Modification of ion chromatographic separations by ionic and nonionic surfactants

James S. Fritz[a,*], Zhu Yan[b,c], Paul R. Haddad[b]

[a]*Ames Laboratory, US Department of Energy and Department of Chemistry, Iowa State University, 332 Wilhelm Hall, Ames, IA 50011, USA*
[b]*Australian Centre for Research on Separation Science, School of Chemistry, University of Tasmania, G.P.O. Box 252-75, Hobart, Tasmania 7001, Australia*
[c]*Department of Chemistry, Zhejing University, Hangzhou 310028, China*

Abstract

New findings are reported on simple ways to modify an ordinary HPLC column to obtain efficient ion chromatographic (IC) separations. Permanently coating a column with an ionic surfactant is known to produce an effective column for IC. We now show that incorporation of a nonionic surfactant in the coating, or coating in separate layers, results in a dramatic reduction of ion retention times and gives sharper peaks. Dynamic coating by incorporating a small amount of an alcohol, diol or zwitterion in the aqueous mobile phase permits good separations of alkanecarboxylic acids. A mobile phase containing a quaternary ammonium cation and a zwitterion anion provides excellent separations of common anions on a silica C_{18} column. An aqueous eluent containing a mixture of a zwitterion 4-(2-hydroxyethyl) acid and methanesulfonic acid can be used in conjunction with a standard cation exchange column. After passing through a membrane suppressor, the mobile phase has a slightly acidic pH, permitting divalent transition metal ions (as well as others) to be detected by conductivity.
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1. Introduction

Ion exchange involves more than just the electrostatic attraction of an analyte ion for the ion-exchange functional group of opposite charge. There is also a hydrophobic attraction effect between the hydrophobic portion of the analyte and the relatively hydrophobic backbone of the ion-exchanger. This latter component can lead to slower kinetics for some analyte ions and broad or asymmetric peaks. It is not uncommon to recommend incorporation of some acetonitrile or methanol in the aqueous eluent to reduce this effect.

A more effective approach is to modify the nature of the stationary phase. One way to accomplish this is by treatment of the stationary phase to make it more hydrophilic and thus more compatible with the aqueous mobile phase. Mobile phase components can also affect the surface of the stationary phase by a dynamic equilibrium. By selecting a component that is somewhat bulkier than is normally used, the amount partitioning onto the stationary phase may be sufficient to effect a significant change in the surface characteristics.

*Corresponding author. Tel.: +1-515-294-5987; fax: +1-515-294-3578.
E-mail address: kniss@ameslab.gov (J.S. Fritz).

The main focus of this work is on simple ways to prepare and modify the stationary phase in ion chromatography (IC). In additional, some novel eluents are proposed. The use of mixed surfactants in preparing IC columns is examined first. Next, methods for separation of alkane carboxylic acids are described in which the primarily aqueous mobile phase contains a small amount of an alcohol, diol or zwitterion. New zwitterion mobile phase components for cation chromatography are proposed, and a new system is described for separation of anions by ion pair chromatography.

2. Experimental

2.1. Instrumentation

The ion chromatograph used in these experiments was a Dionex (Sunnyvale, CA, USA) Model DX-600 column instrument equipped with a GS50 gradient pump, an AS50 Thermal compartment, an AS50 autosampler, a UI 20 universal interface, and a Waters 484 tunable absorbance detector. Data were acquired by using Peaknet 6.2 software installed on a P-III 550 computer. A Dionex AMMS-ULTRA 4-mm or CMMS-ULTRA 4-mm suppressor were used for suppressed conductivity detection in both auto-suppression recycle mode and the external chemical suppression mode.

2.2. Columns

The separation columns used were a Dionex IonPac NS1 (150×4 mm I.D.) polymeric column, Dionex Acclaim 120 C_{18} (100×4.6 mm I.D.), Waters (Milford, MA, USA) Nova-Pak C_{18} (150×3.9 mm I.D.), Phenomenex (Torrance, CA, USA) Synergi 4-MAX-PR 80 Å C_{12} (150×4.6 mm I.D.). The separation column used for carboxylic acid separation was a stainless steel 150×3.9 mm I.D., packed with 7.6-μm derivatized macroporous polystyrene–divinylbenzene (PS–DVB) resin (Dionex) prepared in our laboratory. A Shandon HPLC packing pump was used for column packing. The cation-exchange column used in this work was a Waters (Milford, MA, USA) IC-Pak C M/D column (150×3.9 mm I.D.), which is silica-based and coated with poly-

butadiene–maleic acid copolymer, of particle size approximately 5 μm, and capacity 1.5±0.2 mequiv./g.

2.3. Reagents

Acetonitrile (ACN) was of HPLC grade from Merck (Darmstadt, Germany). The eluent additives and analyte chemicals were of analytical reagent grade and were used as obtained from Aldrich (Milwaukee, WI, USA) or Ajax (N.S.W., Australia). All eluents were prepared daily. Stock solutions were used to prepare all sample solutions by dilution with the mobile phase. A Millipore Milli-Q water system (Milford, MA, USA) was used to further deionize distilled water for all eluents and sample mixtures.

Tetrapropylammonium hydroxide and tetrabutylammonium hydroxide were obtained from Dionex.

2.4. Procedure

Dynamically coated columns were prepared using commercial reversed-phase columns from Dionex and Waters. The columns were coated by passing solutions of 5 mM didodecyldimethylammonium bromide (DDAB), 5 mM cetylpyridinium chloride (CPC), or 5 mM solutions of a nonionic surfactant (Triton X-100, Tween 20 or Brij 35) at 1 ml/min for 1–2 h. In some cases, coating with a mixed solution of ionic and nonionic surfactant was employed. If desired, the progress of coating could be monitored continuously by a UV–Vis detector at 200 nm.

Separations on columns coated with DDAB were performed with 5.0 mM sodium perchlorate at a flow-rate of 2.0 ml/min with direct UV detection of sample ions. Separations on the other columns were performed with 10 mM sodium carbonate as the eluent, a flow-rate of 1.0 ml/min, and a suppressed conductivity detector.

The separation column was equilibrated with eluent until the baseline was stabilized. Sample injection was made at this point. The suppressor was operated at 50 mA using the recycle mode.

Separations on a silica C_{18} column were performed with different zwitterion and ion pair reagents with pH less than 8 at a flow-rate of 1.0 ml/min with direct UV detection of sample anions.

A flow-rate of 1 ml/min was selected for all the chromatographic separations. The separation column was equilibrated with mobile phase until the baseline was stabilized. Sample injections were made at this point. Carboxylic acids were detected by direct conductivity and also by UV detection at 210 nm to confirm peak identification.

Retention factors, k', were calculated according to the expression: $k' = (t_r - t_o)/t_o$. The system dead time, t_o, used to calculate the retention factor k' was measured by injecting water onto the system. Peak asymmetry (A) was calculated using $A = (RW5\% + LW5\%)/(2LW5\%)$, where RW5% and LW5% are widths of the right and left portions of the peak at 5% of the peak height. Theoretical plates were calculated using the half-height method.

3. Results and discussion

3.1. Anion chromatography on columns coated with nonionic and cationic surfactant

Since its introduction in 1975, IC has become the dominant analytical method for determining anions. With modern columns and equipment, complex mixtures of anions, and to a somewhat lesser extent cations, can be analyzed with good efficiency. Nevertheless, the separation power of IC columns, measured in theoretical plates, continues to be lower than that of HPLC columns used for separation of organic analytes. An intriguing possibility is to convert a highly efficient HPLC column into an IC column of equal efficiency by coating with an ion-exchange material. This might be accomplished either by a simple permanent coating procedure or by incorporating the coating material into the mobile phase so that coating occurs by a dynamic equilibrium between the two phases.

Cassidy and Elchuk [1] obtained good separations of anions on poly(styrene–divinylbenzene) columns dynamically coated with cetylpyridinium salicylate. Duval and Fritz [2] used columns coated with CPC and reported that the retention times of anionic analytes were affected by the chemical nature of the solid substrate as well as by the thickness of the CPC coating. Several investigators have separated anions on HPLC columns coated with a cetylpyridinium salt

[3–6]. Polyethyleneimine (PEI)-coated columns [7,8] have also been used effectively for separation of anions. Polymeric layers have also been grafted onto HPLC columns. Schomburg et al. [9] and others [10] used columns coated with poly(butadiene-maleic acid) for effective separation of cations. Connolly and Paull [11] obtained fast ion-chromatographic separation of common inorganic anions using a 3-cm silica C_{18} column permanently coated with DDAB.

We now describe rapid, simple methods for permanently coating an HPLC column with a cationic surfactant to produce an efficient column for separation of anions by IC. Importantly, coating with both a nonionic surfactant as well as a cationic surfactant was found to improve separation efficiency and to reduce the time required for separation.

Based on previously reported research [11], DDAB was used to coat a commercial silica C_{18} column. Sodium perchlorate (5.0 mM) was found to be the most efficient eluent with analyte detection at 200 nm. Although several common inorganic anions were separated in ca. 5 min, organic anions had broad peaks and much longer retention times. However, the same C_{18} column coated with a mixture of DDAB and Tween 20 (a nonionic surfactant) gave much shorter retention times and sharper peaks with the same eluent. For example, the mixed coating reduced the retention time for 4-hydroxybenzoate from 11.7 to 3.1 min, molybdate from 13.5 to 3.4 min, benzoate from 16.5 to 7.5 min, and phthalate from 22.5 to 6.9 min [12]. Thus the presence of a relatively polar surfactant such as Tween dramatically reduces the hydrophobic interaction between these anions and a stationary phase.

Coating a prepacked column expands the volume of the solid particles and can result in a higher back pressure. The DDAB coating solutions were cloudy and appeared to give a relatively thick coated layer. In the next series of experiments, CPC was selected because it is readily soluble in water and gives a clear solution for coating. CPC has previously been used successfully to create columns for anion chromatography [2]. Several nonionic surfactants were also tested to modify the IC performance of coated columns: Triton X-100, Tween 20 and Brij 35. Of these, Triton X-100 was the most promising.

Coating of a packed column with a mixture of a cationic surfactant and a nonionic surfactant resulted in a mixture of the two surfactants in a single adsorbed layer on the surface. When the coating was performed in two steps, namely first with a layer of a nonionic surfactant and then subsequently with the cationic surfactant CPC, a more efficient column resulted. Reversal of the coating sequence (i.e., using CPC first) gave a column with almost no measurable anion-exchange capacity. The most efficient column for anion chromatography was prepared by coating a Phenomenex Synergi column with 5 mM Triton X-100 from 30% acetonitrile and then aqueous 5 mM CPC. The eluent was 2 mM sodium perchlorate and direct UV detection at 210 nm was used. The performance data showed an actual average plate number of 12 900 for the 15-cm column or 86 000 plates/m. The peaks were well shaped with an average asymmetry factor of 1.09. A chromatographic separation is shown in Fig. 1.

The efficiency of our double-coated columns (about 80 000 plates/m) compares well with commercial columns used for ion chromatography. We obtained about 30 000 plates/m for similar anion separations on Dionex AS 14A and As 17, and about 15 000 plates/m on a single-coated silica C$_{18}$ column. The double-coated columns are also very durable. Each of several columns tested lasted at least 360 h of use, including analyses of tap water and water from commercial plants. Similar performance results were obtained by double coating chromatographic columns from three different manufacturers. A column that begins to show a significant loss in efficiency can be washed out with acetonitrile and recoated to restore it to almost its original efficiency.

In a study using anion-exchange columns prepared by coating neutral resin particles with CPC, the retention times of analyte anions were found to be influenced by the chemical nature of the particles [2]. Thus, the retention times of bromide, nitrate and iodide, relative to that of chloride, increased substantially going from Rohm and Haas XAD-1 (polystyrene) to XAD-8 (polyacrylate), while the relative retention times of sulfate and thiosulfate decreased. These findings demonstrate that the underlying material in coated columns also affects chromatographic behavior in ion chromatography. Our present study provides further confirmation of this hypothesis. Ion exchange retention behavior is determined by both the lower neutral surfactant layer and the upper charged surfactant layer acting in concert, and not by the upper layer alone.

3.2. Separation of carboxylic acids

Relatively small, polar organic compounds are often separated by ion-exclusion chromatography. These separations are usually performed on columns of fairly large dimensions packed with a gel resin containing sulfonate or carboxylate groups for separation of acid or neutral analyses, or quaternary ammonium groups for separation of basic analytes [13,14]. According to the generally accepted mechanism, the molecular carboxylic acids partition between the liquid mobile phase and the stagnant water inside the resin gel. Sample anions are excluded from the resin gel by electrostatic repulsion of the resin sulfonate groups. However, several authors have proposed a mixed-mode mechanism in which

Fig. 1. Separation of a standard solution containing different inorganic and organic anions. Conditions: column: Phenomenex Synergi 4 μm MAX-PR 80 Å (150×4.6 mm I.D.); coated first with 5 mM Triton X-100 with 30% ACN and then 5 mM CPC; eluent, 2 mM NaClO$_4$, flow-rate, 1.0 ml/min; detection, UV detection at 210 nm; sample, 50 μl containing 30 μg/ml acetate, 2 μg/ml nitrite, 2 μg/ml bromide, 2 μg/ml nitrate, 2 μg/ml hydroxybenzoate, 30 μg/ml oxalate, 2 μg/ml nitrite, 2 μg/ml bromide, 2 μg/ml nitrate, 2 μg/ml 4-hydroxybenzoate, 30 μg/ml oxalate, 30 μg/ml chromate, 10 μg/ml benzoate, 10 μg/ml phthalate, 30 μg/ml thiocyanate. Peaks: (1) acetate, (2) nitrite, (3) bromide, (4) nitrate, (5) oxalate, (6) iodide, (7) 4-hydrobenzoate, (8) chromate, (9) thiocyanate, (10) phthalate and (11) benzoate.

partitioning also occurs between the samples solutes and the polymeric resin matrix [15,16].

Morris and Fritz [17] showed that the lower alkylcarboxylic acids could be efficiently separated on a porous, but non-gel, PS–DVB packed column using water containing only a small percentage of *n*-butanol as the mobile phase. The *n*-butanol in the mobile phase was believed to coat the surface of the PS–DVB resin and establish a dynamic equilibrium between the mobile and stationary phases. This increased the hydrophilicity of the resin surface and reduced the hydrophobic attraction of the analyses for the resin phase. The separation involved a typical HPLC partitioning mechanism, although there was a secondary ion-exchange effect due to adsorbed carbonic acid. Li and Fritz [18] showed that the lower alkylcarboxylic acids could also be efficiently separated on silica C_{18} using water containing straight-chain alcohol or diol additives (include 1-butanol, 1,2-hexanediol, 1,2-octanediol.

The separation of a series of five alkylcarboxylic acids, formic through *n*-valeric acid, was attempted on a standard silica C_{18} column with water alone as the eluent and with conductivity detection. Although good resolution was obtained, the latest three peaks were very broad and fronted. Then the same separation was run with an aqueous mobile phase containing 10 m*M* of a zwitterion: 2-(*N*-cyclohexylamino)ethanesulfonic acid (CHES), 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (Hepes) or proline. The best results were obtained with Hepes (Fig. 2). Although some peak-fronting is still evident, the presence of a low concentration of zwitterion in the eluent has markedly improved the peak shape and provided a complete separation in <8 min. The background conductance of the 10 m*M* Hepes solution was only 4.0 μS; thus sensitive detection of the carboxylic acids was possible by non-suppressed conductivity.

Addition of methanesulfonic acid (MSA) to a dilute aqueous solution of Hepes resulted in much better peak symmetry but the high background conductivity necessitated the use of chemical suppression with tetrabutylammonium hydroxide. Even so, the background conductivity for 5 m*M* Hepes + 1 m*M* MSA was 82 μS. However, a much lower concentration of zwitterion and acid had a much lower background and still gave good resolution of
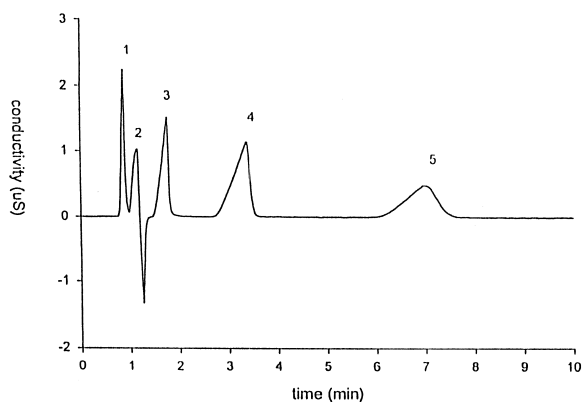

Fig. 2. Chromatographic separations of carboxylic acids on C_{18} column. Conditions: column, Waters Nova-Pak C_{18} column (150× 3.9 mm); eluent, 10 m*M* Hepes in water; flow-rate, 1.0 ml/min; detection, conductivity. Peaks: (1) formic acid (5 μg/ml), (2) acetic acid (10 μg/ml), (3) propionic acid (25 μg/ml), (4) butyric acid (50 μg/ml), (5) *n*-valeric acids (50 μg/ml).

carboxylic acids with excellent peak shape. The separation in Fig. 3 was performed with 0.05 m*M* Hepes + 0.05 m*M* MSA (pH 3.80) and had a conductivity after suppression of only 5.8 μS. In Figs. 2 and 3 common inorganic anions eluted at the dead time.

An aqueous solution of a long-chain zwitterion with amino and sulfonate groups will strongly coat a silica C_{18} column. The zwitterion reagents investigated here all have a molecular mass >200, which
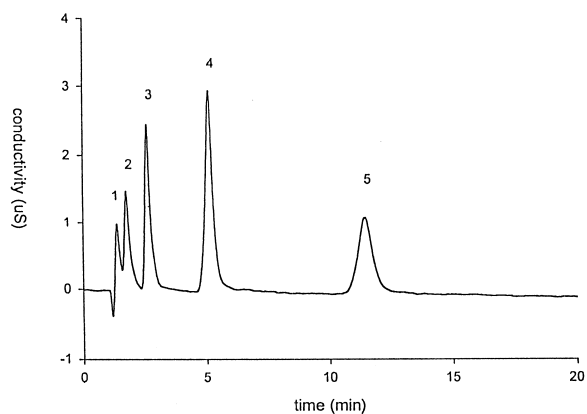

Fig. 3. Chromatographic separation of carboxylic acids. Same conditions as in Fig. 2 except the eluent is 0.05 m*M* Hepes and 0.05 m*M* MSA. Chemically suppressed conductivity detection with tetrabutylammonium hydroxide.

should still be sufficient to form at least a thin coating by a dynamic equilibrium. This coating can alter the surface of the stationary phase and improve the partition characteristics of the analytes.

3.3. Zwitterion eluents for IC

Ion chromatographic separation of cations has traditionally been carried out on columns containing ion-exchangers with a sulfonate functional group. In recent years, however, materials with carboxyl or mixed carboxyl/phosphonate functional groups have become more popular. The net negative charge, and hence the effective cation-exchange capacity of these packings can be reduced by lowering the pH of the eluent, making it easier to elute 2+ cations such as calcium and magnesium. Weakly complexing anionic ligands such as tartrate or citrate are sometimes included in the eluent. Hydrogen ions, which are converted to water when passed through a suppressor, continue to be the main cationic constituent of most eluents used in cation chromatography.

Traditionally, inorganic strong acids or a mixture of an inorganic acid and a diamine have been used for separation and suppressed detection of alkali metal and ammonium cations as well as alkali earth metal cations [19–21]. Detection of transition metal cations by suppressed conductivity has not been feasible because of their precipitation by the hydroxide introduced in the suppressor. Actually, if the pH of eluent can be kept to <7 after suppression, most transition metal ions will not precipitate and can still be detected by suppressed conductivity. Hajos and Szikszay [22,23] proposed the use of acidified histidine as a mobile phase for membrane-suppressed cation chromatography.

The purpose of the present study was to investigate the use of mixtures of an acid, such as MSA, and a zwitterion reagent as eluents for cation chromatography with suppressed conductivity detection. By proper selection of conditions it is possible to separate and detect several transition metal ions as well as alkali metal, ammonium and alkaline earth metal cations.

The chemical structures of zwitterion reagents investigated are given in Table 1. In each case, there is a basic nitrogen and an acidic group, $-SO_3H$ or $-CO_2H$. A proton from the latter group migrates to the nitrogen, giving a $-NH^+$ group and leaving a $-SO_3^-$ or $-CO_2^-$ group. These + and − charges cancel one another making the zwitterion neutral with regard to its net charge. For convenience, we will represent this as Z. Addition of a strong acid to a solution of Z will add another proton to form ZH^+. The acidic ionization constant for $ZH^+ \rightarrow Z$ is represented by k_1. Likewise, the acid ionization for the reaction $Z \rightarrow Z^-$ may be represented by k_2. We used a mobile phase prepared by adding MSA to an aqueous solution containing a moderate excess of a zwitterion, Z. The final solution had a pH between 2 and 4. The two eluent cations are H^+ and ZH^+, but the latter has greater eluting power for cation chromatography. The remaining Z appears to have little, if any, eluting power for cations.
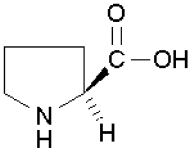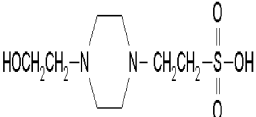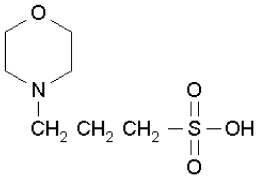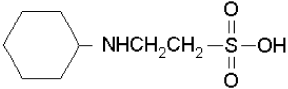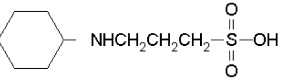
In the suppression mode hydroxide ions are introduced into the eluent stream through the ion-exchange membrane of the suppressor. This results in conversion of the eluent cations, H^+ and ZH^+, to the non-conducting species H_2O and Z. The very weakly acidic nature of k_2 and the buffering capacity of the zwitterion seem to inhibit reaction of Z with hydroxide to form Z^-. The pH of the suppressed eluent can be predicted by the equation for the isoelectric pint, pI:

$$pI = (pk_1 + pk_2)/2.$$

Depending on the particular zwitterion selected, the pI can be between 5 and 9 and the mobile phase pH after suppression will be around 5–9. In order to avoid precipitation of transition metal ions as the hydroxide, the pI of the zwitterion reagent should be toward the lower end of this range.

Eluents containing 5 mM MSA and 10 mM Hepes, proline, 3-(N-morpholino)-2-hydroxypropanesulfonic acid (MOPS) or CHES were used to separate a number of metal cations on a commercial cation-exchange column. The chemical structure of the zwitterion had a profound effect on the retention times of metal cations, and also on the detection sensitivity as indicated by the peak height. At the concentrations used, retention times with Hepes are generally longer than with the other zwitterions. However, peak symmetry and the average number of theoretical plates (3600) were also the most favorable with Hepes. For these reasons, Hepes was

Table 1
Zwitterions used in eluent of ion chromatography and carboxylic acids with C_{18}

Zwitterion	Structure	F.W.	S (g kg^{-1})
L-Proline		115.13	1623
4-(2-Hydroxyethyl)-1-piperazineethanesulfonic acid (Hepes)		238.3	>1639
3-Morpholinopropanesulfonic acid (MOPS)		209.3	>209.3
2-(Cyclohexylamino)ethanesulfonic acid (CHES)		207.3	
3-(Cyclohyexylamino)-1-propanesulfonic acid (CAPS)		221.3	

selected for use in the remaining chromatographic experiments.

The effect of eluent concentration was investigated next using 10 mM Hepes and varying concentrations of MSA. As expected, retention times become progressively longer as the concentration of MSA was decreased. Decreasing the MSA concentration also makes the eluent pH a little less acidic (range 2.82–3.15) and the pH after suppression a little lower (range 5.80–5.50). The latter pH range is sufficiently acidic so that the divalent transition metal ions of Co(II), Zn(II), Cu(II), and Ni(II) can be detected. However, the detection sensitivity of these ions is lower than obtained for the alkali metal ions, Mg^{2+} and Ca^{2+}.

A separation of 10 metal cations was obtained with an eluent containing 7.5 mM MSA and 10 mM Hepes. By decreasing the concentrations of the analyte ions it is possible to also detect Ni^{2+} and Cu^{2+} (Fig. 4). Many of these analyte ions can be separated and readily detected even in the sub-ppm range (~10–25 μM), as shown in Fig. 5.

Linear calibration plots were obtained for all metal ions studied ($r^2 > 0.998$) except for Cu(II) which had a long retention time and broad peak. Linear ranges for the cations were approximately as follows (mg/l): Li$^+$, 0.025–1.0; Na$^+$, 0.06–3.0; NH$_4^+$, 0.05–2.0; K$^+$, 0.1–5.0; Rb$^+$, 0.4–2.0; Cs$^+$, 0.4–20; Mg^{2+}, 0.06–3.0; Ca^{2+}, 0.08–4.0; Sr^{2+}, 0.6–30; Ba^{2+}, 1–50; Co^{2+}, 1–50; Ni^{2+}, 0.06–30; Cu^{2+}, 1–50.
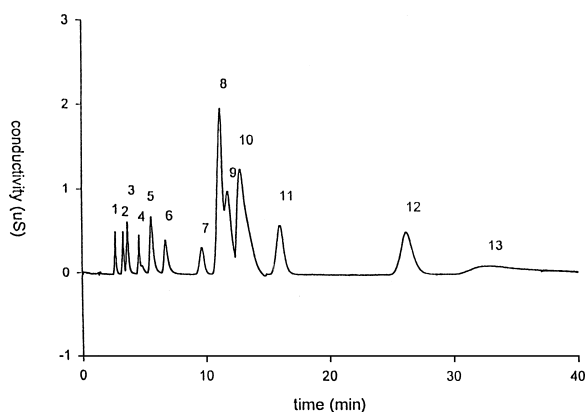
Fig. 4. Separation of a standard solution containing alkali metal ions, alkaline earth metal ions, ammonium and transition metal ions. Waters IC-Pak C M/D column (150×3.9 mm); eluent 6.5 mM MSA–10 mM Hepes sample: 50 μl containing 0.5 μg/ml lithium, 1.5 μg/ml sodium, 1 μg/ml ammonium, 2.5 μg/ml potassium, 10 μg/ml rubidium, 10 μg/ml caesium, 1.5 μg/ml magnesium, 15 μg/ml nickel, 25 μg/ml cobalt, 4 μg/ml calcium, 15 μg/ml strontium, 25 μg/ml barium, 25 μg/ml copper. Peaks: (1) Li^+, (2) Na^+, (3) NH_4^+, (4) K^+, (5) Rb^+, (6) Cs^+, (7) Mg^{2+}, (8) Co^{2+}, (9) Ni^{2+}, (10) Ca^{2+}, (11) Sr^{2+}, (12) Ba^{2+}, (13) Cu^{2+}.
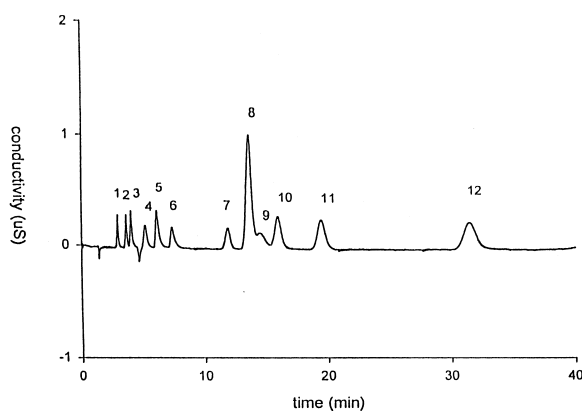

Fig. 5. Separation of a standard solution containing alkali metal ions, alkaline earth metal ions, ammonium and transition metal ions. Conditions same as Fig. 4 except eluent 6.0 mM MSA–9 mM Hepes sample: 50 μl containing 0.2 μg/ml lithium, 0.6 μg/ml sodium, 0.4 μg/ml ammonium, 1.0 μg/ml potassium, 2 μg/ml rubidium, 2 μg/ml caesium, 0.3 μg/ml magnesium, 3 μg/ml nickel, 1 μg/ml cobalt, 0.4 μg/ml calcium, 0.6 μg/ml strontium, 10 μg/ml barium. Peaks: (1) Li^+, (2) Na^+, (3) NH_4^+, (4) K^+, (5) Rb^+, (6) Cs^+, (7) Mg^{2+}, (8) Co^{2+}, (9) Ni^{2+}, (10) Ca^{2+}, (11) Sr^{2+}, (12) Ba^{2+}.

3.4. Ion pair chromatography

As an alternative to conventional ion chromatography, sample ions may be separated on an ordinary HPLC column by adding a large organic ion of opposite charge to the mobile phase. Presumably, this would result in a separation of the paired analyte ions stemming from differences between their mobile stationary phases. A mobile phase containing tetrabutylammonium methanesulfonate in an aqueous solution containing a low percentage of methanol or acetonitrile as a modifier could typically be used for the separation of anions in an analytical sample. The mechanism for a separation has been the subject of some debate. Horvath et al. coined the name ''ion-pair chromatography'' and demonstrated the practicality of this approach [24]. It has been argued that an ion-pair mechanism is incorrect and that ''ion-interaction chromatography'' (IIC) is a more accurate description for this type of ion separation [25].

Although direct or indirect spectroscopy has been the dominant detection mode [26], suppressed conductivity detection may be used in some cases. A micromembrane suppressor is available that is solvent resistant and is permeable to quaternary ammonium ions. The potential of zwitterionic substances as eluents in suppressed anion chromatography should be apparent to anyone who has carefully studied that technique, but the number of publications has been very limited. Ivey [27] reported that residual conductivities as low as $5–10$ μS cm^{-1} could be obtained when using salts of some N-substituted aminoalkylsulfonic acids with a packed suppressor. Irgum [28] obtained background conductivities as low as 0.4 μS cm^{-1} with N-substituted aminoalkylsufonates in membrane-suppressed anion chromatography.

An aqueous mobile phase prepared by adding 2.5–10 mM tetrabutylammonium hydroxide (TBAH) and 5–20 mM of a substituted amino alkylsulfonic acid (designated as Z) can be used to separate anions on an ordinary C_{18} silica column of the type generally used for HPLC. The chemical structures and common names of the zwitterion reagents are given in Table 1. Most likely, TBAH neutralizes a proton on the zwitterion to form Bu_4N^+ and Z^-. After passing through the suppressor, the mobile phase conductance for 2.5 mM TBAH + 5 mM

MOPS is only 7.9 µS, that for 2.5 m*M* TBAH+5 m*M* MSA is 19.8 µS, and that for 2.5 m*M* TBAH+ 5 m*M* Hepes is 3.5 µS.

The retention times of selected anions (Table 2) are affected by the chemical nature of Z. Even CHES and 3-cyclohexylamino-1-propanesulfonic acid (CAPS), which differ in structure by only a single –CH$_2$, show substantial differences in the retention times. MOPS gave the highest number of theoretical plates (Av. *n*=2870 or ~19 000 plates/m) and had the best peak symmetries for the anions studied. The average plate number for MES was 2100, and Hepes gave Av. *n*=1430. For these reasons, MOPS was selected for the remaining studies.

Comparison of tetrapropylammonium hydroxide (TPAH) and TBAH in admixture with MOPS showed that TBAH gave significantly longer retention times and better resolution of anion analytes. The best concentration range for TBAH was found to be 1–5 m*M*, with poor efficiency at concentrations <1.0 m*M*. A molar ratio between 1:2 and 1:4 for TBAH:MOPS (pH 7.2–6.8) was suitable for separations.

The chromatographic separation of 10 anions with a mobile phase containing 5 m*M* TBAH and 10 m*M* MOPS is shown in Fig. 6. The elution order is similar to the retention order for electrostatic ion chromatography (EIC) which is explained in terms of increasing propensity for ion-pair formation [29,30]. However, in Fig. 6 sulfate is eluted after, rather than before, nitrate. The overlap of nitrate and phosphate, and also that of oxalate and chromate,
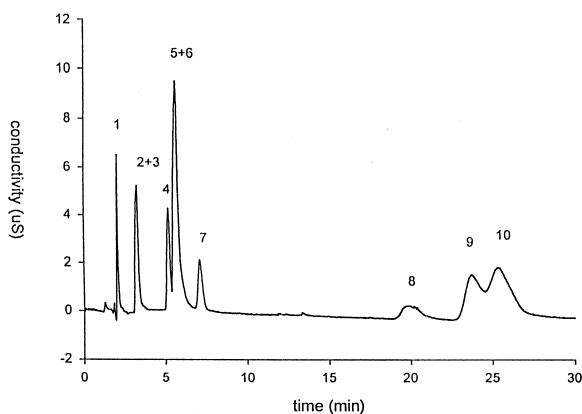

Fig. 6. Separation of a standard solution containing different inorganic and organic anions. Conditions: column, Waters Nova-Pak C$_{18}$ (150×3.9 mm). The eluent was 5 m*M* TBAOH and 10 m*M* MOPS, pH 7.18. Peaks: (1) 5 µg/ml chloride, (2) 10 µg/ml nitrate, (3) 20 µg/ml phosphate, (4) 10 µg/ml sulfate, (5) 20 µg/ml oxalate, (6) 50 µg/ml chromate, (7) 20 µg/ml iodide, (8) 50 µg/ml benzoate, (9) 100 µg/ml thiocyanate, (10) 100 µg/ml perchlorate.

might have been improved by incorporation of a low concentration of methanol or acetonitrile in the eluent.

An excellent separation of mono-, di- and trifluoroacetate is shown in Fig. 7. The separation of additives in lemon juice (diluted 1:10) is shown in Fig. 8. Acetate, tartrate and iodide are well resolved in ca. 7 min, but the benzoate peak has a retention time of ca. 19 min and the citrate peak is too broad for a practical analysis.

Table 2

Retention properties of selected anions with an aqueous mobile phase containing 2.5 m*M* tetrabutylammonium hydroxide plus 5 m*M* of a zwitterion reagent (MOPS, MES or Hepes)

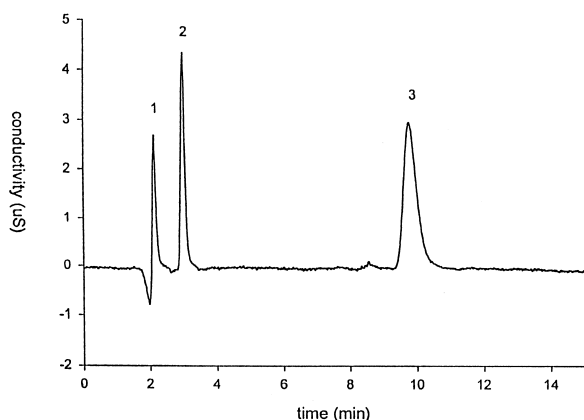| Anion	Zwitterion MOPS, pH 7.1 (min)	Component of mobile phase	
		MES, pH .2 (min)	Hepes, pH 7.6 (min)
Chloride	2.23	2.92	2.66
Nitrite	2.58	3.51	3.11
Nitrate	3.23	4.45	4.13
Phosphate	4.13	3.74	7.24
Iodide	6.46	9.23	8.43
Chromate	7.05	9.15	13.2
Sulfate	7.07	15.4	13.2
Perchlorate	20.3	27.0	24.7
Thiocyanate	20.6	29.7	26.8

Nova-Pak C$_{18}$ column, 150×3.9 mm.

Fig. 7. Separation of a standard solution containing fluoroacetates. Conditions as in Fig. 6. Peaks: (1) 5 μg/ml monofluoroacetate, (2) 5 μg/ml difluoroacetate, (3) 25 μg/ml trifluoroacetate.

In conventional separation of anions by IIC the eluent generally contains a large organic cation (C^+) with a smaller counter anion (E^-). As the eluent is pumped through an HPLC column (PS–DVB or silica C_{18}) the surface becomes coated with C^+ by a dynamic equilibrium. The C^+ on the surface then attracts E^- as the counter ion. Sample anions (A^-) are separated by differences in the extent of the following exchange reaction:

$$\text{Surface } C^+ E^- + A^- \rightleftharpoons = \text{Surface } C^+ A^- + E^-$$
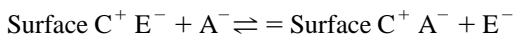
Retention times of the sample anions can be varied by altering the following parameters: type and
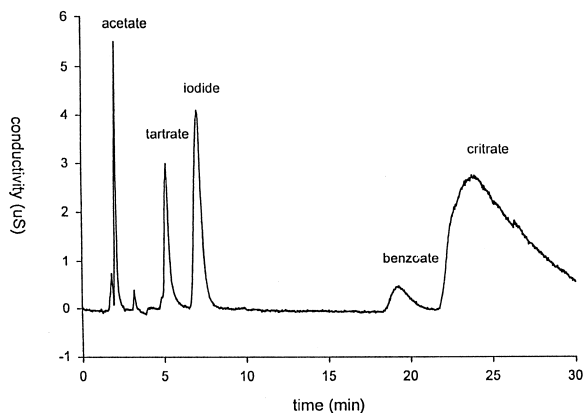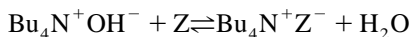

Fig. 8. Separation of food additives in lemon juice (1:10 dilute). Conditions as in Fig. 6.

concentration of C^+, type and concentration of E^-, and the type and concentration of organic solvent in the eluent.

A similar mechanism may apply when the eluent anions are generated from a zwitterion, for example,

$$Bu_4N^+OH^- + Z \rightleftharpoons Bu_4N^+Z^- + H_2O$$

However, the Z^- eluent ions used here are larger and more hydrophobic than the E^- generally used for anion separations. The anion retention times tend to be affected more by the chemical structure of Z^- than by its concentration.

4. Conclusions

This research provides further confirmation that an ordinary HPLC column can be converted to an efficient column for ion chromatography simply by coating it with an ionic reagent. A permanent coating can be applied by passing a solution containing a surfactant through the column. Alternatively, a coating can be applied by a dynamic equilibrium with a less hydrophobic ionic reagent incorporated in the mobile phase. The solid surface just under the ion-exchange sites should be relatively polar in order to obtain rapid exchange reactions and avoid poor peak shape. Thus, applying an initial coating of a nonionic surfactant provides much more efficient ion-exchange behavior than is obtained with a single layer of the ionic surfactant.

Zwitterionic reagents of ca. 200 molecular mass can also be used to modify ion-chromatographic separations. Eluents containing a quaternary ammonium cation and a zwitterionic anion are useful for separation of anions by IIC. An eluent containing ZH^+ and $CH_3SO_3^-$ is useful for cation chromatography with suppressed conductivity detection.

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