 ppm); 4 = acetate (1.1 ppm); 5 = butyrate (55.1 ppm); 6 = unknown.

The last peak in the standard pherogram shown in Fig. 6a, a C_7 sulphonate, is poorly shaped with the benzoate electrolyte while C_8 sulphonate is not eluted at all under these electrolyte conditions. Attempts to use a still lower mobility electrolyte anion than benzoate for longer chain alkylsulphonates proved unsuccessful as resolution was limited by poor peak shape. The approach taken for such low mobility solutes was to use a conventional CZE configuration, *i.e.*, a positive polarity power supply and no electroosmotic flow modifier added to the electrolyte. The use of a very low mobility, UV absorbing electrolyte anion such as naphthalenesulphonate permits the analysis of anionic alkylsulphonates because their migration toward the (positive) anode due to the applied potential is less than the mobility of the bulk electroosmotic

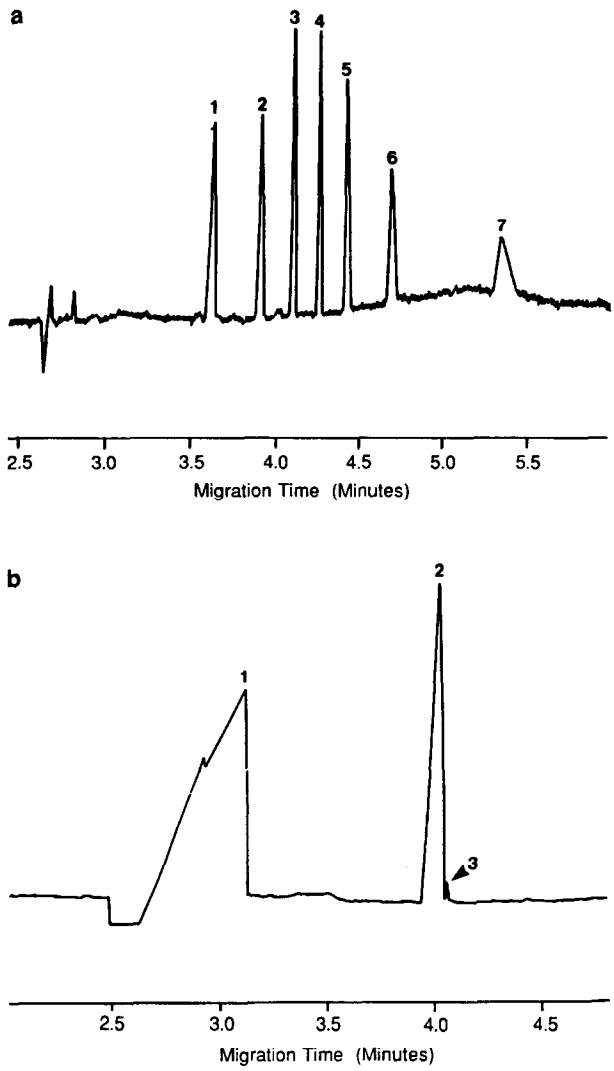

Fig. 6. (a) Electropherogram of C_1–C_7 alkylsulphonate standard with benzoate electrolyte. Conditions as for Fig. 5 except, solutes: 10 ppm each of 1 = methanesulphonate; 2 = ethanesulphonate; 3 = propanesulphonate; 4 = butanesulphonate; 5 = pentanesulphonate; 6 = hexanesulphonate; 7 = heptanesulphonate. (b) Electropherogram of an isopropyl alcohol process extract from a petroleum refinery. Conditions as above except, sample preparation: 1000 × dilution in deionized water; solutes: 1 = sulphate; 2 = propanesulphonate (137.7 ppm); 3 = butanesulphonate (2.4 ppm).

flow toward the (negative) cathode. Hence they can be detected by indirect photometry at the cathodic end of the capillary. A electropherogram of a standard separation of C_4–C_{12} alkylsulphonates obtained using this approach is shown in Fig. 7a. Note that the less mobile anionic species are eluted first under these conditions as they are carried further toward the cathode than more mobile anionic species. Very mobile anions such

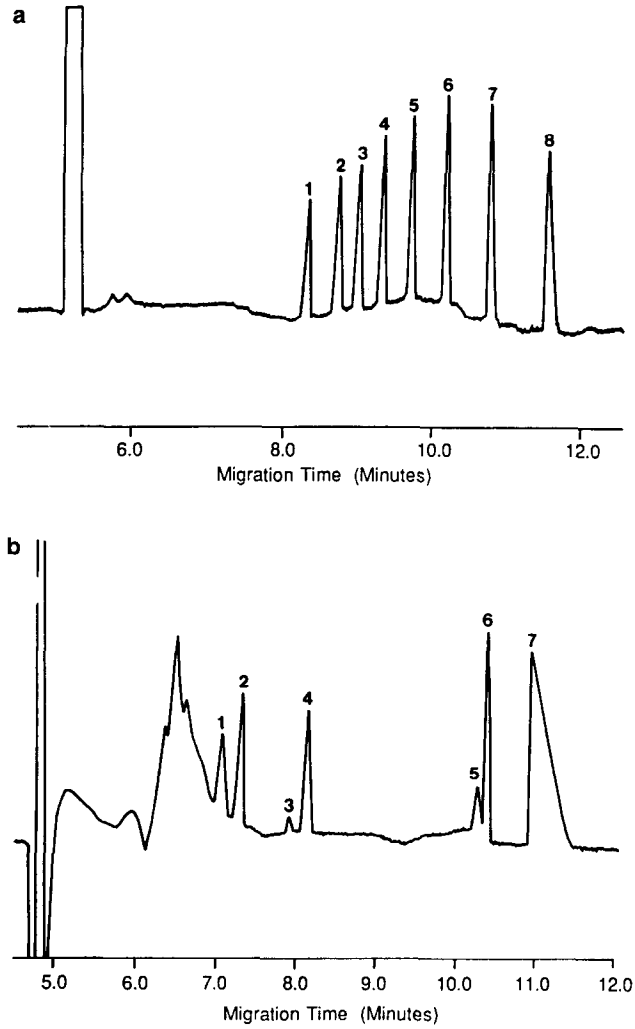

Fig. 7. (a) Electropherogram of C_4–C_{12} alkylsulphonate standard with naphthalenesulphonate electrolyte. Conditions as for Fig. 1 except, power supply: positive; electrolyte: 10 m*M* naphthalenesulphonate with 30% acetonitrile at pH 10.0; solutes: 25 ppm each of 1 = C_{12}-sulphonate; 2 = C_{10}-sulphonate; 3 = C_9-sulphonate; 4 = C_8-sulphonate; 5 = C_7-sulphonate; 6 = C_6-sulphonate; 7 = C_5-sulphonate; 8 = C_4-sulphonate. (b) Electropherogram of an alkylamido glycinate shampoo base. Conditions as above except, sample preparation: 200 × dilution in deionized water; solutes: 1 = C_{10}-sulphonate (27.7 ppm); 2 = C_9-sulphonate (37.0 ppm); 3 = C_7-sulphonate (3.4 ppm); 4 = C_6-sulphonate (25.6 ppm); 5–7 = unknowns.

as chloride do not appear in the electropherogram as their mobility exceeds that of the EOF, hence they are not detected at the cathode. This method can be applied to the determination of several alkylsulphonates in a glycinate shampoo base as shown in Fig. 7b. No sample pretreatment other than dilution was required.

CONCLUSIONS

Inorganic capillary electrophoresis is an powerful separation technique which offers many advantages for the analysis of inorganic and organic acid anions in real samples. Rapid, highly efficient separations with different selectivities (compared to ion chromatography) are obtained from nanoliter sample volumes. A separation can be readily optimized for a particular analysis by choosing an electrolyte anion with a mobility similar to the analytes of interest and changing electrolytes is simply a matter of purging the capillary between successive samples. The utility of the technique was demonstrated by analyzing anionic species such as inorganic anions, organic acids and alkylsulphonates in several complex sample matrices.

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