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Solving complex anion separation problems with a carbonate–hydrogencarbonate gradient

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Abstract

This paper describes the application of a new suppressor to solve complex anion separation problems with carbonate–hydrogencarbonate gradients. In addition to suppressing the mobile phase and enhancing the analyte signal like any other ion chromatography suppressor, the new DS-Plus suppressor removes carbonic acid from the suppressor effluent when carbonate–hydrogencarbonate mobile phases are used. This greatly reduces the background signal, enabling carbonate–hydrogencarbonate gradients. Since carbonate–hydrogencarbonate mobile phase has stronger eluting power than hydroxide or tetraborate, only dilute concentrations are needed to separate most anions. By adjusting the carbonate–hydrogencarbonate ratio in the mobile phase, various anion mixtures including those containing organic and inorganic anions with -1 to -3 charges can be separated on one or two columns, eliminating the needs for columns with special selectivity. © 2002 Elsevier Science B.V. All rights reserved.

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1. Introduction

Although gradient elution has been used widely in HPLC, its application in ion chromatography (IC) with conductivity detectors is limited because most suppressors cannot eliminate the rising background signal as the mobile phase concentration increases [1–4]. Among the gradient methods developed for IC [5–8], sodium hydroxide is the most commonly used mobile phase. Hydroxide is a good mobile phase for gradient applications because it is converted to water in the suppressor. The background conductivity after suppression is near zero throughout the gradient run, minimizing baseline shift. However, hydroxide is a weak mobile phase for anion analysis. It is a monovalent anion and has poor

selectivity for common anion-exchange functional groups. As a result, very high mobile phase concentrations are often required to elute strongly retained anions. In some cases, up to 60 mM mobile phase concentrations are required [9]. Unless an on-line eluent generator is used, pure hydroxide mobile phases are also difficult to prepare and store. Pure hydroxide salts are expensive and difficult to handle, and aqueous hydroxide solutions are readily contaminated by adsorption of carbon dioxide from the atmosphere. Pure hydroxide mobile phases are required for gradient elution or contaminant anions would pre-concentrate on the analytical column during the weaker portion of the gradient and elute as contaminant peaks when the mobile phase strength is increased. A pre-column is often used with sodium hydroxide mobile phases to prevent this problem [8].

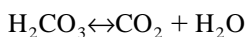
Carbonate/bicarbonate buffer has been the pre-

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ferred mobile phase for suppressor-based IC because it has high selectivity for common anion-exchange functional groups and contains both monovalent and divalent ions. Unlike sodium hydroxide, carbonate–hydrogencarbonate mobile phases offer greater power to control separation selectivity and speed. And because it has a stronger eluting power than hydroxide, only dilute mobile phase concentrations are needed for most separations. The carbonate–hydrogencarbonate salt is also available commercially in its purest form. However, gradient elution with carbonate–hydrogencarbonate mobile phases could not be used with conventional suppressors. Weakly conductive carbonic acid is the suppressor byproduct. As the mobile phase concentration increases, so does the concentration (and conductivity) of the carbonic acid suppressor effluent, producing unacceptable baseline drift.

In solution, carbonic acid exists in equilibrium with dissolved carbon dioxide gas:



In the past, post-suppressor degassing was used to remove dissolved carbon dioxide, and thus the carbonic acid, thereby reducing the background conductivity associated with carbonate–hydrogencarbonate mobile phases [6,10,11]. However, the techniques were impractical for routine use due to system complexity, band broadening, membrane-based suppressor pressure limitations, and chemical resistance constraints. The introduction of a new suppressor technology (Alltech DS-Plus suppressor) [12] overcomes the limitations of previous post-suppressor degassing techniques through the use of highly efficient, low-dead-volume, chemically resistant degassing tubing in conjunction with a continuously regenerated packed bed suppressor that tolerates high back pressures. The objective of this study is to show the power of carbonate–hydrogencarbonate gradients in solving complex anion separations using this new suppressor technology.

2. Experimental

The chromatography system consisted of a Model LC 10Ai HPLC pump (Shimadzu, MD, USA), equipped with a gradient mixer, a Model 530 column

heater, a DS-Plus suppressor, and a Model 550 conductivity detector, all from Alltech (Deerfield, IL, USA). A manual injection valve Model 9125 from Rheodyne® (Rhonert Park, CA, USA) with polyether ether ketone (PEEK) sample loops was used for injection of all samples. Alltech's Novosep A-1 Anion column (150×4.6 mm, 100×4.6 mm) and Allsep A-2 column (250×4.6 mm) were used for separations. The Dionex® (Sunnyvale, CA, USA) ASRS® membrane suppressor and IonPac® AS4A-SC (250×4.0 mm) column were also used. The Alltech Allchrom data system was used to obtain the chromatograms.

Anion standards were prepared by dilution of 1000 mg/l Certified IC standards from Alltech. The mobile phase buffer was prepared using the Alltech's EZ-LUTE buffer concentrates. Deionized water was obtained from Milli-Q (Millipore, Bedford, MA, USA) equipment with a conductivity of 0.054 $\mu\text{S}/\text{cm}$, and was used for preparing all solutions. The coffee and soil extracts were filtered through a 0.2- μm Anotop IC syringe filter (Alltech) before injection.

3. Results and discussion

The DS-Plus suppressor is a continuous, reagent-free solid-phase suppressor [12]. In addition to suppressing the mobile phase, the DS-Plus suppressor removes carbonic acid from the suppressor effluent (carbonate–hydrogencarbonate mobile phase) before detection (patents pending). This eliminates substantially all the residual carbonic acid background conductivity associated with carbonate–hydrogencarbonate mobile phases. This eliminates or minimizes the water dip that interferes with early eluting peaks, improving the quantitation of early eluting analyte peaks [13]. This also enhances detection sensitivity with carbonate–hydrogencarbonate mobile phases compared to other suppressors, and minimizes baseline shift with carbonate–hydrogencarbonate gradients. Thus, this system uses the superior separation power of carbonate–hydrogencarbonate mobile phases while retaining the best characteristics of hydroxide mobile phases (suppressed background conductivity near zero, gradient elution capability) [12].

The DS-Plus suppressor is the only suppressor currently available that allows for gradient elution with carbonate–hydrogencarbonate mobile phase. Fig. 1 shows a gradient separation of hydrophobic anions along with common anions using a carbonate–hydrogencarbonate gradient. As shown in Fig. 1b, without post-suppression degassing the baseline rapidly drifts off-scale as the carbonic acid concentration increases. As shown in Fig. 1a using the DS-Plus suppressor, the baseline shift using the same

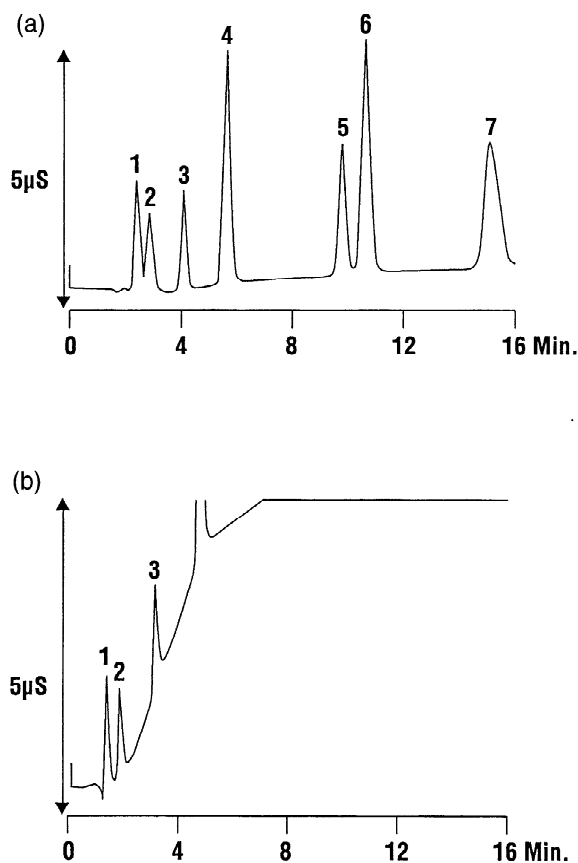


Fig. 1. Baseline shift during carbonate–hydrogencarbonate gradient: (a) with DS-Plus suppressor, (b) with suppressor without carbon dioxide degassing. Peak identification: 1=fluoride (2 mg/l), 2=chloride (2 mg/l), 3=nitrate (5 mg/l), 4=phosphate (10 mg/l), 5=iodide (20 mg/l), 6=thiocyanate (20 mg/l), 7=thiosulfate (20 mg/l). Column: IonPac[®] AS4A-SC, 250×4.0 mm; mobile phase: (A) water, (B) 10 mM sodium carbonate, 1 mM *p*-cyanophenol; gradient: 30% B to 50% B in 3 min, to 70% B in 12 min, to 30% B in 2 min. Flowrate: 1.5 ml/min, detection: suppressed conductivity; injection volume: 25 μl .

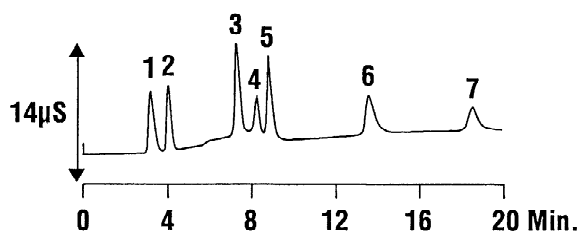


Fig. 2. Separation of monovalent, divalent, and trivalent anions. Peak identification: 1=acetate (5 mg/l), 2=chloride (1 mg/l), 3=phosphate (5 mg/l), 4=sulfate (1 mg/l), 5=oxalate (5 mg/l), 6=perchlorate (5 mg/l), 7=citrate (5 mg/l). Column: Novosep A-1, 100×4.6 mm; mobile phase: (A) water, (B) 10 mM sodium bicarbonate, (C) 10 mM sodium carbonate and 1 mM *p*-cyanophenol; gradient: 17% B and 0% C to 28% B and 22% C in 5 min, to 50% B and C in 5 min, to 100% C in 5 min, hold for 5 min, to 17% B and 0% C in 0.1 min. Flowrate: 1.5 ml/min; detection: suppressed conductivity; injection volume: 100 μl .

carbonate gradient is dramatically reduced. All peaks are easily detected.

Fig. 2 shows the separation of weak acid anions along with common anions and the strongly retained trivalent anion, citrate. The gradient is started with a weaker eluent strength such as sodium bicarbonate.

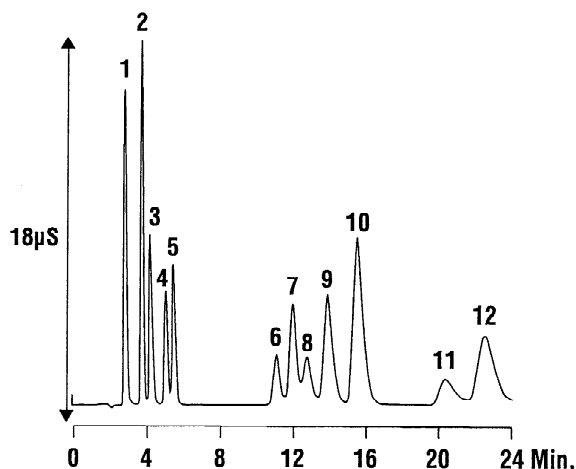


Fig. 3. Isocratic separation of early and late eluting anions. Peak identification: 1=fluoride (1 mg/l), 2=chloride (2 mg/l), 3=nitrite (2 mg/l), 4=bromide (2 mg/l), 5=nitrate (2 mg/l), 6=phosphate (3 mg/l), 7=selenite (10 mg/l), 8=arsenate (10 mg/l), 9=sulfate (3 mg/l), 10=selenate (10 mg/l), 11=tungstate (10 mg/l), 12=molybdate (10 mg/l). Column: Novosep A-1, 150×4.6 mm; mobile phase: 1.9 mM sodium bicarbonate/1.1 mM sodium carbonate. Flowrate: 1.5 ml/min; detection: suppressed conductivity; injection volume: 100 μl .

This enables to resolve the closely eluting acetate and chloride peaks. The mobile phase strength is then ramped up by adjusting the carbonate–hydrogencarbonate ratio to elute other multivalent anions such as phosphate, sulfate and oxalate along with the highly retained perchlorate and citrate anions. Cyanophenol was added to the mobile phase to sharpen the citrate peak. Under isocratic conditions, two separate conditions and possibly columns (ion exchange and ion exclusion) would be needed for this separation. With a hydroxide gradient, a column with special selectivity is needed to achieve this separation, and a much higher mobile phase concentration (up to 60 mM) is needed to elute the trivalent citrate [9].

Since carbonate–hydrogencarbonate has a strong elution power, by combining it with the correct column chemistry, certain complex mixture of anions can be separated with isocratic elution as shown in Fig. 3. However, when weakly retained anions such as acetate and formate are present in the same

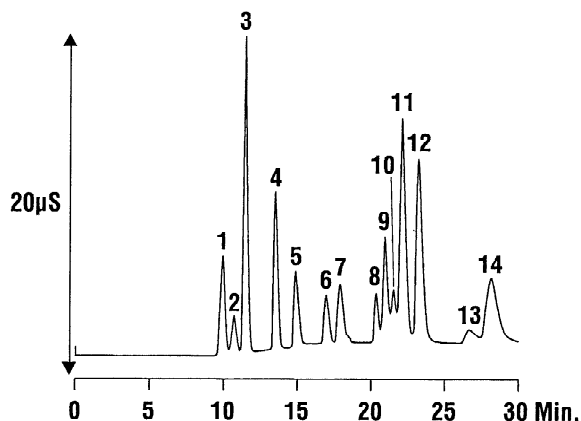


Fig. 4. Gradient separation of early and late eluting anions. Peak identification: 1=fluoride (1 mg/l), 2=acetate (10 mg/l), 3= formate (10 mg/l), 4=chloride (2 mg/l), 5=nitrite (2 mg/l), 6=bromide (2 mg/l), 7=nitrate (2 mg/l), 8=phosphate (3 mg/l), 9=selenite (10 mg/l), 10=arsenate (10 mg/l), 11=sulfate (3 mg/l), 12=selenate (10 mg/l), 13=tungstate (10 mg/l), 14= molybdate (10 mg/l). Column: Novosep A-1, 150×4.6 mm; mobile phase: (A) 1.0 mM sodium bicarbonate, (B) 2.8 mM sodium bicarbonate/2.2 mM sodium carbonate. Gradient: 0% B for 5 min, to 100% B in 10 min, hold for 15 min. Flowrate: 1.0 ml/min; detection: suppressed conductivity; injection volume: 100 μl.

sample, gradient elution may be used to improve resolution, as shown in Fig. 4. Under these gradient conditions, a weaker strength of sodium bicarbonate eluent was used initially to separate fluoride, acetate, formate and chloride. Then a mixture of carbonate and bicarbonate eluent was used to elute the strongly retained anions.

The separation of anions in soil is shown in Fig. 5. Fluoride, acetate, formate, chloride, nitrate, selenite and sulfate are detected in this sample. Other anions such as selenate, tungstate, and molybdate can also be detected with this method.

In Fig. 6, a carbonate–hydrogencarbonate gradient separates the anions commonly found in refinery wastewater during sour gas removal. The eluent strength of the mobile phase is kept as weak as possible to avoid the co-elution of early eluting anions, fluoride and propionate, and also bromate

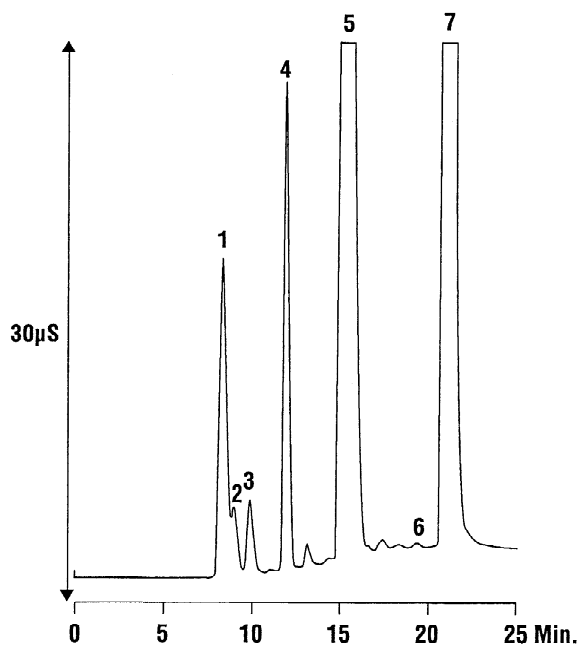


Fig. 5. Anions in soil. Peak identification: 1=fluoride, 2=acetate, 3= formate, 4=chloride, 5= nitrate, 6= selenite, 7= sulfate. Column: Novosep A-1, 150×4.6 mm; mobile phase: (A) 1.0 mM sodium bicarbonate, (B) 2.8 mM sodium bicarbonate/2.2 mM sodium carbonate. Gradient: 0% B for 5 min, to 100% B in 10 min, hold for 15 min. Flowrate: 1.0 ml/min; detection: suppressed conductivity; injection volume: 100 μl.

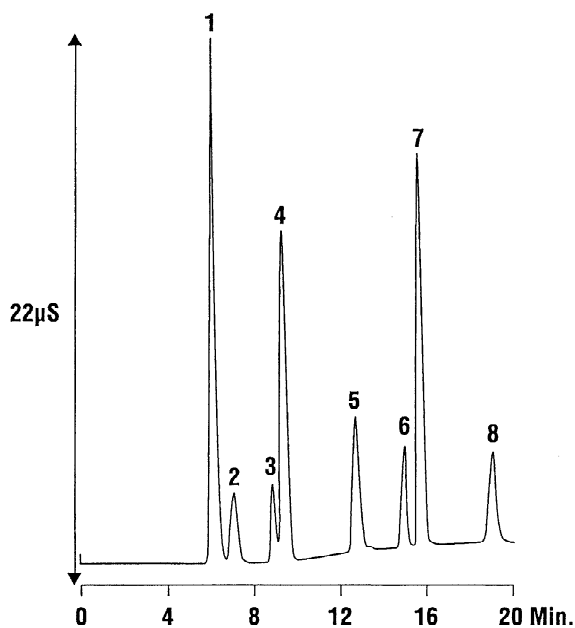


Fig. 6. Anions in refinery wastewater. Peak identification: (10 mg/l of each) 1=fluoride, 2=propionate, 3=bromate, 4=chloride, 5=nitrate, 6=phosphate, 7=sulfate, 8=thiosulfate. Column: Novosep A-1, 150×4.6 mm; mobile phase: (A) 1.0 mM sodium bicarbonate, (B) 5.0 mM sodium carbonate. Gradient: 0%B for 5 min, to 100% B in 10 min, hold for 5 min. Flowrate: 1.5 ml/min; detection: suppressed conductivity; injection volume: 50 μ l.

and chloride. The carbonate concentration is then increased to 5 mM to elute thiosulfate. The excellent peak shape of thiosulfate is obtained without the addition of any organic modifiers.

Gradient elution with carbonate–hydrogencarbonate buffer can also be applied for the analysis of fermentation broth extracts. As shown in Fig. 7, this complex mixture contains weakly retained organic acids, inorganic anions, and strongly retained trivalent anions. Initially, a very weak mobile phase was used to elute the weakly retained organic acids. The carbonate concentration was then increased slowly to 6 mM to elute inorganic anions and moderately retained organic acids. Finally the carbonate concentration was increased to 20 mM to elute the strongly retained trivalent anions. Organic modifier, 1 mM *p*-cyanophenol was added in the carbonate mobile phase to improve the peak shape of the late eluting anions. With isocratic elution, three

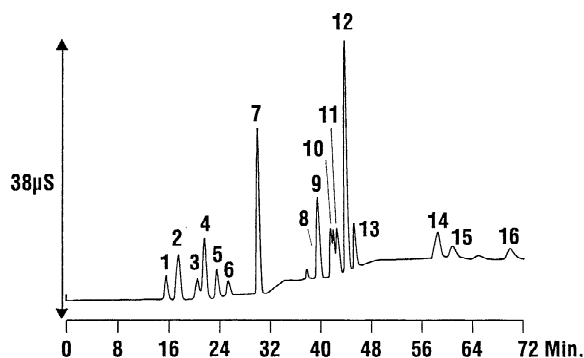


Fig. 7. Analytes found in fermentation broth. Peak identification: 1=lactate, 2=acetate, 3=propionate, 4=formate, 5=butyrate, 6=pyruvate, 7=chloride, 8=nitrate, 9=succinate, 10=malate, 11=phosphate, 12=sulfate, 13=oxalate, 14=citrate, 15=isocitrate, 16=aconitate. Column: Allsep A-2, 250×4.6 mm; mobile phase: (A) water, (B) 10 mM sodium bicarbonate, (C) 20 mM sodium carbonate and 1 mM *p*-cyanophenol; gradient: 21% B and 5% C for 20 min, to 30% B and C in 5 min, hold for 15 min, to 100% C in 5 min, hold for 30 min, to 21% B and 5% C in 0.1 min. Flowrate: 1.5 ml/min; detection: suppressed conductivity; injection volume: 100 μ l.

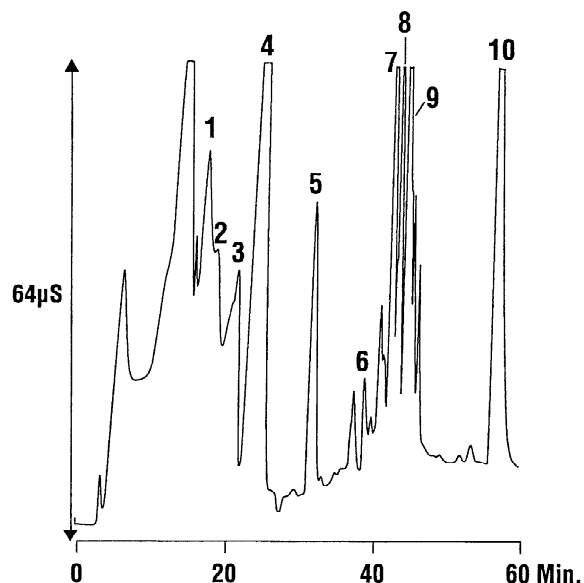


Fig. 8. Organic and inorganic anions in coffee. Peak identification: 1=lactate, 2=acetate, 3=formate, 4=pyruvate, 5=chloride, 6=nitrate, 7=phosphate, 8=sulfate, 9=oxalate, 10=citrate. Column: Allsep A-2, 250×4.6 mm; mobile phase: (A) water, (B) 10 mM sodium bicarbonate, (C) 20 mM sodium carbonate and 1 mM *p*-cyanophenol; gradient: 21% B and 5% C for 20 min, to 30% B and C in 5 min, hold for 15 min, to 100% C in 5 min, hold for 30 min, to 21% B and 5% C in 0.1 min. Flowrate: 1.5 ml/min; detection: suppressed conductivity; injection volume: 100 μ l.

separate conditions and more than one column may be required. With a carbonate–hydrogencarbonate gradient, this 16-anion mixture can be separated within 70 min. Even with a comparatively high concentration of carbonate in the mobile phase, the baseline drift is still low. An application of the same gradient for separation of anions in coffee is shown in Fig. 8.

4. Conclusion

The DS-Plus suppressor simplifies and improves complex anion separations in ion chromatography. Since the DS-Plus suppressor removes carbonic acid from the suppressor effluent, anion separations with carbonate–hydrogencarbonate gradients are possible. By adjusting the carbonate–hydrogencarbonate ratio in the mobile phase, one can perform a wide variety of gradient separations.

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