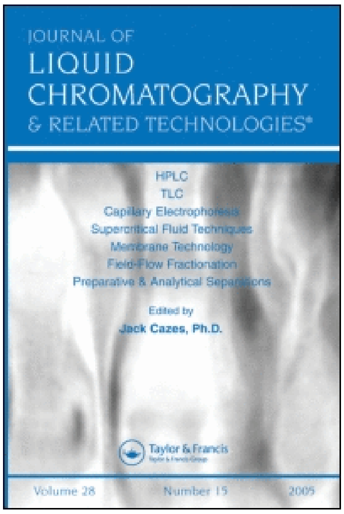

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Roy D. Rocklin[a]; Rosanne W. Slingsby[a]; Christopher A. Pohl[a]
[a] Dionex Corp., Sunnyvale, California

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SEPARATION AND DETECTION OF CARBOXYLIC ACIDS BY ION CHROMATOGRAPHY

Roy D. Rocklin, Rosanne W. Slingsby,
and Christopher A. Pohl
Dionex Corp.
P.O. Box 3603
Sunnyvale, California 94088-3603

ABSTRACT

Common carboxylic acids such as most Krebs cycle acids and other organic acids can be separated by either anion exchange or ion exclusion chromatography and detected by conductivity with chemical suppression. This is accomplished using a new generation of high dynamic capacity suppressors which allow separations on moderate and high capacity resins with concentrated eluants. For both anion exchange and ion exclusion chromatography, cation exchange membrane based suppressors are used to reduce the conductivity of the eluant while enhancing the conductivity of the organic acid analytes. The ability to choose between anion exchange and ion exclusion chromatography expands the versatility of ion chromatography by increasing the analyst's ability to solve co-elution problems.

INTRODUCTION

Carboxylic acids can be separated by several different liquid chromatographic methods. These

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include anion exchange (1), ion exclusion (2), and reversed phase chromatography (3). Although UV absorbance is the most commonly used detection method in HPLC, lack of a strong chromophore on most common aliphatic carboxylic acids results in interference problems and poor sensitivity. As in the determination of inorganic ions, conductimetric detection takes advantage of the ionic nature of the acids. Since eluants consisting of even dilute acids and bases are highly conductive, and since background noise is highly dependent on background conductivity, maximum detection sensitivity can only be achieved when the background conductivity of the eluant is low. The most effective method of reducing background conductivity is chemical eluant suppression. This technique produces at least an order of magnitude improvement in sensitivity as compared to UV absorbance.

Ion chromatography with chemical suppression was introduced in 1975. In the original publication (4), separation and suppression mechanisms were presented for anion and cation exchange separations. The anion method was used primarily for inorganic anions rather than for organic acids for three reasons. First, the major application of ion chromatography was perceived to be inorganic ion determinations. Second, the selectivity provided by the early low capacity separator columns was not sufficient to separate many carboxylic acids of similar charge. Third, many di- and triprotic carboxylic acids could only be eluted by more concentrated eluants than were being used to elute inorganic ions. These eluants would rapidly expend the capacity of the packed bed suppressors, thus making the analysis inconvenient. With the introduction of continuously-regenerated fiber suppressors (5), inorganic ion analysis became much more convenient, as periodic regeneration of the suppressor was no longer necessary. However, fiber suppressors still could not be used for many carboxylic acid separations, since their suppression capacity was not high enough for the eluant concentrations required. As described in this paper, new high efficiency low and medium capacity columns combined with a new generation of micromembrane suppressors capable of suppressing concentrated eluants have made the determination of carboxylic acids by anion exchange practical.

A second method for carboxylic acid determination is ion exclusion chromatography, often called ion chromatography exclusion, or ICE. Using this method, carboxylic acids are separated on cation exchange resins using dilute acid eluants. In the original publication describing ICE, a UV detector was used (2). Conductimetric detection was not used until the introduction of a suppressor filled with cation exchange resin in the silver form (6, 7). This suppressor was used to precipitate chloride from the dilute hydrochloric acid eluant, and lasted only about 40 hours before being fully expended. Due to the high sensitivity obtained, this suppressor is still used, although its principal disadvantage is that the resin can not easily be regenerated, and instead must be replaced. In this paper, a suppression mechanism is described which is based on exchange of highly conductive hydrogen ions in the eluant with much less conductive tetrabutylammonium ions. Using this method, the suppressor is continuously regenerated (8).

EXPERIMENTAL SECTION

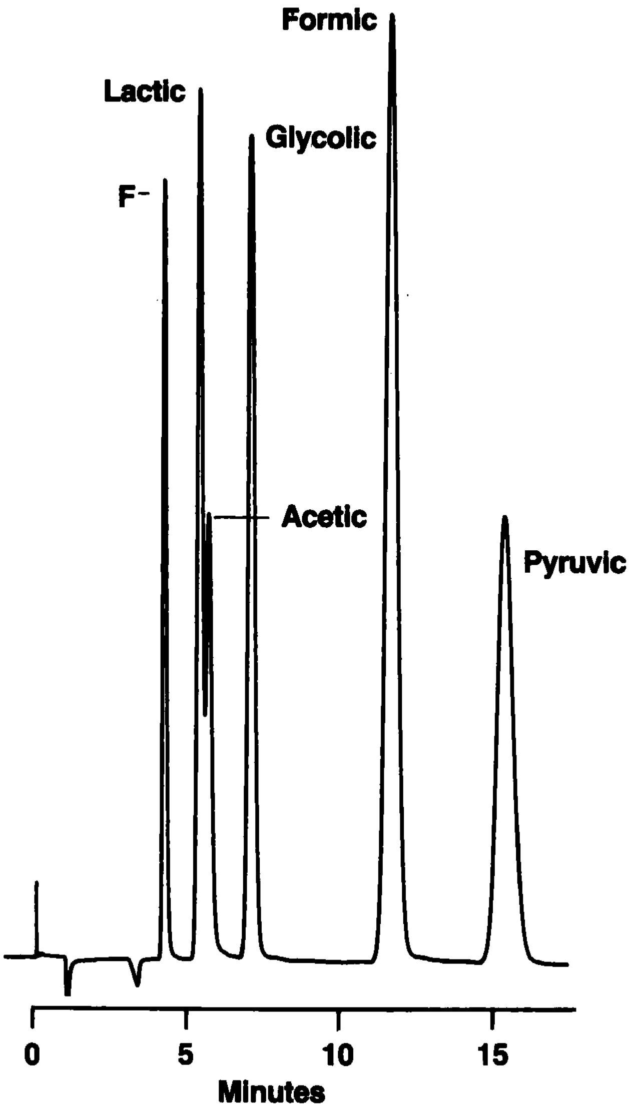
All chromatography was performed on a Dionex 2010i ion chromatograph. This system consists of a pump, a chromatography module, and a conductivity detector. The column dimensions are as follows: HPIC-AS4 (anion exchange): 4 X 250 mm; HPIC-AS6 (anion exchange): 4 X 250 mm; HPICE-AS1 (ion exclusion): 9 X 250 mm. The anion exchange columns differ in their capacity and degree of resin cross-linking. The octanesulfonic acid used was Dionex Ion Sep reagent MPIC-C2, a purified 0.1 N solution. Tetrabutylammonium hydroxide (TBAOH) was obtained from Aldrich as a 40% solution, or from

Southwestern Analytical (Austin, TX) as a 1M electrometric grade solution. All other chemicals were reagent grade. Unless otherwise specified, the sample injection size was 50 µL. The Anion Micromembrane Suppressor was used with 18 mN sulfuric acid regenerant at a flow rate of approximately 10 mL/min. The Anion Fiber Suppressor 2 was used with 10 mM TBAOH regenerant at a flow rate of approximately 3 mL/min.

RESULTS AND DISCUSSION

Anion Exchange

Carboxylic acids determined by anion exchange are separated using medium and low capacity pellicular styrene/divinylbenzene resins. Due to the large retention differences between ions of differing charge, both the ion exchange capacity of columns and eluant concentration must be varied to elute mono-, di-, and triprotic acids. Monoprotic carboxylic acids such as formic, glycolic, and pyruvic acids are weakly retained, but can be separated using a medium capacity column (about 0.36 meq), the AS6. The eluant used is 20 mM sodium borate, $Na_2B_4O_7$. This separation is illustrated in Figure 1. Most diprotic carboxylic acids such as tartaric and succinic are separated on the AS6 using 20 mM sodium carbonate, 2 mM sodium hydroxide as the eluant. This eluant is much stronger

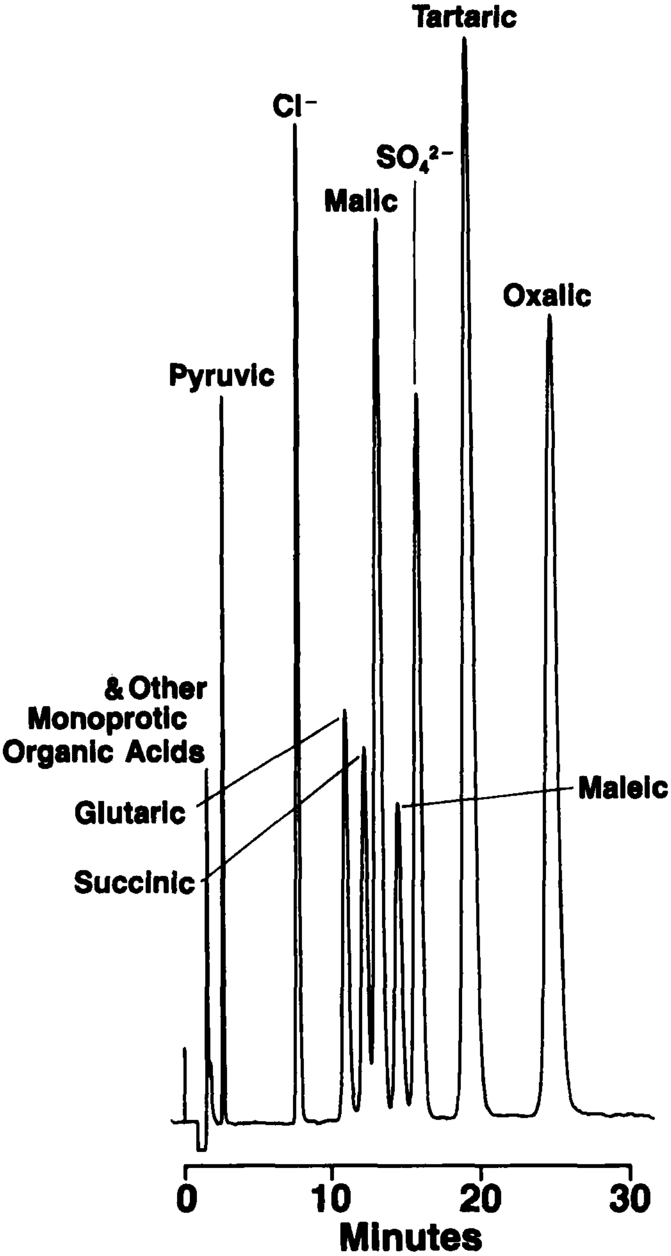


1. Monoprotic organic acids separated by anion exchange. The standard contained 1.0 µg of each acid (0.1 µg F^-). 20 mM $Na_2B_4O_7$ eluant was pumped at 1.5 mL/min through an AS6 separator column and an Anion Micromembrane Suppressor. The detector range was 30 µS.

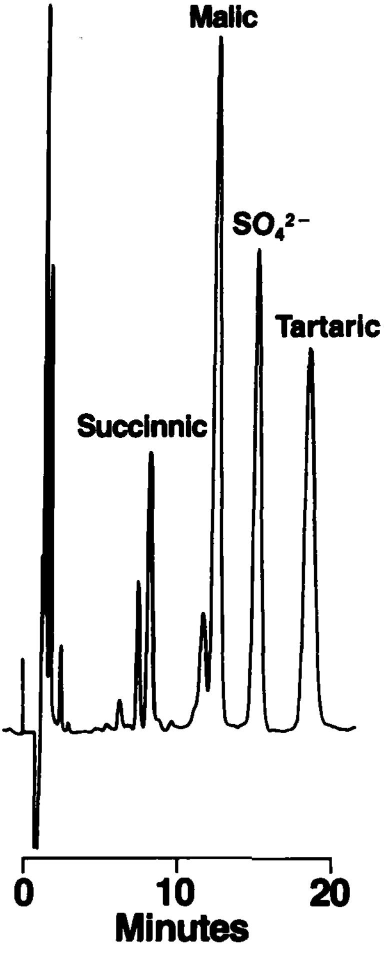
as it contains the divalent displacer ion carbonate. This separation is illustrated using standards in Figure 2, and in wine in Figure 3. Several strongly retained diprotic acids as well as the triprotic acids such as citric acid are separated using the same carbonate/hydroxide eluant but on a column of much lower ion exchange capacity, the AS4 (about 0.028 meq). This separation is illustrated in Figure 4.

As compared to other separation methods such as ICE (described next), anion exchange provides improved selectivity within the three groups of acids listed above. This is particularly true among the stronger acids such as most of the di- and triprotic acids. Of the di- and triprotic acids which are in the Krebs cycle or are commonly found in foods, there are only two groups of co-eluting acids. These are malic and malonic, and isocitric and cis-aconitic. Another advantage of anion exchange separation is that the inorganic anions fluoride, chloride, and sulfate can be determined simultaneously with the organic acids.

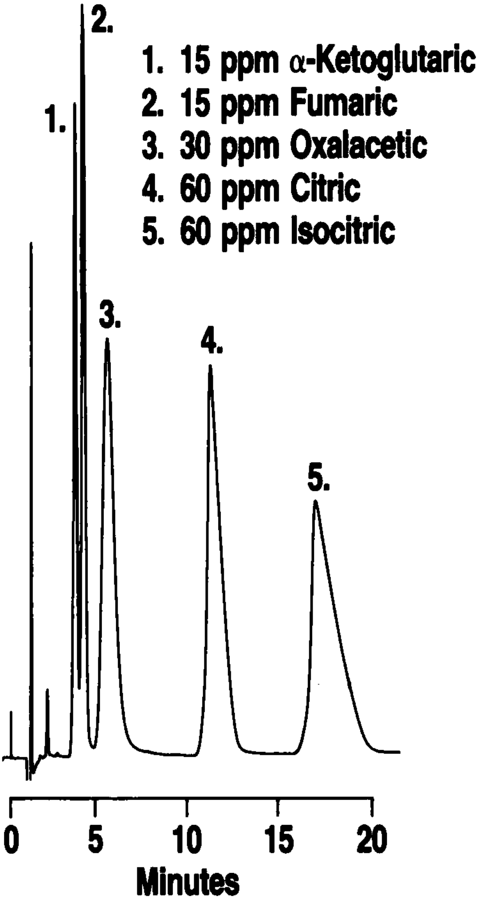
The eluants used to separate carboxylic acids by anion exchange are approximately 5 to 10 times more concentrated than those which have commonly been used in ion chromatography. These eluants can only be suppressed using a new suppressor device, the Anion Micro-Membrane Suppressor (AMMS) (9). Using an eluant



2. Diprotic organic acids separated by anion exchange. 20 mM Na_2CO_3, 2 mM NaOH eluant was pumped at 1.5 mL/min through an AS6 separator and an Anion Micromembrane Suppressor. The detector range was 10 μS.



3. Diprotic acids in wine. 1.5 mL wine was diluted in 100 mL water. Conditions are the same as in fugure 2.



4. Triprotic and strongly retained diprotic organic acids separated by anion exchange. 20 mM Na_2CO_3, 2 mM NaOH eluant was pumped at 1.5 mL/min through an AS4 separator column and an Anion Micromembrane Suppressor. The detector range was 10 µS.

flow rate of 1.0 mL/min., it is capable of suppressing eluants with sodium ion concentrations as high as 200 mM. Detection of the carboxylic acids separated by the methods described here would not be possible without this suppressor.

One disadvantage of anion exchange is that the three groups of carboxylic acids must be determined in separate analyses. Methods to combine these separations using gradient elution are currently being studied. These results will be discussed in a future publication.

Ion Chromatography Exclusion

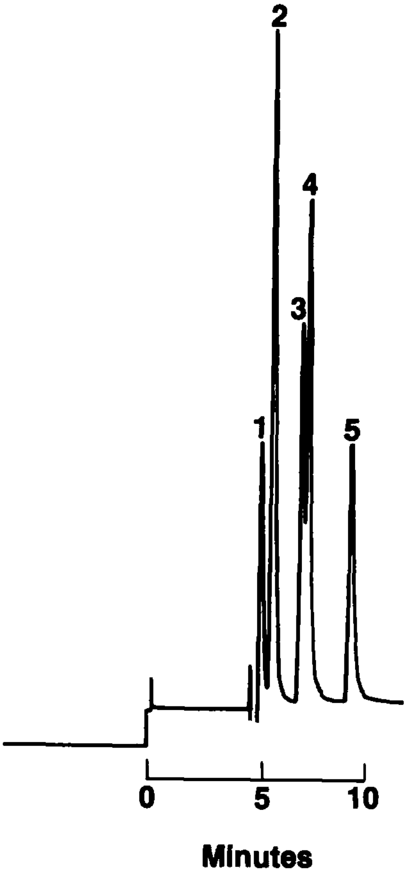
The resin in the HPICE-AS1 ICE separator is a high capacity, fully sulfonated styrene/divinylbenzene cation exchange resin. The separation mechanism is largely Donnan exclusion of the acids. Hydrophobic interactions and steric effects also influence retention. Strong acids (such as mineral acids) are 100% ionized. Since they are anionic, they are excluded from the negatively charged pore structure of the sulfonated resin by Donnan exclusion and elute early in the chromatogram. At the eluant pH used, very weak acids (pK > 5) are neutral, and are more retained because they can penetrate the pore volume of the resin. Acids of intermediate strength such as most carboxylic acids are partially ionized. They generally elute in order of pKa, with stronger acids eluting earlier than weaker acids. The degree of dissociation (and therefore retention) can be controlled by adjusting the concentration of the dilute strong acid eluant.

The conductivity of the dilute strong acid eluant must be reduced prior to detection in order to improve the analyte signal to baseline noise ratio and minimize baseline drift. This is partially accomplished by choosing an acid with an anion of low conductivity. Useful acids include alkyl sulfonic acids such as octanesulfonic acid, and totally fluorinated carboxylic acids such as per-fluoroheptanoic acid, $C_6F_{13}CO_2H$. In addition, the conductivity of the acid eluant is reduced in the suppressor by exchanging the highly conductive hydrogen ions with the much lower conductive tetrabutylammonium ions.

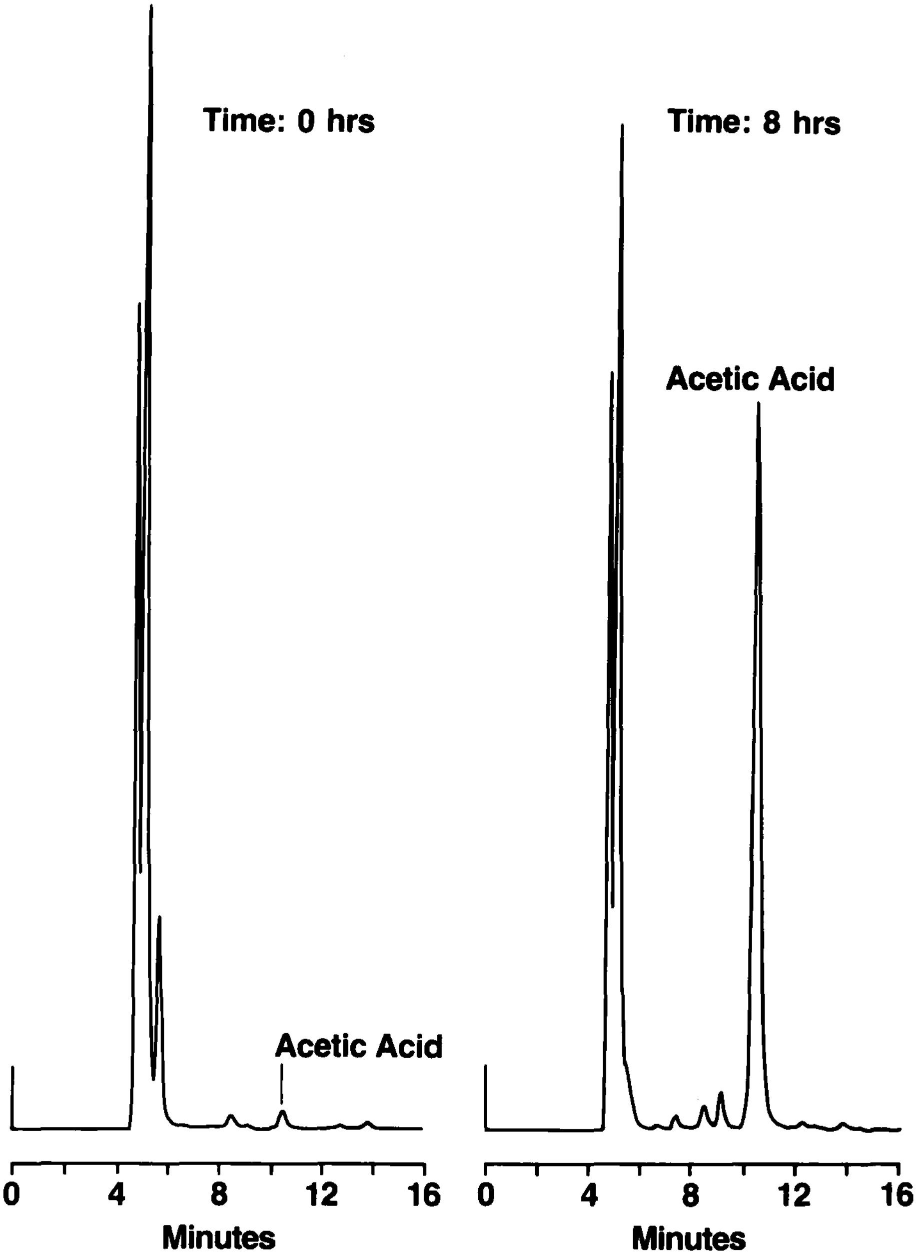
The suppressor is composed of a cation exchange membrane fiber through which the eluant flows, surrounded by the tetrabutylammonium hydroxide regenerant. The driving force for the exchange reaction is the neutralization of hydrogen ions by hydroxide, as well as the concentration gradients across the membrane. Not only is the background conductivity of the eluant suppressed, but the conductivity of the carboxylic acid analytes is enhanced. This is due to the tetrabutylammonium ions replacing the hydrogen counterions, thus ensuring quantitative dissociation of the weaker carboxylic acids.

A chromatogram of five carboxylic acids is illustrated in Figure 5. Separation by ICE is different from anion exchange in several respects. When separating monoprotic carboxylic acids by anion exchange, di- and triprotic carboxylic acids can be retained on the resin indefinitely. Using ICE, these three groups of acids elute in one injection. Since mono-, di-, and triprotic acids can be determined in one analysis, information regarding their presence can be obtained quickly. Another advantage of ICE is that the monoprotic acids acetic, propionic, and butyric are resolved, but co-elute using anion exchange. Also, strong acid anions, such as chloride and sulfate, elute in the void volume. When their concentrations are high, they are less likely to interfere with an analysis using ICE instead of anion exchange.

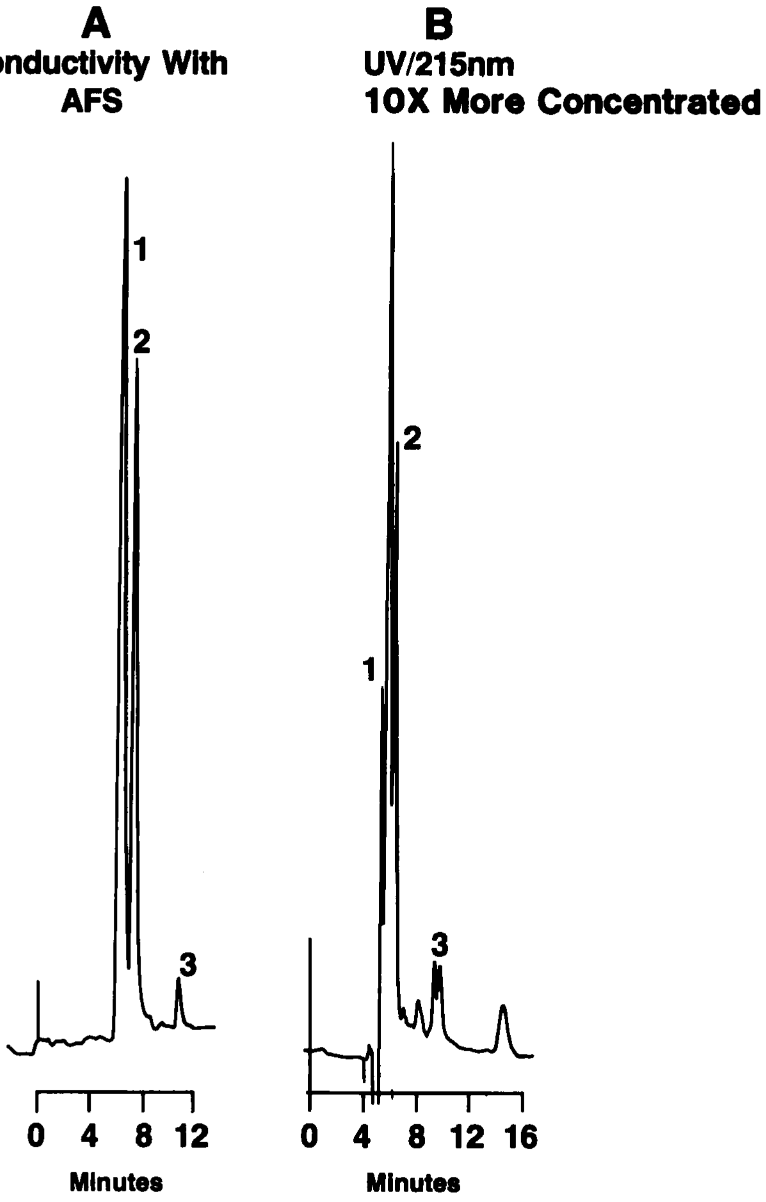
The most common analyses requiring organic acid determinations are of biochemical or food samples. The determination of acetic acid in a fermentation broth is illustrated in Figure 6. This shows how the ICE mode can easily be used to monitor the increase in acetic



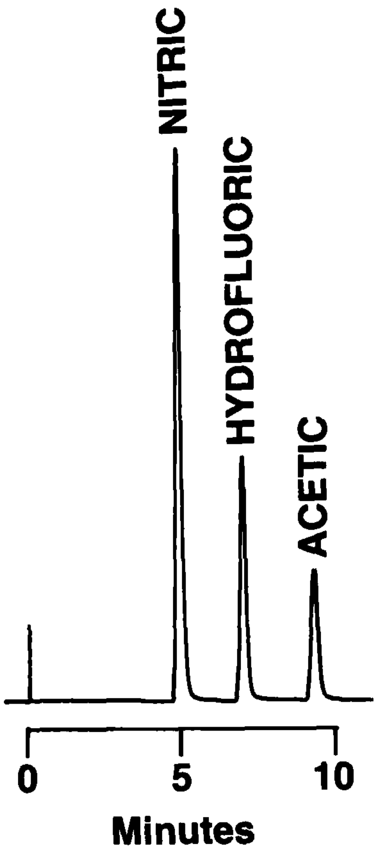
5. 1 µg each of organic acids separated by Ion Chromatography Exclusion. Peak #1 is citric acid; #2 malic; #3 succinic; #4 lactic; #5 acetic. 0.5 mM octanesulfonic acid, 1.5% 2-propanol eluant was pumped at 1.0 mL/min through an HPICE-AS1 separator and an Anion Fiber Suppressor 2. The column temperature was maintained at 50 deg. C.



6. The increase in acetic acid concentration in a fermentation broth is monitored using ICE. The conditions are the same as in figure 5 except the eluant is 1 mM perfluoroheptanoic acid.



7. Comparison of detection modes in the analysis of milk. Peak #1 is citric, #2 is pyruvic, #3 is lactic acid. Chromatogram A demonstrates the increased signal-to-noise ratio and selectivity of suppressed conductivity detection as compared to low wavelength UV (chromatogram B).



8. Analysis of a mixed acid etchant by ICE. The eluant was 1 mM octanesulfonic acid, 5% 2-propanol at a flow rate of 1.0 mL/min. An HPICE-AS1 separator was used with an Anion Fiber Suppressor 2. The column temperature was maintained at 35 deg. C.

acid (an important metabolite) in the broth. In Figure 7 milk acids are separated by ICE and detected by both conductivity and UV absorbance. Conductivity is shown to be much more specific and and at least an order of magnitude more sensitive than UV detection. ICE can also be used to analyze non-biochemical samples. The analysis shown in Figure 8 is of a mixed acid etchant used to etch semiconductors during their manufacture. The nitric acid elutes first, followed by hydrofluoric (pKa 3.45), and then acetic (pKa 4.75). Since the rate at which the semiconductors are etched is dependent on the concentrations of the acids in the mix, it is essential that an accurate analysis be performed. The peak height reproducibility for repeat injections of this sample was measured at 1% or better for each acid (11 injections).

CONCLUSION

Advanced ion exchange columns and membrane-based suppressors have expanded the options available for determination of organic acids in a variety of sample matrices. Now, both anion exchange and ion exclusion separations can be used with continuously regenerated suppressors. Sensitive and specific conductivity detection and selective separation methods provide an additional tool for solving difficult chemical analysis problems. When combined with the ability to determine inorganic ions, these improved methods for carboxylic acids greatly expand the scope of chemical analysis problems for which ion chromatography is the method of choice (10).

REFERENCES

1. Bouyoucos, S. A. "Determination of Organic Anions by Ion Chromatography Using a Hollow Fiber Suppressor", J. Chromatogr. 242, 170, (1982)

2. Turkelson, V. T.; Richards, M. "Separation of Citric Acid Cycle Acids by Liquid Chromatography" Anal. Chem. 50, 1420 (1978)

3. Manning, D. L.; Maskarinec, M. P. "Determination of Low Molecular Weight Carboxylic Acids in Water by HPLC with Conductivity Detection" J. Liq. Chrom. 6, 705 (1983)

4. Small, H.; Stevens, T. S.; Bauman, W. C. "Novel Ion Exchange Chromatographic Method Using Conductometric Detection" Anal. Chem. 47, 1801 (1975)

5. Stevens, T. S.; Davis, J. C.; Small, H. "Hollow Fiber Ion-Exchange Suppressor For Ion Chromatography" Anal. Chem. 53, 1488 (1981)

6. "Analysis of Organic Acids in Coffee, Wine, and Carbonated Beverages" Application notes 19, 21, 25. Dionex Corp., Sunnyvale, CA.

7. Rich, W.; Johnson, E.; Sidebottom, T. U.S. patent #4,242,097

8. Pohl, C.; Slingsby, R.; Johnson, E.; Angers, L. U.S. patent #4,455,233

9. Stillian, J. "A Revolutionary New Suppressor for Ion Chromatography" L. C. Mag., Sept. 1985

10. Franklin, G. O. "Ion Chromatography - An Indispensable Tool" Amer. Lab., June, 1985