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Denver, CO, October 6–9, 1991



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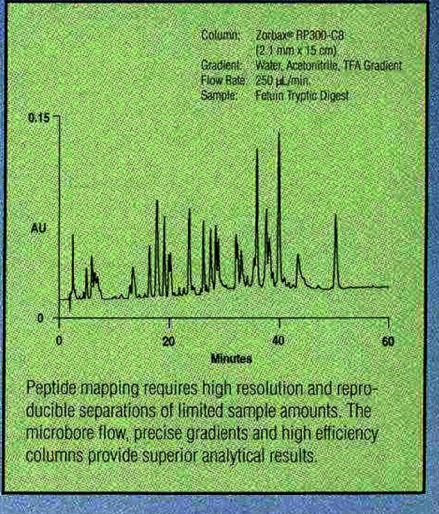


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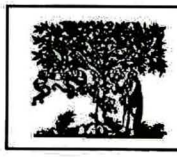

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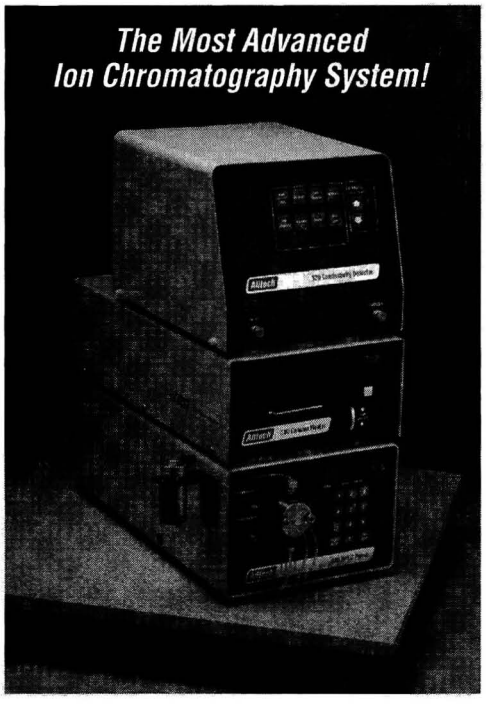

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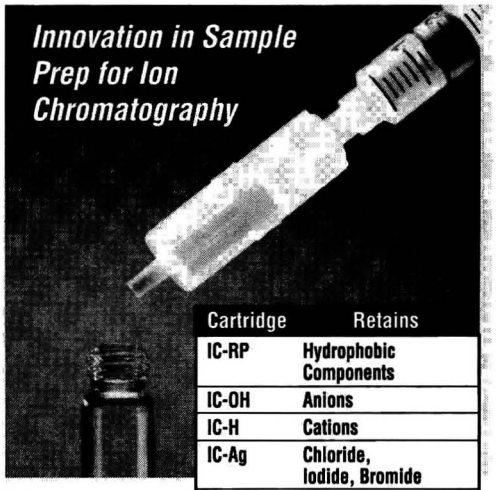

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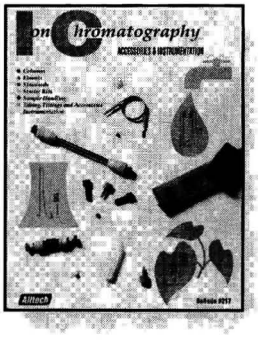


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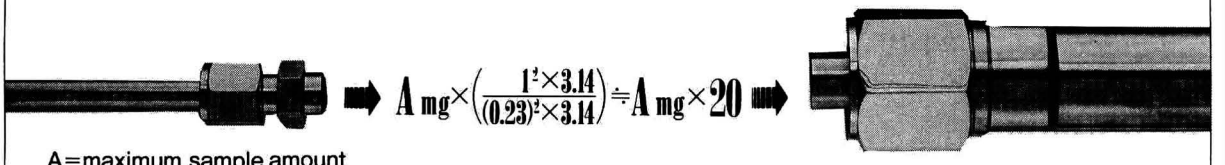


A=maximum sample amount of Analytical column

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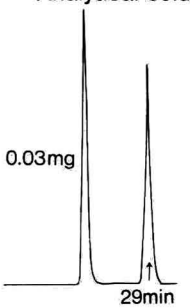



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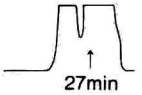


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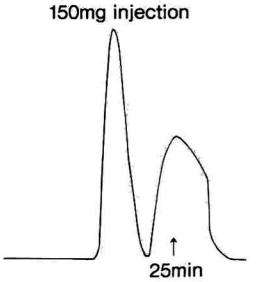


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## SYMPOSIUM VOLUMES




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SYMPOSIUM VOLUME


# INTERNATIONAL ION CHROMATOGRAPHY SYMPOSIUM 1991

*Denver, CO (USA), October 6–9, 1991*

*Guest Editor*

JOHN D. LAMB
(Provo, UT)

VOL. 602 NOS. 1+2 JOURNAL OF CHROMATOGRAPHY JUNE 5, 1992

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INTERNATIONAL ION CHROMATOGRAPHY SYMPOSIUM 1991, DENVER, CO, OCTOBER 6–9, 1991

In articles with more than one author, the name of the author to whom correspondence should be addressed is indicated in the article heading by a 6-pointed asterisk (*).

*Journal of Chromatography*, 602 (1992) 1
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CHROMSYMP. 2561

# Foreword

The *International Ion Chromatography Symposium 1991* (IICS '91) was held at the modern, luxurious Hyatt Regency Tech Center, Denver, Colorado, USA, October 6–9, 1991. IICS '91 was attended by over 240 scientists from 13 countries and 37 US States and featured 70 oral presentations and 29 posters. IICS '91 was the second in the series, following the merging last year of a number of smaller IC conferences. The conference was designed to serve ion chromatographers worldwide by covering a wide variety of topics, including all aspects of the technique.

The symposium opened with a lively plenary lecture by Professor Purnendu (Sandy) K. Dasgupta. Professor Dasgupta's research focused on "Ion Chromatography–*Quo Vadis Domine*?", a personal vision of where IC is headed. It included many innovative concepts in both ion chromatography and capillary electrophoresis. The remainder of the symposium agenda was organized around the following session topics: Fundamental principles and general aspects of ion chromatography, Separations using novel stationary and mobile phases, Novel applications, Sample handling and pre-treatment, Industrial problem solving, Gradient separations, Detection and post-column treatment, and Capillary ion electrophoresis (capillary ion analysis).

A highlight of the meeting was the recognition of two scientists for their outstanding contributions to ion chromatography. The recipients of the Annual Ion Chromatography Achievement Awards were Dr. John Riviello (Dionex Corporation) and Dr. William R. Jones (Waters Chromatography Division of Millipore). Each presented a keynote lecture, Dr. Riviello on Conductometric detection in ion chromatography: a historical perspective, and Dr. Jones on Scope of capillary ion analysis.

On Sunday afternoon, before the conference proceedings began, Dionex and Waters hosted a Short Course in Ion Chromatography. The course was presented to an overflow crowd and was enthusiastically received. After Tuesday's lunch, attendees were treated to an intellectual break as Professor Paul Palmer of Brigham Young University's Physics Department spoke on the topic Cold fusion: what it is and what it isn't.

The success of IICS '91 was due chiefly to the novelty and quality of the technical presentations and the lively contributions of the participants. Thanks are due to the members of the scientific committee (G. K. Bonn, R. M. Cassidy, J. S. Fritz, D. T. Gjerde, P. R. Haddad, P. Jandik, J. D. Lamb, D. J. Pietrzyk, G. Schmuckler, H. Small and J. R. Stillian) for their valuable contributions to the design of the symposium. Special recognition is also due to Janet Strimaitis of Century International for the superb manner in which the meeting was organized, and to Dr. Erich Heftmann of the *Journal of Chromatography* for serving as editor of this proceedings volume. The symposium attendees express special appreciation to the joint corporate sponsors, Dionex Corporation and Waters Chromatography Division of Millipore, for financial support. We look forward to IICS '92, to be held in Linz, Austria, under the chairmanship of Guenther K. Bonn of the Johannes Kepler University.

*Provo, UT (USA)* John D. Lamb
Symposium Chairman

*Journal of Chromatography*, 602 (1992) 3–8
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CHROMSYMP. 2504

# Ion-interaction chromatography: a study of the distribution of *n* -alkylammonium ions on an ODS-2 column

Louis G. Daignault* and D. Paul Rillema

*Department of Chemistry, University of North Carolina at Charlotte, Charlotte, NC 28223 (USA)*

ABSTRACT

The distribution of *n*-alkylammonium ions on a Whatman ODS-2 reversed-phase column was investigated. It was found that only about 24% of the retained *n*-alkylammonium ions act as ion-pairing reagents on the column surface. Approximately 76% of the *n*-alkylammonium ions are electrostatically interacting with the deprotonated surface silanols.

## INTRODUCTION

Recently we reported the distribution of *n*-octylammonium ions sorbed on the surface of an octadecylsilane reversed-phase column [1]. The ion-pairing agent was absorbed in at least two forms. The major amount was attached to the silanol groups; the minor fraction was absorbed as the ionic dihydrogen phosphate salt. This find was at variance with previous workers' interpretations which basically assumed that the amount sorbed on the surface was equal in concentration to the amount in the mobile phase [2–5]. Here we focus our attention on the distribution of various *n*-alkylammonium ions on the octadecylsilane reversed-phase column in order to understand separation efficiency.

## EXPERIMENTAL

The high-performance liquid chromatography (HPLC) system used in this study consisted of a Perkin-Elmer (P-E) Series 2/2 solvent delivery system equipped with a Rheodyne Model 7125 injector valve (20-$\mu$l loop) and a Rheodyne Model 7066 tandem column selector (5 columns and a bypass/flush out tube), a P-E Model LC-75 variable-wavelength detector, and a P-E Model LC Auto control. Recordings of the chromatograms were made on a Fisher Recordall Series 5000. A Whatman Partisil 1025 ODS-2 analytical column with a Whatman CO:PELL ODS guard column was used for the HPLC studies. The ion chromatography system used consisted of a Dionex Series 4000i equipped with a 50-$\mu$l loop, conductivity detector and Dionex anion and cation micro-membrane suppressors. Recordings of the chromatograms and data handling were affected with a Spectra-Physics SP 4290 integrator and an Epson Equity +1 computer equipped with a Spectra-Physics Labnet software program. The anion columns used were the analytical HPIC 4S4A and the guard HPIC 4G4A column (both columns were from Dionex). The pH meter was a Fisher Accumet 915 equipped with an AccupHast pH electrode. The mobile phases were prepared using *n*-butylamine, *n*-hexylamine, *n*-octylamine, HPLC-grade 85% phosphoric acid (Aldrich) and water purified with a Milli-Q reagent grade water system.

## RESULTS

### *Adsorption studies*

The ion-pairing reagents used in this study were *n*-alkylammonium dihydrogenphosphate salts.

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These salts were used for the following reasons: (1) the elution order of the test anions was found to be independent of the counter anion of the ion-pairing reagent; (2) the dihydrogenphosphate ion resulted in an average retention time for the test ions based on chloride ion (20% longer) and sulfate ion (10% shorter); (3) the dihydrogenphosphate ion can act as a pH buffer for the system. The concentration of the ion-pairing reagents in the mobile phases was approximately 30 m*M* so that the experiments could be completed in a reasonable length of time. Initial studies showed that the breakthrough times were inversely proportional to the ion-pairing reagent concentration. The amount of reagent retained by the column was independent of concentration.

Breakthrough studies were used to measure the amount of ion-pairing reagent sorbed on the column. Prior to each breakthrough experiment, the column was washed with a minimum of twenty void bed volumes of both methanol–water (80:20, v/v) and pure methanol to insure a clean column surface. During the cleaning process the column effluent was monitored with the UV detector set at 210 nm to insure the removal of all absorbed species. The breakthrough chromatograms were obtained by first purging the ODS-2 column with Milli-Q reagent-grade water, then filling the tubing up to the head of the column with the mobile phase and then monitoring the column effluent with the UV detector set at 196 nm which is the absorbance maximum for the *n*-alkylamines in a water matrix. The phosphate ion was measured using ion chromatography. The amount of *n*-alkylamine sorbed on the column surface was calculated by measuring the amount of the amine introduced into the column up to the "breakthrough" time minus the amount of amine found in the column effluent prior to "breakthrough". For the *n*-butyl-, *n*-hexyl- and *n*-octylammonium salts, the mobile phases were prepared by mixing 29.50 ± 0.30 mmol of the *n*-alkylamine with 25.00 ± 0.20 mmol of $H_3PO_4$ in 1 liter of Milli-Q reagent-grade water resulting in a mobile phase of pH 6.3. The resulting mobile phase contains approximately 22.50 m*M* *n*-alkylammonium dihydrogen phosphate, 2.50 m*M* di-*n*-alkylammonium hydrogenphosphate and 1.85 m*M* *n*-alkylamine. Analyses of the various mobile phases and effluents prior to breakthrough are listed in Table I.

Two points of interest are shown in Table I. First is the decrease in the pH from 6.3 in the mobile phase to 3.7 in the effluent. This decrease in pH was observed by Hansen *et al.* [3] during their studies on the modification of silica with long-chain quaternary ammonium ions. The decrease was attributed to the quaternary ammonium ions displacing the hydrogen ion of the surface silanols on the silica. The second point of interest is the difference in the *n*-alkylammonium ion and dihydrogenphosphate ion in the effluent of the column for all three of the *n*-alkylammonium salts studied.

The column effluent contained more *n*-alkylammonium ion as the chain length increased, but less dihydrogenphosphate anion, indicating that more

TABLE I

ANALYSIS OF THE MOBILE PHASE AND COLUMN EFFLUENT PRIOR TO THE BREAKTHROUGH (IN m*M*)

Whatman ODS-2 column, flow-rate 1 ml/min.

| | pH | *n*-Alkyl $NH_3^+$ | *n*-Alkyl $NH_2$ | $H_2PO_4^-$ | $HPO_4^{2-}$ | $H_3PO_4$ |
|---|---|---|---|---|---|---|
| *n*-Octyl | | | | | | |
| Mobile phase | 6.3 | 27.50 | 1.84 | 22.63 | 2.51 | – |
| Column effluent | 3.7 | 7.70 | – | 19.53 | – | 0.53 |
| *n*-Hexyl | | | | | | |
| Mobile phase | 6.3 | 27.88 | 1.86 | 22.56 | 2.51 | – |
| Column effluent | 3.7 | 5.14 | – | 19.99 | – | 0.55 |
| *n*-Butyl | | | | | | |
| Mobile phase | 6.3 | 27.90 | 1.86 | 22.10 | 2.46 | – |
| Column effluent | 3.7 | 5.08 | – | 20.98 | – | 0.57 |

TABLE II

AMOUNTS OF THE VARIOUS SPECIES IN THE SYSTEM THROUGH THE BREAKTRHOUGH (IN mmol)

Whatman ODS-2 column, flow-rate 1 ml/min.

| | *n*-Alkyl $NH_3^+$ | *n*-Alkyl $NH_2$ | $H_2PO_4^-$ | $HPO_4^{2-}$ | $H_3PO_4$ |
|---|---|---|---|---|---|
| *n*-Octyl | | | | | |
| Pumped through column | 1.96 | 0.13 | 1.53 | 0.17 | – |
| Column effluent | 0.58 | – | 1.33 | – | 0.04 |
| Retained by column | 1.51 | – | 0.33 | – | – |
| *n*-Hexyl | | | | | |
| Pumped through column | 0.97 | 0.07 | 0.82 | 0.09 | – |
| Column effluent | 0.23 | – | 0.66 | – | 0.02 |
| Retained by column | 0.81 | – | 0.23 | – | – |
| *n*-Butyl | | | | | |
| Pumped through column | 0.75 | 0.05 | 0.59 | 0.07 | – |
| Column effluent | 0.20 | – | 0.52 | – | 0.01 |
| Retained by column | 0.60 | – | 0.13 | – | – |

active ion-pairing reagent was being absorbed on the ODS-2 surface as the chain length increased.

The results of the amount (in mmol) of the various species in the system for the three *n*-alkylammonium salts studied are given in Table II. These data were calculated using the concentrations of the *n*-alkylamine, phosphoric acid, pH and the time of breakthrough [1]. For the *n*-octylammonium salts, 2.09 mmol of the *n*-octylamine and 1.70 mmol of phosphoric acid were used up to the breakthrough point. Of these amounts, only 1.51 mmol of the *n*-octylammonium ion and 0.33 mmol of the phosphate ion were retained on the column. Therefore, the column retained only 72.25% of the *n*-octylammonium ion and 19.41% of the phosphate ion. Using the same analysis for the *n*-hexyl and *n*-butyl salts, it was found that for the *n*-hexyl salts the column retained 77.88% of the *n*-hexylammonium ion and 25.27% of the phosphate ions while for the *n*-butyl reagent, the column retained 75.00% of the *n*-butylammonium ion and 19.70% of the phosphate ion.

TABLE III

AMOUNTS OF THE VARIOUS SPECIES IN THE SYSTEM THROUGH THE BREAKTHROUGH (IN mmol) USING A WHATMAN SILICA COLUMN

| | *n*-Alkyl $NH_3^+$ | *n*-Alkyl $NH_2$ | $H_2PO_4^-$ | $HPO_4^{2-}$ | $H_3PO_4$ |
|---|---|---|---|---|---|
| *n*-Octyl | | | | | |
| Pumped through column | 0.44 | 0.03 | 0.34 | 0.04 | – |
| Column effluent | 0.16 | – | 0.27 | – | 0.01 |
| Retained by column | 0.31 | – | 0.10 | – | – |
| *n*-Hexyl | | | | | |
| Pumped through column | 0.47 | 0.03 | 0.36 | 0.04 | – |
| Column effluent | 0.16 | – | 0.30 | – | 0.01 |
| Retained by column | 0.34 | – | 0.11 | – | – |
| *n*-Butyl | | | | | |
| Pumped through column | 0.47 | 0.03 | 0.38 | 0.04 | – |
| Column effluent | 0.16 | – | 0.30 | – | 0.01 |
| Retained by column | 0.34 | – | 0.11 | – | – |

For these three *n*-alkylamines the ODS-2 column retains about 75% of the amines and 22% of the phosphate; however; the amounts of amine decrease from 1.51 mmol:0.81 mmol:0.60 mmol from *n*-octyl:*n*-hexyl:*n*-butyl.

For a reference, the retention of the three *n*-alkylammonium salts was studied on a Whatman Partisil silica column. The results of these experiments are listed in Table III. By dividing the amount of an ion retained by the column by the total amount pumped through the column, the results given in Table III indicate that the retention of three *n*-alkylammonium phosphate reagents on pure silica is essentially the same: 67.3 ± 1.2% for the *n*-alkylammonium ion and 27.5 ± 1.4% for the phosphate ion. The data further indicate that even on a silica column, the *n*-alkylammonium ion adsorbs on the surface in two forms. The major form is attached without dihydrogenphosphate as the neutralizing anion; the minor form has dihydrogenphosphate anion "attached".

*Separation studies*

To determine the efficiency of various ion-pairing reagents on ODS columns, a series of experiments was carried out using three ion-pairing reagents (*n*-butyl-, *n*-hexyl- and *n*-octylammonium ion) on a Whatman ODS-2 reversed-phase column. The results of these experiments indicated that the *n*-butylammonium ion was not capable of separating the simple anions on the ODS-2 column. All of the test ions (iodate, bromide, nitrate, nitrite and iodide) resulted in a single peak at 3.57 min. However, as shown in Fig. 1, *n*-hexylammonium ion did not separate nitrate and iodide ions, while the *n*-octylammonium ion chromatogram shows fairly good separation of all the test ions. These experiments are summarized in Table IV, which shows a non-linear relation between capacity factor (retention time) and *n*-alkyl chain length at an ion-pairing reagent concentration of 10 m*M*.

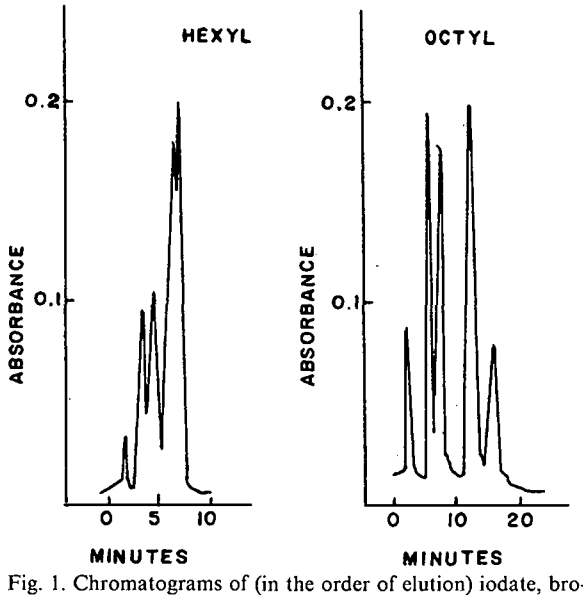


Fig. 1. Chromatograms of (in the order of elution) iodate, bromide, nitrite, nitrate and iodide on an ODS-2 column with *n*-hexylammonium ion (left) or *n*-octylammonium ion (right) as the ion-pairing reagent. Flow-rate 1 ml/min.

TABLE IV

CAPACITY FACTORS OF TEST ANIONS AS A FUNCTION OF THE *n*-ALKYLAMMONIUM ION CHAIN LENGTH ON A WHATMAN ODS-2 COLUMN

Ion-pairing reagent concentration 10 m*M*. Flow-rate 1 ml/min.

| | Capacity factor | | | | |
|---|---|---|---|---|---|
| | $IO_3^-$ | $Br^-$ | $NO_2^-$ | $NO_3^-$ | $I^-$ |
| *n*-Octyl | 0.58 | 0.58 | 0.58 | 0.58 | 0.58 |
| *n*-Hexyl | 0.96 | 1.24 | 1.45 | 1.80 | 1.98 |
| *n*-Butyl | 1.78 | 3.16 | 3.82 | 5.91 | 7.54 |

## DISCUSSION

The experimental observations can be summarized as follows: (1) on a Whatman ODS-2 column,

TABLE V

AMOUNTS OF *n*-ALKYLAMMONIUM DIHYDROGENPHOSPHATE RETAINED AS A FUNCTION OF CHAIN LENGTH ON A WHATMAN ODS-2 COLUMN

| | *n*-Alkylammonium dihydrogenphosphate (mmol) | |
|---|---|---|
| | Total retained | Corrected for silica retention |
| *n*-Octyl | 0.33 | 0.30 |
| *n*-Hexyl | 0.23 | 0.20 |
| *n*-Butyl | 0.13 | 0.00 |

simple inorganic anions cannot be separated using *n*-butylammonium ions, some separation occurs with *n*-hexylammonium ions and acceptable separation occurs with *n*-octylammonium ions, (2) capacity factors, retention, for simple inorganic ions show a non-linear increase as the *n*-alkyl carbon number of the ion-pairing reagent increased from 4 to 8, (3) the Whatman ODS-2 column retains approximately 75% of the *n*-alkylammonium ion and 22% of the phosphate ion that was pumped through the column up to the breakthrough point, (4) the ratio of amounts (mmol) of *n*-alkylammonium ion retained by the ODS-2 column for *n*-octyl:*n*-hexyl:*n*-butyl was 2.51:1.35:1, while the phosphate ratio was 2.54:1.77:1, (5) the amounts (mmol) of *n*-alkylammonium ion and phosphate ion retained by pure silica were essentially independent of the *n*-alkyl chain length.

The equilibria involved are:

$$\text{ODS} + \text{RNH}_3^+ + \text{C}^- \rightleftarrows \text{ODS} \cdots \text{RNH}_3^+ \cdots \text{C}^- \quad (1)$$

$$\text{ODS} \cdots \text{RNH}_3^+ \cdots \text{C}^- + \text{X}^- \rightleftarrows \text{ODS} \cdots \text{RNH}_3^+ \cdots \text{X}^- + \text{C}^- \quad (2)$$

$$-\text{SiOH} + \text{RNH}_3^+ \rightleftarrows -\text{SiO}^- \cdots \text{RNH}_3^+ + \text{H}^+ \quad (3)$$

where ODS is the octadecyl surface of the stationary phase, $\text{RNH}_3^+$ is the mobile phase additive, $\text{C}^-$ is the counter ion and $\text{X}^-$ is the anion being separated.

The first two equilibria are associated with an ion-interaction type of mechanism. These equilibria indicate that the hydrophobic ion-pairing reagent is being absorbed on the ODS surface, followed by a dynamic ion exchange between the solute anion in the mobile phase and the counter ion of the absorbed ion-pairing reagents. Eqn. 3 accounts for the absorption of the ion-pairing reagent on the surface silanol groups. A typical silica surface is estimated to have between 7 and 9.5 $\mu$mol/m$^2$ of surface silanol groups [6]. The deprotonation reaction, $\equiv\text{SiOH} \rightleftarrows \equiv\text{SiO}^- + \text{H}^+$ is reported to have a $pK_a$ value of 7.1 [7]. At a mobile phase pH of 6.3, a Whatman ODS-2 column would contain approximately 1.45 mmol of deprotonated silanol groups.

If one assumes that the active ion-pairing reagent absorbed on the column surface is in the form of the *n*-alkylammonium dihydrogen phosphate salt, then the amount of phosphate ion retained by the column is equivalent to the ion-pairing reagent on the column surface. The data in Table V show that there is a linear relationship between the total amount of dihydrogenphosphate anion retained by the column and the *n*-alkylammonium ion chain length. Also shown is the situation after subtraction of the dihydrogen phosphate ion sorbed on the silica column surface (Table III) from that on the ODS-2 surface. The additional dihydrogenphosphate ion absorbed on the surface of the ODS-2 column increases from near zero for the *n*-butylammonium ion to approximately 0.2 mmol for *n*-octylammonium ion. The fact that the surface-active *n*-alkylammonium ion increases in concentration as the chain length of the *n*-alkyl group increases clearly accounts for the better separation of the test ions with *n*-octylammonium ion in the mobile phase compared to *n*-hexylammonium ion or *n*-butylammonium ion as the carrier.

Table VI lists the amounts of retained *n*-alkylammonium ion without phosphate counter ion as a function of chain length. The relationship is non-linear on the ODS-2 column but, as shown in Table III, is basically a constant on the silica column. We are forced to conclude that either addition of *n*-alkylammonium ion to the silanol groups on the ODS-2 column is chain length dependent or ion-pairing reagents absorbs on the surface by way of yet another mechanism. We are examining this phenomenon in more detail since it may hold the key to our understanding of the non-linear retention behavior of the test ions shown in Table IV.

In conclusion, (1) of the *n*-alkylammonium ions retained by a Whatman ODS-2 reversed-phase column (for *n*-butyl, *n*-hexyl and *n*-octyl) approximately 76% are electrostatically interacting with the deprotonated surface silanols, (2) the amounts of the *n*-alkylammonium ion electrostatically attached to

TABLE VI

AMOUNTS OF ELECTROSTATICALLY ATTACHED *n*-ALKYLAMMONIUM IONS RETAINED AS A FUNCTION OF CHAIN LENGTH ON A WHATMAN ODS-2 COLUMN

| | Amount retained (mmol) |
|---|---|
| *n*-Octyl | 1.18 |
| *n*-Hexyl | 0.58 |
| *n*-Butyl | 0.47 |

the column increase exponentially with chain length, (3) only about 24% of the retained *n*-alkylammonium ions act as ion-pairing reagents on the column surface, (4) the amounts of the ion-pairing reagent on the column increase linearly in the ratio of 1:1.77:2.54 for *n*-butyl:*n*-hexyl:*n*-octyl ammonium ions and (5) the reason for the exponential increase in the capacity factors with *n*-alkyl chain length currently is unknown but may be related to the non-linear retention of *n*-alkylammonium ion on the ODS-2 surface.

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CHROMSYMP. 2521

# Expert system for ion chromatographic methods using dynamically coated ion-interaction separation

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ABSTRACT

The development of an expert system is described for ion chromatographic methods which use ion-interaction chromatography as the separation technique. The object of the system is to help define appropriate starting conditions for the analysis of a desired group of ions. The system is implemented in a rule-based expert system development tool, Xi-Plus. Rules are used which act on certain properties of the sample and on the availability of instrumentation and accessories. With this information the method conditions can be defined for the column, detector and mobile phase. The expert system incorporates a module which allows the user to modify some of the rules in order to avoid problems which arise with expert systems which are too rigid. Many laboratories have their own preferences for columns, etc., which would be difficult for an expert system to predict. The rule change module therefore allows users to customise the system to their own requirements. Two approaches to the knowledge engineering process were employed. The first used the conventional approach of interrogation of an expert (in this case, P. R. Haddad). In the second approach, statistical analyses were applied to a previously compiled database of published ion chromatographic methods. The conclusions from these searches were then examined by the expert to define rules for the expert system. This paper describes this process of knowledge acquisition and some preliminary results on the use of the expert system.

## INTRODUCTION

Expert systems technology is now relatively mature and several systems have been built for analytical chemistry applications [1–5]. Most of these systems have been applied to problems in high-performance liquid chromatographic method development. Systems exist which offer advice on selecting the best starting conditions, and for optimising and validating the chosen method. Some of these systems have also been integrated to form larger systems capable of tackling a number of these problems simultaneously [6,7]. However, no expert system has yet attempted to resolve the unique problems associated with ion chromatography (IC). In this paper we describe such an application of expert systems technology and the approach taken is novel in two respects. First, an extensive library of previously published IC methods is used to assist in the knowledge acquisition process. Second, a module is implemented which allows the user to modify the rules and thus to customise the system to include specific preferences.

The application of IC has grown rapidly since the technique was introduced as an ion-exchange method coupled with conductivity detection for the assay of a series of anions and cations [8]. IC offers reliable methodology for the simultaneous determination of mixtures of ions and has found use in many industrial and environmental applications. Although IC is used widely, expertise in the subject is relatively rare, and this makes it an ideal application for expert systems. Some work has been published on the application of computers to method development for IC [9,10], but this work has not included the application of artificial intelligence.

Knowledge acquisition and engineering is the

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0021-9673/92/$05.00 

most time-consuming stage and is often considered the bottle-neck in the development of expert systems. This stage can require an inordinate amount of time from the domain expert, who is usually a very busy person. It is therefore beneficial if the knowledge engineer can employ techniques which can reduce the involvement of the expert. For this project we were fortunate to have available an extensive database of previously published IC methods, covering the literature up to 1990 [11]. This database was searched systematically and the results were analysed statistically to find the most commonly used method conditions for different applications. These conclusions were then examined by the expert and rules were generated for the expert system.

A failing of some previously developed expert systems has been the inability of the final user to interact with or modify the rule base without compromising the validity of the whole system. To investigate a solution to this problem, a small rule base was programmed which allows the user to alter the conclusions of some of the rules. In this way, the user can interact with the rule base in a controlled manner without altering the overall philosophy of the expert system.

## EXPERIMENTAL

The expert system was implemented in Xi-Plus, which is a rule-based expert system development tool (Expertech, UK). The database was implemented in Reflex (Borland International, CA, USA). These software packages require approximately 10 megabytes of memory, including storage of the database files. An IBM compatible computer with a 486 CPU was employed; however, the system was tested on a 286 and showed satisfactory performance.

## RESULTS AND DISCUSSION

### *Definition of the chemical domain*

Definition of the chemical domain is important to ensure the final success of an expert system. The knowledge for IC can be defined in terms of the following boundaries: (1) separation mechanism; (2) application area; (3) stages in the method devel-

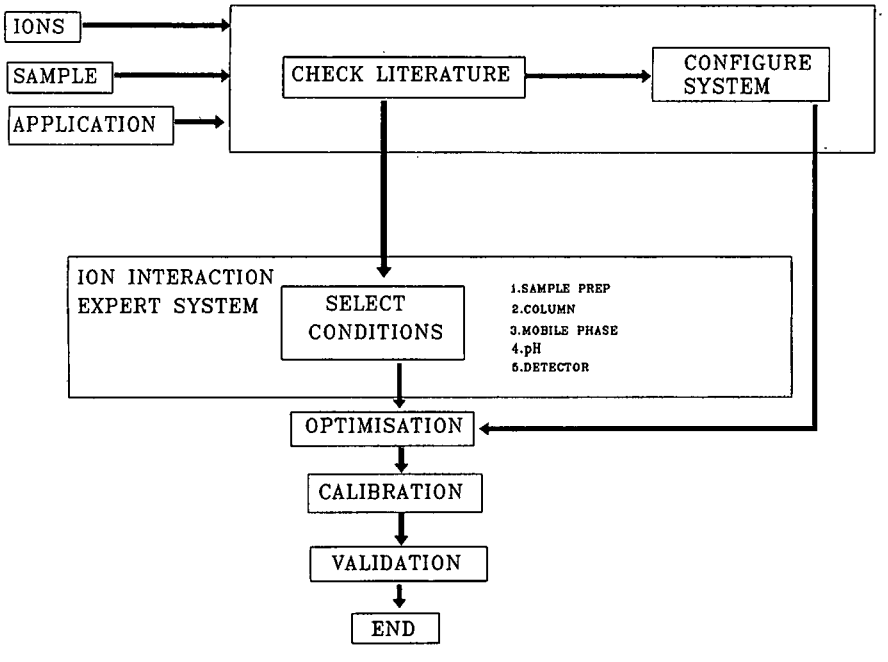


Fig. 1. Stages of method development in IC.

opment process. There are various mechanisms, or separation modes, which can be applied to the analysis of ions. These mechanisms employ different chromatographic conditions to influence different chemistries of the separation process and incude ion-exchange chromatography, ion-interaction chromatography (IIC) (also known as ion-pair chromatography), ion-exclusion chromatography and miscellaneous separation methods, such as reversed-phase liquid chromatography, the use of chelating stationary phases, etc.

IC can be applied to a diverse range of application areas, including environmental, industrial, foods and plants, clinical and pharmaceutical, metals and metallurgical solutions, and treated waters. The sample may be simple and require little or no sample preparation, or can be very complex and require extensive pre-treatment.

The actual development of a method for IC involves several stages, as depicted in Fig. 1. The first stage is to collect any available information on the number and type of ions, the complexity of the sample matrix and the requirements of the application. A check is usually made of the literature in an attempt to locate a previously developed method. If such a method is available it can be configured to a complete method description. Alternatively, the method may require some further optimization. If a method is not available then a first guess must be made for a suitable sample preparation, column, eluent (including pH) and detector. The selected method must now be further optimized for retention, selectivity and instrumental conditions. Finally validation of the method must be performed and a suitable calibration procedure defined. Selection of the calibration method is often an integral part of the validation study.

Quite clearly IC is a very large subject domain and needs to be divided into smaller parts which can be tackled in sufficient depth without requiring excessive computer memory or consultation times. In this project the subject domain has been divided into two stages: the first involves the searching for and configuration of previously published methods, whilst the second involves the development of IC methods using the major retention mechanisms (namely ion-exchange, ion-exclusion and ion-interaction).

This paper describes an expert system which has been built to develop methods which use IIC as the retention mechanism. IIC involves the use of apolar stationary phases with eluents containing a hydrophobic ion (called the ion-interaction reagent, IIR) of opposite charge sign to that of the analyte ions. Two operational alternatives exist. The first (or "permanent coating" method) requires that the column is first equilibrated with the IIR, which is then absent from the eluent during the analysis step. The second (or "dynamic coating" method) uses an eluent containing IIR for both the column conditioning and analysis steps. IIC was selected for expert system development for two reasons. First, the number of literature citations in this area is relatively small (approximately 10% of the total for IC), so that IIC forms an ideal subset for the study of expert systems in IC. Second, IIC has a larger number of experimental variables than any other retention mechanism used in IC and therefore offers the greatest challenge for method development.

*Defining the influencing factors*

In order to define method conditions for the assay of ions by IIC, certain information is required. This information usually encompasses characteristics of the analytes (*e.g.*, whether the solute ion is an anion or a cation and is hydrophobic or hydrophilic), the sample matrix (*e.g.*, the number of ions to be determined and their concentrations, the presence of likely interferences, etc.) and the application (*e.g.*, the need for automation, the detection limits and precision required, etc.). It is also important to recognise that the value of one chromatographic variable can affect decisions on another; for instance some eluents are incompatible with some detection modes. These influences need to be established such that the conditions are selected in an appropriate order.

*Searching the database for relationships*

In order to find any trends or interdependences of chromatographic conditions for published IIC methods, selective searches were made of the previously compiled database of applications. Since there are 343 IIC methods in the database (compared to a total of about 4000 for all separation modes), it was feasible to perform manual searches and subsequent statistical analyses for this number of applications.

TABLE I

CLASSES OF IONS

| Anions | Cations | Organics |
|---|---|---|
| Halides | Ammonium, alkali metals | Carboxylic acids |
| Nitrate, nitrite | Alkaline earth metals | Amines |
| Sulfate | Transition metals | Alkylsulfonates |
| Other | Lanthanides | Other |
| | Other | |

To determine whether the database would reveal preferred conditions for certain classes of ions, it was necessary to clearly classify the ion types. Table I shows the initial classification groups. The database of applications was first searched to find which classes had IIC as the preferred mechanism and the following conclusions were made: (1) all ion classes could be analysed successfully using IIC; (2) IIC appears to be the preferred mechanism for the determination of lanthanides and, to a lesser extent, transition metals; (3) IIC is applied infrequently to the determination of alkali metals, alkaline earth metals and ammonium. The database was then analysed to find the distribution of IIC methods between the two operational modes of dynamic and permanent coating. It was found that permanent coating represented less than 20% of IIC applications and it was therefore decided to concentrate on the dynamic coating procedure. A further factor influencing this decision was that the permanent coating method results in the formation of an ion-exchange stationary phase, so that this method can be correctly classified as ion-exchange chromatography which will be dealt with at a later stage of the project. Initial searches revealed that silica-based columns represented almost 65% of IIC applications. Trends in IIC column usage over the time period 1975–1989 were then examined, which revealed that there has been a trend away from the use of polymer-based columns. This can be explained by the fact that silica columns generally offer better chromatographic efficiency, are widely available and are more rugged than columns packed with polymeric stationary phases. Further searches on column usage for the individual ion classes revealed a similar trend to the overall usage, with the only preference for polymer columns being evident for samples of extreme pH. Over 40 similar searches were performed to ascertain trends in eluent and detector uses and from this a rule base was built for IIC.

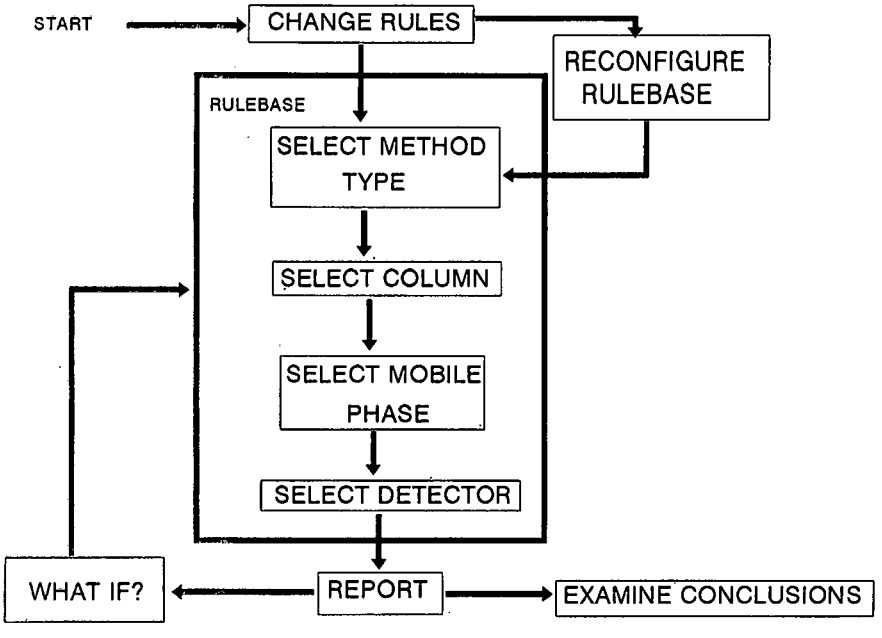


Fig. 2. The IIC expert system.

*Implementation of the expert system*

The knowledge acquired by searching the database was transcribed into rules. An example of such a rule is:

If the ion class is anions
and anions include UV-absorbing species
and anions do not include non-UV-absorbing species
and detectors include ultraviolet spectrophotometer
and sample matrix has an extreme pH
then method is one

This rule selects an outline method for anions which are UV-absorbing and are in a sample matrix with an extreme pH. The way in which these rules are implemented is shown in Fig. 2. This figure also shows how the rule base interacts with the other facilities offered by the development tool. The first module allows the user to change the conditions for activating some of the rules and the conclusions made by these rules. If any modifications are made then the rule base is reconfigured to account for these changes. The actual rules for defining the method conditions are divided into four stages: (1) selection of the method type: this part ascertains the class of ion to be analysed and selects a suitable preliminary method; (2) selection of the column: the column is selected by examining the nature of the sample and its matrix; (3) selection of the eluent: the eluent is determined chiefly by the class of ions for analysis; (4) selection of the detector: the detector is chosen on the basis of the properties of the solute ions and the availability of detector types.

A report is then shown which summarises the chosen method conditions. As an example of the application of the developed expert system, a consultation was made to find a suitable method for the determination of transition metals in a strongly acidic sample. Fig. 3 shows the method printout obtained. The suggested method is workable, but it would be more usual to employ a silica-based $C_{18}$ column, despite the low sample pH. The conclusions reached should therefore be examined by looking at the rules which generated each conclusion and, if necessary, modifying these rules. For example, the rule for selection of a polymer column is based on the sample having an extreme pH value and a polymer column being available, so that some refinement of this rule could be desired by the user. Throughout the consultation, help is available for each query which is made of the user. These help files include hints on the best responses to queries in

**ION INTERACTION EXPERT SYSTEM**

**Print report**

**A polymer-based column is chosen as the most suitable type for this application.**
**There are several columns commercially available, for instance**
**PS-DVB - Hamilton PRP-1**
**XAD-2**
**Dionex MPIC**

**The column length is 30 cm**
**The particle size is 10 μm**
**The internal diameter is 4 to 7 mm**

**The mobile phase recommended for this application is as follows:**
**2 mM sodium octanesulfonate, 50 mM tartaric acid, adjusted to pH 3.5 with sodium hydroxide. Flow-rate of 1 ml/min**
**The octanesulfonate acts as the ion interaction reagent and tartaric acid as the mobile phase ligand.**

**The detection method recommended is spectrophotometry at 530 nm after post-column reaction with PAR**

Fig. 3. Typical method report generated by the expert system.

situations where the information requested is not critical to the specific application under study and the user wishes to maximize the options available to the expert system.

Once the conclusions have been examined it is possible for the user to reconsider answers to any of the queries by using the "What if?" facility. This allows the user to modify any answers and examine the new conclusions. The question "Why?" can also be asked at any stage in the consultation.

## CONCLUSIONS

The method of knowledge acquisition which was employed in this paper, namely the use of a database of literature methods in IC to compile a rule base, enabled us to successfully create an expert system for IIC. The expert agreed with the majority of conclusions which were reached by statistical analysis of the database. This method considerably reduced the amount of time required from the expert, but still resulted in the development of a competent expert system. However, this process proved to be very time-consuming for the knowledge engineer. There are a number of artificial intelligence techniques under development which can generate relationships within databases of examples. These include neural networks and the Quinlan rule generating system. The next stage of this project will be to investigate the potential of these techniques for knowledge acquisition. They will be used to compile rule bases for the mechanisms of ion exclusion and ion exchange. These mechanisms are used more frequently than IIC and thus the number of applications in the database would make it extremely difficult to perform the statistical analyses manually and to ensure that the statistics are free of bias. Furthermore these learning techniques should reduce subsequent validation requirements because they are well defined procedures.

Xi-Plus proved to be a competent expert system development tool, however, it is limited in some respects. It provides few facilities for integration of the expert system with a database and the user interfacing is not extensive. It is therefore likely that other newer expert system tools will be employed for the implementation of the final integrated system.

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CHROMSYMP. 2489

# Applications of an alternative stationary phase for the separation of anions by chemically suppressed ion chromatography

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ABSTRACT

A hydroxymethyl methacrylate-based anion exchanger with quaternary amine functional groups is shown to have a proper selectivity for the separation of common inorganic anions on chemically suppressed ion chromatography systems. Its performance is comparable to the agglomerated pellicular-based anion exchangers commonly used for this purpose. The applications of this new anion exchanger as an alternative stationary phase for chemically suppressed ion chromatography are featured with separations of some simple inorganic and organic anions using both isocratic and gradient elution techniques.

## INTRODUCTION

In chemically suppressed ion chromatography (IC), high conductivity eluents such as carbonate–hydrogencarbonate or hydroxide are used for the separation of anions. Since the pH values of these eluents are considerably high, the stationary phases used for this purpose must be stable within the eluent pH range and have the proper selectivity to separate the anions of interest. A variety of anion exchangers are commercially available for IC analysis of anions [1,2], however, not all are suitable for chemically suppressed IC. Agglomerated pellicular-based anion exchangers are the most common stationary phases used for this purpose [3,4]. The outstanding chromatographic performance of these anion exchangers is well known. Agglomerated ion-exchange resins contain an internal core particle, to which a monolayer of small diameter particles that carry the functional groups is attached. These resins exhibit excellent chromatographic performance due to the very short diffusion paths available to solute ions during the ion exchange process. This material has been used to separate a wide variety of anions using both carbonate–hydrogencarbonate and hydroxide eluents.

This paper describes an alternative stationary phase which is based on hydroxyethyl methacrylate (HEMA). HEMA is a macroporous copolymer of 2-hydroxyethyl methacrylate and ethylene dimethacrylate. The theoretical aspects, the chemical and physical properties of this material, and the use of HEMA-based anion exchangers for the separation of a wide variety of anions using single-column IC methods have been discussed previously [5]. It has been recently shown that this material also can be used for the separation of anions in chemically suppressed IC [6]. In this work, the performance characteristics of HEMA-based anion exchangers are compared to the agglomerated pellicular-based anion exchangers. The application of this material as an alternative stationary phase is shown with the

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separation of simple inorganic and organic anions using both isocratic and gradient elution techniques.

## EXPERIMENTAL

The ion chromatograph used was a Dionex (Sunnyvale, CA, USA) BioLC system. It consists of a gradient program module (Model GPM-1) and a conductivity detector (Model CDM-2). The sample introduction was done with a Rheodyne (Reno, NV, USA) Model 9125 injector. The eluent suppression was achieved using the anion micromembrane suppressor (Model AMMS1) and the regenerant was supplied to the suppressor through an AutoRegen accessory. All data were recorded with a Spectra-Physics (Santa Clara, CA, USA) Model SP 4400 Chromjet integrator.

Separations were carried out on the Alltech (Deerfield, IL, USA) Universal Anion Column (150 mm × 4.6 mm I.D.) packed with 10-$\mu$m particles of HEMA-based anion exchanger with trimethylamine functional groups. The estimated ion-exchange capacity of the anion exchanger is 0.1 mmol/g. For comparison study, a Dionex HPIC AS4A column was used.

All eluents and standards were prepared from reagent-grade chemicals (Aldrich, Milwaukee, WI, USA). HPLC-grade water was used to prepare all eluents, standards and samples. The appropriate eluent compositions as well as the gradient programs used are given as a part of the legends to all figures.

## RESULTS AND DISCUSSION

The characteristics of the HEMA-based anion exchanger as an alternative stationary phase for the separation of anions by chemically suppressed IC is evaluated and compared to the pellicular polystyrene–divinylbenzene-based ion exchangers. The most popular pellicular column, AS4A, made by Dionex is used for this purpose.

One of the most common eluents used for the isocratic separation of anions on the AS4A column is 1.7 m$M$ $NaHCO_3$ and 1.8 m$M$ $Na_2CO_3$. This eluent was chosen to separate the seven common inorganic anions on both columns. Fig. 1A shows the chromatogram obtained on the pellicular-based

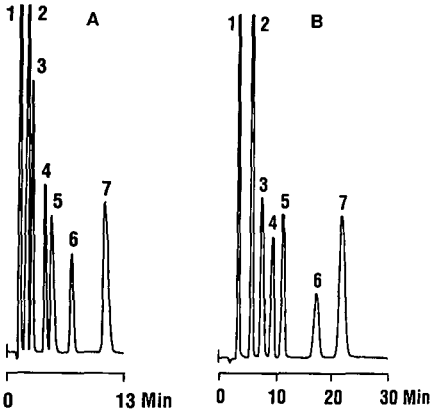


Fig. 1. Separation of the seven common inorganic anions on (A) pellicular-based column (250 mm × 4.0 mm I.D.) and (B) HEMA-based column (150 mm × 4.6 mm I.D.) using 1.7 m$M$ $NaHCO_3$–1.8 m$M$ $Na_2CO_3$ eluent. Flow-rate: 1.0 ml/min; detector: suppressed conductivity, 10 $\mu$S full scale; injection volume: 100 $\mu$l. Peaks: 1 = fluoride (10 ppm); 2 = chloride (20 ppm); 3 = nitrite (20 ppm); 4 = bromide (20 ppm); 5 = nitrate (20 ppm); 6 = phosphate (30 ppm); 7 = sulfate (30 ppm).

column. An excellent separation is obtained for all the anions. Fig. 1B shows the separation of anions on the HEMA-based column. A nice separation is also obtained for all the anions. The selectivity toward the anions for both columns are in the similar order, beginning with fluoride, followed by chloride, nitrite, bromide, nitrate, phosphate and sulfate.

The efficiency reported as number of theoretical plates ($N$) calculated by the half-height method [$N = 5.54 \cdot (t_R/W_{1/2})^2$] for both columns are shown in Table I. Since $N$ values are meaningful only if they are calculated for peaks with moderately large values of capacity factors ($k' > 5$) [7], only nitrate, phosphate and sulfate peaks were used. Same eluent composition (1.7 m$M$ $NaHCO_3$–1.8 m$M$ $Na_2CO_3$) and chromatographic conditions were used for both columns. The results show that the efficiency per column for both columns are comparable. Comparable efficiencies of the shorter (150 mm × 4.6 mm I.D.) HEMA-based column and the longer (250 mm × 4.0 mm I.D.) AS4A column indicate that HEMA-based column has a better resolving power.

However, the total run time is approximately 44% longer on the HEMA-based column. This is

TABLE I

THE EFFICIENCY (NUMBER OF THEORETICAL PLATES PER COLUMN) OF PELLICULAR-BASED AND HEMA-BASED COLUMNS

The efficiency was calculated using half-height method.

| Anion | Efficiency ($N$) | |
|---|---|---|
| | Pellicular-based column (250 mm × 4.0 mm I.D.) | HEMA-based column (150 mm × 4.6 mm I.D.) |
| Nitrate | 2880 | 4780 |
| Phosphate | 3716 | 3184 |
| Sulfate | 4070 | 4092 |

not surprising considering the higher anion-exchange capacity factors, $k'$, as listed in Table II. Early-eluting anions exhibit 2–3-fold increase in $k'$, while the late-eluting anions exhibit 1.5-fold increase. The trifold increase in the $k'$ value for fluoride ion results in longer retention time, thus providing better resolution from the column void volume. In Fig. 1A and as reported elsewhere [8], the fluoride retention time appears at the column void volume using the agglomerated pellicular-based column.

The HEMA-based column is useful for applications which demand the use of columns with higher ion-exchange capacities. Fig. 2 shows the separation of nine anions on both columns. Fluoride and formate peaks are well resolved on the HEMA-based column (Fig. 2A), while on the AS4A column, they are coeluted (Fig. 2B). Since the AS4A column was designed for faster analysis, the eluent

TABLE II

ANION-EXCHANGE CAPACITY FACTORS ($k'$) OF ANIONS ON THE PELLICULAR- AND HEMA-BASED COLUMNS

| Anion | Pellicular-based column | HEMA-based column | $k'_{HEMA}/k'_{pellicular}$ |
|---|---|---|---|
| Fluoride | 0.31 | 1.08 | 3.48 |
| Chloride | 1.23 | 2.46 | 1.95 |
| Nitrite | 1.72 | 3.48 | 2.02 |
| Bromide | 3.08 | 4.58 | 1.49 |
| Nitrate | 3.75 | 5.67 | 1.51 |
| Phosphate | 6.03 | 9.05 | 1.50 |
| Sulfate | 9.54 | 11.64 | 1.22 |

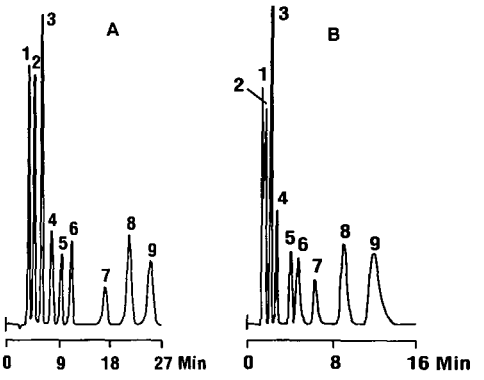


Fig. 2. Separation of nine inorganic and organic anions on (A) HEMA-based column (150 mm × 4.6 mm I.D) and (B) pellicular-based column (250 mm × 4.0 mm I.D.). Peaks: 1 = fluoride (6 ppm); 2 = formate (25 ppm); 3 = chloride (12 ppm); 4 = nitrite (12 ppm); 5 = bromide (12 ppm); 6 = nitrate (12 ppm) 7 = phosphate (18 ppm); 8 = sulfate (18 ppm); 9 = oxalate (40 ppm). Chromatographic conditions as in Fig. 1.

concentration was diluted to give more comparable run times to the HEMA-based column. By reducing the eluent concentration to 0.8 m$M$ $NaHCO_3$–0.9 m$M$ $Na_2CO_3$, the sulfate retention time on the AS4A increases to 31 min, which is approximately 10 min longer than on the HEMA-based column. Under this new conditions, the fluoride and formate peaks are still coeluting. This results show that the HEMA-based column has comparatively better resolving power especially with respect to weakly retained anions such as fluoride and formate.

The isocratic separation of several actual analytical samples are shown in Fig. 3. An eluent composition of 2.8 m$M$ $NaHCO_3$–2.2 m$M$ $Na_2CO_3$ was used for these separations to decrease the overall analysis time by approximately 20%. Under this condition, resolution of all the seven common inorganic anions is still achieved. The coal sample was obtained by oxygen bomb combustion of 1 g of coal in a Parr bomb after pre-purge to remove atmospheric nitrogen. Water was used as the collector solution. The solution was filtered and diluted before injection. The sodium fluoride dental gel and the toothpaste were mixed with deionized water (0.1 g/10 ml), sonicated for 10 min to release the fluoride, filtered and diluted before injection. Resolution of fluoride from the column void volume is achieved in all samples.

HEMA-based anion exchangers can be success-

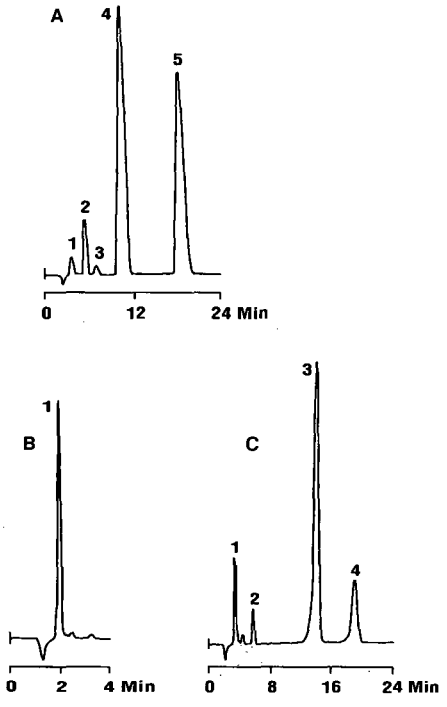


Fig. 3. Separation of anions in actual analytical samples. (A) Coal, peaks: 1 = fluoride; 2 = chloride; 3 = nitrite; 4 = nitrate; 5 = sulfate. (B) Sodium fluoride dental gel; peak 1 = fluoride. (C) Toothpaste; peaks: 1 = fluoride; 2 = chloride; 3 = phosphate; 4 = monofluorophosphate. Column: HEMA-based (150 mm × 4.6 mm I.D.); eluent: 2.8 m*M* $NaHCO_3$–2.2 m*M* $Na_2CO_3$; flow-rate: 1.0 ml/min; detector: suppressed conductivity, 10 $\mu$S full scale; injection volume: 100 $\mu$l.

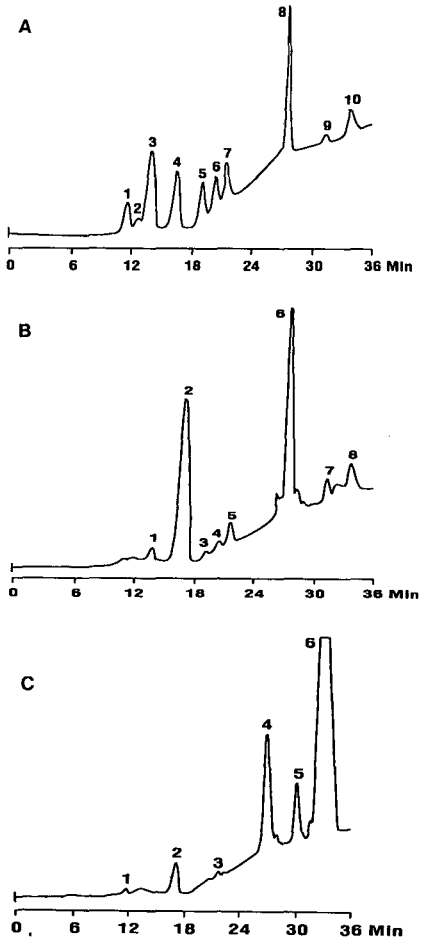


Fig. 4. Gradient elution of inorganic and organic anions. (A) Standard; peaks: 1 = fluoride (0.5 ppm); 2 = acetate (1 ppm); 3 = formate (2 ppm); 4 = chloride (1 ppm); 5 = nitrite (1 ppm); 6 = bromide (1 ppm); 7 = nitrate (1 ppm); 8 = sulfate (1.5 ppm); 9 = phosphate (1.5 ppm); 10 = citrate (10 ppm). (B) Tomato juice; peaks: 1 = formate; 2 = chloride; 3 = nitrite; 4 = bromide; 5 = nitrate; 6 = sulfate; 7 = phosphate; 8 = citrate. (C) Lime juice, peaks: 1 = fluoride; 2 = chloride; 3 = nitrate; 4 = sulfate; 5 = phosphate; 6 = citrate. Eluents: eluent A, deionized water; eluent B, 30 m*M* sodium hydroxide. Gradient program: A–B (95:5) at 0 min, (90:10) at 15 min, (50:50) at 25 min, (40:60) at 35 min, (95:5) at 38 min. Flow-rate: 1.0 ml/min; detector: suppressed conductivity, 10 $\mu$S full scale; injection volume: 50 $\mu$l.

fully employed in gradient elution techniques because of their inherent high capacity and high efficiency. This is demonstrated by the examples shown in Fig. 4. Fig. 4A shows the gradient separation of some inorganic and organic anions with varying charges ($-1$ to $-3$). Fig. 4B and C shows the chromatograms obtained for fruit juices. The fruit juices were diluted and filtered through 0.5 $\mu$m syringe filters prior to injection. No attempt was made to optimize the gradient program and no special precautions were taken in eluent preparation and storage for these analysis. Although this chromatogram does not show much resolving power of the gradient elution technique, the spacings between the peaks do indicate that it is possible to resolve more anions using HEMA-based column. The observed baseline drifts in these examples are not unusual in gradient IC runs. The drifts have been attributed to the problem of contaminants in the eluents which change during the gradient run [8]. When using NaOH as the eluent, contamination by carbonate produced from the absorption of carbon dioxide from the atmosphere can cause a drift in the con-

ductance. NaOH gradients are successful only when special precautions are taken in eluent preparation and storage. The baseline drift can also be eliminated by subtracting the baseline of a blank run using a computer-aided data acquisition.

The HEMA-based anion exchanger has been shown to be a useful alternative to the conventional agglomerated pellicular anion exchangers for the separation of anions by chemically suppressed IC. The HEMA-based columns exhibit higher capacities for all anions and especially for weakly retained anions such as fluoride and formate. It is useful for applications which require the use of columns with higher ion-exchange capacities. It can be used for both isocratic and gradient elution techniques.

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# Direct resolution of organic acid enantiomers on a novel polymer-based stationary phase

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ABSTRACT

A chiral packing for high-performance liquid chromatography was obtained by copolymerization of urea with formaldehyde in the presence of an optically active amino acid derivative. Spherical particles with a mean bead size of 6 μm were evaluated as stationary phases with respect to their chemical stability and chromatographic performance. Electrostatic interactions govern the overall kinetics of the retention in the chromatographic process, so that ion pairing seems to be responsible for the chiral recognition. Increasing the column temperature improves the chromatographic pattern considerably, and the resolution and peak shape are similar to those in the usual high-performance liquid chromatographic separations. The support is easy to pack and yields good column lifetimes. Its application to the resolution of some organic acid racemates is illustrated.

## INTRODUCTION

Most high-performance liquid chromatographic (HPLC) methods for the resolution of enantiomers are done on silica-based packing materials because of their good chromatographic performance and the well established grafting reactions [1–4]. However, these materials have certain disadvantages which limit their use and shorten column lifetimes. These restrictions are related to the Si–O–Si–C bonds, which are unstable in acids and bases, and to the presence of residual surface silanol groups, which results in tailing peaks. As an alternative to silica gel packings, synthetic and natural organic polymers gels can be used as chiral stationary phases (CSPs), and a number of supports with characteristic enantioselectivity for several racemates have been reported [5–11]. These provide excellent chemical stability, but show low column efficiency, and are subject to shrinkage and swelling processes.

In a project aimed at the development of polymer gels that would match silica-bonded CSPs in the resolution of enantiomers and allow the use of eluents over wide ranges of pH and polarity, we undertook the preparation of a novel stationary phase which was obtained by copolymerization of urea with formaldehyde in the presence of L-leucinamide. The resolution of some organic acid racemates into their enantiomers is demonstrated.

## EXPERIMENTAL

### *Apparatus*

The liquid chromatographic system consisted of a Perkin-Elmer (Norwalk, CT, USA) Series 2B solvent-delivery pump equipped with a Rheodyne Model 7125 injection valve, connected to a Jasco (Tokyo, Japan) Uvidec-100-V variable-wavelength detector, combined with a Carlo Erba (Milan, Italy) Mega Series integrator.

Microscopic investigations of the gel particles were performed in the Centro di Studio per la Termodinamica Chimica alle Alte Temperature, CNR (Rome), with a Cambridge 100 scanning secondary electron microscope using the gold-sputtered procedure.

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*Reagents and materials*

Carboxylic acids and barbiturates were purchased from Sigma (St. Louis, MO, USA). L-Leucinamide was obtained from Bachem (Switzerland). All solvents and other reagents were of HPLC or analytical-reagent grade and obtained from Carlo Erba.

*Preparation of chiral packing*

Spherical gel particles were synthesized as follows. About 200 ml of distilled water were acidified with dilute hydrochloric acid, then urea (U) (17.5 g, 0.29 mol), 40% formaldehyde (F) (32 g, 1.06 mol) and L-leucinamide (3.6 g, 0.03 mol) were added. After the pH had been adjusted to 2 with hydrochloric acid, the solution was placed in a high-speed mixer and allowed to react with stirring at *ca.* 300 rpm for 20 h at 24°C. The resulting microspheres were filtered and washed three times with 200–300-ml portions of distilled water by sedimentation and decanting. The sorbent was filtered off under reduced pressure and washed with dilute hydrochloric acid, distilled water and methanol and dried for 2 h at 70°C under vacuum. About 6 g of material were rinsed with 300 ml of acetone and the suspension was placed in a 35.0 × 2.0 cm I.D. glass column. After 5 min a fraction of particles at low sedimentation speed was collected (4.5 g) and used for chromatography. Elemental analysis of the sorbent gave N 29.63, C 31.02, H 5.53%. The particle size distribution (4–7 μm) was calculated from optical micrographs of about 50 randomly selected beads. In addition, a microparticulate material was prepared by mixing urea and formaldehyde according to the above-described procedures. Elemental analysis gave: N 30.83, C 29.34, H 5.48%.

The amount of chiral ligand immobilized on the copolymer was determined by comparing the elemental analysis of the materials prepared in the presence and absence of L-leucinamide. The results indicated that the chiral packing contains at least 2.7% (w/w) of the functional component, corresponding to 0.2 mmol per gram of resin. Because urea reacts with formaldehyde to form a polymeric network according to the following equation [12]:

$$n\ H_2NCONH_2 + n\ HCHO \rightarrow \rightarrow (\text{–}HNCONHCH_2NHCONH\text{–})_n$$

one leucinamide moiety links to the copolymer approximately every 20 urea–methylene units.

The U–F–L-leucinamide sorbent, suspended in 2-propanol, was packed in a stainless-steel tube (250 × 4.6 mm I.D.) at 8000 p.s.i., using *n*-hexane to pressurize the slurry into the column. In order to test the chemical stability of the gel, 0.1 *M* sodium hydroxide solution (pH 13) was passed through the column for 8 h. No reduction in chromatographic or enantioselective performance was observed.

*Chromatographic procedure*

Sample solutions were prepared in methanol at concentrations of *ca.* 1 mg/ml, and 1–3 μl of these solutions were injected. Chromatographic runs were performed with a UV detector set at 230 or 254 nm. The column was thermostated at different temperatures using an HPLC temperature control system (Fiatron, Oconomovoc, WI, USA).

## RESULTS AND DISCUSSION

Stout *et al.* [13] reported the preparation of microspheres obtained by copolymerization of urea and formaldehyde in the presence of silica sol, which was successively removed in order to produce porous organic particles. They concluded that the UF matrix surrounding the pores imprinted by the sol particles is like a foam with micropores of probably 3–12 Å. Other investigators reported on a similar microporous structure present in polystyrene–divinylbenzene copolymers [14].

In this work, urea and formaldehyde combined with L-leucinamide were allowed to copolymerize in the absence of silica, so that presumably a wholly microporous foam structure has to be ascribed to the gel particles, and stronger enantiodiscriminative activity to the superficial chiral sites. The shape and the size distribution of the gel beads are shown in the Fig. 1 and 2, respectively.

A number of organic acid racemates were employed to characterize the resolving properties of the stationary phase. As can be seen in Fig. 3, enantioselectivity ($\alpha$) is strongly influenced by the pH of the buffer in the mobile phase and the size of the substituents bound to the asymmetric carbon atom. It seems that L-leucinamide participates in the formation of zwitterion pairs with the optically active counter ions, operating as a chiral ion-pairing

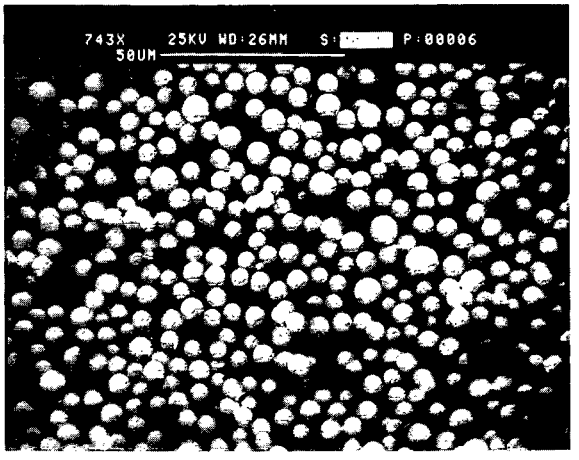


Fig. 1. Scanning electron micrograph of the spherical gel particles.

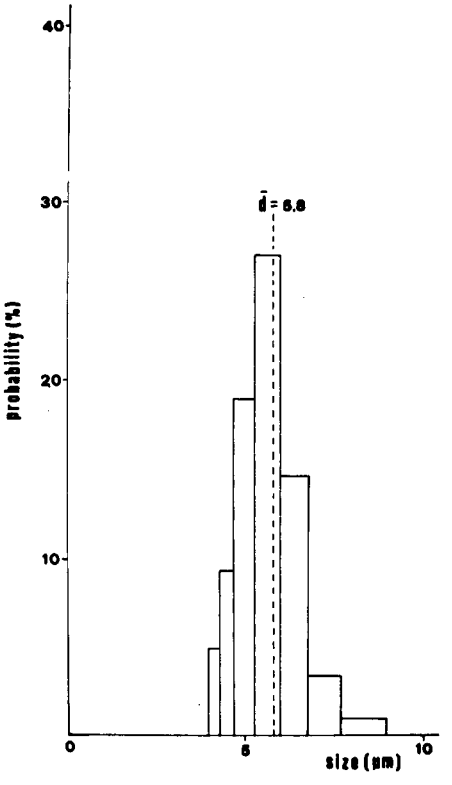


Fig. 2. Particle size distribution of the chiral gel.

agent. This is evidenced by the improved resolution, as with barbiturates, when the amount of methanol in the eluent is increased. The chromatograms of a series of racemates are shown in Figs. 4 and 5. Fig. 4C shows the partial resolution of thiopental. In accord with literature findings, this is the first example reporting the enantiodiscrimination of a barbiturate which does not have the chiral centre in the

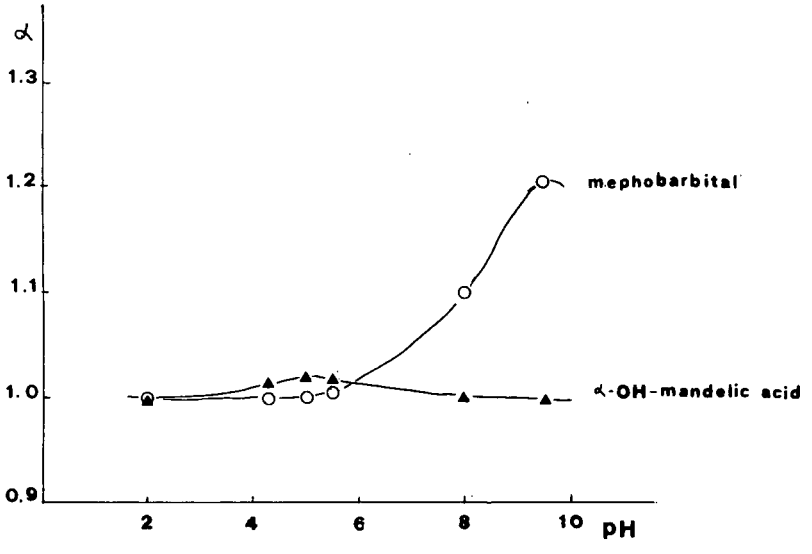


Fig. 3. Plots of $\alpha$ *vs.* pH of the mobile phase containing (▲) 20% or (○) 80% methanol and 0.01 *M* acetate buffer.

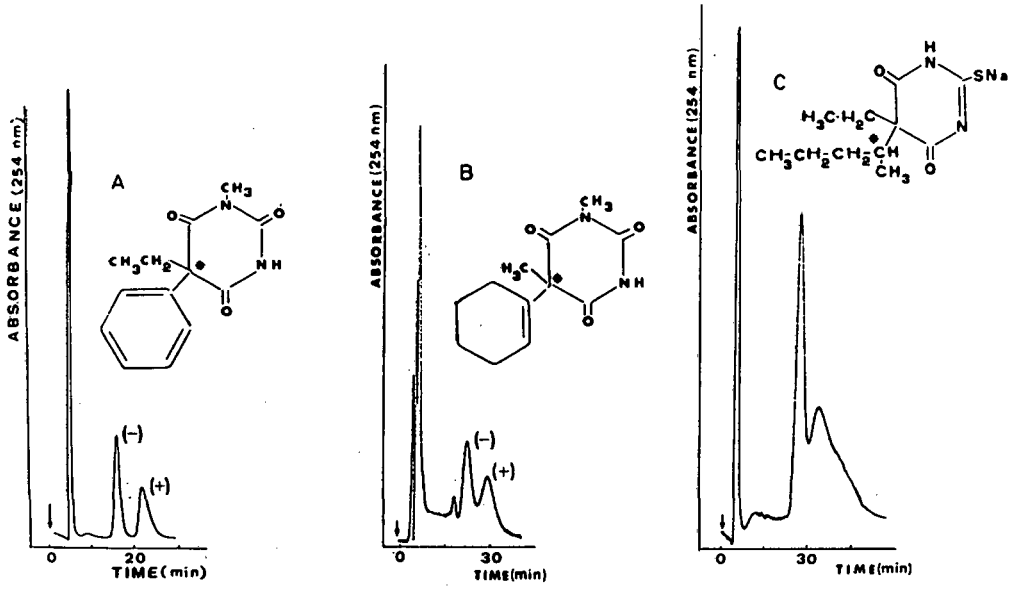


Fig. 4. Resolution of barbiturates on CSP. (A) Mephobarbital; (B) hexobarbital; (C) thiopental. Column temperature: 70°C. Eluent: (A) and (C) methanol–0.01 *M* ammonium acetate buffer (pH 9.5) (85:15, v/v), flow-rate 0.5 ml/min; (B) methanol–0.01 *M* ammonium acetate buffer (pH 9.5) (90:10, v/v), flow-rate 0.7 ml/min.

pyrimidine ring. The chromatographic results are summarized in Table I. A considerable improvement in the chromatographic pattern is brought about by increasing the column temperature. The effect of temperature on the enantioselectivity and the efficiency of the column with mephobarbital as reference is reported in Table II.

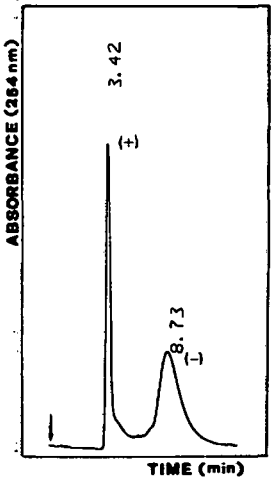


Fig. 5. Resolution of phenyllactic acid enantiomers. Eluent, methanol–0.01 *M* acetate buffer (pH 5.0) (20:80, v/v); flow-rate, 0.5 ml/min; column temperature, 60°C.

TABLE I

CHROMATOGRAPHIC CHARACTERISTICS OF ORGANIC ACID RACEMATES ON UREA–FORMALDEHYDE–L-LEUCINAMIDE POLYMER GEL

Column temperature, 70°C.

| Compound | $k'^a$ | $\alpha^b$ |
|---|---|---|
| Hexobarbital[c] | 9.10 | 1.18 |
| Mephobarbital[c] | 7.42 | 1.32 |
| Thiopental[c] | 8.60 | 1.09 |
| Phenyllactic acid[d] | 0.44 | 1.36 |
| Hydroxymandelic acid[d] | 5.31 | 1.02 |
| α-Hydroxycaproic acid[d] | 6.54 | 1.00 |

[a] Capacity factor of the first-eluted enantiomer.
[b] Separation factor, $k'_2/k'_1$.
[c] Chromatographic conditions as in Fig. 4.
[d] Chromatographic conditions as in Fig. 5.

TABLE II

EFFECT OF TEMPERATURE ON THE ENANTIOSELECTIVITY ($\alpha$) AND EFFICIENCY ($N$) USING MEPHOBARBITAL AS REFERENCE

Flow-rate, 0.6 ml/min; other conditions as in Fig. 4.

| Temperature (°C) | $\alpha^a$ | $N$ |
|---|---|---|
| 23 | 1.26 | 140 |
| 50 | 1.30 | 372 |
| 70 | 1.32 | 405 |

[a] Separation factor, $k'_2/k'_1$.

## CONCLUSIONS

It has been demonstrated that urea and formaldehyde-based gels copolymerized with L-leucinamide combine chemical stability with enantioselectivity for a series of organic acid racemates. Characteristic enantiodiscrimination was shown for cyclic compounds. Because a large number of optically active mono- and polyfunctional amides are commercially available or can easily be synthesized, several new chiral packings with a wide range of resolution could be produced.

## ACKNOWLEDGEMENTS

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# Evaluation of low-conductance eluents for suppressed ion-exclusion chromatography

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ABSTRACT

5-Sulphosalicylic acid was investigated as an eluent for ion-exclusion chromatography with suppressed conductimetric detection and was used for the sensitive determination of weak organic acids. The chromatographic performance of the aromatic eluent was similar to sulphuric acid; 5-sulphosalicylic acid did not give rise to systemic peaks in the chromatograms. The suppressed background conductance of 1.0 m*M* 5-sulphosalicylic acid was decreased by 25% from that found for sulphuric acid at the same concentration and the sensitivity was improved accordingly. The background conductance of the suppressed aromatic acid was about twice that previously reported for 1-octanesulphonic acid. However, 5-sulphosalicylic acid was distinguished by being inexpensive, available at an adequate grade from commercial sources and is chemically stable. For the separation of three small fatty acids the use of 0.1 m*M* sulphuric acid was adequate, resulting in a significant increase in the sensitivity, but no advantage was found in using the low-conductance eluent.

## INTRODUCTION

High-performance liquid chromatography (HPLC) combined with ion-exclusion and conductimetric detection is a versatile method for the determination of weak aliphatic acids that are only slightly absorbing in the ultraviolet. A cation-exchange resin in the $H^+$-form is used. An acidic eluent is used to repress the ionisation of the sample acids, thus improving the entrance into the negatively charged resin. Strong, highly ionised acids pass quickly through the column; they are excluded from the resin phase and elute at the dead volume of the column.

The sensitivity of the conductimetric detection is set by the background conductance of the strong acids used as eluents. Unfortunately, the hydronium ion is one of the most conductive ions and the pH of the eluent cannot be decreased below the $pK_a$ value of the sample acids when good separation is required. One approach to avoid this limitation of the sensitivity is to modify the eluent after passage through the column but before it enters the detector. Rich *et al.* [1] developed a suppressor system to reduce the background conductance from a hydrochloric acid eluent. A cation-exchange column in the $Ag^+$-form removes $H^+$ by exchange with $Ag^+$ and $Cl^-$ by precipitation. An improved device for eluent suppression is the membrane suppressor [2]. A special kind of membrane allows the hydronium ions in the column effluent to be replaced by cations of low mobility, such as the tetrabutylammonium ion. A continuous flow of fresh alkaline regenerant, tetrabutylammonium hydroxide, to the suppressor gives a constant ion-exchange capacity during separation.

Concomitant with eluent suppression, a strong acid with a low-conductance corresponding base can be applied instead of the frequently used hydrochloric or sulphuric acid to improve the sensitivity

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further. Several low-conductance strong acids have been proposed [2–4]. However, they are expensive and some of them are not standard chemical reagents.

In this paper, the use of 5-sulphosalicylic acid as a low-conductance eluent for ion-exclusion chromatography is proposed. The improved sensitivity is compared with that obtained using sulphuric acid. The applicability of other low-conductance eluents for routine analyses is also discussed.

## EXPERIMENTAL

### *HPLC system*

The HPLC system was equipped with a Model 510 dual-piston pump (Waters Assoc.). A Model 6000A high sensitivity noise filter was placed between the pump and the sample injector to reduce the pump pulsation (Waters Assoc.). The injection was performed using a Model 7011 loop valve (Rheodyne) with a 20-$\mu$l sample loop. The temperature of the column was controlled and maintained with a programmable temperature control system (Waters Assoc.). A Model 430 conductivity detector (Waters Assoc.) was used with a cell temperature of 35°C and the time constant switch was set at 0.5 s. Data acquisition from the detector and the determination of retention times, peak heights, areas and numbers of theoretical plates were performed on an IBM- compatible computer using a Model ACI interface and the AI-450 software version 3.0 (Dionex).

### *Column*

An IonPac ICE-AS1 (Dionex) epoxy column 252.5 mm × 9.0 mm with a fully sulphonated polystyrene resin (particle size 7.5 $\mu$m) and a 1500 p.s.i. pressure limit was used. The degree of cross-linking of the resin was 9%. No guard column was used.

### *Suppressor*

An anion micromembrane suppressor for ion chromatography exclusion (AMMS-ICE, Dionex) was installed outside the oven between the column and the detector. The suppressor membrane was continuously regenerated by 5 m*M* tetrabutylammonium hydroxide. A pulse-less flow of regenerant was established by pressurisation of the container with helium. The pressure of the gas was regulated to deliver a regenerant flow of 2.0 ml/min.

### *Mobile phases*

The eluent was sulphuric or 5-sulphosalicylic acid. The concentration of the acids was determined from pH titration after preparation of an approximately 100 m*M* stock solution and were accordingly diluted to the stated concentration. The flow-rate of the eluent was 0.8 ml/min. During chromatographic separation the eluent was degassed with helium.

### *Standard samples*

The standard solutions were prepared from 100 m*M* stock solutions of the organic acids. They were mixed and diluted with the actual eluent to the stated concentrations.

### *Chemicals*

All the chemicals used for standards were of analytical-reagent grade. The sulphuric acid was ACS grade and the 5-sulphosalicylic acid (dihydrate) was of minimum 99.5% purity. The tetrabutylammonium hydroxide was purchased as a 40% solution in water. The eluents and the hydroxide were obtained from Riedel-de Haën (Seelze, Germany).

## RESULTS AND DISCUSSION

In suppressed conductimetric detection the background conductance of the eluent is decreased by the exchange of hydronium ions with tetrabutylammonium ions ($NBu_4^+$) when an AMMS-ICE suppressor membrane is used. The limiting equivalent conductivity [5] of the hydronium ion is 350 $\mu S/cm^2$ equiv., whereas that of $NBu_4^+$ is 19 $\mu S/cm^2$ equiv. The corresponding bases of inorganic strong acids such as hydrochloric, sulphuric, nitric and perchloric acids are small molecules with a relatively high limiting equivalent conductivity. They are 76, 80, 71 and 67 $\mu S/cm^2$ equiv., respectively. Big, bulky ions have a lower conductivity than small ions, as clearly shown by the $H^+/NBu_4^+$ couple. These values show that the most significant improvement of the sensitivity is obtained by suppression of the eluent. However, the sensitivity can be refined by using a low-conductance acid.

The low-conductance acids suitable for ion-ex-

TABLE I

PROPERTIES OF ELUENTS USED IN ION-EXCLUSION CHROMATOGRAPHY

– = Not known or not determined.

| Acid | Compound purchased | Purity (%) | Price[a] (DM) | Concentration (m*M*) | Conductance (unsuppressed) ($\mu$S) | Conductance (suppressed) ($\mu$S) | Conductivity (suppressed) ($\mu$S/cm) |
|---|---|---|---|---|---|---|---|
| Hydrochloric | – | – | – | 1.0 | – | – | 100[b] |
| Perfluoroheptanoic | Acid; Riedel-de Haën (61033) | 95 | 11.38 | 1.0 | – | – | 40[b] |
| 1-Octanesulphonic | Sodium salt; Aldrich (22,156-2) | 98 | 11.25 | 1.0 | 320-330[c,e] | – | 45[b] |
| Sulphuric | Acid; Aldrich (25,810-5) | 95-96 | 0.02 | 0.5 | 368[d,e] | 100[d,e] | – |
| 5-Sulphosalicylic | Acid Riedel-de Haën (33619) | Min. 99.5 | 0.17 | 0.5 | 290[d,e] | 72[d,e] | – |
| Perfluorobutyric | Anhydride; Riedel-de Haën (61444) | 99 | 9.17 | – | – | – | – |
| Tridecafluoroheptanoic | Acid; Aldrich (34,204-1) | 99 | 16.63 | – | – | – | – |

[a] Calculated for 10 l of the eluent containing 1 m*M* hydronium ions, the price is exclusive of ion-exchange materials, preparation time, etc.

[b] From ref. 2, eluent flow-rate 0.8 ml/min.

[c] From ref. 6.

[d] From this work, eluent flow-rate 0.8 ml/min, column temperature 30°C.

[e] These figures were read directly from the "total conductivity" digital display on the Waters detector; however, the unit given at the display is $\mu$S, which to be correct is the unit of the conductance, i.e. the conductivity divided by the detector cell constant. The unit for the conductivity is $\mu$S/cm. The cell constant for the Waters detector is 10 $cm^{-1}$. To allow comparisons of the results from different sources the figures for the two mineral acids with the same concentration of hydronium ions were included.

clusion chromatography include 1-octanesulphonic acid [2–4], perfluoroheptanoic acid [2], perfluorobutyric acid [3] and tridecafluoroheptanoic acid [4]. 1-Octanesulphonic acid is unstable and is only commercially available as the sodium salt. Before use, the salt has to be ion-exchanged. If only mineral acid is added, the sodium salt of this acid will contribute to the background conductivity of the final eluent. Recrystallisation has also been recommended [6]; the purity of sodium octanesulphonate is limited to 98%. The 1-octanesulphonic acid must be freshly prepared monthly owing to its low stability. Perfluoroheptanoic acid is purchased in a limited grade containing 95% of the acid. Perfluorobutyric acid is not, to our knowledge, available commercially, but the anhydride (99% pure) can be obtained and the acid synthesised from this compound. Tridecafluoroheptanoic acid can be purchased with a purity of 99%. It was concluded that most of the chemicals have to be either purified, ion-exchanged or synthesised before they can be used and all the acids are expensive. The properties and current prices are listed in Table I.

The aim of this work was to find a suitable acid without these disadvantages. Until now only aliphatic acids have been widely used in ion-exclusion chromatography (but see Tanaka and Fritz [1]), but 5-sulphosalicylic acid seemed to fulfill the chemical and economic requirements. Initially, the unsuppressed conductance of 0.5 m*M* sulphosalicylic acid was found to be lower than the unsuppressed conductance of 1-octanesulphonic acid [6]. The values

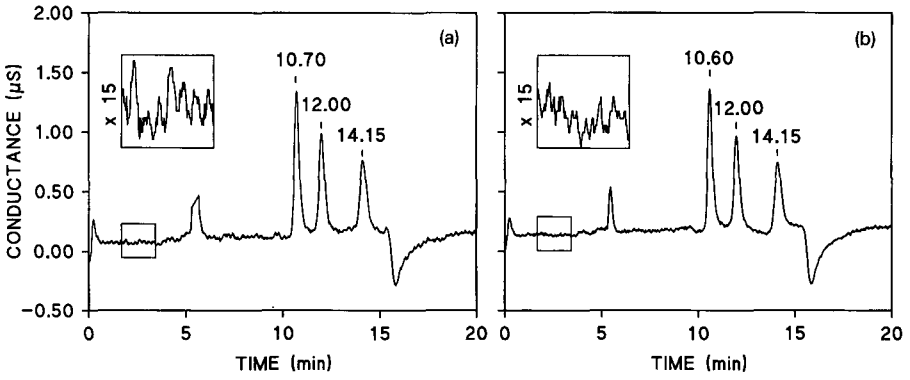


Fig. 1. Ion-exclusion chromatograms of a 10 $\mu M$ standard solution of small fatty acids obtained with (a) 1.0 m$M$ sulphuric acid and (b) 1.0 m$M$ 5-sulphosalicylic acid. The retention times for formate, acetate and propionate were 10.7 (10.6 in Fig. 1b), 12.0 and 14.15 min, respectively. The insets show the baseline noise magnified 15 times. The suppressed background conductances were (a) 200 and (b) 144 $\mu$S. The sample loop was 20 $\mu$l. The flow-rate of the eluent was 0.8 ml/min and the column temperature was 30°C. The range settings of the conductimetric detector were 200 $\mu$S. Owing to the construction of the cell in the detector only the sample ions in the flow cell are measured. Therefore the detector output gives no offset from zero as a result of the background conductance.

are given in Table I. The suppressed background conductances were 72 and 100 $\mu$S for the 0.5 m$M$ eluents of 5-sulphosalicylic and sulphuric acids, respectively. The signal-to-noise ratio ($S/N$) in the chromatogram obtained with 5-sulphosalicylic acid was improved by about 25%, as shown in the insets of Fig. 1. The limit of detection defined as 2 $S/N$ was 2 $\mu M$ for formate and higher for the later eluting acids. The other chromatographic properties of 5-sulphosalicylic acid were identical to sulphuric acid at the same pH as shown in Fig. 1. No systemic peaks were observed.

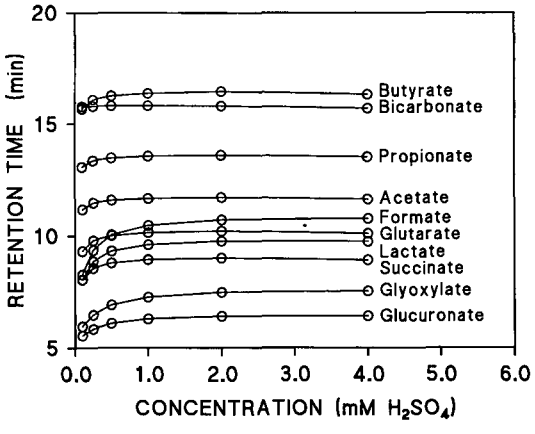


Fig. 2. Retention time of weak organic acids on an ion-exclusion column as a function of the concentration of the eluent. Sulphuric acid was used. Mixtures of weak organic acids with concentrations of 100 or 500 $\mu M$ were injected. The flow-rate of the eluent was 0.8 ml/min. The temperature of the column was 55°C.

A 60% lower background conductivity is claimed to be obtained for perfluoroheptanoic and 1-octanesulphonic acids [2] than that achieved with the aromatic acid. The conductance measured for the unsuppressed 0.5 m$M$ 5-sulphosalicylic acid in this work was less than that given for 1-octanesulphonic acid with the same concentration of hydronium ions, 1.0 m$M$. Both conductances were measured by the same model of detector, therefore some doubt remains about the lowest possible conductance obtainable. However, the expenses for the aliphatic low-conductance acids are considerably higher, about 60–70 times the price for 5-sulphosalicylic acid (see Table I).

The possibility of improving the sensitivity was investigated using a more dilute eluent. Fig. 2 shows that several weak organic acids can be separated with eluents containing 0.2 m$M$ hydronium ions. Fig. 3 shows a chromatogram obtained when 0.1 m$M$ 5-sulphosalicylic acid was used. The background conductances of the sulphuric and 5-sulphosalicylic acids containing 0.2 m$M$ hydronium ions were 27 and 23 $\mu$S, respectively. This small difference gave no measurable difference in the signal-to-noise ratio in the chromatograms (not shown). The resolution between the peaks from formate and acetate was better for the eluents containing 0.2 m$M$ hydronium ions compared to those containing 2.0 m$M$. The limits of detection in the former eluents were set by the digital resolution of the analog-to-digital converter in the data collecting equip-

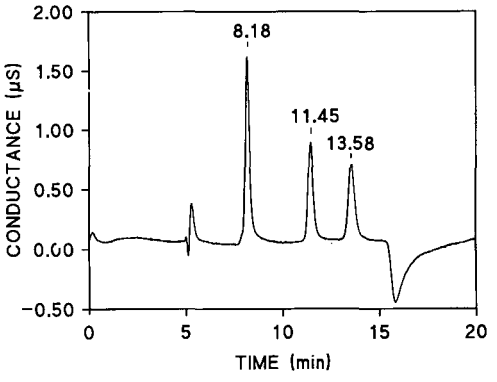


Fig. 3. Ion-exclusion chromatogram of a 10 $\mu M$ standard solution of formate, acetate and propionate obtained with 0.1 m$M$ 5-sulphosalicylic acid as the eluent. The retention times were 8.18, 11.45 and 13.58 min, respectively. The suppressed background conductance was 23 $\mu$S. The range setting of the conductimetric detector was 20 $\mu$S. Other parameters were as described in the legend to Fig. 1.

ment (data not shown), but 1 $\mu M$ formate could be detected.

5-Sulphosalicylic acid is able to form complexes with many cations. The $Ca^{2+}$ present was not precipitated by 5-sulphosalicylate. Therefore this eluent is appropriate for analyses of samples with a high concentration of the cation because $CaSO_4$ is less soluble. The ion-exclusion column is packed with a cation-exchange resin. It is possible that complex formation between 5-sulphosalicylate and the divalent and trivalent cations from the samples might preserve the column in the $H^+$-form for a longer period of time and prolong the life of the column.

In all the chromatograms obtained by application of the membrane suppressor a dip in the baseline with a retention time of 15 min was observed. This did not disappear on injection of the eluent. Hydrogencarbonate eluted at the same retention time and it might be the background content of hydrogencarbonate in the samples which was detected. Detection of butyrate was not possible with a column temperature of 30°C owing to coelution with the negative dip.

## CONCLUSIONS

The chromatograms reported in this paper show the applicability of 5-sulphosalicylic acid as an eluent for ion-exclusion chromatography. The signal-to-noise ratio was reduced by 25% compared with chromatograms obtained with sulphuric acid at the same concentration. A fine detection of weak organic acids in a concentration range down to about 5 $\mu M$ was the result. A further reduction of the background conductivity might be obtained by the use of 1-octanesulphonic acid, but for at least 65 times the price of 5-sulphosalicylic acid and the preparation of the eluent is also more laborious and expensive. The determination of formate, acetate and propionate could be achieved with eluents containing 0.2 m$M$ hydronium ions and the low baseline noise decreased the detection limits to less than 1 $\mu M$ for formate. The 0.1 m$M$ 5-sulphosalicylic acid was selected as eluent for ion-exclusion chromatography for the analyses of small fatty acids as a result of the fine resolution of formate and acetate, the low detection limit and the absence of precipitation of $Ca^{2+}$ salts in the injector. It was found that the additional refinement of the sensitivity possibly obtained by use of the aliphatic low-conductance eluents was less than the costs and, in the case of 1-octanesulphonic acid, the inconvenience of the preparation of the eluent when compared with 5-sulphosalicylic acid. For analyses which require a pH value of the eluent less than 3.7, there is still a need for a lower conductive acid than 5-sulphosalicylic acid, but with the same valuable properties.

## ACKNOWLEDGEMENTS

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# Studies on eluents suitable for use with simultaneous conductivity and direct UV detection in non-suppressed ion chromatography

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ABSTRACT

Simultaneous conductivity and direct UV detection is frequently employed in ion chromatography for the analysis of complex matrices or when samples contain disparate levels of anions. A number of eluents suitable for use with both detection modes were evaluated in terms of their general utility as anion screening eluents for non-suppressed ion chromatography. Octanesulphonate–borate was perhaps the most versatile of the eluents investigated as it had good separation selectivity, gave no system peaks and chloride response could be eliminated when using direct UV detection. Both borate–gluconate and carbonate–hydrogencarbonate also proved to be very useful screening eluents for use with direct UV and non-suppressed and suppressed conductivity detection respectively. Hydroxyde and tartrate–borate were of less utility as general purpose eluents, however both have unique characteristics which make them ideal for selected applications.

## INTRODUCTION

Conductivity is the most commonly used detection mode in ion chromatography (IC); however, the tandem combination of conductivity and direct UV detection is frequently used for the analysis of complex matrices or when samples contain disparate levels of anions [1]. Many of the eluents commonly employed with conductivity detection, such as carbonate–hydrogencarbonate [2,3] or borate–gluconate [4,5], are also applicable for use with direct UV detection. Other species which are suitable for use as eluents with simultaneous conductivity and direct UV detection include alkylsulfonates, such as methane- [6], chloromethane [7] and octanesulfonate [8] and UV-transparent inorganic anions, such as hydroxide [9] and phosphate [10]. Non-suppressed IC offers an advantage over suppressed IC in terms of the wide range of eluents that are applicable with the technique; however, in many cases it is not necessarily clear which eluent is most appropriate for a particular application. This can frequently lead to the chromatographer having to "try" a number of eluents in order to achieve suitable results.

In this paper, several eluents which can be used with simultaneous conductivity and direct UV detection were evaluated with a view toward making the selection of an eluent for a particular application more straightforward. The eluents studied were the commonly used borate–gluconate and carbonate–hydrogencarbonate; hydroxide, tartrate–borate, which gives similar elution characteristics to borate/gluconate eluent [11]; and also an octanesulfonate–borate eluent. The elution characteristics and detection properties of these eluents are discussed and examples of optimal practical applications of each eluent are presented.

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## EXPERIMENTAL

### *Instrumentation*

The liquid chromatograph consisted of a Waters Chromatography Division of Millipore (Milford, MA, USA) Model 510 pump, either a U6K injector or WISP 712 autoinjector, Model 431 conductivity detector, Model 486 UV detector and either an 820 Maxima data station or Model 730 data module. A Waters reagent delivery module (RDM) was added to the system for solid-phase reagent (SPR) conductivity detection. Three methacrylate-based, anion-exchange analytical columns from the Waters IC-Pak Anion range were used; an IC-Pak Anion (50 × 4.6 mm I.D.), an IC-Pak Anion HC (150 × 4.6 mm I.D.) and an IC-Pak Anion HR (75 × 4.6 mm I.D.) column. UV-absorbance spectra were recorded on a Varian DMS 100 scanning spectrophotometer (Palo Alto, CA, USA).

### *Eluents*

The five eluents used in the preliminary evaluations were initially selected to give a similar separation of a mixture of carbonate, chloride, nitrite, nitrate, phosphate and sulfate. The eluents were: borate–gluconate (1.3 m*M* tetraborate, 5.8 m*M* boric acid, 1.3 m*M* gluconate, 5 g/l glycerin, 120 ml/l acetonitrile, 20 ml/l *n*-butanol, pH 8.5), carbonate–hydrogencarbonate (1.2 m*M* carbonate, 1.2 m*M* hydrogencarbonate, pH 10.0), hydroxide (6.0 m*M* hydroxide, pH 11.7), tartrate–borate (3.0 m*M* tartaric acid, 0.4 *M* boric acid, adjusted to pH 4.5 with lithium hydroxide) and octanesulfonate–borate (3.0 m*M* octanesulfonate, 5 m*M* boric acid, adjusted to pH 8.5 with lithium hydroxide). Eluents were prepared daily, filtered and degassed with a Waters solvent clarification kit.

### *Reagents*

Water purified (18 MΩ) using a Millipore Milli-Q water purification system (Bedford, MA, USA) was used for all solutions. Sodium tetraborate, lithium hydroxide and boric acid (all analytical-reagent grade) and glycerin and tartaric acid (both laboratory-reagent grade) were obtained from Ajax Chemicals (Sydney, Australia), as were the analytical-grade sodium salts used for the preparation of all the anion standards. Sodium gluconate (laboratory-reagent grade) was obtained from Fluka (Buchs, Switzerland). Sodium octanesulfonate was obtained from BDH (Poole, UK). Acetonitrile and *n*-butanol (both HPLC grade) were obtained from Waters.

## RESULTS AND DISCUSSION

### *Preliminary investigations*

In considering the requirements of an eluent to be suitable for general purpose use with dual conductivity and direct UV detection, three important criteria emerge. These are sensitivity, selectivity and eluent pH and buffering capacity. The eluent chosen by the chromatographer must firstly permit sensitive detection, *i.e.* have a large difference between the equivalent conductances of the solute and eluent anions in the case of non-suppressed conductivity detection [12] and be (essentially) transparent at the detection wavelength in the case of direct UV detection. An eluent must then have appropriate selectivity for a particular application with the column of choice. Finally, the eluent should be able to be operated at a suitable pH to allow the determination of common weak acid anions, such as phosphate, and also posses sufficient buffering capacity to allow samples with pH values appreciably different to the eluent to be sucessfully chromatographed.

The eluents chosen for this study include three commonly used IC eluents, namely borate–gluconate, carbonate–hydrogencarbonate and hydroxide as well as the less commonly used tartrate–borate [11] and a novel eluent, octanesulfonate–borate. All eluents permit relatively sensitive detection with both non-suppressed conductivity and direct UV detection, with the exception of the combination of carbonate–hydrogencarbonate eluent and conductivity detection. While this eluent is typically used with suppressed conductivity detection [2], for the purposes of comparison, it was used in the non-suppressed mode in the preliminary investigations. The five eluents were initially selected to give a separation of a mixture of carbonate, chloride, nitrite, nitrate, phosphate and sulfate within a reasonable elution time in order to evaluate the selectivity of the eluents. The UV spectrum of each of the eluents was then measured, along with the spectrum of a solution of 100 ppm chloride, as dual conductivity–direct UV detection is frequently employed when

samples contain high levels of chloride. The final stage of the preliminary investigations was to use each eluent for an application for which dual conductivity–direct UV detection IC would be typically employed, the determination of anions in a sewage plant effluent to bay discharge sample.

Fig. 1 shows the UV spectra in the range 190–256 nm for each of the five eluents described in the Experimental section, along with the spectra of a solution of 1000 ppm chloride. These results indicate that octanesulfonate–borate should probably provide the most sensitive detection of UV-absorbing anions, such as the commonly analyzed nitrite and nitrate, in the 200–220 nm region where these anion show appreciable absorbance. Carbonate–hydrogencarbonate, hydroxide and tartrate–borate all appear as though they will permit similar detection sensitivity in the 200–220 nm range, although the hydroxide spectrum was probably effected by the adsorption of carbon dioxide into the solution. The UV spectra also indicate that it should be possible to eliminate any UV response for chloride by appropriate wavelength selection when using an octanesulfonate–borate eluent.

Fig. 2 shows chromatograms of a standard mixture containing 10 ppm chloride, 20 ppm nitrite, 20 ppm nitrate, 30 ppm phosphate and 20 ppm sulfate using each of the five eluents described in the Experimental and an IC-Pak Anion HC column with conductivity and direct UV absorption detection at 214 nm. A review of the chromatograms in Fig. 2 suggests that each of the different eluents has a number

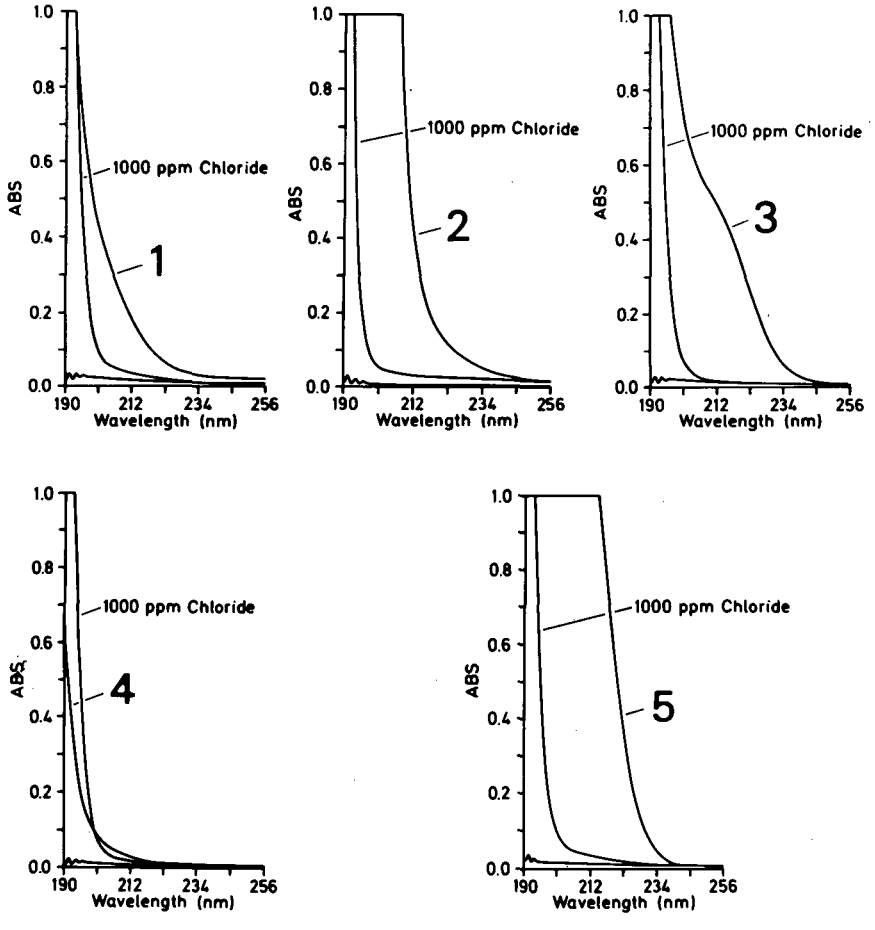


Fig. 1. UV spectra in the range 190–256 nm for the five eluents and 1000 ppm chloride. Eluents (see Experimental): 1 = borate–gluconate; 2 = hydroxide; 3 = tartrate–borate; 4 = octanesulfonate–borate; 5 = carbonate–hydrogencarbonate.

of unique characteristics: (a) Borate–gluconate gave the best overall selectivity of the five eluents for the standard mixture with the methacrylate-based anion exchanger. Hydrogencarbonate and chloride, and to a lesser extent phosphate and sulfate gave negative peaks when using direct UV detection and also the UV response for nitrate was significantly greater than for nitrite with this eluent. (b) Carbonate–hydrogencarbonate was a poor eluent for non-suppressed conductivity detection, as was expected and system peaks [13,14] were observed with both detection modes. Also, phosphate did not elute under these conditions, however this eluent gave excellent sensitivity for nitrite and nitrate with UV detection at 214 nm. (c) Phosphate also did not elute when using an hydroxide eluent and carbonate co-eluted with sulfate under most eluent conditions that allowed a reasonable total tun time with the IC-Pak Anion HC column. Good response was obtained for nitrite and nitrate; and as was the case with the other two eluents above, chloride appeared as a negative peak with UV detection at 214 nm. (d) Tartrate–borate gave the least sensitive response for nitrate when using direct UV detection and was the only eluent where the UV response for nitrite was significantly greater than for nitrate. The phosphate peak gave a negative response with conductivity detection and no carbonate peak appeared at this low eluent pH. Also, nitrate eluted later than sulfate under these eluent conditions. (e) Octanesulfonate–borate gave reasonable selectivity for the anions of interest, but most significantly, it gave good UV response for nitrite and nitrate and virtually no response for the other anions at 214 nm. All the eluents, with the exception of carbonate–hydrogencarbonate, gave similar conductivity response for the anions of interest, although hydroxide (in the indirect mode) gave slightly better sensitivity with conductivity detection than the other eluents.

The five eluents were then used for an application

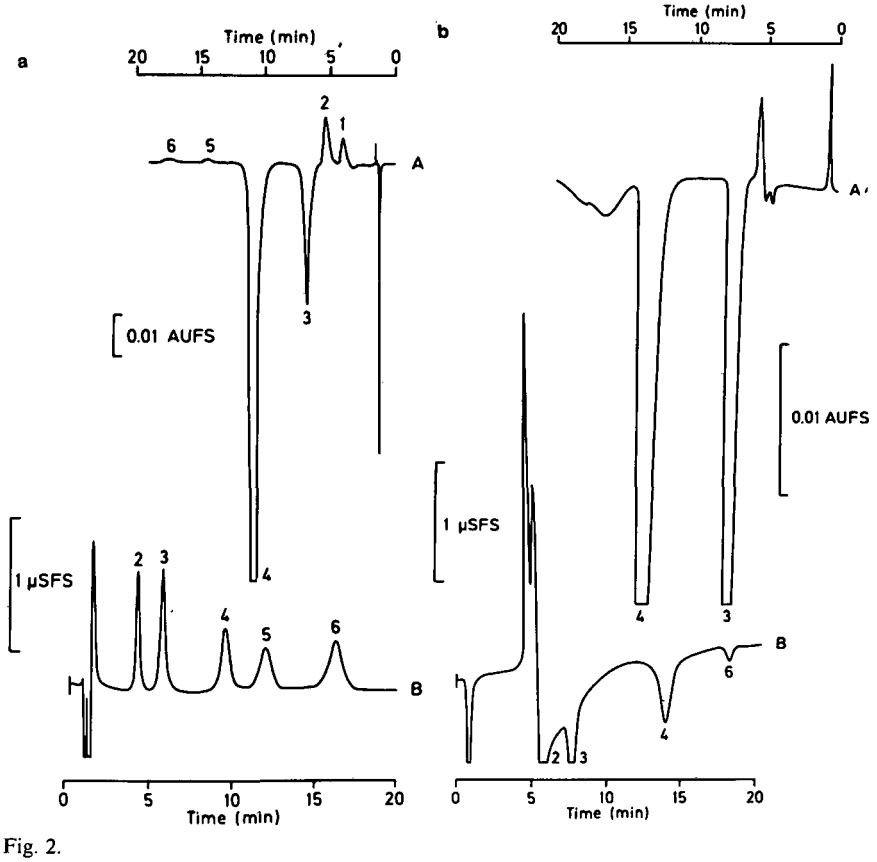


Fig. 2.

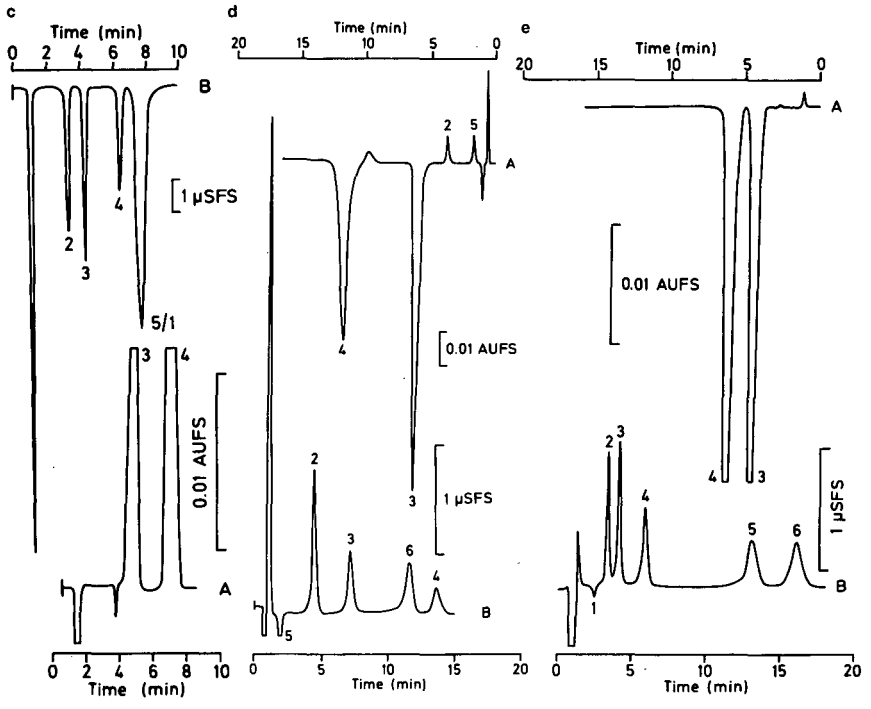


Fig. 2. Chromatograms of a standard anion mixture for the five eluents with conductivity and direct UV absorption detection at 214 nm. Conditions: eluents (details as in Experimental), (a) borate–gluconate; (b) carbonate–hydrogencarbonate; (c) hydroxide; (d) tartrate–borate; (e) octanesulfonate–borate; column, Waters IC-Pak Anion HC; flow-rate, 2.0 ml/min; injection volume, 25 μl; detection, A = UV at 214 nm, 0.05 AUFS and B = conductivity, 5 μSFS. Solutes: 1 = carbonate; 2 = chloride (10 ppm); 3 = nitrite (20 ppm); 4 = nitrate (20 ppm); 5 = phosphate (30 ppm); 6 = sulfate (20 ppm).

for which dual conductivity–direct UV detection would be typically employed, *i.e.* the analysis of anions in a sewage plant effluent to bay discharge sample using the same conditions as described for the standard chromatograms above. The use of dual detectors was important in this application as it allowed the quantitation of nitrite (and nitrate to a lesser extent) by UV at 214 nm and the remaining anions in the sample by conductivity. As would be expected, the same characteristics for each of the eluents as described in the previous paragraph were evident. Fig. 3 shows an example of a typical chromatogram of the discharge sample obtained using an octanesulfonate–borate eluent and dual conductivity and direct UV absorption detection. The higher level of chloride present in the sample (compared to the standards shown in Fig. 2) resulted in a negative chloride peak which interfered with the determination of nitrite by direct UV at 214 nm when using an hydroxide eluent. Also, a negative sulfate peak interfered with the determination of nitrate by direct UV at 214 nm in the sample when using a tartrate–borate eluent. The borate–gluconate and octanesulfonate–borate eluents were the most suitable eluents for this analysis as they allowed the determination of all the anions of interest in the sample, *i.e.* chloride, nitrite, nitrate, phosphate and sulfate with no interferences for any peak using either detection mode.

*Optimal applications of each eluent*

The preliminary investigations above indicated that each eluent had both useful and deleterious features which would influence whether it would be suitable for a particular application. Table I lists the advantages and disadvantages of each of the eluents and Figs. 4–8 show examples of optimal applications for each of the five eluents. These examples were selected to highlight the merits of each of the eluents. Fig. 4 shows a chromatogram of a sanita-

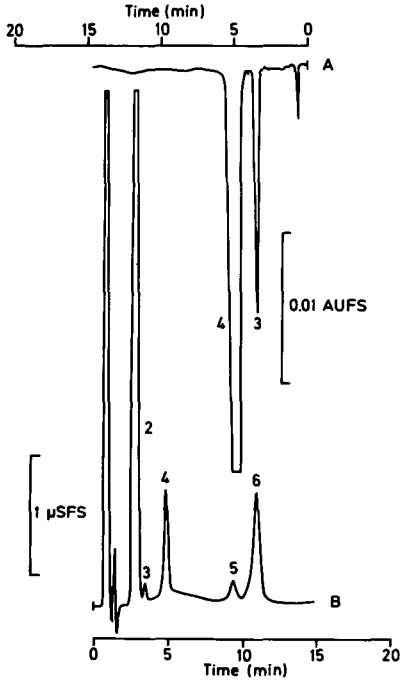


Fig. 3. Chromatogram of anions in a sewage plant effluent to bay discharge sample using octanesulfonate–borate eluent and dual conductivity and direct UV absorption detection at 214 nm. Conditions as for Fig. 2. Solutes: 1 = carbonate; 2 = chloride; 3 = nitrite; 4 = nitrate; 5 = phosphate; 6 = sulfate.

tion effluent sample using borate–gluconate eluent with an IC-Pak Anion HC column and dual conductivity–direct UV detection. Borate–gluconate had the best overall selectivity (at least with methacrylate-based anion-exchangers) of any eluent and as such, was ideally suited to anion screening in samples such as effluents, drinking waters, wastewaters, etc. Fluoride can also be quantitated using these eluent conditions. Extreme pH samples and also samples high in calcium/magnesium may cause system peaks [15] and high levels of bicarbonate may interfere with chloride when using this eluent. As mentioned previously, carbonate–hydrogencarbonate was a poor eluent for use with non-suppressed conductivity detection, however it is the most versatile eluent when used with suppressed (post-column enhancement) conductivity detection [1]. Fig. 5 shows a chromatogram of an Antarctic ice melt sample using carbonate–hydrogencarbonate eluent with an IC-Pak Anion HR column and post-column "enhancement" SPR conductivity detection [16]. This selectivity of this eluent was particularly useful for the analysis of samples containing alkylsulfonates in the presence of chloride and carboxylic acids [17], as demonstrated in Fig. 5. System peaks can be a problem with this eluent and excessive phosphate retention was a problem with many column and eluent concentration combinations. An additional advantage of carbonate–hydrogencarbonate eluents is that their use masks sample carbonate to a large degree, making this eluent particularly suited for the analysis of samples high in carbonate, *e.g.* alkaline fusion or trap samples.

Borate–gluconate and carbonate–hydrogencarbonate were both very versatile for anion screening applications and are the most widely used eluents with non-suppressed and suppressed conductivity detection respectively [1]. Both can be used with direct UV detection, although high levels of chloride can interfere with the determination of nitrite when using this detection mode. Hydroxide was by far the most "difficult" to use of the five eluents studied, hence was of less utility as a general purpose eluent. The high pH means that eluent protection with helium blanketing/sparging or the use of an Ascarite trap was necessary as carbon dioxide adsorption can lead to baseline drift, cycling and also retention time instability. However, this eluent did permit the most sensitive conductivity detection when operated in either the non-suppressed or suppressed mode and it also allowed very low detection limits for nitrite and nitrate when using direct UV detection at 214 nm. Undoubtably, the greatest advantage of hydroxide is that the high eluent pH allows very weak acids ($pK_a > 7$) to be chromatographed using an anion-exchange column. This eluent is often used in conjunction with other detection modes, such as amperometry, for the analysis of weak acid anions, *e.g.* sulfide and cyanide [18]. Another important application of hydroxide eluents is for the analysis strongly acidic samples, such as acid preserved drinking waters. Fig. 6 shows a chromatogram of nitrite-N and nitrate-N (at low μg/l levels) in a sulfuric acid preserved, chlorinated drinking water sample with an IC-Pak Anion column and direct UV detection at 214 nm.

Tartrate–borate was of limited utility as a general purpose eluent as phosphate eluted as a negative peak when using conductivity detection and could

TABLE I

SUMMARY OF THE ADVANTAGES AND DISADVANTAGES FOR THE FIVE ELUENTS

| Eluent | Advantages | Disadvantages |
|---|---|---|
| Borate–gluconate | (1) Excellent overall selectivity<br>(2) pH allows determination of most common anions<br>(3) Good buffer | (1) Chloride may interfere with nitrite at high levels on UV detection<br>(2) Carbonate may interfere with chloride at high levels on conductivity<br>(3) System peaks may be a problem |
| Carbonate–hydrogencarbonate | (1) Reasonable selectivity, especially for alkylsulfonate and carboxylic acid mixtures<br>(2) Good "suppressed" eluent<br>(3) Good buffer<br>(4) No sample carbonate peak | (1) Chloride may interfere with nitrite at high levels on UV detection<br>(2) System peaks may be a problem<br>(3) Phosphate retention can be excessive with some column/eluent combinations |
| Hydroxide | (1) High eluent pH allows for determination of weak acid anions, *i.e.* cyanide<br>(2) Most sensitive eluent with conductivity detection<br>(3) Good sensitivity with UV detection at 214 nm | (1) Chloride may interfere with nitrite at high levels on UV detection<br>(2) Eluent must be helium sparged<br>(3) Baseline drift/cycling a problem<br>(4) Retention time stability a problem<br>(5) $CO_3/SO_4$ resolution can be a problem<br>(6) No buffering capacity |
| Tartrate–borate | (1) Low eluent pH discriminates against organic acid retention<br>(2) Good low-pH buffer<br>(3) No sample carbonate peak | (1) Sulfate may interfere with nitrate at high levels on UV detection<br>(2) Phosphate cannot be quantitated<br>(3) System peaks may be a problem |
| Octanesulfonate–borate | (1) Reasonable overall selectivity<br>(2) pH allows determination of most common anions<br>(3) No chloride interference with UV detection at 214 nm | (1) Carbonate may interfere with chloride at high levels on conductivity |

not be quantitated, however this eluent was particularly suited to the analysis of inorganic anions in samples containing high levels of organic acid anions. Divalent organic acid anions, *e.g.* tartrate, succinate, malate and oxalate, often co-elute with inorganic anions such nitrate and sulfate when using neutral to alkaline eluents. Similarly, short-chained carboxylic acids, such as formate and acetate, are typically weakly retained and can interfere with anions such as fluoride and chloride. These organic acids all eluted at the void volume with tartrate–borate as a result of the low affinity of these species for the column exchange sites combined with the fact that low eluent pH protonates the organic acids, further decreasing their retention. The presence of a high concentration of a weak eluting species such as boric acid also discriminates against the retention of organic acids, hence this eluent was applicable to the analysis of inorganic anions in samples containing high levels of organic acids, such as Bayer liquors, soil and plant extracts. Fig. 7 shows a chromatogram of well resolved chloride and sulfate peaks in a very complex sample matrix, a diluted Bayer liquor (which typically contains up to 30 g/l total organic carbon present as various organic acids at a pH of *ca.* 14) using a tartrate–borate eluent with an IC-Pak Anion HC column and conductivity detection. Lowering of the eluent pH would further decrease the retention of sample organic acids and also increase the sensitivity of conductivity detection [19], however the run times also become much longer with decreasing eluent pH.

A variety of alkylsulfonates have been used previously with dual conductivity–direct UV detection in IC [6–8]; however, these eluents had no buffering capacity and the pH was typically not appropriate to permit phosphate analysis. The addition of a bo-

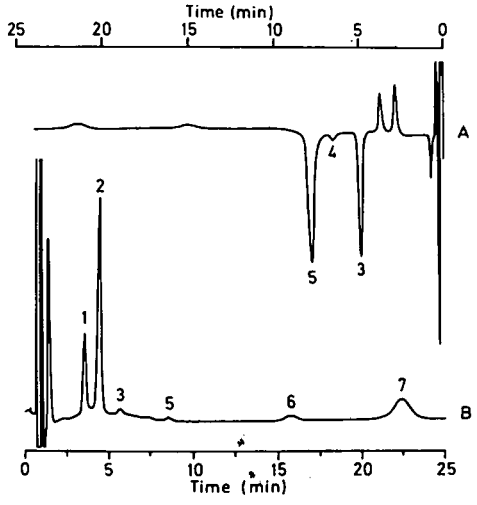


Fig. 4. Chromatogram of a sanitation effluent sample using borate–gluconate eluent and dual conductivity–direct UV detection. Conditions as for Fig. 2, except eluent, 1.3 m*M* tetraborate, 5.8 m*M* boric acid, 1.3 m*M* gluconate, 5 g/l glycerin, 120 ml/l acetonitrile, 20 ml/l *n*-butanol, pH 8.5; injection volume, 100 µl; detection, A = UV at 214 nm and B = conductivity. Solutes: 1 = carbonate; 2 = chloride (10.5 ppm); 3 = nitrite (0.4 ppm); 4 = bromide (0.2 ppm); 5 = nitrate (0.5 ppm); 6 = phosphate (2.5 ppm); 7 = sulfate (7.3 ppm).

rate buffer to octanesulfonate creates a very versatile, anion-screening eluent for IC. Borate itself is a very weak eluent [11] and a concentration of approximately 50 m*M* was required to elute the

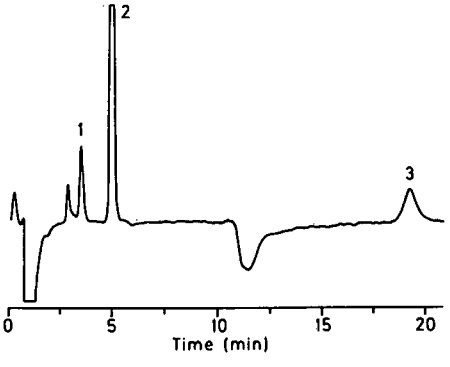


Fig. 5. Chromatogram of an Antarctic ice melt sample using carbonate–hydrogencarbonate eluent and SPR conductivity detection. Conditions as for Fig. 2, except column, Waters IC-Pak Anion HR; eluent, 1.2 m*M* carbonate, 1.2 m*M* hydrogencarbonate; flow-rate, 1.0 ml/min; injection volume, 100 µl; detection, SPR conductivity. Solutes: 1 = methanesulfonate (0.07 ppm); 2 = chloride (0.12 ppm); 3 = sulfate (0.04 ppm).

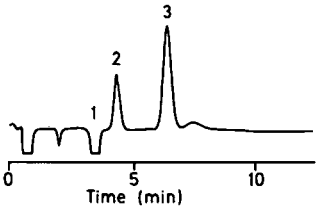


Fig. 6. Chromatogram of nitrite-N and nitrate-N in a sulfuric acid preserved, chlorinated drinking water sample using hydroxide eluent and direct UV detection at 214 nm. Conditions as for Fig. 2, except column, Waters IC-Pak Anion; eluent, 2.5 m*M* hydroxide; flow-rate, 1.2 ml/min; injection volume, 100 µl; detection, UV at 214 nm. Solutes: 1 = chloride; 2 = nitrite-N (0.03 ppm); 3 = nitrate-N (0.07 ppm).

anions with approximately the same retention times as shown in Fig. 2e, hence it buffers the octanesulfonate eluent without significantly effecting its eluting strength. The utility of the octanesulfonate–borate eluent is demonstrated by the analysis of a relatively high chloride matrix, a grass filtration (preliminary treatment) sewage sample, as shown in Fig. 8. The chloride peak, present at approximately 500 ppm, did not interfere with the determination of nitrite, present at 0.5 ppm, by direct UV detection. This eluent has good overall selectivity for the common anions and the fact that chloride has no UV response allows the determination of nitrite by direct UV detection with no interference from as much as a 10 000-fold excess of chloride.

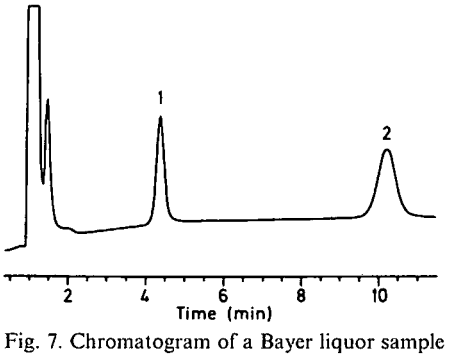


Fig. 7. Chromatogram of a Bayer liquor sample using tartrate–borate eluent and conductivity detection. Conditions as for Fig. 2, except eluent, 3.0 m*M* tartaric acid, 0.4 *M* boric acid adjusted to pH 4.5 with hydroxide; injection volume, 100 µl; detection, conductivity; sample preparation, 1:500 dilution with water. Solutes: 1 = chloride (31 ppm); 2 = sulfate (43 ppm).

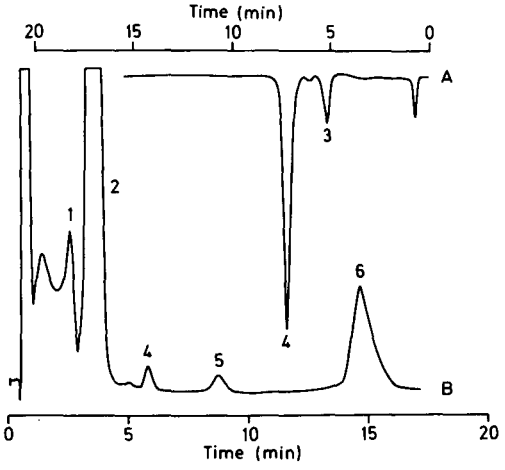


Fig. 8. Chromatogram of a grass filtration (preliminary treatment) sewage sample using octanesulfonate–borate eluent and dual conductivity–direct UV detection. Conditions as for Fig. 2, except eluent, 3.0 m*M* octanesulfonic acid, 5 m*M* boric acid adjusted to pH 8.5 with hydroxide; injection volume, 100 μl; detection, A = UV at 210 nm and B = conductivity. Solutes: 1 = carbonate; 2 = chloride (523 ppm); 3 = nitrite (0.5 ppm); 4 = nitrate (3.1 ppm); 5 = phosphate (58 ppm); 6 = sulfate (124 ppm).

## CONCLUSIONS

The use of dual conductivity and direct UV detection is a very versatile approach for the analysis of complex samples in IC. While no single eluent is appropriate for all applications, octanesulfonate–borate is perhaps the most versatile, general purpose anion screening eluent for non-suppressed IC. This eluent has good separation selectivity, gives no system peaks and chloride response can be eliminated when using direct UV detection. Both borate–gluconate and carbonate–hydrogencarbonate are also very good general purpose eluents for use with direct UV and non-suppressed and suppressed conductivity detection, respectively. The difficulties of using hydroxide eluents detract from their general utility, however the high pH makes it the only eluent suitable for the analysis of weak acid anions such as cyanide and silicate when using anion-exchange separations. The use of tartrate–borate eluents is restricted to analyses not requiring phosphate quantitation, however the eluent selectivity and low pH strongly discriminate against the retention of organic acids, making it ideal for the analysis of inorganic anions in samples containing high levels of organic acids.

## ACKNOWLEDGEMENT

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# Approaches to gradient elution in ion-exclusion chromatography of carboxylic acids

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## ABSTRACT

A range of eluents has been examined with a view to determining which can be used to manipulate the selectivity of retention in ion-exclusion chromatography when applied to the separation of common water-soluble carboxylic acids. The column used was a Bio-Rad HPX-87H Organic Acids column and the eluents examined included water and dilute solutions of sulfuric acid, phosphoric acid, *p*-toluenesulfonic acid, methanesulfonic acid and benzoic acid. Both conductivity and ultraviolet detection were utilized. The use of water alone as an eluent gave poorly shaped peaks, whilst the remaining eluents gave satisfactory peak shape. The best performance was obtained using methanesulfonic acid as eluent. Studies on gradient elution in ion-exclusion chromatography were also undertaken. Three approaches to gradient separation were investigated. The first approach was to utilize a concentration gradient in which the eluent concentration was decreased over the run, thereby increasing the degree of solute ionization and thus solute retention times. This method proved to be of limited utility. The second approach involved increasing the amount of an organic modifier (acetonitrile) in the eluent, and satisfactory gradients were produced by this method. In the third approach, a varying concentration of $\beta$-cyclodextrin was introduced into the eluent as a means of reducing the retention times of aromatic solutes through the formation of inclusion compounds with $\beta$-cyclodextrin. Once again, satisfactory gradient separations were produced with this approach.

## INTRODUCTION

Carboxylic acids can be analyzed chromatographically by employing reversed-phase [1], ion-exchange [2,3] or ion-exclusion [4,5] techniques. The reversed-phase and ion-exchange methods are somewhat limited in that it is difficult to separate low-molecular weight acids using these approaches. On the other hand, ion-exclusion chromatography using a high-capacity cation-exchange resin in the $H^+$ form with an acidic eluent offers separation of a wider range of acids than is possible with either of the alternative methods. Typical ion-exclusion columns designed for the separation of carboxylic acids contain sulfonated polystyrene–divinylbenzene copolymers. Large column dimensions are necessary to provide sufficient occluded mobile phase to permit a reasonable degree of retention of solute acids.

The theory of ion-exclusion chromatography has been discussed by a number of authors [6–8] and it is evident that solutes are retained by a number of mechanisms, including electrostatic effects, adsorption, and perhaps size exclusion. Paramount amongst these is the electrostatic interaction of the solute with the charged functional groups on the resin surface. These functional groups can be considered to comprise a charged membrane separating the flowing mobile phase from occluded, static mobile phase trapped in the pores of the resin. Ionic solutes are rejected, or excluded, from the resin because of their inability to penetrate the charged

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"membrane" and so are eluted at the void volume of the column. In contrast, non-ionic (or weakly ionic) substances may partition between the occluded liquid phase and the flowing mobile phase. The degree of partition determines the extent of retention on the column. Although ion-exchange resins are used, true ion-exchange reactions are not involved.

The degree of retardation increases with decreasing the degree of ionisation (or increasing p*K*a) [7]. Strong acids which are fully ionised are totally excluded and are eluted at the void volume. Very weak acids (p$K_a$ greater than 6.4) tend not to be excluded at all and permeate totally into the resin, giving retention times which are independent of p$K_a$. Many acids with p$K_a$ values intermediate between these extremes show an elution order which can be predicted from p$K_a$ values. However, other acids (particularly aromatic carboxylic acids and long-chain aliphatic carboxylic acids) show retention times which are longer than expected from consideration of their p$K_a$ values alone. In these cases, hydrophobic adsorption effects are considered to contribute to the retention process. Finally, some acids (especially difunctional aliphatic carboxylic acids) show less retention than expected from their p$K_a$ values. For these species, it has been postulated [9–11] that size-exclusion effects may restrict access to the occluded liquid.

Eluents employed in ion-exclusion chromatography can be water [12] or a dilute solution of a strong mineral acid such as sulfuric acid [13] or an aliphatic sulfonic acid [14,15]. Weak acids such as phosphoric acid [16] and benzoic acid [13] are also commonly used. The use of an acidic eluent gives improved peak shape and ensures that the retention time is independent of solute concentration. Organic modifiers have also been employed as eluent components in ion-exclusion chromatography [12]. Solvents such as methanol have little effect on the retention times of low-molecular-weight aliphatic acids, but cause a decrease in the retention time of larger aliphatic acids [12]. Gradient elution in ion-exclusion chromatography has been reported using eluents comprising 6–64% methanol in 0.5 m*M* sulfuric aicd [13]. A further limitation on the type of eluent used is that UV detection (typically in the wavelength range 210–215 nm) is generally employed, and the eluent must be transparent at the detection wavelength. With all of the eluents currently used, the separation selectivity is rather limited, with the pH of the eluent exerting the greatest influence on retention.

The potential of gradient elution has been exploited widely in most forms of liquid chromatography, but has not been used to any significant extent in ion-exclusion chromatography. In this study, selectivity effects in ion-exclusion chromatography arising from variation of the composition of the eluent are investigated. These selectivity effects are then applied to gradient elution of mixtures of aliphatic and aromatic carboxylic acids by ion-exlusion.

## EXPERIMENTAL

### *Instrumentation*

The liquid chromatograph comprised a Millipore-Waters (Milford, MA, USA) M-600 E multisolvent gradient pump and a Waters Model U6K injector. Two detectors were used, namely a Waters Model 480 variable-wavelength UV–VIS detector and a Waters Model Model 430 conductivity detector. The column used was a Bio-Rad (Richmond, CA, USA) Aminex HPX-87H Organic Acids ion-exclusion column (300 × 7.8 mm I.D.) packed with 9-$\mu$m sulfonated styrene–divinylbenzene resin with 8% cross-linking. Chromatograms were recorded on a BBC Goerz Metrawatt (Vienna, Austria) SE 120 chart recorder.

### *Reagents and procedures*

The carboxylic acids used as solutes are listed in Table I, together with the p$K_{a1}$ value for each acid.

TABLE I

p$K_{a1}$ VALUES OF THE CARBOXYLIC ACIDS INVESTIGATED

| Acid | p$K_{a1}$ | Acid | p$K_{a1}$ |
|---|---|---|---|
| Oxalic | 1.23 | Acetic | 4.75 |
| Maleic | 1.83 | Propionic | 4.87 |
| Malonic | 2.83 | Isobutyric | 4.84 |
| Tartaric | 2.98 | Mandelic | 3.66 |
| Citric | 3.13 | Phthalic | 2.89 |
| Malic | 3.40 | Terephthalic | 3.82 |
| Succinic | 4.20 | *p*-Hydroxybenzoic | 4.48 |
| Formic | 3.75 | Benzoic | 4.19 |
| | | Salicylic | 3.00 |

TABLE II

RETENTION TIMES OF CARBOXYLIC ACIDS FOR DIFFERENT ELUENTS (1 m*M*)

| Solute acid | Retention time (min) | | | | | |
|---|---|---|---|---|---|---|
| | Water | $H_3PO_4$ (pH 3.02) | $H_2SO_4$ (pH 3.63) | Benzoic acid (pH 3.62) | *p*-Toluenesulfonic acid (pH 3.00) | Methanesulfonic acid (pH 2.74) |
| Citric | 4.0 | 5.7 | 6.3 | 4.5 | 5.7 | 5.3 |
| Tartaric | 4.0 | 5.7 | 6.8 | 4.5 | 5.7 | 5.5 |
| Malonic | 4.0 | 6.0 | N.A. | 4.5 | 6.3 | 5.5 |
| Malic | 4.0 | 6.6 | 7.6 | 5.3 | 6.7 | 6.3 |
| Succinic | N.A.[a] | 9.2 | N.A. | 7.0 | 9.2 | 8.6 |
| Formic | 6.3 | 9.7 | 10.9 | 7.0 | 10.0 | 9.5 |
| Acetic | 8.7 | 11.7 | 12.1 | 9.5 | 11.3 | 10.9 |
| Isobutyric | N.A. | 15.6 | N.A. | 12.8 | 15.8 | 14.7 |

[a] N.A. = Data not available.

These acids were obtained as analytical-grade reagents and were used without further purification. Eluents were prepared by diluting the required amounts of the eluent acid in a 1-l volumetric flask, followed by degassing using a Bransonic 220 (Branson, CO, USA) ultrasonic bath prior to use. The water used was purified on a Millipore (Bedford, MA, USA) Milli Q water purification system and filtered through a Millipore solvent clarification apparatus using a Millipore type 0.45-$\mu$m HA filter. The eluent flow-rate was fixed at 1.0 ml/min. Working standard solutions were prepared daily from stock solutions of 10 000 ppm (aliphatic) or 1 000 ppm (aromatic) carboxylic acids.

## RESULTS AND DISCUSSION

### *Nature of the eluent acid*

Ion-exclusion chromatography is most commonly performed using sulfuric acid as eluent, with UV detection. This eluent was compared with water, phosphoric acid, methanesulfonic acid, *p*-toluenesulfonic acid and benzoic acid eluents, using both UV and conductivity detection. Table II lists retention times obtained at an eluent strength of 1 m*M* and shows that the elution order was almost identical for each eluent. That is, no selectivity effects arose when the nature of the eluent acid was varied. Detection limits were calculated for each eluent using both UV and conductivity detection. In some cases, a particular detection mode was inappropriate (*e.g.* UV detection with UV-absorbing eluents, such as benzoic acid). Again, no large variations in detection limits were observed for the different eluents tested but methanesulfonic acid offered the best overall performance in terms of separation and chromatographic efficiency and was therefore employed in all further work.

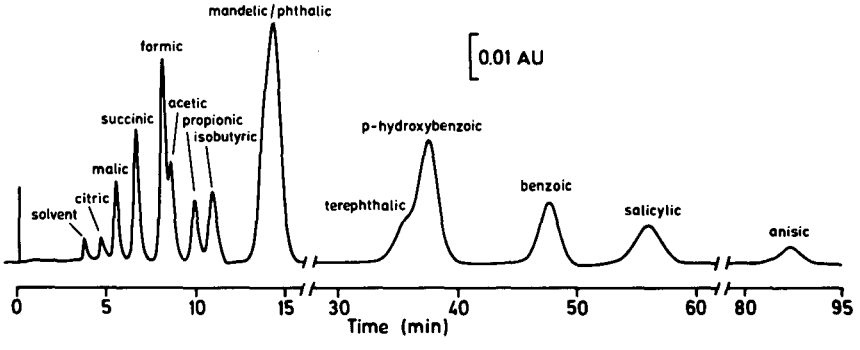


Fig. 1. Isocratic separation of aliphatic (20–500 ppm) and aromatic (5–50 ppm) carboxylic acids. The eluent was 10 m*M* methanesulfonic acid containing 1% (v/v) acetonitrile. Spectrophotometric detection at 210 nm was used.

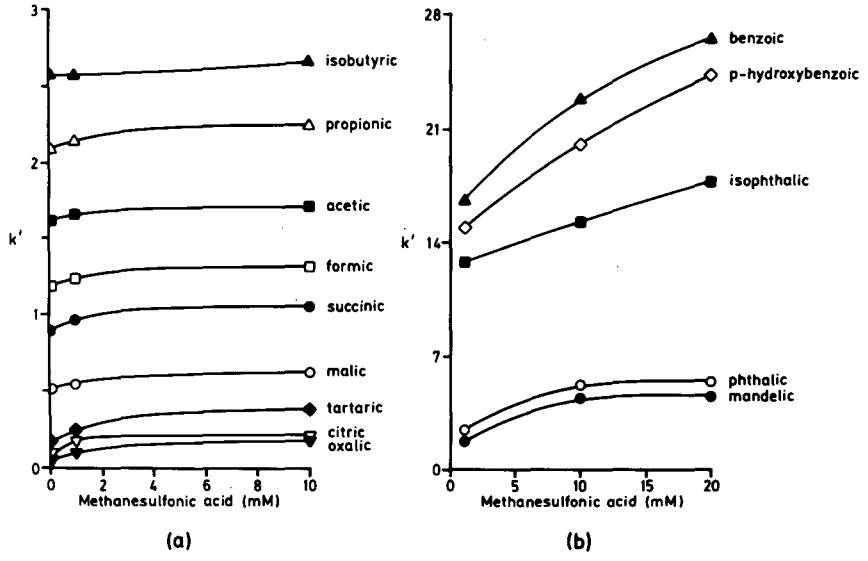


Fig. 2. Effect of the eluent concentration of methanesulfonic acid on the capacity factors of (a) aliphatic and (b) aromatic carboxylic acids. Other conditions as in Fig. 1.

Fig. 1 shows the isocratic separation of fourteen aliphatic and aromatic carboxylic acids using 10 m$M$ methanesulfonic acid [containing 1% (v/v) acetonitrile] as eluent. The aliphatic acids are eluted in the early part of the chromatogram, followed by the aromatic acids which show very strong retention on the column. The retention times of the aliphatic carboxylic acids increase with increasing p$K_{a1}$ and with the exception of the difunctional succinic acid, the elution order can be predicted from the p$K_{a1}$ values. This indicates that the electrostatic ion-exclusion effect predominates for these solutes. The strong retention of aromatic acids is attributable to hydrophobic adsorption effects.

*Effect of eluent concentration*

The effect of concentration of methanesulfonic acid in the eluent on the capacity factors of aliphatic and aromatic carboxylic acids is shown in Fig. 2. Although most of the organic acids are too weak for their ionisation to be affected significantly by pH changes in the range 1.80–3.52, an increase of eluent concentration caused a moderate increase in capacity factors. This result agrees with a previous study [4]. This is a characteristic effect of the pH of the eluent in ion-exclusion chromatographic separation [8]. Stronger acids, such as oxalic and citric acids, show a more pronounced dependence on the eluent concentration. As this parameter is increased (and the eluent pH is lowered) these acids became less ionized, leading to decreased electrostatic (ion-exclusion) repulsion and hence increased retention times. The aromatic acids (Fig. 2b) show quite strong dependence on the concentration of methanesulfonic acid in the eluent.

The effect of the eluent concentration on column efficiency is listed in Table III, which shows that there is a general increase in column efficiency as the

TABLE III

COLUMN EFFICIENCIES AT DIFFERENT METHANESULFONIC ACID CONCENTRATIONS

| Acid | Theoretical plates per column ($N$) | | |
|---|---|---|---|
| | 0.5 m$M$ | 1.0 m$M$ | 10.0 m$M$ |
| Citric | 4 225 | 4 556 | 6 148 |
| Malic | 5 378 | 6 601 | 8 100 |
| Malonic | 4 726 | 5 814 | 7 836 |
| Formic | 13 514 | 14 400 | 16 256 |
| Acetic | 8 251 | 11 556 | 13 275 |
| Propionic | 8 755 | 9 025 | 10 796 |
| Isobutyric | 4 160 | 5 025 | 6 944 |

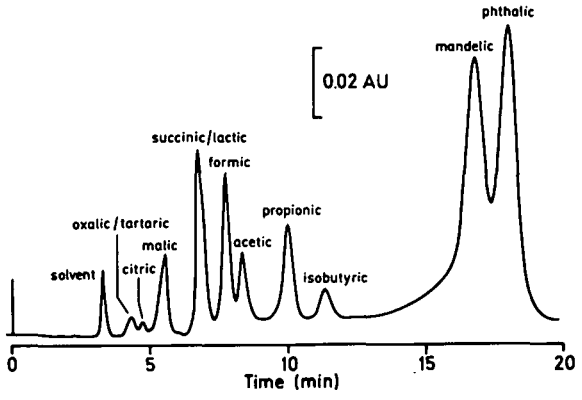


Fig. 3. Gradient elution from 50 to 1 m*M* methanesulfonic acid. Other conditions as for Fig. 1.

eluent concentration is increased. This result is in accordance with a previous study [17]. The plate number ($N$) is strongly influenced by the degree of ionization of the acids, with the relatively strong acids (*e.g.* citric) showing the lowest $N$ values.

The above results suggest that the eluent concentration can exert a limited degree of retention selectivity in ion-exclusion chromatography, especially between the stronger and weaker carboxylic acid solutes. Gradient elution by changing the eluent concentration of methanesulfonic acid should therefore be possible for mono- and dibasic carboxylic acids and the less strongly retained aromatic carboxylic acids. Fig. 3 shows a chromatogram obtained by changing the eluent concentration of methanesulfonic acid over the range 50.0–1.0 m*M*. The gradient employed was a negative gradient, *i.e.* from high to lower concentration of eluent, since this process increases the eluotropic strength. The baseline change is minimal, but the separation time and resolution are improved only marginally in comparison to isocratic separation.

*Effect of organic modifier*

Addition of an organic modifier, such as methanol, acetonitrile or acetone, to the eluent may influence the participation of solute adsorption effects in the retention process. This influence should be greatest for aromatic acids. Fig. 4 shows the retention changes for both aliphatic and aromatic acids caused by the addition of acetonitrile to the methanesulfonic acid eluent. For aliphatic carboxylic acids (Fig. 4a), the capacity factors were reduced slightly by the addition of acetonitrile, whereas very significant changes were observed for the aromatic acids (Fig. 4b). Some eluent selectivity therefore exists, especially between the aromatic and aliphatic carboxylic acids.

Gradient elution was therefore performed by varying the percentage of acetonitrile over the range

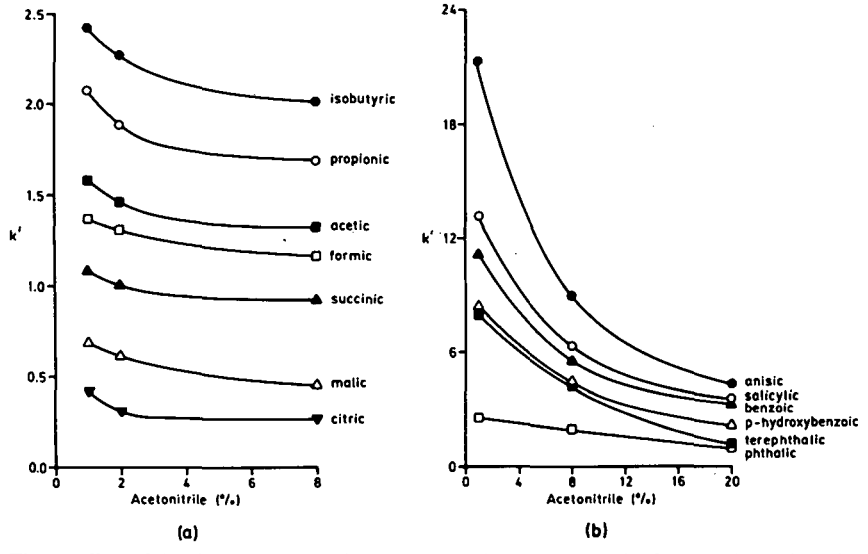


Fig. 4. Effect of the eluent concentration of acetonitrile on the capacity factors of (a) aliphatic and (b) aromatic carboxylic acids. Other conditions as in Fig. 1.

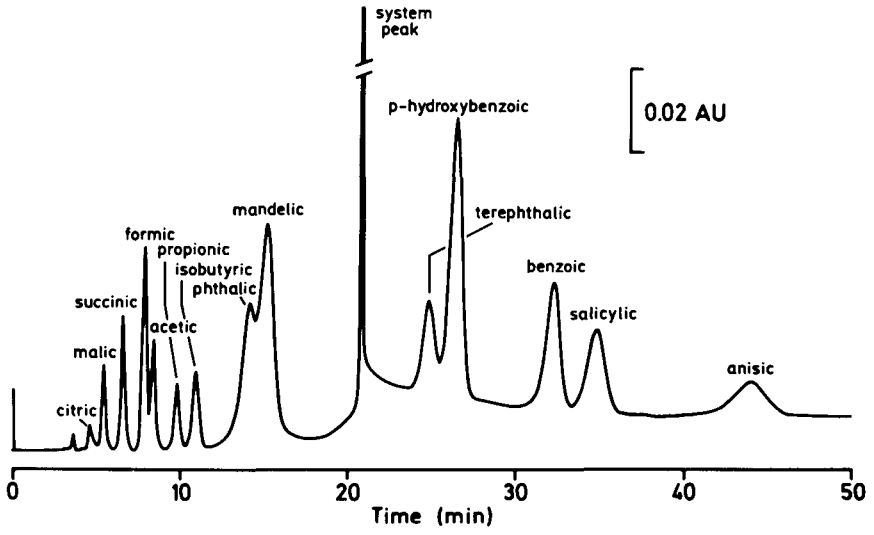


Fig. 5. Gradient elution performed by varying the percentage (1 to 15%) of acetonitrile in 10 m*M* methanesulfonic acid eluent. Other conditions as in Fig. 1.

1–15%, with the methanesulfonic acid concentration being maintained at 10.0 m*M*. The chromatogram obtained is shown in Fig. 5. There is little baseline change and both the separation time and resolution are improved considerably when compared to the isocratic run, especially for the aromatic carboxylic acids. As expected, the separation of the aliphatic acids does not differ greatly from that shown in Fig. 1. A disadvantage of this approach is the presence of an extraneous peak in the chromatogram, the retention time of which was constant regardless of the concentration limits of the gradient or the slope of the gradient ramp. The height of the peak was proportional to the percentage of acetonitrile in the eluent, suggesting that this peak originated from the acetonitrile itself. Such a system peak has been noted previously [17]. It is possible that other organic modifiers could be used to eliminate this peak; however, this was not investigated.

*Effect of β-cyclodextrin in the eluent*

Cyclodextrins (CDs) are cyclic oligosacharides, constructed from α-(1,4)-linked glucose units arranged in a torus, with the most common CDs being α, β- and γ-CD, containing six, seven and eight glucose units, respectively. The structures of these compounds are typified by a central cavity which gives rise to their remarkable ability to form inclusion complexes with various guest molecules. In addition, CDs are stable within a wide range of pH and do not absorb in the full UV region commonly used in chromatographic detection.

For the above reasons β-CD has been investigated as a mobile phase modifier in reversed-phase liquid chromatography (RPLC) [18] and, more recently, in ion-exclusion chromatography [19]. In the latter study, we have shown that the value of the inclusion constant for a solute (*i.e.* the equilibrium constant for the formation of the inclusion complex) can be determined using the ion-exclusion chromatography retention volume of that solute in an eluent containing β-CD. In the present study β-CD is utilized as an eluent modifier in order to reduce the retention times of those solutes forming inclusion complexes. Fig. 6 shows the effect of varying eluent concentrations of β-CD on the retention times of carboxylic acids. It can be seen that retention times of the aliphatic carboxylic acids showed slight decreases (Fig. 6a), whereas those for most of the aromatic acids were decreased significantly (Fig. 6b). This result is in accordance with our previous study [19] wherein aromatic carboxylic acids were found to have much greater inclusion constants than aliphatic acids.

The third approach to gradient elution in ion-

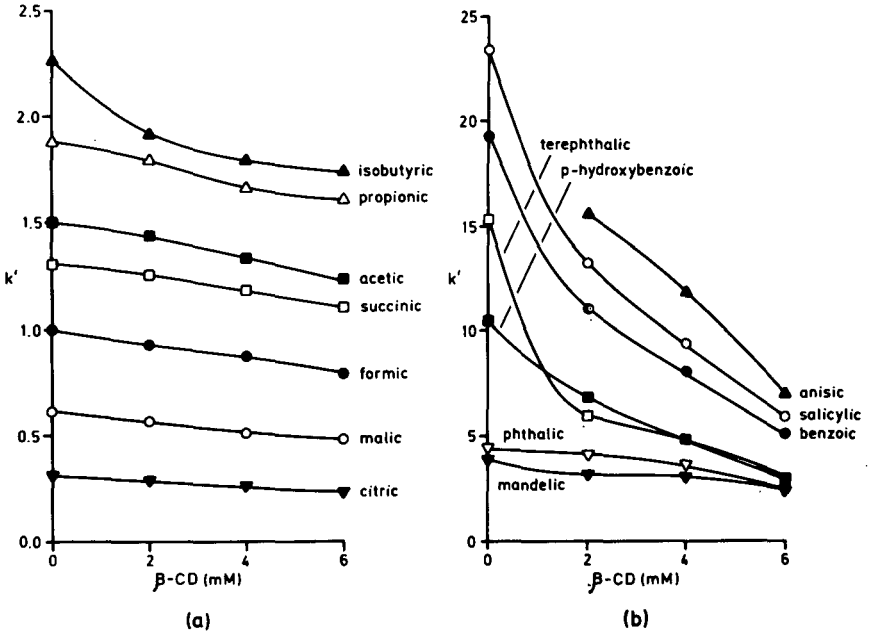


Fig. 6. Effect of the addition of β-cyclodextrin on the capacity factors of (a) aliphatic and (b) aromatic carboxylic acids. Other conditions as in Fig. 1.

exclusion chromatography involved increasing the concentration of β-CD in the eluent. A chromatogram obtained with a gradient from 0 to 6.0 m*M* β-CD in an eluent containing 10.0 m*M* methanesulfonic acid and 2% acetonitrile is shown in Fig. 7. Some baseline drift is apparent (due to the slight absorbance of β-CD at the detection wavelength used), however, the analysis time and resolution are

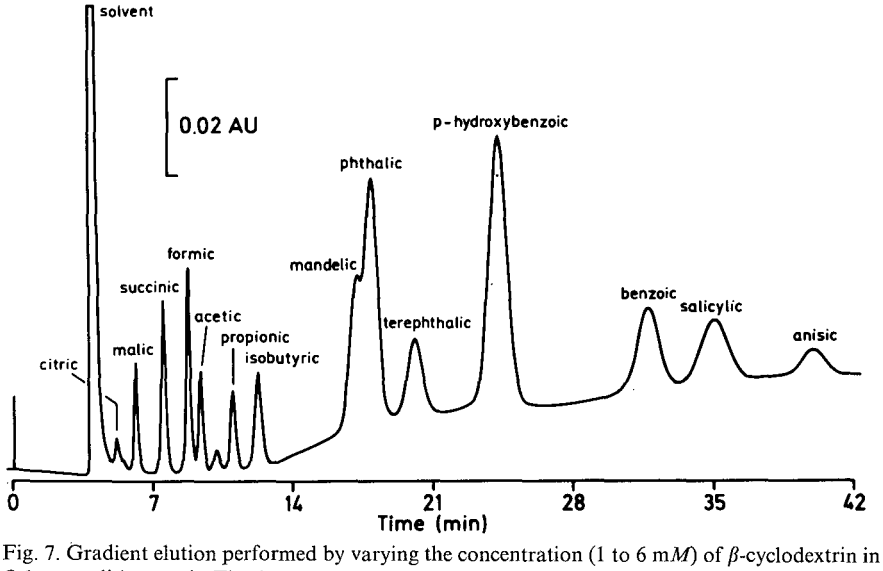


Fig. 7. Gradient elution performed by varying the concentration (1 to 6 m*M*) of β-cyclodextrin in 10 m*M* methanesulfonic acid eluent. Other conditions as in Fig. 1.

superior to those for the other gradients shown in Figs. 3 and 5. Calibration plots were constructed by injecting mixtures of carboxylic acids at various concentration and using the gradient described above. Linear calibration was obtained up to at least 1000 ppm for the aliphatic carboxylic acids and 100 ppm for the aromatic carboxylic acids.

## CONCLUSIONS

This study has shown that methanesulfonic acid is a useful eluent for ion-exclusion chromatography of carboxylic acids. Some eluent selectivity can be attained through variation of the concentration of methanesulfonic acid in the eluent, but greater selectivity effects occur through the addition of acetonitrile or $\beta$-CD to the eluents. Gradient elution in ion-exclusion chromatography can be achieved by varying the concentration of methanesulfonic acid, acetonitrile or $\beta$-CD. The latter two approaches are more effective than the first, but acetonitrile produces a system peak in the chromatogram which may cause interference. The $\beta$-CD gradient causes a significant reduction in the retention times of aromatic acids as a result of the formation of inclusion complexes. Since such complexes are formed only with solutes of appropriate size, the $\beta$-CD gradient is more selective than the alternative approaches investigated in this work.

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# Direct serum injection in ion chromatography on packing materials with a semi-permeable surface

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ABSTRACT

Reversed-phase packing materials with restricted access of proteins to the hydrophobic sites were tested for their applicability in the ion-interaction chromatography of anions. Especially $C_8$-modified silica, which had been coated with a hydrophilic polymer acting as a semi-permeable barrier, could be used successfully for the separation of several anions in a proteinaceous matrix without removing the proteins prior to injection. In combination with UV or conductivity detection, this technique allows the determination of some physiologically important anions in serum samples.

## INTRODUCTION

The determination of anions or cations in biological fluids by ion chromatography (IC) generally requires pretreatment procedures to remove proteinaceous sample components. Direct injection of biological samples on to conventional packing materials may cause denaturation and accumulation of proteins, resulting in severe degradation of the system performance. Various sample preparation steps are possible to remove sample proteins prior to IC, such as protein precipitation or ultrafiltration. In many instances, these sample pretreatment procedures are disadvantageous because of low recoveries, difficulties with reproducibility or consumption of time.

More recently, direct injection of serum samples on to specially prepared columns has been described as an alternative to sample pretreatment. This approach has gained some popularity for the detemination of drugs in biological fluids by reversed-phase high-performance liquid chromatography (HPLC). Typical packings designed for this purpose include internal surface reversed-phase materials [1–3], semi-permeable surface materials [4], dual-zone materials [5], shielded hydrophobic phase materials [6,7] and mixed functional phase materials [8–10]. Common to all these packing materials is the restricted access of proteins to the hydrophobic sites of the particle. Ideally, proteins will elute in the void volume. Although these techniques have successfully proved their advantages for typical reversed-phase HPLC separations, no applications have been reported in IC.

This paper reports the determination of anions in proteinaceous samples using ion-interaction chromatography with semi-permeable surface (SPS) packings. $C_8$-modified silica was used, which had been coated with a hydrophilic polymer. This hydrophilic layer acts as a semi-permeable barrier which prevents proteins from adsorbing irreversibly on the packing. A commercially available SPS phase and laboratory-made phases coated with polyethylene glycol were tested and compared with respect to the separation efficiency for anions in serum samples. Further, an internal surface reversed-phase (ISRP) material was investigated for its applicability in ion-interaction chromatography.

## EXPERIMENTAL

### *Instrumentation*

The chromatographic instrumentation consisted of a Perkin-Elmer (Norwalk, CT, USA) Series 3B

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HPLC pump, a Rheodyne (Cotati, CA, USA) Model 7125 injection valve with a 10- or 20-$\mu$l loop, a Perkin-Elmer LC 75 UV detector and a Waters (Milford, MA, USA) M430 conductivity detector. The following separation columns were used: a 5-$\mu$m SPS RP-8 column (250 × 4.6 I.D.), obtained from Regis (Morton Grove, IL, USA); a column (250 × 4 mm I.D.) packed with $C_8$-modified silica coated with polyethylene glycol according to the procedure given below; and a Pinkerton GFF-S5-80 ISRP column (100 × 4.6 mm I.D.), obtained from Regis.

The mobile phase was prepared by adjusting an aqueous 5, 10 or 15 m*M* octylamine solution to pH 6.5 with phosphoric acid.

Serum samples were passed through a Schleicher & Schuell (Keene, NH, USA) 0.45-$\mu$m Spartan-3 filter prior to injection.

The characterization of coated particles by infrared spectrometry was carried out with a Bio-Rad Labs. (Cambridge, MA, USA) FTS-45 Fourier transform infrared spectrometer.

*Coating of $C_8$-modified silica with polyethylene glycol*

A 10-g amount of polyethylene glycol (PEG) with a molecular weight of 1000 (Carbowax 1000) obtained from Supelco (Bellefonte, PA, USA), was heated to about 100°C and mixed with 3 g of LiChrospher RP-8 silica, 5-$\mu$m particle size, 100-Å pore diameter (obtained from Merck, Darmstadt, Germany). The temperature was raised to 280°C and the suspension stirred for 3 h. The excess of PEG was dissolved by addition of 20 ml of methylene chloride and the particles were separated by centrifugation. This step was repeated three times. Finally, the particles were washed with isopropanol.

## RESULTS AND DISCUSSION

The first investigations dealt with the behaviour of a commercially available SPS $C_8$ column in ion-interaction chromatography. The analysis of proteinaceous samples requires the use of a mobile phase with little or no organic modifier and a pH around neutral to avoid protein precipitation. Skelly [11] suggested a mobile phase containing an octylammonium salt for use in ion-interaction chromatography. In our experiments, a 5 m*M* octylamine solution adjusted to pH 6.5 with phosphoric acid was found to give satisfactory separations of inorganic anions on LiChrospher $C_8$ silica. Therefore, this mobile phase was chosen for separations on the SPS column. The separation pattern of chloride, bromide, nitrite, nitrate, iodide and thiocyanate on the SPS column was similar to that on the uncoated $C_8$ column and the capacity factors were 1.8, 2.5, 2.8, 3.6, 7.1 and 19.5, respectively. This result confirmed that the semi-permeable surface does not interfere with the separation mechanism of ion-interaction chromatography.

In the next step, the separation of an anion standard in a 5% albumin matrix was investigated. Fig. 1 shows a typical chromatogram with UV detection at 200 nm. The peak eluting in the void volume corresponds to albumin. Fractions of the eluate were collected and the protein content was determined by the method of Hartree [12]. These experiments confirmed that the injected protein is eluted quantitatively and no protein is adsorbed irreversibly on the stationary phase. A graph of peak area *versus* concentration showed linearity of the reponse of nitrite, nitrate, bromide, iodide and thiocyanate anions in the albumin matrix in the range 1–100 ppm.

Finally, the direct injection of human serum samples was investigated. The broad peak of the serum proteins and also peaks of serum components eluting near the void volume cause problems for the separation of early-eluting anions such as nitrite, bromide and nitrate if UV detection is used. The separation can be improved to some extent if the concentration of octylamine in the mobile phase is increased to 10 m*M*, but then the retention of anions such as thiocyanate becomes unacceptably long.

Considering that the concentrations of many anions in serum are in the low ppm or ppb range, UV detection obviously is not the optimum detection mode. Nevertheless, one should remember that the aim of this work was the investigation of the general behaviour of proteinaceous samples on SPS $C_8$ silica under ion-interaction chromatographic conditions and not the optimization of sensitivity. Selective and sensitive detection modes are known for several anions of physiological or medical importance, such as postcolumn reaction detection or

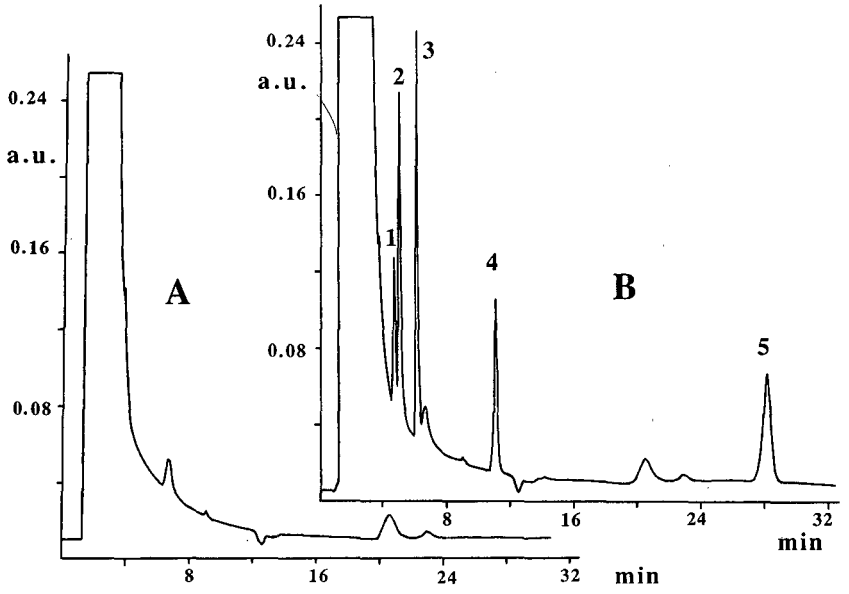


Fig. 1. Chromatogram of a standard mixture of anions (10 ppm each) in a proteinaceous matrix using a Regis semi-permeable surface $C_8$ packing. (A) 5% albumin solution; (B) anion standard in a 5% albumin matrix. Peaks: 1 = bromide; 2 = nitrite; 3 = nitrate; 4 = iodide; 5 = thiocyanate. Injection volume, 20 $\mu$l. UV detection at 200 nm. Mobile phase, 5 m*M* octylamine adjusted to pH 6.5 with phosphoric acid. Flow-rate, 1.0 ml/min.

electrochemical detection, which can easily be combined with direct injection of proteinaceous samples on to SPS phases. In some instances, however, direct UV detection at 210 nm can be used successfully for certain anions, as can be seen from Fig. 2, which shows the determination of iodide and thio-

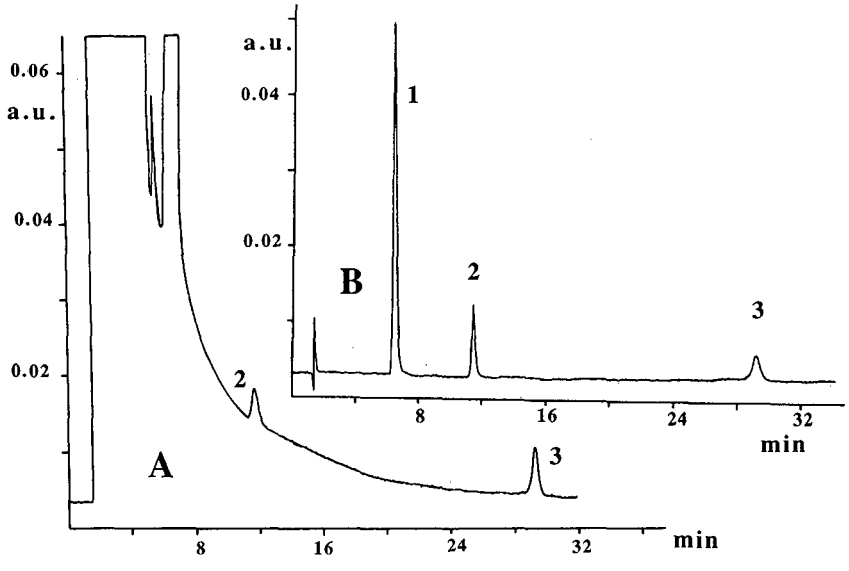


Fig. 2. Determination of anions in human serum. (A) Serum sample; (B) anion standard mixture (5 ppm each). Peaks: (1) nitrate; (2) iodide; (3) thiocyanate. UV detection at 210 nm. Other conditions as in Fig. 1.

cyanate in a serum sample from a subject after treatment with iodide-containing drugs; the thiocyanate concentration is also relatively high, indicating that the subject was a heavy smoker; the peak at the retention time of nitrate is caused by interfering serum components.

In a series of experiments, the coating of RP-8 particles with PEG was investigated in order to produce stationary phases with properties similar to those of commercially available SPS phases. The idea was to bind PEG via its alcohol group to free silanol groups of the silica particle (after partial removal of $C_8$ groups already bound to the silica). The mechanism of the coating procedure described under Experimental is not fully clear. Nevertheless, after extensive washing of the coated phase with different organic solvents there were still PEG groups present at the surface of the particle. This could be verified by diffuse reflectance IR spectrometry. The spectrum obtained after subtraction of a spectrum of RP-8 silica from a spectrum of PEG-coated RP-8 silica showed characteristic bands at wavenumbers of 1457, 1349, 1326, 1299 and 951 $cm^{-1}$, which were identical with the bands of a PEG standard. Therefore, it is unlikely that the polymer is adsorbed only physically. Further information on the extent and nature of PEG binding could not be deduced from the IR measurements carried out so far.

The separation properties of the PEG-coated RP-8 silica were similar to those of the commercially available SPS column, the only difference being the order of elution of sulphate and iodide. Sulphate eluted before iodide on the SPS column but after iodide on the PEG-coated column. During the first injections of proteinaceous samples on to a new PEG-coated column, the elution of the protein was incomplete. Only after the fifth injection was the injected protein eluted quantitatively. Obviously, some active sites for irreversible protein adsorption are still present. Nevertheless, the amount of protein adsorbed seems to be small enough to avoid column deterioration.

Physiological amounts of sulphate can be detected in serum by using conductivity detection. Such measurements are essential for investigations of factors controlling the rate of sulphoconjugation in the organism. Fig. 3 shows a typical chromatogram of a human serum sample containing *ca.* 30 ppm of sulphate. This concentration was found after calibration with aqueous sulphate standards. Comparisons with other established procedures are still to be done in order to assess the accuracy of the method. The main advantage of this HPLC procedure is that no sample pretreatment is necessary except filtration through a 0.45-$\mu$m filter. Therefore, the method is simpler than other HPLC procedures reported recently [13].

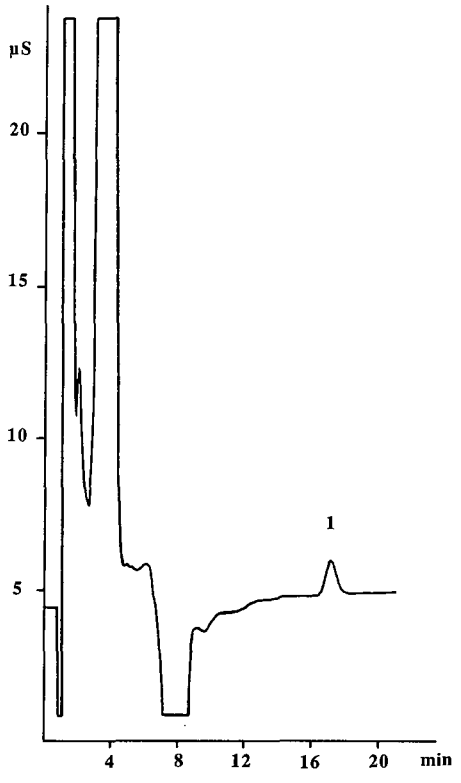


Fig. 3. Determination of sulphate in human serum using a $C_8$ packing coated with PEG and conductivity detection. Peak: (1) sulphate, *ca.* 30 ppm. Flow-rate, 1.5 ml/min. Other conditions as in Fig. 1.

The long-term behaviour of PEG-coated $C_8$ silica was also investigated and compared with an uncoated $C_8$ silica. Seventy replicate 10-$\mu$l injections of a serum sample were carried out. The efficiency of the coated material (number of theoretical plates) remained roughly the same whereas the efficiency of the uncoated material changed in an unpredictable way. The performance tended to decrease dramatically after a few injections, but could

be restored to some extent by extensive washing of the column with water. The uncoated material cannot be recommended for routine analytical work.

Preliminary investigations were carried out on coating a silica-based anion-exchange material with PEG. A 40-$\mu$m Bondesil SAX material (obtained from Analytichem International, Harbor City, CA, USA) was treated in the same way as described above for RP-8 material. The separation of anions was possible using sodium sulphate as eluent, but owing to the particle size it was not possible to fill a high-performance column. Further experiments will be done with a material of smaller particle size and lower capacity, as the capacity of the SAX material is too high to use eluents with low concentration, which are compatible with conductivity detection.

Some investigations were carried out with a commercially available ISRP column. This packing material has a hydrophilic diol phase on the external surface of the silica particle and a hydrophobic phase (glycerylpropylglycyl-L-phenylalanyl-L-phenylalanine) on the internal surface (pores) [1,2]. Serum proteins are excluded from the small pores, whereas small molecules penetrate and may be retained at the hydrophobic sites. Unfortunately, the use of the mobile phase described above containing 5 m*M* octylammonium phosphate as the ion-interaction reagent resulted in almost no retention of anions such as chloride, nitrate, iodide and thiocyanate. A threefold increase in the concentration of the ion-interaction reagent did not improve the separation. Obviously, the hydrophobicity of the internal polypeptide phase is considerably lower than that of typical reversed-phase materials such as $C_8$- or $C_{18}$-modified silica. Therefore, its applicability in ion-interaction chromatography seems to be limited. Nevertheless, the concept of ISRP packings is worth developing further in order to obtain materials with new internal phases of higher hydrophobicity.

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# Dialytic clean-up of alkaline samples prior to ion chromatographic analysis

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ABSTRACT

Membrane-based devices for the neutralization of alkaline samples prior to ion chromatographic analysis are studied. These devices use a cation-exchange membrane fibre (either hollow or packed with polystyrenedivinylbenzene beads) immersed in a hydrogen ion-donating medium. As the sample is passed through the lumen of the fibre, a dialysis reaction involving exchange of sodium ions in the sample with hydrogen ions from the surrounding medium occurs, resulting in total or partial neutralization of the sample. Several hydrogen ion-donating media are evaluated on the basis of neutralization efficiency and penetration of the acid anion through the membrane. Octanesulfonic acid (OSA) at a concentration of 0.1 *M* gave optimal performance. Dual ion-exchange using hydrogen-form cation exchange resins slurried with water or 0.1 *M* OSA are also evaluated. The resin slurries provide much larger neutralization capacities than the acid solutions and are not subject to problems of penetration of the acid anion into the sample. When the resin is slurried in OSA and is stirred occasionally, the total theoretical neutralization capacity of the resin can be achieved.

## INTRODUCTION

Samples of extreme pH (*i.e.* outside the pH range 3–11) often pose problems in ion chromatographic (IC) determinations because of deleterious effects on the column life and performance [1]. In particular, strongly alkaline samples may give distorted analyte peaks, system peaks and severe baseline perturbations due to the effect of the injected sample on the acid–base equilibria existing in the eluent in both suppressed and non-suppressed IC. Whilst these problems can sometimes be circumvented through the use of selective detectors or specially designed eluents, adequate sample clean-up steps are generally required to ensure the ultimate success of the analysis.

Sample clean-up may involve the removal of undesired particulate matter, the reduction in concentration or complete removal of potential interferences, or concentration of the analyte of interest to improve detection. Samples of high ionic strength are often troublesome due to the large response and long recovery times of the conductivity detectors. When the high ionic strength of the sample is due to the presence of elevated levels of sodium hydroxide, simple neutralization of the sample is unsuitable because the resultant high level of the acid anion would also be likely to cause interference problems. However, two alternative methods of sample clean-up are applicable.

The first involves treatment of the sample with a cation-exchange resin in the hydrogen form, which results in replacement of sodium in the sample with hydrogen ions from the resin, leading to neutralization of the sample. This treatment can be accomplished using a batch method wherein the ion-exchange resin is added to the sample, or using a column method wherein the sample is passed through a small column packed with suitable resin [2]. The second approach to sample clean-up uses a dialysis treatment with a suitable membrane. In this case, the membrane is usually functionalized with sulfonic acid groups to impart cation-exchange characteristics and dialysis occurs between the sample solu-

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tion on one side of the membrane and an acidic solution on the other side. Again, exchange of sodium ions for hydrogen ions leads to sample neutralization and the mechanism of operation is identical to that used in membrane-based suppressors employed in suppressed IC. The physical form of the membrane may vary, with flat sheets [3] or hollow fibres [4] being used.

The dialysis process involves the transfer (diffusion) of ions of positive charge through the cation-exchange membrane. Anions possess the same charge sign as the exchange site and, in theory, are excluded from diffusion through the membrane by electrostatic repulsion. The extent of exclusion (referred to as the permselectivity of the membrane) depends on the concentration of the external electrolyte with which the membrane is in equilibrium, and decreases as this concentration is increased [5]. Permselectivity may also decrease when the membrane shows excessive swelling in water, due to the resultant low charge density of functional groups on the membrane. Thus, the concentration of the acidic medium used as a source of hydrogen ions for the dialysis process is limited by the permselectivity of the membrane towards the anion of the acid employed. One method which may be used to overcome this problem is described as "dual ion-exchange" dialysis [6]. Here, an aqueous slurry of cation-exchange resin in the hydrogen form is used as the source of hydrogen ions instead of an acidic solution. The acid anion is now a resin bead and its incursion through the membrane is physically precluded. Moreover, the change in concentration of an analyte ion in the sample solution either through contamination effects, adsorption losses or sample volume change which commonly feature in conventional ion-exchange is also minimized.

In this paper we examine membrane dialysis procedures for treatment of alkaline solutions prior to IC analysis. The efficiency and neutralization capacity of various hollow-fibre cation-exchange membranes used with a range of hydrogen ion-donating media (including cation-exchange resins) are evaluated.

## EXPERIMENTAL

### *Instrumentation*

The ion chromatograph consisted of a Millipore-Waters (Milford, MA, USA) Model 510 pump, Model U6K injector and Model 430 conductivity detector. The column used was a Millipore-Waters IC Pak A anion column, 50 × 4.6 mm I.D., packed with polymethacrylate anion-exchange resin. Chromatography was carried out at room temperature with an eluent flow-rate of 1.2 ml/min.

Two sample treatment devices were constructed. The first, shown in Fig. 1, utilized a length of cation-exchange hollow fibre (30 cm × 1.2 mm I.D. fibre protected by a woven polymer sheath) housed in a 10 cm × 24 mm I.D. glass tubing. A syringe was fitted to the inlet end of the tubing and was used to pass the sample through the membrane, with the effluent being collected. The outside of the fibre was bathed in a solution of a suitable hydrogen ion-donating medium. This apparatus was similar in construction to that described by Jones and Jandik [4]. The second device, shown in Fig. 2, used cation-exchange membrane fibre (345 cm × 0.5 mm I.D.

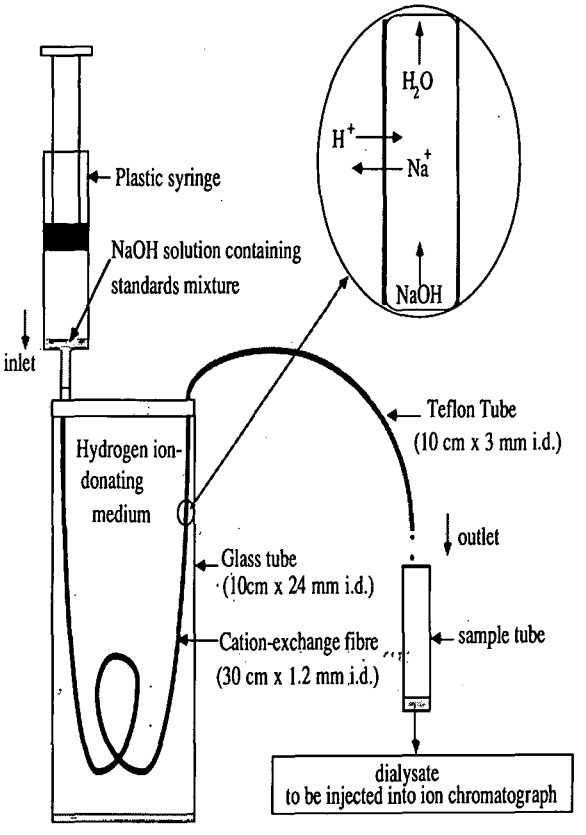


Fig. 1. Dialysis device used for comparison of hydrogen ion donating media.

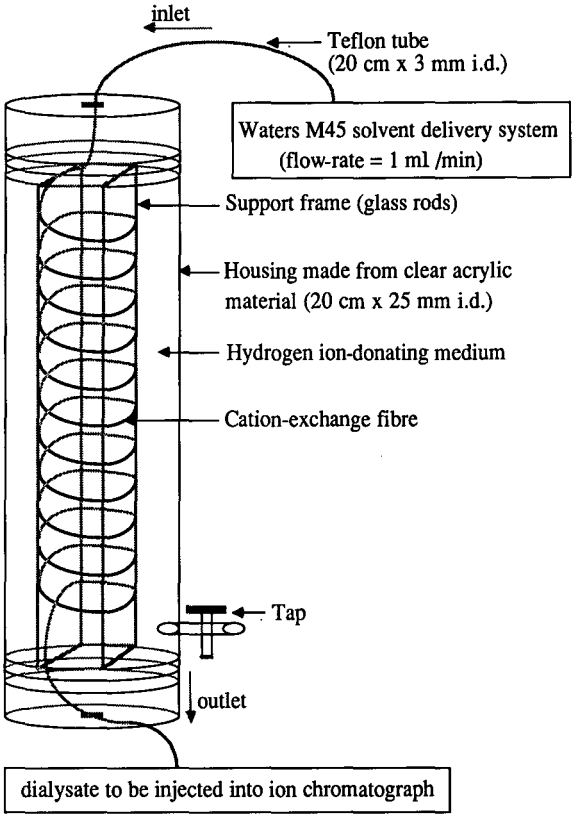


Fig. 2. Dialysis device used for comparison of fibre types and for determination of maximum neutralization capacity.

DuPont Nafion tubing or 160 cm × 0.6 mm I.D. DuPont Nafion tubing packed with polystyrene–divinylbenzene beads) coiled on glass rods and housed in an acrylic container 20 cm × 25 mm I.D. A Millipore-Waters M45 pump was used to pass sample through the membrane fibre and the effluent was collected. Again, the outside of the fibre was bathed in a solution of a suitable hydrogen ion-donating medium.

*Reagents*

All chemicals used were of analytical-reagent grade and the water used in the preparation of standard solutions and eluents was purified on a Millipore (Bedford, MA, USA) Milli-Q water treatment system. Samples and eluents were filtered through a Millipore 0.45-$\mu$m membrane filter and degassed in an ultrasonic bath prior to use. The eluent used for IC analysis of the treated samples contained 1.3 m$M$ sodium tetraborate, 5.8 m$M$ boric acid and 1.4 m$M$ potassium gluconate adjusted to pH 8.5 and made up in water–acetonitrile (88:12).

Standard stock solutions of inorganic anions were prepared by dissolving appropriate amounts of the sodium salts in water. Working solutions of these ions were obtained by diluting the stock solutions to give $10^{-1}$–$10^{-4}$ $M$ sodium hydroxide in the final solution. The concentration of inorganic anions in these solutions was in the range 30–100 ppm.

Hydrogen ion-donating solutions were prepared using 0.1–1.0 $M$ sulfuric acid, methanesulfonic acid (MSA), octanesulfonic acid (OSA), camphorsulfonic acid (CSA) and *p*-toluenesulfonic acid (TSA). All were obtained from Sigma (St. Louis, MO, USA), with the exception of OSA which was prepared by passing a solution of sodium octanesulfonate through a glass column packed with 100 g, Bio-Rad AG 50W-X8 hydrogen form cation-exchange resin, 200–400 mesh. Dual ion-exchange experiments were performed using the following cation-exchange resins, all of which were used in the hydrogen form: Bio-Rad AG 50W-X8, 200–400 mesh; Bio-Rad AG 50W-X2, 200–400 mesh; Amberlite IRC-50, 50–100 mesh and Amberlite IR-120, 50–100 mesh. Resin was pretreated by thorough washing with Milli-Q water.

*Procedures*

The apparatus shown in Fig. 1 was used as follows. Before dialysis, the fibre was flushed thoroughly with Milli-Q water and a plastic syringe was used to pass 1 ml of sample through the fibre at a flow-rate of 1 ml/min. The first 10 drops of dialysate were discarded with the remaining dialysate being collected in 50-$\mu$l fractions for IC analysis. The pH of the samples was measured before and after dialysis using pH indicator sticks. The apparatus shown in Fig. 2 was used in a similar manner, except that a pump was used to deliver the sample at a flow-rate of 1 ml/min. The resin slurries were made by adding 25 ml of either Milli-Q water or 0.1 $M$ OSA into 80 g of resin which had been soaked in Milli-Q water and filtered on a Buchner funnel.

## RESULTS AND DISCUSSION

*Comparison of hydrogen ion donating media*

The first step in the design of a suitable sample

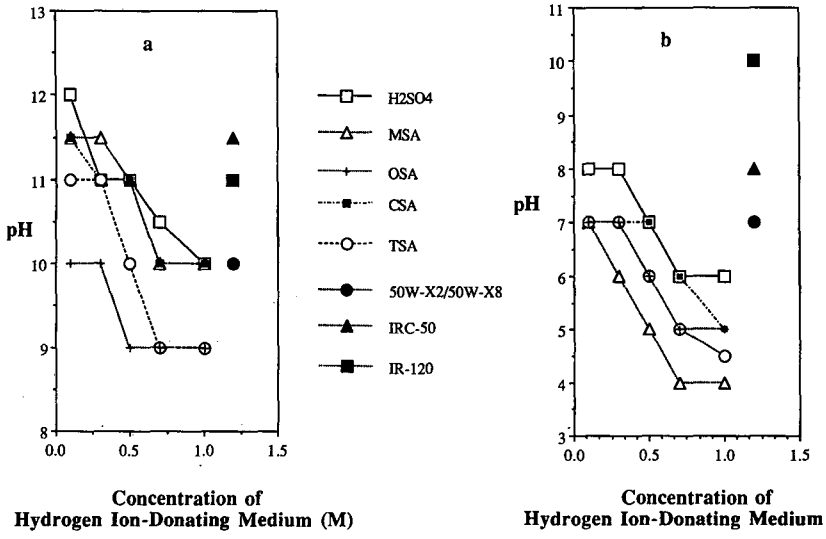


Fig. 3. Dialysate pH from (a) $10^{-1}$ *M* NaOH and (b) $10^{-2}$ *M* NaOH samples using various hydrogen ion donating media. MSA = Methanesulfonic acid; OSA = octanesulfonic acid; CSA = camphorsulfonic acid; TSA = *p*-toluenesulfonic acid.

treatment device was to determine the optimum composition of the hydrogen ion-donating solution. A range of sulfonic acids and cation-exchange resins was compared with sulfuric acid. Fig. 3 shows the pH of 0.1 and 0.01 *M* NaOH solutions after dialysis using the apparatus shown in Fig. 1. In all cases the pH of the sample was lowered significantly and the extent of pH reduction increased when the concentration of the hydrogen ion-donating medium was increased. All of the sulfonic acid solutions were more effective in lowering pH than sulfuric acid and some differences in performance between the different sulfonic acids can be noted. The four cation-exchange resins used in the dual ion-exchange approach were less effective in lowering the sample pH; however, Bio-Rad AG 50W-X8 gave the best performance.

The incursion of the acid anion into the sample must also be considered since anions normally have non zero transport numbers in cation-exchange membranes [6]. Sulfate shows typical penetration behaviour (Fig. 4), with the degree of incursion increasing with concentration of the hydrogen ion-donating medium. At a concentration of more than 0.3 *M*, each of the sulfonic acids was found to penetrate the membrane giving negative peaks in the final chromatogram. The penetration of MSA was most severe, whilst OSA and CSA showed least

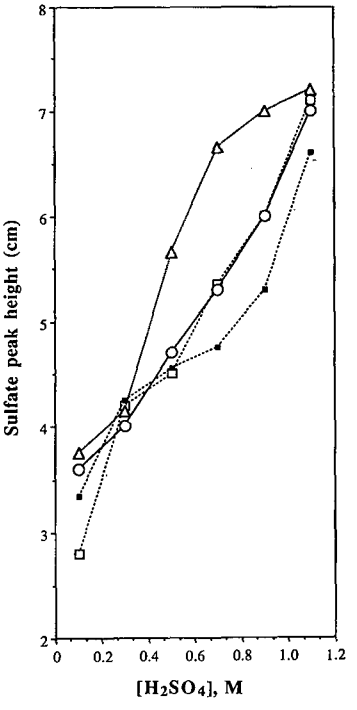


Fig. 4. Sulfate incursion from $H_2SO_4$ medium after dialysis. NaOH concentration: □ = $10^{-1}$ *M*; △ = $10^{-2}$ *M*; ○ = $10^{-3}$ *M*; ■ = $10^{-4}$ *M*.

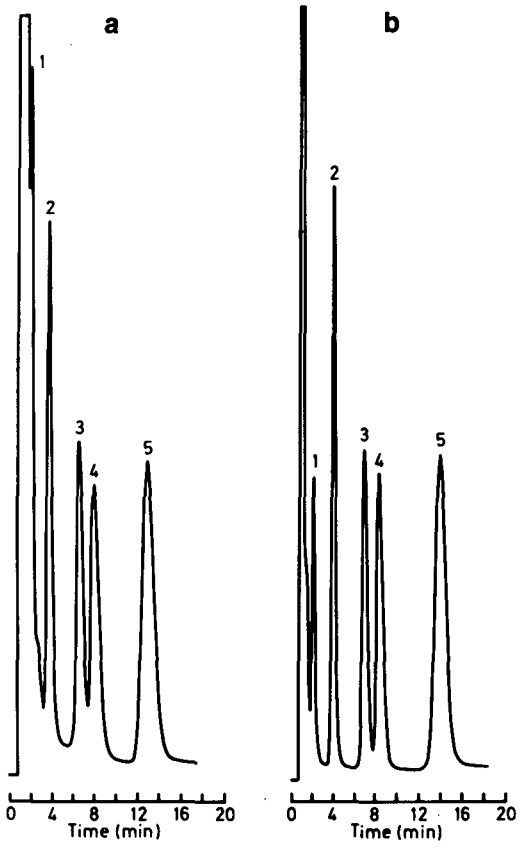


Fig. 5. Chromatogram obtained before (a) and after (b) Donnan dialysis of $10^{-2}$ *M* NaOH solution containing anions. Injection volume: 50 µl. Eluent: gluconate–borate, pH 8.5. Column: Waters IC Pak A, 50 × 4.6 mm I.D. Peaks: 1 = fluoride; 2 = chloride; 3 = bromide; 4 = nitrate; 5 = sulfate.

penetration. The efficacy of sample clean-up by dialysis can be seen in Fig. 5, which shows chromatograms for a mixture of anions in $10^{-2}$ *M* NaOH before and after dialysis with 0.1 *M* OSA. The fluoride peak is partially obscured prior to dialysis but can be quantitated after sample treatment.

Loss of analyte during dialysis is a further important consideration. Average recovery data are given in Table I and show that no significant loss of analyte occurred. High apparent recoveries were observed for fluoride in 0.1 *M* NaOH solution due to the fact that the pH of the dialysed solution was above 9, causing distortion of the fluoride peak. The high recoveries of sulfate when sulfuric acid was used as the hydrogen ion-donating medium were due to penetration of sulfate through the membrane.

*Comparison of fibre types*

Two types of cation-exchange fibres, namely Nafion tubing both with and without polystyrenedivinylbenzene beads, were compared using OSA, CSA and Bio-Rad AG 50W-X8 cation-exchange resin as hydrogen ion-donating media. The results showed that the packed fibre was most efficient since 160-cm fibre of this type performed virtually identically to a 345-cm unpacked fibre. For example, a 0.1 *M* NaOH sample after dialysis with 0.1 *M* OSA, 0.1 *M* CSA and Bio-Rad AG 50W-X8 cation-exchange resin gave pH values after dialysis of 4.5, 4.0 and 4.0, respectively, for the packed fibre, whilst the corresponding pH values for the unpacked fibre were

TABLE I

AVERAGE RECOVERIES (%) OF SOLUTE ANIONS IN $10^{-1}$–$10^{-4}$ *M* NaOH AFTER DIALYSIS TREATMENT

The range derived from 20 replicates is shown in parentheses.

| Medium | $F^-$ | $Cl^-$ | $No_2^-$ | $Br^-$ | $NO_3^-$ | $SO_4^{2-}$ |
|---|---|---|---|---|---|---|
| $H_2SO_4$ | 126 (4) | 103 (14) | 93 (18) | 99 (14) | 103 (18) | 182 (77) |
| MSA | 123 (55) | 101 (19) | 94 (8) | 98 (19) | 98 (17) | 95 (9) |
| CSA | 106 (28) | 99 (14) | 89 (15) | 94 (8) | 93 (19) | 99 (9) |
| TSA | 127 (62) | 97 (14) | 93 (10) | 89 (14) | 90 (7) | 100 (11) |
| OSA | 106 (38) | 94 (12) | 92 (8) | 90 (5) | 94 (11) | 98 (10) |
| AG 50W-X8 | 116 (49) | 96 (9) | 93 (11) | 102 (10) | 95 (7) | 89 (9) |
| AG 50W-X2 | 130 (44) | 92 (9) | 88 (8) | 91 (10) | 90 (6) | 91 (12) |
| IRC-50 | 130 (78) | 93 (13) | 89 (18) | 85 (16) | 86 (12) | 89 (12) |
| IR-120 | 118 (63) | 90 (10) | 90 (10) | 86 (20) | 85 (15) | 88 (9) |

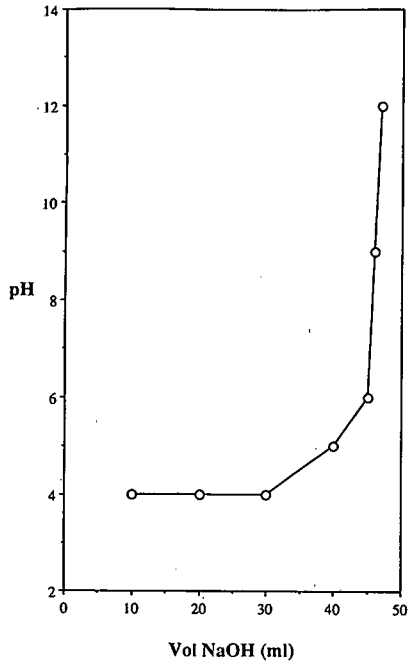


Fig. 6. Breakthrough curve for the packed fibre using 100 ml of 0.1 *M* octanesulfonic acid as hydrogen ion-donating medium and 0.1 *M* NaOH as sample.

each 4.0. The superior performance of the packed fibre is in accordance with the results obtained in previous studies using such fibres [7]. It was also noted that CSA showed greater penetration of the unpacked fibre.

*Neutralization capacity of sample clean-up devices*

Clean-up devices of the type described in this paper will have a finite life (expressed as the volume of sample of known concentration which can be treated) governed by the volume and concentration of the hydrogen ion-donating solution and the diffusion kinetics across the membrane. This lifetime can be determined by breakthrough experiments by measuring the pH of the effluent and noting the volume of sample which may be treated until the pH of the effluent shows a sharp rise. Fig. 6 shows such a breakthrough curve for the packed fibre using 100 ml of 0.1 *M* OSA as hydrogen ion-donating medium and 0.1 *M* NaOH as sample. The breakthrough point is 47 ml, which corresponds to 47% of the total theoretical capacity of the device.

The neutralization capacity cannot be increased substantially simply by increasing the concentration of hydrogen ion-donating medium because of the likelihood of penetration of the acid anion, even when OSA is used. However, resin slurries offer very high theoretical neutralization capacities. For example, direct titration of 5 g of Bio-Rad AG 50W-X8 cation-exchange resin with 0.1 *M* NaOH using phenolphthalein indicator gave an end-point corresponding to 127.5 ml of NaOH. A slurry containing 80 g of resin therefore has a theoretical total neutralization capacity corresponding to 2040 ml of 0.1 *M* NaOH. The problem with such resin slurries is that their neutralization efficiency is low, as shown in Fig. 3. This suggests that the transfer of hydrogen ions from one resin particle to the next and ultimately through the membrane is rather slow when water is used as the slurrying solvent. One possible solution to this problem is to use an acid solution as the slurrying solvent, at a concentration

TABLE II

NEUTRALIZATION CAPACITY OF THE DIALYSIS DEVICE

The 345 cm × 0.5 mm I.D. unpacked Nafion fibre was used.

| Hydrogen ion-donating medium (total volume 100 ml) | Volume 0.1 *M* NaOH neutralized (ml) |
|---|---|
| 0.1 *M* OSA | 47 |
| Bio-Rad AG 50W-X8 resin (80 g) in water | 25 |
| Bio-Rad AG 50W-X8 resin (80 g) in 0.1 *M* OSA | 300 |
| Bio-Rad AG 50W-X8 resin (80 g) in 0.1 *M* OSA, stirred | 2000 |
| As above, regenerated with 1 *M* OSA | 450 |
| 0.45 *M* SPR-$H^+$ reagent, stirred | 220 |

below which penetration of the acid anion through the membrane occurs.

Table II shows the neutralization capacities obtained with a number of resin slurries with the unpacked fibre. Data for the packed fibre showed similar trends but in each case the neutralization capacities were less than those for the unpacked fibre, presumably due to the greater length of the former. From Table II it can be noted that resin slurried in water has a particularly low neutralization capacity, however, this can be increased by a factor of 12 when 0.1 *M* OSA is used as slurrying solvent. Even in the latter case, the neutralization capacity is only approximately 15% of the theoretical total of the hydrogen ions contained in the resin and the OSA slurrying solvent. We found that occasional stirring of the slurry greatly improved performance, with capacity now reaching 97% of the theoretical value. *In situ* regeneration of the exhausted resin was attempted by passing 50 ml of 1 *M* OSA through the device. Although some neutralization capacity was restored, the original performance was not recovered, suggesting that more thorough regeneration is necessary.

The particle size of the resin is also likely to exert some influence on performance. This aspect was not studied in detail, however a solution of ultra-fine (approximately 50 nm in diameter) cation-exchange resin was examined as a hydrogen ion-donating medium. The particular reagent used was Millipore-Waters SPR-$H^+$ reagent, which has been used for post-column addition to IC eluents as a means of reducing their conductance through protonation reactions [8]. When titrated with base, this solution was found to contain 0.45 *M* of $H^+$, so that 100 ml provides a theoretical neutralization capacity corresponding to 0.45 l of 0.1 *M* NaOH. As shown in Table II, the SPR-$H^+$ reagent gave a low neutralization capacity (approximately 50% of the theoretical value), even when the solution was stirred. Examination of the fibre by electron microscopy after use of the SPR-$H^+$ reagent showed particles of resin imbedded in the pores of the fibre, suggesting that physical blockage of the fibre could be responsible for the observed low neutralization capacity.

## CONCLUSIONS

This study has shown that clean-up of strongly alkaline samples prior to IC analysis can be achieved with the aid of membrane fibre devices. The hydrogen ion-donating solution into which the fibre is immersed must be chosen carefully to avoid penetration of the acid anion into the sample. Hydrophobic sulfonic acids, such as OSA and CSA give best results. The neutralization capacity of the clean-up devices can be increased greatly by using a slurry of cation-exchange resin in the hydrogen form as the hydrogen ion-donating medium. If a suitable acid (such as 0.1 *M* OSA) is used as the slurrying solvent and the slurry is stirred during use, the neutralization capacity approximates the theoretical maximum value dictated by the total ion-exchange capacity of the resin.

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# Analysis of oxyhalide disinfection by-products and other anions of interest in drinking water by ion chromatography

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ABSTRACT

The US Environmental Protection Agency is developing regulations for various drinking water disinfection by-products (DBPs). This effort involves developing analytical methods for the DBPs formed as a result of different disinfection treatments and collecting occurrence data for these species. Ion chromatography is one method being used to analyze drinking water samples for the following inorganic DBPs: chlorite, chlorate and bromate. These anions, however, are difficult to separate from common interfering anions of chloride, carbonate and nitrate. A method is therefore presented by which tetraborate/boric acid is used to separate these anions. Method detection limits of the order of 10 μg/l, using conductivity and UV detection were obtained. Stability studies of chlorite showing the effectiveness of ethylenediamine as a preservative and summary data for an occurrence of nitrite, nitrate and the DBP precursor bromide are presented.

## INTRODUCTION

Prior to recent advances in ion chromatography (IC), studies of oxyhalide anion chemistry had been hindered by the lack of methods which could directly measure these anions. Using a recently introduced IC column, research into a reliable and practical IC method for oxyhalide anion measurement has been conducted [1]. However, when these conditions are applied to drinking water matrices, problems are often encountered in resolving low levels of fromate ($BrO_3^-$), nitrite ($NO_2^-$), and chlorate ($ClO_3^-$) from typical background levels of chloride ($Cl^-$), carbonate ($CO_3^{2-}$) and nitrate ($NO_3^-$).

Recently, the oxyhalides of chlorine and bromine have drawn much attention as inorganic disinfection by-products (DBPs). They are being considered for regulation by the US Environmental Protection Agency (EPA) as part of the DBP rule (to be proposed in 1993). Depending on the disinfection process, different oxhalide by-products can form. When chlorine dioxide ($ClO_2$) is used as a disinfectant, the anions chlorite ($ClO_2^-$) and chlorate ($ClO_3^-$) are formed [2]. Chlorination using hypochlorous acid (HOCl) solutions, which can contain $ClO_3^-$ as a product of HOCl disproportionation, may contribute to $ClO_3^-$ contamination in the treated water. When ozone is applied as a disinfectant, bromide ($Br^-$) in the source water initially oxidizes to hypobromous acid (HOBr), which can then further react to form $BrO_3^-$.

$ClO_2^-$ and $ClO_3^-$ have been studied as by-products of disinfection with $ClO_2$ [2]. Pfaff and brockhoff [3] demonstrated $ClO_3^-$ was stable in drinking water, but $ClO_2^-$ was lost within a day. Research has indicated that $ClO_2^-$ reacts with hypochlorite

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($OCl^-$), the aqueous form of chlorine, as follows [4]

$$ClO_2^- + OCl^- \rightarrow ClO_3^- + Cl^- \quad (1)$$

$ClO_2^-$ has also been shown to react with $Fe^{3-}$ by forming an intermediate $[FeClO_2^{2+}]$ and further reacting as follows [5]

$$ClO_2^- + Fe^{3+} \rightarrow [FeClO_2^{2+}] \rightarrow ClO_2 + Fe^{2+} \quad (2)$$

Observations made in the laboratory suggest $ClO_2^-$ degrades upon exposure to light.

When the treatment is chlorination, the disinfectant is added to the water either as chlorine gas or as HOCl solution. Once the gas is dissolved in water it rapidly converts to HOCl by the following reaction [6]

$$Cl_2 + H_2O \rightarrow HOCl + H^+ + Cl^- \quad (3)$$

at which point an equilibrium is established, dependent upon pH and expressed by [6]

$$HOCl \rightleftharpoons OCl^- + H^+ \quad (4)$$

The HOCl acts as the functioning biocide, but will also readily react with fulvic and humic acids, present in the source water, to form a myriad of chlorinated organic DBPs [7]. HOCl can also decompose as is expressed in the following reaction [4]

$$3HOCl \rightarrow 3H^+ + 2Cl^- + ClO_3^- \quad (5)$$

forming chlorate which EPA is also considering for regulation.

The presence of $Br^-$ in the source water can significantly influence the distribution of DBPs in the treated water, since the following reaction occurs upon chlorination [6]

$$Br^- + HOCl \rightarrow HOBr + Cl^- \quad (6)$$

As in eqn. 4 above, an equilibrium is established dependent upon pH and expressed as [8]

$$HOBr^- \rightleftharpoons OBr^- + H^+ \quad (7)$$

HOBr will also readily react with the fulvic and humic acids to form brominated organic DBPs.

Research has shown the oxyhalide bromate ($BrO_3^-$) is formed following ozone treatment of source waters containing measurable levels of Br [8]. Ozone reacts with $Br^-$ as follows [8]

$$Br^- + O_3 \rightarrow OBr^- + O_2 \quad (8)$$

Again, as in eqn. 7, an equilibrium is established between HOBr and $OBr^-$. Depending on the pH of the water, two different addition reactions can occur. In waters where the pH is slightly acidic the predominate species is HOBr. In waters where the pH is neutral or slightly basic the equilibrium shifts to the $OBr^-$ form. This species will further react with ozone to form the oxyhalide $BrO_3^-$ as follows [8]

$$OBr^- + 2O_3 \rightarrow 2O_2 + BrO_3^- \quad (9)$$

This paper presents an improved method for the separation of $BrO_3^-$, $NO_2^-$ and $ClO_3^-$ from $Cl^-$, $CO_3^{2-}$ and $NO_3^-$. In addition, we apply these analytical conditions to the analyses of $NO_2^-$, $NO_3^-$ and $Br^-$. Application data are also included demonstrating the effectiveness of ethylenediamine (EDA) as a preservative for $ClO_2^-$ and the relationship between source water $Br^-$ and the formation of brominated DBPs in chlorinated and $BrO_3^-$ in ozonated waters.

## EXPERIMENTAL

### *Analytical conditions*

Analyses were performed by direct injection using a Waters 712 WISP autosampler and a Dionex 4500i ion chromatograph. Analytical conditions are presented in Table I.

### *Reagents*

Reagents for calibration standards and spiking solutions were purchased as follows: $ClO_2^-$ as sodium chlorite ($NaClO_2$), Novatek, 99.7%; $ClO_3^-$ as

TABLE I

ION CHROMATOGRAPHIC ANALYTICAL CONDITIONS

| | |
|---|---|
| Guard/separation columns | Dionex Ionpac AG9/AS9 |
| Eluent | 30 m*M* NaOH and 120 m*M* $H_3BO_3$ in water |
| Eluent flow | 2.0 ml/min |
| Detection | Dionex CDM-2 conductivity and Waters 484 UV at 195 nm in series |
| Suppressor | Anion micromembrane |
| Suppressor regenerant | 25 m*M* $H_2SO_4$ |
| Regenerant flow | 10 ml/min |
| Sample injection volume | 50 µl |
| Minimum $R^2$ value for calibration curve | 0.9998 |

sodium chlorate ($NaClO_3$), Alfa, 99%; $BrO_3^-$ as potassium bromate ($KBrO_3$), Alfa, ACS grade; $Br^-$ as sodium bomide (NaBr), Alfa, ultrapure; $NO_2^-$ as sodium nitrie ($NaNO_2$), Alfa, ACS grade; $NO_3^-$ as sodium nitrate ($NaNO_3$), Alfa, ultrapure; $Cl^-$ as sodium chlride (NaCl), EM Science, GR grade; $SO_4^{2-}$ as potassium sulfate ($K_2SO_4$), Alfa, ultrapure; and $CO_3^{2-}$ as sodium carbonate ($Na_2CO_3$), EM Science, GR grade.

Instrument calibration standards, spiking solutions, and eluents were prepared in distilled, deionized (DI) water displaying a minimum resistance of 17.8 MΩ cm.

Carbonate eluents were prepared from $Na_2CO_3$ (EM Science, GR grade) and sodium bicarbonate ($NaHCO_3$, Alfa, ACS grade). Borate eluents were prepared from either sodium tetraborate decahydrate ($Na_2B_4O_7 \cdot 10H_2O$, Aldrich, ACS grade) and boric acid ($H_3BO_3$, Aldrich, 99.999%) or sodium hydroxide (NaOH, Aldrich, ACS grade) and boric acid. The suppressor regenerant was prepared from $H_2SO_4$ (Baker, ULTREX ultrapure) diluted to 12.5 m*M* in DI water.

*Data reduction*

Following a sample analysis, the data were processed by the Dionex AI-450 software. Using parameters established in the instrument method of the software, the data were qualitatively and quantitatively compared, based upon retention time, peak height and external standard quantitation, to the calibration standard data.

## RESULTS AND DISCUSSION

*Development of analytical conditions*

To demonstrate the potential problems in resolving $ClO_2^-$, $ClO_3^-$, $BrO_3^-$ and $NO_2^-$, from the interferent anions of $Cl^-$, $CO_3^{2-}$ and $NO_3^-$, a simulated drinking water matrix (SDWM) was prepared in DI water spiked with $NO_3^-$ as N at 3 mg/l, $Cl^-$ at 50 mg/l and $CO_3^{2-}$ at 150 mg/l. These levels were chosen because they represent typical levels found in drinking water samples. This matrix was then spiked at trace levels of $ClO_2^-$, $ClO_3^-$ and $Br^-$ at 0.015 mg/l and $NO_3^-$ as N at 0.0015 mg/l.

This SDWM was analyzed using an eluent of 1.8 m*M* $Na_2CO_3$ and 1.7 m*M* $NaHCO_3$ in water, as specified in ref. 1 (Other than eluent change, all of the conditions outlined in Table I apply.) The chromatogram shown as Fig. 1 shows the poor resolution, due to the SDWM's high ionic strength as compared to that of the carbonate eluent, between $ClO_2^-$ and the positive deflection of the baseline at the expected water dip. Before the baseline can stabilize $ClO_2^-$ elutes and $BrO_3^-$ begins to elute when a second dip disturbs the baseline. Also, the $NO_2^-$ peak is unresolved from the $Cl^-$ peak.

Due to the poor resolution using this eluent strength, a weaker concentration (0.70 m*M* $Na_2CO_3$/1.3 m*M* $NaHCO_3$) was investigated as a means to separate the early eluting peaks. Fig. 2 is the chromatogram generated using this weaker carbonate eluent. Again, the ionic strength of the SDWM distorts the early portion of the chromatogram causing the $ClO_2^-$ peak to be unresolved from the baseline disturbance. The $BrO_3^-$ peak is no longer completely obscured in the second dip and the $NO_2^-$ is somewhat resolved from the $Cl^-$, however, quantitation is still difficult.

These problems, combined with the often erratic baseline and resulting high detection limits, prompted us to look for an alternate eluent. Consultation with other researchers [9], who had reported success with the borate eluent, led us to investigate it as an alternative. Initially, an eluent of 22 m*M* $Na_2B_4O_7$/22 m*M* $H_3BO_3$ was prepared. Fig. 3 shows the chromatogram of the SDWM using this eluent. The resolution was a significant improvement over the carbonate eluents. Due to the high ionic strength of the borate eluent, compared to the SDWM sample, the water dip was again observed.

However, a new problem was created by the elution of the $CO_3^{2-}$ peak following the $Cl^-$. With this eluent, the $CO_3^{2-}$ eluted as a broad peak which is unresolved from $NO_2^-$. In drinking water samples, $CO_3^{2-}$ can exceed 15 ppm. At such high concentrations, as demonstrated in Fig. 3, the $NO_2^-$ peak can not be quantitated. To circumvent this problem the eluent pH was changed from 9.0 to 8.6 by adjusting the tetraborate-to-boric acid ratio. It is believed, this eluent shift converts the $CO_3^{2-}$ to an earlier eluting bicarbonate ($HCO_3^-$) species [9]. By preparing an eluent of 15 m*M* $Na_2B_4O_7$/60 m*M* $H_3BO_3$ the carbonate peak was resolved from the $NO_2^-$ and coeluted with the chloride peak, an anion not of interest to us for our current studies.

After using this eluent for a few days, subtle base-

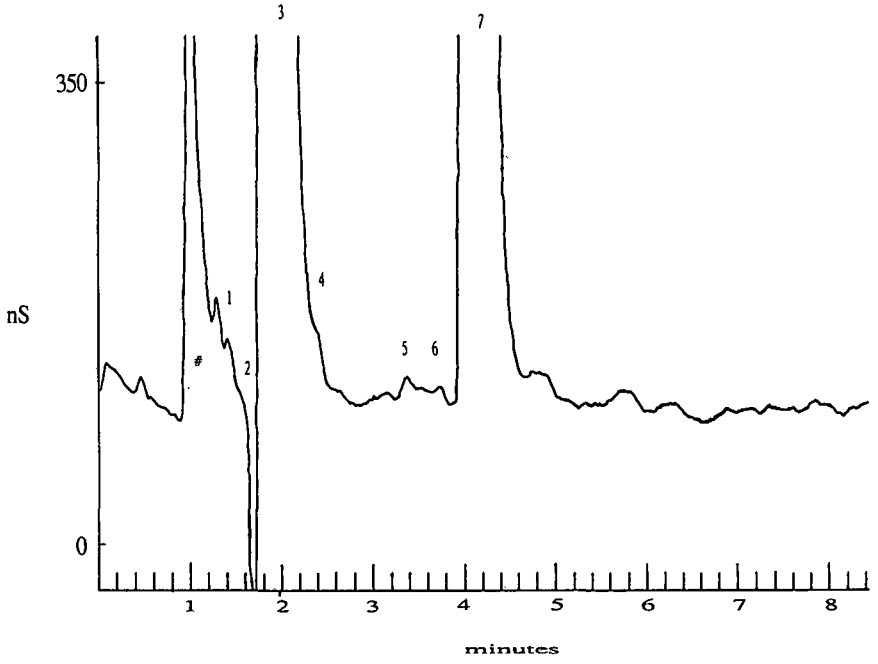


Fig. 1. Simulated drinking water matrix using eluent conditions of 1.8 m*M* $Na_2CO_3$/1.7 m*M* $NaHCO_3$. All other conditions as in Table I. Peaks: 1 = $ClO_2^-$ (0.015 mg/l); 2 = $BO_3^-$ (0.015 mg/l); 3 = $Cl^-$ (50 mg/l); 4 = $NO_2^-$ (0.0015 mg/l as N); 5 = $Br^-$ (0.015 mg/l); 6 = $ClO_3^-$ (0.015 mg/l); 7 = $NO_3^-$ (3.0 mg/l as N). # indicates positive baseline deflection at the expected water dip.

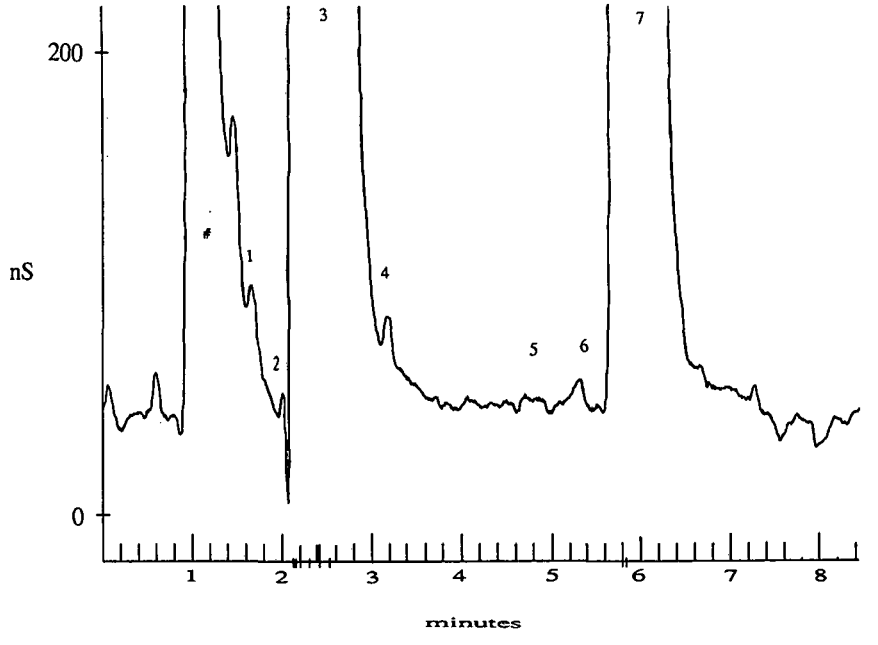


Fig. 2. Simulated drinking water matrix using eluent conditions of 0.70 m*M* $Na_2CO_3$/1.3 m*M* $NaHCO_3$. All other conditions as in Table I. Peaks as in Fig. 1. # indicates positive baseline deflection at the expected water dip.

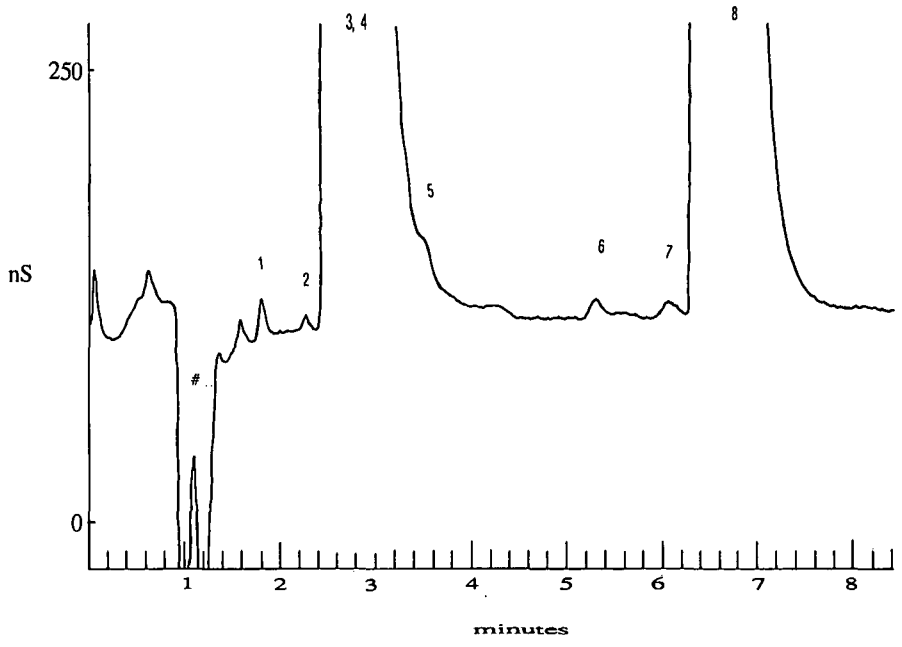


Fig. 3. Simulated drinking water matrix using eluent conditions of 22 m*M* $Na_2B_4O_7$/22 m*M* $H_3B_3$. All other conditions as in Table I. Peaks: 1 = $ClO_2^-$ (0015 mg/l); 2 = $BrO_3^-$ (0.015 mg/l); 3 = $Cl^-$ (50 m/l); 4 = $CO_3^{2-}$ (150 mg/l); 5 = $NO_2^-$ (0.0015 mg/l as N); 6 = $Br^-$ (0.015 mg/l); 7 = $ClO_3^-$ (0.015 mg/l); 8 = $NO_3^-$ (3.0 mg/l as N). # identifies the water dip.

line disturbances were observed. The baseline would gradually drift up approximately 20 nS at the retention time for $BrO_3^-$, which prevented detection of low $BrO_3^-$ concentrations. In an attempt to eliminate this baseline drift, the preparation of the borate eluent was modified by using NaOH and $H_3BO_3$ [9]. By preparing the eluent from 30 m*M* NaOH/120 m*M* $H_3BO_3$, nearly identical chromatographic results were achieved with a more stable baseline and lower background. it was thought the $Na_2B_4O_7$ was a source of unknown contamination, and that by using an alternative preparation, relying on the acid–base reaction of the hydroxide and boric acid, a higher-purity borate eluent could be attained. Using this eluent, the chromatogram shown in Fig. 4 was generated. The resolution, while not ideal, is acceptable considering the SDWM represents a "worst case" scenario. Due to the high ionic character of the SDWM matrix, it would be difficult to analyze under any eluent conditions. With these final conditions (Table I), it is possible to quantitatively measure trace anion concentration in the presence of interferent anions 10 000 times greater in concentration.

Method detection limits (MDLs) [10] under these final method conditions were determined through the analyses of 8 replicate reagent water spikes at approximately 3 times the baseline noise. A statistical MDL was calculated by multiplying the student's *t* value for 7 degrees of freedom, 99% confidence interval (2.998) by the standard deviation. In addition, a noise MDL was estimated by calculating the concentration of an analyte which would generate a peak response equal to 3 times the baseline noise. An actual MDL was reported as the higher value of the statistical MDL or the noise MDL. The results from this MDL calculation showing the statistical MDL, the noise MDL and actual MDL, for each anion of interest, are shown in Table II for the conductivity and UV detectors.

Precision data for the conductivity detector were generated by performing an initial demonstration of capabilities (IDC) where 20 replicate DI water spikes were analyzed over 4 days, 5 samples per day. These data (Table III) represent a maximum relative standard deviation (R.S.D.) of less than 6% and are used to construct control charts to monitor instrument performance. With each set of samples

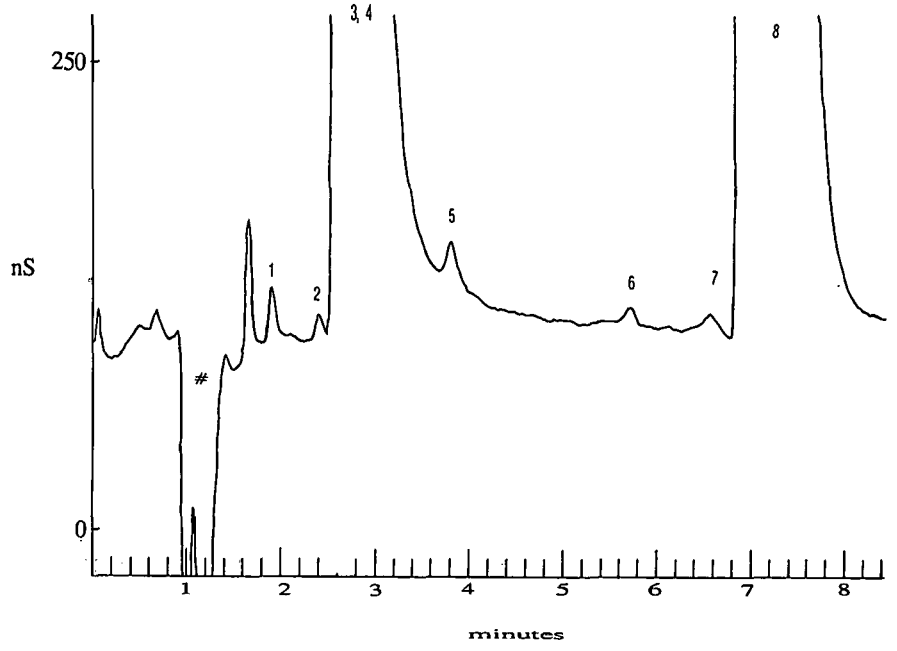


Fig. 4. Simulated drinking water matrix using eluent conditions of 30 m*M* NaOH/120 m*M* $H_3BO_3$. All other conditions as in Table I. Peaks as in Fig. 3. # identifies the water dip.

TABLE II

METHOD DETECTION LIMITS (MDLs) FOR SIX ANIONS OF INTEREST BY IC: CONDUCTIVITY AND UV DETECTORS IN SERIES

Analytical conditions as in Table I. Spiked DI water was used for replicate analyses and signal-to-noise determinations. The actual MDL was the higher value of the noise MDL or the statistical MDL.

| Anion | Spiking concentration (μg/l) | MDL (μg/l) | | |
|---|---|---|---|---|
| | | Statistical[a] | Noise | Actual[b] |
| *Conductivity detection* | | | | |
| $ClO_2^-$ | 10.0 | 3.39 | 2.94 | 3.4 |
| $BrO_3^-$ | 10.0 | 7.31 | 5.92 | 7.3 |
| $N-NO_2^-$ | 2.0 | 1.44 | 0.86 | 1.4 |
| $Br^-$ | 10.0 | 3.92 | 8.34 | 8.3 |
| $ClO_3^-$ | 25.0 | 5.18 | 9.44 | 9.4 |
| $N-NO_3^-$ | 2.0 | 2.41 | 1.42 | 2.4 |
| *UV detection at 195 nm* | | | | |
| $ClO_2^-$ | 10.0 | 6.53 | 9.44 | 9.4 |
| $BrO_3^-$ | 10.0 | 6.51 | 10.3 | 10.3 |
| $N-NO_2^-$ | 2.0 | 2.06 | 0.74 | 2.1 |
| $Br^-$ | 10.0 | 7.58 | 3.48 | 7.6 |
| $N-NO_3^-$ | 2.0 | 2.14 | 0.66 | 2.1 |

[a] Student's *t* value of 2.998 for 8 replicates at 99% confidence level.
[b] Calculated as 3 times baseline noise.

TABLE III

PRECISION RESULTS FROM AN INITIAL DEMONSTRATION OF CAPABILITY

Analytical conditions as in Table I. Replicates (20) were analyzed over 4 days, 5 samples per day, to generate the following statistical data.

| Anion | Spiked concentration μg/l | Mean recovery (%) | Precision (R.S.D., %) |
|---|---|---|---|
| $ClO_2^-$ | 250 | 101.8 | 3.09 |
| $BrO_3^-$ | 100 | 96.6 | 3.87 |
| $N — NO_2^-$ | 500 | 98.7 | 2.55 |
| $Br^-$ | 100 | 99.0 | 5.91 |
| $ClO_3^-$ | 250 | 99.3 | 3.13 |
| $N — NO_3^-$ | 1000 | 98.2 | 2.90 |

analyzed, one DI water spike, at the same levels of the IDC, is analyzed. To confirm the instrument is in control, recoveries must fall within ± 3 times the R.S.D. of the mean. Over time these data are used to rate the performance of the method and can be used to calculate confidence limits.

*Chlorite stability*

Experiments were conducted to study the stability of $ClO_2^-$ in solution with the reactive species $OCl^-$ and $Fe^{3+}$. A 15-day storage study was conducted using three matrices of deionized water, all containing 2.0 mg/l $ClO_2^-$. One matrix contained 1.0 mg/l $Fe^{3+}$, the second 2.4 mg/l $OCl^-$, and the third was free of ny reactive species. The matrices were divided into two groups, preserved with 50 mg/l EDA and unpreserved. Currently, no preservation technique is identified for $ClO_2^-$ as part of EPA Method 300.0 [1], therefore, EDA was studied as a preservative due to its ability to chelate metal ions and because of experimental evidence indicating its ability to remove $OCl^-$ [11]. Analyses were conducted on days 0, 1, 2, 8 and 15. All samples were stored in opaque, brown, high-density polyethylene bottles.

The stability studies involving chlorite demonstrated the effectiveness of EDA as a preservative. In Fig. 5, the results from the stability study of the unpreserved (Fig. 5A) and preserved (Fig. 5B) matrices are presented. These figures graphically illustrate $ClO_2^-$ losses in both the unpreerved $Fe^{3+}$ and $OCl^-$ matrices of 85 and 50%, respectively. No losses were observed in the unpreserved matrix with no reactive species present or in any matrix preserved with EDA.

*$NO_2^-$, $NO_3^-$ and $Br^-$ survey summary*

Thirty-nine community system drinking water wells were sampled for the occurrence of $NO_2^-$, $NO_3^-$ and $Br^-$ [12]. Sites were chosen whch reflected a total $NO_2^-/NO_3^-$ grater than 3.0 mg/l as N based upon occurrence data gathered in the National Pesticide Survey [13]. $NO_2^-$ and $NO_3^-$ are regulated anions with maximum contaminant levels (MCL) of 10 mg/l and 1.0 mg/l in drinking water, respectively [14]. Table IV presents a summary of the analytical results from the analyses of these samples. Every site sampled contained measurable concentrations of both $NO_3^-$ and $Br^-$, however, only one site contained detectable concentrations of $NO_2^-$.

The performance of this analytical procedure for ground water samples was illustrated through these results. Table V shows the precision of duplicate sample analyses and the low relative standard deviation attained.

*$Br^-$ as a DBP precursor*

As an illustration of the importance of monitoring $Br^-$ in source water, six sites were chosen to demonstrate the effect of $Br^-$ on the distribution of trihalomethanes. For three sites, $Br^-$ was not present above the detection limit (At the time of analyses the MDL for $Br^-$ was 20 η/l), while the other three had measurable $Br^-$ concentrations in the source water. This study was conducted prior to the change to a borate eluent, using the 0.7 m*M* $Na_2CO_3$/1.3 m*M* $NaHCO_3$, which gave a higher background conductance resulting in a noisier baseline and higher detection limits. Other than eluent change, all of the conditions in Table I apply. Trihalomethanes was determined by liquid–liquid extrac-

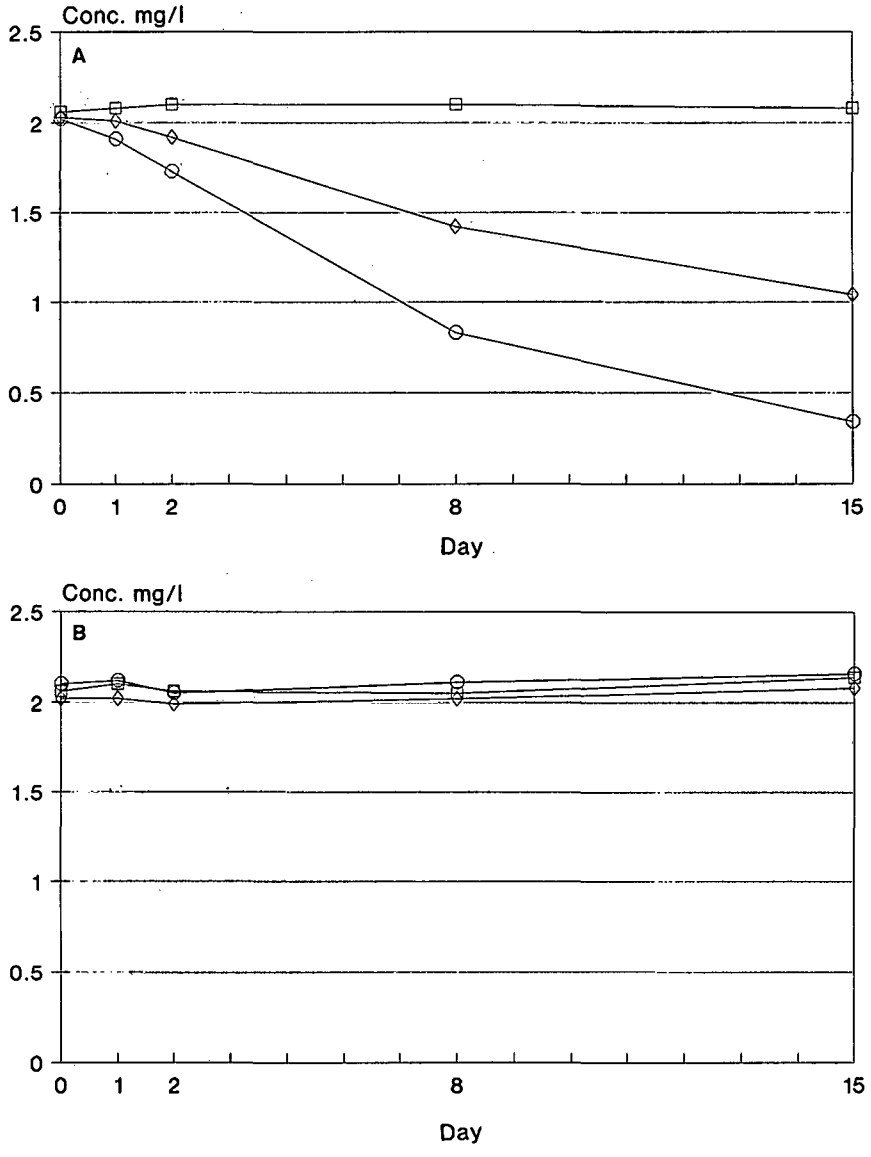


Fig. 5. (A) Results from the $ClO_2^-$ stability study showing degredation in unpreserved matrices when reactive species are present. All matrices initially were spiked with 2.0 mg/l $ClO_2^-$. □ = Unpreserved with no reactve species present; ◇ = unpreserved and spiked with 2.4 mg/l $OCl^-$; ○ = unpreserved and spiked with 1.0 mg/l $Fe^{3+}$. (B) Results from the $ClO_2^-$ stability studyshowing no degradation in any matrices preserved with 50 mg/l EDA. All matrices initially were spiked with 2.0 mg/l $ClO_2^-$. □ = EDA preserved with no reactive species present; ◇ = EDA preserved and spiked with 2.4 mg/l $OCl^-$; ○ = EDA preserved and spiked with 1.0 mg/l $Fe^{3+}$.

tion following EPA method 551 [15] on a gas chromatograph equipped with an electron-capture detector. Table VI presents data showing the relationship between $Br^-$ levels in the source water and the distribution of trihalomethanes in the treated water. As shown in the table, the presence of $Br^-$ in the source water contributes to proportionately higher concentrations of $CHClBr_2$ and $CHBr_3$ in the treated water. It should be pointed out, the levels for chlorinated and brominated DBPs in treated water is additionally effected by various parameters of source water quality.

TABLE IV

SUMMARY OF THE $NO_2^-$, $NO_3^-$ AND $Br^-$ SURVEY

Analytical conditions as in Table I. Thirty-nine community system drinking water wells were sampled for the occurrence of the anions $NO_2^-$, $NO_3^-$ and $Br^-$.

| Analyte | No. of positive samples | Minimum (mg/l) | Maximum (mg/l) | Mean (mg/l) |
|---|---|---|---|---|
| $N-NO_2^-$ | 1 | <0.001 | 0.015 | <0.001 |
| $N-NO_3^-$ | 39 | 0.49 | 13 | 5.3 |
| $Br^-$ | 39 | 0.006 | 1.0 | 0.13 |

TABLE V

PRECISION OF DUPLICATE SAMPLE ANALYSES FOR THE $NO_2^-$, $NO_3^-$ AND $Br^-$ SURVEY

Analytical conditions as in Table I. Of the 39 community drinking water wells sampled, 18 were analyzed in duplicate.

| Analyte | No. of duplicate samples | Precision (R.S.D., %) |
|---|---|---|
| $N-NO_3^-$ | 18 | 1.5 |
| $N-NO_2^-$ | 18 | 0[a] |
| $Br^-$ | 18 | 2.5 |

[a] 17 out of 18 were non-detects.

When the treatment is ozonation, $Br^-$ in the source water can react with the disinfectant to form DBPs. Samples were analyzed from an EPA pilot treatment plant experimenting with ozonation. The source water had a $Br^-$ concentration of 0.037 mg/l and a pH of 7.5 to 8.1. Source water was dosed with $O_3$ at 0.29 mg/l, 1.08 mg/l, 1.94 mg/l, and 3.97 mg/l. Table VII displays the results of the analyses and shows the formation of $BrO_3^-$ at measurable concentrations once the applied ozone dose reaches 1.95 mg/l. The $BrO_3^-$ found in the treated water accounts for the loss of $Br^-$, relative to the level present in the source water, on a mass balance basis, within the confidence limits of the data. This demonstrates $BrO_3^-$ is a possible DBP of ozone treatment. Further studies are planned to better understand the parameters involved in the formation of this DBP.

## CONCLUSIONS

Depending on the type of disinfection employed for drinking water, various inorganic DBPs can be formed. Whether the treatment is with chlorine, chlorine dioxide or ozone, there is a potential for formation of oxyhalide anions of chlorine and bromine. Because these anions are being considered for regulation, a method for monitoring these anions by IC has been refined and is currently being used to collect occurrence data. A borate eluent was shown to provide improved separation over the carbonate eluent for resolving trace levels of $ClO_2^-$, $ClO_3^-$, $BrO_3^-$ and $NO_2^-$ in the presence of high concentrations of $Cl^-$, $CO_3^{2-}$ and $NO_3^-$. The effectiveness of EDA to preerve $ClO_2^-$ concentrations from the reactive species of $OCl^-$ and $Fe^{3+}$ was illustrated. IC has been shown to be an effective analytical tool for

TABLE IV

EFFECTS OF HIGH $Br^-$ IN SOURCE WATER ON TRIHALOMETHANE DISTRIBUTION IN CHLORINATED DRINKING WATER

Analytical conditions as in Table I except the eluent was 0.7 m*M* $Na_2CO_3$/1.3 $NaHCO_3$. This study was conducted prior to a change to the borate eluent. Anions were determined by IC and trihalomethanes were determined by liquid–liquid extraction following EPA Method 551 on a gas chromatograph equipped with an electron-capture detector.

| Site No. | Source water $Br^-$ concentration (mg/l) | Trihalomethane concentration (μg/l) | | | |
|---|---|---|---|---|---|
| | | $CHCl_3$ | $CHCl_2Br$ | $CHClBr_2$ | $CHBr_3$ |
| 34 | <0.02 | 18.3 | 4.00 | 0.40 | <0.20 |
| 36 | <0.02 | 40.6 | 10.2 | 2.04 | <0.20 |
| 52 | <0.02 | 30.4 | 5.84 | 0.58 | <0.20 |
| 41 | 0.058 | 23.8 | 12.9 | 5.09 | 0.96 |
| 43 | 0.108 | 33.5 | 23.5 | 13.8 | 5.92 |
| 46 | 0.180 | 20.3 | 22.5 | 20.1 | 4.72 |

TABLE VII

EFFECTS OF HIGH $Br^-$ IN SOURCE WATER FOLLOWING OZONATION: FORMATION OF $BrO_3^-$

Analytical conditions as in Table I.

| Sample | $O_3$ Dose | | $Br^-$ (μg/l) | $BrO_3^-$ (μg/l) |
|---|---|---|---|---|
| | Applied (mg/l) | Dissolved (mg/l) | | |
| Source | 0.00 | 0.00 | 37 | <7 |
| No. 1 | 0.29 | 0.03 | 36 | <7 |
| No. 2 | 1.08 | 0.47 | 36 | <7 |
| No. 3 | 1.94 | 1.13 | 23 | 15 |
| No. 4 | 3.97 | 2.13 | 12 | 35 |

monitoring inorganic DBPs as well as the presence of $NO_2^-$, $NO_3^-$ and $Br^-$ in drinking water.

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CHROMSYMP. 2513

# Determination of trace anions in hydrofluoric acid by ion chromatography

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ABSTRACT

A method for the chemical analysis of trace phosphate and sulfate found in 4.9% hydrofluoric acid is described. Analysis to sub-ppm levels is required to determine the trace quantities of contaminants in hydrofluoric acid used in the etching process for submicron techologies.

In the laboratory, experiments were conducted to determine the amount of phosphate and sulfate in the hydrofluoric acid. Success was achieved by diluting 49% hydrofluoric acid, and by using a weak eluent of sodium carbonate and sodium bicarbonate in the analysis of these anions to the detection limits of 7.8 μg/l by ion chromatography. The run time is approximately 20 min.

## INTRODUCTION

With the growth of the semiconductor industry, there has been an increase in the need to develop methods for trace anion analysis. This demand has been the justification for a search for more powerful analytical techniques, including chromatographic methods. The result of these efforts has been the basis for developing a method for the detection of anions in hydrofluoric acid.

Because of its rapidity and efficiency in quantifying anions in aqueous samples, the application of ion chromatography (IC) in unification with conductivity detection and an automated system has been an especially important development. This type of chromatography provides accurate data on anions found in 4.9% hydrofluoric acid. This system offers the sensitivity needed to distinguish between anions and generates highly useful results, making it an excellent method for characterizing hydrogenphosphate and sulfate detected in dilute HF [1]. This paper describes the experimental methods used in analyzing $HPO_4^{2-}$ and $SO_4^{2-}$ in HF in the μg/l range. This method was developed using a fully automated system for data collection and presentation.

## EXPERIMENTAL

### *Apparatus*

A Model 4500 series ion chromatograph [2] furnished with a gradient pump (GPM), a conductivity detector (CDM), an autosampler (including plastic vials for 5-ml and filterless caps provided from Dionex) and an eluent degas module (EDM). The columns used were a Dionex IonPac AS4A analytical column, 250 mm × 4 mm I.D., packed with 15-μm polystyrene–divinylbenzene substrate agglomerated with a 0.05-μm diameter anion-exchange aminated latex particles [3], a HPIC-AG4A guard column, tace anion concentator-1 (TAC-1), an anion trap column (ATC) and an anion micromembrane suppressor-II (AMMS-II) was used [4] (Dionex, Sunnyvale, CA, USA).

### *Materials*

Ultrapure dionized distilled water free from interferences at the minimum detection limit of each constituent was used and degassed using the EDM. Reagent-grade sulfuric acid, sodium hydrogen-carbonate, and sodium carbonate were used. Ultrapurity-grade $HPO_4^{2-}$ and $SO_4^{2-}$ standards were used. Reagent-grade HF was used. The volumetric flasks, sample bottles, vials, pipette tips, and beakers are cleaned according to suggested procedures [5,6].

0021-9673/92/$05.00 

*Eluent preparation*

A solution of 0.9 m*M* $Na_2CO_3$ and 0.85 m*M* $NaHCO_3$ is prepared by dissolving 0.382 g $Na_2CO_3$ and 0.286 g NaHCO3 in ultrapure deionized distilled water to a final volume of 1000 ml. Eluent flow-rate was set to 2.0 ml/min at room temperature and degassed using the EDM.

*Standard and regenerant preparation*

Standards were prepared by diluting stock solutions (1000 ppm) of $HPO_4^{2-}$ and $SO_4^{2-}$ to make concentrations, detailed in the figure legends. A regenerant of 0.0125 m*M* $H_2SO_4$ was prepared from reagent-grade concentrated $H_2SO_4$.

*Sample preparation*

HF (49%) was diluted 1:10 (v/v) (1 ml 49% HF to 9 ml ultrapure deionized water) using an automatic plastic pipette, plastic volumetric flasks, plastic pipette tips, and plastic disposable beakers intended for trace level analysis.

*Sample injection*

A 5-ml aliquot of each blank, standard and sample is delivered to its designated vial, capped and placed in the autosampler in a precise order. From the autosampler, the aliquot is delivered to the TAC-1 through the sample port at a flow-rate of 5 ml/2.4 min.

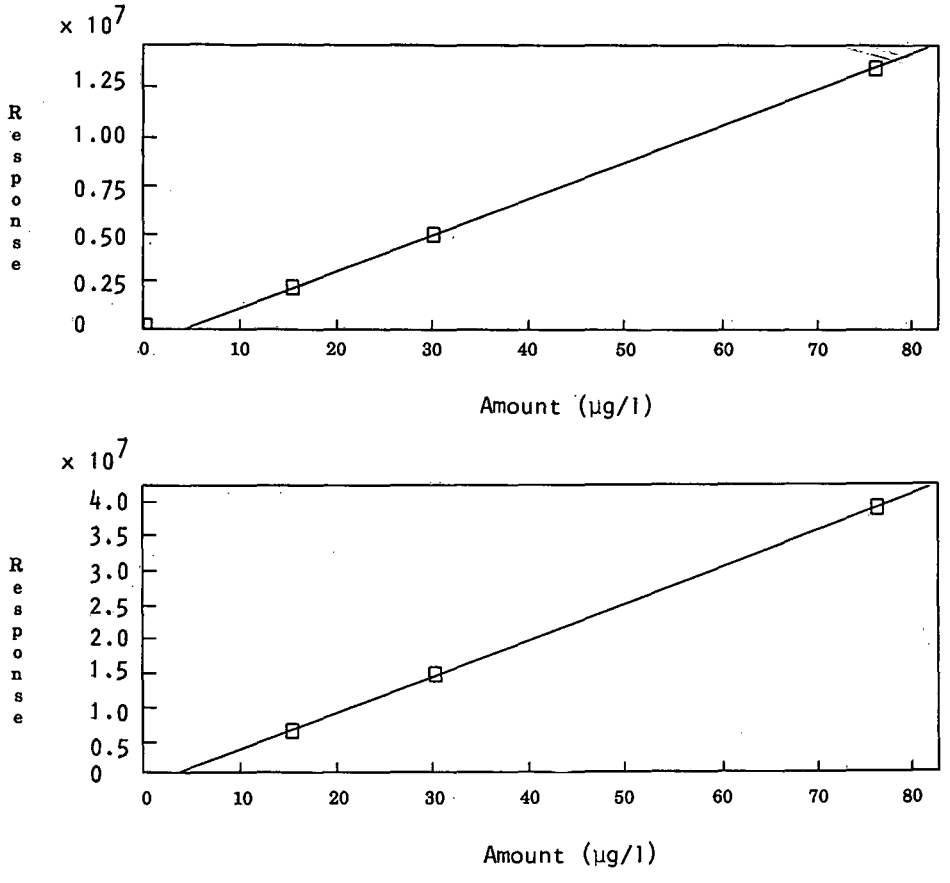


Fig. 1. Calibration curves of phosphate (top) and sulfate (bottom) standards at 15, 30 and 75 µg/l. Dionex AS4A, AG4A, TAC-1 and ATC columns were used. Application conditions included the following: the eluent was 0.9 m*M* $NaHCO_3$ and 0.85 m*M* $Na_2CO_3$ run at a flow-rate of 2.0 ml/min. A conductivity detector was used and the background conductivity was 15 µS. The volume injected was 5 ml. The data acquisition was 18 min.

*Note*

Because small sample volumes are used, contamination is to be scrupulously avoided.

## RESULTS

Relatively high concentration of fluoride interferes with the determination of ions such as chloride and nitrate. Sample dilution will not overcome this interference.

The minimum detectable concentration of an anion is a function of sample size and conductivity scale used. The concentrator column was used for the acquisition of ultratrace level ions. A direct injection of the sample insured the reproducibility of $HPO_4^{2-}$ and $SO_4^{2-}$. The operation provided a decrease in background noise necessary for a low detection limit [7]. Since the signal to noise level is dependent on the sensitivity of the $HPO_4^{2-}$ and $SO_4^{2-}$ ions at ultratrace levels, several approaches to decrease background noise required automatic injection of samples, degassed eluents, and no liquid leaks. A stable temperature is also required because temperature fluctuations have been shown to affect precision, sensitivity and reproducibility of conductivity detection in IC [8–11].

In IC, anions are detected by controlling the eluent that increases resolution. In the presence of high levels of $F^-$, this ion was eluted as a very large peak which masked the detection of ions eluting before 7 min [12,13]. A weak eluent is used to increase

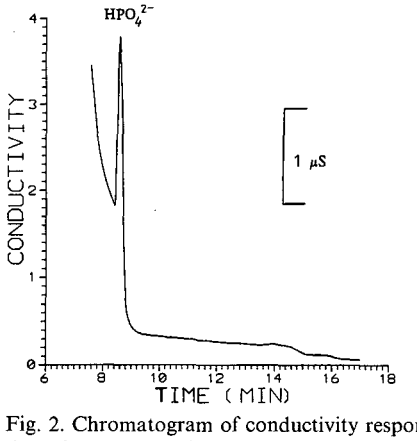


Fig. 2. Chromatogram of conductivity response showing detection of approximately 30 µg/l of phosphate in 49% HF solution diluted by 1:10 (v/v) to 4.9% HF. All other application conditions as in Fig. 1.

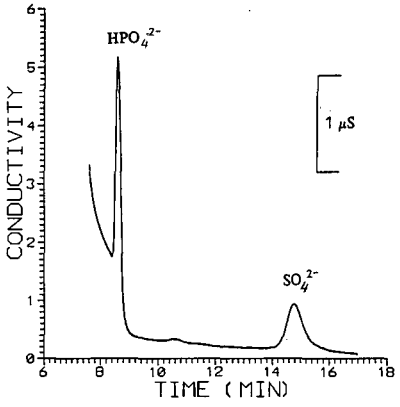


Fig. 3. Chromatogram of conductivity response showing detection of phosphate and sulfate after 4.9% HF is spiked with 75 µg/l of phosphate and sulfate respectively. All other application conditions as in Fig. 1.

elution time to reveal anions after $F^-$ has completely eluted [14]. As this occurs, the column overloads with the $F^-$ for approximately 7 min. After complete elution of $F^-$ late eluters such as $HPO_4^{2-}$ and $SO_4^{2-}$ are detected. In analyzing a sample of 4.9% HF, 30 µg/l of $HPO_4^{2-}$ were detected, and no $SO_4^{2-}$ was detected. After each run, the system needed a rinse before running the next sample.

The concentration of $HPO_4^{2-}$ and $SO_4^{2-}$ is measured from calibration curves from the standards (see Fig. 1). The instrument detection limit of $HPO_4^{2-}$ and $SO_4^{2-}$ was determined to be 7.8 µg/l after calculating $3\sigma(dc/dx)$ (where $c$ = peak height and $x$ = noise ratio); therefore, the sample of 4.9% HF $<7.8$ µg/l of $SO_4^{2-}$. Ion chromatograms of 4.9% HF and a spike HF sample are provided in Figs. 2 and 3. A standard addition method was used to verify the quantification of $HPO_4^{2-}$ and $SO_4^{2-}$ ions because of the overabundance of $F^-$ present. Recoveries of $HPO_4^{2-}$ and $SO_4^{2-}$ were in the range of 95–110%.

## DISCUSSION

The limits in IC operations are restricted by the difference between the retention times and concentrations of the ions being analyzed. The adjustment of the strength of sodium carbonate and hydrogencarbonate combinations significantly controls the retention times of the ions being chromatographed

[14]. The sensitivity for ions at ultratrace levels required a means that provided a decrease in background noise necessary for a low detection limit. The chromatogram of $HPO_4^{2-}$ and $SO_4^{2-}$ displayed a fit baseline for precise quantitation.

ACKNOWLEDGEMENT

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# New approach to the analysis of low levels of anions in water

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ABSTRACT

Optimized electromigrative sample introduction improves detection limits for anions in capillary electrophoresis. Reproducible results are achieved for micromolar and nanomolar levels of concentration. The new method offers shorter runtimes, improved resolution and greater simplicity in comparison with ion chromatography. The technique was applied to water samples from the power industry. Trace levels of anions are monitored routinely in water for steam generation in conventional and nuclear power plants. Reproducible and accurate results are presented for pure water samples containing typical concentrations of anions as well as for more specific types of samples, such as water compositions in the primary and secondary circuits of a nuclear power plant.

## INTRODUCTION

The detection limits of capillary electrophoresis (CE) with optimized indirect UV detection and hydrostatic sample introduction were shown to be in the micromolar range for common inorganic anions [1–3]. The optimization of indirect UV detection consists of finding carrier electrolyte components of high molar absorptivities and matching their ionic mobility to that of the analyte anions.

The detection limits could be improved even farther, from the micromolar into the nanomolar range, by the application of electromigrative sample introduction under isotachophoretic conditions [4]. Isotachophoretic conditions are achieved by adding a suitable amount of a component of low ionic mobility to the sample. Under properly chosen conditions, the carrier electrolyte acts as an isotachophoretic leading electrolyte, whereas the sample additive fulfills the function of a terminating electrolyte. Analytes of ionic mobilities within the range bracketed by the respective ionic mobilities of the leading and terminating electrolytes are preconcentrated in the narrow zones at the entrance to the separation capillary. As imposed by the Kohlrausch Regulation Function [5], the concentration of ions in the separated isotachophoretic zones is adjusted very close to the concentration of the leading electrolyte (*i.e.* carrier electrolyte). This represents a considerable preconcentration factor with carrier electrolyte in the millimolar range and original concentrations of sample components in the picomolar or nanomolar range.

Nanomolar concentrations of anions are monitored routinely in water for steam generation in power plants, ultrapure water used in semiconductor manufacturing and in many other different types of industrial water samples. In the past ten to fifteen years ion chromatography (IC) has become a method of choice for the analysis of trace anions in water samples. However, it is now widely recognized that a complete analysis of anions in water can be achieved only with relatively complex techniques such as coupled separation modes [6,7] or gradient IC [8,9,10]. Only so was it possible to separate various carboxylates from common inorganic anions or, for example, to analyze trace concentrations of anions in the presence of high concentrations of boric acid [6]. The complexity of IC systems required in many industrial situations prompted us to investigate the usefulness of newly developed CE methodologies for industrial water samples.

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## EXPERIMENTAL

### *Instrumentation*

The Quanta 4000 capillary electropherograph (Waters Chromatography Division of Millipore, Milford, MA, USA) with a negative power supply and the twelve-position carousel was used in all experiments. The 75 $\mu$m I.D. AccuSep (Waters) fused-silica capillary had dimensions of 52 cm (to detection point) and 60 cm (total length). The separations were followed and preceded by 2 min purging of the capillary with the carrier electrolyte to remove the large water peak, migrating at the speed of the electroosmotic flow, which would otherwise reach the point of detection at *ca.* 7 min. Polyethylene vials (2 ml) from Sun Brokers (Wilmington, NC, USA) were utilized as both sample and electrolyte containers. The UV detector settings were 254 nm and 0.1 s time constant. The data were acquired by a Maxima 820 chromatography work station (Waters) using the 20 Hz data acquisition rate.

### *Chemicals*

The sodium octanesulfonate used as an additive to water samples prior to preconcentration was obtained from Kodak (Rochester, NY, USA) and purified by recrystallization in 18 M$\Omega$ water. The ultrapure water for recrystallization of octane sulfonate and for the preparation of all solutions used in this study was from a Milli-Q laboratory water-purification system (Millipore, Bedford, MA, USA).

The chromate electrolyte used for capillary electrophoresis was prepared by dilution of two different concentrated stock solutions. The first concentrate contained 100 m*M* analytical-grade $Na_2CrO_4$ (Mallinckrodt, Paris, KY, USA) and 0.34 m*M* Ultrex sulfuric acid (J. T. Baker, Phillipsburg, NJ, USA). The second concentrate was electroosmotic flow modifier (OFM-BT, Waters Chromatography Division of Millipore), a 20 m*M* solution of a cationic surfactant. All electrolytes for the analysis of low levels of anions were prepared by diluting 10 ml of the first concentrate and 2.5 ml of the second concentrate to 100 ml with 18-M$\Omega$ water. Standard mixtures were prepared by diluting 100 $\mu M$ (inorganic anions) and 1000 ppm (carboxylic acids) stock solutions. Weighed amounts of salts rather than acids were taken for preparation of stock solutions.

Boric acid utilized to prepare compositions similar to primary water in nuclear power plants was of gold label purity (J. T. Baker). Morpholine for simulation of secondary water was an ACS reagent (Aldrich, Milwaukee, WI, USA).

### *Electromigrative trace enrichment procedure*

The final dilutions of standards and samples were placed into 100-ml polypropylene volumetric flasks (VWR, Boston, MA, USA) presoaked 24 h in 18M$\Omega$ water. An addition of 100 $\mu$l of 70 m*M* stock solution of purified octanesulfonate was made to each 100.0 ml volume of a sample in volumetric flasks. Disposable laboratory gloves and disposable polyethylene pipettes (Samco Pipettes, Cole Parmer, Chicago, IL, USA) were essential in helping to prevent contamination during the transfer of additive containing samples from the 100-ml volumetric flasks into the 2-ml polypropylene sample vials prior to actual analysis. The sample vials had also to be soaked in 18 M$\Omega$ water for 24 h before their use in trace anion analysis. At the 70 $\mu M$ level of the octanesulfonate additive, two 45-s electromigrative injections at 5 kV yielded comparable ($\pm$ 5%) levels for all analyte anions. At 35 $\mu M$ of the additive only one electromigrative injection was possible from the 2-ml sample vials. The second injection gave *ca.* 20% lower values than the first injection. The whole sequence, consisting of electromigrative trace enrichment followed by transfer of the capillary to an electrolyte vial and electrophoretic separation, can be carried out automatically by the Quanta 4000. Up to six samples can be analyzed unattended.

## RESULTS AND DISCUSSION

### *Optimized conditions for water analysis*

As previously reported [4], a combination of electromigrative trace enrichment, capillary electrophoresis and indirect UV detection, yields detection limits in the nanomolar (ppb) range[a]. The first such results for the seven common inorganic anions were obtained with an electrolyte containing among other things 5 m*M* chromate. The resolution of fluoride and phosphate after trace enrichment (Fig. 7 in ref. 4) is much less complete than in conjunction with hydrostatic injection (Fig. 2 in ref. 4).

[a] Throughout this article the American billion ($10^9$) is meant.

The loss of resolution is caused mainly by the shift of phosphate to shorter migration times. Such a shift can be explained as a consequence of an increase of pH of the chromate electrolyte inside the capillary during the isotachophoretic steady state. An explanation of the underlying mechanism is the subject of another report currently in preparation by the authors. For an easier quantitation of fluoride and phosphate after electromigrative preconcentration it is possible to improve the resolution of the two peaks by increasing the concentration of chromate in the electrolyte [11]. As illustrated by the two electropherograms obtained with hydrostatic sample introduction and shown in Fig. 1, the resolution of fluoride and phosphate is greatly improved in 10 m*M* chromate. A disadvantage of the higher chromate concentration is the incomplete separation of the sulfate–nitrite peak pair. Since nitrite is absent in most industrial pure water samples, the separation in 10 m*M* electrolyte with its improved resolution of phosphate and fluoride is of a higher practical value than that with 5 m*M* chromate. Fig. 2 is an electropherogram in 10 m*M* chromate after an electromigrative trace enrichment. The fluoride and phosphate peaks are sufficiently separated not only from each other, but also from an additional anion, formate. The formate anion is frequently encountered in pure water samples and the ability to analyze it further increases the usefulness of the higher concentration electrolyte. Other anions frequently present in water samples, such as oxalate, formate, acetate and propionate, are also well separated under these conditions. The two unidentified minor peaks at 3.10 and 3.18 min are impurities stemming from the carboxylate standards. The largest peak at 4.14 min belongs to the carbonate. The concentration of carbonate could not be controlled under the conditions of our experiment and consequently the concentrations of that anion were not evaluated. However, as discussed in one of the following paragraphs, the migration time of the omnipresent carbonate peak can be useful in normalizing of migration time variations caused by fluctuations of certain matrices of industrial water samples.

Another advantage of increased chromate concentration is the higher trace enrichment recovery for analyte anions. The effect is illustrated by the plot of peak areas for one μequivalent per liter of sulfate, nitrate and chloride *versus* m*M* chromate (Fig. 3). The sensitivity almost doubles for those anions in going from 5 m*M* to 10 m*M* chromate. This finding also represents experimental evidence supporting the original assumption of sample pre-

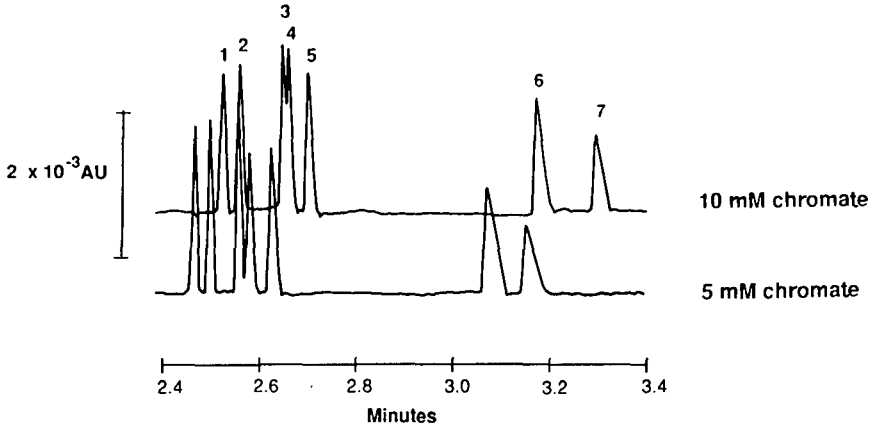


Fig. 1. Influence of the concentration of carrier electrolyte anion. Both separations were obtained after 30 s hydrostatic injection of the same standard mixture at 10 cm height. The standard mixture contained 7.9 ppm bromide (peak 1), 3.5 ppm chloride (peak 2), 4.8 ppm sulfate (peak 3), 4.6 ppm nitrite (peak 4), 6.2 ppm nitrate (peak 5), 1.9 ppm fluoride (peak 6) and 3.2 ppm phosphate (peak 7). Carrier electrolyte concentration was 0.5 m*M* NICE-Pak OFM anion-BT, 5 m*M* (lower trace) or 10 m*M* (upper trace) sodium chromate. The electrolyte was adjusted to pH by an addition of a suitable volume of dilute sulfuric acid. The 75-μm I.D. fused-silica capillary had dimensions of 60 cm (total length) and 52 cm (sample entry to detector). The separations were carried out with −20 kV separation voltage and the peaks were detected by indirect photometric detection at 254 nm.

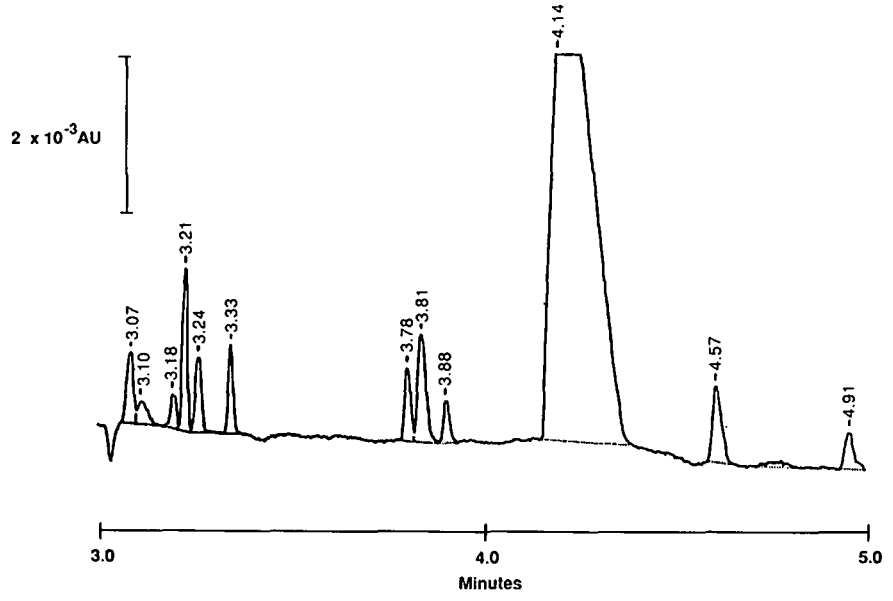


Fig. 2. Trace analysis of common inorganic and organic anions in pure water. The separation was recorded after an electromigrative trace enrichment, 45 s at 5 kV and with 75 $\mu M$ octanesulfonate additive in the sample (see Experimental for a detailed description of the electromigrative trace enrichment procedure). The sample contained 3.5 ppb chloride (3.07 min), 4.8 ppb sulfate (3.21 min), 6.2 ppb nitrate (3.24 min), 5 ppb oxalate (3.33 min), 1.9 ppb fluoride (3.78 min), 5 ppb formate (3.2 min), 3.2 ppb phosphate (3.88 min), 5 ppb acetate (4.57 min) and 5 ppb propionate (4.91 min). The carrier electrolyte consisted of 10 m$M$ sodium chromate and 0.5 m$M$ NICE Pak OFM anion-BT, adjusted to pH 8 with dilute sulfuric acid. The separation voltage was −15 kV. The capillary dimensions and detection technique were the same as in Fig. 1.

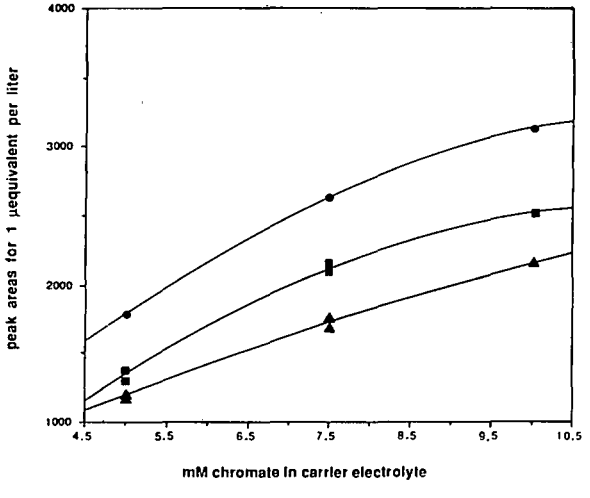


Fig. 3. Influence of chromate concentration in the carrier electrolyte on the sensitivity (peak areas) for three anions. Analytical conditions were as indicated in Fig. 2, except for the concentration of chromate in the electrolyte which was 5, 7.5 or 10 m$M$. The analyte anion concentrations were 48 ppb sulfate (●), 62 ppb nitrate (▲) and 35 ppb chloride (■).

concentration due to an isotachophoretic steady state [4].

According to the Kohlrausch Regulation Function however, the relationship between the concentration of sample zones on one the hand and the chromate concentration on the other hand (Fig. 5 in ref. 4) should be linear under the conditions of isotachophoretic trace enrichment. The non-linear behavior above *ca.* 7.5 m$M$ is most likely caused by an increased rate of electroosmotic flow leading to a decreasing efficiency of the electromigrative trace enrichment.

The applied voltage of 15 kV in Fig. 2 is lower than the 20 kV utilized in our initial work. The lower voltage was adapted to eliminate low-frequency baseline oscillations caused by overheating of the electrolyte. An improved sensitivity at 15 kV separation voltage and 45 s trace enrichment time is compared with an electropherogram using 20 kV separation voltage and 30 s trace enrichment time in Fig. 4. Under the latter set of conditions the peak

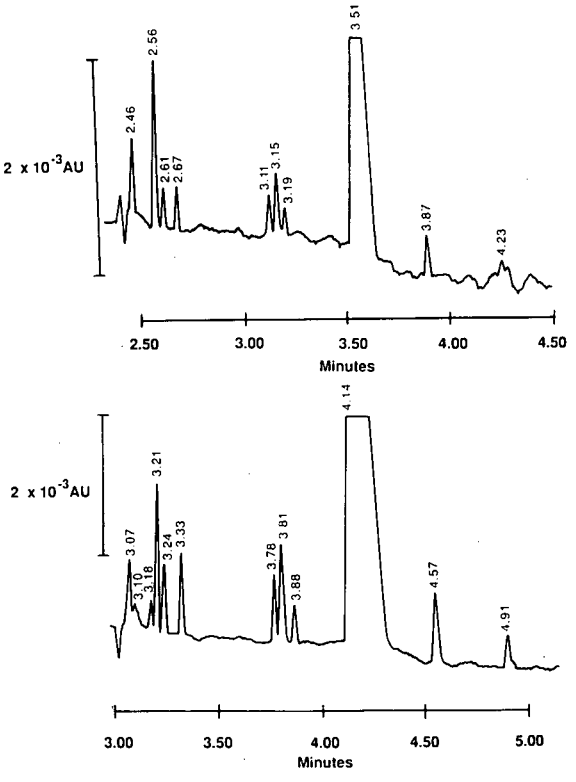


Fig. 4. Influence of separation voltage and sampling time on the sensitivity for traces of common inorganic and organic anions. Analytical conditions, except for separation voltage and preconcentration time, are given in Fig. 2. The separation voltage was 20 and 15 kV for the upper and lower separation, respectively. The upper chromatogram was obtained after electromigrative trace enrichment lasting 30 s, while the lower electropherogram is the result of a 45-s long trace enrichment. The peak identities are the same as in Fig. 2.

for 5 ppb propionate (4.23 min) is approaching its detection limit. At the lower separation voltage of 15 kV the sensitivity has clearly increased beyond the factor of 1.5 given by the longer trace enrichment time. The long-drawn and relatively regular ocillations lasting about 0.25 min, that are clearly present at 20 kV, are not encountered in the 15-kV electropherogram. Table I gives the values of detection limits determined under the optimized conditions for water samples.

IC is capable of similar or better detection limits in matrix-free pure water samples, but it requires trace enrichment times much longer than 45 s — typically 5–10 min.

TABLE I

DETECTION LIMITS FOR TRACE ANIONS IN WATER

Trace enrichment at 5 kV and 45 s. See Experimental for a complete description of analytical procedure.

| Anion | ppb |
|---|---|
| Chloride | 0.5 |
| Sulfate | 0.3 |
| Nitrate | 0.8 |
| Oxalate | 0.6 |
| Fluoride | 0.3 |
| Formate | 0.5 |
| Phosphate | 0.3 |
| Acetate | 0.8 |
| Propionate | 0.6 |

It is also appropriate to comment on the interesting selectivity of CE separations of anions in comparison with IC. The complete separation of short-chain alkyl carboxylates from fluoride, simultaneously with anions strongly retained on ion-exchange columns, such as sulfate and oxalate (Fig. 2), is achieved within only *ca.* 6 min using comparatively simple instrumentation. The same kind of separation by IC takes always longer than 15 min and requires either coupled separation modes or gradient elution.

*Quantitation of trace anions in aqueous samples*

The usefulness of the method for quantitative monitoring of anions in pure water was evaluated by means of calibration and reproducibility experiments. As shown in Table II, valid calibration plots could be obtained for all anions separated in Fig. 2.

TABLE II

CALIBRATION DATA BETWEEN 0.1 TO 1 $\mu M$ FOR NINE ANIONS IN WATER

| Anion | Correlation coefficient | Slope | $y$ intercept |
|---|---|---|---|
| Chloride | 0.999 | 8423 | 394 |
| Sulfate | 0.998 | 21 159 | 649 |
| Nitrate | 0.999 | 8832 | −11 |
| Oxalate | 0.999 | 18 520 | −116 |
| Fluoride | 0.999 | 7796 | 223 |
| Formate | 1.000 | 9700 | 795 |
| Phosphate | 0.993 | 12 686 | −17 |
| Acetate | 0.999 | 8503 | 888 |
| Propionate | 0.997 | 7781 | 293 |

The calculated correlation coefficients for analyte concentrations between 0.1 and 1 $\mu M$ were in the range of 0.993 to 1.000. The $y$ intercept values were the highest for sulfate, formate and acetate. All three anions were subsequently detected as contaminants in the purified octane sulfonate employed as an additive for the electromigrative trace enrichment [11]. Additional purification steps planned for the octanesulfonate can thus be expected to decrease the values of $y$ intercepts in future experiments.

The values of slope were similar within ± 10% for monovalent and divalent anions, respectively. A "universal" calibration by one anion standard can thus be expected to give useful, approximate results in equivalents per liter for all known and unknown peaks in the electropherogram. The possibility of "universal" calibration with indirect detection in IC was first evaluated by Yeung [12]. The above calibration data indicate that a "single standard calibration" may also be possible in CE, if indirect detection is employed.

The precision of six consecutive determinations was evaluated at 0.5 $\mu M$ for inorganic anions and 0.4–0.5 $\mu M$ carboxylates. In each analytical run only one electromigrative trace enrichment was carried out from a 2-ml volume of standard anion mixture containing 70 $\mu M$ sodium octanesulfonate. All analytical conditions were as described in the Experimental section. The results, calculated as relative standard deviations (R.S.D.) of peak areas along with the anion concentrations in the standards used for the repetitive runs, are listed in Table III.

TABLE III

PRECISION DATA FOR SIX CONSECUTIVE RUNS

| Anion | ppb | R.S.D. (%) |
|---|---|---|
| Chloride | 17.5 | 4.3 |
| Sulfate | 24 | 3.3 |
| Nitrate | 31 | 2.7 |
| Oxalate | 25 | 2.9 |
| Fluoride | 9.5 | 2.5 |
| Formate | 25 | 2.0 |
| Phosphate | 15.5 | 2.6 |
| Acetate | 25 | 7.5 |
| Propionate | 25 | 7.2 |

*Trace anions in water samples containing high levels of other components*

In many industrial situations trace anion determinations have to be performed in aqueous samples containing much larger concentrations of various ionic and non-ionic components. Nuclear pressurized water reactors (PWRs) provide two important examples of such water samples.

The first example is so-called primary water (PW). PW is an aqueous solution of 500–2000 ppm boron added as boric acid (2855–11420 ppm $H_3BO_3$), it also contains maximum 3 ppm Li, added as LiOH. The boric acid can be expected to fluctuate strongly within the specified limits. Our study was carried out at a constant level of 1.7 ppm Li and at several different levels of boron. Values of 5 ppb are recognized as normal levels for the most common inorganic anions: chloride, sulfate, nitrate and fluoride. The maximum admissible levels for any of the inorganic anions are approximately 40 ppb.

Given the relatively high concentration of boric acid, we first had to clarify the role of borate anion in the electromigrative trace enrichment. A possibility that could not be dismissed without a clarifying experiment, is that through a pH increase in the electrolyte due to isotachophoresis, sufficient amounts of borate would be generated to act as a terminating electrolyte contributing to the trace enrichment of other anions according to the mechanism postulated in our earlier work [4]. The series of three enrichment experiments is depicted in Fig. 5. The presence of boric acid in the sample did not contribute to preconcentration of analyte anions. The analysis of spiked levels became possible only after an addition of octanesulfonate resulting in 25 or 75 $\mu M$ levels in the sample before the preconcentration step.

The feasibility of calibration for standard anion concentrations ranging from 0.1 to 2 $\mu M$ in a constant PW matrix was evaluated next. The corresponding calibration data are summarized in Table IV. As in the case of matrix free, pure water samples (Table II), meaningful calibraton plots can also be obtained for water samples containing high levels of boric acid. The single standard calibration, for example by chloride, appears to be feasible for normalities (equivalent per liter) of the remaining three anions, with absolute values of errors not exceeding 10%. The calibration equations for chloride and

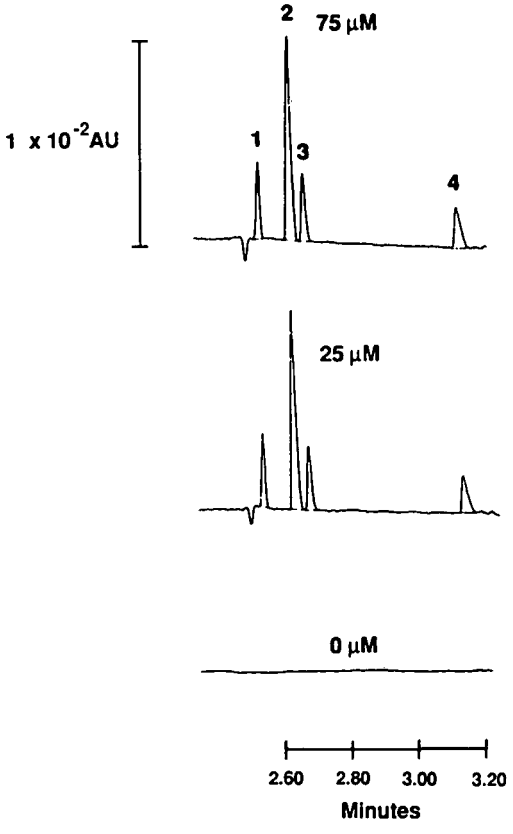


Fig. 5. Results of electromigrative trace enrichment from samples containing 5710 ppm boric acid (1000 ppm boron). The trace enrichment was carried out first at 5 kV, 30 s, without any addition of octanesulfonate (bottom trace). The upper two separations were generated also at 5 kV, 30 s, but with 25 (electropherogram in the middle) and 75 $\mu M$ octanesulfonate (upper electropherogram) in the sample solution. The peak identities and concentrations were as follows: chloride 35 ppb (peak 1), sulfate 48 ppb (peak 2), nitrate 62 ppb (peak 3) and fluoride 19 ppb (peak 4). All other analytical conditions were as indicated in Fig. 2.

TABLE IV

CALIBRATION IN PRIMARY WATER (1000 ppm BORON AND 1.7 ppm LITHIUM)

0.1 to 2.0 $\mu M$ anions.

| Anion | Correlation coefficient | Slope | $y$ intercept |
|---|---|---|---|
| Chloride | 0.999 | 2161 | 541 |
| Sulfate | 1.000 | 4307 | 1020 |
| Nitrate | 1.000 | 1930 | 158 |
| Fluoride | 0.999 | 1875 | 200 |

sulfate show higher values of $y$ intercepts than those for nitrate and fluoride. This indicates a presence of noticeable levels of sulfate and chloride impurities from the octanesulfonate additive or from the boric acid.

Can variations of boric acid concentration be expected to affect the reproducibility of measured migration times and peak areas of trace anions present in primary water samples? The plot of measured migration times *versus* boron concentration for the four anions and carbonate is presented in Fig. 6A. The actual variations of the migration times within the range of 500 to 2000 ppm boron are relatively minimal. However, a clear trend toward longer migration times is observed within a broader range from 500 to 3000 ppm boron. The increasing boron concentration in the samples causes decreasing sample pH (pH 7.3 at 500 ppm, pH 6.85 at 1000 ppm, pH 5.65 at 2000 ppm), if the concentration of lithium hydroxide remains constant. In boric acid solutions, the lower pH increases electrical resistance of the sample segment in the initial stages of the CE separation. The higher resistance leads to a slower increase of current immediately after the application of separation voltage and in consequence to slower migration times.

The observed shifts of migration times can be eliminated by normalization. We have carried out a normalization by dividing the migration times of sulfate, nitrate, fluoride and chloride at a given concentration of boron by the migration time of the carbonate peak obtained in the respective separations. The plot in Fig. 6B shows constant values of normalized migration times throughout the entire range of 500 to 3000 ppm boron. Carbon dioxide equilibrates at several hundreds ppb in water. After the trace enrichment of anions from samples exposed to the atmosphere, a peak for carbonate is always present in the electropherogram and no addition of an internal standard has to be made to obtain a reference peak for migration times. An automatic normalization of migration times by calculated ratios of analyte *versus* reference value can be performed by most internal standardization routines in commercial data acquisition and reduction software.

The data for variations of peak areas with changing boron concentrations are presented in Fig. 7. With increasing boron concentration the response

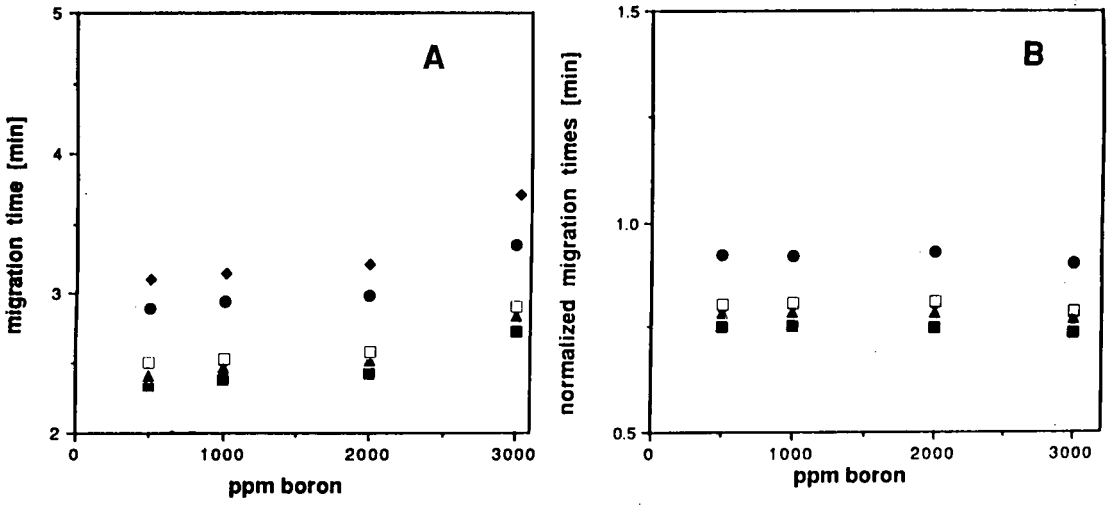


Fig. 6. Changes of migration times with changing concentration of boron (added as boric acid). (A) Directly measured migration times *versus* ppm boron; (B) migration times divided (normalized) by migration times of the carbonate peak at the same concentration of boron. The concentration of Li (added as LiOH) was kept constant at 1.7 ppm. The anion concentrations were also constant at the following levels: ■ = chloride, 35 ppb; ▲ = sulfate, 48 ppb; □ = nitrate, 62 ppb; ● = fluoride, 19 ppb. The level of carbonate (◆) was as obtained by adsorption from the atmosphere (several hundred ppb) and quantitative evaluation of peak areas was made for this anion. The trace enrichment was carried out at 30 s, 5 kV and 75 $\mu M$ octanesulfonate. All other conditions were as in Fig. 2.

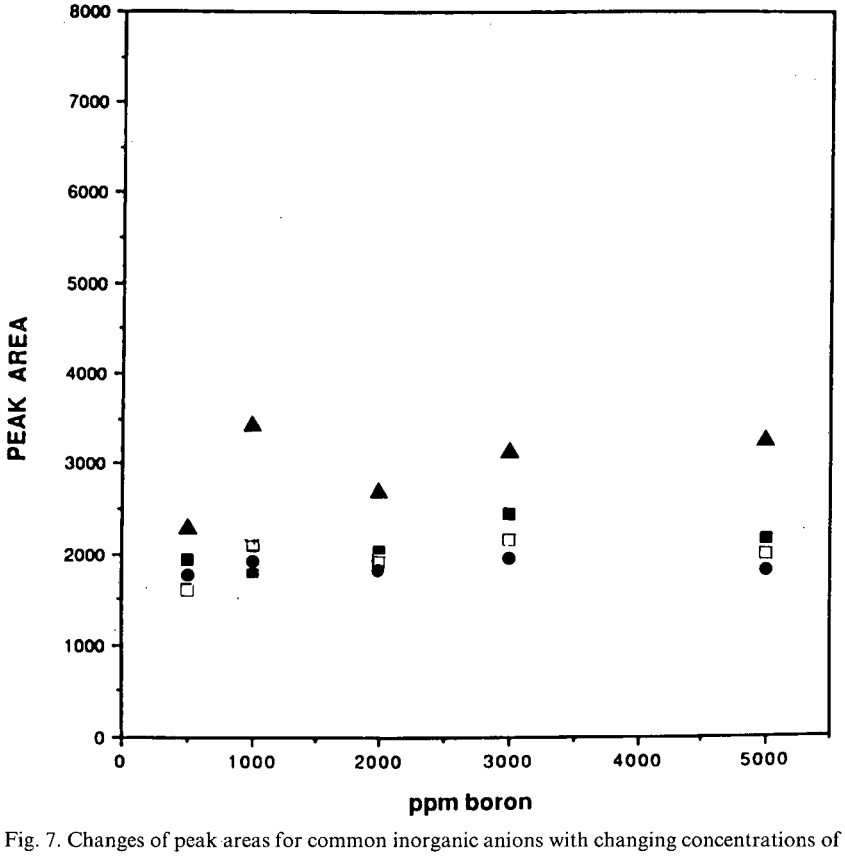


Fig. 7. Changes of peak areas for common inorganic anions with changing concentrations of boron (added as boric acid). The samples were prepared as indicated in Fig. 6 and analytical conditions were as indicated in Fig. 2, except for the trace enrichment conditions which were modified to 5 kV and 30 s. ■ = chloride (R.S.D. 10.9%); ▲ = sulfate (R.S.D. 14.0%); □ = nitrate (R.S.D. 6.7%); ● = fluoride (R.S.D. 3.2%).

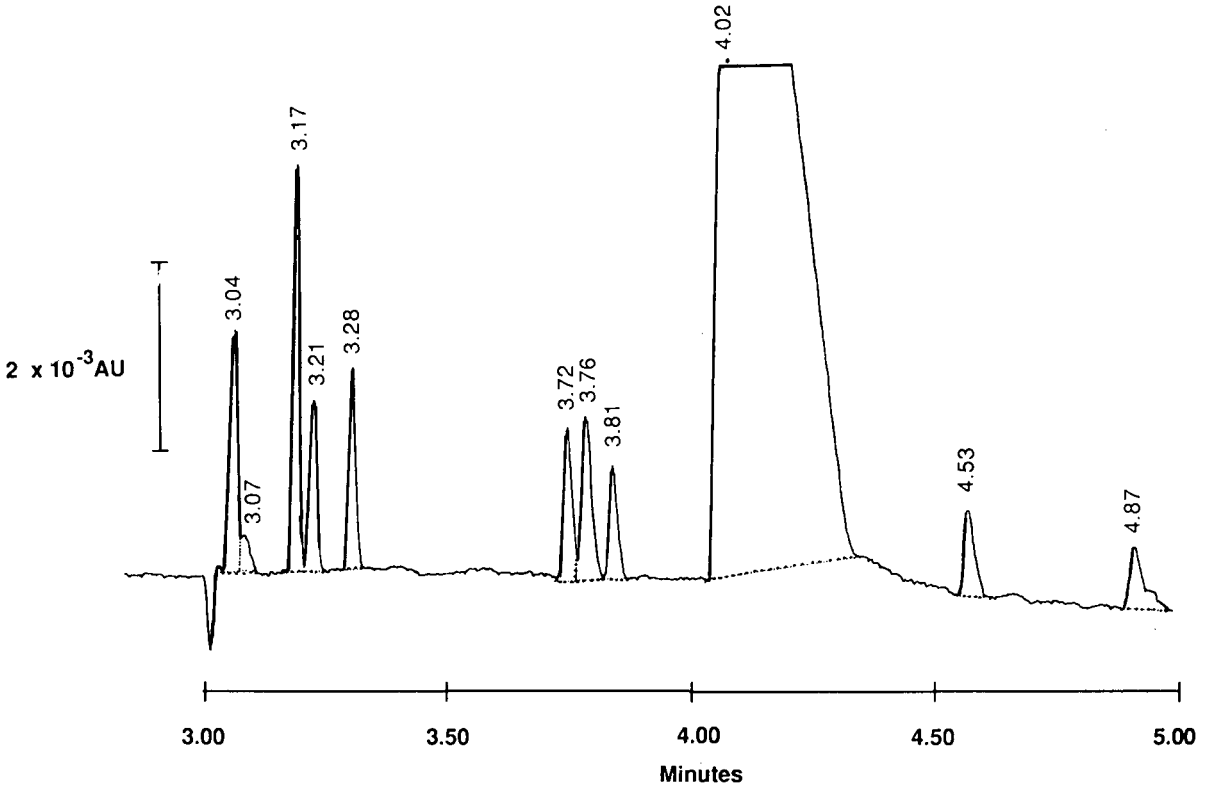


Fig. 8. Trace anions in the presence of 3 ppm morpholine. The separation was generated after electromigrative trace enrichment, 45 s at 5 kV and with 75 $\mu M$ octanesulfonate additive in the sample. A complete description of the procedure is given in the Experimental section. The sample contained 7 ppb chloride (3.04 min), 9.6 ppb sulfate (3.17 min), 12 ppb nitrate (3.21 min), 10 ppb oxalate (3.28 min), 3.8 ppb fluoride (3.72 min), 10 ppb formate (3.76 min), 6.2 ppb phosphate (3.81 min), 10 ppb acetate (4.53 min) and 10 ppb propionate (4.87 min). The carrier electrolyte consisted of 10 m$M$ sodium chromate and 0.5 m$M$ NICE Pak OFM anion-BT, adjusted to pH 8 with dilute sulfuric acid. The separation voltage was $-15$ kV. The capillary dimensions and detection technique were the same as in Fig. 1.

for sulfate and chloride increases more rapidly than that of nitrate and fluoride. The higher values of $y$ intercepts for sulfate and chloride, obtained in calibration plots at 1000 ppm boron (Table IV), support the preliminary conclusion of observed increases of peak areas for these two anions being caused by a relatively higher levels of these impurities in boric acid. The actual level of variance would than be less than 10% for 500 to 5000 ppm boron as indicated by the R.S. D. values of nitrate and fluoride. All practically possible samples of primary water (500 to 2000 ppm boron, *ca.* 1.7 ppm Li, detection limit up to 40 ppb inorganic anions) could thus be analyzed using a calibration at a single level of boron.

The second investigated example of an industrial water sample containing elevated levels of ionic matrix is the secondary water (SW) in PWR. SW normally contains ammonia or morpholine at *ca.* 3 ppm. Also present are the reaction products (amines, carboxylates, etc.) of the basic additives and ion-exchange resins. Maximal admissible levels of inorganic anions and carboxylates are at 40 ppb. Normally encoutered concentrations of these ionic impurities are less than 5 ppb. An electropherogram of all anions usually monitored in SW, obtained in the presence of 3 ppm morpholine, is shown in Fig. 8.

The separations in the presence of morpholine are virtually identical with those obtained in the absence of the additive, the only noticeable differences being firstly, the larger areas of carbonate peaks due

to higher pH of the samples, and secondly, a slight shift towards longer migration times in morpholine-containing samples in comparison to pure water samples. Despite that, the calibration and precision data in synthetic SW samples are very similar to those shown in Tables II and III for matrix-free pure water samples and are for that reason not presented here.

## CONCLUSIONS

The CE approach to the analysis of trace anions offers shorter run times per sample, improved resolution of sample components and a greater simplicity in comparison with IC. Validity of calibration curves is demonstrated in pure water samples containing common anions at trace levels as well as for some more specific samples such as water compositions in a nuclear power plant.

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# Determination of trace anions in concentrated acids by means of a moderate-capacity anion-exchange column

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ABSTRACT

The development of a moderate-capacity anion-exchange resin has resulted in new ion chromatography (IC) methods for the determination of trace anions in concentrated acids. Suppressed microbore IC offers a higher suppression capability, which allows higher-capacity analytical columns to be used. As a result, higher-capacity columns permit higher concentrations of acids to be injected into the column without overloading, thus improving trace anion detection limits. In addition, using a selective matrix elimination method, high concentrations (% levels) of weak acids (*e.g.* hydrofluoric acid and acetic acids) may be eliminated prior to analysis by IC.

## INTRODUCTION

The determination of trace inorganic constituents in concentrated reagent is important in a variety of chemical and semiconductor processes. Historically, the levels of impurities have been dictated by available analytical methodologies. In the semiconductor industry, the requirements for lower impurities levels are exceeding the limits of current analytical methods. Such a case is the determination of trace anions in concentrated acids. In many cases, new specifications are being set with impurities at the low and sub-$\mu$g/l levels in concentrated acids.

The determination of trace anions in concentrated acids has been a difficult analytical challenge. Labor-intensive manual preconcentration methods have been required prior to analytical measurements. Typical preconcentration procedures involve evaporation of a specific volume of sample for 6 to 10 h on a hot plate before transfer to a volumetric flask and analysis. Most of the wet chemistry methods of analysis used are semiquantitative and insensitive. For example, turbidity assays have been used to determine chloride and sulfate contaminations in semiconductor-grade hydrofluoric acid [1]. Colorimetric methods have been used for the determinations of nitrate and phosphate in the same acid. Also, a class 100 clean room environment is normally required during sample pretreatment.

Since the invention of ion chromatography (IC) in 1975 [2], IC has not been applied to sample matrices of extreme ionic strength due to low column capacity and the low ionic strength of the eluents used in most analytical separation. Several-fold dilution of the concentrated acids is usually required in order to determine the trace anion contaminants, usually compromising the detection limits. A separation of trace component form the sample matrix on a moderate to a high capacity anion exchanger is the method of choice; however, a strong eluent is required which will overload the suppression capacity of the suppressor.

Suppressed microbore IC offers analysts a solution for trace anion determination in concentrated acids. Greater suppression capacity can be achieved at the low flow rates of the system [3–5]. Improved background suppression allows higher capacity analytical columns to be used. As a result, higher-capacity columns permit higher concentration of acids to be injected onto the column without overloading, thus improving trace anion detection limits.

This paper will describe the determination of trace anions in concentrated acids using a moderate

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capacity anion exchnage column. Using a selective matrix elimination method, the high concentration of weak acids (% levels) such as hydrofluoric acid and acetic acid can be eliminated prior to analytical determination by IC.

## EXPERIMENTAL

### *Chromatographic system*

All chromatography was performed on a Dionex DX-300 system with only minor modifications. The system consisted of a microbore advanced gradient pump (AGP), a liquid chromatography module (LCM-3), a single piston pump (DQP), and a conductivity detector (CDM-II).

Three types of columns were used in the system. An IonPac AS10 (250 × 2 mm) was used for analyte separation. An IonPac AC10 (50 mm × 2 mm) was used for sample pretreatment and matrix elimination prior to sample analysis. Two ATC-1 columns were employed for eluent purification. The ATC-1 (trap column) columns were cleaned by pumping 0.5 *M* sodium hydroxide in water–methanol (50:50) at 5.0 ml/min for 15 min followed by rinsing with 200 ml deionized water. One of the two ATC-1 columns was placed between the DQP outlet check valve and the LCM-3. This column was used to purify methanol–water (70:30) eluent. The other ATC-1 column was placed between the water eluent bottle and the AGP. The DQP pump flow-rate was set at 1.0 ml/min.

The DX-300 system was modified as shown in Fig. 1. An additional inert double stack four-way slider valve (5000 p.s.i.) was placed between a rotary injection valve and the analytical column. The rotary valve and four-way slider valve were controlled by control 5 and 6 on the AGP. The DQP was used to pump methanol–water (70:30) eluent to the rotary injection valve and pass through the IonPac AC10 concentrator located on the four-way slider valve. The NaOH eluent was delivered by the AGP to the concentrator column and to the analytical column. An anion micromembrane suppressor (AMMS, 2 mm format) was employed for eluent suppression. The sulfuric acid regenerant was delivered to the AMMS by a Dionex AutoRegen system.

### *System operation*

The chromatographic conditions are listed in Table I. At 0.0 min, valves 5 and 6 were OFF. The sample was loaded into the sample loop while the DQP was pumping methanol–water (70:30) to the

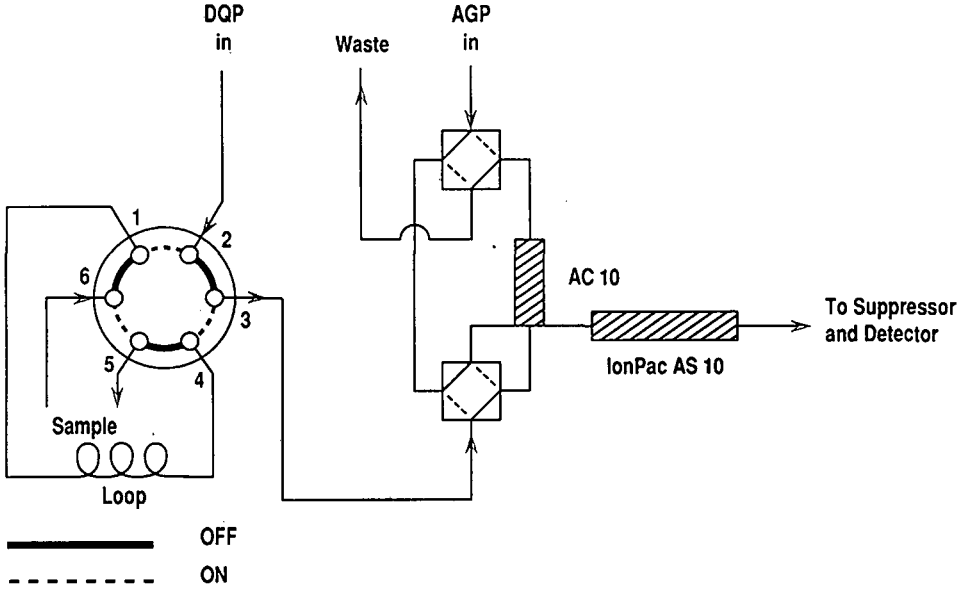


Fig. 1. Microbore suppressed IC system configuration.

TABLE I

CHROMATOGRAPHIC CONDITIONS

| | | | | |
|---|---|---|---|---|
| Columns | IonPac AS10 (2 mm)<br>IonPac AG10 (2 mm)<br>IonPac AC10 (2 mm) | | | |
| Trap Column | ATC-1 (2 required) | | | |
| Eluent | E1 deionized water<br>E2 400 m*M* NaOH | | | |
| *AGP Program* | | | | |
| Time (min) | E1 | E2 | 5INJ | 6AUX |
| 0.0 | 75 | 25 | 0 | 0 |
| 2.5 | 75 | 25 | 1 | 0 |
| 12.0 (Begin sampling) | 75 | 25 | 0 | 1 |
| Rinsing reagent | methanol–water (70:30) | | | |
| Flow-rate | 0.25 ml/min | | | |
| Injection volume | 10 $\mu$l | | | |
| Detection | Suppressed conductivity | | | |
| Regenerant | 25 m*M* sulfuric acid | | | |
| Regenerant flow-rate | 10 ml/min | | | |
| Suppressor | AMMS ( 2mm) | | | |

IonPac AC 10 column. During this time, the AGP was pumping 100 m*M* NaOH to the IonPac AS10 column. At 2.5 min, valve 5 was switched ON and valve 6 kept OFF. Concurrently, the methanol–water eluent delivered by the DQP flushed the sample loop and passed through the IonPAC AC10 column. At this point, most weak acids (*e.g.* acetic acid, hydrofluoric acid) would be eluted off the concentrator to waste while strongly retained species such as chloride, nitrate and sulfate would be concentrated by the concentrator. At 12.0 min, valve 5 was switched OFF and valve 6 switched ON. The IonPAC AC10 was switched in line with the IonPac AS10 at which point the retained anions were eluted to the analytical column. The analysis time was approximately 25 min.

*Reagents*

The high-purity sodium hydroxide, 30% Suprapur NaOH (VWR Scientific), was used for the entire study. First, 960 ml of 18-M$\Omega$ water was degassed in a clean 1-l eluent bottle by vacuum degassing while sonicating (15 min). Then, 53.4 g (40.2 ml) of 30% Suprapur NaOH was added to the solution and mixed well. HPLC-grade methanol (Baker) was used to prepare methanol–water (70:30) eluent.

## RESULTS AND DISCUSSION

A moderate-capacity anion exchanger, IonPac AS10, allows for the separation of trace anions in high-ionic-strength matrices. The IonPac AS10 is a macroporous pellicular resin which consists of a highly cross-linked inert solid core penetrated by channels through which solutions can flow. This core is coated with a thin layer of anion-exchange particles. The anion-exchange particles (65 nm diameter) are small enough to coat the inside of the macroporous resin (200 nm pore size). This results in good mass transfer, while providing higher capacity and higher efficiency of the AS10 resin compared to the conventional surface-aminated type macroporous anion exchanger [4].

Suppressed microbore IC allows greater suppression of high eluent concentration than the standard IC format due to the lower flow-rate of the system. In the standard 4 mm format, the suppressor can be used to suppress at least 100 m*M* NaOH eluent at a flow-rate of 1.0 ml/min and regenerant flow of 10 ml/min. While the microbore IC operates at 0.25 ml/min and the suppressor regenerant flow-rate remains unchanged, the resulting combination allows the use of strong eluent concentrations of at least 300 m*M* NaOH [3]. Improved background suppression allows higher capacity analytical columns to be used. Consequently, higher concentrations of acids (% levels) can be injected onto the column without overloading, thus improving trace anion detection limits.

*System blank caused by high-ionic-strength matrices*

The matrix effect remains a serious problem in the determination of trace anions in concentrated reagents. The term "matrix effect" is defined as the interference imposed by the matrix on the analytical separation and detection. In this case, the matrix disrupts the equilibrium concentrations of trace anion contaminants between the eluent and the ion-exchange column, causing the elution of those contaminants (system blank) along with the analyte anions. Consequently, the major contaminants commonly found in the eluent used are a limiting factor in analyzing trace anions.

The system blank was observed when samples of HF at various concentrations were analyzed by direct injection. The NaOH gradient used started at

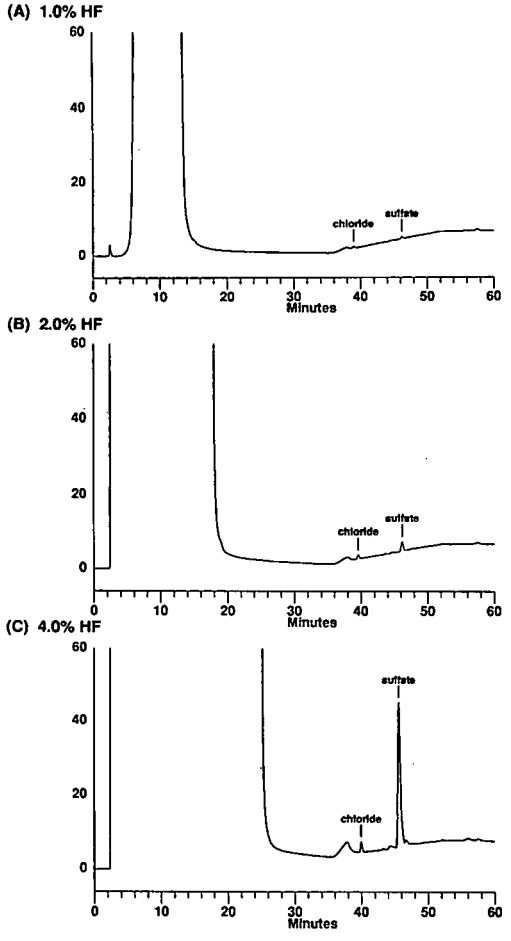


Fig. 2. Analysis of various HF concentrations. (A) 1% HF, (B) 2% HF, (C) 4% HF. Injection volume: 10 μl; column: IonPac AS10 (250 mm × 0.2 mm); suppressor: Microbore AMMS; eluent: time 0.0 —10 m*M* NaOH, time 30 min —10 m*M* NaOH, time 45 min —200 m*M* NaOH; eluent flow: 0.25 ml/min; regenerant: 25 m*M* sulfuric acid; regenerant flow: 10 ml/min; detector: conductivity (CDM-II).

10 m*M* for 30 min where the majority of fluoride was eluted off the column. Then, the analytes of interest were separated when the NaOH concentration increased to 200 m*M* in 15 min. The equilibration time used before the next analysis was 15 min, making the total analysis time of 75 min per sample. Fig. 2 shows the chromatograms of various HF concentrations using NaOH gradient. The results showed that increasing HF concentration from 0.125% to 0.25% HF (2×), chloride concentration increased from 0.11 mg/l to 0.21 mg/l (2×). However, increasing HF concentrations from 1% (0.58 *M*) to 2% (1.16 *M*) and to 4% (2.32 *M*), chloride concentrations found did not increase proportionally to HF concentrations. Similar behavior was found in the case of sulfate. Fig. 2 also shows that sulfate concentration increases more than 100 times, from 0.58 mg/l (A) to 61 mg/l, (C), when HF concentration increases from 1% to 4%. From this experiment, it was concluded that chloride and sulfate contaminants in NaOH contribute to the system blank when analyzing high ionic strength samples.

TABLE II

SPIKE/RECOVERY OF CHLORIDE IN HF USING DIRECT INJECTION METHOD

| HF (%) | $Cl^-$ in HF[a] (mg/l) | Spiked (mg/l) | Found (mg/l) | Corrected (mg/l) | Recovery (%) |
|---|---|---|---|---|---|
| 0.25 | 0.041[b] | 0.039 | 0.080 | 0.039 | 100 |
| 0.50 | 0.082[c] | 0.078 | 0.272 | 0.190 | 250 |
| 1.0 | 0.164[c] | 0.156 | 0.754 | 0.590 | 380 |
| 2.0 | 0.328[c] | 0.312 | 1.208 | 0.880 | 280 |
| 3.13 | 0.513[c] | 0.487 | 1.953 | 1.440 | 290 |
| 4.17 | 0.684[c] | 0.649 | 2.683 | 2.0 | 310 |

[a] Chloride in original sample.
[b] Determined experimentally.
[c] Estimated from concentration of chloride in 0.25% HF.

The "system blank" caused by high-ionic-strength sample can be explained as follows. At equilibrium where the analytical column is in the hydroxide form, the chloride present in he NaOH eluent is also equilibrated in the column. The concentration of chloride in the stationary phase is determined by the distribution coefficient of the anion exchanger for chloride relative to hydroxide and the level of chloride impurity in the eluent used. Upon direct injection of concentrated HF, the high concentration of $F^-$ behaves like an eluent, eluting "blank" chloride from the stationary phase along with the "analyte" chloride. Table II illustrates the determination of trace chloride in various HF concentrations. All analytical results presented in Table II are based upon aqueous calibration standards. The 0.25% HF sample was first analyzed and found to contain 0.041 mg/l chloride. Then, the sample was spiked with 0.039 mg/l chloride, the result

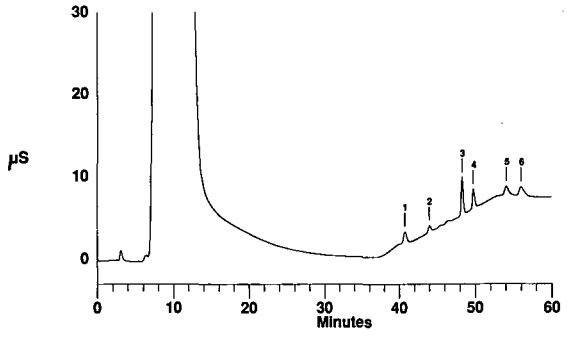


Fig. 3. Analysis of spiked acetic acid by direct injection. Sample concentration: 0.5 *M* acetic acid; 1 = chloride (2 mg/l); 2 = nitrite (2 mg/l); 3 = sulfate (4 mg/l); 4 = phosphate (10 mg/l); 5 = bromide (2 mg/l); 6 = nitrate (4 mg/l). Chromatographic conditions as for Fig. 2.

showed a good recovery reflecting the fact that the 0.25% HF did not cause the system blank and the concentration of chloride in 0.25% is accurate. At more than 0.5% HF, the recoveries of chloride were more than 250%. All the experimental values were corrected in proportion with the chloride concentration found in 0.25% HF. For example, 0.082 mg/l chloride was used to subtract the experimental value of 0.272 mg/l giving 0.19 mg/l recovery in 0.5% HF. Therefore, the direct injection method using the analytical-grade NaOH (as specified in experimental section) is only quantitative for HF concentrations less than or equal to 0.25% since the system blank induced by the HF at various concentrations is not consistent and is sometimes more than twice the analyte concentration and the system blank increases when sample concentration is increased.

Figs. 3 and 4 show the direct injection of 0.5 *M* acetic acid and hydrochloric acid. By using a gradient separation on the IonPac AS10 column, the analytes were cleanly separated from the anion matrix. However, the results of the analysis were not quantitative due to the system blank caused by the acid matrices.

The detection limits by direct injection method are between 0.04 to 0.08 mg/l for chloride, sulfate, phosphate, bromide and nitrate. Since the analyte loading is limited by the system blank, several-fold

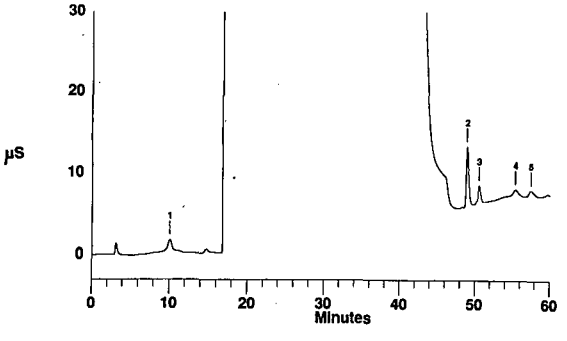


Fig. 4. Analysis of spiked hydrochloric acid by direct injection. Sample concentration: 0.5 *M* hydrochloric acid; 1 = fluoride (1.5 mg/l); 2 = sulfate (5 mg/l); 3 = phosphate (5 mg/l); 4 = bromide (1.5 mg/l); 5 = nitrate (3.0 mg/l). Chromatographic conditions as for Fig. 2.

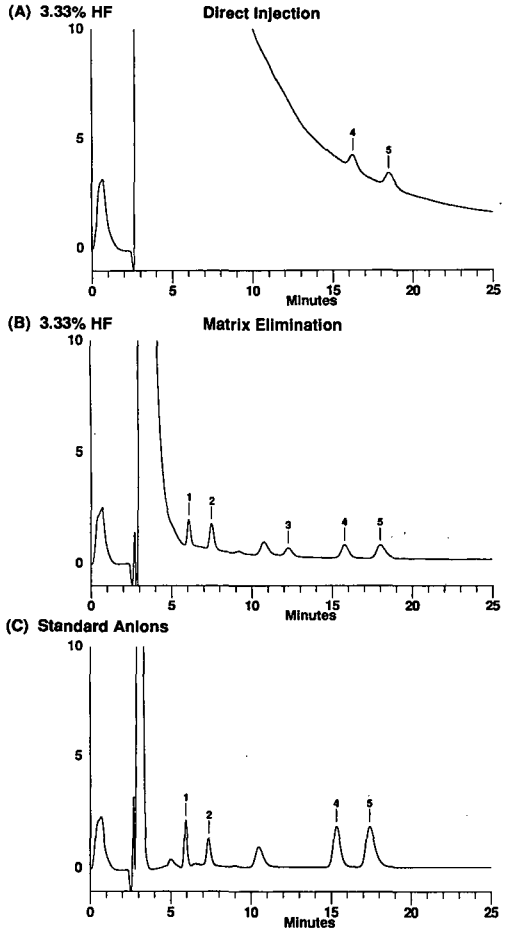


Fig. 5. Comparison of direct injection and matrix elimination methods of 3.33% HF. (A) Direct injection of 3.33% HF, (B) matrix elimination of 3.33% HF and (C) standard anions: 1 = chloride; 2 = sulfate; 3 = phosphate; 4 = bromide, 5 = nitrate.

dilution of concentrated acid is required and it compromises the detection limits of this method. For instance, the concentrated HF (50% or 28.9 *M*) Must contain at least 10 mg/l of anions of interest in order to be analyzed by direct injection method. Therefore, other means of sample preparation techniques were explored to eliminate the sample matrix and to increase analyte loading thus improving the detection limits.

*Matrix elimination prior to IC analysis*

Sample pretreatment techniques prior to analytical measurement have long been used to eliminate the matrix effect. Unfortunately, most of the anion-exchange resins are not selective for anions of the same charge. The matrix elimination method using anion-exchange resin prior to analytical technique was reported [6]. However, this method is limited to low matrix concentration due to the low capacity of the anion exchange pretreatment column. Another approach is the separation of matrix components from analytes based upon hydrophobicity differences. Weak acids such as hydrofluoric acid, formic acid, propionic acid and acetic acid have p$K_a$ values between 3.0 and 4.8. At low pH, they remain protonated and uncharged. In solvents these acids are even weaker. Using an organic solvent-containing solution such as methanol–water as a wash, the matrix component at low pH can be removed prior to analytical measurement.

This matrix reduction of weakly retained anions was performed by the IonPac Anion Concentrator 10 (AC10). The IonPac AC10 is a microporous styrene–divinylbenzene copolymer resin that is agglomerated with a quaternary amine-functionalized latex and has the same selectivity as the IonPac AS10. The acid sample (10 $\mu$l) was injected onto the AC 10 which had previously been equilibrated with methanol–water eluent. The weak acid matrix component was eluted with methanol while chloride, sulfate, bromide and nitrate were retained in the column (phosphate is not quantitatively retained under these conditions). As long as the acid matrix remains protonated, it does not cause the system blank in the concentrator column. The retained anions were then eluted from the AC10 to the IonPac AS10 where they were separated. Although the matrix component may not have been completely eliminated using the AC10, it was reduced to the point that it did not cause the matrix effect on the analytical column. Since the matrix concentration was greatly reduced, an isocratic separation could be applied. Using 100 m*M* NaOH eluent, the analysis time was reduced from 75 to 30 min. Fig. 5 shows the comparison of the direct injection and the matrix elimination method for a 3.33% HF matrix. The HF samples of up to 4.9% were successfully analyzed by this technique (see Fig. 6).

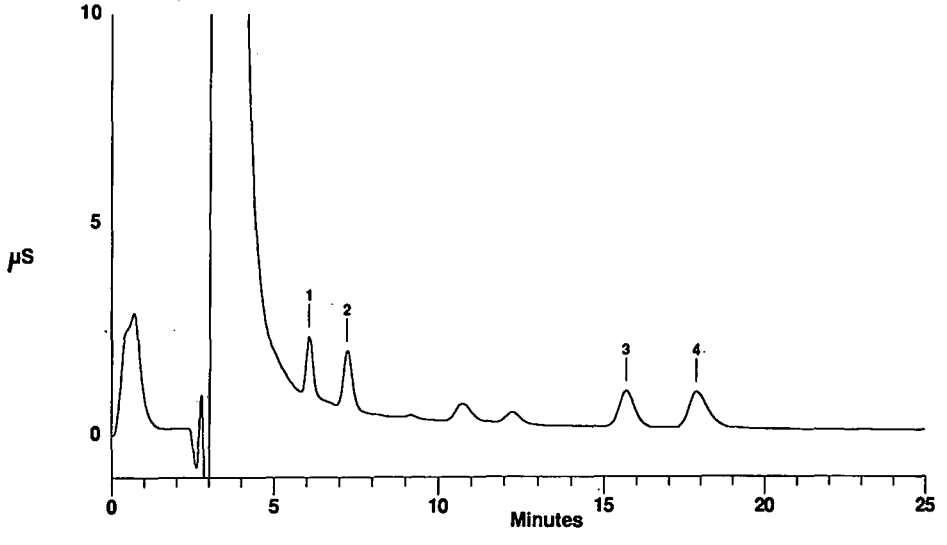


Fig. 6. Anion analysis in 4.9% HF by matrix elimination. 1 = Chloride (0.25 mg/l); 2 = sulfate (1.0 mg/l); 3 = bromide (0.25 mg/l); 4 = nitrate (2.5 mg/l).

TABLE III

SPIKE/RECOVERY OF ANIONS IN 3–5% HF USING MATRIX ELIMINATION METHOD

All values are average of 4 replicates.

| Anion | HF (%) | Spike (mg/l) | Found (mg/l) |
|---|---|---|---|
| Chloride | 3.33 | 0.46 | 0.53 ± 0.01 |
| | 4.17 | 0.57 | 0.57 ± 0.03 |
| | 5.00 | 0.69 | 0.67 ± 0.02 |
| Sulfate | 3.33 | 0.86 | 0.98 ± 0.03 |
| | 4.17 | 1.07 | 1.01 ± 0.02 |
| | 5.00 | 1.29 | 1.17 ± 0.07 |
| Nitrate | 3.33 | 1.66 | 1.79 ± 0.02 |
| | 4.17 | 2.08 | 2.10 ± 0.07 |
| | 5.00 | 2.50 | 2.50 ± 0.04 |
| Bromide | 3.33 | 1.66 | 1.75 ± 0.03 |
| | 4.17 | 2.08 | 1.89 ± 0.04 |
| | 5.00 | 2.50 | 2.42 ± 0.06 |

In order to evaluate the matrix effect on the concentrator and the analytical column using matrix elimination method, an experiment similar to that used to generate the data in Table II was performed. Knowing that the IonPac AC10 column capacity is estimated at 1.0 μequiv. per column, a breakthrough study of chloride in varying HF concentrations was also studied. The HF concentrations ranging from 0.25% to 16.67% were used to determine the dynamic range of this technique. Table III summarizes the spike/recovery of anions in various HF concentrations reflecting excellent recoveries for all anions studied. Table IV shows the chloride breakthrough study revealing the dynamic range of the matrix elimination method. HF concentrations greater than 6.0% (10-μl loop) begin to elute chloride off the IonPac AC10 concentrator. All analytical results presented in Tables III and IV are based upon aqueous calibration standards. The aqueous standards were treated the same as the acid samples. The chromatographic conditions used are listed in Table I. The detection limits based upon a signal-to-noise ratio of 3 are 25 to 50 μg/l for most anions.

TABLE IV

SPIKE/RECOVERY OF CHLORIDE IN 0.25% TO 16.67% HF USING MATRIX ELIMINATION METHOD

All values are blank corrected.

| HF (%) | Spike (mg/l) | Found (mg/l) | Recovery (%) |
|---|---|---|---|
| 0.25[a] | 0.028 | 0.028 | 100 |
| 0.50 | 0.056 | 0.060 | 107 |
| 1.00 | 0.112 | 0.130 | 116 |
| 2.00 | 0.224 | 0.270 | 120 |
| 3.13 | 0.350 | 0.370 | 105 |
| 4.17 | 0.466 | 0.510 | 115 |
| 6.25 | 0.700 | 0.670 | 95 |
| 8.33 | 0.933 | 0.750 | 80 |
| 10.42 | 1.166 | 0.780 | 66 |
| 12.50 | 1.400 | 0.820 | 59 |
| 16.67 | 1.865 | 0.840 | 26 |

[a] 0.25% HF contains 0.041 ± 0.007 mg/l chloride.

## CONCLUSIONS

An improved method for the determination of trace anions in concentrated weak acids has been developed. Using microbore IC with chemical eluent suppression, trace anions can be determined in high-concentration weak acids such as acetic acid and hydrofluoric acid using a moderate-capacity anion-exchange column. Due to the system blank caused by the high ionic strength of the sample, the eluent impurity was found to be a limiting factor when determining trace anions in concentrated acid. An alternative method, a matrix elimination method, has also been developed. This method involves eliminating most of the weak acid using methanol–water eluent on an IonPac AC-10 concentrator column prior to analytical separation. The retained anions of interest are eluted from the AC10 concentrator and separated on the moderate capacity IonPac AS10 column. The detection limits for most anions are 25 to 50 μg/l.

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# Separation and indirect visible detection of inorganic and organic analyte cations on dye-coated stationary phases

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ABSTRACT

The separation and indirect visible detection of inorganic and organic analyte cations on dye-coated stationary phases was studied. The dye employed in this study, Thymol Blue, is typically used for pH titrations and is composed of hydrophobic groups and a fixed charge site. The mobile phase variables that were found to affect analyte cation retention, resolution and selectivities are: concentration of dye, concentration of organic modifier, mobile phase pH, type and concentration of countercation and/or ligand, and ionic strength. Two different types of reversed-phase packings were used in this study: polymer-based stationary phases and silica-based ODS stationary phases. Detection of the analyte cations was accomplished by indirect visible detection at 428 nm.

## INTRODUCTION

The addition of a hydrophobic counterion to the mobile phase for the separation of inorganic and organic analyte ions has been studied over the past several years, and models have been developed to explain the interactions that take place between an analyte ion, the counterion, and the stationary phase [1–4].

One model that has been successfully used to describe the interactions between an analyte ion and the mobile phase counterion is ion-interaction chromatography [4–15]. A hydrophobic ion (ion-interaction reagent) that contains a fixed charge site is added to the mobile phase. The ion-interaction reagent (IIR) is sorbed on the stationary and forms a charged double layer. The primary layer is composed of the sorbed hydrophobic counterion while the co-ion occupies the diffuse secondary layer. The analyte ions of interest are then separated in the diffuse secondary layer, based on selectivity differences between ions.

Several recent reports deal with the separation of inorganic and organic ions and mobile phases that contain a dye. The dyes that were studied are commonly used for pH titrations. A Brilliant Green-coated stationary phase was used for separating aliphatic acids [16], while another study dealt with the separation of organic and inorganic anions, where Methylene Blue was added to the mobile phase [17]. Inorganic anions were separated on a Methyl Green-coated column [18], while the separation of metal ions was studied with several different dyes [19]. One paper described the separation and indirect invisible detection of inorganic and organic anions with a mobile phase that contains Ethyl Violet [20]. Both polymer- and silica-based stationary phases were studied, using Ethyl Violet mobile phases.

In the present study, the hydrophobic dye is used as both the IIR for separating the inorganic and organic analyte cations and for their indirect visible detection. This paper describes the mobile phase variables that affect the separation of inorganic and organic analyte cations on the dye-coated stationary phases. The results obtained are discussed.

## EXPERIMENTAL

### *Chemicals*

HPLC-grade acetonitrile was obtained from Baxter Scientific Products (McGraw Park, IL, USA).

HPLC-grade water was obtained by passing de-ionized water through a Nanpure water purification unit. Thymol Blue, citric acid, tartaric acid, inorganic salts, guanidines, and metal salts were obtained from Aldrich (Milwaukee, WI, USA). All chemicals were of reagent grade.

*Apparatus*

The instrumentation used in this study consisted of a Hewlett-Packard liquid chromatography system, Model 1090. The columns used were: a 150 × 4.1 mm I.D. Hamilton (Reno, NV, USA) PRP-1 column, a 150 × 4.6 mm I.D. PLRP-S column from Polymer Labs. (Amherst, MA, USA), and a 5 μm, 150 × 4.6 mm I.D. B&J OD5 column from Baxter Healthcare Corp. (McGraw Park, IL, USA). The PRP-1 column contains a spherical, 10-μm poly(styrene–divinylbenzene) packing. The PLRP-S column is composed of a spherical, 5-μm poly(styrene–divinylbenzene) packing. Flow-rates of 1.0 ml/min and aqueous analyte samples of *ca.* 500 μg/ml and sample aliquots of 50 μl were used. Inlet pressures of 500–1500 p.s.i. were observed. A wavelength of 428 nm was used for the indirect visible detection.

*Mobile phase preparation*

The Thymol Blue dye was quantitatively transferred (appropriate volume of a 0.01 *M* Thymol Blue solution) to a beaker that contained the aqueous buffer solution. The desired pH was achieved by adding acid or base. The aqueous solution was diluted to the appropriate volume and the organic modifier was then added. The solution was mixed and then filtered through a 0.45-μm PTFE membrane.

*Column loading*

Column loading was determined by running the mobile phase through the column and UV–visible detector until the breakthrough occurred. The number of μmoles of dye adsorbed on the stationary phase was calculated from the breakthrough volume [7]. The column was then allowed to equilibrate for an additional 30–60 min.

## RESULTS AND DISCUSSION

Two major equilibria can be used to describe the retention of inorganic and organic analyte ions on reversed stationary phases with mobile phases that contain a hydrophobic ion of opposite charge [7,8,12–15]. Eqn. 1 describes the first equilibrium that takes place, where the hydrophobic counterions sorbs on the stationary phase. The second equilibrium (eqn. 2) describes the interaction that takes place in the diffuse secondary layer between the analyte ion and the co-ion that is associated with the retained hydrophobic ion.

$$A + TB^- + C^- + M^+ \rightleftharpoons A \cdots TB^- {}^+M + C^- \quad (1)$$

$$A \cdots TB^- {}^+M + X^+ + C^- \rightleftharpoons A \cdots TB^- {}^+X + C^- + M^+ \quad (2)$$

A represents the stationary phase, $TB^-$ represents an ion-interction reagent (UV-active counteranion) in the mobile phase, $M^+$ is the counteraction associated with the IIR, the buffer and/or added inert electrolyte, $C^-$ is an anion associated with the countercation and/or the analyte cation, and $X^+$ is the analyte cation. The variables that have been found to affect the separation of ions are: the reversed stationary phase, the type and concentration of the IIR, the concentration of organic modifier, the type and concentration of countercation and/or buffer in the mobile phase, and the mobile phase pH. The inorganic analyte cations that were studied in this paper exhibited little or no retention on the stationary phases in the absence of the IIR, whereas the organic analyte cations show some retention, depending on the hydrophobicity of the organic analyte cation.

An advantage of the IIR used in this study is that it contains a chromophoric group which allows the indirect visible detection of the analyte cations. Care must be taken to keep the absorbance in the UV–visible detector below 0.8 A.U.F.S. when using indirect visible detection. If the detector absorbance exceeds 0.8 AUFS, the detector is outside of its linear working range. How indirect visible detection works can be explained by differences in the relative concentrations of the IIR in the effluent. As an analyte cation travels down the column (eqns. 1 and 2), the concentration of the UV-absorbing IIR band changes relative to the background absorbance. The concentration of the IIR in the band either increases, due to its removal from the column and provides a positive chromatographic peak, or it de-

creases, due to its uptake on the column, in which case a negative chromatographic peak is produced. The IIR in the mobile phase is responsible for both the retention of the analyte cations and in the indirect visible detection of the analyte cations.

*Effect of Thymol Blue concentration*

The first mobile phase parameter studied was the concentration of Thymol Blue and its effect on analyte cation retention. The amount of Thymol Blue adsorbed on the stationary phase was found to increase as its concentration in the mobile phase increased. The amount of Thymol Blue adsorbed on the stationary phase was found to be similar to that of low-capacity cation exchangers [12–14,21] and to mobile phases that contain ion-interaction reagents, such as alkylsulfonate salts [12–14].

As the concentration of Thymol Blue in the mobile phase increased, a corresponding increase in the amount of Thymol Blue adsorbed on the stationary phase was observed. This, in turn, leads to a higher number of cation exchange sites available on the stationary phase and should lead to higher analyte cation retention. A comparison of two different mobile phase concentrations of Thymol Blue is shown in Fig. 1. Chromatogram I shows the separation when 0.1 m*M* Thymol Blue was used, while chromatogram II shows the separation when the concentration of Thymol Blue was increased to 0.2 m*M*. Although the higher concentration of Thymol Blue provided longer retention times, the lower concentration of Thymol Blue provided better separation and better sensitivity. Therefore, lower concentrations of Thymol Blue were used.

*Mobile phase variables: effect on Thymol Blue adsorption*

The concentration of organic modifier was found to affect the amount of Thymol Blue adsorbed on the stationary phase. The amount of Thymol Blue adsorbed on the stationary phase decreased as the mobile phase concentration of organic modifier was increased. This, in turn, leads to a decrease in the number of cation-exchange sites present on the stationary phase and a corresponding decrease in analyte cation retention.

Ionic strength will also affect the amount of Thymol Blue adsorbed on the stationary phase [3,7,12, 13,15]. As the mobile phase ionic strength was increased, a corresponding increase in the amount of Thymol Blue adsorbed on the stationary phase was observed. The increase in the amount of Thymol Blue adsorbed showed an increase in the apparent number of cation-exchange sites present. The amount of Thymol Blue adsorbed on the stationary phase increased until an ionic strength of about 0.120 (0.075 m*M* NaCl) was reached, when the number of cation-exchange sites leveled off. Even though more cation-exchange sites are present at higher mobile phase ionic strengths, analyte cation retention decreased due to increased competition for the cation-exchange sites from the higher concentration of countercations (see eqn. 2).

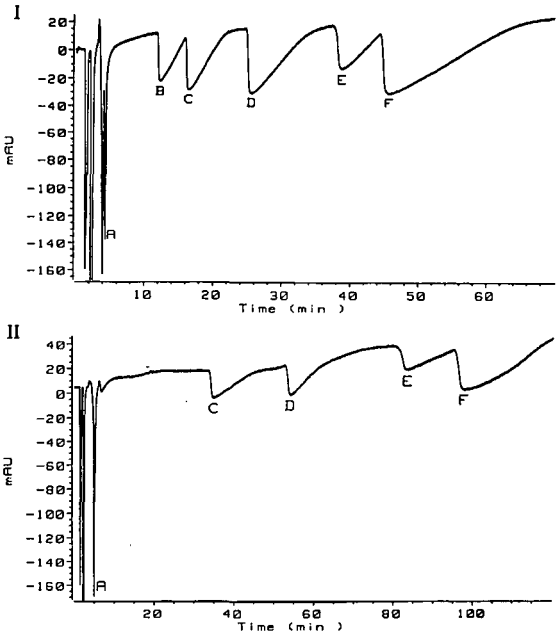


Fig. 1. Separation of several metal cations on a PLRP-S stationary phase at different mobile phase concentrations of Thymol Blue. Mobile phase: (I) 0.1 m*M* Thymol Blue, 15.0 m*M* tartrate (pH 3.7), acetonitrile–water (10:90); (II) as I except 0.2 m*M* Thymol Blue. A = $Cu^{2+}$; B = $Zn^{2+}$; C = $Ni^{2+}$; D = $Co^{2+}$; E = $Fe^{2+}$; F = $Mn^{2+}$.

*Effect of organic modifier on cation retention*

Fig. 2 illustrates how analyte cation retention was affected by the concentration of acetonitrile that covered a range of 7.5% to 20%. As the mobile phase concentration of acetonitrile was increased, the amount of Thymol Blue adsorbed on the stationary phase decreased. This, in turn, leads to a

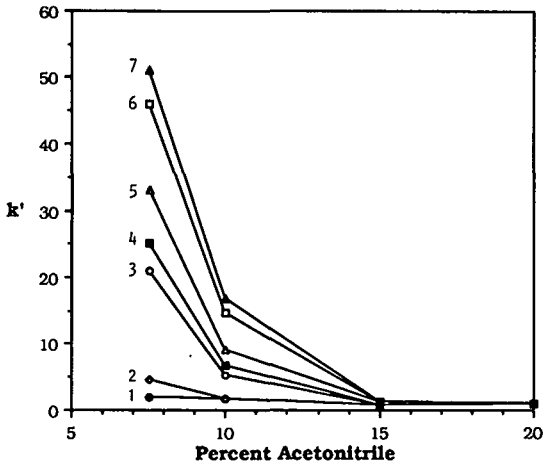


Fig. 2. Effect of acetonitrile concentration on analyte cation retention ($k'$ = capacity factor). Mobile phase conditions: 0.1 m$M$ Thymol Blue, 10.0 m$M$ tartrate (pH 3.7) in acetonitrile–water. Curves: 1 = $Fe^{3+}$; 2 = $Cu^{2+}$; 3 = $Zn^{2+}$; 4 = $Ni^{2+}$; 5 = $Co^{2+}$; 6 = $Fe^{2+}$; 7 = $Mn^{2+}$.

lower number of apparent cation-exchange sites present and lower analyte cation retention. Resolution of the analyte cations was better at lower concentrations of acetonitrile due to the higher number of apparent cation-exchange sites present on the stationary phase. At lower concentrations of acetonitrile, breakthrough volumes for Thymol Blue were found to be extremely high. A mobile phase that contained 5% acetonitrile required over 2 l of eluent to be passed through the column before the breakthrough occurred.

*Effect of pH*

Mobile phase pH plays a very important role in the retention and separation of metal cations. Complexation takes place between the ligand in the mobile phase and the metal cations. The metal–ligand complexation was found to be affected by the mobile phase pH. Fig. 3 shows the effect of pH on transition-metal retention. Retention times changed dramatically over the pH range of 3.0 to 5.0 (ionic strength held constant). In this study, citric acid was the ligand used. Similar results were also obtained when tartrate was used. As the pH of the mobile phase was increased, metal retention decreased. This is attributed to complexation taking place between the ligand and the metal cation. Optimal conditions for metal cation retention and resolution were found between pH 3.5 and 4.0.

A similar study was also made for the alkaline-earth metals and for several simple guanidines. Retention of the alkaline-earth metals was found to be affected in the same way that the transition metals were: increasing the mobile phase pH led to lower retention times. As the pH of the mobile phase was increased from 3.5 to 7.0, retention of the alkaline-

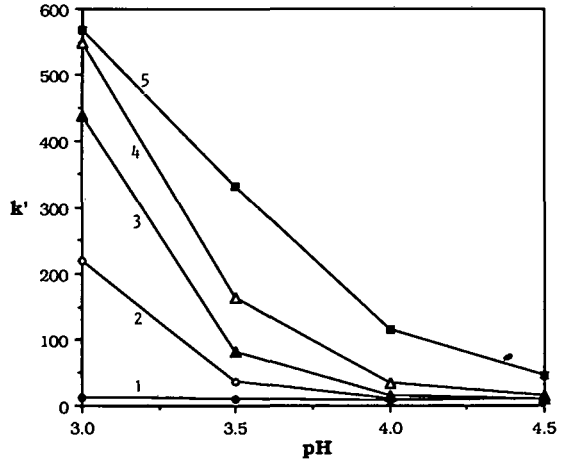


Fig. 3. Effect of mobile phase pH on analyte cation retention. Mobile phase conditions: 0.1 m$M$ Thymol Blue, 3.0 m$M$ citrate, acetonitrile–water (10:90). Curves: 1 = $Fe^{3+}$; 2 = $Cu^{2+}$; 3 = $Ni^{2+}$; 4 = $Zn^{2+}$; 5 = $Fe^{2+}$.

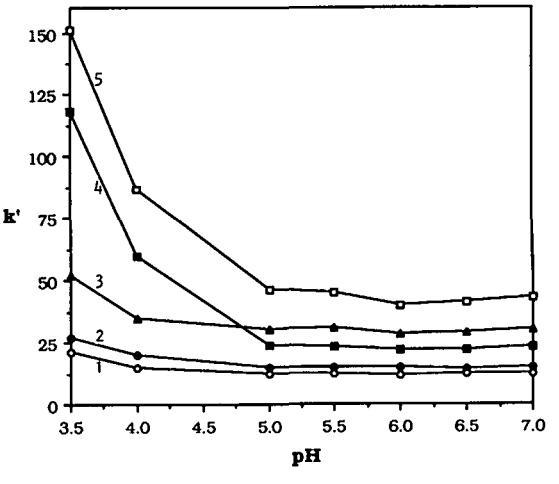


Fig. 4. Effect of mobile phase pH on analyte cation retention. Mobile phase conditions: 0.1 m$M$ Thymol Blue, 15.0 m$M$ tartrate, acetonitrile–water (10:90). Curves: 1 = guanidine; 2 = 1-methylguanidine; 3 = 1-ethylguanidine; 4 = $Ca^{2+}$; 5 = $Mg^{2+}$.

earth metals decreased; however, selectivities did not change. The simple guanidines initially decreased in retention with increasing pH and then leveled off at pH 5.0. The effect of mobile phase pH on the retention of the alkaline-earth metals and simple guanidines, is shown in Fig. 4.

*Effect of ligand concentration*

The concentration of ligand in the mobile phase plays a key role in the separation of the metal cations. The complexation that takes place between the metal cation, the adsorbed IIR, and the mobile phase ligand control retention and resolution. If a ligand were not added to the mobile phase, the metals would have very high retention due to the strong complexation with the sorbed IIR. If IIR were absent but the mobile phase ligand was present, the metal cations would show little or no retention.

The results observed for the Thymol Blue–citrate or tartrate mobile phases and the metal cations indicated that the ligand concentration had a major affect on metal retention. At low concentrations of ligand, retention of the metals was very high. As the concentration of ligand was increased, metal retention decreased. Elution orders were found to remain the same over the ligand concentration range. Elution orders for the transition metals with either a citrate or a tartrate mobile phase were: $Fe^{3+} < Cu^{2+} < Zn^{2+} < Ni^{2+} < Co^{2+} < Fe^{2+} < Mn^{2+}$. Elution orders for the alkaline-earth metals were different, depending on the ligand used. For a citrate mobile phase the elution order was $Mg^{2+} < Ca^{2+} < Sr^{2+} < Ba^{2+}$, whereas when tartrate was added to the mobile phase the elution order was $Ca^{2+} < Sr^{2+} < Ba^{2+} < Mg^{2+}$. This difference in elution order is apparently due to differences in the complexation between Thymol Blue, alkaline-earth metals, citrate, and tartrate. The complexation between magnesium and citrate is substantially stronger than that of magnesium and tartrate. Fig. 5 shows the separation of the alkaline-earth metals with a mobile phase containing tartrate.

*Effect of ionic strength*

Sodium chloride was added to the mobile phase in order to determine the effect of ionic strength on cation retention. Retention of the cations decreased as the concentration of sodium chloride was increased. This is attributed to increased competition

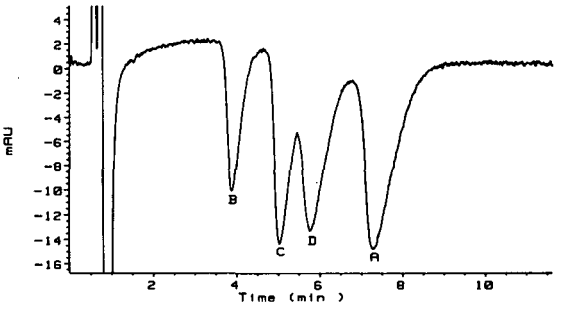


Fig. 5. Separation of alkaline-earth metals on a Hamilton PRP-1 column. Mobile phase conditions: 0.1 m*M* Thymol Blue, 25.0 m*M* tartrate (pH 4.5), acetonitrile–water (10:90). A = $Mg^{2+}$; B = $Ca^{2+}$; C = $Sr^{2+}$; D = $Ba^{2+}$.

for the cation-exchange sites (eqn. 2). As previously stated, the amount of Thymol Blue adsorbed on the stationary phase increases with increasing ionic strength, and this leads to an increase in the apparent number of cation-exchanges sites. However, this did not lead to an increase in cation retention, since competition for the cation-exchange sites was increased due to the higher concentration of sodium ions present.

*Separation on an ODS column*

A silica-based ODS column was coated with Thymol Blue and used for the separation of different cations. The separations on the ODS column were better than those on the polymer-based columns. One problem with the silica-based columns, however, was the lack of ruggedness. The columns did not last as long as the polymer-based columns. Fig. 6 shows the separation of several transition metals

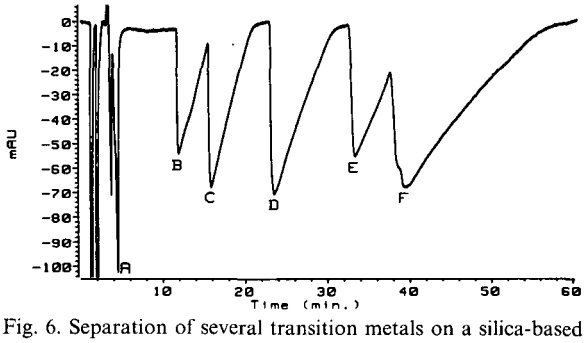


Fig. 6. Separation of several transition metals on a silica-based ODS column (B&J OD5). Mobile-phase conditions: 0.1 m*M* Thymol Blue, 15.0 m*M* tartrate (pH 3.7), acetonitrile–water (12.5:87.5). A = $Cu^{2+}$; B = $Zn^{2+}$; C = $Ni^{2+}$; D = $Co^{2+}$; E = $Fe^{2+}$; F = $Mn^{2+}$.

on a silica-based column. When this is compared with the metal separation on the polymer-based column (Fig. 1), the more efficient silica-based column provided better peak shape and a better separation. The results for the different mobile phase variables on the silica-based column were similar to those observed for the polymer-based columns.

## CONCLUSIONS

A Thymol Blue-coated stationary phase provided acceptable separations of the inorganic and organic cations studied. The mobile phase variables affecting cation retention were identified and studied. Good separations of all of the different cations studied were obtained. The elution order of $Mg^{2+}$, $Ca^{2+}$, $Sr^{2+}$ and $Ba^{2+}$ depend on the ligand used. When a tartrate mobile phase was used, $Mg^{2+}$ was eluted after $Ba^{2+}$. Elution orders for the transition metals did not change when different ligands were used.

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CHROMSYMP. 2558

# New thiohydrazones for complexation and chromatographic determination of metal ions

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ABSTRACT

A general scheme is outlined for rapid determination of metal cations by complexation and subsequent high-performance liquid chromatographic separation. The synthesis and general properties are described for several new thiohydrazone complexing reagents. Solubility considerations suggest that the metal complexes have a positive charge. Excellent chromatographic separations are obtained for mixtures of up to seven metal ion complexes. Addition of a positively charged additive to the eluent is shown to have a significant effect on both the retention times and sharpness of the chromatographic peaks. Separation of the metal complexes on resins with a permanent charge is also shown to be feasible.

## INTRODUCTION

Reaction of a thiosemicarbazide or a substituted thiosemicarbazide with a carbonyl compound results in a product with strong chelating properties for metal ions that have an affinity for sulfur. Especially strong chelating reagents are formed by reaction of two molecules of the semicarbazide with a 1,2-dicarbonyl compound. Thiosemicarbazones have been used as chemical spray reagents for metal ions separated by thin-layer chromatography [1] and as color-forming reagents for spectrophotometric determination of $Co^{2+}$ [2], iron [3], $Cu^{2+}$ [4], uranium [5] and other metal ions [6]. Main and Fritz [7] employed a reagent made by reacting a thiosemicarbazide with 2-acetylpyridine for complexation and chromatographic separation of metal ions.

An attractive and rapid way to determine metal ions is to add an appropriate complexing agent to the sample and separate the metal complexes by high-performance liquid chromatography (HPLC). Several review articles have been published on this technique [8–13]. Many of the published methods use reagents that form water-insoluble complexes and thus require a preliminary solvent extraction step. It is better to employ a reagent that forms complexes with a reasonable solubility in water. It is also necessary to use a reagent that forms complexes with sufficient stability to avoid decomposition during the chromatographic separation. One of the new reagents described in this paper has the necessary properties and has been used for the chromatographic separation of several metal ions.

We first evaluated a reagent made by reaction of semicarbazide with glyoxal that was reported to form highly colored complexes with silver(I) and mercury(II) even in highly acidic solutions [14]. We confirmed this behavior but found both the reagent and its metal complexes to be sparingly soluble in water and water-organic solvent mixtures that are usually employed as eluents in HPLC separation. However, more water-soluble reagents with good chelating and color-forming properties can be prepared by a similar reaction by varying the chemical structures of both reactants. In this paper, the preparation and properties of several new reagents are described. One of the new reagents is chosen for complexation and chromatographic separation of several metal ions.

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## EXPERIMENTAL

*Synthesis and characterization of thiohydrazones*

To synthesize reagent IV, 1.55 g of 4-(4-dimethylaminophenyl)-3-thiosemicarbazide (Fairfield) and approximately 250 ml of absolute ethanol were added to a 500-ml round-bottom flask. The solution was heated to 80°C while refluxing in an oil bath to help dissolve the thiosemicarbazide. Then 0.33 ml of diacetyl dissolved in 10 ml hot ethanol was added to the flask dropwise. A few drops of acetic acid were also added to the flask as the catalyst. The whole mixture was refluxed at 70°C for 5 h and the resulting yellow precipitate was washed by hot ethanol and collected.

Complexing reagents I and II were synthesized from their different starting materials in a similar manner. To synthesize reagent III, absolute ethanol was replaced by dimethylformamide as solvent and reaction temperature was further raised to above 100°C owing to the low reactivity of the conjugated 1,2-dicarbonyl compound.

Since reagent IV turned out to be the best overall (see Results and Discussion section), later experiments were all based on reagent IV.

The following procedures were used ot characterize reagent IV. The melting point was measured by a Thomas "Uni-Melt" melting point unit with thermometer reader and illuminator. The infrared spectrum was taken on an IBM IR/98 (film). The NMR spectrum was obtained on a Nicholet NT-300 instrument using dimethyl sulfoxide as the solvent and a chemical shift standard. The mass spectrum was taken on a Kratos MS-50 employing a high-resolution mass spectrometer. Results for characterization of this reagent are summarized in Table I.

TABLE I

CHARACTERIZATION OF DIACETYL DI-(4-DIMETHYLAMINOPHENOL)-3-THIOSEMICARBAZONE

| Melting range (°C) | IR maxima ($cm^{-1}$) | Major mass spectral lines ($m/z$) | Proton shift of NMR (ppm) |
|---|---|---|---|
| 232–234 | 3287 | 471.2 | 9.86 |
| | 3194 | 261.1 | 7.34–7.38 |
| | 1653 | 235.1 | 6.78–6.81 |
| | 1605 | 196.1 | 3.43 |
| | 1578 | 179.1 | 2.98 |
| | 1526 | 137.1 | 2.58 |
| | 1475 | 121.1 | 2.38 |
| | 1337 | | |
| | 1256 | | |
| | 1178 | | |
| | 1132 | | |
| | 810 | | |

*Preparation of solutions*

Metal ion stock solutions were prepared from their chloride or nitrate metal salts and kept in HCl or $H_2SO_4$ media. Chromatographic samples were prepared by taking an aliquot of stock metal solution and adding enough reagent. The pH value of the solution is controlled either at 2.0 (0.01 $M$ $HClO_4$) or 4.6 (160 m$M$ acetate). Finally, the solution was diluted by deionized water and HPLC-grade acetonitrile to make the metal complex concentration $10^{-5} - 10^{-4}$ $M$. Whenever possible, the volume percentage of acetonitrile in sample solution was made the same as the percentage in the eluent.

The UV–VIS spectra of product VI and its three metal complexes were taken at a pH 5.0 in a 50 m$M$ acetate buffer. Both spectra were blanked with same buffer. The concentration of metals ranged from 1.0 $10^{-6}$ $M$ to $1.0 \cdot 10^{-5}$ $M$.

*HPLC studies*

The chromatographic system consisted of a LKB 2150 HPLC pump, LKB 2156 HPLC controller, Rheodyne 7125 injector (20-$\mu$l injection loop), and a Kratos Spectroflow 783 UV–VIS detector. Most of the separations were performed on columns from Sarasep of different dimensions, packed with 10-$\mu$m polystyrene–divinylbenzene resins (RP-80). An anion-exchange column (Sarasep AN-1, 50 × 4.6 mm) was used for one group of separations. Other columns used were a PLRP-S 100A 5-$\mu$m polystyrene–divinylbenzene (150 × 4.6 mm) column from Polymer Labs. and a $C_{18}$ silica (250 × 4 mm) column from E. Merck.

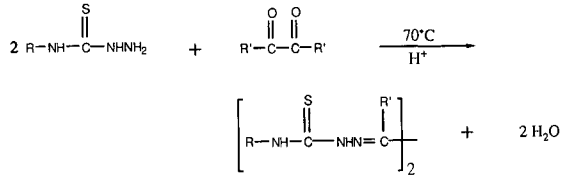
Eluents were prepared from Fisher HPLC-grade acetonitrile and water purified with a Barnstead Nanopure II system on a percent volume basis. Acids and organic amine salts were all reagent grade or bettere. The pH values of eluents were controlled by 0.01 $M$ $HClO_4$ or acetate buffer. The eluent components were mixed, filtered with 0.2-$\mu$m nylon 66 filters (Rainin) and degassed before use in

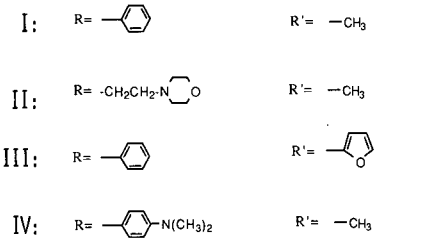
the chromatographic system. The flow-rate was 1 ml/min. The detection wavelength was 474 nm.

## RESULTS AND DISCUSSION

### *Preparation and properties of chelating reagents*

The synthetic method used is given by the following equation:



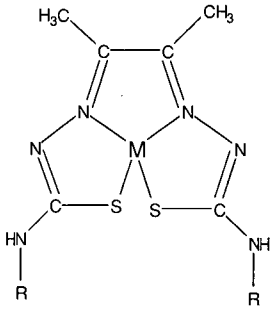


Four different reagents of this type were prepared and tested. Reagent I gave strongly colored complexes with several metal ions. Its solubility in water–organic solvent mixtures was much better than the reagent where both R and R′ = H but a rather high percentage of organic solvent was still needed to form soluble metal complexes.

Reagent II had very good solubility but most of the metal complexes were colorless which could be caused by the loss of conjugation from benzene ring. Preparation of a reagent III was attempted but apparently failed because of the low reactivity of the conjugated 1,2-dicarbonyl compound.

Reagent IV was the best of the reagents. It formed stable, colored complexes with several metal ions. Both the reagent and the metal complexes were very soluble in acidic aqueous solution owing to protonation of the dimethylamino groups. However, in basic solution the acidic hydrogens were lost and the complexes became less soluble than those of reagent I.

The structure of similar metal complexes has been studied [15]. With divalent metal ions a hydrogen atom is displaced from each of the two –NH groups, thus forming neutral complexes of the following structure.



However, the diphenylamino groups in reagent IV are protonated in acidic solutons, leading to charged metal complexes (probably 2+). According to Budesinsky and Svec [14] in the case of the $Ag^+$ complex, coordination with only the sulfur atoms is likely.

The qualitative color-forming properties of reagent IV are summarized in Table II. Spectra of reagent IV and three of its metal complex are portrayed in Fig. 1. The molar absorptivities and optimum analytical wavelengths of several metal complexes are listed in Table III. All of the molar absorptivities are high enough to detect very low concentrations of metal ions.

### *Chromatographic separations*

Spectrophotometric determination of a metal ion is apt to be subject to numerous interferences unless

TABLE II

COLOR-FORMING PROPERTIES OF REAGENT IV

| Metal ions | Acidic (pH 2–3) | Neutral (pH 5–6) |
|---|---|---|
| $Hg^{2+}$ | Yellow | Yellow |
| $Ag^+$ | Yellow | Golden |
| $Cu^{2+}$ | Rust | Rust |
| $Co^{2+}$ | Rust | Brown |
| $Ni^{2+}$ | Yellow | Golden |
| $Pd^{2+}$ | Green | Brown |
| $Bi^{3+}$ | Yellow | Purple |
| $Fe^{2+}$ | Purple | Golden |
| $Fe^{3+}$ | Purple->golden | Golden |
| $In^{3+}$ | | Yellow |
| $Zn^{2+}$ | | Yellow |
| $Cd^{2+}$ | | Yellow |

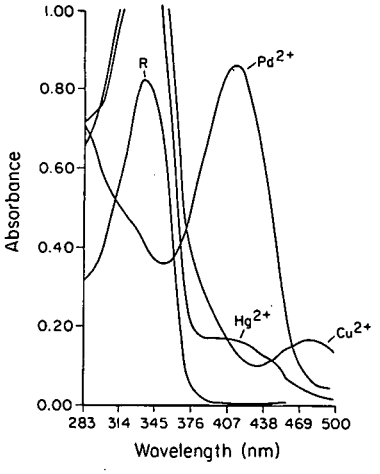



Fig. 1. Spectra of reagent IV and some of its metal complexes. Hg(II) and Pd(II) complexes were $1.0 \cdot 10^{-5}$ *M*. The Cu(II) complex was $1.7 \cdot 10^{-6}$ *M*.

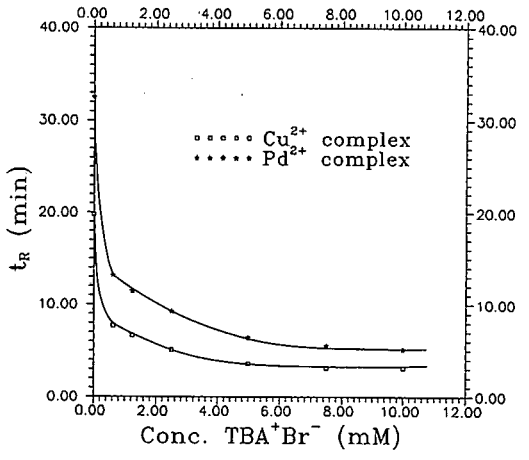



Fig. 2. The effect of tetrabutylammonium (TBA) concentration on the retention time of metal complexes. The eluent was acetonitrile–water (25:75) containing 0.01 *M* perchloric acid. A 50 × 4.6 mm RP-80 column was used. The sample contained $1.0 \cdot 10^{-5}$ *M* Cu(II) or $3.8 \cdot 10^{-5}$ *M* Pd(II) plus an excess of reagent.

the color-forming reagent is highly selective for that particular metal ion. Complexation of a group of metal ions, followed by HPLC separation of the individual metal complexes, can be an attractive alternative approach.

We first tried the separation of metal complexes of reagent IV on a short polymeric resin column using an acetonitrile–water eluent containing some perchlorate ion to form an ion pair with the positively charged complexes. It was difficult to adjust the acetonitrile content of the eluent so that good separations were obtained. Sharp but early and unresolved peaks were obtained for a mixture of several metal ions. Lowering the acetonitrile content of the eluent resulted in later and better resolved peaks, but the peaks were too broad for really good separations.

The addition of a quaternary ammonium salt ($Q^+$), such as tetraethylammonium bromide or tetrabutylammonium chloride, to the eluent resulted in lower retention times and substantially narrower peaks. Good resolution of several metal ions in a mixture was possible. These effects are shown in Figs. 2 and 3 in which retention time ($t_R$) and peak

TABLE III

ANALYTICAL WAVELENGTHS AND MOLAR ABSORPTIVITIES FOR METAL COMPLEXES OF REAGENT IV IN AQUEOUS SOLUTION AT pH 5.0

| Metal ion | Analytical wavelength (nm) | Molar absorptivity |
|---|---|---|
| $Ag^+$ | 410–420 | $2 \cdot 10^4$ |
| $Cd^{2+}$ | 450 | $2.8 \cdot 10^4$ |
| $Cu^{2+}$ | 474 | $9.1 \cdot 10^4$ |
| $Hg^{2+}$ | 404 | $1.9 \cdot 10^4$ |
| $Pd^{2+}$ | 420 | $7.3 \cdot 10^4$ |
| $Zn^{2+}$ | 410–420 | $2 \cdot 10^4$ |

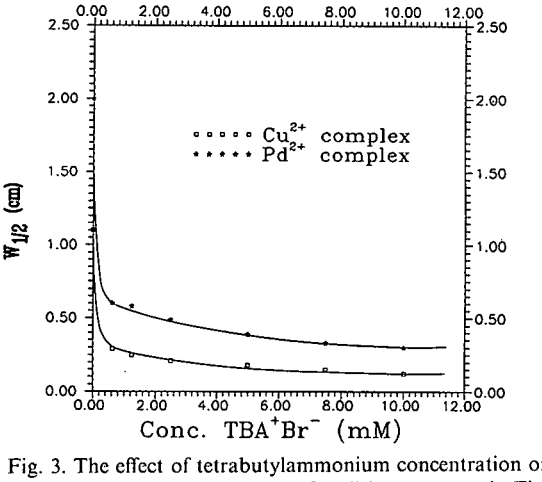



Fig. 3. The effect of tetrabutylammonium concentration on the peak width of metal complexes. Conditions same as in Fig. 2.

width ($W_{1/2}$) respectively, are plotted against the concentration of $Q^+$ added to the eluent.

The chain length of the $R_4N^+$, as well as the concentration added, also affects the chromatographic behavior of the positively charged metal complexes. Incorporation of a tetraethylammonium salt in the eluent has a substantially lower effect on retention time and peak width than does a tetrabutylammonium salt at the same concentration.

In HPLC the addition of an ion *opposite* in charge to a charged sample ion has often been used to increase retention via an ion-pair or ion-interaction mechanism. However, the addition of an ion of the same charge as the sample ion to the eluent has rarely been used. Miura [16] added tetrabutylammonium bromide to assist the separation of vanadium and other transition metal ion complexes by azo dyes on a silica column. Bidlingmeyer *et al.* [17] and others [18] demonstrated the effect of an ionic additive to the eluent for HPLC separations of simple, charged organic compounds.

The principle involved is also explained briefly by Haddad and Jackson [19]. The added $Q^+$ undergoes an equilibrium between the liquid mobile phase and the solid, stationary phase. Increased $Q^+$ concentration in the mobile phase or use of $Q^+$ with more carbon atoms would both increase the amount of $Q^+$ on the surface of the stationary phase. The presence of $Q^+$ on the solid surface tends to repel the positively charged sample ions and thereby reduce

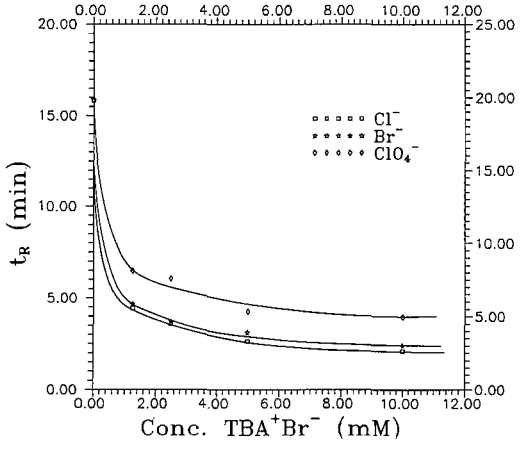



Fig. 4. The effect of different counter ions on the retention time of copper(II). Conditions as in Fig. 2 except for varying the counter ion.

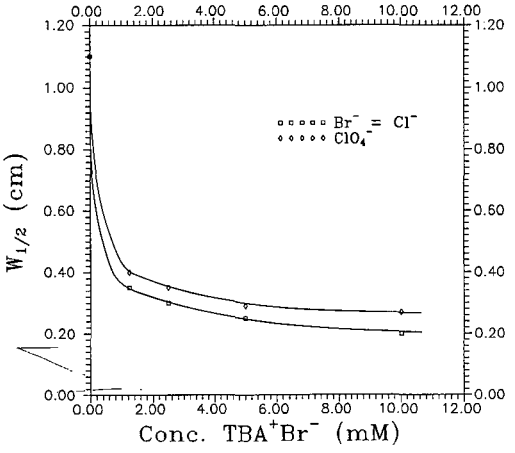



Fig. 5. The effect of different counter ions on the peak width of copper(II). Conditions as in Fig. 4.

their retention times. However, this repulsion effect is less than the attraction of the organic sample ions for the porous resin, thus ensuring that the sample ions are still retained sufficiently by the solid resin phase. For a neutral analyted, adding $Q^+$ in the eluent essentially has no effect on the retention time and peak width.

Since the sample complexes are cationic in the pH range used, it follows that the chemical nature of the counter anion would have an effect on the HPLC separation. To test this, different anions were added to the eluent. Fig. 4 shows that retention times are longer with perchlorate than with bromide or chloride. However, Fig. 5 shows that the peak width is slightly greater with perchlorate.

If the mechanism postulated for the addition of $Q^+$ to the eluent is correct, it would follow that incorporation of a fixed positive charge on the resin surface would have a similar effect. Fig. 6 shows an excellent separation of several metal complexes on a low-capacity anion-exchange resin (containing permanent $Q^+$ groups). The retention times are shorter and the peaks are sharper than when the same separation is attempted on a neutral resin column.

The chromatographic separation shown in Fig. 7 was obtained on a column only 5 cm long and packed with 10-$\mu$m neutral resin. The eluent contained an added quaternary ammonium salt. Separations with longer columns did not show very much improvement over those obtained with shorter columns. Fig. 8 shows a separation obtained with

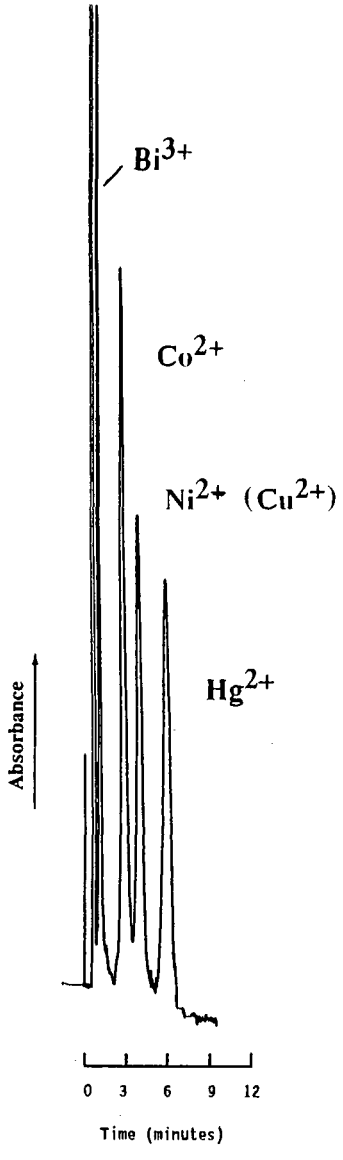



Fig. 6. Separation of metal complexes of reagent IV on a 50 × 4.6 mm AN-1 anion-exchange column. Eluent is acetonitrile–water (25:75) containing 0.01 *M* perchloric acid. Detection at 474 nm.

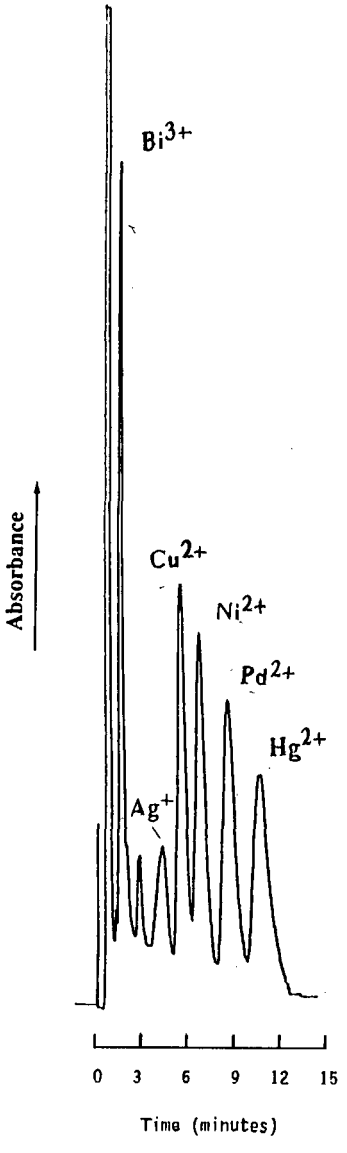



Fig. 7. Separation of metal complexes on a 50 × 4.6 mm neutral RP-80 polymeric resin column with 2.5 m*M* tetraethylammonium bromide added to the eluent. Other separation conditions as in Fig. 6.

a 250 × 4.6 mm column using 10 m*M* tetraethylammonium chloride as the additive.

Separations with a reversed-phase silica $C_{18}$ column were reasonable, but generally not as good as those obtained with a polymeric resin column. Fig. 9 shows the separation of complexes of indium(III), copper(II) and mercury(II) using a 250 × 4 mm $C_{18}$ column. This separation was at a somewhat higher pH than the other separations, and a much higher acetonitrile content was required in the eluent.

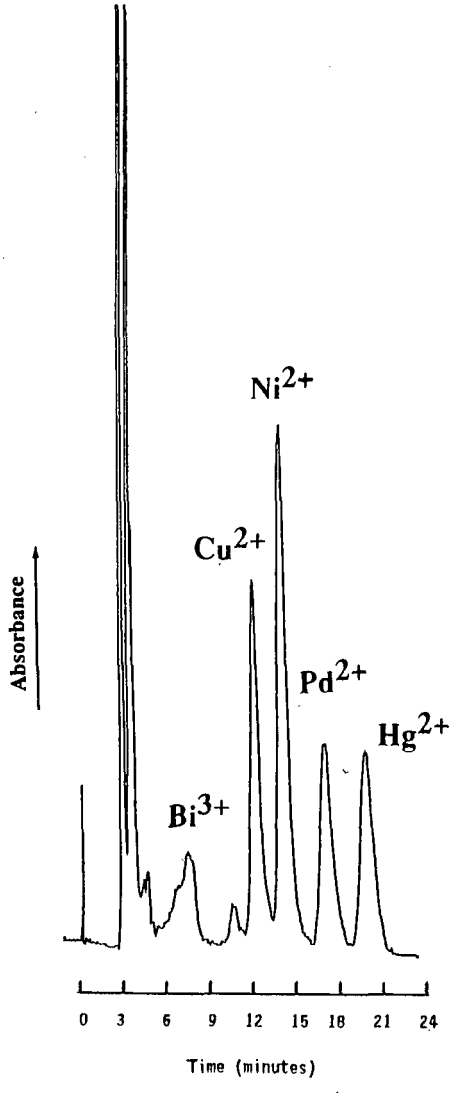



Fig. 8. Separation of metal complexes on a 250 × 4.6 mm RP-80 column using acetonitrile–water (30:70) containing 0.01 *M* perchloric acid and 10 m*M* tetraethylammonium chloride.

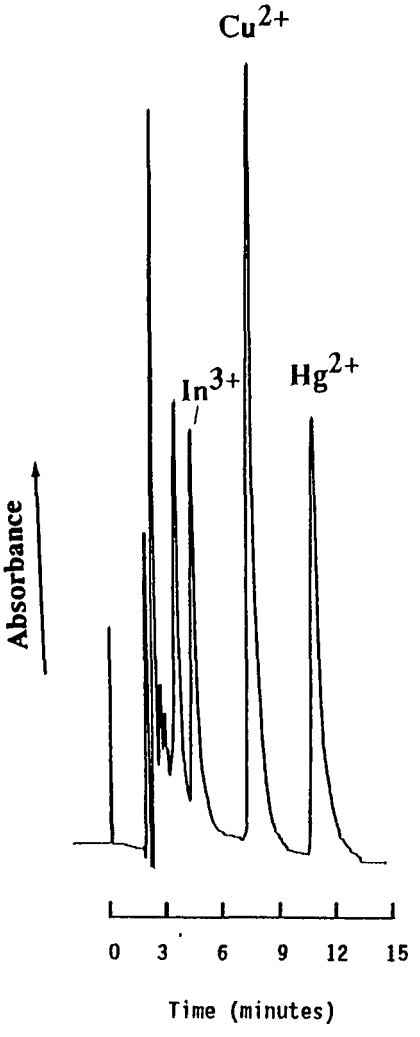



Fig. 9. Separation of reagent IV complexes on a 250 × 4 mm $C_{18}$ silica column. Eluent is acetonitrile–water (75:25) containing 5 m*M* tetrabutylammonium bromide and buffered with 160 m*M* acetic acid–sodium acetate pH 4.6.

*Scope of chromatographic separations*

Chromatographic separations were obtained for most of the elements that form stable complexes with our thiosemicarbazone reagent (reagent IV) at an acidic pH values. Ions such as zinc(II) and cadmium(II) form thiosemicarbazone complexes, but a more alkaline pH is required. Unfortunately, reagent IV and its complexes are deprotonated at higher pH values and are much less soluble in water and in water-organic solvent mixtures. Reagent I might be a better candidate for separations carried our at a neutral or alkaline pH because the reagent and its complexes both have a reasonable solubility in acetonitrile–water mixtures.

## CONCLUSIONS

Semicarbazone reagent IV is an excellent color-forming reagent. It forms highly colored complexes

with a number of metal ions and has the added advantage that both the reagent and its metal complexes are very soluble in water at acidic pH values. Complexes of several metal ions with reagent IV are also well separated on a short HPLC column if an appropriate quaternary ammonium salt is added to the eluent. This should be a convenient and practical way to determine certain metal ions in analytical samples. The complexes are easily formed simply by adding the reagent to the aqueous sample and adjusting the pH. The chromatographic step takes only a few minutes. At acidic pH values, reagent IV forms complexes with only a few metal ions, thereby imparting considerable selectivity to the chromatographic determination of these ions.

ACKNOWLEDGEMENTS

We thank Doug Gjerde of Sarasep Inc. for a gift of the polymeric resins used in HPLC separation of the metal complexes. We also acknowledge helpful discussions with George Kraus and Walter Trahanowsky regarding synthesis of the chelating reagents.

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# Ion chromatography of metal cations on carboxylic acid resins

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ABSTRACT

The goal of the present work is to study cation separations on weak-acid resins that are easily synthesized and carry the exchange group on the crosslinking benzene ring of the resin or on a short spacer arm from the ring. The synthesis and general properties of two carboxylic resins are described. A higher exchange capacity is required for effective ion chromatographic separations than that needed for sulfonated resins. A number of excellent separations are reported using eluents containing ethylenediammonium cations plus a weak complexing agent. The effect of pH on retention times of metal ions is reported.

## INTRODUCTION

Perhaps the best method for separating 2+ and 3+ metal cations by ion chromatography is through the use of complexing eluents. Sevenich and Fritz [1] obtained excellent separations using an ethylenediammonium tartrate eluent in conjunction with a conductivity detector. Further selectivity is possible with the addition of a second chelating reagent (such as EDTA) to the sample only [2]. Others have obtained excellent separations of various metal ions using eluents containing tartrate, citrate, oxalate, 2-hydroxyisobutyrate, or salts of pyridyldicarboxylic acid [3–7].

Virtually all ion chromatographic separations of metal cations have been carried out on various strong-acid ion exchangers containing the sulfonic acid group. These strongly acidic ion exchangers maintain complete ionic capacity over a large pH range of 2–12. A weak-acid exchanger, such as the carboxylic acid, loses its ionic capacity as the pH goes below the $pK_a$ of the functional group. The pH affects the separation of the metal cations thus adding an additional parameter for optimization. Kolla *et al.* [8] prepared an efficient carboxylic acid resin by coating a polybutadiene–maleic acid resin onto the surface of 5 $\mu$m porous silica. Using this resin, they obtained simultaneous separations of monovalent and divalent cations.

The preparation and use of two new carboxylic acid resins are described in the present research. Both resins are prepared by simple Friedel–Crafts addition reactions. One has the carboxyl group attached to the benzene ring of a spherical polystyrene–divinylbenzene (PS–DVB) resin via a spacer arm of three carbon atoms. The carboxyl group is attached directly to the benzene ring in the other resin. Excellent ion chromatographic separations of metal ions are possible using these resins in conjunction with any of several complexing eluents.

## EXPERIMENTAL

### *Apparatus*

The chromatographic system consisted of several components. An Eldex model AA-94 pump (Eldex Labs, Menlo Park, CA, USA) set to deliver eluent at a flow-rate of 1.0 ml/min, a 7125 Rheodyne injector (Rheodyne, Berkeley, CA, USA) equipped with a 10-$\mu$l loop, a Wescan ICM II ion analyzer (Wescan Instruments, Santa Clara, CA, USA) with conductivity detection at a constant temperature, a

0021-9673/92/$05.00 

Fisher Recordall series 5000 recorder (Fisher Scientific/Instrument Lab., Itasca, IL, USA) and a Hitachi D-2000 integrator (EM Science, Cherry Hill, NJ, USA) were used for all separations. A Shandon HPLC packing pump (Shandon Southern, Sewichley, PA, USA) was used for column packing.

*Reagents and chemicals*

The spherical PS–DVB resins (Sarasep, Santa Clara, CA, USA) used in this experiment consisted of two sizes; 10-$\mu$m resin with 80 Å pore size and 415 $m^2/g$ surface area, and 5-$\mu$m resin with similar pore size and surface area. The resin was washed with water, acetonitrile and methanol, and then dried.

The reagents and solvents used for the derivatization reactions were reagent grade. Stock solutions of the metals were prepared from reagent-grade chloride salts. The concentrations of metal ions in the salt solutions injected ranged from $1.2 \cdot 10^{-4}$ to $1.1 \cdot 10^{-3}$ *M*. Eluents were prepared daily using reagent-grade solutions of ethylenediamine and the specified organic acid. A Barnstead Nanopure II system (Sybron Barnstead, Boston, MA, USA) was used to further deionize distilled water for all eluents and sample mixtures. Adjustments in pH were made with reagent grade solutions of sodium hydroxide and hydrochloric acid.

*Synthetic procedures*

The carboxylic acid functional groups were introduced into the benzene ring of PS–DVB resins by the following procedures:

*(1) $-COCH_2CH_2CO_2H$ derivative (resin I).* Mix 2.5 g of resin with 3.6 g of succinic anhydride and 50 ml of tetrachloroethane in a 100-ml round-bottomed flask. Stir for 15 min, then add 10.7 g of anhydrous aluminum chloride and reflux at 45°C for 24 h. Pour the product into a methanol–ice solution to quench the reaction. Collect the resin by filtration, wash with methanol, 1 *M* hydrochloric acid and water, then dry. The exchange capacity was found to be 0.60 mequiv./g by acid–base titration of the carboxylic acid.

*(2) $-CO_2H$ derivative (resin II).* Mix 2.5 g of resin with 8.0 ml of phenyl chloroformate and 50 ml of tetrachloroethane in a 100-ml round-bottomed flask. Stir for 15 min, then add 10.0 g of anhydrous aluminum chloride and reflux at 100°C for 4 h. Pour the product into a methanol–ice solution. Isolate the resin, wash with methanol, then dry. Hydrolysis of the ester is accomplished by refluxing the resin for 1 h in a 1 *M* sodium hydroxide–ethanol solution. Collect the resin, wash with methanol, 1 *M* hydrochloric acid and water, then dry. The exchange capacity was found to be 0.39 mequiv./g by acid-base titration of the carboxylic acid.

## RESULTS AND DISCUSSION

*Preparation of resins*

Resin I was prepared by reacting a porous, cross-linked polystyrene resin with succinic anhydride in a Friedel–Crafts reaction. The chemical structure of the derivatized resin can be written as $P–C_6H_4COCH_2CH_2CO_2H$ (P = polymer). Resin II was prepared by reacting the polystyrene resin with phenyl chloroformate in a Friedel–Crafts reaction, followed by hydrolysis to the carboxylic acid. The chemical structure can be written as $P–C_6H_4CO_2H$. Coppock [9] showed that phenyl chloroformate was required in the Friedel-Crafts reaction of aromatic hydrocarbons to obtain the expected aryl ester of the aromatic hydrocarbon. Alkylation of the hydrocarbon occurs with the use of alkyl chloroformates.

At first, resin I was prepared under very mild reaction conditions to give a carboxylic acid capacity $<0.1$ mequiv./g, which is within the range that is widely used for the sulfonic acid resins used in ion chromatography. However, this carboxylic acid resin failed to give any useful separations. Another batch of resin I was prepared under different reaction conditions to give a resin of much higher carboxylic acid capacity (0.60 mequiv./g). This resin (and others of similar capacity) was used very successfully for ion chromatographic separations.

*Optimization of separation conditions*

Earlier work with columns of sulfonated polymeric resins demonstrated that good separation of several divalent metal ions could be obtained using weakly complexing eluents of ethylenediammonium tartrate [1,2]. Almost all of the present work was done with carboxylic acid resin I. Separations of several divalent metal ions were obtained using eluents containing the ethylenediammonium 2+ cation and one or two of the following complexing anions: citrate, pyridine-2,6-dicarboxylate (PDA),

oxalate, hydroxybutyrate and tartrate. The first three of these were found to give the best separations. The ethylenediammonium 2+ cation acts as a "pusher" to move the divalent metal ions down the column. The complexing anions act as "pullers" to aid the separation of metal ions based on the formation constant of each metal ion with that particular anion.

Experiments designed to optimize separation conditions showed that the ethylenediammonium cation and a complexing anion were both needed in the eluent. Broad peaks and few separations were obtained using the ethylenediammonium cation with only a non-complexing anion. Addition of any of the complexing anions tightened the chromatographic peaks considerably. However, very poor separations were obtained when the eluent contained a complexing anion in conjunction with a monovalent cation such as sodium. The presence of ethylenediammonium, or some other divalent cation, seemed to be necessary. Using an eluent consisting of ethylenediammonium and any of the mentioned complexing anions, there is a noticeable later eluting system peak at lower pH values (pH 4.0 to 4.8). The retention time of this system peak is well beyond that of any of the metal ions in a mixture and adds no interference. The majority of work reported here was at pH 5.0 and higher where the system peak is no longer noticed and can be ignored. The system peak never appeared in later chromatograms when doing successive injections.

The effect of varying pH was investigated using the ethylenediammonium oxalate eluents. The results in Table I show the logarithm of adjusted retention time to be a linear function of pH ($t'_R$ increasing as the pH assumes a higher value). The metal ions studied fell into two distinct groups, one with a slope very close to 0.5 and the other with a slope of approximately 1.0. We have no explanation for these differences in slope except that some of the metal ions might form metal hydrogen oxalate complexes and the others simple metal oxalate complexes.

The pH study was conducted in the pH range where oxalate was predominantly in the 2− form. A complexing anion of a weaker acid would be converted more completely to the fully deprotonated forms as the pH is increased. This would increase its chelating ability and could therefore lead to lower $t'_R$ values with increased pH.

TABLE I

LINEAR REGRESSION DATA FOR PLOTS OF LOG $t'_R$ (ADJUSTED RETENTION TIME) *VS.* pH USING AN ETHYLENEDIAMMONIUM OXALATE ELUENT

| Metal ion | pH range | Slope | Intercept | Correlation coef. |
|---|---|---|---|---|
| $Ni^{2+}$ | 5.4–6.8 | 1.03 | −5.80 | 0.9994 |
| $Co^{2+}$ | 5.0–6.8 | 0.50 | −2.74 | 0.9822 |
| $Zn^{2+}$ | 5.0–6.4 | 1.07 | −5.80 | 0.9944 |
| $Fe^{2+}$ | 5.4–6.8 | 0.53 | −2.75 | 0.9802 |
| $Pb^{2+}$ | 4.0–5.4 | 0.98 | −4.06 | 0.9948 |
| $Cu^{2+}$ | 4.2–5.8 | 0.97 | −4.88 | 0.9952 |
| $Cd^{2+}$ | 4.0–5.6 | 0.87 | −3.52 | 0.9976 |
| $Mn^{2+}$ | 4.2–7.2 | 0.52 | −2.31 | 0.9986 |
| $Mg^{2+}$ | 4.0–7.2 | 0.50 | −2.10 | 0.9991 |
| $Ca^{2+}$ | 4.0–7.2 | 0.50 | −1.89 | 0.9962 |
| $Sr^{2+}$ | 4.0–7.2 | 0.51 | −1.86 | 0.9977 |
| $Ba^{2+}$ | 4.0–7.2 | 0.51 | −1.76 | 0.9980 |

Increasing the concentration of complexing anion in the eluent leads to more complete complexation of metal ions and thus to lower $t'_R$ values. The value of $t'_R$ for any given metal ion seems to be determined primarily by the formation constant of the metal–anion complex, although this is modified by the affinity of the resin for the uncomplexed metal cation. The importance of metal-anion formation constants is demonstrated by the PDA anion. PDA has a rigid planar structure which forms very strong complexes with transition metals leading to fast chromatographic elution. Alkaline earths, however, form weaker complexes allowing excellent chromatographic separation. The order of elution of metal ions for all complexing anions studied was found to be that of decreasing complex formation constants (see Table II).

An earlier study [1] with complexing eluents in conjunction with sulfonated resins showed that log $t'_R$ is inversely proportional to log $\alpha_M$, where $\alpha_M$ is the concentration ratio of free metal cation to the total metal cation in solution. This paper [1] also showed that log $t'_R$ is a linear function of the logarithm of the ethylenediammonium concentration in the eluent.

*Citrate systems*

An eluent containing 1.0 m*M* ethylenediammonium ion and slightly lower concentrations of citrate

TABLE II

LOGARITHMS OF FORMATION CONSTANTS OF SELECTED METAL COMPLEXES

| Reagent | Log formation constant | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | $Cu^{2+}$ | $Ni^{2+}$ | $Co^{2+}$ | $Zn^{2+}$ | $Mn^{2+}$ | Mg | $Ca^{2+}$ | $Sr^{2+}$ | $Ba^{2+}$ |
| Citric acid | 5.60 | 5.11 | 4.83 | 4.70 | 3.70 | 3.25 | 3.18 | 2.81 | 2.55 |
| PDA | 8.80 | 6.60 | 6.35 | 6.43 | 4.70 | 2.02 | 4.30 | 3.50 | 3.13 |
| Oxalic acid | 4.53 | 3.70 | 3.25 | 3.43 | 2.60 | 2.10 | 1.66 | 1.25 | 1.02 |

at pH 5.4 was found to give some useful separations. Keeping the ethylenediammonium concentration and the pH constant, the citrate concentration of the eluent was varied from 0.2 to 0.6 m$M$ in 0.1 m$M$ increments. Poor separations were obtained at 0.2 m$M$ and 0.6 m$M$ citrate; the best separation was obtained at 0.3 m$M$ citrate.

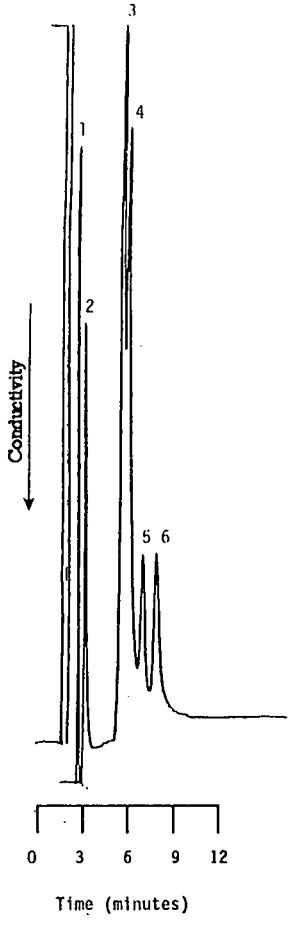


Fig. 1. Chromatographic separation on succinic acid-derivatized PS–DVB resin (resin I) column (50 × 4.6 mm I.D.). Eluent conditions: 1.0 m$M$ ethylenediammonium, 0.30 m$M$ citrate (pH 5.4). Peaks: 1 = $Co^{2+}$; 2 = $Zn^{2+}$; 3 = $Mg^{2+}$; 4 = $Ca^{2+}$; 5 = $Sr^{2+}$; 6 = $Ba^{2+}$.

Fig. 1 shows the separation of several metal ions at 0.3 m$M$ citrate. The $Ni^{2+}$ peak is covered by the injection peak. The $Co^{2+}$ and $Zn^{2+}$ peaks are well resolved even though the ratio of the logarithmic citrate formation constants is only 1.03 (Table II). The $Ca^{2+}$ and $Sr^{2+}$ peaks (ratio of log consants = 1.13) and $Sr^{2+}$ and $Ba^{2+}$ peaks (ratio of log constants = 1.10) are resolved but the resolution of the $Mg^{2+}$ and $Ca^{2+}$ peaks is poor, probably due to the very small ratio of their logarithmic formation constants (= 1.02).

*Oxalate systems*

Preliminary experiments showed that good separations of several metal ions could be obtained with an eluent containing 1.0 m$M$ ethylenediammonium ion and 1.0 m$M$ oxalate. The separation of $Ni^{2+}$ (or $Co^{2+}$), $Zn^{2+}$, $Mn^{2+}$, $Mg^{2+}$, $Ca^{2+}$ and $Ba^{2+}$ was then optimized with respect to pH. Virtually no separation was obtained at pH 4.0 to 4.2 and the system peak was close to the metal ion peaks. By pH 4.4 some separation had begun and the system peak was well removed from the vicinity of metal ion peaks. The best separation occured around pH 5.3 with all six peaks being well separated (Fig. 2). Further increases in pH brought longer retention times, and by pH 5.8 the quality of the separation had deteriorated noticeably. At pH 6.4 almost no separation was obtained.

The metal ion retention times were next reduced by increasing the oxalate concentration from 1.0 m$M$ to 1.6 m$M$. With this eluent, the optimum pH was approximately 5.8. It was possible to separate all seven metal ions in a mixture as shown in Fig. 3.

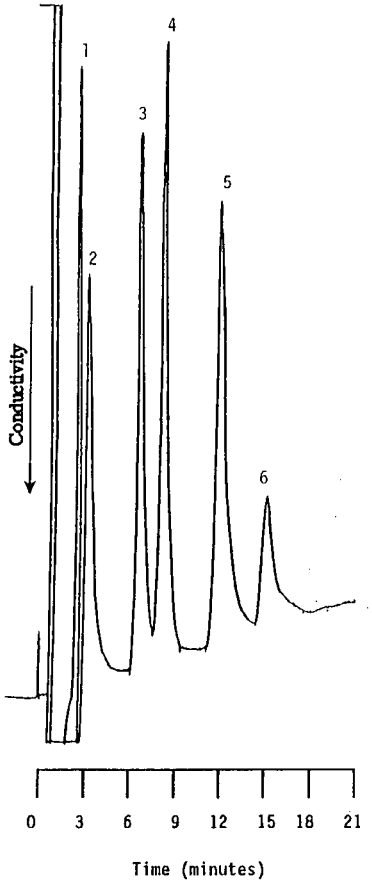


Fig. 2. Chromatographic separation on resin I column (100 × 4.6 mm I.D.). Eluent conditions: 1.0 m*M* ethylenediammonium, 1.00 m*M* oxalate (pH 5.3). Peaks: 1 = $Co^{2+}$; 2 = $Zn^{2+}$; 3 = $Mn^{2+}$; 4 = $Mg^{2+}$; 5 = $Ca^{2+}$; 6 = $Ba^{2+}$.

The use of resin II was next investigated. A short series of optimization experiments suggested the following conditions for separation: 0.75 m*M* ethylenediammonium, 1.5 m*M* oxalate and pH 4.5 to 5.0. Fig. 4 shows an excellent and rapid separation of $Zn^{2+}$, $Mn^{2+}$, $Mg^{2+}$, $Ca^{2+}$ and $Ba^{2+}$. $Ni^{2+}$ was partially separated from the $Zn^{2+}$ peak.

These separations are similar to those obtained in Fig. 3 using resin I except for the pH which is lower for the separations using resin II. Based on the acid dissociation constants for the monomers of similar chemical structure, the acid strength of the carboxylic acid should be somewhat stronger for resin II. Except for this, we could not discern any major differences in the two resins for chromatographic separations.

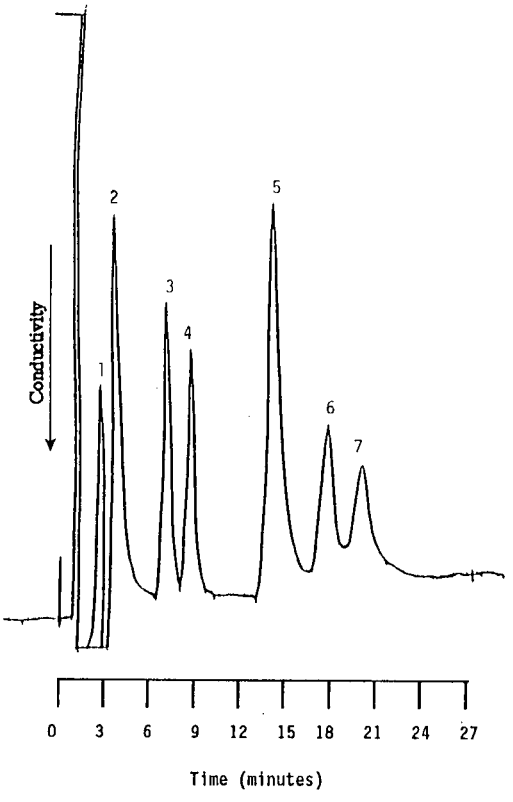


Fig. 3. Chromatographic separation on resin I column (100 × 4.6 mm I.D.). Eluent conditions: 1.0 m*M* ethylenediammonium, 1.60 m*M* oxalate (pH 5.8). Peaks: 1 = $Co^{2+}$; 2 = $Zn^{2+}$; 3 = $Mn^{2+}$; 4 = $Mg^{2+}$; 5 = $Ca^{2+}$; 6 = $Sr^{2+}$; 7 = $Ba^{2+}$.

*PDA systems*

PDA forms more stable complexes with most elements than oxalate or citrate. There is a reasonable difference in the formation constants of the PDA complexes listed in Table I. Preliminary optimization experiments showed that an excellent separation of $Mg^{2+}$ and the three alkaline earths could be obtained with an eluent containing 1.0 m*M* ethylenediammonium and 0.05 or 0.1 m*M* PDA, adjusted to pH 5.4 (see Fig. 5). The order of elution is unusual in that $Mg^{2+}$ elutes after the alkaline earths. In other chromatographic systems, $Mg^{2+}$ elutes before $Ca^{2+}$. Increasing the PDA concentration in steps from 0.025 m*M* to 0.02 m*M* decreases the retention times of $Ca^{2+}$, $Sr^{2+}$ and $Ba^{2+}$ but has little effect on the retention time of $Mg^{2+}$. This effect was explained following theoretical considerations in earlier work by Sevenich and Fritz [1] in

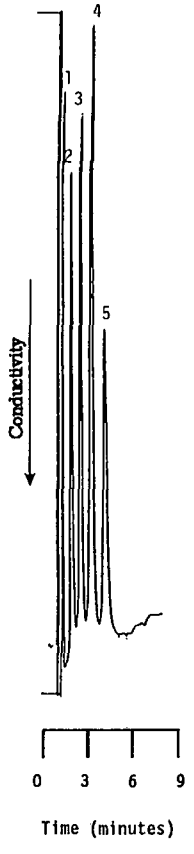


Fig. 4. Chromatographic separation on benzoic acid-derivatized PS–DVB resin (resin II) column (100 × 4.6 mm I.D.). Eluent conditions: 0.75 m*M* ethylenediammonium, 1.5 m*M* oxalate (pH 4.5). Peaks: 1 = $Zn^{2+}$; 2 = $Mn^{2+}$; 3 = $Mg^{2+}$; 4 = $Ca^{2+}$; 5 = $Ba^{2+}$.

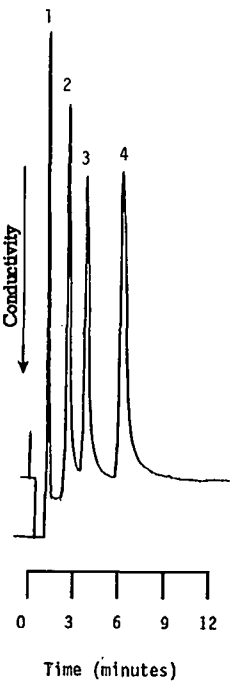


Fig. 5. Chromatographic separation on resin I column (50 × 4.6 mm I.D.). Eluent conditions: 1.0 m*M* ethylenediammonium, 0.10 m*M* PDA (pH 5.4). Peaks: 1 = $Ca^{2+}$; 2 = $Sr^{2+}$; 3 = $Ba^{2+}$; 4 = $Mg^{2+}$.

which a similar ethylenediammonium tartrate eluent was used. They derived a logarithmic equation relating adjusted retention time to the fraction of the metal ion in solution that exists as the free metal cation ($\alpha_M$). The equation was tested for a number of cations and linear plots were obtained when the concentration of the complexing anion in the eluent was varied and log $t'_R$ was plotted against log $\alpha_M$. In the present work using an ethylenediammonium–PDA eluent, when the concentration of PDA was increased from 0.025 to 0.200 m*M*, the following changes in $\alpha_M$ values were calculated: $Ca^{2+}$ (0.495 to 0.095); $Sr^{2+}$ (0.863 to 0.438); $Ba^{2+}$ (0.937 to 0.649); $Mg^{2+}$ (0.995 to 0.860). Linear plots were obtained for $Ca^{2+}$, $Sr^{2+}$ and $Ba^{2+}$ when plotting log $t'_R$ against log $\alpha_M$. In each case the value of $\alpha_M$ decreased by 30–40% for the increased PDA concentration. $Mg^{2+}$, however, showed only a 3.5% decrease in $\alpha_M$ for the same increase in PDA concentration. This very weak complexation of $Mg^{2+}$ by the PDA accounted for the negligible effect on the retention time of $Mg^{2+}$.

Using a longer column than that used to obtain Fig. 5, the retention times of $Ca^{2+}$, $Sr^{2+}$, $Ba^{2+}$ and $Mg^{2+}$ are somewhat increased allowing separation of some additional early-eluting ions. Fig. 6 shows an excellent separation of $Zn^{2+}$, $Na^+$, $Ca^{2+}$, $Sr^{2+}$, $Ba^{2+}$ and $Mg^{2+}$ at pH 5.4 on a 10-cm column. Very similar chromatograms were obtained for samples in which $Co^{2+}$ or $Cu^{2+}$ was substituted for $Zn^{2+}$ and $K^+$ or $NH_4^+$ was present instead of $Na^+$. The same desired effect was achieved by increasing the pH from 5.4 to 5.8 using the shorter column. An equally good and somewhat faster separation was obtained at pH 5.8 on a 5-cm column.

Linear calibration curves were obtained for the ions separated by plotting peak area *vs.* concentra-

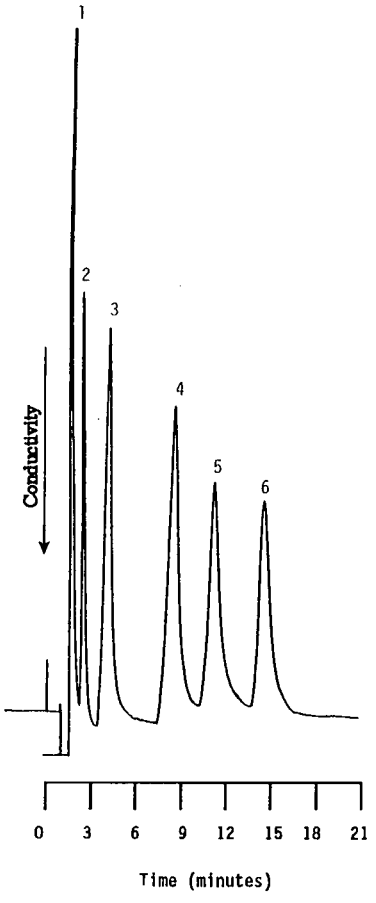


Fig. 6. Chromatographic separation on resin I column (100 × 4.6 mm I.D.). Eluent conditions: 1.0 m*M* ethylenediammonium, 0.05 m*M* PDA (pH 5.4). Peaks: 1 = $Zn^{2+}$; 2 = $Na^{+}$; 3 = $Ca^{2+}$; 4 = $Sr^{2+}$; 5 = $Ba^{2+}$; 6 = $Mg^{2+}$.

tion. The curves remained linear over the studied concentration range of $10^{-4}$ to $10^{-2}$ *M*. The desirable goal of separating an alkali metal from several divalent metal ions in a single run has thus been achieved. Unfortunately, we were not able to separate $Na^{+}$, $K^{+}$ and $NH_4^{+}$ from one another. The sum of these monovalent ions can be obtained but this would be difficult to quantify because of the differing responses of the conductivity detector for these ions. A dicarboxylic derivatized resin could possibly accomplish this task.

ACKNOWLEDGEMENTS

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# High-performance ion chromatographic separation of uranium and thorium in natural waters and geological materials

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ABSTRACT

An ion chromatographic separation of uranium and thorium is described. The method uses a cation-exchange resin for the separation of uranium and thorium from other common metal interferences. Detection of uranium and thorium is accomplished using post-column derivatization with arsenazo III followed by spectrophotometric detection. In addition to direct injection of sample, a method for concentrating uranium and thorium from complex matrices will be presented. Using direct injection, detection limits for uranium and thorium in simple matrices is 20 μg/l for uranium and 60 μg/l for thorium using a 50-μl sample loop. The use of a chelating resin for selective sample concentration lowers the detection limit to 1.0 μg/l for uranium and 3.0 μg/l for thorium when concentrating 5.0 ml of sample. Sample concentration on a selective chelating resin also extends the applicability of the method for the analysis of complex matrices.

## INTRODUCTION

Uranium and thorium are naturally occurring elements found at trace levels in the environment. The routine determination of trace amounts of uranium and thorium is challenging due in part to the lack of a simple and specific colorimetric test which is not prone to interferences by other metal ions. The natural radioactivity of uranium and thorium is the basis of some common methods of analysis. The determination of uranium in aqueous samples is often done using a radiochemical method [1]. In this method, the uranium is co-precipitated from solution with ferric hydroxide, separated from iron by open column anion exchange using a hydrochloric acid eluent, evaporated and converted to a nitrate salt and finally the alpha activity is measured. Thorium may be analyzed in clean matrices using flame atomic absorption [2]. The flame atomic absorption method requires the use of a nitrous oxide–acetylene flame for analysis. Other accurate means for uranium analysis include neutron activation analysis [3], and inductively coupled plasma mass spectrometry (ICP-MS) [4]; however these techniques are not well suited to routine analysis, and may suffer interferences from other metals present in the matrix.

The use of ion chromatography for the separation of metals is well documented. Early work focussed on open column separations of metals by both anion and cation exchange using acid eluents [5,6]. These open column ion-exchange separations used detection based on fraction collection and off-line derivatization and detection. The development of on-line detection schemes led to the first modern high-performance ion chromatograph with continuous detection for metal separations [7]. High-performance separations on low-capacity cation-exchange resins and anion-exchange separations of metal complexes have allowed the rapid and accurate determination of many transition metals and lanthanide metals [8]. Separations of a wide variety of metal ions in addition to uranium and thorium have also been determined using reversed-phase columns in the ion-pairing mode [9,10]. More recently, highly selective chelating resins have been used for on-line concentration of metal ions combined with selective elution of potentially interfering matrix components

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[11]. The coupling of selective sample concentration with high-performance ion chromatographic separations has increased the scope of sample types that can be analyzed for trace metals using ion chromatography. This paper will describe the development of a high-performance ion chromatographic separation based on cation exchange. The discussion will cover the separation mechanism, detection scheme and use of chelating resins for selective sample preconcentration.

## THEORETICAL

### *Separation*

The separation of uranium and thorium by cation-exchange chromatography is complicated by the large differences in distribution coefficients of the two analytes. Uranium is generally present as a divalent cation ($UO_2^{2+}$) while thorium exists as the tetravalent $Th^{4+}$ species and therefore considerable differences are observed in their relative affinity for a cation-exchange resin (Tables I–III). With both HCl and $HNO_3$ eluents, a relatively low acid concentration will elute $UO_2^{2+}$ as an anionic complex, but a much higher concentration of acid ($>3$ *M*) is required to elute thorium. Although an acid gradient can be used for the separation, this approach will cause difficulties in detection as will be described in the *Detection* section.

The distribution coefficient for thorium in a sulfuric acid eluent is much lower than for a comparable concentration of hydrochloric or nitric acid, presumably due to the formation of a thorium–sulfate ($ThSO_4^{2+}$) complex which reduces the affinity of thorium for the ion exchanger. To resolve uranium and thorium without the use of an acid gradient, a sulfate gradient can be used to elute thorium from the resin following the elution of uranium with HCl. A sodium sulfate gradient maintaining a constant concentration of HCl (0.6 *M*), will resolve uranium and thorium from the potential interferences $Fe^{3+}$, $Ca^{2+}$, $Hf^{4+}$, $ZrO^{3+}$ and the lanthanide metals. The separation takes place at constant pH which is necessary in order to maintain baseline stability throughout the separation. Note also that $Fe^{3+}$ and thorium have similar distribution coefficients in sulfuric acid. The use of HCl eluent followed by a sodium sulfate gradient separates ferric ion from thorium by exploiting the differences in selectivity with the HCl eluent.

TABLE II

WEIGHT DISTRIBUTION COEFFICIENTS AT DIFFERENT CONCENTRATIONS OF $HNO_3$ ON CATION-EXCHANGE RESIN

From ref. 6.

| Cation | $HNO_3$ Concentration (*M*) | | | | |
|---|---|---|---|---|---|
| | 0.2 | 0.5 | 1.0 | 2.0 | 3.0 |
| $UO_2^{2+}$ | 262 | 69 | 24.4 | 10.7 | 7.4 |
| $Th^{4+}$ | $>10^4$ | $>10^4$ | 1180 | 123 | 43 |
| $Fe^{3+}$ | 4100 | 362 | 74 | 14.3 | 6.2 |

### *Detection*

The detection of uranium and thorium is accomplished by postcolumn addition of a color-forming

TABLE I

WEIGHT DISTRIBUTION COEFFICIENTS AT DIFFERENT CONCENTRATIONS OF HCl ON CATION-EXCHANGE RESIN

From ref. 5.

| Cation | HCl concentration (*M*) | | | | |
|---|---|---|---|---|---|
| | 0.2 | 0.5 | 1.0 | 2.0 | 3.0 |
| $UO_2^{2+}$ | 860 | 102 | 19.2 | 7.3 | 4.9 |
| $Th^{4+}$ | $>10^5$ | $10^5$ | 2049 | 239 | 114 |
| $Fe^{3+}$ | 3400 | 225 | 35.4 | 5.2 | 3.6 |

TABLE III

WEIGHT DISTRIBUTION COEFFICIENTS AT DIFFERENT CONCENTRATIONS OF $H_2SO_4$ ON CATION-EXCHANGE RESIN

From ref. 6.

| Cation | $H_2SO_4$ concentration (*M*) | | | | |
|---|---|---|---|---|---|
| | 0.1 | 0.25 | 0.5 | 1.0 | 1.5 |
| $UO_2^{2+}$ | 118 | 29.2 | 9.6 | 3.2 | 2.3 |
| $Th^{4+}$ | 3900 | 264 | 52 | 9.0 | 3.0 |
| $Fe^{3+}$ | 2050 | 255 | 58 | 13.5 | 4.6 |

complexing agent followed by spectrophotometric measurement of the metal complex. The postcolumn reagent used is arsenazo III (Fluka, Ronkonkoma, NY, USA) which has been described in many reports as a colorimetric reagent for uranium and thorium [12–14]. The greatest impediment to the use of arsenazo III has been its lack of selectivity for uranium and thorium. Arsenazo III will form colored complexes with many metals including iron, calcium, zirconium, hafnium and the lanthanide series [12]. The use of an ion chromatographic separation prior to detection with arsenazo III eliminates many of the interferences that prevent direct use of arsenazo III as a colorimetric reagent for uranium and thorium. Additionally, the selectivity of arsenazo III increases with decreasing pH. Although many metals will react with arsenazo III at moderate pH, in an acidic environment the number of metals which form stable complexes with arsenazo III is much more limited [12]. Therefore, metals such as iron and calcium which are often present in high levels relative to uranium and thorium, pose a less severe interference due to their diminished response with arsenazo III at low pH. The complex of arsenazo III with a metal ion absorbs strongly at 660 nm while the free arsenazo III absorbs rather weakly at that wavelength. The background absorbance of free arsenazo III observed at 660 nm is pH dependent, with the absorbance decreasing as the pH is lowered [15]. This requires the use of a constant pH separation to prevent a downward sloping baseline that would accompany an acid gradient. The constant pH separation was discussed in the preceding section on separation. In addition to arsenazo III, the postcolumn reagent contains acetic acid and Triton X-100, a nonionic surfactant. Both components are added to stabilize the solution and prevent the adsorption of arsenazo III and arsenazo–metal complexes on the polymeric membrane reactor and mixing coil.

*Preconcentration*

The determination of uranium and thorium in a simple, low-ionic-strength matrix can be accomplished by direct injection of the sample. In many cases however, the levels of uranium and thorium may be very low (less than 50 μg/l) or may be present in a high ionic strength matrix. A high ionic strength matrix may compromise the analysis by overloading the separator column with high levels of alkali and alkaline earth metals. Additionally, these same interferences may saturate a conventional ion-exchange concentration column resulting in poor concentration efficiency from a high ionic strength matrix. The use of chelating resins as selective preconcentrators has been previously described for the selective preconcentration of transition metals from high ionic strength matrices [11]. Uranium and thorium can be selectively preconcentrated on an iminodiacetate chelating resin at pH 5.5. At this pH, the chelating resin is highly selective for transition and post-transition metals relative to alkali and alkaline earth metals. Alkaline earth metals which are weakly retained by the chelating resin are subsequently eluted to waste by an ammonium acetate wash of the resin. The ammonium acetate wash is performed at pH 5.5 where the selectivity of the resin for uranium and thorium relative to alkaline earth metals is optimum. The chelating resin, containing a weak acid functional group, has very low selectivity for most metals at low pH. Therefore, the concentrated uranium and thorium, as well as other concentrated transituon and post-transition metals, can be efficiently eluted from the concentrator column with the acid eluent used for the analytical separation. The use of chelating resins for sample pretreatment not only selectively concentrates uranium and thorium from high ionic strength matrices, but also eliminates alkali metals, alkaline earth metals and anions which were present in the original matrix.

## EXPERIMENTAL

*Experiment*

All chromatography was performed on a Dionex (Sunnyvale, CA, USA) 4500i ion chromatograph equipped with two quaternary gradient pumps (GPM-II), a reagent delivery module (RDM), and a variable wavelength UV–VIS detector (VDM-II). The entire flow path of the ion chromatograph was metal free, permitting the use of acid eluents. A Dionex IonPac CS-2 (250 × 4 mm) cation analytical column was used for the chromatography. The concentrator was a Dionex MetPac CC-1 (50 × 4 mm) containing an iminodiacetate-functionalized chelating resin. Postcolumn reagent addition and

mixing was done using a Dionex membrane reactor followed by a short delay coil to allow for complete reaction of the postcolumn reagent with the analytes. Data was collected and processed using Dionex AI-450 software.

The separation was accomplished on a Dionex CS2 column using a 15 minute linear gradient from 0.6 *M* HCl to 0.6 *M* HCl–0.5 *M* $Na_2SO_4$. The gradient was generated by proportioning with a gradient pump from reservoirs containing (1) 2.0 *M* HCl, (2) 1.0 *M* sodium sulfate and (3) deionized water. The eluent flow-rate was 1.0 ml/min. The postcolumn reagent consisted of 0.3 m*M* arsenazo III, 0.5 *M* acetic acid and 0.1% Triton X-100. The postcolumn flow rate was 0.5 ml/min and the reagent was added pneumatically using a Dionex membrane reactor with a mixing coil. Detection was by absorbance in the visible region at 660 nm.

*Chemicals*

Concentrated hydrochloric acid used to prepare the HCl eluent and glacial acetic acid for the postcolumn reagent were trace-metal grade from Fisher Scientific (Pittsburgh, PA, USA). Sodium sulfate eluent was prepared from anhydrous sodium sulfate (Fisher Scientific). Arsenazo III and Triton X-100 used for the postcolumn reagent were from Fluka (Ronkonkoma, NY, USA). Ultrapure 2.0 *M* ammonium acetate, pH 5.5 (for elution of alkaline earth metals from the concentrator column) was from Dionex. Deionized water (18 MΩ) was used to prepare all reagents and standards. 1000 ppm uranium and thorium atomic absorption standards were used as primary standards (Aldrich, Milwaukee, WI, USA).

## RESULTS AND DISCUSSION

*Direct injection*

The chromatography of uranium and thorium is illustrated in Fig. 1. The separation was accomplished using a 15 minute linear gradient from 0.6 *M* HCl to 0.6 *M* HCl–0.5 *M* $Na_2SO_4$. These were the conditions for all chromatograms run by both direct injection and with preconcentration. The separation was run at 1.0 ml/min for the eluent, and the postcolumn reagent was mixed in at 0.5 ml/min. The stability of the baseline is due to the pH remaining constant throughout the sodium sulfate gradient.

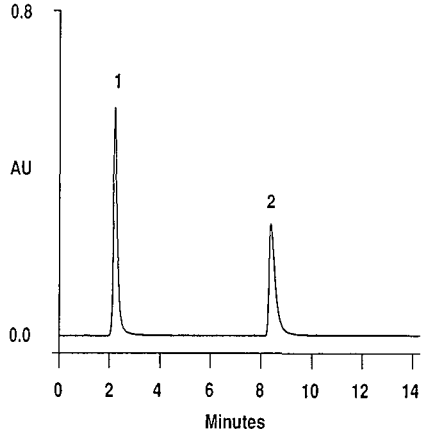


Fig. 1. Cation-exchange separation of uranium and thorium in reagent water. Direct injection (50 μl) of (1) 40 ppm uranium (as $UO_2^{2+}$) and (2) 20 ppm thorium. Dionex CS2 column, 15-min gradient from 0.6 *M* HCl to 0.6 *M* HCl–0.5 *M* $Na_2SO_4$. Eluent flow-rate 1.0 ml/min. Postcolumn reagent: 0.3 m*M* arsenazo III, 0.5 *M* acetic acid, 0.1% Triton X-100. Postcolumn flow-rate 0.5 ml/min. Visible detection at 660 nm.

Fig. 2 shows the analysis of an acid digested phosphate rock sample by direct injection. Although the sample contained percent levels of calcium, aluminum and iron, uranium was resolved from the major metal interferences. The sample was diluted 500× and a 50-μl sample loop was injected. The

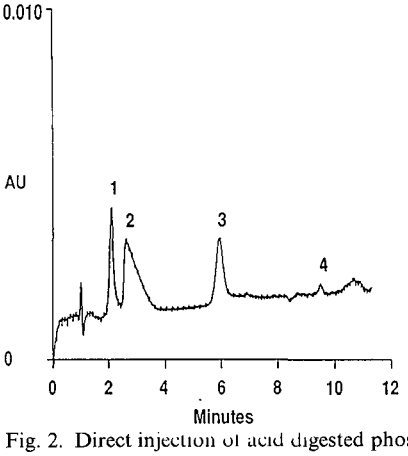


Fig. 2. Direct injection of acid digested phosphate rock sample, National Institute of Standards and Technology (NIST) Standard Reference Material (SRM 120c). Dilution of 500×. Conditions as in Fig. 1. Peaks: 1 = uranium; 2 = calcium (48.02% in rock as CaO); 3 = iron(III) (1.02% in rock as $Fe_2O_3$); 4 = thorium. Uranium: ion chromatography 108 ± 3 μg/g; certified value, 114.48 ± 1.7 μg/g ($n$ = 4).

value determined from this direct injection was close to the certified value of uranium in SRM 120c. A small thorium peak was detected, but no value for thorium was specified for SRM 120c. The determination of thorium may be problematic in the presence of several common inorganic ions. Fluoride, iodate, oxalate and phosphate all form insoluble precipitates with thorium even in strongly acidic (6 *M*) solutions [16]. This raises serious concerns that thorium analysis in many matrices which contain the previously mentioned ions may not be quantitative. Note that despite the high levels of calcium and iron in the rock sample, uranium was still resolved from both elements. Fig. 2 illustrates the need for concentration in uranium and thorium analysis. While the peaks are resolved from other metals in the sample, the calcium is very near the uranium peak. Additionally, the level of uranium (220 $\mu$g/l) is approaching the minimum detection limit for direct injection with a 50-$\mu$l sample loop. The low level of uranium as well as the calcium interference can be addressing by selective preconcentration on a chelating resin.

Fig. 3 illustrates the valving required to concentrate uranium and thorium on a chelating resin and selctively elute alkaline earth metals (chelation concentration). All aspects of the concentration step were controlled by a microprocessor-based gradient pump (chelation concentration pump). This pump was used to load sample onto a concentrator column as well as to control the valves in the system. A 5 ml sample loop was loaded with the raw sample buffered to pH 5.5 with an aliquot of 2.0 *M* ammonium acetate (ultrapure). The contents of the sample loop was loaded onto the chelation concentrator column (MetPac CC-1) by the chelation concentration pump. Loading the sample in this manner serves two functions. First, the pump loads the buffered sample onto the column where the uranium and thorium are retained. Second, because the chelation concentration pump is pumping 2.0 *M* ammonium acetate (pH 5.5), it selectively elutes the alkaline earth metals to waste while leaving the concentrated uranium and thorium on the concentrator column. Following the elution of the alkaline earth metals to waste a valve was actuated which places the chelation concentra-

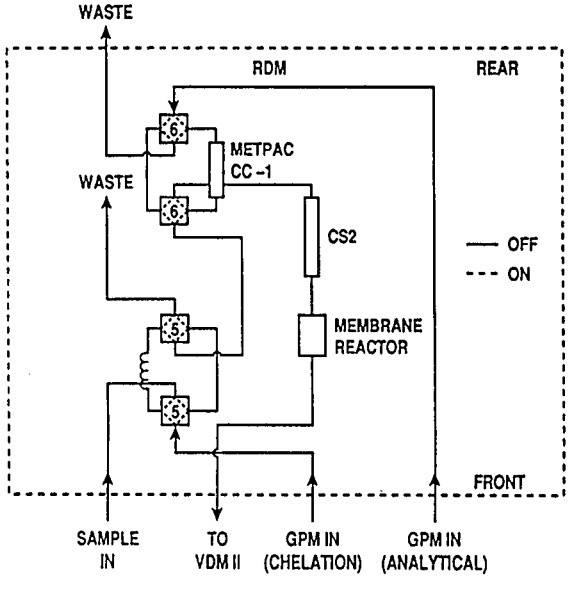


Fig. 3. Schematic diagram of system used for separation of uranium and thorium with on-line preconcentration using chelating resin. The valving is contained within the reagent delivery module. Two pumps are used, one to perform the analytical separation and one to perform the concentration and matrix elimination steps. The valves are controlled by the chelation concentration pump microprocessor.

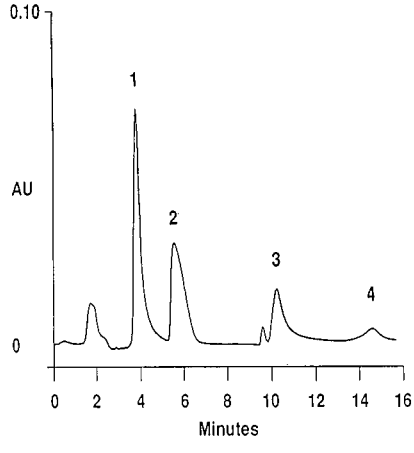


Fig. 4. Acid-digested phosphate rock sample using preconcentration on chelating resin. NIST Standard Reference Material (SRM 120c). Dilution of 500×. A 5.0-ml volume of buffered sample concentrated on MetPac CC-1 concentrator. Concentrator column washed with 6 ml of 2.0 *M* ammonium acetate prior to injection. Chromatographic conditions as in Fig. 1. Peaks: 1 = uranium (111.2 ppm in rock); 2 = iron(III) (1.02% in rock as $Fe_2O_3$); 3 = zirconium, hafnium and lanthanide metals; 4 = thorium (7.3 ppm in rock). Uranium: chelation ion chromatography, 111.2 ± 2.2 $\mu$g/g; certified value, 114.48 ± 1.7 $\mu$g/g. Thorium: chelating ion chromatography, 7.3 ± 0.6 $\mu$g/g; no certified value ($n$ = 4).

tion column in line with the analytical pump flow path. The uranium and thorium were eluted to the CS2 column where the previously described separation takes place.

Fig. 4 is an example of a chromatogram generated using preconcentration of uranium and thorium on a chelating resin. The sample, NIST SRM 120c, is the same sample shown by direct injection in Fig. 2. In Fig. 4, the sample was run by buffering the digested rock to pH 5.5 using ultrapure 2.0 *M* ammonium acetate and concentrating 5.0 ml of buffered sample on a chelating resin. Following sample loading, the chelating column was washed with ultrapure 2.0 *M* ammonium acetate to elute the alkaline earth metals to waste. Unlike Fig. 2 where a high level of calcium was observed near the uranium peak, using selective preconcentration of uranium and thorium followed by selective elution of the matrix, calcium was eliminated. Some metals present in the sample at low concentrations are concentrated with uranium and thorium. Among these metals which are concentrated and also detected by arsenazo III are iron, zirconium, hafnium and the lanthanides. None of these components interfere with the separation of uranium and thorium. The increase in retention time for uranium and thorium using the concentration method (Fig. 4) relative to direct injection (Figs. 1 and 2) is a result of the added capacity in the system due to the presence of the concentrator column. Note that relative to direct injection (Fig. 2), using preconcentration (Fig. 4) results in a greatly enhanced signal to noise ratio for uranium as well as higher accuracy when compared using the NIST certified value.

Fig. 5 shows the analysis of seawater for uranium. The sample was a seawater standard reference material from the Canadian Marine Analytical Chemistry Standards Program (National Research Council of Canada). The sample, NASS-2 (open ocean seawater) has certified values for trace elements including uranium. The certified value for uranium in NASS-2 is 3.00 μg/l. The sample was run using 5.0-ml, 10.0-ml and 20.0-ml sample loops for concentration. The results of the analysis agreed with certified values for uranium in all cases (Fig. 5). The reproducibility of the method, as evidenced by the standard deviation, was good, particularly when larger quantities of sample were concentrated.

## CONCLUSIONS

A cation-exchange separation of uranium and thorium has been developed. The separation uses a hydrochloric acid eluent with a sodium sulfate gradient to efficiently separate uranium and thorium as well as several interfering metals. Detection based on postcolumn addition of acidic arsenazo III provides a sensitive and specific detection scheme for uranium and thorium. The detection limit by direct injection is 20 μg/l for uranium and 60 μg/l for thorium using a 50-μl sample loop. The use of chelating stationary phases for sample preconcen-

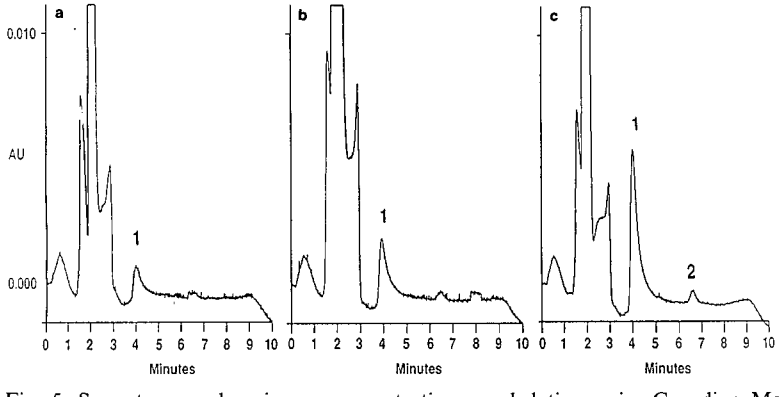


Fig. 5. Seawater sample using preconcentration on chelating resin. Canadian Marine Analytical Chemistry Standards Program (National Research Council of Canada) seawater sample NASS-2. Dilution 2× with ammonium acetate buffer. Concentration on MetPac CC-1 concentrator of (a) 5.0, (b) 10.0 and (c) 20.0 ml of sample. Concentrator column washed with 6 ml of 2.0 *M* ammonium acetate prior to injection. Chromatographic conditions as in Fig. 1. Peaks: 1 = uranium (certified value 3.00 μg/l); 2 = iron(III). Uranium values from ion chromatography: (a) 3.14 ± 0.25 μg/l; (b) 3.12 ± 0.18 μg/l; (c) 3.02 ± 0.04 μg/l ($n$ = 4).

tration has led to an enhancement of detection limits for uranium and thorium as well as elimination of potential interferences. Using a chelating resin and concentrating 5.0 ml of sample, the detection limit has been extended to 1.0 μg/l for uranium and 3.0 μg/l for thorium. Additionally, the use of chelating resins for concentration has greatly increased the scope of matrices which may be analyzed for uranium and thorium without significant interference from the matrix.

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# Dual-column techniques for the simultaneous analysis of anions and cations

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ABSTRACT

A dual-column technique for the simultaneous analysis of anions and cations is described. The technique involves the use of the conventional ion chromatography equipment with the addition of a switching valve. Two columns (an anion and a cation column) are used as the separator columns. By using an eluent that contains both anion and cation driving ions, the simultaneous separation of anions and cations can be accomplished with one injection, one pump, and one detector. Two eluents are developed for the simultaneous analysis of anions, monovalent cations and divalent cations. The detection limits for most ions are below 400 μg/l.

## INTRODUCTION

Ion chromatographic (IC) analysis of anions and cations are performed mostly independent of one another using separate columns, eluents and detectors. For samples that require the determination of both anions and cations, the use of separate methods tend to be expensive and time consuming. In order to simplify the analysis, several techniques for the simultaneous analysis of both anions and cations have been investigated. One technique uses a dual-channel instrument in which the anions and cations are determined via separate channels [1]. Using this approach, two separate pumps, eluents, columns and detectors are required. Another technique uses chemical derivatization procedures in which the cations are converted to anions and separated on the anion column, together with the common inorganic anions [2,3]. This approach requires only conventional IC equipment, however, it is limited to cations that can form anionic complexes with the eluent. Another technique uses a series of three columns and two detectors in order to perform the separation and detection [4,5]. This method uses a suppressor column to remove the cations from the system after they have been detected in the first detector and before the anions are detected in the second detector preventing peak overlap between the anions and cations. Another approach uses two columns connected in series followed by a differential conductivity detector [6]. This approach is simpler than the previous approaches, however, the ion-exchange capacities of the two columns and the ionic strength of the eluent must be carefully controlled to provide the appropriate retention times and to prevent peak overlapping between the anions and cations.

The use of a single ion-exchange column which exhibits both anion- and cation-exchange capacity has also been used. Pietrzyk and Brown [7] used a column containing alumina and silica microparticles. At an eluent pH of about 5, alumina acts as an anion exchanger and silica acts as a cation exchanger, thus allowing simultaneous separation of anions and cations. A mixed-bed ion-exchange column containing polystyrene–divinylbenzene resin with quaternary amine and sulfonic acid functionalities has also been used [8]. By using an eluent that contains both anion and cation eluting ions, the simultaneous separation of inorganic anions and cations can be performed with one injection, one pump, one column and one detector. The advantage of this approach is that the analysis can be accomplished using existing single-column ion

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chromatography systems without the need for additional equipment. However, since the column is made by mixing two type of ion exchangers, column-to-column reproducibility is hard to achieve.

An improved method for the simultaneous analysis of anions such as fluoride, chloride, nitrite, bromide, nitrate, phosphate and sulfate and cations such as sodium, ammonium, potassium, magnesium and calcium was developed [9]. This dual-column technique uses one injection valve, one pump, an anion column, a cation column, a switching valve and a conductivity detector. By changing the eluent composition, the simultaneous analysis of anions and monovalent cation, or anions and divalent cations can be achieved. This report extends the applications of the dual-column technique to allow simultaneous analysis of both monovalent, and divalent cations in one run along with the anions.

## EXPERIMENTAL

Chromatography was performed on an Alltech (Deerfield, IL, USA) IC system which consists of a Model 325 metal-free pump, a Rheodyne 9125 metal-free injection valve (100-$\mu$l sample loop), a Model 320 conductivity detector and a Timberline (Boulder, Co, USA) column heater. The temperature of the column heater and the conductivity detector cell was maintained at 35°C. A Rheodyne Model 9000 metal-free switching valve combined with the Alltech universal valve actuator was used to direct the eluent flow through or around the cation separator column. A Spectra-Physics (Santa Clara, CA, USA) SP 4400 Chromjet integrator was used to record all data. The Alltech Universal Anion Column (150 mm × 4.6 mm I.D., 100 mm × 4.6 mm I.D. and 50 mm × 4.6 mm I.D.) and the Alltech Universal Cation Column (100 mm × 4.6 mm I.D.) were used as the separator columns.

### *Reagents*

Only reagent-grade chemicals (Aldrich, Milwaukee, WI, USA) were used for standard an eluent preparations.

Two eluents were used: phthalic acid and a mixture of phtalic acid and 1,2,4,5-benzenetetracarboxylic acid. Stock solution of phthalic acid (200 m*M*) was prepared by dissolving the ACS reagent-grade chemicals in methanol. This solution was diluted with deionized HPLC-grade water (Alltech) to make 5 m*M* phthalic acid eluent. Phthalic acid–benzenetetracarboxylic acid eluent was prepared by diluting the phthalic acid stock solution to 3 m*M* and adding 1,2,4,5-benzenetetracarboxylic acid to make 0.3 m*M* solution.

### *Procedure*

Fig. 1 shows the system configuration. This technique consists of three steps:

*Step 1.* At position A where the eluent is passed through both anion and cation columns, a sample is injected. Since cations are not retained on the anion column, they will rapidly pass through the anion column.

*Step 2.* Once the cations reach the inlet of the cation column, the valve is switched to position B. At this position, the cation column is bypassed, trapping the sample cations at the inlet of the cation column. All the anions are separated and detected by the conductivity detector.

*Step 3.* When all the anions have eluted from the anion column, the valve is switched back to position

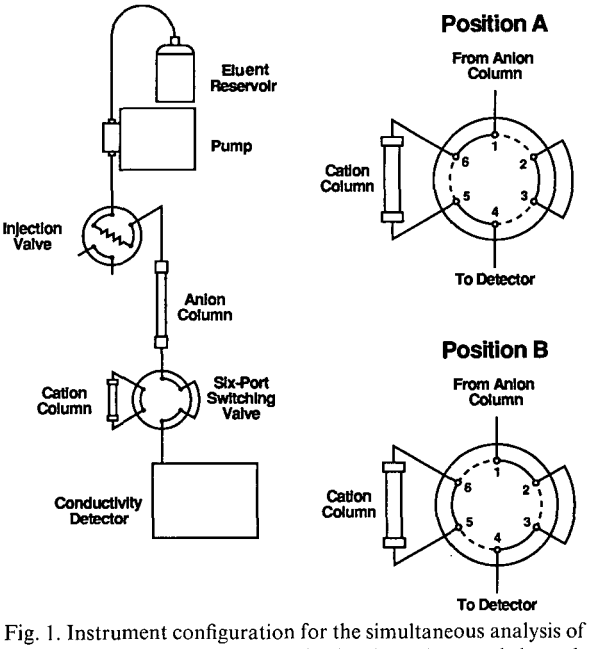


Fig. 1. Instrument configuration for the simultaneous analysis of anions and cations. At position A, the eluent is passed through both anion and cation columns. At position B, the cation column is bypassed.

A redirecting the eluent to separate the cations that are retained at the inlet of the cation column.

The timing of step 2 is very important. If the valve is switched too early, the cations may not reach the cation column. The cations that do not reach the cation column will not be separated and pass rapidly through the anion column in the void volume. If the timing is delayed too long, the cations may be separated on the cation column and eluted along with the other anions. This may cause peak overlapping (anion and cation) and inaccurate results. The exact time to switch the valve in step 2 can be determined easily by injecting an anion standard on an anion column with the valve at position B (the cation column is bypassed). Since cations are not retained on the anion column, they will pass rapidly through the anion column in the column void volume along with other non-retained components. The first peak (solvent peak) on a chromatogram is attributed to these non-retained components. The retention time at which the solvent peak returns to baseline is used as the exact time to switch the valve from position A to position B. The timing for step 3 is not as critical as step 2. The valve may be switched at any time after all the anions are eluted from the anion column. The retention time for the cations (and the total analysis time) is dependent on the timing of step 3. If the valve is switched immediately after all the anions are eluted, the retention times for the cations (and the total analysis time) will be shorter. If it is delayed, the retention times and the whole analysis time will be longer. The switching valve may be operated manually, or once the proper timing is determined, automatically using an electronically actuated switching valve and data system.

## RESULTS AND DISCUSSION

In the earlier works, three eluents were developed to separate anions and monovalent cations, or anions and divalent cations [9]. The simultaneous separation of both monovalent and divalent cations along with the anions could not be achieved using these eluents. The goal of this work was to develop a method for the simultaneous determination of anions, monovalent cations and divalent cations. The determination of both group I and group II cations is important in a variety of samples.

The two most important criteria when developing a simultaneous method for analyzing anions and cations are choosing the appropriate column and eluent. The column must be able to separate the species of interest. The eluent must be able to elute both the anions and the cations of interest. When a conductivity detector is used, the difference in the equivalent conductance between the eluent and the solutes must be as large as possible in order to observe the signals. The only column that is capable of separating both monovalent and divalent cations under isocratic condition is the silica-based polymer-coated stationary phase developed by Schomburg *et al.* [10]. The Alltech Universal Cation Column which is packed with similar stationary phase (silica-based column coated with polybutadiene–maleic acid copolymer) was used to separate the cations. Anions are separated on the Universal Anion Column. This column, which is packed with hydroxyethylmethacrylate-based anion exchanger, has been shown to be useful for the separation of anions using a wide variety of eluents [11].

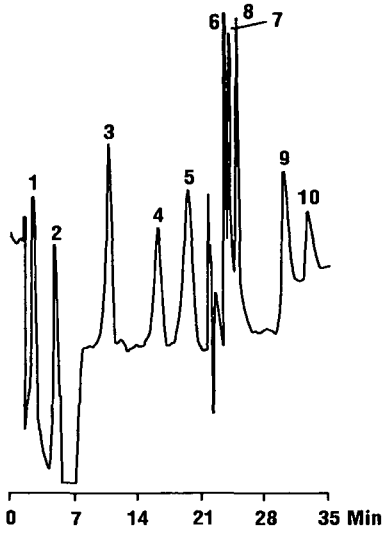


Fig. 2. Simultaneous analysis of anions, monovalent cations and divalent cations using 5 m*M* phthalic acid eluent. Column: Alltech Universal Anion Column (150 mm × 4.6 mm I.D.) and Alltech Universal Cation (100 mm × 4.6 mm I.D.). Eluent flow-rate: 1.0 ml/min. Detector: conductivity, 1.0 μS full scale. Peaks: 1 = fluoride (10 ppm); 2 = phosphate (20 ppm); 3 = chloride (10 ppm); 4 = bromide (20 ppm); 5 = nitrate (20 ppm); 6 = sodium (6 ppm); 7 = ammonium (4 ppm); 8 = potassium (12 ppm); 9 = magnesium (3 ppm); 10 = calcium (5 ppm).

It has been reported that the retention and separation of monovalent cations on the polybutadiene–maleic acid copolymer-coated stationary phase is achieved through conventional cation-exchange mechanisms, while divalent cations are separated through coordination with maleic acids [10,12]. Effective eluents for the separation of cations on this stationary phase are organic acids such as citric, phthalic and salicylic, which are capable of forming complexes with calcium and magnesium [10]. A mixture of nitric acid and ethylenediaminetetraacetic acid has also been used [12]. Experiments in our laboratory showed that the isocratic separation of monovalent and divalent cations can also be accomplished using nitric acid, hydrochloric acid and trifluoroacetic acid. Therefore, complexing eluent is not required to achieve these separations. Since phthalic acid is the most common eluent used with the Universal Anion Column, phthalic acid was chosen as the eluent for the simultaneous anion/cation analysis in this study. Fig. 2 shows the chromatogram of the anion and cation standards using 5 m*M* phthalic acid as the eluent. Using this eluent, fluoride phosphate, chloride, bromide, nitrate, sodium, ammonium, potassium, magnesium and calcium are separated and detected simultaneously. Phthalate is the driving ion for the anions, hydronium ion is the driving ion for the monovalent cations and the divalent cations are retained and separated through the formation of coordination complexes with phthalic acid and maleic acid on the stationary phase. Since the equivalent conductance for the anions is higher than the equivalent conductance for phthalate ion, anions are detected as positive peaks. However, the equivalent conductance for the cations is lower than the equivalent conductance for the hydrogen ion (phthalic acids); thus, the cation peaks are detected as negative peaks (decrease in conductance). The polarity of the detector is reversed after the valve is switched back from position B to position A to make the cation peaks appear as positive peaks. At the pH of the eluent (approximately 2.8) used, phosphate is present as dihydrogenphosphate and eluted between fluoride and chloride. At this pH, phthalate is present mostly in $-1$ charge and not capable of eluting divalent anions such as sulfate. The separation of some real samples using this eluent are shown in Fig. 3. The apple and grape juices are diluted and filtered through the Anotop IC (Alltech) disposible syringe filters before injection. Cereal sample was extracted with deionized HPLC-grade water and filtered through a syringe filter before injection. As shown in Fig. 3A and B, acetate can also

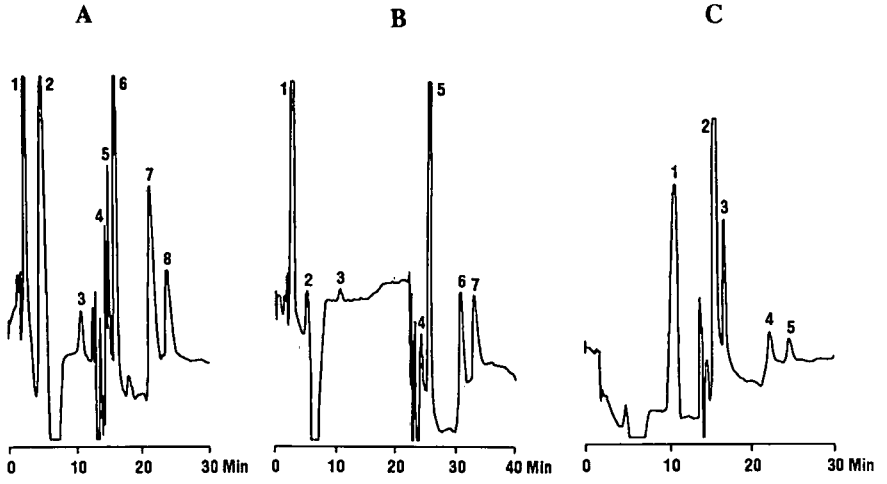


Fig. 3. Simultaneous separations of anions and cations in grape juice, apple juice and cereal using 5 m*M* phthalic acid eluent. Other chromatographic conditions as in Fig. 2. (A) Grape juice; peaks: 1 = fluoride; 2 = phosphate; 3 = chloride; 4 = sodium; 5 = ammonium; 6 = potassium; 7 = magnesium; 8 = calcium. (B) Apple juice; peaks: 1 = fluoride; 2 = phosphate; 3 = chloride; 4 = sodium; 5 = potassium; 6 = magnesium; 7 = calcium. (C) Cereal; peaks: 1 = chloride; 2 = sodium; 3 = potassium; 4 = magnesium; 5 = calcium.

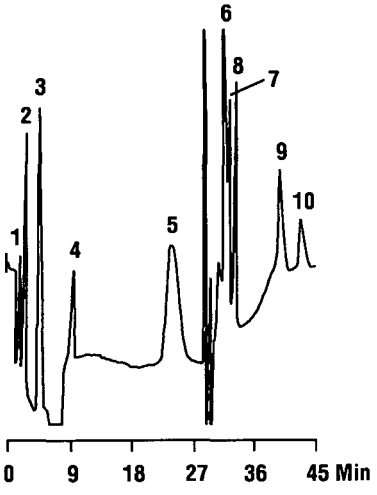


Fig. 4. Simultaneous analysis of anions, monovalent cations and divalent cations using 3 m*M* phthalic acid–0.3 m*M* benzenetetracarboxylic acid eluent. Column: Alltech Universal Anion Column (100 mm × 4.6 mm I.D.) and Alltech Universal Cation (100 mm × 4.6 mm I.D.). Eluent flow-rate: 1.0 ml/min. Detector: conductivity, 1.0 μS full scale. Peaks: 1 = fluoride; 2 = phosphate; 3 = chloride; 4 = nitrate; 5 = sulfate; 6 = sodium; 7 = ammonium; 8 = potassium; 9 = magnesium; 10 = calcium.

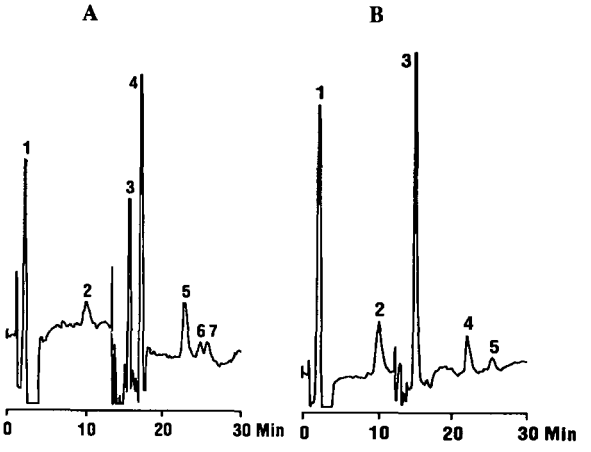


Fig. 5. Simultaneous separation of anions and cations in orange juice and bottled drinking water using 3 m*M* phthalic acid–0.3 m*M* benzenetetracarboxylic acid eluent. Column: Alltech Universal Anion Column (50 mm × 4.6 mm I.D.) and Alltech Universal Cation column (100 mm × 4.6 mm I.D.). Eluent flow-rate: 1.0 ml/min. Detector: conductivity, 1.0 μS full scale. (A) Orange juice; peaks: 1 = chloride; 2 = sulfate; 3 = sodium; 4 = potassium; 5 = magnesium; 6 = iron; 7 = calcium. (B) Bottled drinking water; peaks: 1 = chloride, 2 = sulfate; 3 = sodium; 4 = magnesium; 5 = calcium.

be analyzed in the same run. If fluoride is present in the sample, it will coelute with acetate. Phosphate and nitrite will also coelute under this condition. Other organic acids such as citric and maleic may also present in fruit juices. At the eluent pH used, they are not ionized and eluted in the column void volume. The total analysis time in Fig. 3B is approximately 10 min. longer than the total analysis time in Fig. 3A. This is an example of poor timing in step 3, where the valve was not switched immediately after all the anions are eluted. In Fig. 3C, since chloride is the only anion present in the sample, the valve was switched from position B back to position A immediately after the chloride is eluted. This reduces the total analysis time from 35 to 25 minutes.

In order to determine sulfate in the same run, another eluent was developed. Phthalic acid may be used at a higher pH in order to elute sulfate. However, at higher pH, the carboxyl functional groups on the cation column becomes more ionized and will strongly retain the monovalent and divalent cations. In order to be able to elute the cations, low-pH must be used. Benzenecarboxylic acids have been shown to be a useful eluent for separating anions in non-suppressed IC [13]. Tetraprotic acids such as 1,2,4,5-benzenetetracarboxylic acid can be effective eluents for divalent anions even at low pH values. Fig. 4 shows the separation of anions and cations using 3 m*M* phthalic acid–0.3 m*M* benzenetetracarboxylic acid as the eluent. Benzenetetracarboxylic acid is added to increase the strength of the eluent for eluting divalent anions. Using this eluent, fluoride, phosphate, chloride, nitrate, sulfate, sodium, ammonium, potassium, magnesium and calcium can be analyzed in the same run in approximately 45 min. When a smaller number of ions present in the sample, the total analysis time can be shorten by using a shorter column as shown in Fig. 5A and B. In Fig. 5, only chloride and sulfate are separated on the anion column. In addition to the cations mentioned earlier, iron(II) can also be analyzed in the same run. As with the 5 m*M* phthalic acid eluent, the polarity of the detector must be reversed after the valve is switched back from position B to position A to make the cation peaks positive.

A slight baseline shift occurs when the cation column is switched in and out of the flow stream. This baseline shift may be due to differences in the distri-

TABLE I

LINEAR REGRESSION ANALYSES AND SIMPLE CORRELATION COEFFICIENTS OF THE POTASSIUM NITRATE AND CALCIUM BROMIDE CALIBRATION PLOTS

$y$ represents the peak area; $c$ represents the concentration of ions.

| Ions | Regression equation | Correlation coefficient ($r$) |
|---|---|---|
| Nitrate | $y = 0.39 + 0.08c_{\text{nitrate}}$ | 1.00 |
| Potassium | $y = 0.25 + 0.10c_{\text{potassium}}$ | 1.00 |
| Bromide | $y = -0.06 + 0.06c_{\text{bromide}}$ | 1.00 |
| Calcium | $y = -0.03 + 0.20c_{\text{calcium}}$ | 1.00 |

bution coefficient for the eluent components on the anion and cation stationary phases [14]. When the cation column is bypassed, an ion-exchange equilibrium is established between the anion driving ion and the anion exchanger. When the valve is switched, a second equilibrium is established between the cation driving ion and the cation exchanger, thus, a baseline shift results. However, this shift does not adversely affect the linearity or precision of this method. Linear regression analyses and simple correlation coefficients of the calibration plots of peak area against ionic concentration for potassium nitrate (0–100 ppm nitrate) and calcium bromide (0–100 ppm bromide) using 5 m$M$ phthalic acid as the eluent are listed in Table I. These results are comparable to those obtained in normal anion and cation chromatography using separate columns and eluents.

A standard solution containing various anions and cations was analyzed to determine the reproducibility of the method using one of the eluents developed. Table II lists the relative standard deviations (R.S.D.) for seven replicate injections using 5 m$M$ phthalic acid eluent. The R.S.D. ranged from 0.60 to 3.00%. This is, again, comparable to single-column IC analysis of anions and cations using separate columns and eluents.

TABLE II

REPRODUCIBILITY OF THE SIMULTANEOUS SYSTEM USING 5 m$M$ PHTHALIC ACID ELUENT

| Ions | Concentration (ppm) | R.S.D. (%) ($n=7$) |
|---|---|---|
| Fluoride | 10 | 1.05 |
| Chloride | 10 | 2.90 |
| Bromide | 20 | 1.90 |
| Nitrate | 12 | 3.00 |
| Potassium | 19 | 1.30 |
| Magnesium | 3.2 | 2.02 |
| Calcium | 5.0 | 0.60 |

TABLE III

DETECTION LIMITS WITH 5 m$M$ PHTHALIC ACID ELUENT

| Ions | Detection limits ($\mu$g/l) |
|---|---|
| Fluoride | 50 |
| Phosphate | 0.28 |
| Chloride | 300 |
| Bromide | 380 |
| Nitrate | 560 |
| Potassium | 20 |
| Magnesium | 40 |
| Calcium | 14 |

The detection limits for various ions (expressed as minimum detectable concentration) with 5 m$M$ phthalic acid eluent are shown in Table III. The numbers were obtained based on a 100-$\mu$l injection volume and were calculated as a threefold signal-to-noise ratio at the baseline. The detection limits for most ions are less than 0.4 mg/l.

## CONCLUSIONS

The technique developed in this study is useful when the determination of both anionic and cationic fractions of the sample are required. Instead of performing two chromatographic analyses using two different eluents, this technique offers a simpler, cheaper, and faster method for the determination of anions and cations. Only conventional IC equipment with the addition of a switching valve is required. Using 5 m$M$ phthalic acid eluent, fluoride, phosphate, chloride, bromide, nitrate, lithium, sodium, ammonium, potassium, magnesium and calcium can be analyzed simultaneously. The 3 m$M$ phthalic acid–0.3 m$M$ benzenetetracarboxylic acid eluent allows for the determination of fluoride, phosphate, chloride, nitrate, sulfate, lithium, sodium, ammonium, potassium, magnesium, iron and calcium. One major advantage of this method is that it allows the analyst to minimize the run time depending on the nature of the sample.

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CHROMSYMP. 2546

# Application of ion chromatography to corrosion studies

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ABSTRACT

The corrosion of metals is controlled by the chemical composition of the solution at the metal surface. During localized corrosion, the composition of the solution close to the metal/solution interface can become very different from the bulk solution composition due to the occurrence of electrochemical and chemical reactions at the surface. Considering the controlling influence of this solution, determining its composition would be indispensable for improving the understanding of metallic corrosion. Analysis of the transition metal dissolution products of stainless steel under uniform corrosion, pitting corrosion and crevice corrosion conditions has been accomplished.

## INTRODUCTION

The corrosion of metals is controlled by the chemical composition of the solution at the metal surface. This solution can often have a radically different composition from that of the bulk. Such a situation can develop on metal surfaces due to restricted mass transport and its coupling to the electrochemical and chemical reactions which cause corrosion. One example where this is of importance is localized corrosion.

Localized corrosion of metals and alloys in aqueous environments is an especially damaging form of environmental attack due to its unpredictability, the difficulties involved in its detection, and the limited efficacy of mitigation strategies. Since localized corrosion is the major corrosion problem for many highly alloyed materials, efforts aimed at understanding its various forms have been the focus of much attention. It has become generally accepted that the cause of the propagation of localized corrosion sites (*e.g.*, pits, crevices, cracks) is the development of an highly aggressive environment in the occluded cell which comprises the area of attack. While there have been recent advances in the modelling and simulation of such environments, the measurement of the development of the chemistry of these sites has not been possible. Hence, the observed improvement of the localized corrosion resistance of stainless steels with the addition of various alloying elements (such as nitrogen and molybdenum) and solution inhibitors (such as sulfate and nitrate) has remained unexplained.

While there are a variety of types of localized corrosion phenomena (*e.g.*, crevice corrosion, pitting, intergranular attack, stress-corrosion cracking) they share a number of commonalties. Foremost among these is the development of an extremely aggressive solution locally which causes high rate, localized attack. It is becoming increasingly clear that much can be understood about the different types of localized corrosion by considering them different manifestations of the same basic phenomena [1–3]. For example, pitting can be viewed as crevice corrosion on a smaller scale, with either micropores or surface heterogeneities acting to form the crevice. The controlling factor in localized corrosion is the development and maintenance of the aggressive chemistry at the local site. Despite its importance, little is known about this chemistry.

Crevice corrosion occurs on a metal or alloy at locations where the bulk solution has restricted access. This can occur at the meeting points of flanges and gaskets. The rate of attack inside the crevice

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formed by the gasket can be orders of magnitude higher than the rate on the surfaces that are boldly exposed to the bulk electrolyte. In order to understand this type of attack, a better insight into the chemistry of the localized site and how it changes with time is required. However, the sampling of the most important solution is the most difficult due to the restricted geometry. By their very nature, crevices contain small volumes of electrolyte. Past work [1,3] has preliminarily characterized the solution chemistry for an established localized corrosion site in stainless steels as a low pH, concentrated metal chloride solution. However, a great deal of important information concerning the solution is missing. For example, the presence and concentration of the various alloying elements in the localized corrosion site is not well characterized. In addition, the valence of some species in these solutions is also uncertain. The excellent mass sensitivity of ion chromatography, along with its ability to speciate the ionic state of the elements, makes it extremely attractive for studying the localized corrosion behavior of metals and alloys. Its ability to handle small injection volumes also makes it amenable to cases of analysis of the small volumes associated with localized corrosion.

This paper reports the initial results of the application of ion chromatography (IC) to the localized corrosion of stainless steels. The effects of solution and alloy composition as well as potential on the transition metal content of articial pits are shown. In addition, the extraction and analysis of extremely small volumes of solution from a real crevice formed by pressing a deformable material onto stainless steel is demonstrated.

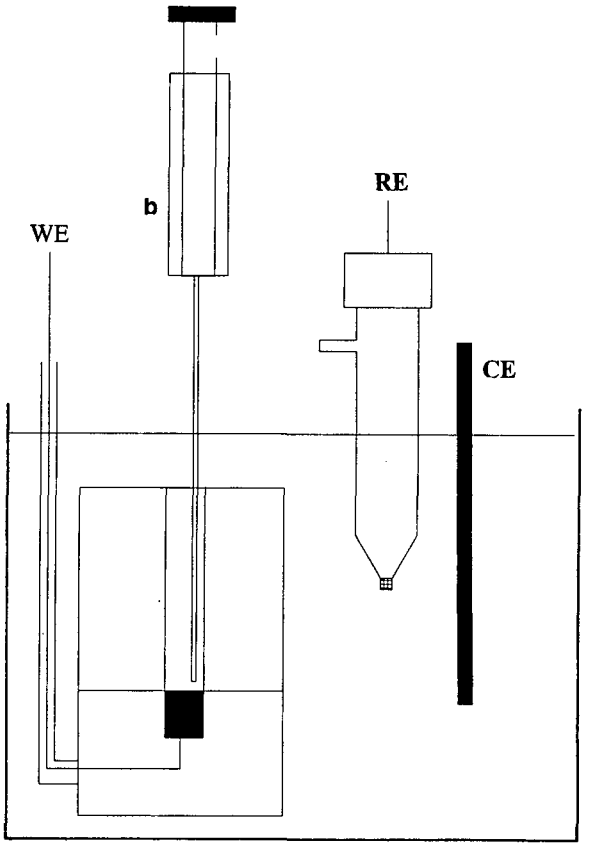


Fig. 1. Schematic of artificial pit apparatus. WE = Working electrode connection to voltage control device (potentiostat). The stainless-steel specimens were the working electrodes. RE = Reference electrode connection to potentiostat. A saturated calomel electrode was used. CE = Counter electrode connection to potentiostat. A graphite rod was used. b = 1-ml syringe used to withdraw solution from artificial pit cavity. Clamping device used to hold artificial pit together not shown.

## EXPERIMENTAL

### *Electrochemical testing*

Fig. 1 shows a schematic of the experimental arrangement. A 6-mm diameter rod of stainless steel (SS, Type 304 or 316) was mounted in a room temperature curing epoxy. Electrical connection was made to the specimen via a previously spot-welded wire. The surface was ground flat and polished with silicon carbide paper of successively finer grit size, ending with 600 grit. An artificial pit was formed by placing another cylinder of the epoxy, with a 6-mm hole drilled through it, on top of the mounted stainless steel so that the hole was concurrent with the specimen surface. The upper cylinder was held in place via a clamping device. The entire artificial pit was then placed in a beaker containing the test solution (either 1 *M* hydrochloric acid or 1000 mg/l hydrochloric acid + 1000 mg/l sodium chloride). This "lead in pencil" arrangement insures that the dissolution products which form during the test remain in the artificial pit cavity due to the restricted diffusion. A more detailed description of the artificial pit method can be found elsewhere [4]. The length of the upper cylinder (25 mm) as compared to the electrode diameter leads to an estimate of 100

h for the diffusion time, if one dimensional diffusion is assumed to occur. No test reported here lasted longer than 2 h.

A saturated calomel (SCE) reference electrode and a graphite rod to be used as a counter electrode were also placed in the beaker as shown in Fig. 1. The beaker was open to air. A Princeton Applied Research Model 173 Potentiostat with a Model 276 Computer Interface applied a set potential between the working electrode (the stainless steel) and the reference electrode. All potentials are quoted *versus* SCE. A typical experiment consisted of first immersing the artificial pit and polarizing it to a set potential for a set period of time. During this time, the current necessary to maintain the potential was recorded. At the end of the set time, a 1-ml syringe was used to remove 700 $\mu$l of the artificial pit solution. This sample was placed in a 1-ml vial. It was diluted as necessary with 18 M$\Omega$ cm water from a Barnstead purification system.

*Chromatography*

For all experiments, a Waters Action Analyzer was used. The system is equipped with a Model 486 tunable absorbance detector, along with a reagent delivery module. A Rheodyne injector was used with a 100-$\mu$l fixed loop. For the analysis of transition metals, a gradient method was developed to allow for the simultaneous determination of $Fe^{3+}$, $Cr^{3+}$, $Cu^{2+}$, $Pb^{2+}$, $Zn^{2+}$, $Ni^{2+}$, $Co^{2+}$, $Fe^{2+}$ and $Mn^{2+}$. This method is a gradient adaptation of a method developed for transition metals by Cassidy and Elchuk [5] in which separation occurs on a $C_{18}$ column that is dynamically coated with sodium octanesulfonate. The initial eluent is a pH 3.4, 20 m*M* tartrate buffer, with 2 m*M* sodium octanesulfonate and 5% acetonitrile. After 4.5 min at 0.8 ml/min isocratically, the tartrate concentration is increased parabolically to 35 m*M* over the next 15.5 min as shown in Fig. 2. During this time the other conditions (pH, flow-rate, octanesulfonate and acetonitrile concentrations) are kept constant. Post-column derivatization was done with PAR [0.2 m*M* 4-(2-pyridylazo)resorcinol, 3 *M* $NH_4OH$, 1 *M* acetic acid]. It was combined with the eluent via nitrogen gas pressure at 0.5 ml/min. The resulting metal ion–PAR complex was detected by measuring the absorbance at 500 nm.

Calibration curves were generated which included within their range the concentrations expected in the test solutions. All calibration curves showed excellent linearity, with correlation coefficients of greater than 0.995 for concentration ranges from 0.01 to 2 ppm for all ions for which analyses were made. Further details of the development and statistical analysis of the method will appear elsewhere [6].

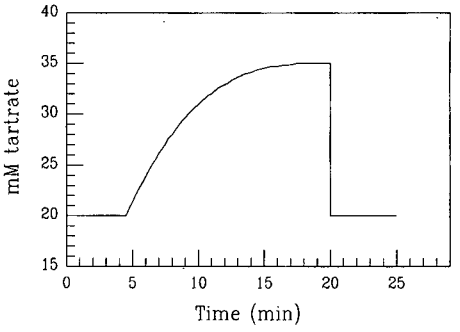


Fig. 2. Tartrate gradient profile used for the transition metal analysis. The concentrations of the other constituents were held constant throughout the analysis: pH 3.4, 2 m*M* sodium octanesulfonate and 5% acetonitrile. The eluent flow-rate was 0.8 ml/min, and the post-column reagent flow-rate was 0.5 ml/min.

## RESULTS AND DISCUSSION

Fig. 3 shows the chromatogram of a standard solution consisting of 1 mg/l of each of the metals indicated in the figure. Related work [7] has shown that the sensitivity of this approach allows 10 $\mu$g/l or less of each metal to be detected. It is clear from

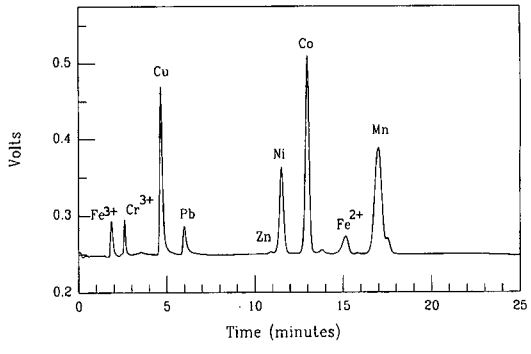


Fig. 3. Chromatogram of 1 ppm each of the transition metals labeled. The voltage cited is the output of the absorbance detector. The standard was made in high purity water from atomic absorption standards. The small Zn peak is an impurity.

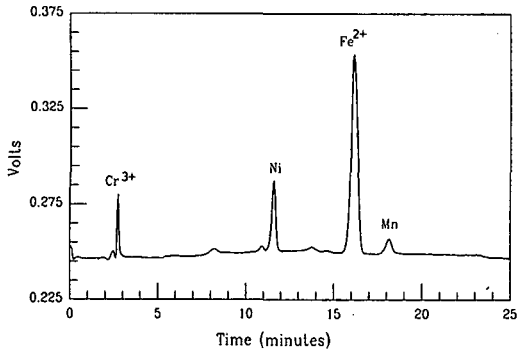


Fig. 4. Chromatogram of solution removed from artificial pit apparatus with 304 SS in 1 *M* hydrochloric acid, −0.1 V (SCE).

this figure that detection of the different transition metals in solution is straightforward. For iron, it is also possible to discriminate between the ferrous and ferric states.

Since it is generally accepted that the environments within localized corrosion sites of stainless steel have low pH and a high [$Cl^-$], some occluded cell experiments were performed in 1 *M* hydrochloric acid. The chromatogram which results from analysis of the solution extracted from an artificial pit of 304 SS held at −0.1 V (SCE) is shown in Fig. 4. An analysis of the relative abundance of the different metals in the extracted pit solutions is shown in Table I, along with the results for 304 SS held at a higher potential. It is immediately clear that Cr is underrepresented in the solution compared to its concentration in the alloy (18%), while Ni (10% in alloy) and Mn (< 2% in alloy) have been preferentially dissolved.

The results of potentiostatically holding 304 SS at +0.7 V (SCE) for 30 min in a less aggressive solution (1000 mg/l sodium chloride + 1000 mg/l hydrochloric acid) on the solution composition within the artificial pit are shown in Fig. 5. Pitting, rather than uniform dissolution, occurs under these conditions. The large amount of manganese relative to its concentration in the base alloy again indicates preferential dissolution of Mn (most likely from MnS inclusions), while again $Cr^{3+}$ is underrepresented when compared to its concentration in the alloy. It should be noted that under these conditions (a higher pH compared to the 1 *M* hydrochloric acid), some Cr may have dissolved but precipitated as chromium oxide, and would therefore not be detected. The origin of $Fe^{3+}$ is most likely the oxidation of the $Fe^{2+}$ by dissolved oxygen. Changes in the experimental apparatus to allow for deaeration are planned. The splitting of the $Fe^{2+}$ peak only occurs when there is a high concentration of $Cl^-$ in the injected sample and may be due to the presence of a ferrous chloride complex. This would be analogous to the aluminum fluoride complexes previously detected chromatographically [8].

A 304 SS sample was partially covered with a piece of PTFE to form a crevice through the application of pressure from the clamping device. This simulates the type of crevice corrosion testing used [9], as well as the crevice formed by a gasket, for example. It was also felt to provide a challenging test for the experimental approach, as the crevice volume in this case would be well less than 1 μl. The sample was then polarized to +0.2 V (SCE) where crevice corrosion initiated almost immediately. After 3700 s, the sample was removed from the solution, the clamp carefully loosened and the two faces of the crevice rinsed with 300 μl of 18 MΩ cm water. This solution was then analyzed for transition metals after a 4× dilution with the chromatogram

TABLE I

PERCENTAGE OF TRANSITION METALS IN ALLOYS AND IN EXTRACTED ARTIFICIAL PIT SOLUTIONS

| Metal | 304 alloy composition | 316 alloy composition | 304, 1 *M* HCl, −0.2 V (SCE) | 316, 1 *M* HCl, −0.2 V (SCE) | 304, 1 *M* HCl, −0.1 V (SCE) |
|---|---|---|---|---|---|
| $Cr^{3+}$ | 18 | 17 | 7.3 | 7.1 | 10.0 |
| $Ni^{2+}$ | 10 | 12 | 18.1 | 17.8 | 14.6 |
| $Fe^{2+}$ | 68 | 65 | 68.2 | 68.0 | 73.4 |
| $Mn^{2+}$ | 1.5 | 2.0 | 6.4 | 7.1 | 2.0 |

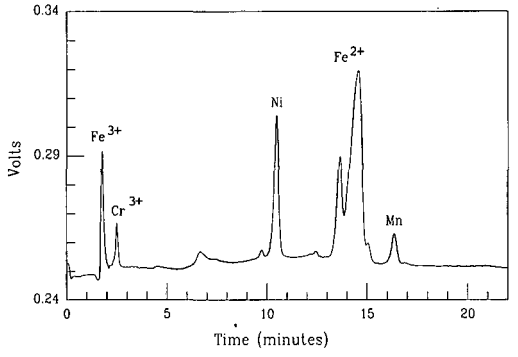


Fig. 5. Chromatogram of solution removed from artificial pit apparatus with 304 SS in 1000 mg/l sodium chloride + 1000 mg/l hydrochloric acid, +0.7 V (SCE). The splitting of the $Fe^{2+}$ peak was found to occur with high concentrations of $Cl^-$ in the sample.

shown in Fig. 6 resulting. The presence of $Cu^{2+}$ indicates that this trace alloying element will dissolve under crevice corrosion conditions if the potential is high enough. It was not detected in any of the 1 *M* hydrochloric acid tests [$E = -0.2$ V (SCE)], indicating that the potential inside at least a portion of the crevice was sufficiently above the $Cu/Cu^{2+}$ reversible potential to allow significant dissolution. In addition, a small amount of cobalt was detected. While not an intentional alloying addition to Type 304 SS, cobalt often enters the alloy as part of the scrap metal from which much of the Type 304 SS is now made. These results indicate that it also preferentially dissolves from the alloy under crevice

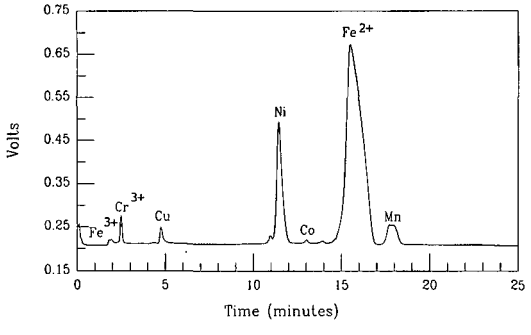


Fig. 6. Chromatogram of solution removed from actual 304 SS crevice in 1000 mg/l sodium chloride + 1000 mg/l hydrochloric acid, +0.2 V (SCE). Note the detection of $Cu^{2+}$ and $Co^{2+}$, indicating preferential dissolution of these elements under crevice corrosion conditions.

corrosion conditions. The slightly distorted peak shape for the $Fe^{2+}$ is due to the relative high concentration of this species.

## CONCLUSIONS

IC has been shown to be capable of determining the transition metal content of occluded cells of stainless steels. The simultaneous analysis of iron (both ferrous and ferric), chromium, copper, nickel, cobalt and manganese is possible to the 10 $\mu$g/l level. In this way, the relative dissolution rates of different components have been determined under both pitting conditions and uniform dissolution conditions. In neither case is dissolution stoichiometric, with Mn and Ni being preferentially dissolved and Cr preferentially retained in the alloy. In addition, it has been shown that solutions can be extracted and analyzed from crevices consisting of PTFE and 304 stainless steel.

Future work will focus on the following areas: (a) working with smaller, well-defined crevice volumes, (b) studying the effects of alloying elements such as Mo and N on the initiation and propagation stages, (c) investigating the role of the dissolution products of MnS inclusions in the initiation of crevice corrosion, and (d) extending the technique to aluminum-based and nickel-based alloys.

## ACKNOWLEDGEMENTS

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# Forensic applications of coupling non-suppressed ion-exchange chromatography with ion-exclusion chromatography

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ABSTRACT

Non-suppressed ion-exchange chromatography coupled with ion-exclusion chromatography separates carboxylic acids and inorganic anions in one isocratic run. The sample is injected into an ion-exclusion column, which provides separation of weak acid anions. The void volume of the ion-exclusion column is collected on-line and injected into an anion-exchange column, which separates the strong acid anions found there. Samples are described in which inorganic anion as well as carboxylic acid interferences were removed by coupled ion chromatography. Additionally, the practicality of the coupled system for forensic sample screening is discussed.

## INTRODUCTION

Forensic investigations performed by the National Forensic Chemistry Center (NFCC) frequently require immediate analysis of samples ranging from relatively pure drugs to complex food matrices. Limited time is available for method development. Often, a particular sample matrix has not been worked with previously, nor is it likely to be encountered again. In some cases, analysis of a specific poisonous substance is required. More frequently, inorganic anions and carboxylic acids found in forensic samples are used with the results of other analytical techniques to link or differentiate suspect samples. Which analyte(s) will prove useful is often unknown.

Ion chromatography is a very useful technique for the rapid analysis of multiple ionic analytes in a wide variety of sample matrices. Dilution and filtration is often the only sample preparation necessary; however, in complex matrices the trace analysis of contaminants by ion chromatography can be hindered by the presence of major sample components. While carboxylic acids or other ions may be interferences in the analysis of inorganic anions, their elimination (*e.g.* by solid phase extraction) may also result in analyte losses. For example, the use of a strong cation-exchange resin in the silver form to remove high levels of chloride would simplify the analysis of fluoride and acetate but would also eliminate iodide. In addition, when sample volume is limited, extensive sample pretreatment is not a viable approach. In some forensic cases, only a few hundred microliters of diluted sample have been available for ion chromatographic analysis.

Excellent separations of weak carboxylic acids and inorganic anions in a single analysis have been obtained using gradient ion chromatography [1]. However, gradient analysis requires precisely controlled re-equilibration time between runs, and carbonate contamination of sodium hydroxide eluents can cause baseline drift and poor retention time reproducibility [1]. In many of the forensic samples we have analyzed, there are large differences in the levels of some ions, especially between those naturally occurring and contaminants. When there are disparate levels of ions which have similar retention times, it may be necessary to alter gradient ramps after an initial run to obtain adequate separations in forensic samples, therefore increasing analysis time.

Another approach to the separation of carboxylic acids and inorganic anions is coupling ion exclusion to anion exchange. A system using suppressed ion chromatography was described by Rich *et al.* [2]. However, $Cl^-$ could not be quantified since hydrochloric acid used as ion-exclusion eluent caused a $Cl^-$ system peak. Additionally, packed-bed suppressors in the Ag form were used [2], however packed-bed suppressors suffer from down-time and cause band broadening. Modern membrane suppressors also could not remove the system peak caused by the ion-exclusion eluent (hydrochloric acid or octane sulfonic acid).

A similar system using non-suppressed coupled ion chromatography was developed by Jones *et al.* [3]. The sample is injected onto an ion-exclusion column which provides separation of weak acid anions. The void volume of the ion-exclusion column is collected on-line and injected into an anion-exchange column which can separate strong acids found there. In this way, strong acid anions are analyzed free from carboxylic acid interference. The non-suppressed coupled system does not have a system peak since the eluent for ion exchange is the sodium salt of the acid used for ion exclusion.

In this work, the non-suppressed coupled system was evaluated using actual samples encountered in our lab. Examples will be given to demonstrate the usefulness of this coupled system for samples in which an inorganic anion would interfere in the analysis of a carboxylic acid, as well as cases where an excess of a carboxylic acid would normally hinder the analysis of an inorganic anion by ion exchange. Additionally, the practicality of the coupled system for sample screening will be discussed.

## EXPERIMENTAL

### *Apparatus*

The instrumentation used included a Waters (Millipore, Milford, MA, USA) Model 625 pump with system controller, Model 510 pump, two Model 431 conductivity detectors, a Model 700 autosampler, and an automated six-port switching valve. System control and data acquisition were accomplished with a Maxima 825 data station and WD24 interface board (Millipore).

A Waters Ion-Exclusion column (300 × 7.8 mm I.D.) with IC-Pak ion-exclusion Guard-Pak precolumn insert and an IC-Pak A anion-exchange column (50 × 4.6 mm I.D.) were used.

### *Coupled ion chromatography*

Samples were injected onto the ion-exclusion column via the autosampler (100 $\mu$l injected) which also triggered the automatic switching program and data acquisition from both detectors. The transfer time of the ion-exclusion void volume peak was determined from injections of 100 $\mu$l distilled deionized water. The automated switching valve was triggered to inject a 500-$\mu$l fraction of the ion-exclusion void volume onto the anion-exchange column at the time that the void volume peak from the ion-exclusion column was detected (retention time, $t_r$ = 4.65 min). The timed events program used has been described elsewhere [4]. The eluents used were 1 m*M* octanesulfonic acid (pH 3) and 3 m*M* octanesulfonate (pH 6) for the ion-exclusion and anion-exchange separations, respectively. Experiments with anion-exchange only separations used 3 m*M* octanesulfonate eluent. A flow-rate of 1 ml/min was used in all cases.

### *Reagents, standards and samples*

Eluents for ion exclusion and ion exchange were prepared from the same lot of octanesulfonic acid sodium salt (98%, Aldrich, Milwaukee, WI, USA) to minimize sulfate contamination in the ion-exclusion eluent. Concentrated octanesulfonic acid eluent was prepared as described by Jones *et al.* [3] by mixing the sodium salt with cation-exchange resin (Bio-Rad AG 50W-X12, 200–400 mesh, hydrogen form) and filtering through a 0.2-$\mu$m filter (Anodisc 47, Alltech, Deerfield, IL, USA).

Individual 1000 mg/l standards were prepared from weighed amounts of salts. Standard mixtures for calibration were prepared by dilution of stock single-ion standards.

Samples were prepared by dilution in 18 M$\Omega$ distilled deionized water and filtration through 0.2-$\mu$m filter. Food samples were also passed through activated $C_{18}$ cartridges (Maxi-Clean, Alltech) prior to injection.

## RESULTS AND DISCUSSION

Fig. 1 illustrates the separation of a mixture of fifteen inorganic anions and carboxylic acids

obtained using coupled ion chromatography. Ion-exclusion separations are based not only on Donnan exclusion (neutral species migrate through the water phases toward the resin core, whereas ionized species are excluded), but are also based upon steric exclusion, and adsorption effects [5,6]. Thus, fluoroacetate, fluoride, glycolate, formate, acetate, propionate, adipate, azide and butyrate elute generally in order of increasing $pK_a$ (Table I and Fig. 1a). However, glycolate ($pK_a$ 3.83) elutes before formate ($pK_a$ 3.75). Strong acids iodate, chloride, nitrate, iodide and sulfate are separated by anion exchange, generally by size and charge (Table I and Fig. 1b). Retention time reproducibility of ten replicate injections on the same day was better than 0.6% relative standard deviation for all compounds except adipate (1.2%). Repeatability of response and linear range are presented in Table I. Detection limits (defined as three times the standard deviation of baseline data points in a blank) range from 0.007 mg/l for fluoride to 1 mg/l for butyrate. Trace enrichment prior to separation can be used to lower detection limits [3]; however, typically sample volume is limited in our work, and thus only detection limits obtainable by direct injection are reported. Repeatability of peak areas ranged from 0.8 to 2.8% R.S.D. at concentrations from 20 to 200 times the detection limit.

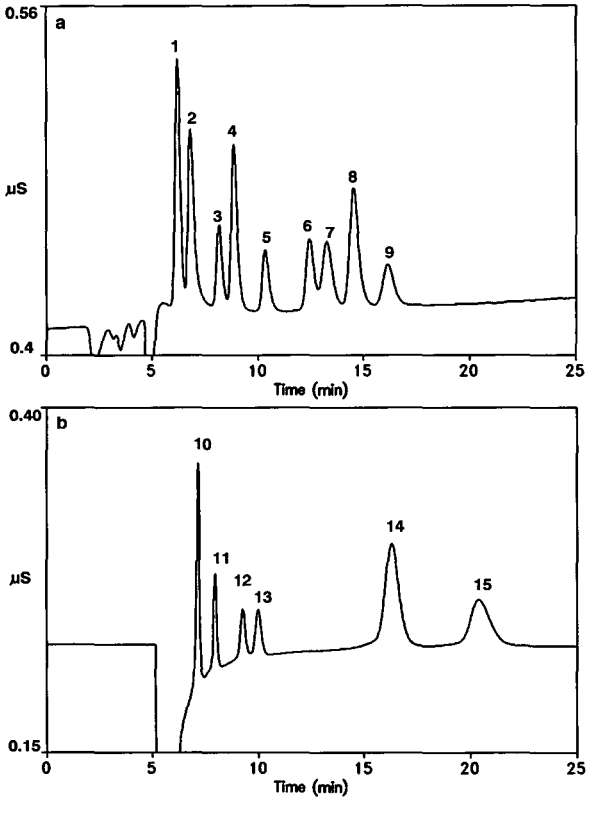


Fig. 1. Separation of carboxylic acids and inorganic anions by the coupled system. (a) Ion-exclusion chromatogram; peaks: 1 = fluoroacetate (1.26 ppm); 2 = fluoride (0.5 ppm); 3 = glycolate (2 ppm); 4 = formate (2 ppm); 5 = acetate (10 ppm); 6 = propionate (20 ppm); 7 = adipate (20 ppm); 8 = azide (11.6 ppm); 9 = butyrate (20 ppm). (b) Anion-exchange chromatogram; peaks: 10 = iodate (50 ppm); 11 = chloride (1 ppm); 12 = bromide (2 ppm); 13 = nitrate (2 ppm); 14 = iodide (20 ppm); 15 = sulfate (5 ppm).

A positive peak was observed in the void volume of the ion-exclusion chromatogram at higher concentrations of inorganic anions (*e.g.* 20 mg/l $Cl^-$ or 100 mg/l $Br^-$). This is not an indication of non-reproducible transfer onto the anion-exchange column at higher concentration. Rather, it occurred when the conductance of the inorganic anions was sufficient to be observed above the negative signal of water in the ion-exclusion chromatogram. Nitrite and phosphate were not determined using this system. According to Jones *et al.* [3], nitrite and phosphate are not quantitatively transferred to the anion-exchange column in the 500-µl fraction chosen in this system and are not well retained by ion-exclusion mechanisms.

*Inorganic anion interferences*

The forensic analysis of pharmaceuticals is often complicated by high levels of chloride which can typically mask trace levels of weak carboxylic acids such as acetate or formate. In one poisoning case, traces of acetate and formate were readily detected in a drug which was a hydrochloride salt using the coupled system. Since $Cl^-$ eluted in the void volume of the ion-exclusion column, the coupled system effectively removed $Cl^-$ allowing the detection of acetate and formate which were separated by ion exclusion. Other ions of interest, such as $I^-$ and $SO_4^{-2}$ were determined in the same run on the anion-exchange column.

Salad dressing is another matrix in which chloride can interfere in the analysis of acetate (from vinegar). As stated above, the strong acid anion $Cl^-$ does not interfere with the weak acid acetate in the coupled system. Additionally, $F^-$ ($pK_a$ 3.18) and

TABLE I

LINEARITY, RELATIVE STANDARD DEVIATION (R.S.D.) AND DETECTION LIMITS

| Compound | $pK_a$[a] | Linearity correlation coefficient (concentration range) | R.S.D. (area) (%) ($n = 10$) (concentration) | Detection limit (mg/l) |
|---|---|---|---|---|
| *Weak acids* | | | | |
| Fluoroacetate | 2.59 | 0.9997 (0.09–3.8 ppm) | 0.8 (1.3 ppm) | 0.015 |
| Fluoride | 3.18 | 0.995 (0.05–2 ppm) | 1.6 (0.5 ppm) | 0.007 |
| Glycolate | 3.83 | 0.9989 (0.1–10 ppm) | 1.8 (2 ppm) | 0.06 |
| Formate | 3.75 | 0.9987 (0.05–2 ppm) | 1.1 (2 ppm) | 0.011 |
| Acetate | 4.76 | 0.9985 (0.5–50 ppm) | 2.2 (10 ppm) | 0.22 |
| Propionate | 4.78 | 0.9998 (0.65–65 ppm) | 2.2 (20 ppm) | 0.40 |
| Adipate | 4.42 | 0.9997 (2–200 ppm) | 0.9 (20 ppm) | 0.90 |
| Azide | 4.72 | 0.9999 (0.5–50 ppm) | 1.1 (12 ppm) | 0.30 |
| Butyrate | 4.82 | 0.9996 (2–200 ppm) | 2.4 (20 ppm) | 1.0 |
| *Strong acids* | | | | |
| Iodate | 0.80 | 0.9972 (1–100 ppm) | 1.4 (50 ppm) | 0.60 |
| Chloride | −6.1 | 0.9986 (0.1–5 ppm) | 1.8 (1 ppm) | 0.03 |
| Bromide | −9 | 0.9997 (0.15–15 ppm) | 1.1 (2 ppm) | 0.075 |
| Nitrate | −1.38 | 0.9999 (1–100 ppm) | 2.1 (2 ppm) | 0.08 |
| Iodide | −9.5 | 0.9979 (1–100 ppm) | 2.3 (20 ppm) | 0.40 |
| Sulfate | *ca.* −3 | 0.9997 (0.5–50 ppm) | 2.8 (5 ppm) | 0.20 |

[a] From ref. 7.

azide ($pK_a$ 4.72) contamination in salad dressing were determined in the same analysis (Fig. 2). While acetate, fluoride, and azide can all be determined by anion exchange, interferences can occur due to co-elution with other common anions under typical isocratic conditions. Also, disparate levels of closely eluting species may require weaker eluents and therefore longer run times, or gradient analysis. Since the three species are anions of weak acids they are retained and separated on the ion-exclusion column away from the strong acid anion, $Cl^-$.

In another case, saline eye solution suspected of containing HF was analyzed by ion chromatography. Elemental analysis by inductively coupled plasma-optical emission spectroscopy (ICP-OES) was to be used in order to trace the source of HF. High levels of HF can damage an ICP torch, thus quantitation of $F^-$ was necessary to determine the minimum dilution of the sample for ICP-OES analysis. As saline eyedrops contain percentage levels of sodium chloride and boric acid, a weak eluent would have been needed for isocratic anion exchange in order to resolve fluoride from the major sample components. No changes in operating conditions were necessary for the analysis of $F^-$ by coupled chromatography, enabling a rapid solution to the problem. The sample was not found to contain HF as suspected, but instead contained sulfuric acid. Although low levels of $F^-$ would have been detected, the source of the low pH was easily determined in one run. Sources of $H_2SO_4$ rather than HF were then investigated.

*Carboxylic acid interferences*

In forensic analyses, it is helpful to determine trace contaminants in a relatively pure substance. A drug of abuse was analyzed by anion exchange; however, the major component obscured the beginning of the chromatogram as shown in Fig. 3a. When analyzed on the coupled system, the major peak was retained on the ion-exclusion column (which was consistent with the drug's identity) while trace levels of $Cl^-$, $NO_3^-$, and $SO_4^{-2}$ were now observed in the anion-exchange chromatogram. These anions, in conjunction with trace element patterns, were used to determine lot to lot variability.

Carboxylic acids are common in foods. Pyruvate

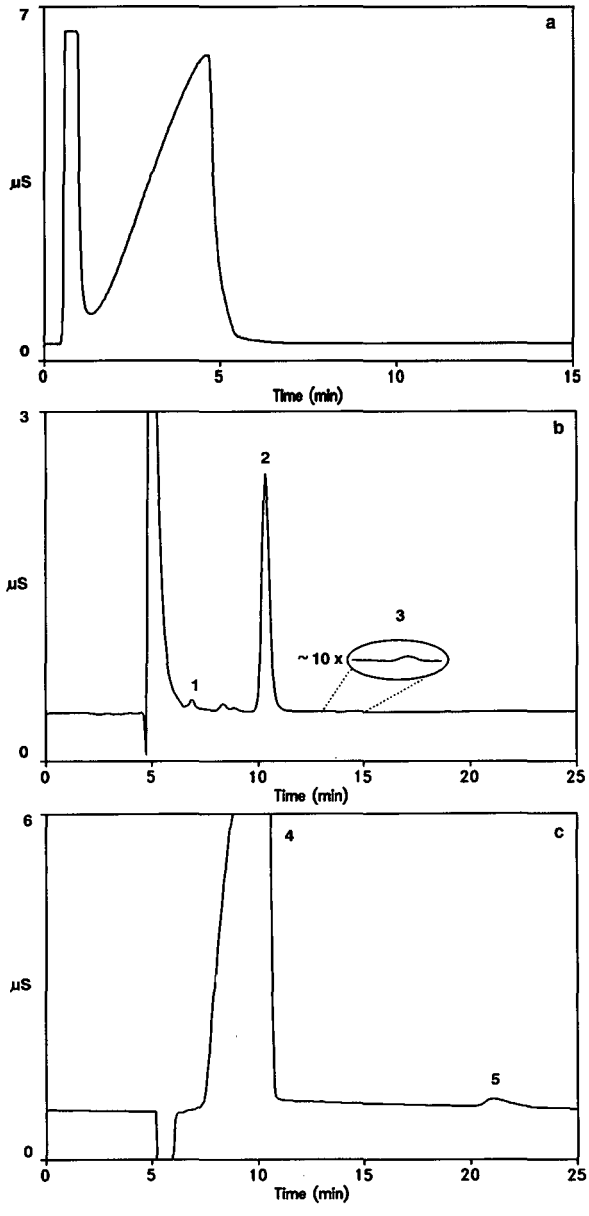


Fig. 2. Sample chromatograms for salad dressing, spiked with 5 ppm fluoride and 6 ppm azide. (a) IC Pak-A column only, 3 m*M* octanesulfonate at 1 ml/min; (b) ion exclusion, and (c) anion exchange in the coupled system. Peaks: 1 = fluoride; 2 = acetate; 3 = azide; 4 = chloride; 5 = sulfate.

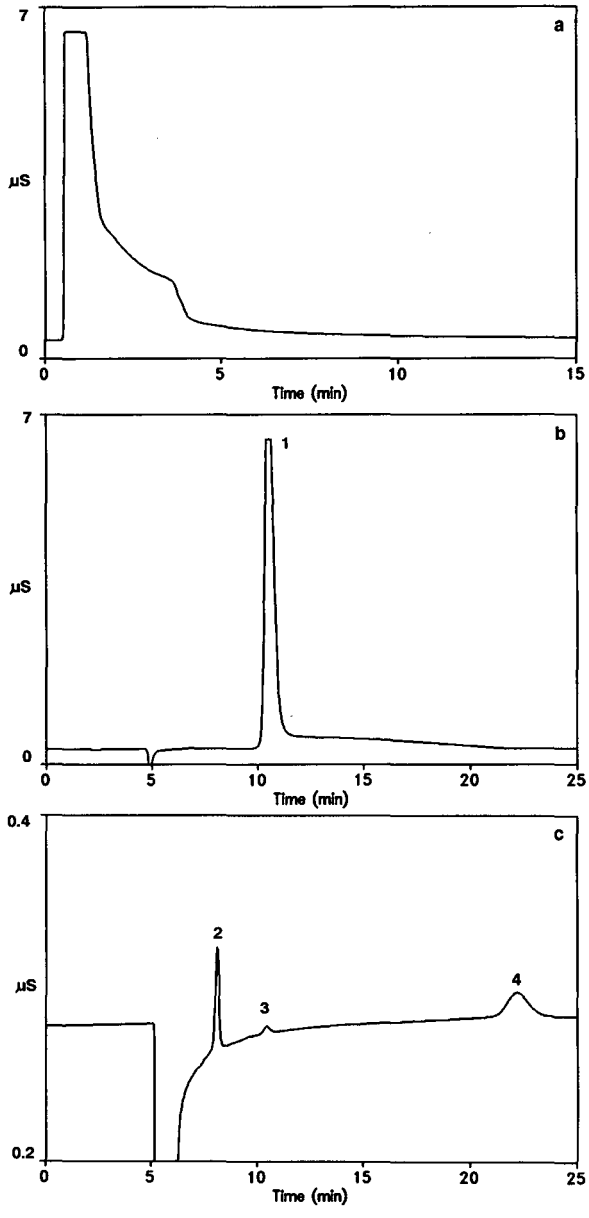


Fig. 3. Comparison between anion-exchange and coupled system in the analysis of a drug of abuse. (a) IC Pak-A column only, 3 m*M* octanesulfonate at 1 ml/min; (b) ion exclusion, and (c) anion exchange in the coupled system. Peaks: 1 = active ingredient; 2 = $Cl^-$; 3 = $NO_3^-$; and 4 = $SO_4^{-2}$.

and lactate elute near the void volume in isocratic anion exchange, complicating the detection of $F^-$ in milk. While $F^-$ can be determined with an ion-selective electrode, analysis by coupled ion chromatography enables the detection of additional anions such as $Cl^-$ which may indicate reconstitution.

Pyruvate, lactate and fluoride were separated by ion exclusion and $F^-$ in milk was determined at a spike level of 2 μg/ml.

*Practicality of the coupled system for sample screening*

Several anions are toxic and can be screened for in a variety of matrices using coupled ion chromatography. Sodium azide is a commonly used preservative in clinical laboratories and at least two deaths have been reported following accidental ingestion [8]. Azide is difficult to resolve from $Br^-$ and $NO_3^-$ in anion exchange separations [9]. However, using coupled chromatography, the weak acid azide ($pK_a$ 4.72) was separated by ion exclusion, while strong acids $Br^-$ ($pK_a$ −9) and $NO_3^-$ ($pK_a$ −1.38) were separated by anion exchange (Fig. 1; peaks 8, 12 and 13, respectively). Thus, even high levels of $Br^-$ or $NO_3^-$ do not interfere in the analysis of azide. While adipate may co-elute with azide via ion exchange, they are separated by ion exclusion. Nitrates in such complex matrices as meat, cheese or forages would not interfere with azide analysis.

Whereas in some cases emphasis is on obtaining the maximum information available, detection of specific anions is also necessary. In anion-exchange separations fluoroacetate and fluoride elute near the void volume along with other weak acids and small inorganic anions. Although both compounds are toxic, fluoroacetate is more poisonous than fluoride, necessitating separation of these species. Fig. 4 illustrates the separation of weak acids fluoroacetate and fluoride by ion exclusion, as well as other toxic anions lactate, azide, and chlorate in buttered popcorn. High levels of $Cl^-$ observed in the anion-exchange chromatogram would have obscured the weak acids fluoroacetate, fluoride, and lactate in an isocratic anion exchange only separation. Popcorn was freeze dried, ground, and then extracted with distilled deionized water. These analytes have also been determined in seafood.

In some cases a suspect sample may be compared to a matrix control to detect contamination, or sometimes the sample composition is completely unknown. For example, a liquid in an unlabelled bottle collected by an investigator was identified as lactated ringers solution, a veterinary solution, with the help of coupled ion chromatography. The detection of lactate and $Cl^-$ provided much of the

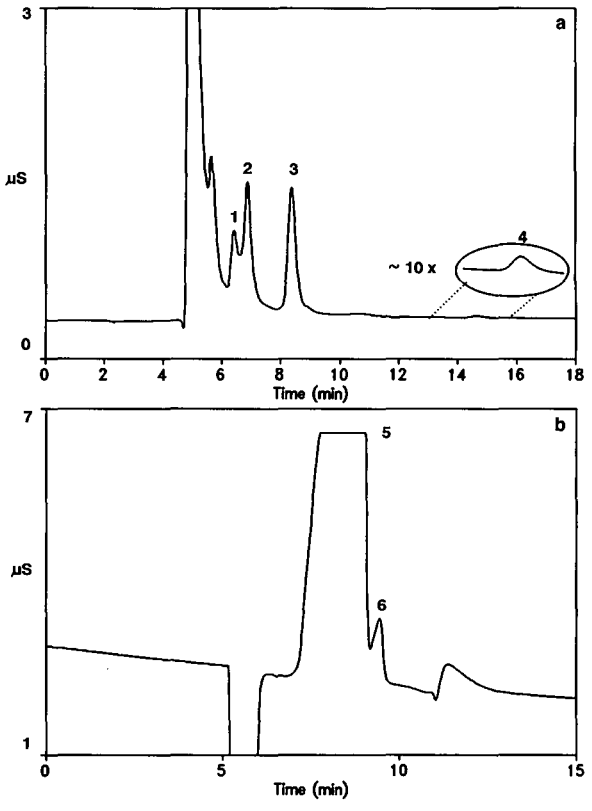


Fig. 4. Analysis of contaminants in buttered popcorn by the coupled system: (a) ion exclusion, (b) anion exchange. Peaks: 1 = fluoroacetate; 2 = fluoride; 3 = lactate; 4 = azide; 5 = chloride; 6 = chlorate.

evidence necessary to identify the solution. More commonly, however, it is not possible to identify by ion chromatography alone a peak which occurred in the suspect sample. Yet, the two modes of separation used in conjunction with one another provides valuable information about $pK_a$ as well as the size/charge of the analyte. An unknown peak detected in the ion-exclusion chromatogram would indicate a weak acid, generally eluting in order of increasing $pK_a$ (such as those in Fig. 1a). Whereas an unknown peak detected on the anion-exchange chromatogram would indicate a strong acid, such as those in Fig. 1b. An early-eluting peak by ion exchange would tend to be a small singly charged anion; a late-eluting peak would tend to be a larger multi-charged anion. Using the information from

the two modes of separation, the focus of investigation by other techniques can then be narrowed.

In other cases, although the sample is not suspected of containing toxic substances, information is required to link or dissociate samples of interest. For example, a juice sample which smelled strongly of liquor was investigated. While it was not possible to identify the liquor using ion chromatography, the coupled system was used to determine the amount of juice in the sample. A peak observed in the ion-exclusion chromatogram of the complaint sample and a control juice was not detected in any of the liquors suspected as the source of the liquor in the juice. That peak, malic acid (retention time 6.6 min), was quantitated and used to calculate the percentage of juice in the sample. This information facilitated the identification of the liquor by gas chromatography and high-performance liquid chromatography.

## CONCLUSIONS

While no one method can solve all ion chromatographic separation problems, the coupled system has become a useful part of the overall scheme at the National Forensic Chemistry Center where a variety of analytical techniques are used to solve cases as quickly as possible. Weak acid and strong acid anions are separated on the coupled system, often eliminating matrix interferences. The two modes of separation (ion exclusion and anion exchange) used in conjunction with one another provide valuable information about $pK_a$ as well as the size/charge of an analyte. Additional information obtained using the two modes may narrow the focus of investigations of unknowns by other analytical techniques. Additionally, potentially useful information is not lost in sample preparation.

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# Analysis of commercial explosives by single-column ion chromatography

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ABSTRACT

Commercial explosives utilize inorganic salts as an inexpensive and effective oxidizing agent. Important performance criteria of commercial explosives are gathered from the analysis of these salts in the formulation. An optimum ratio of inorganic salts to fuel oil needs to be maintained to produce complete combustion and maximum energy. This information is quickly and accurately provided by an ion chromatographic method described here. The anion analysis is performed on an hydroxyethylmethacrylate-based macroporous copolymer-based anion-exchange column, which provides good peak shapes for nitrate. Analyzing both monovalent and divalent cations in a single chromatographic step is made possible by new cation-exchangers. The dual-column method for the simultaneous determination of anions and cations was applied to the analysis of nitrate, ammonium, hexamethylenetetramine, calcium and magnesium in a single chromatographic analysis of less than 35 min. A comparison between the traditional, wet chemistry methods commonly used for commercial explosive analysis and this new ion chromatographic method is presented.

## INTRODUCTION

Commercial explosives used in mining and construction utilize ammonium nitrate as an inexpensive and effective oxidizing agent. Fertilizer grade ammonium nitrate is mixed with fuel oil to formulate a safe non nitroglycerin alternative to dynamite. This ammonium nitrate–fuel oil mixture (ANFO) is easy to manufacture at a low cost. ANFO is very hydroscopic and is used only in large-diameter boreholes because it will not sustain a detonation in small-diameter holes. These limitations of ANFO led to the development of explosives with better water resistance and excellent performance. These aqueous-based ammonium nitrate explosives include slurries and water-in-oil emulsions. Greater water resistance and sensitivity of slurries and emulsions is provided by the intimacy between fuel and oxidizer. Due to their low cost, excellent performance and superior safety characteristics, ANFO and other ammonium nitrate explosives now account for 96.6% of the total commercial explosives market [1].

The optimum ratio of ammonium nitrate to fuel oil in ANFO, 94.5:5.5, is necessary to stoichiometrically balance the combustion process. With this ratio there will be sufficient oxygen in the formulation to react with all the fuel. Complete combustion is achieved at this level to produce the highest energy. The theoretical reaction is shown in eqn. 1 [2].

$$3NH_4NO_3 + CH_2 \rightarrow CO_2 + 7H_2O + 3N_2 \quad (1)$$

In the formulation of aqueous-based slurries and emulsions a large amount of ammonium nitrate must be used to react with a small amount of fuel to maintain this necessary oxygen balance. This ratio of ammonium nitrate to fuel in emulsion is approximately 90:10. Dry ammonium nitrate is added to the emulsion to provide the additional oxygen necessary to balance the combustion process. Ingredients of commercial explosive are selected to vary the energy, detonation sensitivity, oxygen balance, rheology and stability. Other oxidizer salts that are used include sodium nitrate, calcium nitrate, sodium perchlorate and amine nitrates. By varying the ingredients it is possible to customize commercial to meet specific requirements.

It is this customization that necessitates the anal-

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ysis of the inorganic salts in the formulated product. Formulations that do not perform as expected are analyzed to confirm that the desired oxygen balance is met. In addition to troubleshooting, analysis of the oxidizer salts is necessary for process control and quality assurance. The traditional wet chemical methods, which are both tedious and time consuming, are being replaced by chromatographic methods. In the past, these chromatographic methods included the analysis of the anions and cations of the inorganic salts on separate columns. Monovalent cations have been separated on cation-exchange columns with a dilute mineral acid eluent. Divalent cation analysis was achieved with the same column by changing to a divalent eluent. For example, a single-column ion chromatography (IC) method was used to determine cations in commercial explosives. A Wescan Cation/R poly(styrene–divinylbenzene) (PS–DVB) column, was used in conjunction with 3.9 m*M* nitric acid to provide the separation of sodium, ammonium, and monomethylamine. The same column was used with ethylenediamine for the determination of calcium [3]. Chemically suppressed IC methods have also been used for the determination of anions and cations in water-based explosives and residues. A UV detector was used in line with the conductivity detector to provide detection of perchlorate and nitrate on an anion exchange column using a $NaHCO_3$–$NA_2CO_3$ eluent. Monovalent and divalent cations were separated on a cation-exchange column with hydrochloric acid and hydrochloric acid–phenylenediamine eluents, respectively [4].

A new single-column IC method for the analysis of ammonium nitrate in a commercial emulsion and slurry explosives using new anion and cation column technology and a dual-column technique will be compared to the traditional wet chemical analysis. The nitrate analysis is performed on an anion-exchange column packed with a hydroxyethyl methacrylate-based macroporous copolymer. This column provides improved peak shapes for polarizable anion, such as nitrate, when compared with PS–DVB based anion columns [5]. The ability to separate monovalent and divalent cations present in commercial explosives in one isocratic run is provided by a commercial version of a silica-based polymer coated stationary phase developed by Schomburg *et al.* [6]. A dual-column technique for the simultaneous analysis of anions and cations is used to obtain the total inorganic composition of the formulation in one chromatographic run of approximately 35 min duration.

## EXPERIMENTAL

### *Chromatography*

All chromatographic equipment, columns and reagents, unless stated otherwise, were obtained from Alltech Assoc. (Deerfield, IL, USA). Chromatography was performed on a system which consists of a Model 325 metal-free high-performance liquid chromatography (HPLC) pump, a 9125 injection valve (Rheodyne, Reno, NV, USA) with a 100-$\mu$l sample loop, a column oven (Timerline, Boulder, CO, USA) and an Alltech Model 320 conductivity detector. A Rheodyne Model 9000 switching valve mounted to a Universal Valve Actuator was used to direct the flow through or around the cation-exchange column. An SP 4400 Chromjet integrator (Spectra-Physics, Santa Clara, CA, USA) was used to record the separation and control the switching valve. The Universal Anion Column (50 mm × 4.6 mm I.D.) packed with hydroxyethyl methancrylate copolymer anion exchanger and a Universal Cation Column (100 mm × 4.6 mm I.D.) packed with a silica coated with polybutadiene–maleic acid copolymer were used to provide the separation.

### *Distillation apparatus*

An all-glass still was used for the distillation of the ammonia in the traditional wet chemistry methods. It consisted of a 2-l flask, connected to a spray trap, water-cooled condenser, and a long delivery tube which extends to the bottom of a 250-ml Erlenmeyer flask.

### *Reagents*

All reagents used for the traditional wet and chromatographic methods were prepared using NBS- or ACS-grade materials. The eluent used for the chromatographic separation was phthalic acid. A stock solution was prepared by dissolving the ASC-grade reagent in methanol. This solution was diluted to make 5 m*M* phthalic acid. The pH of this solution, approximately 2.9, was not adjusted. All standards, eluents and dilutions were prepared with deionized HPLC-grade water.

*Samples and sample preparation*

Samples of commercial explosives were obtained from commercial suppliers along with the quantitative results of the determination of ammonia by the traditional wet chemical method. Prior to chromatographic or wet chemistry analysis, 10 g of the commercial explosives were homogenizing and dissolved in 100 ml of HPLC-grade methanol. These solutions were diluted and filtered before chromatographic determination. No other sample preparation was necessary. The traditional wet chemical method utilized aliquots of the explosive–methanol solution.

*Procedures*

*Traditional wet chemical methods.* The ammonium nitrate concentration is determined by a distillation procedure for ammonia nitrogen in water based on the EPA Method 350.2 [7]. This distillation method covers the determination of ammonia-nitrogen exclusive of total Kjeldahl nitrogen. An aliquot of the digested explosive sample is weighed into a Kjeldahl flask. A 50-ml volume of a 50% ammonium hydroxide solution is added to adjust the pH of the sample. The flask is filled with 220 ml of water and distilled into 2% boric acid. The ammonia in the distillate is titrated with 0.05 *M* sulfuric acid to the endpoint determined by bromoceresol green indicator. The volume of titrant is corrected by titrating a blank to the same endpoint.

*Chromatographic method.* The Universal Anion Column was used with an eluent of 5 m*M* phthalic acid to determine the nitrate concentration in the commercial explosives. The Universal Cation Column was used with the same eluent to provide the separation of monovalent and divalent cations. A dual-column technique for the simultaneous analysis of anion and cations, described previously [8,9], was used to provide a complete ionic quantification of the commercial explosive formulation in one chromatographic run. With the eluent passing through both columns, the sample is injected. The anions in the sample are retained on the anion-exchange column but the cations will pass through the column unretained. Once the cations reach the head of the cation column, the switching valve is actuated and the eluent bypasses the cation column. This allows the anions to be separated on the anion column and passed on to the detector. After the anion analysis is complete the switching valve diverts eluent through the cation column, to separate and elute the cations left at the inlet of the column.

## RESULTS AND DISCUSSION

Standard wet chemical methods are often used for the quantification of the inorganic salts in commercial explosives. Although these standards methods have been well studied and can be accurate and reproducible, the analysis is time consuming taking approximately up to 5 h per sample depending on the composition. The ammonium nitrate concentration is determined by the EPA method of distillation and titration. This method determines the concentration of ammonia in the sample. In calculating the ammonium nitrate present in the sample, it is assumed that all of the ammonia is present in the form of ammonium nitrate.

IC provides a method of the analysis of inorganic oxidizer salts in commercial explosives that can be rapid and sensitive. The ammonia concentration can be determined by IC in only 6 min. The anion analysis of nitrate in a commercial emulsion explosive on the Universal Anion Column using 5 m*M* phthalic acid eluent is shown in Fig. 1. The analysis of ammonia in the emulsion explosive separated on

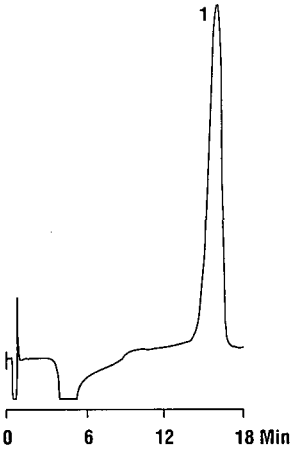


Fig. 1. Nitrate analysis of commercial emulsion explosive. Chromatographic conditions: column, 100 mm × 4.6 mm I.D.; packing, Universal Cation; eluent, 5 m*M* phthalic acid, pH unadjusted; flow-rate, 1 ml/min; detector, conductivity; range, 1 μS cm full scale. Peak 1 = nitrate.

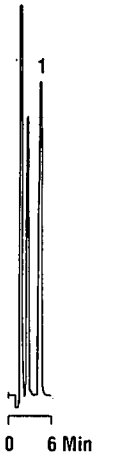


Fig. 2. Ammonia analysis of commercial emulsion explosive. Chromatographic conditions as in Fig. 1. Peak 1 = ammonium.

the Universal Cation Column using the same eluent is shown as Fig. 2. The cation analysis can also determine any secondary salts, such as sodium, organic amines, calcium and magnesium in the formulation. Fig. 3 displays the separation of ammonia, hexamethylenetetramine, magnesium and calcium in reagent water.

Although the use of IC with the new anion- and cation-exchange column allows a rapid and complete analysis of the ions present in the formulation, performing the anion and cation analysis independently can be time consuming. A dual-column technique for the simultaneous analysis of anion and cations provides the analysis of ammonium and nitrate in 20 min. A complete ionic quantification of the commercial explosive formulation, including anions and monovalent and divalent cations, can be obtained in one chromatographic run of less than 35 min. The simultaneous analysis of ammonium, hexamethylenetetramine and nitrate using this dual-column technique for the analysis of a commercial slurry explosive is shown in Fig. 4.

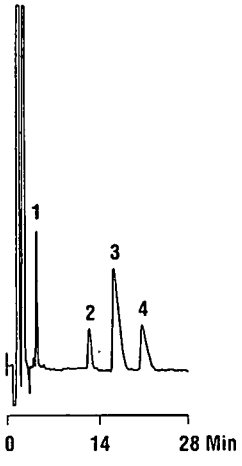


Fig. 3. Cation analysis of oxidizer salts. Chromatographic conditions as in Fig. 1. Peaks: 1 = ammonium; 2 = hexamethylenetetramine; 3 = magnesium; 4 = calcium.

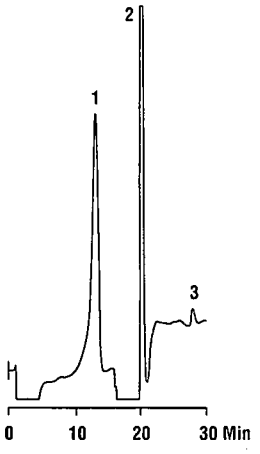


Fig. 4. Simultaneous analysis of anions and cations in commercial slurry explosive. Chromatographic conditions: anion column, 50 mm × 4.6 mm I.D.; anion packing, Universal Anion; cation column, 100 mm × 4.6 mm I.D.; cation packing, Universal Cation; eluent, 5 m*M* phthalic acid, pH unadjusted; flow-rate, 1 ml/min; detector, conductivity; range, 1 $\mu$S cm full scale. Peaks: 1 = nitrate; 2 = ammonium; 3 = hexamethylenetetramine.

The results of the traditional wet method for the analysis of ammonium in the emulsion explosive are shown in Table I. The chromatographic results of the analysis of a commercial emulsion explosive containing ammonium nitrate as an oxidizer salt is shown in Table II. The ammonium nitrate concentration of a commercial slurry explosive was also analyzed. The results of the analysis can be found in Table III. These chromatographic results were obtained by comparing each peak area to a three-point external standard calibration curve. Instead of assuming that all the ammonia present is present as the ammonium nitrate salt, IC can determine both the anion and cations. Using this method the exact concentration of the ammonium nitrate in the

TABLE I

TRADITIONAL WET METHOD RESULTS: AMMONIUM NITRATE ANALYSIS IN COMMERCIAL EMULSION EXPLOSIVE

| Analyte | Relative S.D. (%) (n = 2) | % of ion in sample | % of $NH_4NO_3$ in sample |
|---|---|---|---|
| Ammonia | 1.45 | 14.16 | 62.97 |

formulation can be confirmed. As shown in Table II, there is excellent correlation between the single-column and dual-column method results for the analysis of ammonium and nitrate. These results indicate that as much as 0.6% or 4 m*M* of the ammonium was present in the formulation may be in a form other than ammonium nitrate. Without the ability to analyze the nitrate concentration this apparent discrepancy would not have been identified. The value for ammonium found by traditional wet chemical methods and both single-column and dual-column chromatographic methods correlate well within the experimental precision of both methods.

A 6-min cation-exchange separation of ammonium can replace the traditional wet chemical method that can take as much as 40 min. The complete analysis of ammonium and nitrate can be achieved in 20 min using the dual-column technique. The sample throughput of this dual-column IC method can be

TABLE II

CHROMATOGRAPHIC RESULTS OF AMMONIUM NITRATE ANALYSIS IN COMMERCIAL EMULSION EXPLOSIVE

Dilution 110.6 mg sample/l.

| Analyte | Method | Concentration | | Relative S.D. (%) (*n* = 3) | % of ion in sample | % of $NH_4NO_3$ in sample |
|---|---|---|---|---|---|---|
| | | ppm | m*M* | | | |
| Ammonium | Cations only | 16.33 | 0.91 | 0.22 | 14.76 | 65.51 |
| | Dual column | 16.52 | 0.92 | 0.25 | 14.93 | 66.38 |
| Nitrate | Anions only | 55.09 | 0.88 | 1.18 | 49.81 | 64.28 |
| | Dual column | 545.54 | 0.88 | 1.69 | 49.31 | 63.62 |

TABLE III

CHROMATOGRAPHIC RESULTS OF AMMONIUM NITRATE ANALYSIS IN COMMERCIAL SLURRY EXPLOSIVE

Dilution 161.3 mg sample/l.

| Analyte | Method | Concentration | | Relative S.D. (%) (*n* = 3) | % of ion in sample | % of $NH_4NO_3$ in sample |
|---|---|---|---|---|---|---|
| | | ppm | *M* | | | |
| Ammonium | Dual column | 2348 | 0.130 | 0.05 | 14.55 | 64.70 |
| | Cations only | 2338 | 0.130 | 0.84 | 14.49 | 64.32 |
| Nitrate | Anions only | 8335 | 0.134 | 1.75 | 51.68 | 66.67 |
| | Dual column | 8242 | 0.133 | 1.09 | 51.10 | 65.93 |

as much as 20 commercial explosive samples per 8-h day. Automation of the IC system with the addition of a autosampler and an automated switching valve can allow unattended operation for continuous analysis.

If calcium, magnesium or organic amines are present in the commercial explosives IC can replace hours of individual traditional wet chemical methods. The analysis of these components in the sample can be obtained in 35 min with the dual-column technique. The traditional wet chemistry method for calcium and magnesium is ASTM Method D 511-88 [10]. This complexometric titration with EDTA requires that two solutions of the sample be analyzed, one at pH of 10 for the determination of calcium plus magnesium and one at pH of 12 for the determination of calcium alone. Magnesium is then determined by the difference between these two measurements. Some amines will interfere with the traditional wet chemistry method for the determination of ammonia. These include amines in the formulation that will breakdown to ammonia during the distillation process, such as methylammonium salts of hexamethylenetetramine. IC provides the ability to determine ammonium and amines simultaneously.

In addition to the increased sample throughput with IC, the variety and consumption of reagents is reduced compared to traditional wet chemistry methods. The consumption of reagents for the dual-column method is less than 40 ml of 5 m*M* phthalic acid per sample. The reagents necessary for the digestion and distillation of one sample can be as much as 50 ml of 50% sodium hydroxide, 50 ml of 2% boric acid, 25 ml of 0.1 *M* HCl. The IC method eliminates the formulation of these reagents and the time consuming process of standardizing the titrant.

## CONCLUSION

The necessity of analyzing the inorganic oxidizer salts in commercial explosives is made less consuming by IC methods. The anion analysis is performed on an hydroxyethyl methacrylate-based macroporous copolymer anion-exchange column to provide good peak shapes for nitrate. The analysis of both mono and divalent cations in the formulations can be achieve with the new cation-exchange column. The dual-column technique provides the simultaneous determination of the mono and divalent cation along with all anions in the commercial explosive. This technique allows for the analysis of ammonium nitrate in 20 min and a complete analysis of the formulation within 35 min. There is good correlation between the traditional wet chemistry and dual-column IC method results for the analysis of ammonium nitrate. Time and money are saved by the rapid analysis time, multicomponent analysis, lower reagent consumption and elimination of titrant standardization.

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CHROMSYMP. 2503

# Matrix elimination in ion chromatography by "heart-cut" column-switching techniques

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ABSTRACT

Are there occasions when a "dilute and shoot" approach to ion chromatographic analysis is appropriate? How can you deal with column overloading? What about system abuse? This paper describes applications where a simple "dilute and shoot" directly or coupled with "heart-cut" column switching techniques can make "dilute and shoot" a reality. A fully automated "dilute and shoot" method using the "heart-cut" technique has been developed for sulfite analysis in a variety of matrices. "Dilute and shoot" methods were also developed for acetate and trifluoroacetate in peptides. Preliminary results are also report herein for the determination of trace anions ($\mu$g/l) in concentrated hydrochloric acid, with minimal sample preparation.

## INTRODUCTION

A technique was previously described to effect matrix elimination in liquid chromatography mechanically [1] rather than chemically via sample pretreatment. The technique uses two 4-way valves inserted before and after the pre-column. The pre-column is the first column the sample sees after injection and can be a guard column, a separator column or an entirely different type of column than the separator column, *e.g.*, a reverse phase pre-column and an ion exchange separator column. The valves are configured such that the bulk of the matrix is diverted to waste and only a "heart-cut" (H-C) of the analyte of interest is transferred to the separator column. The H-C column-switching technique can be extremely effective in either eliminating or at least simplifying sample preparation. This paper describes a variety of applications where a "dilute and shoot" approach has been successfully employed.

## EXPERIMENTAL

The sulfite analyses were conduction using three AS-2 anion-exchange columns (Dionex) with a 1.5 m*M* sodium carbonate, 1.3 m*M* sodium bicarbonate and 0.076% (v/v) formaldehyde eluent. The experimental details of the ion chromatography (IC) procedure for sulfite analysis in the analgesic formulations and food items etc. are given in ref. 1. The food samples were purchased from the local grocery store. Samples with high sulfite content were prepared in the usual manner, 4 g/100 ml, and diluted as required with 0.76% (v/v) formaldehyde. Instrumental details of the H-C technique are also described in Ref. 1. See Fig. 1 for valve plumbing connections.

### *Heart-cut column-switching procedure*

Initially, the system is assembled without the separator column in order to determine the H-C timing interval. Standards are injected and the time for the onset of the analyte peak and complete elution of the analyte peak noted. This time interval is the H-C. The entire system is then equilibrated, after re-inserting the separator column, and the analysis conducted in a four step sequence as follows:

*(1) Divert initial portion of chromatogram to waste.* The sample is injected with the first valve closed, eluent flows through pre-column to the second valve which is opened to waste.

*(2) Introduce H-C to the separator column and*

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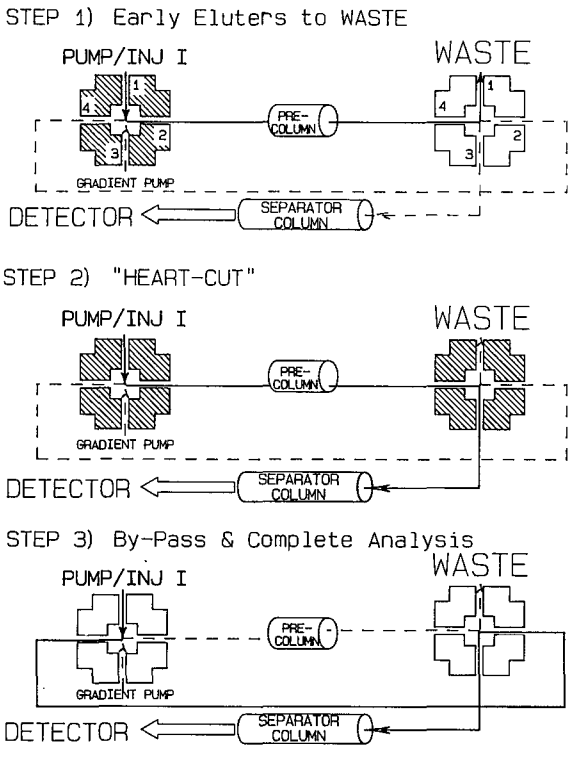

Fig. 1. Heart-cut valve configuration.

*detector.* At the pre-determined time interval, the onset of the H-C, the second valve is closed to divert the pre-column effluent to the separator column.

*(3) By-passing the pre-column and detecting the analyte.* Following the H-C interval, when the entire H-C has been eluted from the pre-column, the first valve is opened to divert the eluent stream to the second valve. The second valve is immediately opened to allow eluent from the first valve to flow into the separator column, by-passing the pre-column.

*(4) Pre-column clean-up.* After the sulfite H-C was transferred to the separator column, a step gradient program was used to flush the pre-column of later eluters. A three-phase, 1.5-min each, gradient program of methanol, concentrated eluent (0.05 *M* each NaOH, $Na_2CO_3$) and polished water was run followed by re-equilibration back to the carbonate–formaldehyde eluent in time for the next injection. A gradient clean-up was not required for the other applications described. The pre-column was cleaned through simple mobil phase elution and the next analysis begun, step 1.

The IC system was a Dionex 4500i dual-channel chromatograph with an automated sampler and a pulsed electrochemical detector (PED), used in the conductivity mode. A 50 $\mu$L loop, an AMMS Dionex suppressor with 25 m*M* $H_2SO_4$ regenerant at 3 ml/min and Dionex anion-exchange columns were used throughout. Acetate analysis was conducted on an AG-7 column with 20 m*M* borate eluent at 1 ml/min. The TFA and anions in concentrated HCl were conducted via H-C analysis using an AG-4A pre-column and a AS-4A separator column with 2 m*M* $Na_2CO_3$/0.75 m*M* $NaHCO_3$, eluent at 2 ml/min.

All chemicals were Mallinckrodt AR and the water was polished (deionized water further purified through a Millipore Milli-Q filtration system). Acetate: dissolve 1 mg of peptide (Desmopressin or Calcitonin Acetate) in a 2-ml volumetric flask and dilute to volume with water. TFA: dissolve 2 mg of peptide in a 2-ml volumetric flask and dilute to volume with water. Anions/HCl: add 10 ml of 1% (w/v) sodium carbonate and 200 ml of concentrated HCl to a 400-ml beaker and evaporate to dryness on a steam bath. Transfer the residue, quantitatively, to a 10-ml volumetric flask with successive additions of water and dilute to volume.

## RESULTS AND DISCUSSION

### *Sulfite*

The previously described sulfite analysis in analgesic formulations [1] has been fully automated. In the earlier work, late eluters were left on the pre-column and flushed from the column after the analysis was complete, with three successive 500 $\mu$l injections each of methanol, concentrated eluent (0.05 *M* NaOH, $Na_2CO_3$) and polished water. By employing a second pump and injector, the pre-column clean-up was conducted immediately after the H-C of the analyte was transferred to the separator column. The latest revision utilizes a step gradient program, of 1.5 min each, methanol, concentrated eluent and polished water, respectively, which lends itself to a fully automated basis (Fig. 1).

The generic utility of the sulfite "dilute and shoot" method arises from the fact that the bulk of the matrix is removed from the analysis, therefore, we should be able to extent this method to the analysis of sulfite in a variety of matrices. The US Food

and Drugs Administration (FDA) has established a 10 ppm labelling limit for sulfite in food items and has recommended that drug manufacturers monitor their products at the same level. Reliable methodology for the determination of the metastable sulfite ion at the level of interest presents a formidable analytical challenge [2–4]. The FDA 10 ppm sulfite labelling limit mandated that appropriate methodology be developed. An accurate reliable means of analysis, not susceptible to matrix interference problems and, of course, rapid, would be ideal. The H-C method approaches this ideal. A comparison was made using our H-C method and the IC method of the USA Association of Official Analytical Chemists (AOAC) [5]. The same food items described in a collaborative AOAC study were analyzed by the H-C method. In the author's opinion, the H-C analysis using conductivity detection was demonstrated to be superior to the AOAC method which uses electrochemical detection, however, it should be noted, no attempt was made to run the AOAC method. The conclusions are based on the literature discussion of the problems encountered with the AOAC method. Major shortcomings described for the AOAC method include: ruggedness, accuracy (based on spike recovery data), precision, stability of the analyte and susceptibility to matrix interference. In our comparison, accuracy was accessed based on spike recoveries at three levels.

In the AOAC's analysis of corn starch, seven of the collaborators failed to detect any sulfite while two other collaborators found 15 and 21 ppm in duplicate sample analyses. The conflicting findings were termed false positives due to inadequate chromatography, "interferences from the matrix". The findings were rejected on statistical grounds. H-C analysis of corn starch found sulfite comparable to the rejected levels, 23 ppm with a 95% average recovery. Our study was also extended to wheat starch where none was detected, <2 ppm with a 94% average recovery (Table I).

The AOAC study found consistently higher results obtained by the IC method, about 400 ppm, relative to the Monier–Williams [6] wet chemical procedure, about 290 ppm, for instant mashed potatoes. The H-C analysis found 175 ppm with a 84% recovery (Table II).

Comparable results were obtained by both IC methods for wine coolers, 19 ppm AOAC *vs.* 42 ppm H-C with a 98% recovery. In the H-C case, the sample was a red sangria wine cooler. The AOAC sample was non-specific; however, a general problem of colored samples was noted. Our method was also extended to a sample of white wine which contained considerably more sulfite, as expected, 132 ppm with a 109% average recovery (Table III).

A problem with interference was encountered for the lemon juice analysis. An unknown peak circum-

TABLE I

COMPARISON OF IC METHODS FOR STARCH ANALYSIS

| Sample designation | AOAC method | | H-C method | |
|---|---|---|---|---|
| | Found (ppm) | Recovery (%) | Found (ppm) | Recovery (%) |
| *Corn starch* | 0 | – | | |
| 10 ppm spike | 5.5–29 | 55–112 | | |
| 30 ppm spike | 22–45 | 72–112 | | |
| *Corn starch (average 23 ppm)* | | | 22 | – |
| 5 ppm spike | | | 27 | 85 |
| 10 ppm spike | | | 33 | 105 |
| 15 ppm spike | | | 37 | 94 |
| Duplicate sample preparation | | | 24 | – |
| *Wheat starch* | | | <2 | – |
| 5 ppm spike | | | 4.9 | 99 |
| 10 ppm spike | | | 9.0 | 90 |
| 15 ppm spike | | | 14 | 93 |
| Duplicate sample preparation | | | <2 | – |

TABLE II

IC DETERMINATION OF SULFITE IN INSTANT MASHED POTATOES

| Sample designation | AOAC method | | H-C Method | |
|---|---|---|---|---|
| | Found (ppm) | Recovery (%) | Found (ppm) | Recovery (%) |
| *Instant mashed potatoes* | 341–436 | – | | |
| 80 ppm spike | 411–609 | 84–219 | | |
| 400 ppm spike | 558–1038 | 56–164 | | |
| *Instant mashed potatoes (average 175 ppm)* | | | 195 | – |
| 250 ppm spike | | | 371 | 79 |
| 500 ppm spike | | | 597 | 84 |
| 750 ppm spike | | | 850 | 90 |
| Duplicate sample preparation | | | 155 | – |

TABLE III

SULFITE IC ANALYSIS OF WINE

| Sample designation | AOAC method | | H-C Method | |
|---|---|---|---|---|
| | Found (ppm) | Recovery (%) | Found (ppm) | Recovery (%) |
| *Wine cooler* | 9–29 | – | | |
| 10 ppm spike | 20–45 | 39–213 | | |
| 30 ppm spike | 36–65 | 67–139 | | |
| *Red sangaria wine cooler (average 42 ppm)* | | | 39 | – |
| 50 ppm spike | | | 92 | 99 |
| 100 ppm spike | | | 141 | 99 |
| 150 ppm spike | | | 185 | 95 |
| Duplicate sample preparation | | | 45 | – |
| *White wine (average 132 ppm)* | | | 133 | – |
| 50 ppm spike | | | 187 | 108 |
| 100 ppm spike | | | 244 | 112 |
| 150 ppm spike | | | 293 | 107 |
| Duplicate sample preparation | | | 131 | – |

TABLE IV

SULFITE IC ANALYSIS OF LEMON JUICE

| Sample designation | AOAC method | | H-C Method | |
|---|---|---|---|---|
| | Found (ppm) | Recovery (%) | Found (ppm) | Recovery (%) |
| *Lemon juice* | 12–30 | – | | |
| 10 ppm spike | 21–52 | 70–299 | | |
| 30 ppm spike | 36–90 | 71–201 | | |
| *Lemon juice (average 155 ppm)* | | | 158 | – |
| 250 ppm | | | 372 | 87 |
| 500 ppm spike | | | 583 | 86 |
| 750 ppm spike | | | 877 | 96 |
| Duplicate sample preparation | | | 153 | – |

TABLE V

SULFITE IN A COLA SOFT DRINK BY H-C ANALYSIS

| Sample designation | Found (ppm) | Recovery (%) |
|---|---|---|
| *Cola soft drink* | <2 | – |
| 5 ppm spike | 4.8 | 96 |
| 10 ppm spike | 9.3 | 93 |
| 15 ppm spike | 13 | 85 |
| Duplicate sample preparation | <2 | – |

vented accurate quantitation. We speculated that the unknown might be organic in nature and were prompted to substitute a PRP-1 reversed-phase column for the AS-2 pre-column. Such a multi-dimensional scheme was previously found to be very effective at dealing with organics [1]. The interference was removed from the subsequent chromatograms and accurate quantitation was achieved, 155 ppm with a 90% average recovery. The AOAC analysis reported 21 ppm, however, the sample we analyzed was labelled "contains sulfite" (Table IV).

The AOAC method could not analyze caramel colored samples. The H-C method appears to be color blind. A cola soft drink was readily analyzed, <2 ppm with a 91% average recovery (Table V).

The actual sulfite content in the samples analyzed in this study *vs.* the specific samples used in the AOAC collaborative study are inconsequential. What this study has demonstrated is the superiority of the H-C method which can be attributed to maintaining analyte stability, using a more rugged detector and eliminating matrix complications via H-C column switching techniques. The net result is that the H-C "dilute and shoot" sulfite method is a more accurate, precise, reliable, rapid, generic procedure; suitable for a broad diversity of sample types (foods, beverages and pharmaceuticals) and should facilitate testing to ensure compliance with the FDA's 10 ppm limit.

*Peptides*

Mallinckrodt Specialty Chemical Company's R&D is developing a peptide product line which includes calcitonin and desmopressin acetate. A request was made to develop methods for the determination of acetate and trifluoroacetate (TFA) in the final products. A "dilute and shoot" approach was developed for each.

The acetate determination was accomplished using a weak eluter system, 20 m*M* borate eluent on an AG-7 anion-exchange column. The bulk of the matrix was retained on the column and eventually

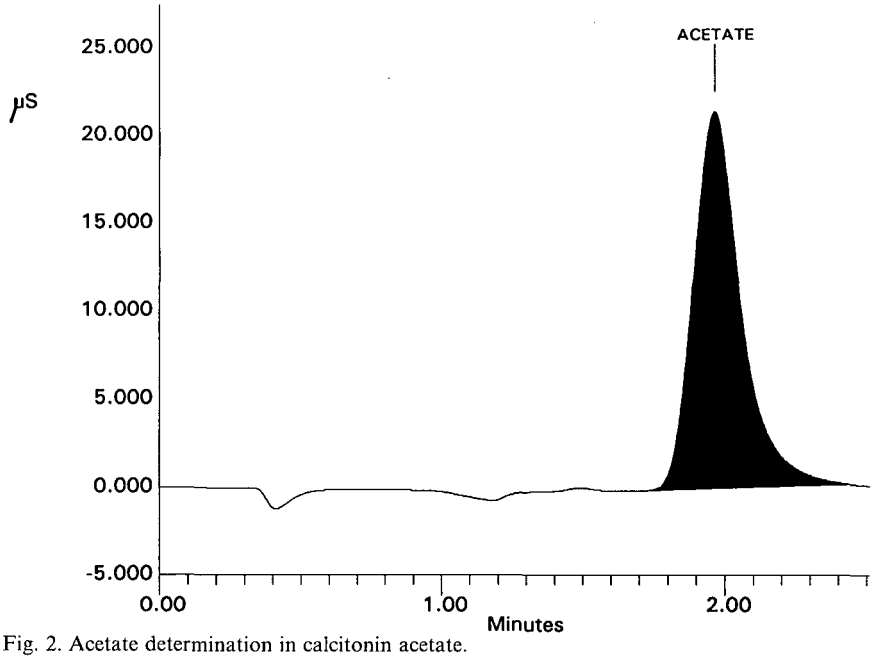


Fig. 2. Acetate determination in calcitonin acetate.

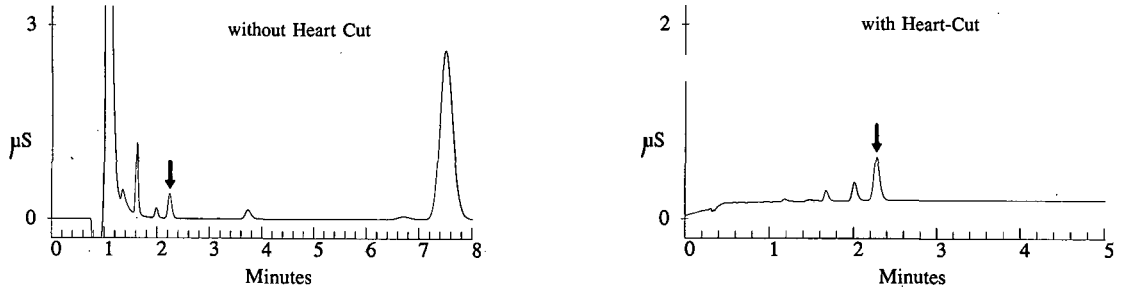


Fig. 3. TFA determination in calcitonin acetate with and without heart-cut column switching.

flushed off with concentrated eluent after the analyses were completed. Since the bulk of the matrix was retained on the column, no H-C column switching was required (Fig. 2). The TFA analysis was accomplished via H-C column switching to eliminate the bulk of the matrix and reduce the analysis time about 40% (Fig. 3).

*Anions in hydrochloric acid*

A direct "dilute and shoot" approach for trace

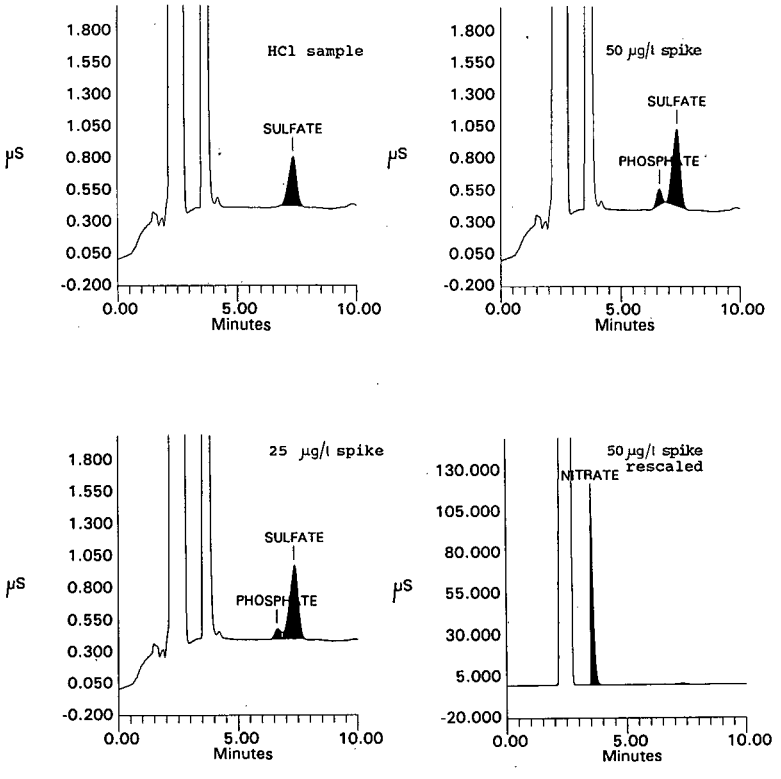


Fig. 4. Trace anions in concentrated hydrochloric acid. Experimental conditions: after determining the heart-cut for the interfering anion, chloride, relative to the anions of interest, sulfate and phosphate, the IC system was configured as described using a AG-4A pre-column and a AS-4A separator column. The valve configuration was the same as step 1 in Fig. 1, from 0–0.5 min, then step 2 for the remainder of the analysis. The eluent was 2 m*M* sodium carbonate and 0.75 m*M* sodium bicarbonate at 2 ml/min. A 50-μl loop was used. An AMMS suppressor was used with 25 m*M* sulfuric acid regenerant at 3 ml/min. Detection was accomplished with a PED detector (Dionex) in the conductivity mode.

anions ($\mu$g/l) in concentrated hydrochloric acid was unsuccessful, even using our H-C technique, however, a tentative procedure has been developed which keeps the sample preparation to a minimum. The sample was treated with sodium carbonate, evaporated to dryness, the residue re-dissolved and analyzed using H-C column switching. The reagent blank interferences with accurate quantitation of sulfate. Impurities in the sodium carbonate are suspected. It should also be noted that contamination of the glassware was a problem in achieving consistent results. The glassware used in this study was soaked in fresh polished water daily for several days. Sulfate and phosphate spikes were made at 25 $\mu$g/l of each and 50 $\mu$g/l of each. Recoveries were 50% and 112% for sulfate and phosphate, respectively, at the 50 $\mu$g/l level. No phosphate was detected in the sample. The sample also appears to contain about 5 mg/l nitrate, which would have gone undetected without the H-C analysis (Fig. 4).

A higher-purity carbonate or perhaps high-purity $NaHCO_3$ or NaOH could probably address the interference problem from the reagent blank. A glassware protocol is also in order to avoid contamination. A set of dedicated columns is mandated to routinely achieve the requisite sensitivity. Ideally a dedicated system would also be used. Further development is obviously required to establish a rugged routine procedure, however, these preliminary results support the feasibility of the present approach to trace anions in concentrated hydrochloric acid.

## CONCLUSIONS

A "dilute and shoot" approach to sample preparation in IC can be successfully employed without necessarily suffering the ill effects of column overloading and system abuse. The H-C technique [1] previously described can eliminate or simplify the requisite sample pre-treatment. This approach was demonstrated for sulfite analysis in a variety of different matrices and revised to a fully automated routine procedure, amenable to a quality control laboratory; acetate and TFA analysis in peptides; for $\mu$g/l anion analysis in high-purity concentrated hydrochloric acid; as well as other examples described in ref. 1.

## ACKNOWLEDGEMENT

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# Qualitative corrosion monitoring by on-line ion chromatography

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ABSTRACT

The corrosion mechanism for secondary side systems of pressurized water reactors has always been difficult to monitor and quantify. Ion chromatography may be the method to evaluate the corrosion phenomena using on-line systems.

Two separate ion chromatography methods have been developed to monitor manganese, a component of carbon steel and stainless steel used in secondary system piping. The first method employs step gradient cation-exchange separation with chemically suppressed conductometric detection. The second method employs traditional transition metal analysis using an analytical column followed by post-column reagent addition and mixing with visible detection at 520 nm.

The presence of manganese was verified using corrosion product monitors. Each monitor has a 0.45-$\mu$m particulate filter followed by three cation-exchange membrane filters for the determination of soluble metallic species. Atomic absorption spectrophotometry was employed to analyze the processed filters.

Results for the corrosion monitors and the ion chromatography methods were compared to attempt validation of all methods and to establish practical methods for on-line corrosion monitoring.

## INTRODUCTION

In the nuclear industry an understanding of the mechanisms and rate of corrosion is required to maximize plant reliability and minimize operational costs. In addition this is necessary to fulfill the utilities' obligation to the general public for safe operation of the plant.

Evaluation of corrosion processes in domestic nuclear plants requires an understanding of the plants operational parameters. Boiling water reactors (BWRs) boil water surrounding the uranium core to form steam that drives the turbines and electrical generator. Condensed steam is returned to the system, and as a result of this recycling, non-volatile anionic and cationic impurities concentrate in the reactor and recirculation water. Other impurities are introduced into the cycle by corrosion of metal surfaces; this corrosion is minimized by maintaining feedwater dissolved oxygen at 20–50 ng/ml. Metallic corrosion impurities can deposit on core surfaces, and become radioactive via neutron activation. Heavy corrosion deposits on the fuel can cause cladding failures and release radioactive fission products into the coolant. Corrosion impurities adhering to core surfaces and other metal surfaces are slowly released during steady-state operation, but can be rapidly released during large power transients or from significant changes in system chemistry. The radioactive deposits not only affect

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heat transfer efficiency but are also a potential radiological hazard to personnel performing maintenance and testing.

Pressurized water reactor (PWR) plants have a primary loop of water circulating through the nuclear core in a system under 2250 p.s.i. pressure and at 296°C wihout boiling. In a secondary loop treated water is circulated through large heat exchangers, called steam generators, where heat is removed from the primary loop, causing the secondary water to become steam, which drives the turbine and electric generator. Because many construction metals are used in the secondary loop, the secondary water must be ultrapure and treated to minimize corrosion. Corrosion products, consisting mainly of insoluble iron species, accumulate or deposit in the steam generators as sludge. This sludge accumulation results in low-flow, steam blanketed areas where ultra-trace ionic impurities present in the feedwater can concentrate by many orders of magnitude resulting in a highly corrosive local environment. Resulting damage to steam generator components can cause forced outages, tube plugging (loss of output) and ultimately, replacement of steam generators at a cost in the millions of dollars. To put the problem in perspective, the Electric Power Research Institute recommends a maximum sludge pile depth of between 0.42 and 0.85 cm [1]. To achieve these goals, utilities may spend US$ 8000–10 000 *per kg* to reduce steam generator sludge. The condensate (condensed steam) flows through mixed bed ion exchangers to remove impurities, then is pH adjusted with ammonium hydroxide or morpholine, and hydrazine for removal of oxygen. The condensate becomes feedwater which is heated by a series of heaters before being introduced into the steam generators.

Domestic BWRs and PWRs usually operate continuously for an eighteen month fuel cycle. If the correct chemistry and operational controls are not enforced for this period, corrosion of plant systems, especially feedwater piping, could be significant. These corrosion products must be minimized and removed from steam generators to prevent loss of heat transfer efficiency in PWRs and minimized in BWRs to prevent cladding defects in the fuel. Corrosion transport studies have been conducted by the Electric Power Research Institute [2] to help domestic nuclear plants understand corrosion mechanisms and to recommend treatment to minimize corrosion impacts. To date, these studies have measured amounts of the major corrosion products, iron and copper. Limited solubilities of these corrosion products in the feedwater complicates taking a representative sample, requiring concentrating of these impurities from large sample volumes, and a lengthy lab analysis. Methods of many steps tend to introduce error and are not timely for making system corrections on a "real time" basis.

The importance of corrosion control continues to drive the need for a fast, reliable method of corrosion monitoring. In support of this quest, this study evaluates manganese which, as a component of carbon steel [3], can also be accurately determined in the pg/ml level by on-line ion chromatography (IC).

## EXPERIMENTAL

### *Instrumentation*

All chromatography in this study was performed using a Dionex (Sunnyvale, CA, USA) series 8100 ion chromatograph. Channel one was set up for transition metal analysis and channel two was set up for the simultaneous determination of monovalent and divalent cation analysis. The ion chromatograph was interfaced with a multichannel data acquisition and control system consisting of a Dionex Autoion 300 computer interface, Hewlett-Packard (Palo Alto, CA, USA) System 300 desk top computer and Hewlett-Packard model 2930 printer. Sample delivery was accomplished using a Dionex sample selection module. Dionex AI 300 software provided data acquisition, data reduction and control of the ion chromatograph.

Corrosion data was collected using NUS (Pittsburgh, PA, USA) corrosion product monitors with Millipore (Bedford, MA, USA) 0.45-$\mu$m particulate filters and Gelman Sciences (Ann Arbor, MI, USA) cation-exchange membrane filters. After acid digestion, the filter solutions were analyzed for iron and manganese using a Perkin-Elmer (Norwalk, CN, USA) Model 5100 atomic absorption spectrophotometer for flame analysis methods. For trace analysis, the Model 5100 was coupled with an HGA-600 Zeeman graphite furnace.

### *Reagents and standard solutions*

The chemicals used in this study were of anal-

ytical-reagent grade unless otherwise noted. Standards were either commercially available atomic absorption standards, or prepared by dissolving the appropriate amounts of salts in deionized water, then stored in polypropylene containers. The water used was plant-produced demineralized water passed through a Barnsted Nanopure (Barnsted/Thermolyne, Dubuque, IA, USA) water system.

The eluent used for cation analysis was a mixture of dilute Ultrex (J. T. Baker, Philipsburg, NJ, USA) hydrochloric acid and 2,3-diaminopropionic acid monohydrochloride (DAP) (Fluka, New York, NY, USA). Tetrabutylammonium hydroxide (TBAOH) (Southwestern Analytical, Austin, TX, USA) was used as the regenerant.

The eluent used for transition metal analysis contained pyridine-2,6-dicarboxylic acid (PDCA), (Kodak, Rochester, NY, USA), glacial acetic acid (J. T. Baker), and sodium acetate (EM Science, Gibbstown, NJ, USA). The post-column reagent contained 4-(2-pyridylazo)resorcinol (PAR) (Fluka, New York, NY, USA), glacial acetic acid and ammonium hydroxide (J. T. Baker).

*Columns*

All columns used in this study were manufactured by Dionex. The columns used for cation analysis were an IonPac CS10 analytical column, a CG10 guard column, and a trace cation concentrator 2 (TCC-2) column. To reduce eluent impurities when a gradient was employed, a cation trap column was placed on the outlet of the eluent pump prior to the injection valve. The trap column function is to "smear out" eluent impurities by preventing the impurities from building up on the analytical column and eluting as a peak as the gradient program is run.

The columns used for transition metal analysis were an IonPac CS5 analytical column, a CG5 guard column, a CG2 concentrator column followed by a mixing tee and a 1500-$\mu$l beaded reaction coil. The post-column reagent was introduced to the eluent at the mixing tee then allowed to react in the beaded reaction coil and detected on the visible detector at 520 nm.

## PROCEDURES

Standards and samples were analyzed for manganese by cation and transition metal IC. The cation channel was operated by employing a gradient program in both the ammonia and morpholine matrix. The conditions were an eluent flow-rate of 1.0 ml/min starting with 20 m*M* HCl/0.35 m*M* DAP and after injection a 3-min ramp to 50 m*M* HCl/14 m*M* DAP. A cation micromembrane suppressor II (CMMS-II) was used to lower the background conductivity to between 2 and 6 $\mu$S. The regenerant was 100 m*M* tetrabutylammonium hydroxide with a flow-rate of 10 ml/min. employing a Dionex AutoRegen system. The overall backpressure of the system ranged between 900 and 1200 p.s.i.g.

The transition metal channel consisted of an eluent which contained 6 m*M* PDCA/50 m*M* sodium acetate/50 m*M* acetic acid at a flow-rate of 1.0 ml/min. The post-column reagent was introduced at the mixing tee at a flow-rate of 0.5 ml/min and contained 0.2 m*M* PAR/3.0 *M* ammonium hydroxide/1.0 *M* acetic acid. This mixture was allowed to react in a 1500-$\mu$l beaded reaction coil and the resultant metal complexes were detected on a visible detector at 520 nm.

Corrosion product filters were acid digested and analyzed by atomic absorption spectrophotometry in accordance with Electric Power Research Institute guidelines [4]. The calibration standards were prepared from separate stock $\mu$g/ml standard solutions and placed in the standard preparation module for dilution to calibration levels. The sample preparation module allows for automatic *in situ* preparation of trace (pg/ml) standards to prevent contamination from handling. The precision of the ion chromatography methods was measured continuously by the AI 300 software for up to 202 data points before the file started overwriting. All statistical data were reviewed by use of the trend file function.

In all methods a three-level multicalibration was performed. For the transition metal analysis of manganese 10 ml of standard and sample were concentrated. For the cation analysis of manganese 25 to 50 ml of sample and standard were concentrated except for samples containing up to 7 mg/ml morpholine. In standards and samples containing morpholine only 15 ml could be concentrated before the concentrator began to overload. The corrosion product filters were placed in-line and the average sample volume on each filter was approximately 100 l.

## RESULTS AND DISCUSSION

The detection of manganese by ion chromatography has received some attention in the past [5–7]; however, the use of manganese as an indicator of corrosion in power plants is a novel idea. The presence of significant quantities of manganese was first observed in the secondary system of the Surry Power Station. An unknown peak which interfered with magnesium was observed in chromatograms obtained with the Dionex Fast Cation column set. This same peak was also observed at the US-TVA Sequoyah Power Station. Dionex personnel [8] suggested that this unknown peak was probably due to manganese. The identity of the unknown peak was later confirmed by spiking samples with manganese standards.

The on-line ion chromatographs were calibrated for manganese in accordance with the conditions discussed in the experimental section. Initial relative standard deviations (R.S.D.) of standards and samples were unacceptably high (45%). A review of the phase diagram [9] for manganese showed the sample pH was critical in order to ensure that manganese remained in the $Mn^{2+}$ form (see Fig. 1).As a result, all standards and samples were acidified to a pH of *ca.* 4 with Ultrex-grade hydrochloric acid. The acidification step was performed on line using the Dionex sample preparation module. Acidification reduced the R.S.D. to approximately 20%. The on-line ion chromatographs were operated under these conditions for the rest of the study. Fig. 2 shows an example of the second level calibration of the cation channel using the CS10 column an morpholine matrix. Fig. 3 shows an example of the second-level calibration of the transition metal channel. The linearity of the manganese response at ultra-trace (pg/ml) levels using the CS10 method is demonstrated in Fig. 4.

A significant quantity of manganese was detected in the steam generator blowdown samples during a "hideout return" study conducted at the Surry Power Station Unit 1 in late 1990. The process known as "hideout" results from the concentration of impurities by boiling in flow-restricted regions of PWR steam generators (S/G) such as S/G crevices. Impurities which are concentrated in these regions during power operation will "return" from hideout to the steam generator bulk water during shutdown and subsequent cooldown. Both the cation (CS10) channel and transition metals channel showed concentrations as high as 100 ng/ml. GEBCO Engineering was contracted by the Viginia Electric and Power Company to assist in the evaluation of hideout

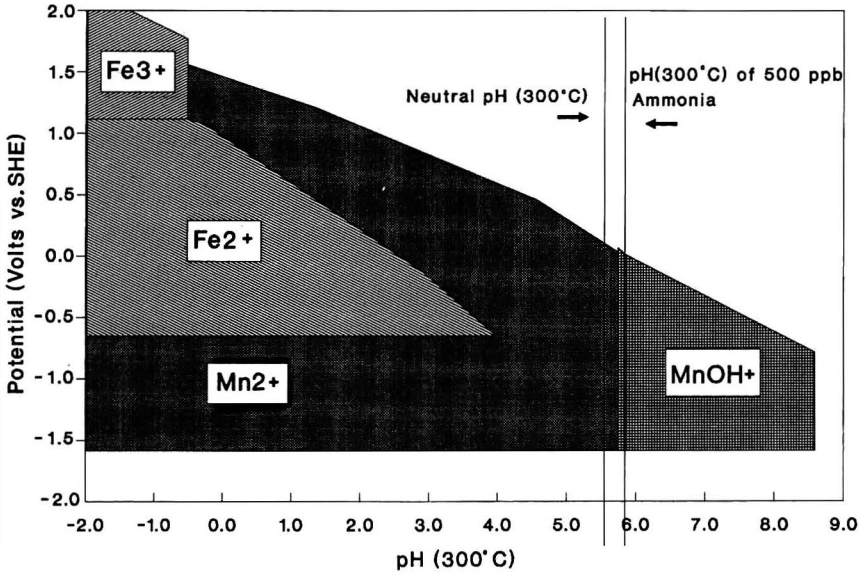


Fig. 1. Partial phase diagrams for iron and manganese at 300°C. SHE = standard hydrogen electrode; ppb represents ng/ml.

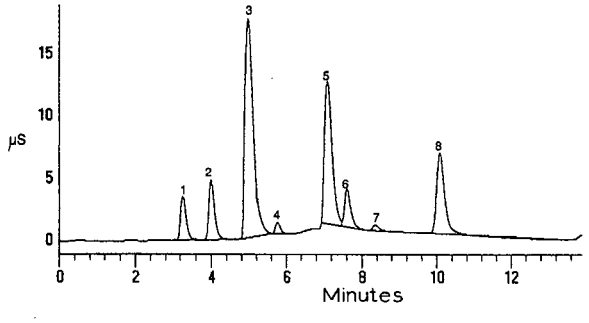


Fig. 2. Autocal 2 in a morpholine–ammonia matrix. Peaks: 1 = lithium (1 ng/ml), 2 = sodium (1 ng/ml); 3 = ammonia (150 ng/ml); 4 = potassium (1 ng/ml); 5 = morpholine (5 μg/ml); 6 = magnesium (2 ng/ml); 7 = manganese (2 ng/ml); 8 = calcium (2 ng/ml).

return data. In their report to Virginia Power, GEBCO suggested that based on their analysis of the data, there was a very strong possibility that the manganese observed during hideout return had originated from corrosion of secondary system piping and components. To attempt to confirm this hypothesis the following test plan was designed to obtain the necessary plant data: (1) conduct mass balance studies to confirm the source of manganese in the secondary system; (2) confirm the identity of manganese in plant samples using alternate analytical methods; (3) perform concurrent monitoring of secondary samples using on-line IC and corrosion product (CP) sampling and analysis by atomic absorption spectrophotometry; (4) attempt to establish a relationship between total iron (using CP

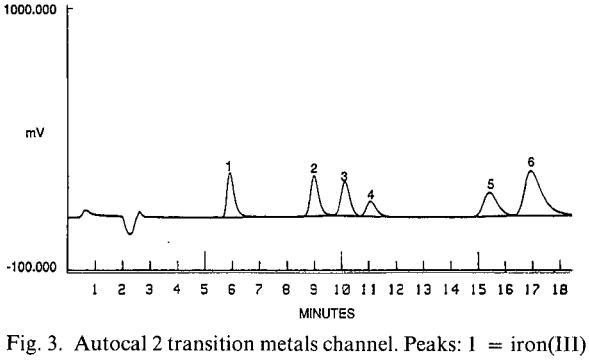


Fig. 3. Autocal 2 transition metals channel. Peaks: 1 = iron(III) (2.5 ng/ml); 2 = copper(II) (2.5 ng/ml); 3 = nickel(II) (2.5 ng/ml); 4 = zinc(II) (2.5 ng/ml); 5 = manganese(II) (5.0 ng/ml); 6 = iron(II) (5.0 ng/ml).

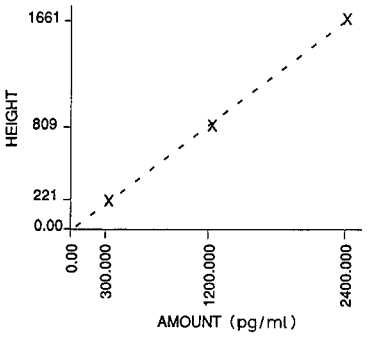


Fig. 4. Manganese low level calibration plot.

analysis) and soluble manganese (using on-line IC) in final plant feedwater.

A mass balance study was conducted at Surry Unit 1 in April, 1991 just three months after unit startup from a refueling outage. The results of this study are shown in Fig. 5. The purpose of the mass balance study was to account for the manganese in the secondary system on a mass transport basis. This approach is used in power plants to identify sources of impurities since many streams are combined to make up the final feedwater. The mass balance identifies the quantitative contribution of each of these streams to the feedwater. From Fig. 5, it was apparent that a significant quantity of the manganese in the final feedwater was contributed by the feedwater piping and components, *i.e.*, the feedwater mass transport rate was nearly double the sum of the polisher effluent and heater drains mass flow-rates. This data strongly indicated that the manganese in the secondary system was originating from corrosion of carbon steel components and therefore should be indicative of total secondary system corrosion.

During the Surry Unit 2 shutdown in the spring of 1991, a second hideout return study was conducted. As in the previous (Unit 1) study, significant concentrations (> 100 ng/ml) of manganese were detected in steam generator blowdown samples analyzed using on-line cation and transition metal IC. To conclusively confirm the presence of manganese, grab samples were collected during the hideout return test period and analyzed using atomic absorption spectrophotometry (AAS). As shown in Fig. 6, the grab sample–AAS results agreed well with the on-line IC data. Figs. 7 and 8 are examples of

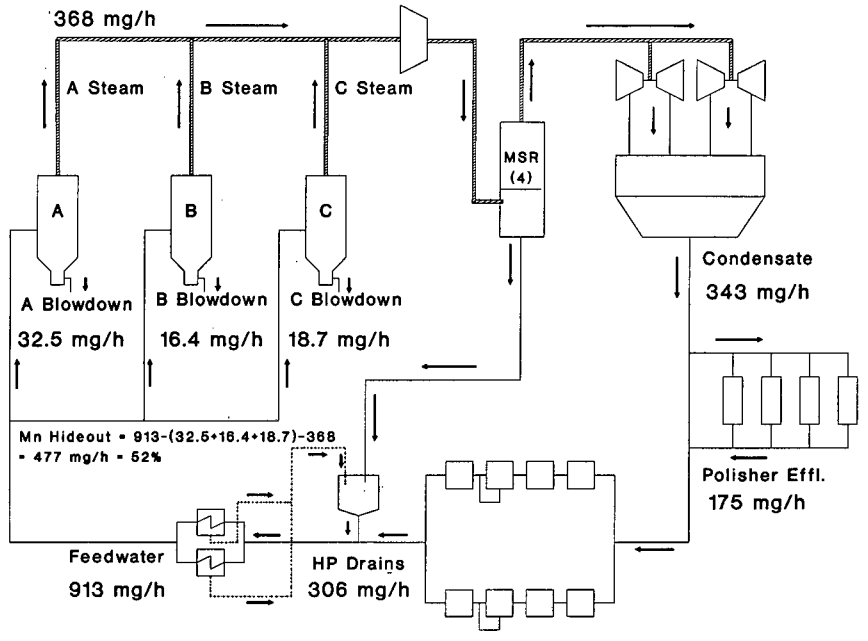


Fig. 5. Surry Unit 1 manganese mass transport from April 21–25, 1991 IC Data.

Unit 2B steam generator blowdown chromatograms of the cation and transition metals channels respectively during this study.

Concurrent monitoring of secondary system samples for manganese by conventional corrosion filter product methods previously described, and on-line IC commenced in April, 1991 and continued through July, 1991. As stated in item 3 above, the purpose of this portion of the study was to determine the long-term stability of the manganese data and the relationship between soluble manganese (IC) data and corrosion product iron data to determine whether a correlation could be established. Since the IC methods measure only soluble manganese, it was critical to first determine the percentage of soluble *versus* total manganese in plant feedwater samples.

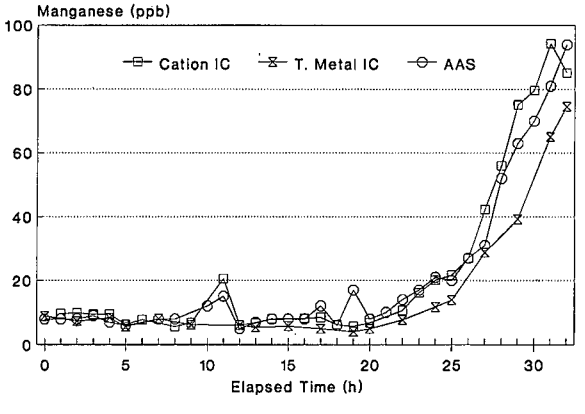


Fig. 6. 2C steam generator manganese hideout return comparison of cation IC, transition (T.) metal IC and atomic absorption spectrophotometry (AAS).

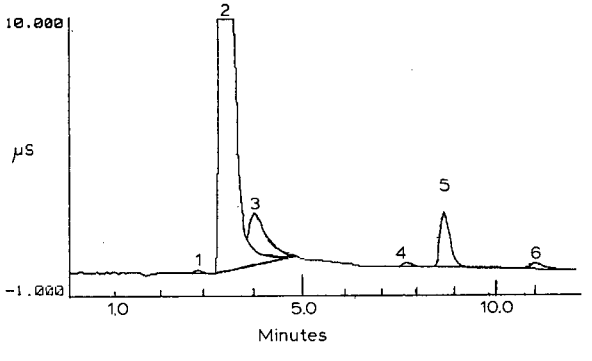


Fig. 7. Surry 2B steam generator cation method. Peaks: 1 = sodium (99.0 pg/ml); 2 = ammonium (125.1 ng/ml); 3 = potassium (139.0 pg/ml); 4 = magnesium (91.0 pg/ml); 5 = manganese (3.21 ng/ml); 6 = calcium (24.9 pg/ml).

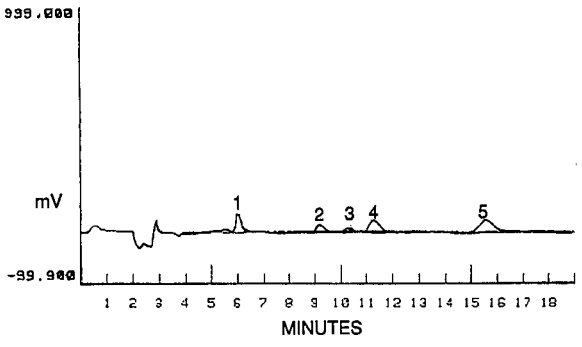


Fig. 8. Surry 2B steam generator transition metals method. Peaks: 1 = iron(III) (1.797 ng/ml); 2 = copper (527.0 pg/ml); 3 = nickel (228.0 pg/ml); 4 = zinc (3.391 ng/ml); 5 = manganese (3.809 ng/ml); 6 = iron(II) (below detection limit).

If the soluble/total manganese ratio was not *ca.* 1.0, or varied significantly, further efforts would not have been warranted. Fig. 9 shows manganese solubility data for the study period from April through July, 1991 as measured by conventional corrosion product filter methods. This data clearly indicated that manganese in the feedwater was >90% soluble in contrast to iron which was <40% soluble.

A second key consideration was the relative agreement between manganese results obtained by on-line IC and corrosion product filter analysis. Fig. 10 shows the results of samples analyzed by both methods during the period from April 22–May 13, 1991. Each IC data point represents the average of all samples analyzed during the 24-h period (typically 2 to 4). Because of the time-consuming nature of the corrosion product method and the necessity to pass very large volumes of sample through the particulate and ion exchange filters to obtain adequate sensitivity, fewer data points were obtained for the corrosion product method. Considering the ultra-trace sample concentrations (200 to 300 pg/ml) in feedwater, agreement between the two methods was quite good however, there was a bias between the two methods. This was not surprising considering the differences is not only the analytical techniques, but also the sampling methods. In addition, the IC method was obviously much better able to detect day-to-day fluctuations on a real-time basis.

As outlined in item 4 above, the final phase of the study was to attempt to determine whether a correlation could be established between the on-line IC manganese data and the corrosion product iron data. In order to establish such a correlation, it was critical that the ratio of soluble manganese to total

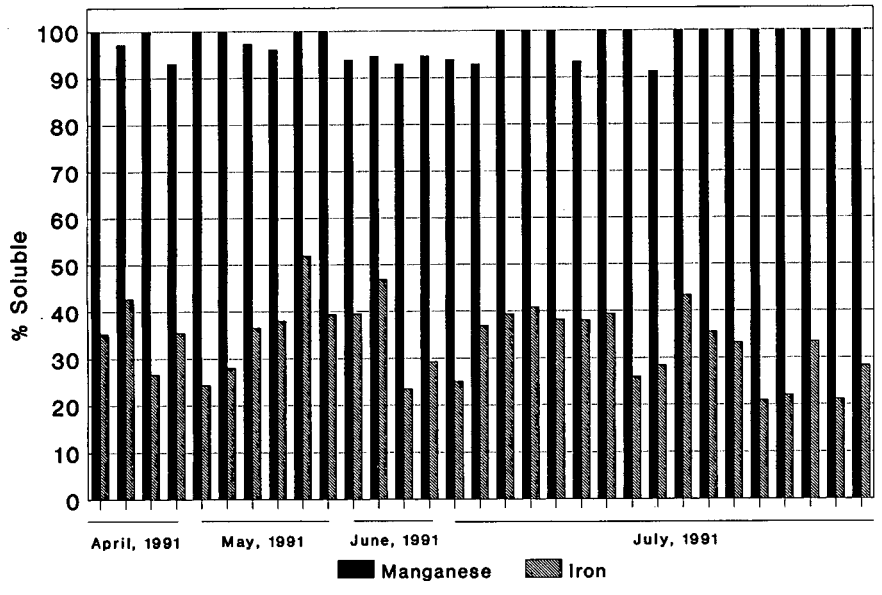


Fig. 9. Surry Unit 1 feedwater manganese and iron solubility April 22–July 30, 1991.

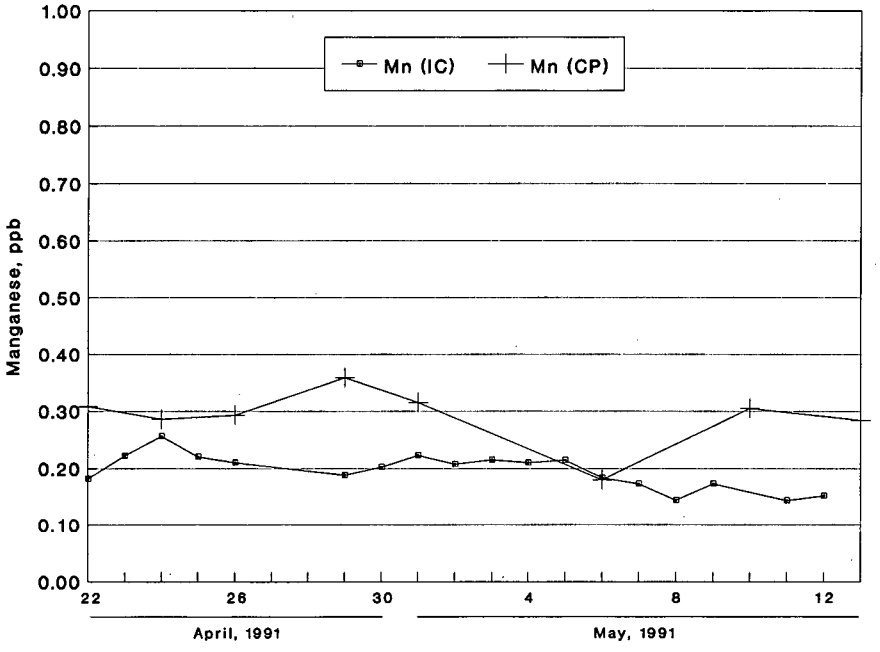


Fig. 10. Surry Unit 1 feedwater manganese: comparison of IC and corrosion product data.

iron remain relatively constant under steady-state plant conditions. The final phase of the study was to use the on-line IC manganese data to predict the total feedwater iron concentration and compare these predictions to measured values obtained from corrosion product analyses. A correlation constant was established using the total manganese to iron ratio and an offset value to account for the consis-

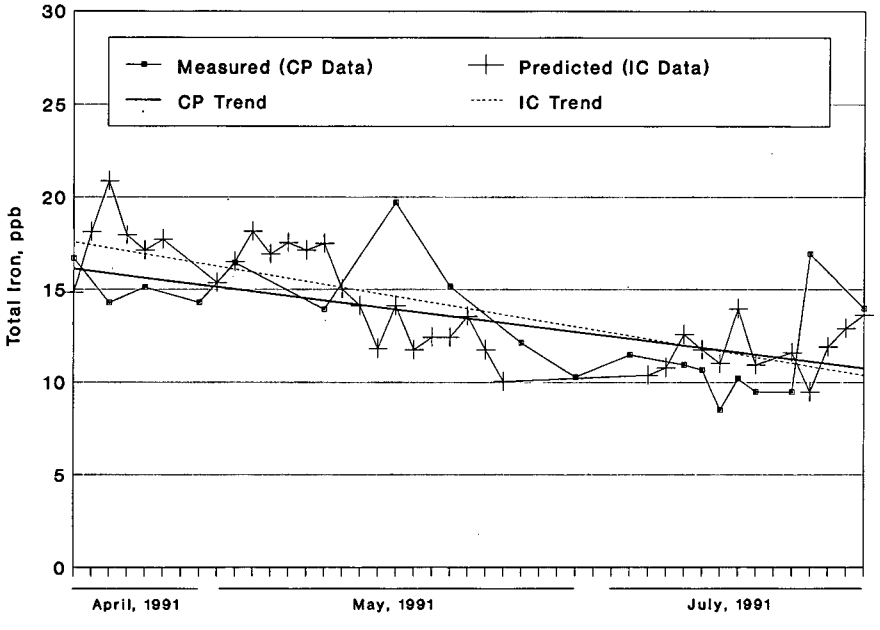


Fig. 11. Surry Unit 1 feedwater under steady-state conditions.

tent difference in manganese results obtained by on-line IC and the corrosion product method. Total iron was predicted from the IC data by simply multiplying the IC result by the correlation constant. Fig. 11 shows the results of these comparisons for the three-month study period. While there were significant differences between some of the individual predicted and measured values, the overall agreement was excellent. Trend lines for the predicted and measured iron concentrations were nearly identical.

## CONCLUSIONS

On the basis of the data presented above, the authors preliminary conclusion was that on-line monitoring of soluble manganese could be used effectively as an indicator of feedwater corrosion product transport in the secondary system of the Surry Station. This is due to the >90% solubility of manganese *versus* the <40% solubility of iron observed during the study. Testing is currently underway at the US-TVA Sequoyah and Browns Ferry Power Stations to determine whether similar correlations can be established for these plants. As stated previously, the use of morpholine for pH control at Sequoyah (*vs.* ammonia at Surry) complicates the chromatography and limits the sample preconcentration volume. At the Browns Ferry Station (BWR) the only additive is oxygen in the feedwater which may influence the solubility of manganese as seen in Fig. 1. Research is underway to attempt to resolve these difficulties and to improve sensitivity and R.S.D.s. It is strongly emphasized that the correlation between soluble manganese and total iron is highly plant-specific since the alloy composition of plant piping and components varies considerably among plants of similar design. The authors are strongly encouraged however, by the results obtained to date. The ability to monitor corrosion of power plant systems on an on-line, real-time basis would provide power plant chemists with a means of assessing potential damage to critical plant components on a timely basis, and to evaluate the effectiveness of alternate water treatment schemes.

## ACKNOWLEDGEMENTS

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# Ion chromatographic analysis of inorganic molecular metal–oxygen cluster compounds

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ABSTRACT

An ion chromatographic technique for the measurement of the concentrations of metal–oxygen cluster anions and the determination of their elemental compositions has been developed. The method depends on the decomposition of the heteropoly anions into their simpler constituent ions by the addition of a basic hydroxide and the subsequent separation and analyses of these constituent ions. Although the susceptibility of the metal–oxygen cluster anions to decomposition in basic solutions is well known and ion chromatography has been supplied to the analysis of tungstate, molybdate and phosphate ions the application of the former observation and the latter method in combination for the analyses of metal–oxygen cluster anions has not been reported previously. The analytical method described here thus constitutes the first reported application of ion chromatography for the determination of concentration and elemental composition of metal–oxygen cluster anions. The method additionally offers the possibility of application in the study of the mechanism of the synthesis and decomposition of the metal–oxygen cluster anions.

## INTRODUCTION

Inorganic molecular metal–oxygen cluster compounds possess interesting structures and unusual properties [1,2] and as a result of the latter have found application in areas as diverse as medicine and heterogeneous catalysis.

The metal–oxygen cluster compounds which have received the greatest attention as heterogeneous catalysts are those frequently referred to as 12-heteropoly oxometalates with anions of Keggin structure. The anion has a tetrahedron ($MO_4$) at its centre which is enveloped by twelve octahedra ($XO_6$) and share oxygen atoms to produce a stoichiometry of $[(XO_4)(M_{12}O_{36})]^{n-}$ (Fig. 1).

These heteropoly anions can be found with a wide variety of cations, from the proton itself to relatively large organic species. With the proton as cation, solid acids are formed while with certain of the monovalent cations (*e.g.* $NH_4^+$) microporous secondary structures are formed [3].

A variety of methods have bene employed for the study of metal–oxygen cluster compounds in both solid and solution form [2]. Electromotive force methods [4] and polarography [5] have been applied for many years and more recently various NMR techniques [6,7] and UV and IR spectroscopy [6]

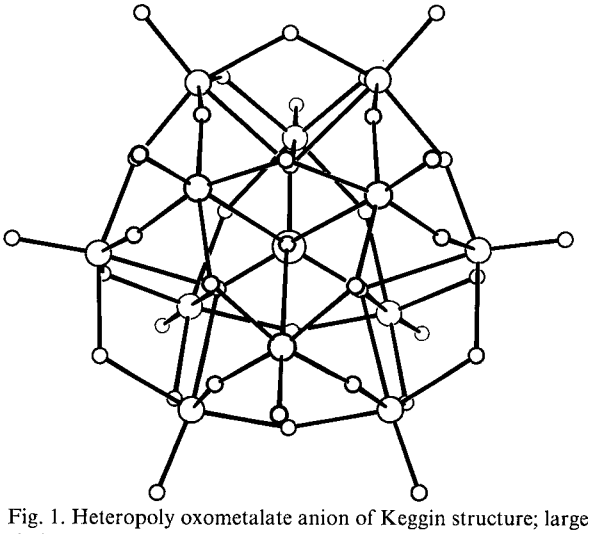

Fig. 1. Heteropoly oxometalate anion of Keggin structure; large circles: central atom (P) and peripheral metal atoms (W or Mo); small circles: oxygen atoms.

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have been utilized. Of course X-ray diffractions has for many years been an important tool for structural identification [8]. However, relatively little work has been reported on the application of chromatographic methods, undoubtedly as a consequence of the large size and complex nature of the heteropoly anions. Recently, however, Kirk and Finke [9] have reported the development of a high-performance liquid chromatographic method for the separation of highly charged polyoxometalates which promises to have considerable value in analytical applications.

The present work is concerned with the analysis of the 12-heteropoly anions of Keggin structure by the use of ion chromatography. Although it is possible, in principle, to analyze these as discrete entities by ion chromatography, for various reasons including their size and relative instability in the presence of bases, it seems preferable to decompose these into their smaller constituent ions and analyze for the latter.

The structural instability of the metal-oxygen cluster anions in basic solutions is well known and, in general, the oxyanion products of the decomposition have been identified. Further, ion chromatographic methods are available for the separation and analyses of their constituent oxyanions such as phosphates, molybdates and tungstates. However, there are no reports of the use of the decomposition of the metal–oxygen cluster anions in combination with the ion chromatographic analyses of the decomposition products as a technique for the determination of the concentration of these cluster anions. The present work demonstrates the validity of this method not only for the determination of the concentrations of the metal–oxygen cluster anions but also as a means of obtaining the elemental compositions of the cluster anions. Consequently the present method appears to be superior to one involving the analyses of the cluster anions as discrete entities. Further, as will become apparent subsequently, the technique offers interesting possibilities for the study of the mechanism of the preparation and decomposition of these anions.

The variables of importance thus are of two kinds, those involved with the chemistry related to the decomposition of the heteropoly anions, and those relating to the separation of the constituent species on the chromatographic column.

## EXPERIMENTAL

Cation and heteropoly anion analyses were made with a Dionex series 4500i ion chromatograph equipped with a conductivity detector. Cation analyses used the Dionex Fast cation separation columns and an eluent consisting of 20 m*M* HCl and 0.3 m*M* 2,3-diaminopropionic acid monohydrochloride (DAP). Background conductivity suppression was achieved using a Dionex cation micromembrane suppressor column and the closed loop Autoregen system with 0.1 *M* tetrabutylammonium hydroxide as the regenerant. Chromatograms were recorded on a Spectra-Physics ChromJet integrator.

Anion chromatography employed a Dionex AG5 guard column and AS5 separator column with a 10 m*M* $NaCO_3$–10 m*M* NaOH eluent. The background conductivity was reduced using an anion micromembrane suppressor column with a 30 m*M* $H_2SO_4$ regenerant.

The present report focuses on 12-tungstophosphoric and 12-molybdophosphoric acids and their salts of monovalent cations. Thus, for the anion analyses potassium phosphate (Baker), sodium molybdate (BDH) and sodium tungstate (Aldrich) were employed for calibration purposes. These were dried at 110°C and stored in a desiccator prior to use. Aqueous solutions of accurately known concentrations were prepared from triply distilled deionized water. In order to avoid saturation of the ion chromatography columns all solutions were carefully diluted to concentrations in the 0–10 ppm range.

Aqueous solutions each containing two anionic components, namely phosphate and molybdate or the former and tungstate were prepared in order to provide an independent confirmation of the calibrations.

Finally, aqueous solutions of known concentration of the heteropoly oxometalate were treated with an aqueous solution of either lithium or sodium hydroxide to decompose the heteropoly anion and were then subjected to analysis.

## RESULTS AND DISCUSSION

Data obtained in the calibration for the phosphate, molybdate and tungstate ions were fitted to a polynomial the coefficients and the standard devia-

TABLE I

CALIBRATIONS: POLYNOMIAL[a] COEFFICIENTS AND STANDARD DEVIATIONS

| Anion | Coefficients | | | | S.D. ($\times 10^4$) |
|---|---|---|---|---|---|
| | $a$ ($\times 10^5$) | $b$ ($\times 10^4$) | $c$ ($\times 10^2$) | $d$ ($\times 10^4$) | |
| $PO_4^{3-}$ | −7.28 | −5.35 | 3.97 | 31.15 | 9.48 |
| $MoO_4^{2-}$ | 0.104 | −0.384 | 2.12 | 8.47 | 35.4 |
| $WO_4^{2-}$ | −1.84 | 8.56 | 2.03 | −13.57 | 27.15 |

[a] $y = ax^3 + bx^2 + cx + d$

tions for which are shown in Table I. Retention times of approximately 4.4, 5.1 and 8.9 min for tungstate, molybdate and phosphate ions were obtained under the present operating conditions. The phophate graph is linear up to a concentration of 0.12 mmol/l. The molybdate and tungstate calibration curves on the other hand, show two distinct linear ranges over the concentration range considered. It is interesting to note the differences in the detector response to the three species considered in the present study. Whereas the response for the phosphate was *ca.* 230 arbitrary units/(mmol/l), the response for the transition species was much lower. For the molybdate calibration curve, the response factor below 0.6 mmol/l was *ca.* 50 but at the highest measured concentrations this value fell to 46. More dramatic concentration effects were observed for the tungstate calibration. Two distinct regions were observed: one below 0.12 mmol/l with a response factor of 41, and one at higher concentrations with a response factor of 37.

The large differences in the response factors can be explained in part by the ionic mobility of the anions. The size, charge distribution, and concentration of the anion will be the factors that most strongly influence the response characteristics. While the formal charge of the three species is the same (−2) the sizes of the ions vary as indicated by the metal atom-oxygen bond lengths of 1.54, 1.76 and 1.79 Å for $HPO_4^{2-}$, $MoO_4^{2-}$, and $WO_4^{2-}$ respectively. The larger size of the transition metal oxyanions presumably leads to a smaller charge density and reduced mobility and hence a lower detector response. The differences between the response factors of the two transition metal species is not readily explained although the additional f-shell electrons in the tungstate ion are expected to exert an influence.

In order to provide independent tests of the calibration, aqueous solutions containing two ionic species, either the phosphate and the molybdate or the phosphate and the tungstate anions were prepared. After suitable dilution to concentrations within the range of calibration, aliquots were passed through the ion chromatograph and concentrations of the two components were obtained from the peak areas and the calibration plots. The measured concentrations of phosphate and molybdate and of phosphate and tungstate in solutions containing the former or latter pair of species were compared with the expected concentrations from the preparation. The coefficients for the linear equation employed to fit the data from the analysis of solutions containing known concentrations of each of two anions are shown, together with the standard deviation, in Table II. The measured concentrations are in good agreement with the expected values. It is evident that suitable precision is attainable provided that changes in the detector response as a function of concentrations are taken into account.

As pointed out in the introduction the analyses of the heteropoly oxometalates are based on the preliminary decomposition of the heteropoly anion in aqueous solution followed by the separation and analysis of the constituent ions by ion chromatography. Although the heteropoly anions are unstable with respect to this decomposition process in basic solutions, the range of pH within which the 12-het-

TABLE II

LINEAR EQUATION[a] COEFFICIENTS AND STANDARD DEVIATIONS FROM CALIBRATION TESTS WITH TWO ANION COMPONENT SOLUTIONS

| Analysis anion | Second anion | Coefficients | | S.D. ($\times 10^3$) |
|---|---|---|---|---|
| | | $a$ | $b$ ($\times 10^3$) | |
| $PO_4^{3-}$ | $WO_4^{2-}$ | 0.988 | 1.12 | 1.52 |
| $PO_4^{3-}$ | $MoO_4^{2-}$ | 0.966 | 0.70 | 2.28 |
| $WO_4^{2-}$ | $PO_4^{3-}$ | 0.974 | 1.11 | 4.84 |
| $MoO_4^{2-}$ | $PO_4^{3-}$ | 0.998 | −3.88 | 5.92 |

[a] $y = ax + b$

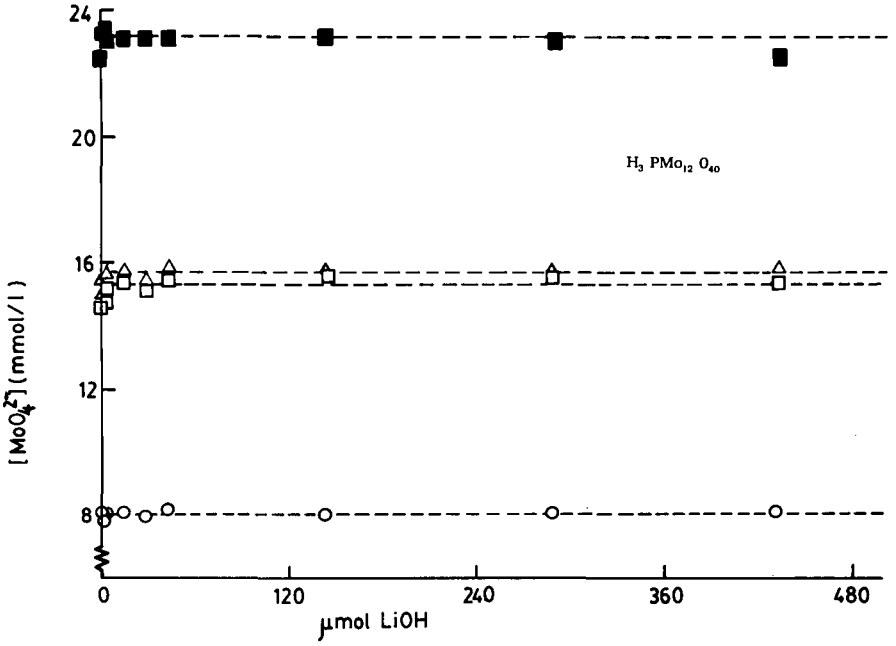


Fig. 2. Measured concentrations of molybdate ions from aqueous solutions of 12-molybdophosphoric acid after addition of lithium hydroxide. (Dashed lines are expected quantities.) Expected concentrations: ■ = 23.21 mmol/l; △ = 15.74 mmol/l; □ = 15.36 mmol/l; ○ = 8.03 mmol/l.

eropoly structure is retained intact is dependent upon the nature of both the central and peripheral atoms [10]. Measurements of the $^{31}P$ chemical shifts of the major species present in aqueous solutions of the two heteropoly anions considered here have shown that for each of the two 12-heteropoly anions, the saturated Keggin anion was found to exist as the single constituent species only in strongly acidic solutions of pH less than 2. At higher solution pH degradation of the Keggin anion to lacunary species was observed as indicated by the large changes in the measured chemical shifts. Only after strongly alkaline conditions were reached was the complete degradation of the Keggin anion to simple oxyanions assured.

In the present work hydroxides have been employed as convenient and non-interfering bases for purposes of affecting the decomposition of the heteropoly anions. To evaluate the influence of the cation in the base, lithium and sodium hydroxides were separately employed. A number of aqueous solutions of 12-molybdophosphoric acid ($H_3PMo_{12}O_{40}$) and of 12-tungstophosphoric acid ($H_3PW_{12}O_{40}$) were prepared, the base added and analyses were performed. Typical results for 12-molybdophosphoric acid and lithium hydroxide are shown in Fig. 2. Although not shown the expected results are obtained with addition of relatively small quantities of either base.

Further, addition of much larger quantities of either base has no effect on the analytical results. With either alkali the measured concentration of molybdate is in good agreement with that expected from complete decomposition of the heteropoly anion. It should be noted that the results for four separate and distinct solutions of 12-molybdophosphoric acid are shown in Fig. 2. The facile decomposition of the 12-molybdophosphate anion following small additions of either hydroxide confirm the previous reports of the relative instability of this anion in aqueous solutions [2].

Aqueous solutions of 12-tungstophosphoric acid ($H_3PW_{12}O_{40}$) were prepared, base was added and analyses were performed. The results with lithium hydroxide are reported in Fig. 3. The differences between the molybdenum and tungsten-containing acids are clearly evident from a comparison of this figure with Fig. 2. With both LiOH and NaOH and

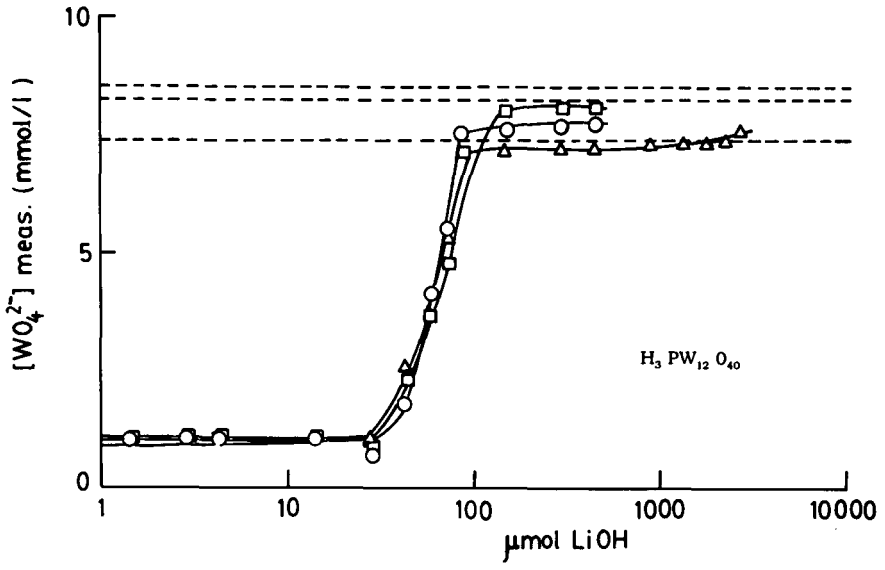


Fig. 3. Measured concentrations of tungstate ions from aqueous solutions of 12-tungstophosphoric acid after addition of lithium hydroxide. (Dashed lines are expected quantities.) Expected concentrations: □ = 8.57 mmol/l; ○ = 8.30 mmol/l; △ = 7.44 mmol/l.

the tungsten-containing acid the quantities of tungstate ion measured remain relatively constant as the base is added up to approximately 30 μmol. The small quantity of tungstate detected in the solutions containing the smallest quantities of base suggest that some decapping of the Keggin anion has occurred with subsequent formation of the lacunary species. As the base concentration increases, the increase in the measured quantity of tungstate indicates further decomposition of the lacunary anion to the constituent anions. Finally for the highest quantities of base, the measured tungstate concentrations approach the expected values.

It is clear from the foregoing that the anion of the tungsten-containing heteropoly acid is more resistant to changes in pH than that of the molybdenum-containing acid. Thus, the Keggin structure of the anion of the former acid is retained over a wider range of pH than that of the latter. This is consistent with the results obtained from the earlier NMR studies [10].

The ion chromatographic method for analysis of the metal-oxygen cluster compounds through decomposition to their constituent ions is evidently a versatile method which promises to have applicability to a wide variety of elementary compositions of these solids. It is important to note that the present method not only permits the measurement of the concentration of the metal–oxygen cluster anions but in addition provides information on the elemental composition of these anions. Equally interesting and valuable, however, is the ancillary information obtainable from the method on the structural stability of the cluster anions, a subject of considerable importance in this area of chemistry.

## CONCLUSIONS

A method has been developed for the analysis of metal–oxygen cluster anions containing a single peripheral metal element, although in principle it is capable of extension to diperipheral cluster anions. The present technique offers the advantage of generating two sets of information, the concentration as well as the elemental composition of the anion. Further, the method has applicability to studies of the mechanism of the synthesis and decomposition of the cluster anions.

## ACKNOWLEDGEMENT

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# Ion chromatography as a tool for optimization and control of fermentation processes

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ABSTRACT

Information concerning the mineral content of fermentation broth and microorganisms is important for the optimization of fermentation processes. Ion chromatographic methods were implemented for measurement of anions and cations in the range 0.01–100 ppm and transition metals in the range 0.01–100 ppb. An IonPac AS5A column and an OmniPac PAX-500 column were compared for anion measurements. Acid-digested samples were used for the determination of transition metals to overcome problems with complex binding by organic compounds. A rapid method for acid digestion with nitric acid in closed vessels and microwave heating is described. These methods were implemented to follow a fermentation process with methanotrophic bacteria. Acetate was found to accumulate when natural gas was used as a substrate. A washout experiment showed magnesium to be growth limiting when the concentration was below 60 ppb.

## INTRODUCTION

The cultivation of microorganisms requires a complex mineral medium reflecting the mineral content of the microorganisms. The expense of minerals is generally not considered to be important in laboratory-scale fermentations or when a high-cost product is produced on a large scale on the basis of a complex mixture of substrates. The tolerable concentration of most minerals is high, and normal practice is to prepare media containing minerals in excess. The mineral expenses can be of importance, however, when a cheap product is to be produced on a large scale. Production of single-cell protein (SCP) from natural gas is such an example. Minerals fed to the process must be balanced by minerals utilized for SCP production to reduce mineral costs and to allow reuse of the process water without a risk of build-up of minerals. Reuse of process water is necessary to minimize the requirement for fresh water and to minimize the amount of waste water.

Natural gas is considered to be a cheap carbon and energy source for the production of SCP [1]. It consists mainly of methane, which can be utilized for growth by methanotrophic bacteria. In addition to methane, natural gas also contains higher hydrocarbons, such as ethane and propane. The ethane content is between 2 and 20% and the propane content between 0.4 and 13%, depending on the location of the gas field [2]. Methanotrophic bacteria can oxidize many different hydrocarbons [3], but only methane is utilized for growth. Ethane and propane are mainly oxidized to acetic acid and propionic acid, respectively, which inhibit the growth of methanotrophic bacteria and are therefore important fermentation parameters.

Methanotrophic bacteria have a special requirement for copper for optimum growth [4]. Like other transition metals, copper is poisonous at high concentrations and the range between the optimum and

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a poisonous copper concentration is very narrow when SCP production from natural gas is considered. This stresses the importance of proper measurement and control of the copper concentration during the growth of methanotrophic bacteria. The determination of copper and other transition metals in fermentation broth can be hindered by complex formation with organic components. These organic components can be metabolites excreted in the fermentation broth by the bacterial cells or material from lysed cells. To remove these interferences it is necessary to decompose the organic materials in the sample before measurement. Degradation of organic materials is also necessary when samples containing microorganisms are to be analysed for minerals. Organic material can be decomposed by ashing at 550–600°C for several hours [5] or by acid digestion in closed vessels heated in a microwave oven [6].

This paper describes methods for ion chromatographic determinations of anions, cations and transition metals in fermentation broth and microorganisms. It compares the Dionex IonPac AS5A separator column with the Dionex OmniPac PAX-500 analytical column for anion determination and describes a rapid method for acid digestion of samples in closed vessels with microwave heating. The methods were used to follow and optimize a fermentation process with methanotrophic bacteria.

## EXPERIMENTAL

### *Chromatographic systems and eluents*

*Anions.* A Dionex (Sunnyvale, CA, USA) system 4500i ion chromatograph equipped with a gradient pump module, a conductivity detector and an anion MicroMembrane suppressor (AMMS) was used for anion measurement. The sample loop was 25 μl. The IonPac AS5A-5μ separator column (250 mm × 4 mm I.D., 5-μm particle size) and the IonPac AG5A guard column (50 mm × 4 mm I.D., 5-μm particle size) or the OmniPac PAX-500 analytical column (250 mm × 4 mm I.D., 8.5-μm bead diameter) and the OmniPac PAX-500 guard column (50 mm × 4 mm I.D., 8.5-μm bead diameter) were used for the separation of anions as stated. An anion trap column (ATC-1) was placed between the gradient pump and the injection valve to remove anionic contaminants from the eluent. The eluent flow-rate was 1 ml/min. The AMMS suppressor was continuously regenerated using 25 m*M* sulphuric acid as regenerant solution. The regenerant flow-rate was 15 ml/min.

Anions were separated on the IonPac AS5A-5μ separator column by use of a modification of the gradient elution method described by Dionex [7] (see Table I). A modification of the gradient elution method described by Dionex [8] was used for anion separation on the OmniPac PAX-500 analytical column (see Table I).

*Monovalent and divalent cations.* Alkali and alkaline earth metals were measured with the Dionex DX-100 ion chromatograph. The system was equipped with a conductivity cell placed in a thermal stabilizer, an IonPac CS 10 analytical column (250 mm × 4 mm I.D., 8.5-μm particle size), an IonPac GC guard column (50 mm × 4 mm I.D., 8.5-μm particle size) and a cation MicroMembrane suppressor (CMMS). The sample loop was 25 μl.

The cations were separated isocratically using a 40 m*M* hydrochloric acid–4 m*M* DL-2,3-diaminopropionic acid (DAP–HCl) as eluent [9]. The eluent flow-rate was 1 ml/min. The CMMS was continuously regenerated with 0.1 *M* tetrabutylammonium hydroxide as regenerant solution. The regenerant flow-rate was 10 ml/min. The regenerant solution was continuously recycled using the Dionex AutoRegen accessory.

*Transition metals.* Transition metals were measured as described [10] on a Dionex Model 4500i ion chromatograph equipped with a variable-wavelenth detector. Isocratic chelation ion chromatography was used. The samples were adjusted to pH 5.3–5.6 by addition of 2 ml of 2.0 *M* ammonium acetate solution. The pH of acid-digested samples was adjusted by addition of 10 ml of 2.0 *M* ammonium acetate to 20 ml of digest. Sample volumes of 20 ml were used for measurements.

*Data system.* All ion chromatographic systems were connected to an IBM AT compatible computer by a Dionex AI-450 Model II data system.

### *Preparation of standards and samples*

Anion and cation standards were prepared at the 0.01, 0.1, 1, 10 and 100 ppm (mg/l) levels and transition metals standards at the 0.01, 0.05, 0.1, 1, 10 and 100 ppb (μg/l) levels. All standards were prepared from pure reagents (analytical-reagent grade). Anion standards were stored at −20°C to

TABLE I

CONDITIONS FOR THE ANION COLUMNS

Eluent composition: A = pure water; B = 0.75 m*M* NaOH; C = 200 m*M* NaOH; D = 20% methanol. The methanol content of eluent D was reduced to 10% and 2% in experiments with 5% and 1% methanol in the eluent, respectively.

| Column | Time (min) | A (%) | B (%) | C (%) | D (%) | Comments |
|---|---|---|---|---|---|---|
| AS5A | 0.0 | 0 | 100 | 0 | 0 | Inject sample |
| | 5.0 | 0 | 100 | 0 | 0 | Start gradient ramp |
| | 15.0 | 0 | 85 | 15 | 0 | |
| | 30.0 | 0 | 57 | 43 | 0 | |
| | 30.1 | 0 | 0 | 100 | 0 | Start wash |
| | 35.0 | 0 | 0 | 100 | 0 | |
| | 35.1 | 0 | 100 | 0 | 0 | Equilibrate |
| | 48 | 0 | 100 | 0 | 0 | Ready to load |
| PAX 500 | 0.0 | 50 | 0 | 0 | 50 | Inject sample |
| | 0.1 | 50 | 0 | 0 | 50 | Start gradient ramp |
| | 10.0 | 42 | 0 | 8 | 50 | |
| | 25 | 28 | 0 | 22 | 50 | |
| | 30 | 0 | 0 | 50 | 50 | |
| | 37 | 0 | 0 | 50 | 50 | |
| | 37.1 | 50 | 0 | 0 | 50 | Equilibrate |
| | 50.0 | 50 | 0 | 0 | 50 | Ready to load |

prevent deterioration of acetate and formate. The other standard solutions were prepared daily from 1000 ppm (cations) or 1 ppm (transition metals) stock solutions. The stock solutions were renewed every month.

Fermentation broth samples, *i.e.*, samples of fermentation liquid centrifuged (3000 *g*, 10 min) to remove bacterial cells (biomass), were filtered (0.45-$\mu$m filter, type HV, Millipore) before measurements of anions and cations, and acid digested before measurement of transition metals. Fermentation liquid samples containing bacterial cells were acid digested before measurement of anions, cations and transition metals.

*Microwave acid digestion*

A CEM (Matthews, NC, USA) MDS-2000 microwave sample preparation system equipped with a pressure-measuring and control device was used for acid digestion.

Sample solutions of 20 ml were placed in 100-ml Teflon PFA vessels and 0.5 ml of 65% nitric acid was added unless stated otherwise. The vessels were closed, placed in the microwave oven and heated according to Table II. Usually two samples were digested at a time but it was possible to digest up to twelve samples in one run by increasing the heating time.

Before the acid digestion of samples for transition metals measurements, it was necessary to clean the Teflon containers carefully to reduce the background. The Teflon vessels were thoroughly washed and filled with 5 ml Suprapur nitric acid. The vessels were heated according to Table II and the digest was discarded. The procedure was repeated and the vessels were used for sample digestion.

TABLE II

POWER AND HEATING TIME FOR MICROWAVE DIGESTION OF SAMPLES

| Parameter | Initial heating | Heating | Cooling |
|---|---|---|---|
| Power (W) | 650 | 325 | 0 |
| Time (min) | 5 | 5 | 15 |
| Maximum pressure (p.s.i.) | 100 | 150 | – |

*Chemicals*

Hydrochloric acid, acetic acid, 2-dimethylaminoethanol (all from Merck), and sodium hydrogencarbonate (Riedel-de Haën) were of analytical-reagent grade. Ammonia solution (25%) and nitric acid (65%) were of Suprapur grade (Merck). D,L-Diaminopropionic acid monohydrochloride (DAP) was obtained from Sigma and pyridine-2,6 dicarboxylic acid (PDCA) from Aldrich. Ammonium acetate (2.0 *M*) and ammonium nitrate (0.1 *M*) were both Dionex class 100 reagents and monosodium 4-(2-pyridylazo)recorcinol monohydrate (Dionex) was >95% pure. All other chemicals were of analytical-reagent grade. Ultra-pure water (18.2 MΩ/cm resistivity at 25°C), obtained by use of a Milli-Q water purification system (Millipore), was used throughout.

## RESULTS AND DISCUSSION

*Determination of anions, cations and transition metals*

The gradient method for measurement of anions with the IonPac AS5A column described by Dionex [7] was initially used. At first we had many problems in applying the method. The chromatograms showed a peak with a retention time ($t_R$) of 18 min and irreproducible gradient ramps with a high ($\gg$4 μS) change in the background conductance. Eventually we found that the problems were caused by carbonate contamination. Atmospheric carbon dioxide trapped by the basic eluents caused a build-up of carbonate in the system. The problems were solved by installation of an anion trap column before the injection valve, by replacing the plastic eluent containers with glass containers (atmospheric carbon dioxide can diffuse through the walls of a plastic container), and by washing with 0.2 *M* NaOH for 5 min after each run. These modifications gave reproducible gradient ramps with about a 4 μS change in background conductance. This change is slightly higher than the 1–3 μS mentioned by Dionex [7].

The IonPac AS5A and the OmniPac PAX-500 columns gave nearly identical results when acetate, formate, chloride, nitrate, sulphate and phosphate were measured (see Fig. 1 and Table III). The calibration graphs were linear in the tested range of 10 ppb–100 ppm, except for sulphate and phosphate,

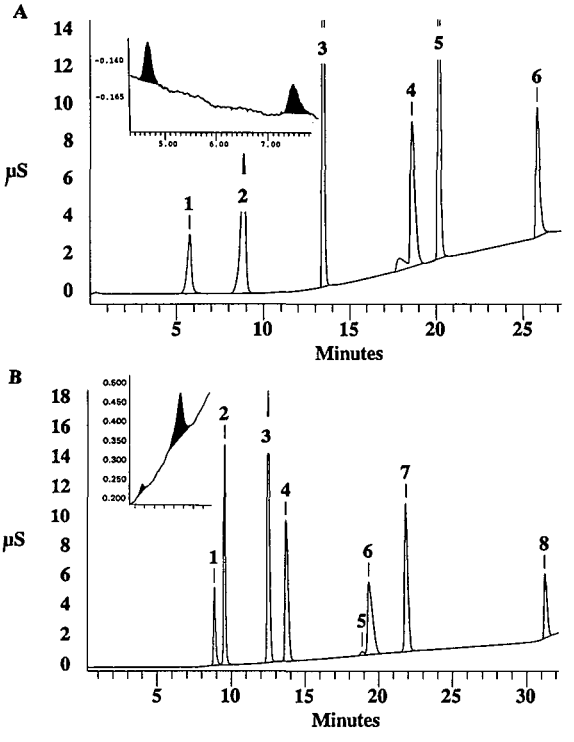


Fig. 1. (A) Chromatogram obtained on the IonPac AS5A column for a 10 ppm standard solution. Inset: chromatogram of 10 ppb standard solution of acetate and formate. Peaks: 1 = acetate; 2 = formate; 3 = chloride; 4 = nitrate; 5 = sulphate; 6 = phosphate. (B) Chromatogram obtained on the OmniPac PAX-500 column of a 10 ppm standard solution. Inset: chromatogram of a 10 ppb standard solution of acetate and formate. Peaks: 1 = acetate; 2 = formate; 3 = chloride; 4 = nitrite; 5 = carbonate; 6 = nitrate; 7 = sulphate; 8 = phosphate.

which showed deviations from linearity at low concentrations owing to the high background level of these anions. The correlation coefficients were above 0.998 in all instances. The linearity of the phosphate and sulphate calibration graphs was extended to 10 ppb when their signals were background corrected. The detection limit for all the measured anions was below 10 ppb. The AS5A column had a lower detection limit for acetate than the PAX-500 column (insert in Fig. 1 and Table III). Five repeated injections of a 10 ppm standard solution showed relative standard deviations (R.S.D.s) below 3% for both columns. The AS5A column performed slightly better than the PAX-500 column. Analysis of fermentation broth showed more

TABLE III

LINEAR RANGE OF CONCENTRATIONS TESTED, LINEAR CORRELATION COEFFICIENTS, SIGNAL-TO-NOISE RATIOS, RETENTION TIMES AND RELEVANT R.S.D.

| Species | Linear range (ppm) | $r^a$ | S/N[b] | R.S.D.[c] (%) | R.S.D.[d] (%) | $t_R$[e] (min) | R.S.D.[f] (%) |
|---|---|---|---|---|---|---|---|
| *Anions measured with the IonPac AS5A-5μ column* | | | | | | | |
| Acetate | 0.01–100 | 0.999 | 10 | 0.7 | 8.3 | 4.98 | 6.1 |
| Formate | 0.01–100 | 1.000 | 8 | 0.9 | 3.9 | 7.79 | 6.0 |
| Chloride | 0.01–100 | 1.000 | 40 | 1.0 | 3.1 | 13.4 | 0.55 |
| Nitrate | 0.01–100 | 1.000 | –[g] | 0.8 | 4.0 | 19.3 | 0.31 |
| Sulphate | 0.01–100 | 1.000 | – | 2.8 | 5.9 | 20.0 | 0.51 |
| Phosphate | 0.01–100 | 1.000 | 6 | 1.4 | 6.0 | 25.6 | 0.54 |
| *Anions measured with the OmniPac PAX-500 column* | | | | | | | |
| Acetate | 0.01–100 | 0.998 | 4 | 1.86 | – | 8.43 | 3.1 |
| Formate | 0.01–100 | 0.999 | 25 | 2.15 | – | 9.17 | 2.3 |
| Chloride | 0.01–100 | 1.000 | 8 | 2.21 | – | 11.9 | 2.5 |
| Nitrate | 0.01–100 | 1.000 | 4 | 2.57 | – | 19.3 | 1.6 |
| Sulphate | 0.01–100 | 1.000 | 6 | 2.21 | – | 21.4 | 2.0 |
| Phosphate | 0.01–100 | 1.000 | 3 | 2.76 | – | 30.9 | 1.6 |
| *Cations* | | | | | | | |
| Sodium | 0.01–100 | 1.000 | 8 | 4.4 | 16.3 | 2.16 | 3.9 |
| Ammonium | 0.01–100 | 0.997 | 2 | 1.2 | 10.1 | 2.38 | 0.9 |
| Potassium | 0.01–100 | 1.000 | 2 | 4.5 | 18.0 | 2.84 | 3.5 |
| Manganese | 0.01–100 | 0.999 | 4 | 4.6 | 22.4 | 9.1 | 6.6 |
| Calcium | 0.01–100 | 1.000 | 4 | 4.1 | 20.3 | 17.3 | 7.2 |
| *Transition metals* (ppb) | | | | | | | |
| $Fe^{3+}$ | 0.05–100 | 0.995 | – | 3.70 | – | 5.5 | 5.0 |
| $Cu^{2+}$ | 0.01–100 | 1.000 | – | 0.63 | – | 9.3 | 6.4 |
| $Ni^{2+}$ | 0.10–100 | 0.997 | 3 | 1.60 | – | 10.4 | 6.3 |
| $Zn^{2+}$ | 0.50–100 | 0.998 | – | 2.13 | – | 11.2 | 6.3 |
| $Co^{2+}$ | 0.05–100 | 1.000 | 3 | 0.57 | – | 12.7 | 6.2 |
| $Mn^{2+}$ | 0.10–100 | 0.999 | 5 | 2.09 | – | 15.3 | 5.8 |

[a] Linear correlation coefficient. The peak area of phosphate and sulphate was background corrected before calculation of the correlation coefficient.
[b] Signal-to-noise ratio of 10 ppb (anions and cations), 0.05 ppb ($Co^{2+}$) or 0.01 ppb ($Ni^{2+}$ and $Mn^{2+}$) standard solutions.
[c] Relative standard deviation of five replicates of 10 ppm (anions and cations) or 10 ppb (transition metals) standard solutions.
[d] Relative standard deviation of nine measurements of 10 ppm standard solutions (anions) or 28 measurements of 100 ppm standard solutions (cations) on different days.
[e] Mean retention time for a period of 9 days (anions on the IonPac AS5A column), 5 days (anions on the OmniPac PAX-500 column), 28 days (cations) or 5 days (transitions metals).
[f] Relative standard deviation of the retention times.
[g] – = Not determined.

peaks on the OmniPac PAX-500 column than on the IonPac AS5A column. One of these peaks was found to be citrate, the others were not identified. The OmniPac PAX-500 column was tested with 1, 5 and 10% of methanol in the eluent. Neither the gradient ramp nor the elution time of the anions tested were affected by the methanol level. Propionate and butyrate were found in separate experiments to elute with $t_R$ 8.7 and 9.0 min, respectively. An improved separation of the short-chain organic acids on the OmniPac PAX-500 column was obtained by isocratic elution with 0.5 m*M* NaOH during the first 18 min. The $t_R$ values were then 10.5, 11.3, 12.8 and 16.7 min for acetate, propionate, butyrate and formate, respectively. The total elution time was in that case extended to 1 h.

The linearity of the calibration graphs and the low R.S.D.s found for all the anions indicate that quantification by peak area instead of by peak height is sufficiently accurate and reproducible even for compounds with resolution below 1.

The stabilities of the anion standard solutions were initially tested. The levels of formate and acetate in the standards decreased from day to day when the standards were stored at room temperature in glass containers, even when the pH was increased to 10 by addition of NaOH. However, the levels of formate and acetate were found to be constant for several months when the standards were stored at −20°C.

Sodium, ammonium, potassium, magnesium and calcium were separated on the IonPac CS10 column with detection limits of about 10 ppb set by the pumping noise of the DX-100 system (Table III). The R.S.D. of the peak areas was below 5% for five repeated injections. The day-to-day R.S.D. in peak area was 10–20% and stressed the need for a daily calibration. The retention times were fairly constant with a day-to-day R.S.D. of 1–7%.

Transition metals were measured by the isocratic chelation ion chromatographic method with a detection limit of 0.01–0.05 ppb (Table III). The signals for manganese, cobalt, iron, nickel and copper were linear in the range 0.1–100 ppb and for zinc in the range 1–100 ppb. The R.S.D. for five 10 ppb samples was below 2.5% except for iron, which had an R.S.D. of 3.7% owing to a high background level.

*Microwave acid digestion*

Acid digestion was initially tested on samples of pure water and samples of fermentation liquid containing 10 g dry weight of biomass per litre. The medium used for the fermentation contained 75 ppm of $Mg^{2+}$ and 41 ppm of $Ca^{2+}$. At first, 5 ml of 65% nitric acid was used for the digestion of 20 ml of sample. However, cation chromatograms of the digest showed noisy baselines with a negative peak which had the same retention time as calcium and the anion chromatograms of the digest were totally dominated by the nitrate peak. Therefore, the release of magnesium and calcium was tested using different amounts of nitric acid for the digestion. Cation chromatograms of the digest showed the same amount of magnesium and calcium when 0.125–2 ml of nitric acid were used (Table IV). The measured concentrations were close to the levels of magnesium and calcium in the fermentation medium, showing a 100% recovery of magnesium and calcium even with the very small addition of nitric acid.

TABLE IV

RELEASE OF MAGNESIUM AND CALCIUM BY NITRIC ACID DIGESTION OF MICROORGANISMS

20-ml fermentation liquid samples containing 10 g dry weight per litre were digested with the indicated amount of nitric acid. Magnesium and calcium were measured using ion chromatography.

| $HNO_3$ (ml) | $Mg^{2+}$ (ppm) | $Ca^{2+}$ (ppm) |
|---|---|---|
| 0.125 | 74.8 | 40.7 |
| 0.25 | 76.0 | 40.8 |
| 0.5 | 70.4 | 39.3 |
| 0.5 | 83.9 | 42.1 |
| 1.0 | 79.9 | 41.6 |
| 2.0 | 84.8 | 46.2 |
| 2.0 | 75.4 | 39.2 |
| Mean | 77.9 | 41.4 |
| R.S.D. (%) | 6.7 | 5.7 |

Anion chromatograms of acid-digested biomass with 0.5 ml of nitric acid could be obtained after a 1:10 dilution of the digest. The dilution was necessary owing to the high content of nitrate from the nitric acid added for the digestion. Fig. 2 shows an

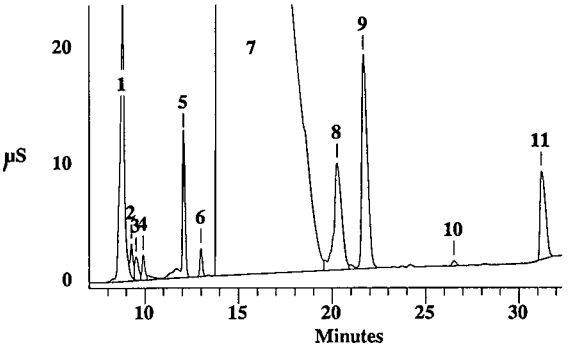


Fig. 2. Anion chromatogram of an acid-digested fermentation liquid sample containing about 10 g dry weight of biomass per litre. The anions were separated on the OmniPac PAX-500 column. Peaks: 1 = acetate; 2 = propionate; 3 = butyrate; 4 = formate; 5 = cloride; 6 = nitrite; 7 = nitrate; 8 = unknown; 9 = sulphate; 10 = unknown; 11 = phosphate.

anion chromatogram of a digested sample of biomass. The peaks were identified according to their retention times. The existence of formate, acetate and propionate was confirmed by gas chromatographic–mass spectrometric studies. The organic acids revealed that the bacterial cells were only partly digested. The digest was turbid and formed a light grey precipitate within a few hours. The turbidity of the digest was unaffected by an increase in the digestion time from 5 to 15 min at 150 p.s.i. A clear digest without organic acids was obtained when 2 ml of nitric acid were used for the digestion. However, this also raised the detection limit as it had to be followed by an extra dilution to reduce the nitrate peak and 0.5 ml of nitric acid was used for digestion in the further studies.

Cation and anion spike recovery studies were performed with a fermentation liquid sample of unknown ion composition (Table V). The recovery was 87–115%. The deviation form 100% can be explained by the R.S.D. of the determination of anions and cations combined with the dilution factor. These experiments confirmed that the use of closed Teflon vessels and microwave heating for nitric acid digestion prevents any loss of analyte due to volatilization or adsorption on the container walls, as previously shown by Patterson *et al.* [6].

Transition metals in the digest could be measured after neutralization with 2.0 *M* ammonium acetate solution. The necessary amount of ammonium acetate was found to be 10 ml for a 20-ml sample. Initially we found a high and variable background of transition metals in the digest of pure water. To reduce this background we found it necessary to apply a special cleaning procedure to the Teflon vessels before each acid digestion (see Experimental). The ion chromatographic determination of copper in acid-digested samples was compared with results obtained by potentiometric stripping analysis (PSA). The PSA method is applicable to measurements of copper in biomass and fermentation liquid without prior degradation of organic materials and has been described elsewhere [11]. The same level of copper was found by the two methods. This indicated that the acid-digestion procedure was suitable for the determination of transition metals. Fig. 3 shows a chromatogram of an acid-digested fermentation broth sample. The concentrations of copper, zinc, cobalt and manganese were 7.8, 16.5, 1.7 and 4.0 ppb, respectively. Iron was observed as $Fe^{2+}$ and $Fe^{3+}$ ($t_R$ = 5 min) but was not quantified.

*Determination of cations in the fermentation broth*

Fermentation broth often contains metabolic by-products and complex organic components that might interfere with the chromatographic determination of cations [5]. We found the same level of sodium, potassium, magnesium and calcium in un-

TABLE V

RECOVERY OF SODIUM, POTASSIUM, MAGNESIUM, CALCIUM, CHLORIDE, SULPHATE AND PHOSPHATE FROM SAMPLES DIGESTED WITH 0.5 ml OF NITRIC ACID

| Species | Standard[a] (ppm) | Sample[b] (ppm) | Spiked sample[c] (ppm) | Recovery[d] (ppm) |
|---|---|---|---|---|
| Sodium | 47.2, 50.2 | 77,6, 71.7 | 123, 135 | 54.3 |
| Potassium | 37.5, 41.1 | 292, 274.5 | 318, 346 | 48.9 |
| Magnesium | 50.7, 51.2 | 55.8, 52.7 | 99.8, 104 | 47.7 |
| Calcium | 52.6, 50.9 | 41.0, 40.1 | 88.4, 88.6 | 48.0 |
| Chloride | –[e] | 59.9, 63.6 | 113.4, 125 | 57,5 |
| Sulphate | – | 264, 266 | 307, 310 | 43.5 |
| Phosphate | – | 251, 251 | 292, 297 | 43.5 |

[a] 10 ml of 100 ppm standard solution + 10 ml of water.
[b] 10 ml of fermentation liquid sample of unknown ion concentrations + 10 ml of water.
[c] 10 ml of fermentation liquid sample + 10 ml of 100 ppm standard solution.
[d] Mean of the measured concentrations of spiked samples minus measured concentrations of samples.
[e] – = Not determined.

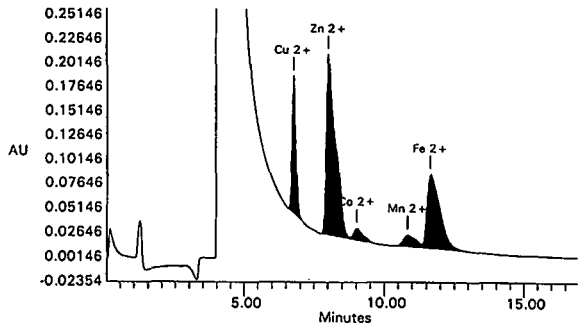


Fig. 3. Chromatogram of an acid-digested fermentation broth sample.

treated as in acid-digested samples of fermentation broth. Hence, it is not necessary to digest fermentation broth before the determination of these cations. In contrast, the level of transition metals measured was clearly influenced by the method of sample treatment prior to chromatographic analysis.

Transition metals are known to form stable complexes with several inorganic and organic compounds. We tested the influence of EDTA, oxalate, cyanide, formate and citrate on the chelation ion chromatographic detection of iron, copper, nickel, zinc, cobalt and manganese. Table VI shows the measured concentrations of the different transition metals when the sample contained one of the complex-forming components. EDTA showed a very strong interference with the chelation ion chromatographic technique. The negative values for iron, copper, zinc and manganese are caused by the binding of background contaminants by EDTA. Oxalate, cyanide and citrate showed a moderate interference with the chelation ion chromatographic technique. Oxalate and cyanide reduced the iron and nickel peaks, but increased the zinc peak. The increased zinc peak is probably due to zinc contamination of the oxalate and cyanide solutions. Citrate showed a different pattern, with a strong reduction of the copper peak and a weaker reduction in the amounts of iron, zinc, cobalt and manganese detected. The results clearly demonstrated that many complex-forming compounds interfere with the chelation ion chromatographic technique and emphasize the need for removal of these interferences.

*Fermentation experiments*

Fig. 4 shows chromatograms of two fermentation broth samples, one taken before and the other taken 4 h after a switch of substrate from methane (99.95% methane) to natural gas (about 91% methane and 4.7% ethane). Fig. 5 gives the concentrations of formate, acetate and citrate during the experiment. The acetate concentration was very low before the switch from methane to natural gas (*ca.* 2 ppm) but showed a steady increase after the switch. This indicated that the bacteria could oxidize ethane to acetate and that they were unable to utilize acetate. The level of citrate was not affected by the substrate switch. The oscillating behaviour of formate was unexpected and needs further investigation.

Acetate has been found to inhibit the growth of methanotrophic bacteria [12]. To optimize the growth on natural gas it is necessary to prevent ace-

TABLE VI

INFLUENCE OF DIFFERENT COMPONENTS ON THE ION CHROMATOGRAPHIC DETECTION OF TRANSITION METALS

The samples contained 12.5 ppb of each transition metal and 5 m*M* of one of the complex-forming components. Values are in ppb.

| Complex-forming component | $Fe^{3+}$ | $Cu^{2+}$ | $Ni^{2+}$ | $Zn^{2+}$ | $Co^{2+}$ | $Mn^{2+}$ |
|---|---|---|---|---|---|---|
| Phosphate | 12.5 | 12.5 | 12.5 | 12.5 | 12.5 | 12.5 |
| EDTA | −0.9 | −0.7 | 0.0 | −3.1 | 0.0 | −0.4 |
| Oxalate | 5.0 | 14.3 | 8.6 | 27.7 | 11.7 | 13.1 |
| Cyanide | 6.8 | 11.6 | 6.9 | 40.0 | 12.0 | 12.5 |
| Formate | 12.2 | 12.2 | 12.3 | 18.9 | 12.0 | 11.6 |
| Citrate | 8.6 | 0.8 | 11.6 | 4.9 | 7.7 | 9.3 |

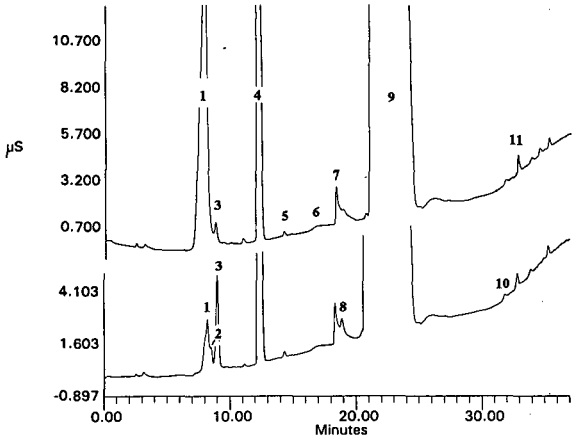


Fig. 4. Anion profile for fermentation of methane and natural gas. The lower curve is a chromatogram of a fermentation broth sample taken from a steady-state methane fermentation. The upper curve is from the same fermentation but 4 h after a shift from methane to natural gas. The OmniPac PAX-500 column and gradient elution with 10% methanol were used for the separation of anions. Peaks: 1 = acetate; 2 = propionate; 3 = formate; 4 = chloride; 5 = nitrite; 6 = carbonate; 7 = unknown; 8 = nitrate; 9 = sulphate; 10 = phosphate; 11 = citrate.

tate accumulation. One possibility is to use a culture consisting of acetate-utilizing bacteria in addition to the methanotrophic bacteria. This was tested by adding *Comamonas acidovorans*, an acetate-utilizing bacterial strain, to the fermenter after 22 hours. This reduced the acetate concentration to about 6 ppm within 12 h (results not shown), confirming the concept.

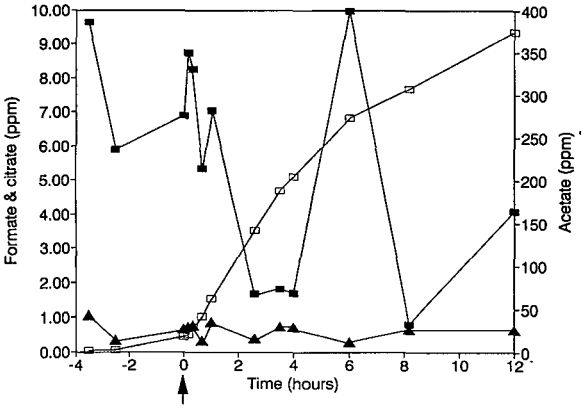


Fig. 5. (■) Formate, (□) acetate and (▲) citrate concentrations in the fermentation broth of a culture of methanotrophic bacteria. The arrow at time zero indicates a change of substrate from methane to natural gas.

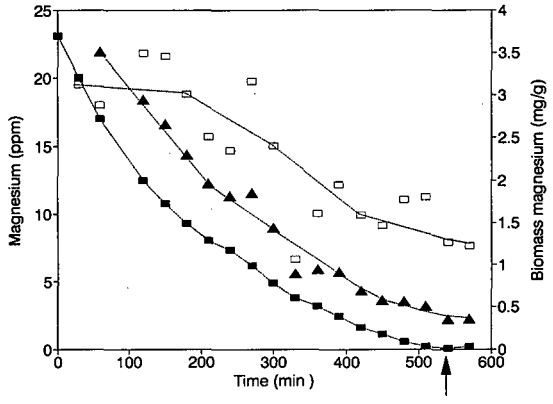


Fig. 6. Magnesium concentration in fermentation broth and fermentation liquid samples with 2 g dry weight of biomass per litre during a washout experiment. At time zero the medium flow was changed to a medium flow without added magnesium. The steady-state growth rate was 0.2 $h^{-1}$. The arrow indicates a sudden stop in growth rate. ■ = Magnesium in fermentation broth; ▲ = magnesium in acid-digested fermentation samples; □ = calculated magnesium content of the microorganisms (difference between fermentation sample and fermentation broth divided by the dry weight).

Fig. 6 shows a magnesium washout experiment. At time zero the feed was changed from a medium with magnesium to a medium without magnesium. The magnesium concentration in the fermenter was followed by ion chromatographic measurements of fermentation broth samples and of acid-digested fermentation liquid samples. The growth rate was constant and about 0.2 $h^{-1}$ during the first 9 h. The arrow indicates a sudden stop in the growth of the bacteria due to magnesium limitation. The limiting concentration of magnesium was 0.06 ppm. The magnesium content of the biomass was reduced from about 3 to about 1.2 mg/g biomass during the washout. This showed a relationship between fermentation broth magnesium concentration and biomass magnesium content.

This experiment showed that the concentration of magnesium in the fermentation broth has to be above 0.06 ppm to maintain optimum growth conditions and that the magnesium content of the final product to some extent can be controlled by the magnesium content of the fermentation broth. Low-magnesium SCP can be produced by keeping the magnesium content of the fermentation broth close to 0.06 ppm whereas high-magnesium SCP can be produced by keeping the magnesium concentration of the fermentation broth above 10 ppm.

## CONCLUSIONS

Anions, alkali metals and alkaline earth metals in the fermentation broth could be determined by ion chromatography after simple removal of the bacterial cells. Determination of transition metals required that complexing compounds were removed. Acid digestion in a microwave oven was a rapid method for removal of complexing compounds and was also useful when preparing samples containing biomass for the chromatographic determination of inorganic anions, cations and transition metals. A total mineral determination of a sample could be obtained in less than 100 min with the combination of a microwave acid digestion and ion chromatography. The implementation of these methods in a fermentation process with methanotrophic bacteria showed that acetate accumulated when natural gas was used as a substrate and that magnesium became the growth-limiting substrate when the magnesium concentration of the fermentation broth was below 60 ppb. These examples show ion chromatography to be a useful tool for the determination of minerals and organic acids in fermentation broth and for the determination of minerals in fermentation liquid and in biomass.

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# Separation of nucleotides and nucleosides by gradient macrocycle-based ion chromatography

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ABSTRACT

Adsorption of the macrocyclic cryptand *n*-decyl-2.2.2 (D2.2.2) to the matrix of the reversed-phase polystyrene Dionex MPIC column generates a novel mixed-mode chromatographic column on which both ionic and hydrophobic interactions can occur. To the aqueous eluent are added cations that bind dynamically to the adsorbed macrocycle, forming positively charged ion-exchange sites. The hydrophobic tail and the MPIC column matrix provide the basis for hydrophobic interactions. Experiments have been carried out to characterize the use of this column in separating both nucleotides and nucleosides. The influence of eluent cation concentration, type of eluent cation and anion, eluent pH and organic solvent are demonstrated. Excellent resolution of nucleotides and nucleosides was achieved under different conditions, due to the respective differences in the mode of retention between the two compound types. A chromatographic gradient was designed which facilitates the simultaneous determination of both species.

## INTRODUCTION

The qualitative and quantitative determination of nucleotides and nucleosides in biological samples is very important for research in nucleic acid biochemistry. The need for separating and quantifying hydrolysates of nucleic acids and free nucleotides has spurred the development of high-performance liquid chromatography (HPLC) for this application. The most commonly used separation techniques involved in the analysis of nucleosides and nucleotides are reversed-phase[1–9], ion-pair[10–15] and ion-exchange[16–21] chromatography.

The conventional ion-exchange method usually allows for the determination of the nucleotides. But this method is not effective for the separation of nucleosides and nucleobases [22]. On the other hand, reversed-phase chromatography is useful for the separation of these molecules, although there are problems in resolving the weakly retained nucleotides [23,24]. Thus, the column-switching technique has been used to separate nucleotides and nucleosides simultaneously in the same analysis [25]. First, the nucleotides are separated on an anion-exchange column. Then, after all the nucleotides are eluted, the $C_{18}$ hydrophobic column is switched into line for separation of the nucleosides and nucleobases. Bischoff and McLaughlin [26–31] developed a mixed-mode chromatography for this purpose. In their experiments a chromatographic matrix which contains sites for both ionic and hydrophobic interactions was used for the separation of the nucleic acid compounds. The mixed-mode matrix could be produced by the addition of hydrophobic moieties to an anion-exchange resin, or the introduction of sites for ionic interactions onto a hydrophobic support. Using this column, oligonucleotides and tRNA molecules were separated with high resolution.

Capillary electrophoresis is another powerful technique for the separation of nucleotides, especially for the separation of oligonucleotides [32]. Nguyen *et al.* [33] developed a method for the separation and quantitation of nucleotides in fish tissues using capillary eletrophoresis. The analysis of three major nucleotides was completed within 15 min. Because there is no negative charge on nucleosides, these are not directly separated by capillary

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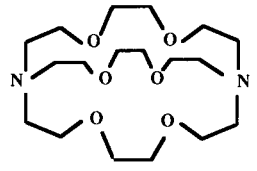
electrophoresis. Cohen *et al.* [34] solved this problem by using micellar solutions and metal additives to partition nucleosides within the micelles.

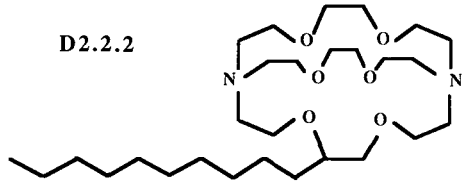
Macrocyclic ligands, such as crown ethers, have been used to separate cations [35–38], based on the size-selective binding of metal and other cations [39,40]. Anions have also been separated using bis- and polymeric crown ethers covalently bonded or polymerically coated on silia [41–43]. In our laboratory, we have developed a novel method for employing macrocyclic ligands as exchange sites in the analysis of inorganic anions using chemically suppressed ion chromatography [44]. The column was prepared by coating macrocycles on commercially available $C_{18}$-derivatized silica or polystyrene columns. The aqueous eluent contains a cation that has an affinity for the immobilized macrocycle, causing the formation of stationary positively charged cation-macrocycle complex exchange sites. Inorganic anions were eluted from the column by $OH^-$ eluent.

Josic and Reutter [45] developed a stationary phase with crown ether for the separation of nucleic acids and proteins. The chromatographic column sorbent was prepared by immobilization of the crown ether 1,10-diaza-18-crown-6 to different porous and non-porous epoxy activated supports. In the presence of potassium ions, the column could be used for the separation of both nucleic acids and proteins. In experiments with standard proteins the influence of pH and the role of loading the column with potassium ions were demonstrated. The retention time is dependent on the size of the nucleic acids. Nucleotides were not retained by this column.

We report here a novel method for the separation of nucleotides and nucleosides by macrocycle-based chromatography. The macrocyclic ligand which we used for the experiment is *n*-decyl-2.2.2 (D2.2.2) cryptand. A Dionex MPIC column was loaded with D2.2.2, yielding a stationary phase having a mixed-mode chromatographic matrix on which both ionic and hydrophobic interactions can occur. The D2.2.2 complexes with a metal ion, forming the desired sites for electrostatic interactions. The hydrophobic tail of the D2.2.2 molecule and the MPIC column matrix provide the sites for hydrophobic interactions. Nucleotides and nucleosides were separated simultaneously on this column. Both ionic




D2.2.2



and hydrophobic interactions were demonstrated, and excellent resolution for nucleotides and nucleosides in one analysis was achieved.

## EXPERIMENTAL

### *Materials*

Macrocyclic ligand D2.2.2 was purchased from EM Science. Reagent-grade nucleotide and nucleoside compounds were obtained from Sigma. HPLC-grade methanol was obtained from Fisher Scientific. Eluent water was purified to 18 MΩ-cm resistivity using a Milli-Q purification system (Millipore). Eluents were degassed by helium purging or sonication. All the other chemicals used to prepare eluents were of analytical grade.

### *Methods*

All the chromatographic separations were performed on a Dionex 2000i (isocratic) or 4000i (gradient) liquid ion chromatograph equipped with a Dionex variable-wavelength UV–VIS detector set at 254 nm. Chromatograms were plotted on a Dionex 4270 integrator and collected using the Spectra-Physics Labnet computer system. A Hewlett-Packard Deskjet Plus printer and 7470A plotter were used for hard copy data presentation. Dionex Autoion 400 software was also used to collect data.

The following columns were used: Dionex MPIC IonPak NS1 (25 cm × 4.6 mm I.D.), polystyrene–divinylbenzene; Dionex OmniPak PAX-500 (25 cm × 10 mm I.D.); Spherisorb 10-μm ODS-2 (25 cm × 4.6 mm I.D.), $C_{18}$ on silica.

The eluents used to separate the nucleotides and nucleosides were aqueous solutions containing varying amounts of salts, acid or base to control

pH, and methanol. Unless otherwise indicated, all chromatograms were made at a flow rate of 1.0 ml/min.

*Preparation of D2.2.2 columns*

A 0.1 ml solution of D2.2.2 [50% (w/v) D2.2.2 in toluene] was put into 50 ml of methanol–water (55:45, v/v) which was degassed by sonication for 10 min. The column had been rinsed first with methanol–water (90:10, v/v) for 2 h. Then the column was rinsed with methanol–water (55:45, v/v) for 30 min. The D2.2.2 was loaded onto the column by recycling the D2.2.2 solution through the column for 3 h.

## RESULTS AND DISCUSSON

*Comparison of column performance with and without D2.2.2*

The retention of nucleotides of the MPIC column without macrocyclic ligand was poor. All the nucleotides eluted immediately after the dead volume of the column with poor resolution even when using pure water as the eluent. In this case, the repulsion effect of the negative charges located at the phosphate moiety must predominate over hydrophobic attraction to the stationary phase. When potassium chloride solution was used as the eluent instead of water, the retention of nucleotides was increased, and some resolution of the three nucleotides used to test the system was achieved when the concentration of KCl in the eluent reached 1 *M*. The peak shapes were poor and the column efficiency was not high. This effect of the addition of salt to the eluent can be explained by formation of weak ion pairs between the metal ion and the ionic nucleotides, which reduces the net negative charges of the solutes, and enhances their retention [46,47].

When the macrocyclic ligand D2.2.2 was adsorbed to the same stationary phase, a dramatic improvement in nucleotide separation was achieved, as shown in Fig. 1. After the column loaded with D2.2.2, the nucleotides do not elute at all with pure water as the mobile phase. Since higher concentrations of salt in the eluent were necessary to reduce the retention of the nucleotides on this column, it was concluded that an ion-exchange mechanism applies, as demonstrated previously for inorganic anions.

*Effect of salt*

The effects of variation in the eluent potassium (KCl) or lithium (LiCl) concentrations on the nucleotide capacity factor using the MPIC/D2.2.2 col-

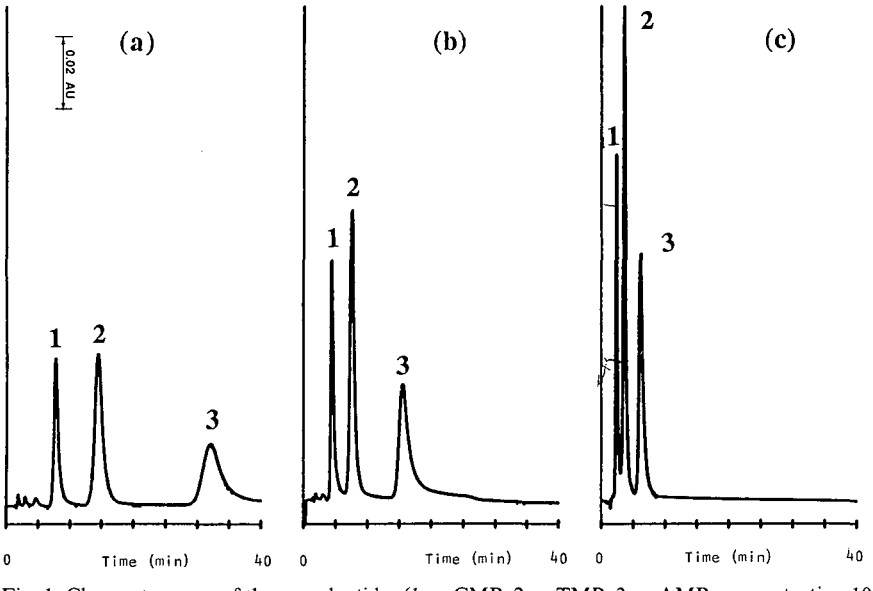



Fig. 1. Chromatograms of three nucleotides (1 = CMP; 2 = TMP; 3 = AMP; concentration 10.0 $\mu M$, 20-$\mu$l injection loop) on MPIC column loaded with D2.2.2. Conditions: UV, 254 nm, flow-rate, 1.0 ml/min. Eluent: (a) 40 m$M$ KCl; (b) 60 m$M$ KCl; (c) 100 m$M$ KCl.

umn was investigated. Retention was consistently higher with $K^+$ than with $Li^+$, in keeping with the much stronger affinity of D2.2.2 for $K^+$. When the concentration of metal ions in the mobile phase was low, the retention of nucleotides was high, especially for the purine nucleotides and the di- and triphosphate nucleotides. The capacity factors ($k'$) for the nucleotides were dramatically decreased when the potassium concentration in the eluent was increased. This result corresponds to conventional anion-exchange chromatography, which adds veracity to the concept of an ion-exchange mechanism for the separation of nucleotides on this macrocycle-loaded column. If ion exchange were the only mechanism for nucleotide retention, nucleotides would not be significantly retained or separated in the absence of a cation which binds with the macrocyle, as we observed with inorganic anions [44]. However, when AMP, CMP, TMP and GMP samples (made free of cation by treatment with $H^+$-loaded ion-exchange resin) were injected into the macrocycle column using pure water eluent, significant retention was still observed. Thus, it is clear that another retention mechanism is at work, as described below.

The population of ion-exchange sites on the stationary phase of the column can be altered simply by changing the mobile phase cation. The higher the binding constant between D2.2.2 and the cation, the higher the column capacity. Fig. 2 shows the effect of changing the cation in the mobile phase on the retention of four nucleotides. Based on this result, we postulated that a cation gradient for the separation of organic anions such as nucleotides was possible, just as was previously achieved for inorganic anions. However, other factors also needed to be explored for this system. Specifically, the presence of hydrophobic interactions with the column, albeit relatively weak, offered possibilities of varying eluent organic content. Furthermore, UV detection in this case made it possible to use anions other than $OH^-$ ion, which is necessary for suppressed conductimetric detection.

The choice of eluent anion has a great influence on the separation of the nucleotides. For eluent halide anions, the order of decreasing retention of nucleotides is $F^- > Cl^- > Br^- > I^-$, which results from the order of affinity of the $K^+$–D2.2.2/MPIC column among these eluent anions, $I^-$ being most

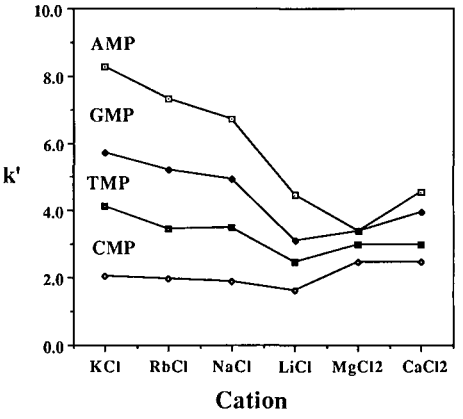



Fig. 2. Variation of nucleotide capacity factor with different eluent cations. Column D2.2.2 on MPIC, eluent 100 m*M* chloride.

strongly attracted to the column. This result is not surprising, since competition of the mobile phase anion with sample anions is the main mechanism for elution of the anionic nucleotides.

*Effect of pH*

The capacity factor of nucleotide retention increases with the pH of the eluent. The pH was varied while holding $[K^+]$ constant by adding variable amounts of KCl, KOH, and/or HCl to the eluent. This result can be explained by the variation with pH of the charges on the acid and base sites of the nucleotide molecules. The phosphate groups increase in negative charge at higher pH, while the amino groups on the nucleobases are neutralized. Capacity increases with pH because the net negative charge of the molecule increases. The other factor influenced by pH is the loading of the macrocyclic compounds with potassium ions [46], since these molecules are weak bases and at low pH, $K^+$ must compete with $H^+$. However, since both the $H^+$–D2.2.2 complex and the $K^+$D2.2.2 complex carry the same charge, this effect must be small.

*Effect of organic solvent*

Addition of an organic solvent to the mobile phase can be used to vary the retention of nucleotides based on the hydrophobic interaction. Indeed, varying the percentage of organic solvent in the eluent illustrates the relative strength of hydro-

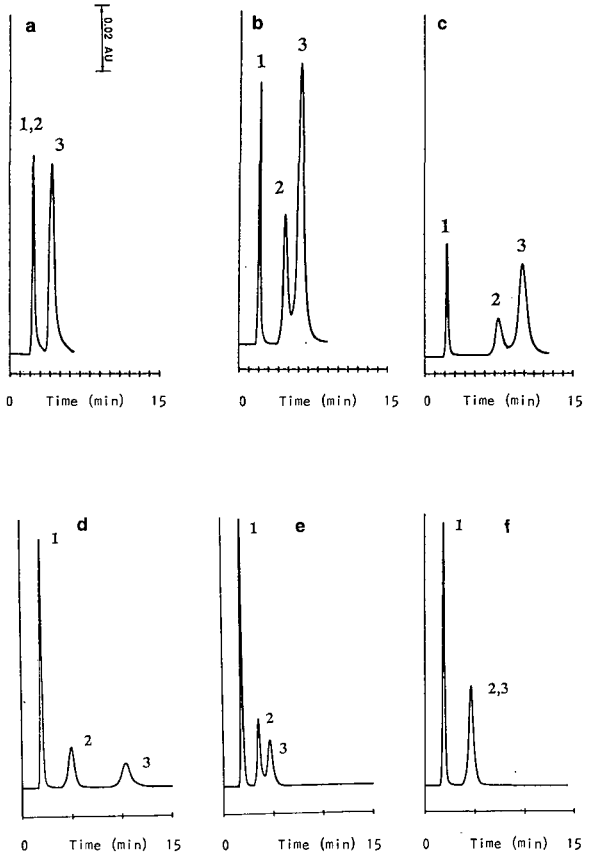



Fig. 3. (a–c) Separation of nucleotides (1 = CMP; 2 = AMP; 3 = TMP; concentration 10.0 $\mu M$, 20 $\mu$l) on D2.2.2/MPIC column. Eluent: 20 m$M$ KCl with (a) 20%, (b) 10% or (c) 5% methanol. (d–f) Separation of nucleosides (1 = cytidine, 2 = thymidine, 3 = adenosine; concentration 10.0 $\mu M$, 10 $\mu$l) on D2.2.2/MPIC column. Eluent: (d) water; (e) water–methanol (90:10); (f) water–methanol (80:20).

phobic interactions with the column. Fig. 3 shows the influence of organic solvent on nucleotide separations on the MPIC/D2.2.2 column. It is interesting to compare this effect on nucleotide retention with the effect on nucleoside retention, since the corresponding nucleoside constitutes the organic moiety of each nucleotide which is prone to hydrophobic interaction with the column. The capacity factors for both nucleotides and nucleosides decrease with increasing percentage of methanol in the mobile phase. This result implies that hydrophobic interactions between nucleosides or nucleotides and the stationary phase do take place, and

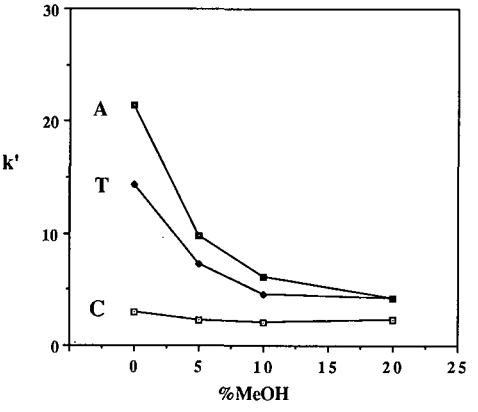



Fig. 4. Variation of nucleoside capacity factor with eluent methanol (MeOH) concentration. Column: D2.2.2 on MPIC. Eluent: water–methanol.

that two mechanisms of retention, *i.e.*, a combination of electrostatic and hydrophobic interactions, come into play with nucleotides. The effect on nuclosides is larger than that of nucleotides, because of the different electron charge in the molecules.

*Separation of nucleosides*

Nucleosides are commonly separated on reversed-phase columns. Thus, as expected, the macrocycle-loaded column is also very good for the separation of nucleosides since hydrophobic interactions are possible. Excellent resolution of three test nucleosided was achieved, as shown in Fig. 4. The effect of salts and organic solvent in the eluent is different for nucleosides than for nucleotides, since there is no phosphate group in these molecules.

The effect of salt concentration in the eluent for nucleosides is much smaller than that for nucleotides because the ion-exchange separation mechanism does not apply.

pH is an important factor which influences the retention of nucleosides because of the variation of net electron charge in the molecules. Higher pH enhances the retention of nucleosides, as is true for nucleotides. Hydrophobic interaction is the predominant separation mechanism. The hydrophobic tail of the cryptand and column matrix provides the site for these interactions. Thus, it is observed that the MPIC/D2.2.2 mixed-mode is very good for the separation of negatively charged or neutral compounds in one analysis.

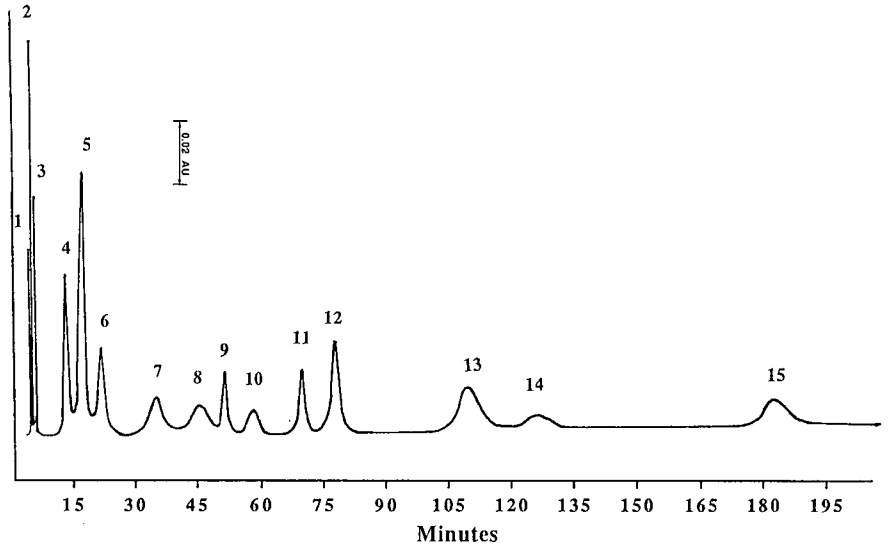



Fig. 5. Gradient chromatogram showing separation of 15 nucleotides and nucleosides. Conditions: UV 254 nm; flow-rate, 1.0 ml/min; concentration of KCl solution 100 m*M*; all concentrations are 10.0 $\mu M$, injection volume, 20 $\mu$l; [KCl] programs: 0–5.0 min: 100% water; 5.0–20 min: 5% KCl solution; 20–150 min: 100% KCl solution. Peaks: 1 = Cytidine; 2 = deoxycytidine; 3 = thymidine; 4 = cytidine-5′-monophosphate; 5 = deoxycytidine-5′-monophosphate; 6 = thymidine-5′-monophosphate; 7 = guanosine; 8 = adenosine + deoxyguanosine; 9 = uridine-5′-monophosphate; 10 = deoxyadenosine; 11 = inosine-5′-monophosphate; 12 = guanosine-5′-monophosphate; 13 = adenosine-5′-monophosphate; 14 = deoxyguanosine-5′-monophosphate; 15 = deoxyadenoside-5′-monophosphate.

*Simultaneous separations of nucleotides and nucleosides*

When the macrocycle columns are combined to separate both nucleosides and nucleotides simultaneously, the resolutions and the peak shape are not very good, especially for the long retented nucleotides. Thus, a gradient was adopted, yielding good resolution for 16 nucleotides and nucleosides as shown in Fig. 5. A KCl concentration gradient program was used to achieve this excellent separation. The chromatogram shows that the gradient is very important for good resolution, especially for the 2-deoxy and oxy nucleotides and nucleosides. Pure water as eluent is good enough to separate nucleosides, but different concentrations of potassium chloride in the eluent are necessary for the separation of the nucleotides and the achievement of good peak shape.

It is conceivable that not only salt concentration gradients can be used to achieve these separations, but also the pH and temperature. The cation gradient described here is a novel gradient separation method we can use which is based on the macrocycle-loaded column because of the change in column ion-exchange capacity when cation is changed in the eluent. So, only macrocycle-based chromatography has cation gradient for the separation of compounds. The cation gradient is one of the advantages of macrocycle-based chromatography.

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# Liquid chromatography and postcolumn indirect detection of glyphosate

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ABSTRACT

Glyphosate [N-(phosphonomethyl)glycine] and its metabolite aminomethylphosphonic acid (AMPA) were separated and detected by a postcolumn indirect detection strategy. Separation can be done on a cation-exchange column, where glyphosate elutes before AMPA, or on an anion-exchange column, where the elution order is reversed. Detection was achieved by using a fluorescent $Al^{3+}$–morin postcolumn reagent. When the postcolumn reagent combines with the column effluent in a mixing tee, the fluorescence decreases in the presence of both analytes. Variables affecting the postcolumn indirect fluorescence detection were established and optimized; the major factors were postcolumn pH and volume and temperature of the postcolumn reaction coil. Detection limits, defined as three times the background noise, for glyphosate and AMPA separated on an anion-exchange column were 14 and 40 ng, respectively.

## INTRODUCTION

Glyphosate [N-(phosphonomethyl)glycine) (**I**), is a widely used broad-spectrum, non-selective, post-emergence herbicide and there is great interest in and a need to determine the herbicide and its metabolite aminomethylphosphonic acid (AMPA) (**II**), in physiological, water, plant, food and soil samples.

$HO_2CCH_2NHCH_2P(O)(OH)_2$

**I**

$H_2NCH_2P(O)(OH)_2$

**II**

Procedures for their determination have been reviewed [1]. Although gas chromatographic procedures [1,2–4] continue to be of interest, in general they suffer from tedious sample preparation because of the need to convert the analytes into volatile derivatives. For this reason and the requirement for a better detection limit, liquid chromatographic (LC) procedures have been developed [5–13].

Three LC approaches have been used to achieve the separation. As glyphosate and AMPA are acidic, they can be separated by anion exchange [5,8,10,11]. However, they are not easily detected without derivatization except at low UV wavelength (< 200 nm) [5], where the detection limits are not favorable. Postcolumn derivatization at the amine function using *o*-phthalaldehyde (OPA) [6,8,11] can be applied, but glyphosate must first be chemically modified on-line following the separation prior to forming the OPA derivative. On the other hand, reversed-phase liquid chromatography (LC) [12,13] and ion interaction LC [9] can be used, provided that glyphosate and AMPA are derivatized precolumn. Other reagents used to derivatize the amine function are 9-fluorenylmethyl chloroformate [7,10], 1-fluoro-2,4-dinitrobenzene [9] and *p*-toluenesulfonyl chloride [12,13]. In each instance detection is possible at a high UV wavelength. The derivatization reactions will provide favorable detection limits. However, the reactions can be more complex, particularly if done precolumn, and can suffer from a lack of reproducibility.

Morin (3,5,7,2′,4′-pentahydroxylflavone) will complex with $Al^{3+}$ and many other metal ions to form a highly fluorescent solution which is the basis for the fluorimetric determination of the metal ions [14]. In the presence of phosphate or $F^-$ the emitted fluorescence of the $Al^{3+}$–morin decreases and the decrease can be correlated with the amount of

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phosphate [15] or $F^-$ [14] in the sample. This indirect fluorimetric chemistry can be used postcolumn to detect phosphate [16] and $F^-$ [17] following a liquid column chromatographic separation with detection limits of 15 and 2 ng, respectively, at a signal-to-noise ratio of 2:1.

The use of $Al^{3+}$–morin as a postcolumn reagent is an indirect detection (ID) because the decrease in $Al^{3+}$–morin fluorescence in the analyte band relative to the fluorescent background is being monitored. This differs from other ID strategies used in LC because in these instances equilibrium effects that occur within the column between the analyte, a detector-active component in the mobile phase and the stationary phase are the basis for the ID [18–20]. A postcolumn ID and the effects of the experimental and detector variables have only rarely been used and/or evaluated [16,17,21].

In the procedure described here, which can be used for the determination of both glyphosate and AMPA, an $Al^{3+}$–morin solution is combined with the column effluent and the decrease in fluorescence is recorded when either analyte, both of which compete favorably with morin to form the $Al^{3+}$ complex, passes through the column. There were four major aims: to demonstrate that postcolumn ID is a viable and sensitive detection strategy in LC separations, to establish the parameters that influence postcolumn ID particularly as they apply to the use of $Al^{3+}$–morin as a postcolumn reagent, to show that the postcolumn ID with $Al^{3+}$–morin is easily carried out and provides low detection limits for the determination of glyphosate and AMPA and to demonstrate that the $Al^{3+}$–morin reagent is a selective reagent for ID.

## EXPERIMENTAL

### *Reagents*

Morin hydrate and $Al(NO_3)_3$ were obtained from Aldrich, glyphosate and AMPA from Sigma and all other acids, bases and salts from EM Science. LC-grade water was prepared by passing laboratory distilled, deionized water through a Milli-Q Plus system (Millipore). USP-grade 95% ethanol was used. Prepacked 150 mm × 4.1 mm I.D. PRP-X100 (10 μm) anion-exchange columns, and 250 mm × 4.1 mm I.D. PRP-X400 (7 μm) cation-exchange columns were obtained from Hamilton.

### *Instrumentation*

The LC system consisted of a Spectra-Physics M 8800 pump, a Rheodyne Model 7125 injector and a Kratos Model 9000-9501 fluorescence detector equipped with a Kratos FSA 113 coated mercury lamp, a Kratos FSA 404 excitation (400–470 nm) filter and a Kratos high-pass emission filter (50% transmission at 480 nm). The detector response was recorded on a strip-chart recorder and collected on a Spectra-Physics Model 4270 integrator coupled with a Spectra-Physics WINner software package. Peak areas are reported as relative integrator units. The postcolumn system consisted of a Varian M 2010 pump to deliver the $Al^{3+}$–morin solution through a pulse damper made from an empty 250 mm × 8.0 mm I.D. column, a 150 mm × 4.6 mm I.D. column containing 80-mesh glass beads and a coil of 2 m × 0.508 mm I.D. stainless-steel tubing. The $Al^{3+}$–morin reagent stream and the column effluent were combined through a Lee Visco mixing tee (No. 344790 SN152). The connection between the tee and the detector was a reaction coil made from 4.57 m × 0.76 mm and 1.52 m × 1.02 mm I.D. PEEK tubing (Upchurch Chromatography), which provided an internal volume of 3300 μl and was woven to minimize postcolumn peak broadening. The coil temperature was maintained at 55°C with a DuPont M 851201-901 temperature-controlled column oven.

### *Procedures*

Analyte stock standard solutions (1 mg/ml) were prepared by dissolving known amounts in LC water. Serial dilutions of the stock standard solutions were done to obtain calibration standard solutions. The mobile phases were aqueous 25 m*M* $NaNO_3$ (pH 9.5, adjusted with dilute NaOH) for anion-exchange separations and 10 m*M* $HNO_3$ (pH 2.0) for cation-exchange separations at flow-rates of 1.0 and 0.50 ml/min, respectively. Sample aliquots were injected by syringe in amounts of 10 μl or less.

The postcolumn $Al^{3+}$–morin solution was prepared by pipetting aliquots of morin, $Al(NO_3)_3$ and acetic acid from stock solutions and combining these with 95% ethanol and LC water and aged overnight to ensure reproducible formation of the $Al^{3+}$–morin complex. The reagent was stable for at least 2 months when stored in a closed container. The solution contained 4.0 μ*M* $Al(NO_3)_3$, 21 μ*M* morin,

27.5 $\mu M$ acetic acid and ethanol–water (4:1) and was delivered to the tee at 0.50 ml/min. Dilute NaOH or $NHO_3$ was added to the $Al^{3+}$–morin solution to produce a pHof 4.3 when the column effluent and the postcolumn $Al^{3+}$–morin solution were combined in the reaction coil.

## RESULTS AND DISCUSSION

Glyphosate and AMPA are readily separated by ion exchange. If the mobile phase pH is about 9.5, glyphosate will be more highly retained on an anion exchanger [5,8,10,11] because of its additional acidic site. As the pH is decreased into the acidic range, both are converted into cations, as both contain a basic amino group, and on a cation exchanger the AMPA cation is more highly retained than the glyphosate cation. Therefore, it is possible to carry out the separation so that the glyphosate is eluted either first (by cation exchange) or second (by anion exchange). This choice is particularly important if the primary objective of the analysis is to determine either AMPA or glyphosate in the presence of the other as a trace component.

The other variables affecting the retention of glyphosate and AMPA on ion exchangers are typical of ion-exchange separations. Thus, increasing the mobile phase counter-ion concentration or switching to a counter ion of higher ion-exchange selectivity decreases the retention. Under the recommended separation conditions outlined in the following discussion, mobile phase conditions were optimized for separations on the anion- and the cation-exchange analytical columns to achieve baseline resolution and to minimize mobile phase component effects on the postcolumn detection chemistry.

Although glyphosate and AMPA are readily separated, detection with a favorable detection limit is not easily done without derivatization. When highly fluorescent $Al^{3+}$–morin solution and either glyphosate or AMPA are combined, a decrease in the fluorescence of the $Al^{3+}$–morin is observed. Further, as the amount of either analyte is increased, the fluorescence of the $Al^{3+}$–morin decreases in proportion to the amount of analyte added, provided that the $Al^{3+}$–morin is in excess. The decrease in fluorescence occurs occurs because the analyte competes favorably with morin to form the $Al^{3+}$–analyte complex over the $Al^{3+}$–morin complex, as shown in the equation

$$Al^{3+}\text{–(morin)}_n + mG \rightleftharpoons Al^{3+}\text{–}G_m + n\text{morin} \quad (1)$$

using glyphosate, G, as the example. The stoichiometry of the two complexes, which is not identified in eqn. 1, will also determine the degree of change in fluorescence. Further, the change in fluorescence is selective as only analytes that form $Al^{3+}$ complexes will cause a fluorescence decrease.

By combining an $Al^{3+}$–morin solution with the column effluent from either an anion- or a cation-exchange separation, it should be possible to detect when glyphosate or AMPA (also phosphate) emerges from the column because within these analyte bands the fluorescence should decrease. Further, the fluorescence decrease should be proportional to the amount of glyphosate or AMPA (also phosphate) present in the separated bands. To achieve postcolumn indirect fluorescence detection (PCIFD), the detector is set at the wavelength of fluorescence and the detector electronic offset is used to zero the detector signal as the postcolumn fluorescent solution passes through the detector. Therefore, the analyte, when it appears, is indicated by a negative peak. The postcolumn concentration of the $Al^{3+}$–morin solution must be in excess to provide a decrease in fluorescence that occurs rapidly and with an appreciable change but yet low enough that the background fluorescence does not exceed the offset capability of the detector and/or provide a background noise level that limits the ability to detect the change in fluorescence with appropriate sensitivity and accuracy.

The variables affecting PCIFD can be divided into two types. One group, which primarily affects the background fluorescence, includes the pH for the postcolumn reaction, the buffer concentration, the postcolumn solvent composition, the $Al^{3+}$ to morin mole ratio and the concentration of the $Al^{3+}$–morin. The second group will have a major effect on the rate of the postcolumn reaction; these include the postcolumn reaction temperature and volume. After a series of preliminary experiments designed to establish the qualitative effect of each variable, each was carefully evaluated over a defined range while all other factors were held constant. The preliminary experiments also demonstrated that a column flow-rate of 1.0 ml/min and a postcolumn

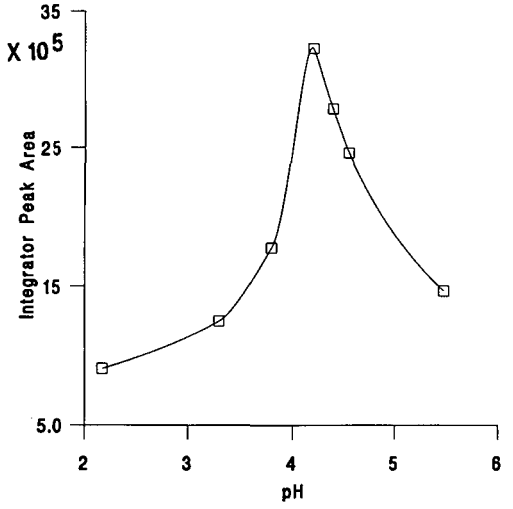


Fig. 1. Effect of postcolumn reaction pH on glyphosate peak area. Mobile phase, 20 m*M* $NaNO_3$ (pH 9.5); PRP-X100 anion-exchange column. The postcolumn solution contained 4.0 $\mu M$ $Al^{3+}$, 21 $\mu M$ morin, 25 m*M* acetate buffer and ethanol–water (4:1) and the temperature was 26°C.

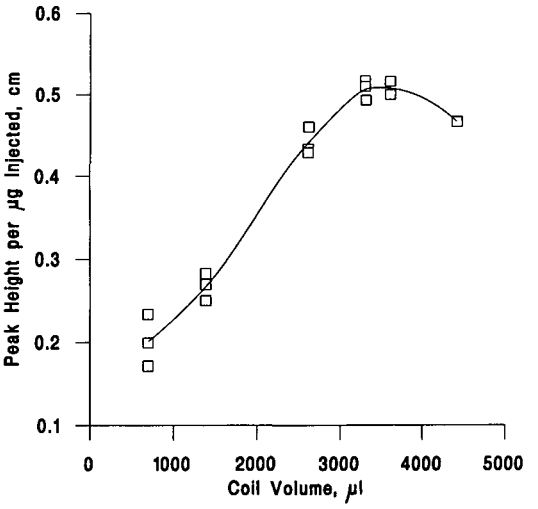


Fig. 2. Effect of postcolumn reaction coil volume on glyphosate peak area. Conditions as in Fig. 1.

$Al^{3+}$–morin solution flow-rate of 0.50 ml/min were optimum for the glyphosate and AMPA sample sizes being separated and detected. The purpose in optimizing each variable was to obtain an accurate, reproducible change in fluorescence that would correspond to the best detection limits for glyphosate and AMPA.

Of the seven postcolumn variables, those which had the greatest effect on fluorescence change and therefore require careful control over narrow limits in order to achieve the lowest detection limits were reaction pH, postcolumn reaction temperature and postcolumn volume. Fig. 1 shows that a maximum peak area (largest change in fluorescence decrease) using glyphosate as the test analyte is obtained when the postcolumn pH is about 4.3. In these experiments all other postcolumn conditions, including the $Al^{3+}$–morin concentration reaction coil length, volume and temperature and flow-rate are held constant while the pH of the acetate buffer is changed. The optimum pH remains the same when the postcolumn mixing volume and/or temperature is elevated. As the pH is shifted above or below 4.3, the peak area (degree of fluorescence decrease) decreases sharply. It is essential that the postcolumn $Al^{3+}$–morin solution contains a buffer of sufficient capacity to overcome the mobile phase pH if the latter is significantly different from 4.3. When the mixing coil beyond the mixing tee is increased in length, while other variables are held constant, the reaction of the analyte with the $Al^{3+}$–morin is more complete, and the decrease in fluorescence (increase in peak area) becomes larger. However, the increased volume of the tubing becomes a factor, and band-broadening effects start to reduce the peak area. As shown in Fig. 2, when the postcolumn coil volume is 3300 $\mu$l, the reaction temperature is 26°C and the flow-rate of the combined column effluent and postcolumn reagent solution is 1.5 ml/min, the maximum peak area is obtained. In this study, two lengths of PEEK tubing of 0.76 and 1.02 mm I.D. were connected in order to adjust the coil length easily. For routine appliations a single PEEK tube of small I.D. yielding 3300 $\mu$l should be used. The PEEK tube should also be woven to increase mixing and reduce band broadening. Using the same conditions, but raising the temperature, indicates (see Fig. 3) that the optimum reaction coil temperature for glyphosate and AMPA is about 55°C. At higher temperatures the peak area begins to decrease because the fluorescence intensity of the $Al^{3+}$–morin starts to decrease at higher temperatures.

When the effects of postcolumn reaction solvent composition was evaluated, the maximum peak area for glyphosate and AMPA was obtained when the ethanol to water ratio was about 2:3. Similarly, the

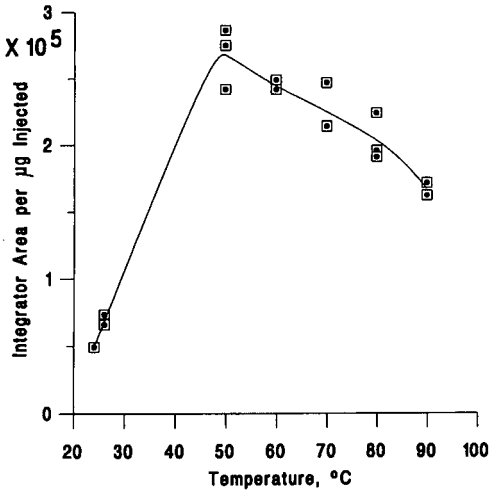


Fig. 3. Effect of postcolumn reaction temperature on glyphosate peak area. Conditions as in Fig. 1 with a reaction coil of 3300 µl and a postcolumn pH of 4.3.

maximum peak area was produced when the $Al^{3+}$ to morin ratio was 1:6 and the $Al^{3+}$ concentration was about 1.4 µ*M* in the mixing tee. As the acetate buffer concentration was increased from 10 to 100 m*M* in the postcolumn $Al^{3+}$–morin solution, the peak area decreased by about 40%. However, at about 10 m*M* after mixing in the tee, a modest change in the buffer concentration produced only minor effects on the peak area. In general, the effects of postcolumn solvent composition, $Al^{3+}$–morin ratio and concentration and buffer concentration on glyphosate and AMPA are similar to those on peak areas for other analytes detected by the PCIFD stategy [16,17].

Fig. 4 illustrates the separation of a mixture of about 50 ng of phosphate, 400 ng of glyphosate and 540 ng of AMPA by cation exchange and a nitric acid eluent with PCIFD using the $Al^{3+}$–morin reagent. Because of the acidic mobile phase, the postcolumn $Al^{3+}$–morin pH was adjusted with dilute NaOH so that a pH of 4.3 was obtained when the column effluent and postcolumn solution were combined in the mixing tee. The phosphate, which was included in the sample because phosphate would be present in physicological samples and is detected by PCIFD [16], is not retained on the cation

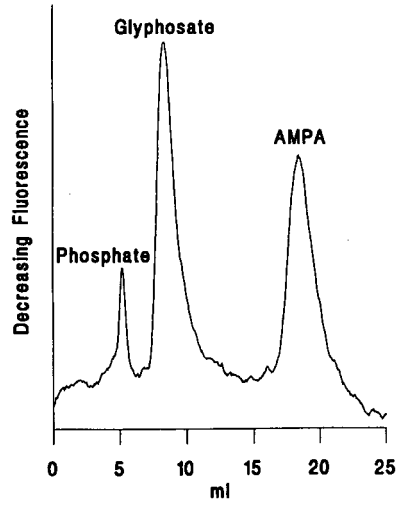


Fig. 4. Separation on a cation exchanger. Mobile phase 10 m*M* $HNO_3$ and PRP-X400 cation-exchange column using the postcolumn reaction conditions as in Fig. 3 with a postcolumn reaction temperature of 55°C. Flow-rate, 0.50 ml/min.

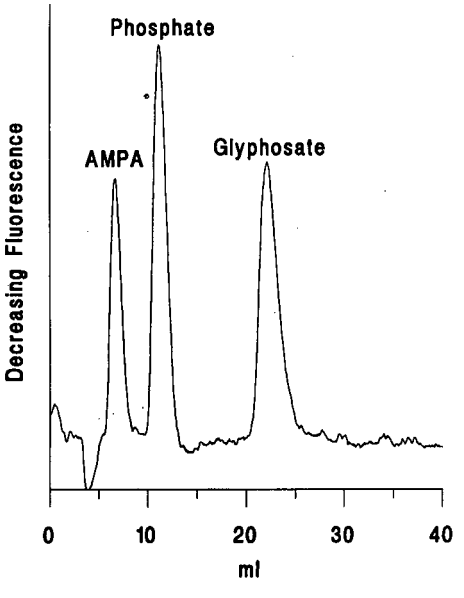


Fig. 5. Separation on an anion exchanger. Mobile and stationary phase conditions as in Fig. 1 except 25 m*M* $NaNO_3$ and postcolumn reaction conditions as in Fig. 4. Flow-rate, 1.0 ml/min.

exchanger and it is detected in the void volume where other common anions also appear. A commercially available 250-mm long cation-exchange column was used and baseline separation of glyphosate and AMPA is possible with a shorter column.

When an anion-exchange column is used for the separation, the elution order is reversed and AMPA appears before phosphate. This is illustrated in Fig. 5 where a mixture of 900 ng of AMPA, 313 ng of phosphate and 340 ng of glyphosate are separated. AMPA and glyphosate are readily resolved over the pH range 8–10; above pH 10 the retention of both increases sharply. The location of the phosphate peak is also sensitive to the mobile phase pH and at pH 9.5 (see Fig. 5) the peak appears between those of AMPA and glyphosate. If the pH is decreased the phosphate peak shifts towards the AMPA peak. Other common inorganic anions, such as $Cl^-$ and $NO_3^-$, that may be present in the sample will not interfere as they are not detected by PCIFD. Fluoride, if present, would be detected [17] in the void volume, where it is eluted, whereas $SO_4^{2-}$, which causes a fluorescence decrease, does not interfere.

PCIFD is both a sensitive and a selective detection strategy. This is illustrated in Fig. 6 using an anion-exchange column and mobile phase conditions where $F^-$ and $Cl^-$ are retained on the column. The $F^-$ peak, which corresponds to 0.33 $\mu$g of NaF, is barely detected by conductance whereas $Cl^-$ as NaCl (11.5 $\mu$g), which is 35 times larger than $F^-$ as NaF, is readily detected. PCIFD, with the detector connected in series with the conductance detector, provides a significant $F^-$ peak area by comparison and is also selective as it does not respond to $Cl^-$. In addition, the large $Cl^-$ excess does not affect the elution time of $F^-$ on the anion-exchange column.

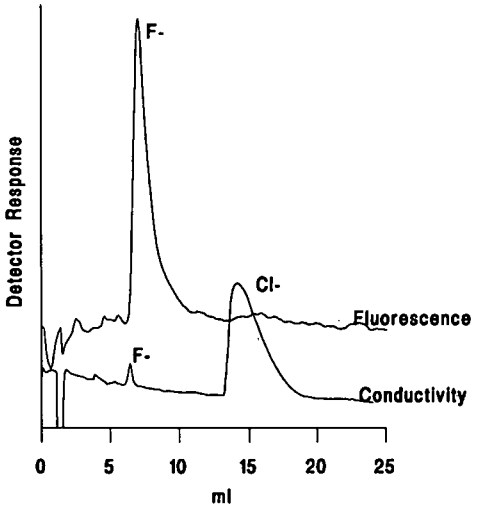


Fig. 6. Sensitivity and selectivity of postcolumn indirect fluorescence detection using $Al^{3+}$–morin compared with conductivity detection. Mobile phase 4.0 m*M* NaOH and postcolumn indirect detection conditions as in Fig. 4 except for a postcolumn reaction temperature of 26°C. Column, PRP-X100; flow-rate, 1.0 ml/min.

When a woven stainless-steel tube of similar inside diameter and volume as the PEEK tube was used as the reaction coil, the peak areas were similar but the peak heights were reduced and the peaks were broad and tailed. This is illustrated by comparing Figs. 4 and 5. In Fig. 4 where a stainless-steel coil was used the peaks are broader than in Fig. 5 where a PEEK coil was used. Apparently, glyphosate and AMPA undergo adsorption and/or react with the stainless-steel coil at elevated temperatures. Both are capable of exhibiting ligand properties and can form stable metal ion ligand complexes [22] which would contribute to broadening.

Calibration graphs were constructed with glyphosate and AMPA standards using an anion-exchange column, 10-$\mu$l injections and the mobile phase conditions outlined in Fig. 5. For glyphosate the straight line corresponded to the equation integrator area counts = 14.1 + 13 300 (nmol injected) with a correlation coefficient of 1.00; for AMPA it was integrator area counts = 3600 + 2560 (nmol injected) with a correlation coefficient of 0.99. No attempt was made to determine the upper limit of linearity. The slope for glyphosate is almost five times greater and the increased sensitivity for the glyphosate determination over AMPA arises because more $Al^{3+}$–morin undergoes a reaction with glyphosate under the postcolumn reaction conditions than for an equivalent amount of the AMPA. Hence, the fluorescence decrease for glyphosate is larger. Another factor contributing to the increased sensitivity is that glyphosate is probably a better ligand than AMPA for $Al^{3+}$. The precision for the data used to establish the glyphosate calibration graph, for example, was better than 3% (relative standard deviation) at each experimental point on the graph. The detection limits for the anion-exchange separation where the decrease in the

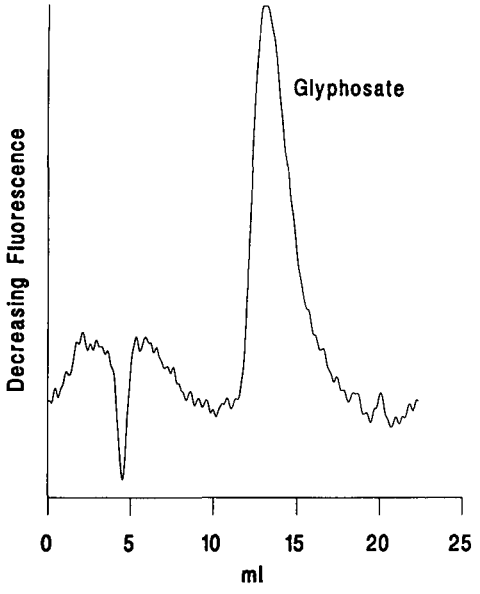


Fig. 7. Separation of glyphosate in a commercial herbicide. Conditions as in Fig. 5 except 40 m*M* $NaNO_3$.

fluorescence signal was three times the noise were found to be 14 ng of glyphosate and 40 ng of AMPA. For glyphosate separated on the cation exchanger according to the conditions in Fig. 4, the detection limit as defined above was about 28 ng of injected analyte.

Fig. 7 illustrates the separation and detection of glyphosate in a commercially available herbicide sample on an anion-exchange column. The glyphosate was listed in the product as 0.96% as the isopropylamine salt. A small aliquot of the commercial sample was passed through a Millipore Sep-Pak $C_{18}$ cartridge and a Millipore 0.2-$\mu$m filter to remove the sample matrix. When a 3-$\mu$l neat aliquot of this sample was injected, the glyphosate, which overloaded the column, was readily detected. Diluting the sample 1:10 with water and injecting a 4-$\mu$l aliquot provided the chromatogram shown in Fig. 7. Comparison of peak area with the calibration graph indicated that the glyphosate concentration in the sample was about 0.96%.

## CONCLUSIONS

Postcolumn indirect detection is a viable detection strategy and other postcolumn reactions should be adaptable to indirect detection. Like other indirect detection strategies, the background signal developed in the postcolumn reaction must be low enough that the signal can be zeroed by the detector offset electronics. When using an $Al^{3+}$–morin solution as a postcolumn reagent, a decrease in fluorescence is used to indicate the amounts of glyphosate, AMPA and phosphate in the sample. The elution order can be reversed depending on whether the separation is carried out on an anion or a cation exchanger. The detection is sensitive and simple, and the procedure does not suffer from the experimental problems usually associated with postcolumn derivatization reactions. The $Al^{3+}$–morin indirect detection of glyphosate and AMPA is selective, as few other analytes will cause a fluorescence decrease, and therefore even if they are not separated from glyphosate and AMPA they will not interfere in the detection.

## ACKNOWLEDGEMENT

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CHROMSYMP. 2562

# Regulated methods for ion analysis

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ABSTRACT

Ion chromatography is one of the analytical techniques that has been approved by the United States Environmental Protection Agency (EPA) for the determination of inorganic ions such as nitrite and nitrate in drinking water. Advantages of ion chromatography methodology include separation before detection, increased sensitivity, simple sample preparation, and faster analysis time compared to non-chromatography techniques.

This paper offers a discussion of approved ion chromatography methods 300.0 and B-1011 as well as other methods that are being currently reviewed and also new methodologies for the future.

## INTRODUCTION

Concern for our environment has grown significantly throughout the world. Everywhere, the fear of polluting our globe is a continuing problem. There is a need for strict control of toxic substances and close monitoring of their presence in the environment in order to prevent contamination and protect our natural resources.

The United States Environmental Protection Agency (EPA) has established regulations and methodology for inorganic contaminants under the Safe Drinking Water Act. Fluoride, nitrite and nitrate are listed as primary pollutants since they can cause adverse health effects. Ion chromatography (IC) has become a well established technique for the determination of nitrite and nitrate in drinking water [1]. The EPA has approved IC methods 300.0 and B-1011 for the analysis of nitrite and nitrate in drinking water [2].

Chloride and sulfate are listed as secondary contaminants because they are organoleptic (affect the smell, taste, or appearance of water). They are not monitored by the EPA, hence, a laboratory can use any method (IC, ion selective electrode, flow injection analysis, etc.) to analyze for these analytes.

The ability of IC to separate the analytes of interest from interferences provides a dinstinct advantage over other analytical techniques in terms of detection, sensitivity and is capable of multi-elemental analysis. This paper reviews methods 300.0 and B-1011 and describes other ion chromatographic methods under current evaluation by the EPA. It also proposes new a method for the future.

## EXPERIMENTAL

### *Instrumentation*

The liquid chromatograph consisted of a Waters (Waters Chromatography Division of Millipore, Milford, MA, USA) 500 or 600 Series pump, a Model 431 conductivity detector, a Model 441, 484, 486 or 490 UV detector, a pneumatic reagent delivery module (RDM), WISP auto sampler or a Rheodyne 710 manual injector, and either a Waters 840 or 860 data station. The analytical columns used were a Waters IC-Pak Anion (50 × 4.6 mm I.D.), Waters IC Pak Anion HR (75 × 4.6 mm I.D.), or Waters IC Pak Anion HC (150 × 4.6 mm I.D.), methacrylate-based anion exchanger.

The capillary electrophoresis system employed was the Waters Quanta 4000 with a negative power supply and an Hg lamp for 254-nm detection. The separation was carried out on a Waters AccuSep polyimide-coated fused-silica capillary (60 cm × 75 μm I.D.). While the Quanta 4000 is capable of both hydrostatic and electromigration injections, the hydrostatic sample introduction mode (10 cm for 30 s)

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was used in this work. Data acquisition was performed with a Waters 860 data station. Detector time constant was set at 0.1 s and data acquisition rate was 20 points/s.

*Reagents*

Water (18 MΩ) (Millipore, Bedford, MA, USA) was used to prepare all solutions. Analytical-grade chemicals, sodium gluconate, boric acid, gluconic acid, lithium hydroxide monohydrate, ammonium sulfate and diphenylcarbohydrazide were obtained from Aldrich, Milwaukee, WI, USA and sodium tetraborate decahydrate and sodium chromate tetrahydrate were obtained from Mallinckrodt, Paris, KY, USA. Glycerin, ammonium hydroxide, and sulfuric acid were obtained from J. T. Baker, Phillipsburg, NJ, USA. HPLC-grade solvents were obtained as follows: acetonitrile and methanol from J. T. Baker and *n*-butanol from Aldrich. CIA-Pak OFM anion BT, is propriety chemical obtainable from Waters.

All standard mixtures were prepared by diluting 1000 ppm stock solutions containing a single anion. Eluents and carrier electrolytes were prepared fresh daily, filtered and degassed using a Millipore solvent carification kit prior to use.

## RESULTS AND DISCUSSION

*EPA Method 300.0*

The original version of method 300.0 was approved for nationwide use as an alternate test procedure for the measurement of nitrate by ion chromatography for National Interim Primary Drinking Water Regulation (NIPDWR) compliance monitoring in 1984 [3]. Since IC can detect several anions simultaneously, chloride and sulfate were also included in the method, however only as secondary contaminants. Since levels of secondary contaminants are not legally enforceable. IC was recommended for chloride and sulfate measurements. The method was updated in 1989 to incorporate new column and hardware advances (300.0 Method A) [4].

The separation of a seven-anion standard mixture shown in Fig. 1 was generated using a Dionex AS4A column, sodium hydrogencarbonate–sodium carbonate eluent, anion suppressor device and conductivity detector [5]. Although the chromatogram

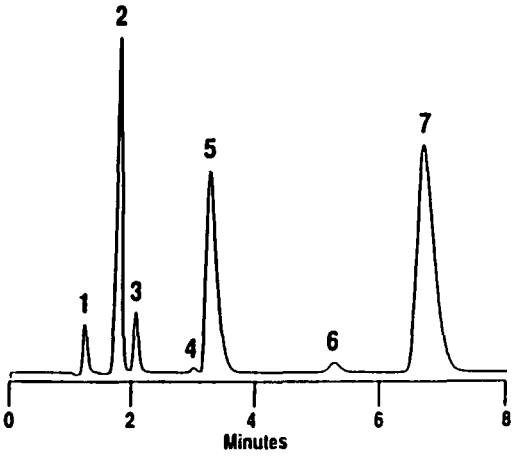


Fig. 1. Separation of a standard anion mixture using the Dionex AS4A column. Peaks: 1 = fluoride (2 ppm); 2 = chloride (20 ppm); 3 = nitrite (2 ppm); 4 = bromide (2 ppm); 5 = nitrate (10 ppm); 6 = phosphate (2 ppm); 7 = sulfate (60 ppm). Chromatogram was taken from EPA test method 300.0.

shows seven common anions, method 300.0 is only approved for nitrite and nitrate in chlorinated drinking water.

Recently there has been a joint EPA–ASTM collaborative study of an extension of EPA Method 300.0 [6] for expansion to both primary and secondary contaminants in drinking and waste water. The method is currently under committee review.

*EPA Method B-1011*

In 1987, method B-1011, *The determination of nitrite/nitrate in water using single column ion chromatography* [7] was recommended to the Office of Drinking Water (ODW) by the Environmental Monitoring Systems Laboratory (EMSL), Cincinnati, OH, USA as equivalent to EPA method 300.0 for nitrate. The method was published in the *Federal Register* as a proposed new method at the same time as the EPA published the *National Primary and Secondary Drinking Water Regulations, Proposed Rule*, in 1989. Formal EPA approval was accomplished when method B-1011 was published in the *National Primary and Secondary Drinking Water Regulations* on January 30, 1991.

There are conflicting opinions on whether to use single (EPA method B-1011) or dual (EPA method 300.0) column IC for nitrate analysis. The EPA evaluated data from a comparability study for both

of the methods and concluded that they both were successful in analyzing nitrate, *i.e.*, precision, accuracy and acceptance limits were met [2].

The upper chromatogram of Fig. 2 contains a separation of nitrite and nitrate in a chlorinated drinking water sample using method B-1011 which includes a Waters IC-Pak Anion column, lithium hydroxide eluent and UV detector. By changing the detection mode to ultraviolet absorbance, the effects of interferences are eliminated and both nitrite and nitrate are easily detected since they both absorb at 214 nm.

The real utility of UV detection is for drinking water samples that are not chlorinated. The EPA requires that non-chlorinated drinking water samples be preserved by an addition of sulfuric acid until the sample pH is less than 2 [1]. This could add greater than 1000 ppm sulfate to the sample. The lower chromatogram is Fig. 2 shows a ground water sample containing over 200 ppm calcium carbonate and preservation with sulfuric acid added 1500 ppm sulfate. The sample was diluted 1:100 to avoid column overloading by such a high sulfate level and then chromatographed.

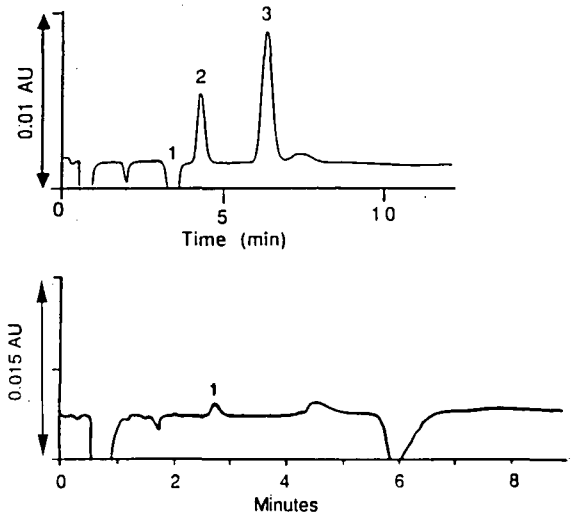


Fig. 2. Upper chromatogram: Analysis of nitrite and nitrate in chlorinated drinking water using EPA method B-1011. Conditions, column: Waters IC-Pak Anion, eluent: 2.5 m*M* lithium hydroxide, flow-rate: 1.2 ml/min, detection: UV at 214 nm. Peaks: 1 = chloride; 2 = nitrite–N (32 $\mu$g/l); 3 = nitrate–N (68 $\mu$g/l). Lower chromatogram: Use of EPA method B-1011 for the analysis of non-chlorinated drinking water ($H_2SO_4$ preserved). Same conditions except eluent: 5 m*M* lithium hydroxide. Peak 1 = Nitrate–N (3.75 $\mu$g/l).

One would not have been able to use conductivity detection for this sample due to the vastly different anion concentrations and the significant conductivity response of sulfate. There was no problem analyzing it using UV detection. In fact, the EPA's Laboratory Certification Manual states that, due to the close elution times for nitrate and sulfate anions, conductivity detection methods may not be used to analyze for nitrate in samples preserved with sulfuric acid [1].

*EPA Method A-1000*

Also in 1987, method A-1000, *Conductivity Detection of Anions Using Single Column Ion Chromatography* [7] was forwarded to the EPA Environmental Monitoring Systems Laboratory, Cincinnati, OH, USA. Method A-1000 was cited in the Manual for the Certification of Laboratories Analyzing Drinking Water, as a recommended method for the determination of chloride and sulfate in 1989 [1]. A chromatrogram of a standard mixture of seven anions in water using a Waters IC-Pack Anion column, borate–gluconate eluent, and conductivity detection is given in Fig. 3.

*Waters test method B-1012 for nitrite/nitrate in wastewater*

Waters submitted data collected by Enwright Environmental Laboratories [8] to the EPA Environmental Monitoring Systems Laboratory, Cincinna-

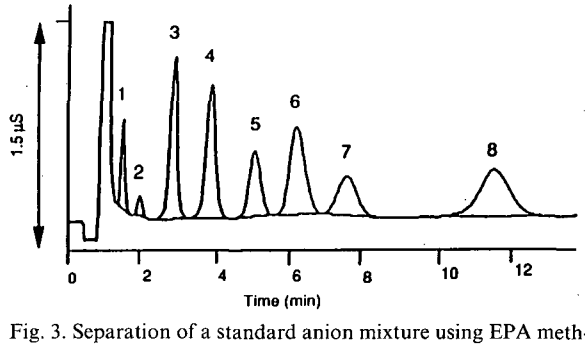


Fig. 3. Separation of a standard anion mixture using EPA method A-1000. Conditions, column: Waters IC-Pak Anion, eluent: borate–gluconate, flow-rate: 1.2 ml/min, detection: conductivity. Peaks: 1 = fluoride (1 ppm); 2 = hydrogencarbonate; 3 = chloride (2 ppm); 4 = nitrite (4 ppm); 5 = bromide (4 ppm); 6 = nitrate (4 ppm); 7 = phosphate (6 ppm); 8 = sulfate (4 ppm).

ti, OH, USA, in 1990. A Waters Chromatography single-column IC method for nitrite and nitrate was compared to the EPA approved cadmium reduction method 353.3, in order to obtain alternate test procedure (ATP) approval. The analysis of nitrite and nitrate in a sample obtained from a sewage treatment plant is shown in Fig. 4. Method B-1012 prescribes the use of a Waters IC-Pak Anion HC column, modified borate/gluconate eluent, and UV detection in series with conductivity detection. The sample was diluted 1:4. The reported results of the analysis were based on data from the UV detector, rather than the conductivity detector, due to better sensitivity and fewer interferences for nitrite and nitrate determination. However, simultaneous detection is advantageous because if offers more information per analysis. This test method is currently being reviewed by the EPA.

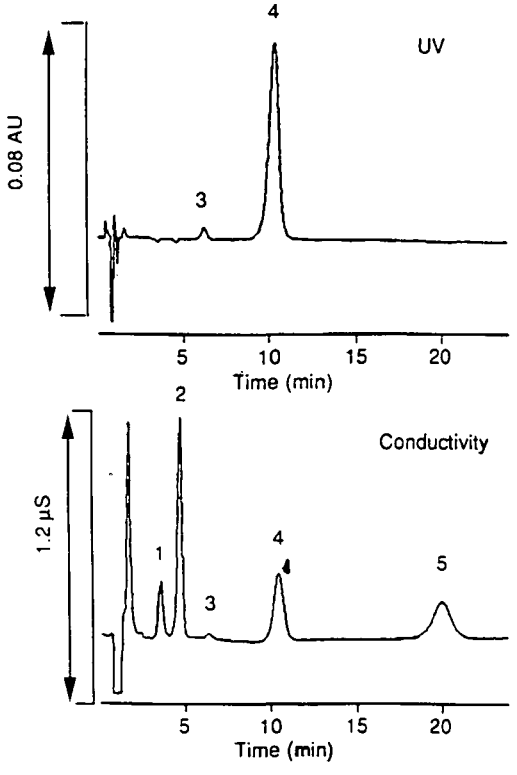


Fig. 4. Analysis of nitrite and nitrate in wastewater by Wates test method B-1012. Conditions, column: Waters IC-Pak Anion HC, eluent: modified borate–gluconate, flow-rate: 2.0 ml/min, detection: upper chromatogram; UV at 214 nm, lower chromatogram; conductivity. Peaks: 1 = hydrogencarbonate; 2 = chloride; 3 = nitrite (0.27 ppm); 4 = nitrate (8.95 ppm); 5 = sulfate.

*EPA Method 218.6*

The EPA has established regulations and methodology for hexavalent chromium Cr(VI) due to its adverse health effects even at trace levels. Atomic absorption; furnace technique and inductively coupled plasma are EPA approved methods for the determination of chromium in drinking water [2]. However, these methods are only marginally sensitive and selective when used for the analysis of complex matrices such as industrial waste water.

A joint EPA–ASTM collaborative study of EPA method 218.6 [9], the analysis of hexavalent chromium in reagent, drinking, and waste water using IC with post-column derivatization and UV–VIS detection at 530 nm, was completed in December, 1990. The ASTM subcommittee D19.05 on inorganics in water approved the data in June, 1991. Fig. 5 is an example of a 10-µg/l hexavalent chromate standard under the test method conditions: a Waters IC-Pak Anion HC column, ammonium sulfate–ammonium hydroxide eluent, post-column reagent diphenylcarbohydrazide–methanol–sulfuric acid, and a UV–VIS detector. The advantages of

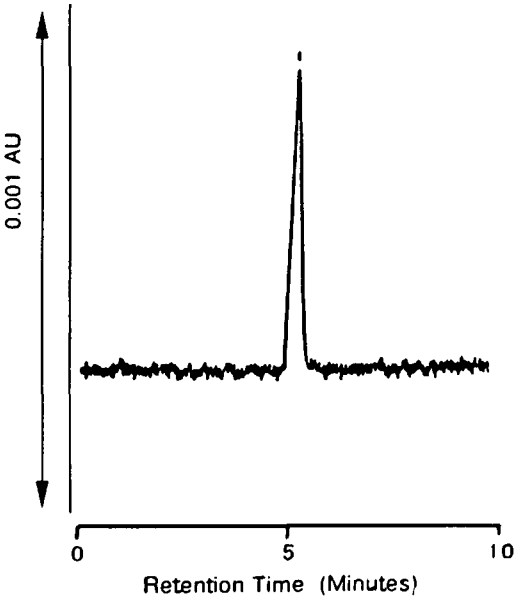


Fig. 5. Separation of a chromate standard using EPA method 218.6. Conditions, column: Waters IC-Pak Anion HC, eluent: 25 m*M* ammonium sulfate–10 m*M* ammonium hydroxide, flow-rate: 1.5 ml/min, post-column reagent: diphenylcarbohydrazide–methanol–sulfuric acid, detection: UV at 530 nm. Peak: 1 = hexavalent chromate (10 µg/l).

TABLE I

CHROMATE ANALYSIS USING POST-COLUMN DERIVATIZATION AND UV

All data expressed as μg/l $Cr^{6+}$. TV = true value, PCD = post-column derivatization.

| Sample | TV | PCD at 530 nm | UV at 365 nm |
|---|---|---|---|
| *Reagent water* | | | |
| 1 | 8.0 | 10.4 | 10.1 |
| 2 | 20.0 | 21.5 | 22.5 |
| 3 | 40.0 | 41.8 | 40.7 |
| 4 | 100 | 104 | 100 |
| 5 | 800 | 782 | 787 |
| *Wastewater* | | | |
| 6 | 20.0 | 21.8 | 18.6 |
| 7 | 100 | 99.9 | 107 |
| 8 | 140 | 148 | 151 |
| 9 | 800 | 798 | 800 |
| 10 | 960 | 906 | 960 |

this method include better sensitivity and no interferences. Although post column derivatization with UV–VIS detection at 530 nm is stipulated in the method, direct UV detection at 365 nm can also be employed. Table I shows data taken from Waters contribution to the collaborative study of the EPA method 218.6 for chromate. A comparison of the true values of chromate for ten samples to values obtained from both detection techniques shows a good correlation of the results. This suggests that direct UV detection at 365 nm can be simpler alternative to post-column derivatization for non-compliance monitoring of hexavalent chromium.

*Test methods for disinfection by-products*

The EPA is developing regulations for various disinfection by-products (DBPs) in drinking water. Because of its sensitivity and precision, IC is a good choice for analyzing the by-products of chlorine dioxide and ozone oxidation (*i.e.*, chlorite, chlorate and bromate) [10].

EPA method 300.0 contains a "Method B" for oxyhalides [4]. However, since these analytes are not regulated by the EPA any method can be used. Waters test method for Oxyhalides, A-119 [11] is described in Fig. 6 as an extension of EPA Method A-1000. This method incorporates a Wates IC-Pak

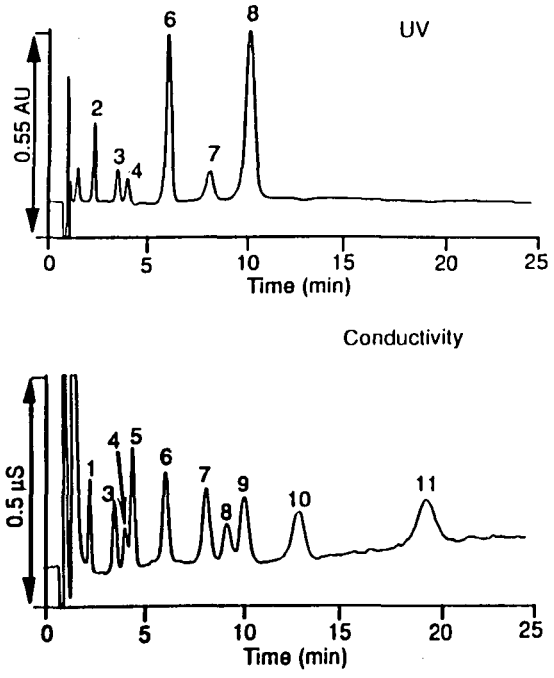


Fig. 6. Separation of a standard anion mixture using Waters test method for oxyhalides. Conditions, column: Waters IC-Pak Anion HC, eluent: borate–gluconate, flow-rate: 2.0 ml/min, detection: upper chromatogram; UV at 214 nm, lower chromatogram; conductivity. Peaks: 1 = fluoride (1 ppm); 2 = iodate (4 ppm); 3 = chlorite (4 ppm); 4 = bromate (4 ppm); 5 = chloride (1 ppm); 6 = nitrite (2 ppm); 7 = bromide (4 ppm); 8 = chlorate (4 ppm); 9 = nitrate (4 ppm); 10 = phosphate (6 ppm); 11 = sulfate (4 ppm).

Anion HC column, borate–gluconate eluent, and a UV detector followed by a conductivity detector. The two chromatograms are the result of a simultaneous detection of an eleven-anion standard mixture in water. UV detection allows one to take advantage of UV absorption properties of iodate, chlorite, bromate, nitrite, bromide, and nitrate. In the chromatogram obtained with conductivity detection, iodate coelutes with fluoride, bromate and chloride are partially separated, and it is difficult to resolve nitrate from chlorate. Thus iodate, bromate, nitrite, and nitrate are best quantitated by UV, while the other anions could best be quantitated by conductivity.

*Test method for inorganic and organic halides using capillary ion electrophoresis*

Capillary ion electrophoresis (Waters' trade

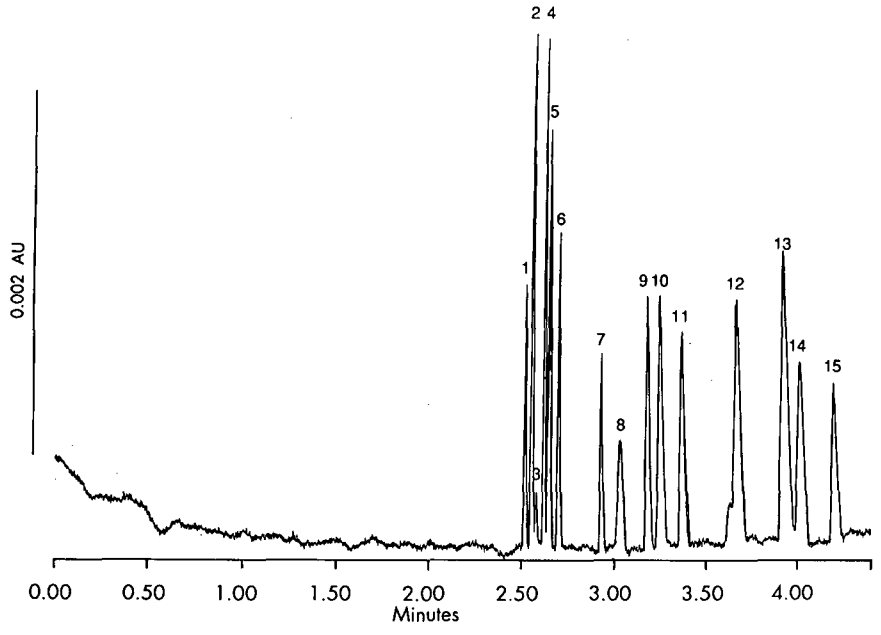


Fig. 7. Electropherogram of a standard anion mixture using a proposed Waters test method for the analysis of anions in water by capillary ion electrophoresis. Conditions, capillary: Waters AccuSep 60 cm × 75 μm I.D. fused silica, electrolyte: 5 m*M* chromate with 0.3 m*M* CIA-Pak OFM anion-BT (patent pending) at pH 8, potential: 20 kV at 18 μA (negative), detection: 254 nm indirect, injection: hydrostatic (10 cm for 30 s). Peaks: 1 = bromide (4 ppm); 2 = chloride (2 ppm); 3 = iodide (4 ppm); 4 = sulfate (4 ppm); 5 = nitrite (4 ppm); 6 = nitrate (4 ppm); 7 = chlorate (4 ppm); 8 = perchlorate (4 ppm); 9 = fluoride (1 ppm); 10 = phosphate (4 ppm); 11 = chlorite (4 ppm); 12 = carbonate (4 ppm); 13 = acetate (5 ppm); 14 = monochloroacetate (5 ppm); 15 = dichloroacetate (5 ppm) (Courtesy of William R. Jones, Millipore Waters Chromatography).

name: Capillary Ion Analysis, CIA) is a branch of Capillary electrophoresis optimized for the rapid analysis of low-molecular-weight anions and cations that separates ions according to their mobility in electrolytic solutions [12,13]. Capillary ion electrophoresis is a powerful separation technique that offers rapid, highly efficient separations with different selectivities (compared to IC) obtained from nanoliters of sample volume [14]. Fig. 7 illustrates a proposed Waters Test Method for the analysis of anions in water by CIA. This method utilizes a Waters Quanta 4000, Waters AccuSep capillary, chromate/CIA-Pak OFM anion BT electrolyte, and indirect UV detection. The electropherogram demonstrates the ability of capillary ion electrophoresis to analyze primary and secondary contaminants as well as other anions of environmental concern, in less than 5 min. It would require four different methods to analyze these components by IC at considerably longer run times.

## CONCLUSIONS

EPA methods for primary and secondary contaminants using ion chromatography offer several advantages. The ability to chromatographically separate various anion species from interferences before detection. Detector versatility enables one to mask interferences/coeluting peaks and increase sensitivity. Sample preparation typically involves just a dilution and filtration step prior to injection into the IC.

The capillary ion electrophoresis method will be submitted for ASTM–EPA consideration. Capillary ion electrophoresis offers a significant improvement over IC in efficiency and analysis time. The unique selectivity provides an alternative solution to coelution problems that occur with IC.

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CHROMSYMP. 2566

# Determination of reaction by-products in high-molecular-weight polysulfonated scale inhibitors by ion chromatography

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ABSTRACT

In the inhibition of oilfield barium sulfate mineral scale, it is counterproductive to increase the concentration of scaling anions in the system. Sulfate and other potentially scaling ions are produced during the sulfonation of organic polymers. Upon injection into a high-barium, oil-producing formation, these ions tend to form large quantities of sparingly soluble salts. Such precipitates damage oil-producing zones and reduce oil productivity. It is therefore necessary to determine the concentrations of these anions prior to application.

An ion chromatographic technique has been developed for the simultaneous determination of hydroxyethylsulfonate, sodium vinylsulfonate, chloride and sulfate reaction by-products. This technique can be used to analyze monomer, polymer and brine solutions. Linearity ranges were determined for these ions.

## INTRODUCTION

In the production of oil and gas from a porous rock matrix, deposition of mineral scales either within the rock matrix or oil well pipework can reduce oil flow to a trickle. Common mineral scales are barium sulfate, calcium carbonate and calcium sulfate. Changes in pressure or temperature or the mixing of different geologically stable, but incompatible brines generally results in some scale precipitation [1–3].

When millions of gallons of oil and natural pore water are removed from a reservoir each day, the overall reservoir pressure soon declines. Lower reservoir pressure means slower flow from oil wells and decreased production. Thus, water is often injected into the reservoir to replace oil and maintain pressure. The most abundant water source is sea water that contains close to 3000 mg $l^{-1}$ of sulfate. After a short period of time, the sulfate-rich sea water has migrated from the point of injection to a producing well. In the turbulent high-flow region close to the producing well, the injected water mixes with the natural pore water, which contains calcium and/or barium ions (up to 5000 mg $l^{-1}$). The resultant precipitate of barium or calcium sulfate will severely damage an oil well.

Scale inhibitors are organic molecules which retard the kinetics of precipitate growth. They do not sequester or chelate very well but sterically interfere with active crystal growth sites. Scale inhibitors are injected into a producing oil well (not an injection well) to adsorb onto the porous matrix. When the oil well is allowed to flow back, scale inhibitor is produced along with oil, natural pore water and the incompatible injected water. It is thus, obviously, counterproductive to inject a scale inhibitor which contains ions incompatible with brines known to be present in the reservoir. Inhibitors containing high levels of phosphate, sulfate or carbonate are likely to form precipitates when pumped into a wellbore rich in calcium or barium ions. The damage can easily cost millions of dollars a day in lost oil production.

It is therefore critical to establish safe levels of the aggressive anion prior to using any scale inhibitor

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and to reject batches which may damage an oil reservoir. Using gradient ion chromatography (IC), a procedure was developed using a three-eluent system with a two-step gradient program. This procedure simultaneously separates hydroxyethylsulfonate (HES), sodium vinylsulfonate (SVS), chloride and sulfate reaction by-products. A methanol extraction process [4] is one of the purification processes used to clean up scale inhibitor prior to field use. The IC procedure was used to evaluate the effectiveness of this process.

## EXPERIMENTAL

### *Instrumentation*

The method presented was performed on a Dionex 4500i ion chromatograph equipped with a gradient pump [plumbed with polyether ether ketone (PEEK) tubing and fittings], conductivity detector (CDM-2), eluent degas module (EDM-2), autosampler and AI-450 software. The columns used were: (1) anion trap column (24 × 9 mm, high-capacity anion-exchange resin in the hydroxide form); (2) Dionex OmniPac PAX-100 [5] column (250 mm × 4 mm, an alkanol quarternary amine anion-exchange resin); and (3) Dionex OminiPac PAX-100 guard (50 × 4 mm).

The columns were used with a Dionex Anion MicroMembrane Suppressor (AMMS-II) operating via a Dionex AutoRegen System.

### *Reagents*

Inorganic standards were prepared from Johnson Matthey/Aesar ultra dry, 99.99% sodium chloride and Puratonic grade of sodium sulfate (99.999%). HES was prepared from Aldrich isethionic acid, sodium salt, 98% that had been freeze dried. SVS was prepared from vinylsulfonate monomer, sodium salt (30% in water) manufactured by Hoechst, Frankfurt, Germany. Eluents were prepared from Baker 50% sodium hydroxide, Burdick & Jackson methanol (HPLC grade), Burdick & Jackson acetonitrile (HPLC grade) and distilled water passed through a Millipore Milli-Q system. All eluents were degassed with helium and kept under a blanket of helium at all times.

Stock solutions of approximately 1000 μg/g were prepared for each of the four components. A set of mixed standards of chloride and sulfate was prepared. Individual standards for HES were prepared fresh as they may contain small amounts of impurities and the composition changes with time. The individual standard for SVS must also be prepared fresh as it may polymerize over time.

### *Sample preparation*

The twelve samples analyzed in this study were high-molecular-weight polysulfonated scale inhibitors (polymeric sulfonates, PSs). These were obtained from three separate sources. Additionally, the procedure can be utilized for the analyses of monomer and brine samples. By-products/contaminants of interest were sulfate, chloride, HES and SVS. Carbonate and phosphate ions were not involved in this chemistry. The samples were diluted on a weight/weight basis using water, to allow each component to be within the calibrated range. Where possible, an appropriate dilution was chosen to obtain data for all four components in a single run.

### *Procedure*

The gradient IC conditions are listed in Table I.

## RESULTS AND DISCUSSION

Calibration graphs for the four components of interest were performed using five standards, ranging from about 1 to 500 μg/g. The graph was ex-

TABLE I

GRADIENT IC CONDITIONS

Columns: anion trap column, OmniPac PAX-100 and PAX-100 guard; suppressor: anion micromembrane suppressor-II (AMMS-II); autoregen: 50 m*M* sulfuric acid, flow-rate: 11.0–11.5 ml/min; injection: 5 μl; detection: conductivity, range: 0–1000 μS.
Eluent 1: 0.7 m*M* sodium hydroxide in water–methanol (92.5:7.5).
Eluent 2: 150 m*M* sodium hydroxide in water–methanol (95:5).
Eluent 3: acetonitrile–water (90:10).

Gradient program

| Time (min) | Flow-rate (ml/min) | Eluent 1–eluent 2–eluent 3 | Comment |
|---|---|---|---|
| 0.0 | 1.0 | 100:0:0 | Equilibrate |
| 5.1 | 1.0 | 100:0:0 | Load |
| 6.0 | 1.0 | 100:0:0 | Inject |
| 23.0 | 1.0 | 50:20:30 | Ramp 1 |
| 30.0 | 1.0 | 20:40:40 | Ramp 2 |

TABLE II

CALIBRATION DATA

| No. | HES | | SVS | | $Cl^-$ | | $SO_4^{2-}$ | |
|---|---|---|---|---|---|---|---|---|
| | μg/g | Peak Area | μg/g | Peak Area | μg/g | Peak Area | μg/g | Peak Area |
| 1 | 0.000 | 0.00000 | 0.000 | 0.00000 | 0.000 | 0.00000 | 0.000 | 0.00000 |
| 2 | 0.970 | $1.03400 \cdot 10^6$ | 1.057 | $1.08700 \cdot 10^6$ | 0.947 | $6.13480 \cdot 10^6$ | 0.992 | $4.15500 \cdot 10^6$ |
| 3 | 9.972 | $1.43064 \cdot 10^7$ | 10.613 | $1.68784 \cdot 10^7$ | 10.056 | $7.60814 \cdot 10^7$ | 10.047 | $4.41524 \cdot 10^7$ |
| 4 | 50.129 | $7.80384 \cdot 10^7$ | 75.806 | $1.37095 \cdot 10^8$ | 48.603 | $4.17717 \cdot 10^8$ | 48.245 | $2.38115 \cdot 10^8$ |
| 5 | 96.794 | $1.61984 \cdot 10^8$ | 128.480 | $2.38665 \cdot 10^8$ | 100.210 | $8.81126 \cdot 10^8$ | 100.070 | $5.20342 \cdot 10^8$ |
| 6 | 479.360 | $8.95821 \cdot 10^8$ | 570.240 | $1.16871 \cdot 10^9$ | 500.570 | $4.66363 \cdot 10^9$ | 502.430 | $2.80486 \cdot 10^9$ |

Correlation coefficients: HES $R^2 = 0.999$, SVS $R^2 = 0.999$, chloride $R^2 = 1.000$, sulfate $R^2 = 1.000$

tended through the origin although the chromatogram was not run. The calibration data in Table II illustrate good linearity for all four components of interst (correlation coefficients of 0.999 to 1.000).

Upon analyses, PSs from two of the three sources were nearly identical in by-product contamination. Typical chromatograms for PS samples are found in Fig. 1. The PS from one source contained HES, chloride and sulfate but not SVS. It also contained several unidentified peaks, possibly other by-products. The peak at 4.8 min is removed by the addition of barium chloride. The PSs from the two other sources contained all four contaminants and also some unidentified peaks. These samples also contained a peak at 18.9–19.2 min. This peak is suspected to be ethionic acid, which is formed from the hydrolysis of carbyl sulfate, a monomer precursor [6–10]. Both structures are shown in Fig. 2. In common with sulfate, the compound is precipitated with barium and is retained in the non-polymer phase during various organic liquid–liquid extrac-

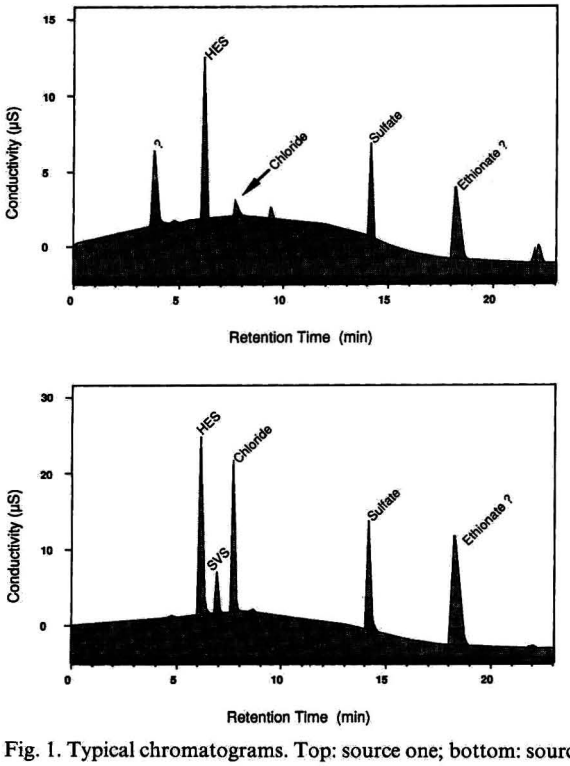


Fig. 1. Typical chromatograms. Top: source one; bottom: source two.

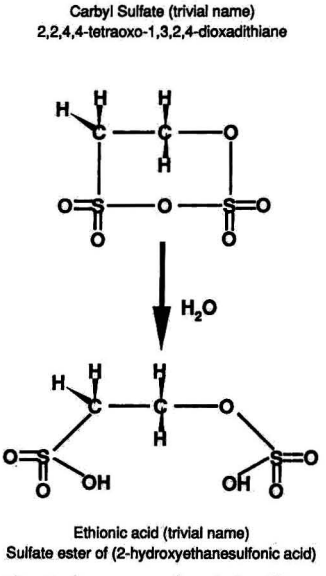


Fig. 2. Structure of carbyl sulfate and its hydrolysis product.

TABLE III
COMPARISON OF SULFATE BY SEC AND IC

| Sample | Sulfate (%) | |
|---|---|---|
| | SEC | IC |
| 1 | 3.31 | 0.60 |
| 2 | 0.65 | 0.28 |
| 3 | 0.48 | 0.29 |
| 4 | 2.45 | 0.55 |
| 5 | 1.95 | 0.66 |
| 6 | 1.35 | 0.31 |
| 7 | 1.59 | 0.47 |
| 8 | 1.77 | 0.29 |
| 9 | 1.87 | 1.48 |
| 10 | 1.71 | 0.35 |
| 11 | 2.97 | 0.56 |
| 12 | 3.00 | 0.67 |

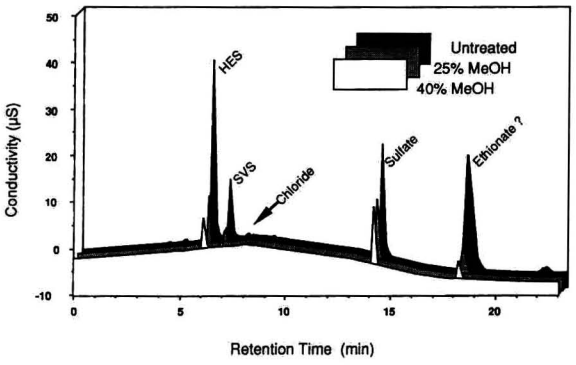


Fig. 3. Effectiveness of methanol extraction procedure.

tions. This compound was co-eluted with sulfate by size-exclusion chromatography (SEC). Comparison of the sulfate determination by SEC *versus* IC is shown in Table III. Because of the coelution problem during SEC separation, there is no correlation between sulfate by SEC and sulfate by IC.

The effectiveness of the methanol liquid–liquid extraction [4] was evaluated by using IC to analyze the products of a 25% and a 40% methanol extraction in addition to the untreated product. Comparison chromatograms are shown in Fig. 3. Both of the extractions partially remove sulfate, chloride, HES and SVS. The 25% and 40% extractions removed similar amounts of sulfate. However, the 40% methanol extraction removed larger proportions of all remaining contaminants. Both extractions removed some of the suspected ethionic acid, again confirming its chemical similarity to sulfate.

The precision of the method was investigated from the sample preparation to the final calculation. The polymer studied contained all four contaminants plus the suspected ethionic acid. A 200-fold dilution was chosen in order to obtain data for five contaminants in a single run. These higher dilutions significantly extend the life of the columns. These data are presented in Table IV. This method shows remarkably good precision for such large dilutions of a high-molecular-weight sulfonate. Ethionic acid

TABLE IV

PRECISION STUDY RESULTS

All replicates were diluted 1:200 by weight.

| Replicate | [HES] (µg/g) | [SVS] (µg/g) | [$Cl^-$] (µg/g) | [$SO_4^{2-}$] (µg/g) | Ethionate? (area) |
|---|---|---|---|---|---|
| 1 | 27 845 | 2590 | 5289 | 6323 | 42 534 |
| 2 | 28 109 | 2545 | 5328 | 6381 | 42 974 |
| 3 | 28 180 | 2567 | 5362 | 6364 | 42 852 |
| 4 | 28 259 | 2577 | 5383 | 6399 | 43 313 |
| 5 | 28 182 | 2543 | 5324 | 6372 | 43 231 |
| 6 | 28 219 | 2528 | 5309 | 6338 | 43 053 |
| 7 | 28 315 | 2636 | 5377 | 6382 | 43 475 |
| 8 | 28 455 | 2669 | 5363 | 6402 | 43 356 |
| 9 | 28 292 | 2651 | 5355 | 6407 | 43 266 |
| 10 | 28 061 | 2659 | 5388 | 6406 | 43 265 |
| Mean + S.D. | 28 192 ± 156 | 2596 ± 50 | 5348 ± 32 | 6377 ± 27 | 43 132 ± 267 |
| Relative S.D. (%) | 0.6 | 1.9 | 0.6 | 0.4 | 0.6 |

is not readily available; therefore, the precision was determined by comparative peak area.

## CONCLUSIONS

Gradient IC simultaneously detected HES, SVS, chloride and sulfate in PS samples, monomer and brine samples. Because of excellent linearity to about 500 $\mu$g/g, concentrations of all four components were determined with good precision. This procedure demonstrates the effectiveness of methanol extraction in removing unwanted contaminants.

Currently, sulfate contamination cannot be determined by any of the traditional methods usually employed. Sulfate by SEC cannot be correlated with the IC data and, thus, cannot be used in the evaluation of products. IC remains the only viable method in this matrix. PS is currently being squeezed without formation damage in the world's most severe scaling system in the North Sea. The polymer may be appropriate for use on three similar fields.

## ACKNOWLEDGEMENTS

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CHROMSYMP. 2565

# Applications of capillary ion electrophoresis in the pulp and paper industry

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ABSTRACT

Alkaline (kraft) pulping of wood accounts for more than 60% of paper production. Anions critical to the performance of the kraft pulping process are presently determined by wet chemical methods and more recently by ion chromatography. Chemical recovery and liquor regeneration efficiencies are evaluated by monitoring these ions: $Cl^-$, $S_2O_3^{2-}$, $SO_3^{2-}$, $SO_4^{2-}$, $S^{2-}$, $OH^-$, and $CO_3^{2-}$, $Na^+$ and $K^+$.

Capillary ion electrophoresis is a novel analytical technique that is optimized for the rapid monitoring of charged species. The technique is applied for the first time on kraft process streams using indirect UV detection at 254 nm and 214 nm to monitor the charged species. The results are used to evaluate important process variables such as sulfidity (white liquor), reduction efficiency (recovery furnace performance), and causticization efficiency (slaker performance).

This paper presents results obtained by off-line monitoring of ions found in the white, black and green liquors from kraft mills. The potential of capillary ion electrophoresis as a cost effective technique for rapid process processing of kraft pulping liquors is suggested.

## INTRODUCTION

Chemical pulping of wood to obtain cellulose for the papermaking industry is a mature technology [1]. It consists of mixing inorganic chemicals with wood chips to separate lignin from the wood fibers. In the kraft pulping process lignin is removed by cooking wood chips in a aqueous solution of NaOH and $Na_2S$ (white liquor). This white liquor contains smaller amounts of $Na_2CO_3$, $Na_2SO_4$, $Na_2S_2O_3$ and $Na_2SO_3$. The spent liquor (black liquor) containing organic and inorganic anions is concentrated and burned in a Tomlinson recovery furnace to obtain a smelt of $Na_2CO_3$ and $Na_2S$. The molten sodium salts are dissolved to form the green liquor which is reacted with $Ca(OH)_2$ to regenerate the white liquor. Fig. 1 illustrates the unit operations and chemical recovery cycle used in existing kraft mills.

In addition to monitoring the temperature/time profile of chip digestion, careful control of liquor composition is critical to all phases of the operation. The composition of the liquors obtained from the combustion of the black liquor and regeneration of the white liquor will reveal whether targeted performance schedules have been met. To a great extent, it is the performance of the recovery operation which determines the quality of the product and the favorable economics of the kraft process.

Chemical components critical to the quality of pulping and make up of the liquors are the anions: hydroxide, sulfide, carbonate, sulfate, chloride, thiosulfate, sulfite. A significant portion of mill time is spent monitoring the concentrations of these anions to determine process performance. Analyses for these anions are performed off-line by various gravimetric and titrimetric procedures. Often on-line titrators and conductivity techniques are used to monitor the alkalinity and specific conductance respectively of the liquors.

Innovations in ion chromatography [2,3] and its

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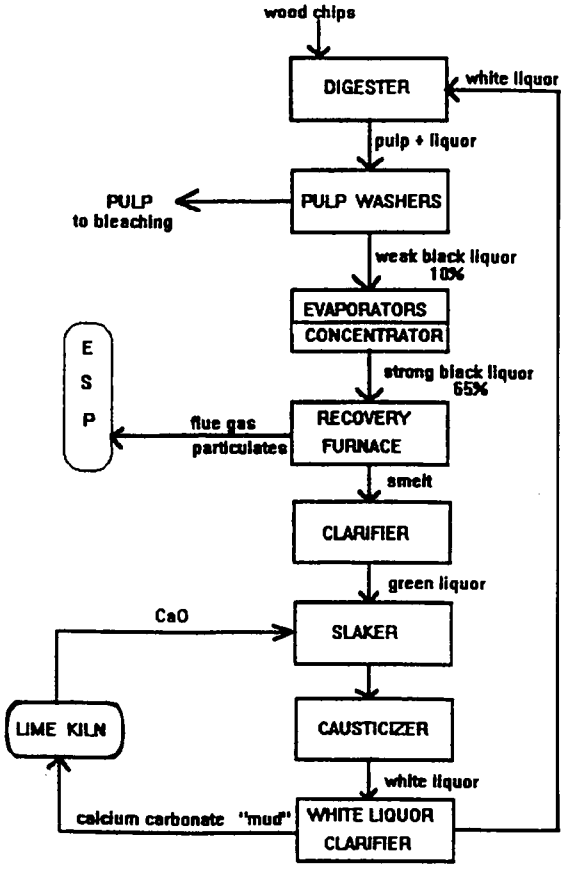


Fig. 1. Schematic illustrating the kraft process and the chemical recovery loop for the liquors. The cycle begins with the combustion of the black liquor in the recovery furnace. Recovered chemicals are converted to the green liquor. White liquor is regenerated in the causticizer and returned to the digester. ESP = Electrostatic precipitation.

application to process liquors [4–6] were significant in that analyses were simplified and ion specific. However, separation of all anions cannot be performed with one method. For example, the organic and inorganic anions present in the black liquor cannot be separated in one run. Furthermore, the determination of the cations in the liquors requires a different column and instrument stabilization before analysis.

Electrophoresis has developed into a powerful analytical tool for macromolecules [7,8]. In particular, capillary zone electrophoresis (CZE) with on-column detection is recognized as an important separation methodology for low molecular weight species. State-of-the art reviews on CZE have been published [9,10]. The application of CZE to the analyses of small inorganic ions is exceptional because of selectivity, versatility, and the short time (minutes) required for development of a method [11].

Simplicity, speed, low operating costs, small sample size and ease of automation are some of the instrumental attributes to be considered for any analytical technology that has the potential for process monitoring. Capillary ion electrophoresis fulfils some of these criteria. Real time analyses for process monitoring and control at kraft plants should reduce operating costs, lower environmental emissions, and increase throughput.

This paper discusses results obtained by application of capillary ion electrophoresis to the analyses of kraft process liquors. It will be shown that CZE can monitor these complex process streams with little sample manipulation and with high speed compared with existing analytical methodologies.

## EXPERIMENTAL

### *Instrumentation*

The capillary electrophoresis system was a Quanta 4000 (Waters, Milford, MA, USA) equipped with a negative power supply for separation of anions. The applied voltage was 20 kV. For cations a positive power supply was used. Fused-silica capillaries of 60 cm × 75 μm I.D. and 60 cm length were obtained from Waters (AccuSep capillaries). The external capillary temperature was at 23°C. The detector window was formed by burning off a 10-mm section of the outer polyimide coating. Indirect UV detection was performed with a Hg lamp and a 254-nm optical fitler. For determination of sulfide a zinc lamp with a 214 nm optical filter was used. Sample introduction into the capillary was by a 30-s hydrostatic injection from a height of 10 cm.

Electropherograms were recorded and processed with a Waters 860 Data Station and Waters SIM Interface. Data processing was performed with Waters Maxima 820 data station.

### *Reagents*

Purified water (18 mΩ, Milli-Q; Millipore, Bedford, MA, USA) was used for all electrolytes, standards, and sample preparation. Containers for all

preparations were made of polyethylene. The anion standards were prepared by diluting 1000-ppm stock solutions containing a single anion.

The chromate electrolyte was prepared from a concentrate containing 100 m*M* $Na_2CrO_4$ (Mallinckrodt analytical-reagent grade) and 0.069 m*M* $H_2SO_4$ (J. T. Baker, Ultrex grade). Electroosmotic flow (EOF) modifier for the reversal of the direction of EOF was obtained as a 20 m*M* concentrate from Waters (Nice-Pak OFM Anion-BT). The carrier electrolyte was prepared from 5 m*M* chromate and 0.5 m*M* electroosmotic flow modifier. The pH of the electrolyte was adjusted with 100 m*M* NaOH. Liquor samples were obtained from the respective process streams and were diluted 1:1000 with water and injected immediately. The $Na_2S_9 \cdot H_2O$ was obtained from Anachemia and used for making fresh sulfide solutions.

## RESULTS AND DISCUSSION

### *Chemical recovery*

As Fig. 1 illustrates wood chips are cooked in a digester with an aqueous solution of NaOH and $Na_2S$ (white liquor) to remove the lignin. After bulk delignification the cellulose fibers are separated from the spent liquor (black liquor) and washed. The raw pulp is fed to the bleaching plant to complete the removal of lignin and to obtain a high quality pulp.

The chemical recovery cycle begins by concentrating the black liquor to about 70% solids. This concentrated black liquor is combusted in the recovery furnace to reclaim $Na_2CO_3$, $Na_2S$ and to generate process steam. The smelt ($Na_2CO_3$ + $Na_2S$) is dissolved to form the green liquor. This liquor is reacted with $Ca(OH)_2$ to regenerate the white liquor (NaOH + $Na_2S$) which is returned to the digester. The precipitated lime mud ($CaCO_3$) is fed to the lime kiln to recover CaO which is used for the next cycle of liquor regeneration.

### *Kraft liquor anion analysis at 254 nm*

An eleven-anion standard consisting of inorganic and organic ions is shown in Fig. 2. The concentration of each anion is 10 ppm. The pH of the standard sample was 10.6.

Fig. 3. shows the electrophoretic separation of a strong black liquor sample obtained in less than 8 min. The inorganic anions are separated in less than 5 min. The sodium salts of the organic acids are the degradation products from the lignin and elute after the inorganics. Traditional wet chemical methods require several hours to perform the same analysis for the inorganic anions. Procedures for the analyses of liquors by suppressed ion chromatography (IC) have been developed [5,6,12]. The analysis by IC require two different methods in order to obtain similar results. CZE requires one method and therefore can deliver the analysis of this liquor in less time than IC.

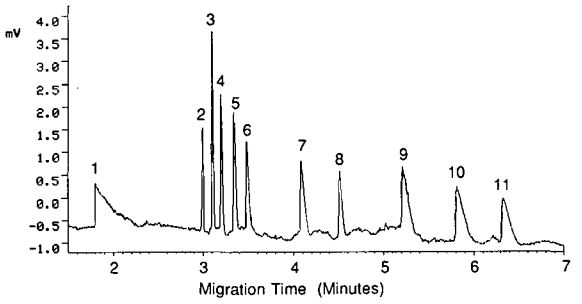


Fig. 2. Electropherogram of eleven-anion standard at 254 nm. Peaks: 1 = hydroxide; 2 = thiosulfate; 3 = chloride; 4 = sulfate; 5 = oxalate; 6 = sulfite; 7 = formate; 8 = carbonate; 9 = acetate; 10 = propionate; 11 = butyrate.

Fig. 4 shows the electropherogram for a white liquor sample obtained by indirect UV detection. The presence of thiosulfate, sulfate and carbonate in the liquor are the "dead load" of the operation

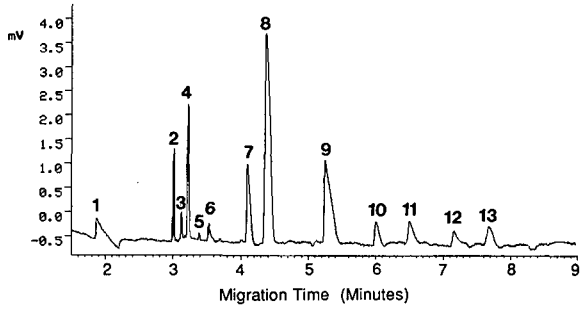


Fig. 3. Electropherogram of kraft strong black liquor. Conditons: Fused-silica 60 cm × 75 $\mu$m capillary; voltage 20 kV (negative); 5 m*M* chromate electrolyte with Nice-Pak OFM Anion BT at pH 10.6; injection: hydrostatic for 30 s at 10 cm, indirect UV detection at 254 nm. The sample was diluted 1:1000 with water. Peaks: 1 = hydroxide; 2 = thiosulfate; 3 = chloride; 4 = sulfate; 5 = oxalate; 6 = sulfite; 7 = formate; 8 = carbonate; 9–13 = sodium salts of organic acids.

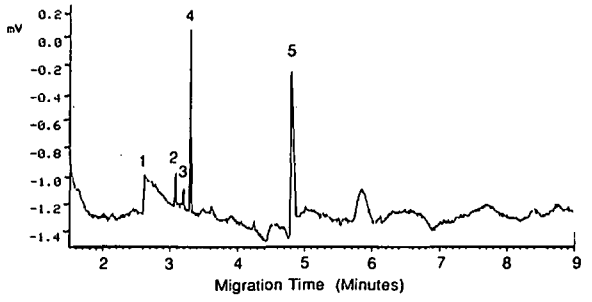


Fig. 4. Electropherogram showing the separation and indirect detection at 254 nm of a white liquor. The sample was diluted 1:10 000 with water; other conditions as in Fig. 2. Peaks: 1 = hydroxide; 2 = thiosulfate; 3 = chloride; 4 = sulfate; 5 = carbonate.

since these ions do not contribute to lignin removal. The amount of each ion correlates with the performance of the recovery furnace and slaker operations. A high thiosulfate and sulfate indicates poor reduction efficiency in the recovery furnace. A high carbonate content of the liquor following causticization operation indicates poor slaker performance.

Fig. 5 and 6 show electropherograms for samples from the digester at 45 and 75 min respectively. The increase of the organic acids (sodium salts) is evidence for lignin degradation. Analysis of this liquor can show the loss in sulfidity and alkalinity during the cooking of the wood chips.

*Kraft liquor cation analysis at 214 nm*

An electropherogram of the cations found in the black liquor is presented in Fig. 7. As expected sodium is the principal cation found in all liquors

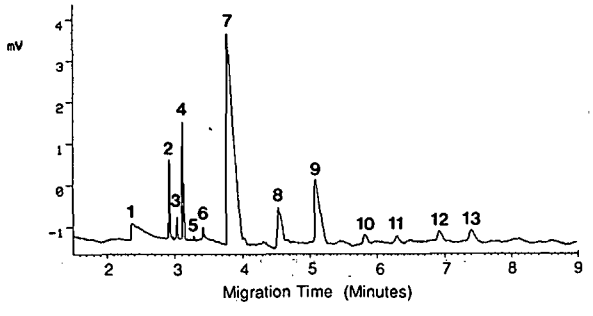


Fig. 5. Electropherogram of digester liquor after 45 min of pulping. Conditions as in Fig. 2: Peaks: 1 = hydroxide; 2 = thiosulfate; 3 = chloride; 4 = sulfate; 5 = oxalate; 6 = sulfite; 7 = formate; 8 = carbonate; 9–13 = sodium salts of organic acids.

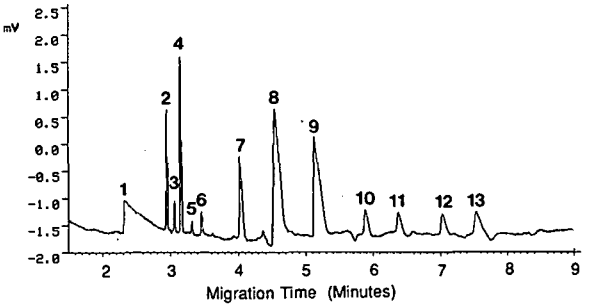


Fig. 6. Electropherogram of digester liquor after 75 min of pulping. Peaks as in Fig. 5.

(about 20%). Sodium is measured in order to assess where in the process there is significant soda loss. The potassium ion (less than 1%) is from the wood. Its accumulation in the liquor affect the combustion characteristics of the black-liquor fuel going to the recovery furnace.

*Kraft liquor anion analysis at 214 nm*

A spectrum comparing the absorbance of the chromate electrolyte with sodium sulfide at pH 11 is shown in Fig. 8. At 214 nm the hydrosulfide ion absorbs more strongly than the electrolyte at 214 nm. At 254 nm the absorptions are reversed with the result that the residual absorption of the hydrosulfide ion make it less sensitive to indirect photometric detection.

A six-anion standard is shown in Fig. 9. The hydrosulfide and thiosulfate ions are measured directly at this wavelength while the other ions are detected indirectly and with opposite polarity.

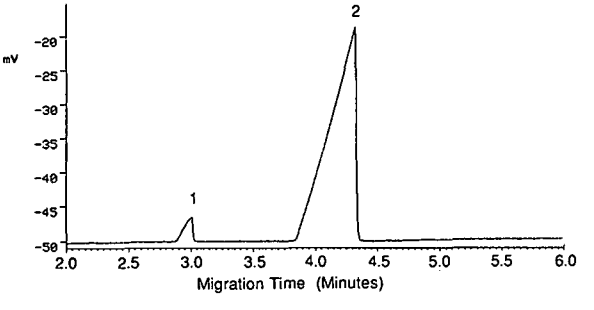


Fig. 7. Electropherogram for cations in black liquor. Carrier electrolyte 5 m*M* Waters UV Cat 1, 6.5 m*M* hydroxyisobutyric acid, pH 4.4, hydrostatic injection 30 at 10 cm, indirect UV detection at 214 nm. Peaks: 1 = potassium; 2 = sodium. Sample dilution was 1:1000 with water.

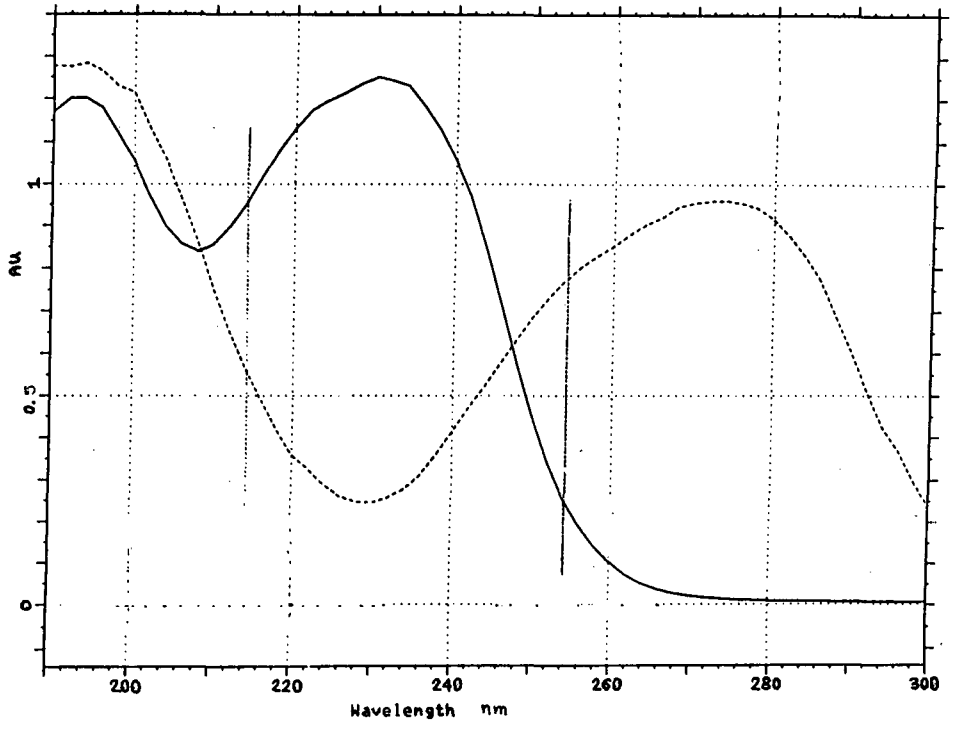


Fig. 8. Spectral overlay of chromate electrolyte (-----) and sodium sulfide (———) at pH 11.

Analysis for carbonate, sulfide and sulfate ions is difficult to accomplish in one run by IC. These ions are critical for monitoring the performance of the recovery furnace and regneration of the white liquor. Fig. 10 shows the electropherogram for a green liquor sample. The significance of this analysis is that the reduction efficiency of the recovery furnace can be monitored. Information on the reduction efficiency allows adjustment to be made to the recovery furnace either via black liquor feed or

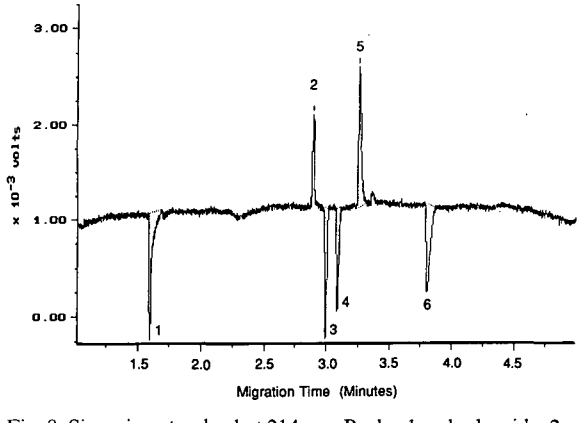


Fig. 9. Six-anion standard at 214 nm. Peaks: 1 = hydroxide; 2 = thiosulfate; 3 = chloride; 4 = sulfate; 5 = hydrosulfide; 6 = carbonate. Thiosulfate and hydrosulfide detected directly at this wavelength.

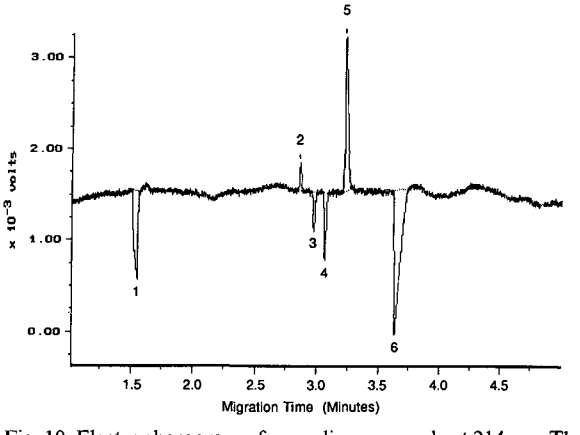


Fig. 10. Electropherogram of green liquor sample at 214 nm. The sample was diluted 1:10 000 with water; other conditions as in Fig. 9. Peaks: 1 = hydroxide; 2 = thiosulfate; 3 = chloride; 4 = sulfate; 5 = hydrosulfide; 6 = carbonate.

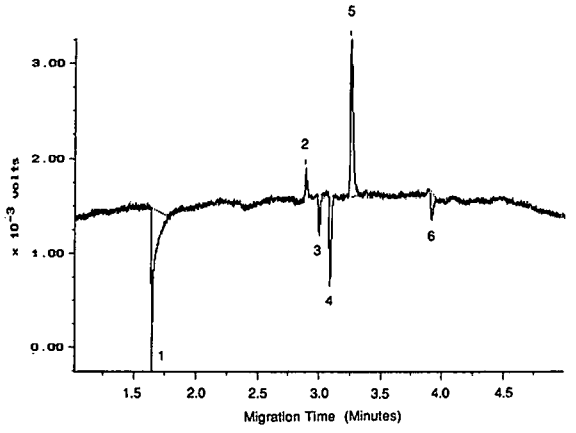


Fig. 11. Electropherogram of white liquor sample using 214 nm detection. Conditions as in Fig. 9. Peaks: 1 = hydroxide; 2 = thiosulfate; 3 = chloride; 4 = sulfate; 5 = hydrosulfide; 6 = carbonate.

air feed to the furnace. Thus, optimization and troubleshooting can be performed on the recovery system at a faster rate than could be performed by IC. Process problems can be addressed effectively and the operation run more evenly, resulting in lower operating costs.

Fig. 11 is from a white liquor sample with a pH for the diluted sample of 11.8. At this pH the principal species is the $HS^-$ ion and therefore the peak in the corresponding electropherogram is identified as this ion [13]. Sulfide has been detected at 215 nm by IC [12].

Table I summarizes the repeatability of the migration time of the anions that were analyzed at pH 10.6 and 11. As expected the migration times decrease with increasing pH. The hydroxide ion has the largest deviation. This is probably due in part to its higher mobility compared to the chromate ion (198 *vs.* 85) making it (OH) more susceptible to local electric field variations. The peak shape asymmetry (tailing) is opposite to that predicted for an analyte with higher mobility than the electrolyte [14].

Mixing of the green liquor with $Ca(OH)_2$ converts $Na_2CO_3$ to NaOH and $CaCO_3$. The recausticization efficiency can be calculated by monitoring the carbonate and hydroxide ions. Table II lists the important ions which make up the various liquors and their significance to the process. The causticization efficiency for this liquor was found to be 81.8% while the plant value determined by titration was 84.5%. For the sulfidity we obtained 33.7% and the plant value was 24.5%. The higher value obtained for the sulfidity is due to the greater uncertainty in

TABLE I

REPEATABILITY OF MIGRATION TIMES FOR SAMPLE ANIONS AT pH 10.6 AND 11

Mean values of three analyses ± relative standard deviation (R.S.D.). Conditions: 5 m*M* chromate electrolyte with 0.5 m*M* electroosmotic flow modifier. Liquor samples were diluted 1:1000 with water.

| Anion | pH 10.6 | | pH 11 | |
|---|---|---|---|---|
| | Migration time (min) | R.S.D. (%) | Migration time (min) | R.S.D. (%) |
| Hydroxide | 2.7 | 4.5 | 1.7 | 6.9 |
| Thiosulfate | 3.0 | 2.8 | 2.9 | 2.0 |
| Chloride | 3.1 | 2.4 | 3.0 | 2.0 |
| Sulfate | 3.2 | 2.2 | 3.1 | 0.56 |
| Carbonate | 4.7 | 2.9 | 3.7 | 3.1 |

TABLE II

PROCESS SIGNIFICANCE OF ANIONS MONITORED AT KRAFT MILLS

The correlation of anions to the kraft process variables such as sulfidity, reduction and causticization efficiencies are given below. Chloride and oxalate are potential corrosion indicators.

| Anions/stream | Black | White | Green | Process significance |
|---|---|---|---|---|
| Hydroxide | | × | × | Caustization performance |
| Sulfide | × | × | × | Liquor quality |
| Carbonate | | × | × | Slaker performance |
| Sulfate | × | | × | Furnace performance |
| Thiosulfate | × | | × | Oxidation |
| Sulfite | × | | × | Oxidation |
| Chloride | × | × | × | Corrosion |
| Oxalate | × | | | Scaling |

*Process variables (chemicals in g/l):*
Reduction (%) = $100 \cdot Na_2S / (Na_2S + Na_2SO_4)$
Sulfidity (%) = $100 \cdot Na_2S / (Na_2S + NaOH)$
Causticization (%) = $100 \cdot NaOH / (NaOH + Na_2CO_3)$

determination of the hydroxide ion by the present CZE methodology. Further work will address the quantitation of the hydroxide anion and its peak shape asymmetry.

## CONCLUSIONS

Table II shows the ions that were monitored by CZE and their significance to the kraft pulping process. The advantages of this analytical technique are speed, simplicity, significant time savings, and minimal consumption of reagents for the analyses. Process diagnostics and troubleshooting can be implemented rapidly. Method development is faster than any currently available analytical technique and at a lower operating cost. All ions can be monitored conveniently at 214 nm.

## ACKNOWLEDGEMENT

We thank Mr. Brian Chalmers of Howe Sound Pulp & Paper Ltd. (Port Mellon, B.C., Canada) for the opportunity to conduct these experiments on site. We are grateful to Eileen Blair of St. Anne-Nackawic Pulp Co. and Vic Morandini of Celgar Pulp Company for providing liquor samples.

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# Bonded-phase capillaries and the separation of inorganic ions by high-voltage capillary electrophoresis

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## ABSTRACT

Both a $C_1$ and a $C_{18}$ saturated hydrocarbon have been bonded to 75-$\mu$m capillaries for high-voltage capillary electrophoresis separations. The performance of these bonded phases has been compared with unbonded capillaries under a variety of experimental conditions. The bonded phases were prepared by a flow-through procedure at room temperature, and the reproducibilities of the electroosmotic flow for the $C_1$ and $C_{18}$ capillaries were 6 and 3%, respectively. Both of the bonded phases reduced interactions between the silica surface and positively charged ions, but for larger hydrophobic ions sorption and peak tailing were observed on the $C_{18}$ phase. In the presence of sodium dodecylsulfate and sodium decanesulfonate it was possible to control the electroosmotic flow over a wide range. The separation of lanthanide metal ions is illustrated, and improved resolution and reduced surface interactions are shown for the bonded phases.

## INTRODUCTION

High-voltage capillary electrophoresis (CE) is a technique for the separation of charged species in small capillaries at electric field strengths in the range of 10 to 30 kV. The small-diameter capillaries dissipate heat efficiently and can provide very efficient separations. This technique is growing rapidly, and has been the subject of several recent review articles [1–3]. Fused-silica capillaries have been used widely in CE. Since the walls of silica capillaries are normally negatively charged in aqueous solution from the ionization of surface silanol groups, positive counterions are present in the double layer adjacent to the capillary walls. Some of these counterions induce a flow at the wall, and as long as the capillary is small, all of the liquid in the capillary will flow with these ions. This flow is termed electroosmotic (EO) flow, and it has a flat velocity distribution across the capillary, except for a few nanometres at the capillary surface [4,5]. Thus the net rate of elution of any ion is the sum of the EO flow and the electrophoretic mobility of the ion; neutral compounds will be carried at the velocity of the EO flow. Therefore, the rate of electroosmotic flow can affect separation time and resolution [1]. The chemical properties of the interface can also affect analyte sorption, which results in band broadening, and in some cases the peak is lost completely. Thus it would be useful to have the ability to control the chemial and physical properties of the silica–electrolyte interface.

The majority of CE separations have been performed in untreated fused-silica capillaries. Untreated capillaries can sorb solutes by electrostatic and/or chemical interactions, especially in the case of large molecules like proteins. Several research groups have tried to eliminate adsorption by coating the capillary wall with a polymer, or by covalently bonding organic phases to the surface. The composition of surface phases used to reduce protein sorption have included the following: methylcellulose [6], acrylamide [7,8], trimethylsilane [9], epoxydiols [10], maltose [10], polyethylene glycol [11,12], poly(vinylpyrrolidine) [13], arylpentafluoro [14], polyethyleneimine [15], OV-1 [16], and Carbowax 20M [16]. Recently, commercially-prepared bonded phases have been introduced, but, to a large part, the properties of these phases are proprietory information [17]. In addition to the reduction of the

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sorption of proteins, the goal of several studies of surface treatment has been to reduce EO flow [12]. In large capillaries this is required to reduce band broadening due to parabolic flow [12]. For smaller diameter capillaries elimination of EO flow will maximize resolution arising from differences in electrophoretic mobilities. The most effective coatings for elimination of EO flow have been large molecules, such as methylcellulose [6], acrylamide [7] [8], polyethylene glycol [11,12] and poly(vinylpyrrolidine) [13].

Another way to manipulate EO flow is via surfactant interaction. When surfactants are added to the electrolyte, even at concentrations below the critical micelle concentration, they adsorb at the surface of the capillary. This adsorption changes the surface properties and can have dramatic effects on the separation. Altria and Simpson [18] briefly studied the effect of cationic surfactants, with carbon chain lengths from 1 to 16, on the EO flow, and found that the flow decreased linearly with the log of surfactant concentration. Other studies [19,20] have used cationic surfactants to reverse the electroosmotic flow for the separation of anions. Foret *et al.* [21] have used a non-ionic surfactant to eliminate electroosmotic flow. In principle, only cationic and non-ionic surfactants adsorb sufficiently on the silica surface to be of practice use; presumably, anionic surfactants are repelled by the negative charge of the dissociated silanol groups. However, if the surface is first bonded with a hydrophobic phase, the anionic surfactant will sorb on the surface by hydrophobic interaction. In practice, all types of surfactants should sorb onto the surface, hopefully to an extent that can be reproducibly controlled by the concentration of the surfactant in the electrolyte and by the electrolyte composition. Thus it should be possible to quickly evaluate the effects of different surface loadings and different charge densities on the performance of the capillary for a variety of surfactants. In the studies here, sodium dodecylsulfate (SDS) and sodium decanesulfonate (SDECS) have been used to manipulate EO flow on three types of capillaries: unbonded, a highly-hydrophobic bonded phase, and a weakly-hydrophobic bonded phase.

The lanthanide series of metal ions is one of the classes of ions that we have chosen to evaluate these bonded-phase capillaries. Lanthanides are of importance in nuclear science, high-efficiency magnets, and as geological tracers. Efficient separation processes are important for these metal ions, and liquid chromatographic techniques have been employed for this purpose [22–25]. Although capillary electrophoresis has become accepted for the separation of organic species, the separation of inorganic compounds has not gained widespread acceptance [26]. Recently, Foret *et al.* [26] separated lanthanides with a polyacrylamide coated capillary, with α-hydroxyisobutyric acid (HIBA) as a selective complexing counterion and creatinine for indirect UV detection. The purpose of the present studies was to evaluate the potential of surfactants and bonded phase with lanthanides as the test solute ions.

## EXPERIMENTAL

### *Apparatus and electrophoresis*

A Waters Quanta 4000CE system (Millipore Waters, Milford, MA, USA) equipped with a positive high-voltage power supply was used. Polyimide-coated fused-silica capillaries, 60–70 cm in length with an I.D. of 75 μm, were obtained from Polymicro Technology (Phoenix, AZ, USA). The window of the on-column detector cell was created by burning a small section (*ca.* 0.5 cm) of the polyimide-coating off with a match, and excess residue was then wiped off with methanol or acetone. The sample was injected hydrostatically with the capillary inlet lifted 9.8 cm higher than the capillary outlet for a few seconds. The electrolyte was monitored at 214 nm. The electropherogram was recorded and evaluated on a PC computer with a Waters SIM interface and Waters Baseline 820 software.

### *Chemicals*

All solutions were prepared from water that was distilled, deionized and then distilled again (Corning Mega-Pure system, MP-6A & D2; Corning, NY USA). The stock acetate buffer was prepared by mixing two acetate buffers that had different pH values but the same total concentration of acetate, to give the final desired pH value of 4.6. This buffer always contained a total concentration of free acetate of 0.01 mol/l. The pH was measured with a combination glass electrode calibrated with pH 4.0 and 7.0 buffers (Hydrion dry buffers; Aldrich, Mil-

waukee, WI, USA). The electrolytes containing surfactants were obtained by mixing the stock acetate buffer with a 0.01 mol/l stock solution of the surfactant; SDS (99%; Sigma, St. Louis, MO, USA) or SDECS (98%, Aldrich). This approach gave a constant ionic strength if the volume change on mixing was negligible. All surfactant solutions were at concentrations below the critical micelle concentration. The background electrolyte for lanthanide separations was 9 mmol/l benzylamine (BDH, Toronto, Canada), 4 mmol/l hydroxyisobutyric acid (98%, Aldrich) and 20 mmol/l acetic acid (BDH). The pH value of this solution was 4.60. The benzylamine (BDH) was purified by distillation under vacuum. All solutions were filtered through a 0.2-$\mu$m nylon-66 membrane syringe filter (Cole-Parmer, Chicago, IL, USA) prior to use.

Samples of $1.0 \cdot 10^{-4}$ mol/l benzylalcohol (analytical reagent, BDH) were used to determine the EO flow. Benzyltrimethylammonium chloride was reagent grade (97%, Aldrich). Lanthanide samples were obtained from Alfa (Danvers, MA, USA) as nitrate salts (Dy, Er, Gd, Ce), chloride salts (La, Pr, Yb) and as oxides (Nd, Sm, Lu, Tm, Ho, Eu). Oxides were dissolved in an excess of 0.5 mol/l nitric acid, evaporated to dryness, and redissolved in water to form a 0.01 mol/l stock solution. Test samples ($1.0 \cdot 10^{-4}$ mol/l) were prepared by dilution of 20 $\mu$l of the 0.01 mol/l stock solution to 2 ml in the electrolyte. All injected samples were filtered through a 0.2-$\mu$m nylon-66 membrane syringe filter prior to injection. The trimethylchlorosilane (99.9%) and dimethyloctadecylchlorosilane were obtained from Hüls Petrarch Systems (Bristol, PA, USA). Imidazole (99%, Aldrich) was dried under vacuum ($\approx$20 mmHg) for two days. The reaction solvents, N,N-dimethylformide (DMF, certified ACS, Fisher) and dichloromethane (analytical grade, BDH) were distilled over calcium hydride 2 h prior to use.

*Preparation of bonded phases*

The capillaries were etched with 1.0 ml/l sodium hydroxide for 3 h at room temperature, rinsed with water for 10 min, flushed overnight with 1.2 mol/l hydrochloric acid to remove $Na^+$ from wall and to produce free silanol groups, washed with water for a few hours to remove excess acid, rinsed with methanol for 0.5 h, and then dried at 160°C for 3 h by gentle flushing with nitrogen.

A schematic of the arrangement used for the preparation of the bonded phases is shown in Fig. 1. Solutions of trimethylchlorosilane in dried DMF or dimethyloctadecylchlorosilane in dried dichloromethane were prepared in 1.5-ml polypropylene centrifuge tubes (Cole-Parmer); dried imidazole was added as an acid acceptor. The concentration of the silane (0.01 mol/l) in one capillary volume was over five times higher than that required for complete coverage of the surface silanols in one capillary, and the concentration of the base was double the concentration of the silane [27]. These reactant mixtures were filtered through a 0.2-$\mu$m nylon-66 membrane syringe filter into antoher centrifuge tube, and a 100-$\mu$l eppendorf pipette tip was inserted into the cap of the tube (Fig. 1). The capillary was inserted through the pipette tip into the solution, and a small cotton ball was put around the capillary in the bottom of the tip, followed by calcium chloride and more cotton to cover on the top of the calcium chloride. The silane solution with the capillary inserted was held to a height of *ca.* 30 cm above the other end of the capillary. The silane solution was sucked through the dried capillary with a syringe connected with a length of polyethylene tube (I.D. 0.38 mm), and the solution was allowed to run through the capillary by gravity (see (Fig. 1)

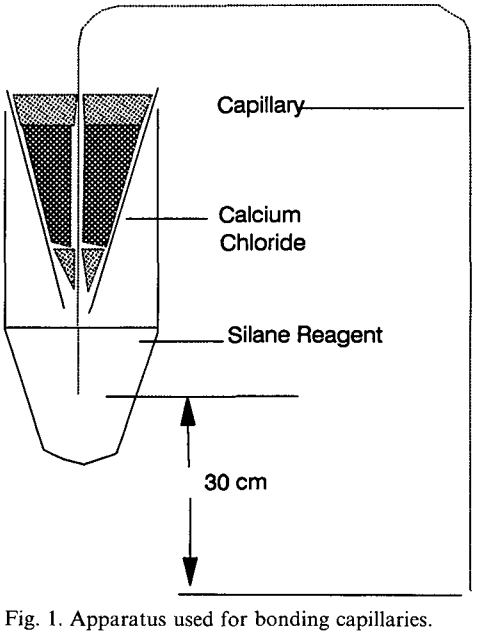


Fig. 1. Apparatus used for bonding capillaries.

for 24 h. After reaction, each capillary was rinsed with the pure reaction solvent (DMF for $C_1$ and dichloromethane for $C_{18}$), followed by methanol, water, and then with the aqueous buffer overnight.

*Preparation of Carbowax 20M-coated capillary*

The procedure used for coating the capillary with Carbowax 20M was based on procedures reported for liquid and gas chromatographic packings [28,29]. The capillary was washed with 1.0 mol/l hydrochloric acid for 3 h, rinsed with water for 0.5 h, and methanol for 0.5 h. The capillary was then dried at 150°C by gentle flushing with nitrogen for 3 h, filled with a 6% (w/w) Carbowax 20M chloroform solution, connected to a gas chromatograph, flushed with nitrogen at 50°C for 3 h, and then at 270°C for 16–18 h. The capillary was then washed with methanol, filled with the aqueous buffer and allowed to stand overnight.

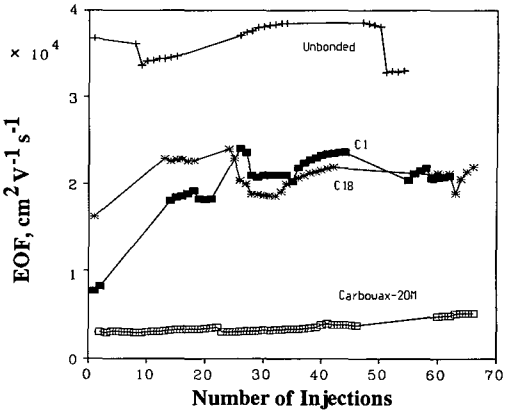


Fig. 2. The electroosmotic flow (EOF) coefficient as a function of number of injections. Experimental conditions: 9 mmol/l benzylamine, 4 mmol/l HIBA and 20 mmol/l acetic acid at pH 4.60; electric field, 460 V/cm; injection, 6 s at a differential height of 9.8 cm.

## RESULTS AND DISCUSSIONS

*Preparation of bonded phase*

The first preparation tried was a 24-h reaction in an ultrasonic bath [30], but it was difficult to seal the capillary, and the temperature of the water in the bath rose to 50°C, which caused bubbles to form in the capillary. Even with the higher-boiling solvent, DMF [31], it was found that this procedure was too complicated. Since Jones [32,33] had shown that the yield of the silanization did not appreciably change from room temperature to reflux, a room temperature reaction was used with the silane solution flowing through the capillary overnight. Imidazole was chosen as the acid acceptor because a study of several organic bases showed [31] that it gave the fastest reaction rate. The bonding procedure was repeated three times (3 × 24 h) on the same capillary, and it was found that the EO flow did not change when reaction times above 24 h were used. Consequently, all capillaries were made to react for 24 h.

*Stability and reproducibility of bonded and unbonded capillaries*

The EO flow was monitored with the uncharged molecule, benzylalcohol ($1.0 \cdot 10^{-4}$ mol/l) at pH 4.60 for bonded and unbonded capillaries for a total of up to 65 injections (shown in Fig. 2), and it was found that the EO flow was essentially stable after 12 injections. The electroosmotic flow for both the $C_1$ and $C_{18}$ phases was decreased by about 35% relative to that for the unbonded capillary, which is similar to that reported for a commercially available bonded phase [17]. The Carbowax 20M coating gave a very low EO flow, but there was a slight increase with time, indicating a possible slow loss of the polymer. The portions of the curves in Fig. 2 that do not have data points represent periods when the capillary was used for other tests with electrolytes containing surfactants. At the end of each of these test periods the capillaries were washed with acetonitrile–water 1:1 overnight, and then placed in the acetate buffer. The curves in Fig. 2 show that this rather drastic change in elecrolyte composition could be made with little change in EO flow. To achieve this reproducibility, however, careful attention had to be given to the purity of the solutions and the treatment of the containers used. During initial studies large and irreproducible variations in EO flow were observed when different batches of electrolytes of the same composition were used (even in the absence of surfactants). It was eventually found that the changes in EO flow could be controlled if all materials were cleaned in alcoholic potassium hydroxide and the use of soaps to clean containers was eliminated. Such irreproducible behaviour is not uncommon in CE and this behaviour

TABLE I

AVERAGE ELECTROOSMOTIC FLOW COEFFICIENT AND ITS STANDARD DEVIATION ON $C_1$ AND $C_{18}$ CAPILLARIES

The electrolyte is an acetate buffer (acetate concentration of 0.01 mol/l) with a pH of 4.60, and the electric field is 500 V/cm.

| No. | Capillary | Number of measurements | Average of EOF ($cm^2V^{-1}s^{-1}$) | R.S.D. of EOF (%) |
|---|---|---|---|---|
| 1 | $C_{18}$ | 19 | $2.27 \cdot 10^{-4}$ | 13 |
| 2 | $C_{18}$ | 20 | $2.23 \cdot 10^{-4}$ | 4.4 |
| 3 | $C_{18}$ | 20 | $2.36 \cdot 10^{-4}$ | 12 |
| 4 | $C_1$ | 20 | $2.35 \cdot 10^{-4}$ | 11 |
| 5 | $C_1$ | 20 | $2.09 \cdot 10^{-4}$ | 6.9 |
| 6 | $C_1$ | 20 | $2.30 \cdot 10^{-4}$ | 10 |
| Average of capillaries 1–3 | | | $2.29 \cdot 10^{-4}$ | 3 |
| Average of capillaries 4–6 | | | $2.25 \cdot 10^{-4}$ | 6 |

is likely caused by the sorption of small amounts of impurities onto the capillary surface.

To evaluate the reproducibility of the chemical bonding procedure, three capillaries were treated with $C_1$ and $C_{18}$ reagents. The average EO flow coefficient and the standard deviation for each of the capillaries are shown in Table I. The standard deviations of the EO flows for the capillaries ranged from 4 to 13%; much larger deviations were observed if proper experimental procedures were not maintained (see above). The relative standard deviations for the results between individual capillaries was considered to be very good, at 3% and 6% for $C_1$ and $C_{18}$ bonded phases, respectively (see Table I). These results show that the procedures used in this study can produce reproducible bonded phases.

The ability of the bonded phase to shield cations from interactions with the silica surface of the capillary was also studied briefly with the organic cation, benzyltrimethylammonium chloride. Although it is difficult to make an absolute comparison because of differences in EO flows and elecrolyte composition, the results indicated that the $C_1$ bonded phase had the best shielding properties, with a peak symmetry (peak-tail/peak-front) of 2.9. The peak symmetry for the unbonded phase was 4.9, and for the $C_{18}$ phase, 4.3. The large value for the $C_{18}$ phase is likely due to hydrophobic interactions as this broadening was not observed with inorganic cations.

*Effect of the ionic strength on electroosmotic flow and column efficiency*

It is known that EO flow decreases with an increase in ionic strength on unbonded capillaries, due to changes in the thickness of the charged double layer at the capillary–electrolyte interface [34]. While the structure of the double layer is uncertain for bonded phases, the presence of salts still can affect EO flow as shown by the results in Fig. 3. Column efficiencies (HETP) for benzylalcohol as a function of ionic strength of the electrolyte for bonded and unbonded capillaries are shown in Fig. 4. The column efficiency decreased with ionic

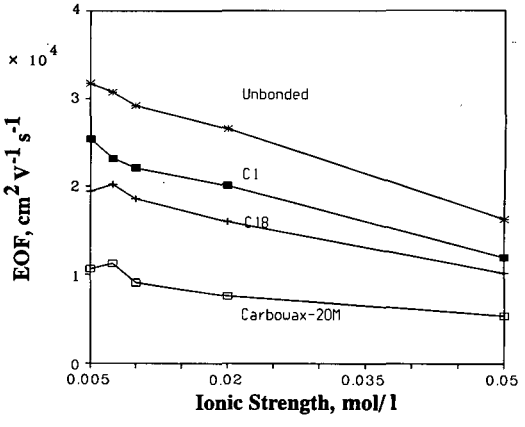


Fig. 3. The electroosmotic flow (EOF) coefficient as a function of ionic strength. Experimental conditions: acetate buffer at pH 4.60; electric field, 200 V/cm; injection time, 6 s.

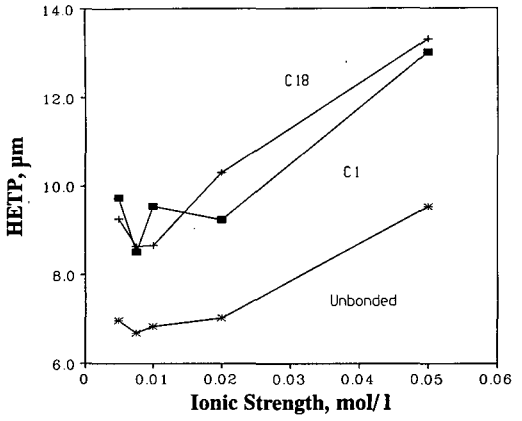


Fig. 4. Column efficiency as a function of ionic strength. Experimental conditions as in Fig. 2. Test solute is $1 \cdot 10^{-4}$ mol/l Ce (III).

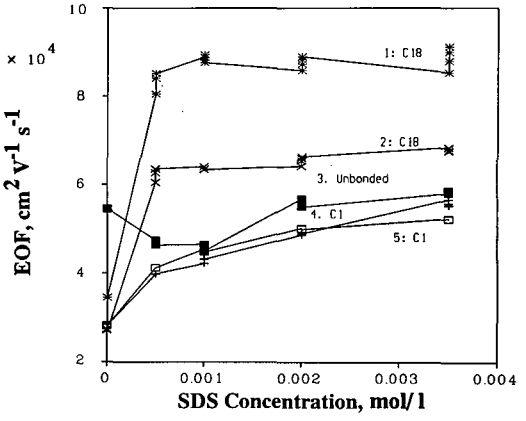


Fig. 6. The electroosmotic flow coefficient as a function of concentration of SDS. Experimental conditions as for Fig. 5.

strength, and this was primarily due to an increase in longtitudinal diffusion with longer separation times.

*Effect of anionic surfactants on the electroosmotic flow on the bonded and unbonded capillaries*

When capillary surfaces are bonded with hydrophobic phases, hydrophobic anionic surfactants can be adsorbed on the surface. Since the EO flow is inversely proportional to the ionic strength, the effect of different concentrations of SDECS and SDS surfactants was studied at constant ionic strength. The results in Fig. 5 and Fig. 6 show that it is possible to control the EO flow over a wide range by the addition of surfactants to electrolytes used in bonded-phase capillaries. The EO flow in the unbonded capillary was not appreciably affected by the presence of the negatively charged surfactants. This is expected due to the negatively charged surface of the silica surface. All of the bonded phases exhibited an increase in EO flow as surfactant was added, but for the $C_1$ phase this was only appreciable for the longer-chain surfactant. Only in the case of the $C_{18}$ phase was it possible to surpass the EO flow of the unbonded capillary, and as the surfactant was added the EO flow increased quickly to reach a plateau value, presumably caused by saturation of the surface with the surfactant. Both of the $C_1$ capillaries exhibited similar EO flow patterns. However, for the $C_{18}$ capillaries quite different flow-rates were observed. Since EO flow is sensitive to small changes in the composition of the interface, it is possible that small changes in bonding density for the $C_{18}$ phase will affect the sorption of surfactant to a greater extent than for $C_1$ phases.

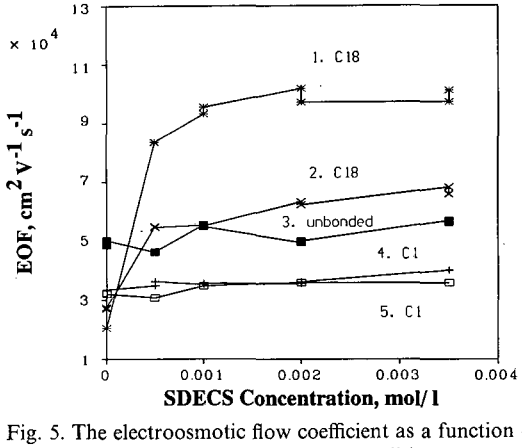


Fig. 5. The electroosmotic flow coefficient as a function of concentration of SDECS. Experimental conditions: acetate buffer with ionic strength 0.01 mol/l and pH 4.60; electric field, 200 V/cm; other condition as for Fig. 2.

*Lanthanide separations on the bonded and unbonded capillaries*

Lanthanide separations on $C_{18}$ and $C_1$ capillaries were obtained with an electrolyte containing 4 mmol/l HIBA, 9 mmol/l benzylamine and 20 mmol/l acetic acid; benzylamine was used for indirect detection. The separations for a $C_{18}$ and an unbonded capillary are shown in Fig. 7. The $C_{18}$

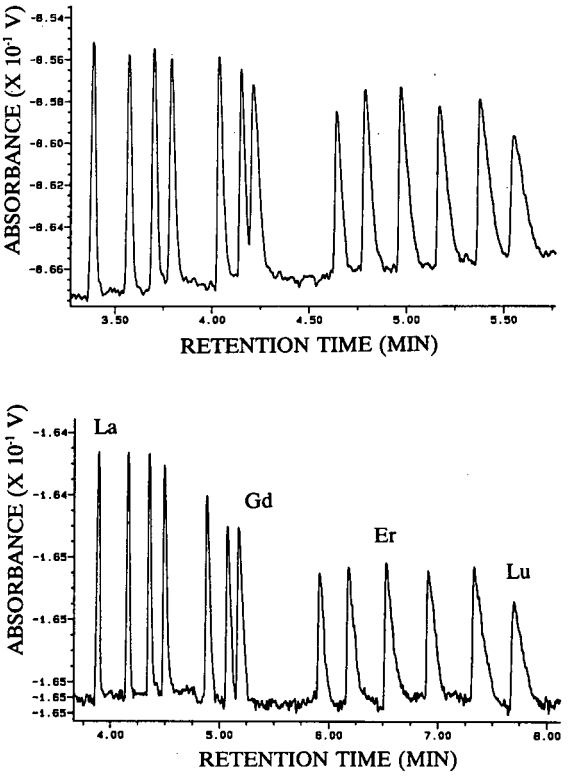


Fig. 7. Electropherogram of lanthanide separation on the $C_{18}$ (bottom) and unbonded capillaries (top). Experimental conditions: concentration of lanthanides is $1.0 \cdot 10^{-4}$ mol/l; detection at 214 nm; other conditions as for Fig. 1.

TABLE II

HEIGHT EQUIVALENT TO A THEORETICAL PLATE (HETP) FOR LANTHANIDES

| Lanthanide(III) | HETP ($\mu$m) | | |
|---|---|---|---|
| | Polyamide [26] | $C_{18}$ | Unbonded |
| La(III) | | 4.9 | 4.8 |
| Ce(III) | | 3.9 | 4.6 |
| Pr(III) | | 3.7 | 4.5 |
| Nd(III) | | 4.2 | 5.2 |
| Sm(III) | | 5.7 | 4.4 |
| Eu(III) | | 5.2 | 4.6 |
| Gd(III) | | 9.0 | 9.4 |
| Tb(III) | 10 | | |
| Dy(III) | 12 | 6.9 | 5.8 |
| Ho(III) | 15 | 9.6 | 7.4 |
| Er(III) | 16 | 12.7 | 8.6 |
| Tm(III) | 18 | 13.5 | 9.8 |
| Yb(III) | 20 | 14 | 12 |
| Lu(III) | 28 | 18 | 16 |

phase seems to show a slightly enhanced separation with the resolution of Eu(III) and Gd(III) being 1.2 on the $C_{18}$ capillary and 0.81 on the unbonded capillary. It should be noted that most lanthanides exhibit tailing peaks, which becomes worse at longer retention times. This broadening is not due to surface interactions, but is a result of differences between the electrophoretic mobilities of the analyte and elecrolyte ions. When the concentration of electrolyte is much larger that the concentration of analyte, or when the solute and the co-ion have close effective mobilities, symmetric peaks should be observed. This type of broadening can be worse in indirect detection, since the concentration ratio of background electrolyte to sample ion may be smaller than for direct detection methods. The electrophoretic mobility of protonated benzylamine is between Ce(III) and Pr(III), so that the Ce(III) peak is expected to exhibit slight fronting and the later eluting ions are expected to tail. When the peaks were expanded it was found that the peaks for Ce(III) on the unbonded capillary tailed, but on the $C_{18}$ and $C_1$ capillaries slight fronting was observed. This indicates that surface interactions are present in the separation of lanthanide cations in unbonded capillaries.

An initial comparison of the HETP values shown in Table II suggests that the overall column efficiencies for the $C_{18}$ capillary are smaller than for the unbonded capillary. However, it is not possible to make a direct comparison because of the differences in retention time. The effect of band broadening processes will be related to the length of time spent in the capillary, and thus it may be more meaningful to compare peaks having similar retention time. If this is done in Fig. 7 it can been that the bonded phase exhibits improved efficiency, but even this comparison is not strictly correct. What is clear however, is that there is an improvement in resolution and a decrease in surface interactions. When these results are compared to those obtained on a polyamide coated capillary (see Table II) the results with the bonded phase were as good, if not better.

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CHROMSYMP. 2505

# Capillary electrophoretic determination of alkali and alkaline-earth cations in various multiple electrolyte solutions for parenteral use

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ABSTRACT

Simultaneous quantitation of sodium, potassium, calcium and magnesium in parenteral solutions is reported. A weakly complexing α-hydroxyisobutyric acid added to the carrier electrolyte aids in electrophoretic separation of cations. The cations are visualized indirectly at 214 nm using a strongly absorbing electrolyte co-ion, UV-Cat I. The method was optimized to enhance resolution between peaks, and limits of quantitation were determined. Effects of high sodium content on quantitation of other ions present at ppm levels are investigated.

## INTRODUCTION

The earliest report of electrophoretic separations of inorganic cations appeared in 1967 [1]. In 1981 Nukatsuka *et al.* [2] described the use of α-hydroxyisobutyric acid (HIBA) as a complexing agent in isotachophoretic separation of lanthanides. Complex-forming equilibria between lanthanides, HIBA and acetic acid were studied by Hirokawa *et al.* [3]. In 1990 Foret *et al.* [4] reported separation of rare earth metals, and lithium, sodium, potassium and magnesium by capillary zone electrophoresis using HIBA as the complexing co-ion and a creatinine-acetate buffer allowing for indirect photometric detection of analytes. In 1991 Waters researchers applied a similar principle to the separation of alkali and alkaline-earth cations and presented their findings at two symposia [5,6]. A comprehensive paper was published recently [7].

This paper describes our attempt to develop a practical capillary electrophoresis (CE) method to analyze various parenteral solutions for sodium, potassium, magnesium and calcium simultaneously. Such solutions are currently analyzed by flame photometry for sodium and potassium, by atomic absorption for calcium and magnesium or by ion chromatography.

## EXPERIMENTAL

### *Apparatus*

Waters Quanta 4000 capillary electrophoresis system with a 20 sample carousel, positive power supply and a Zinc lamp detector (214 nm) was used in all analyses. Accusep fused-silica capillaries, 60 cm × 75 μm, were also supplied by Waters. Data were collected by Hewlett-Packard LAS 3357 at a rate of 16 Hz.

### *Materials*

"UV-Cat 1", the carrier electrolyte co-ion was obtained from Waters. HIBA was purchased from

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Aldrich, Milwaukee, WI, USA. Test articles were prepared from analytical-reagent grade chemicals from various sources. Water used was distilled and deionized on Barnstead NANOpure II system. Standard solutions were prepared by diluting 1000 ppm atomic absorption standards obtained from Ricca (Arlington, TX, USA).

*Methods*

Carrier electrolyte contained 5 m*M* UV-Cat 1 and from 6.5 to 40 m*M* HIBA, pH 4.4, adjusted with N,N-diethylethanolamine purchased from Aldrich. Samples were introduced hydrostatically, 10 cm height for 30 s. Positive voltage of 20 kV was applied and the current was from 5.4 to 27 $\mu$A depending on HIBA concentration. Detector time constant was either 0.3 or 1 s (see below).

## RESULTS AND DISCUSSION

*Resolution optimization*

Fig. 1 shows a typical separation of potassium, calcium, sodium and magnesium under conditions described in ref. 7. Some solutions which we intended to analyze contained 150 times as much sodium as any other cation, which could potentially

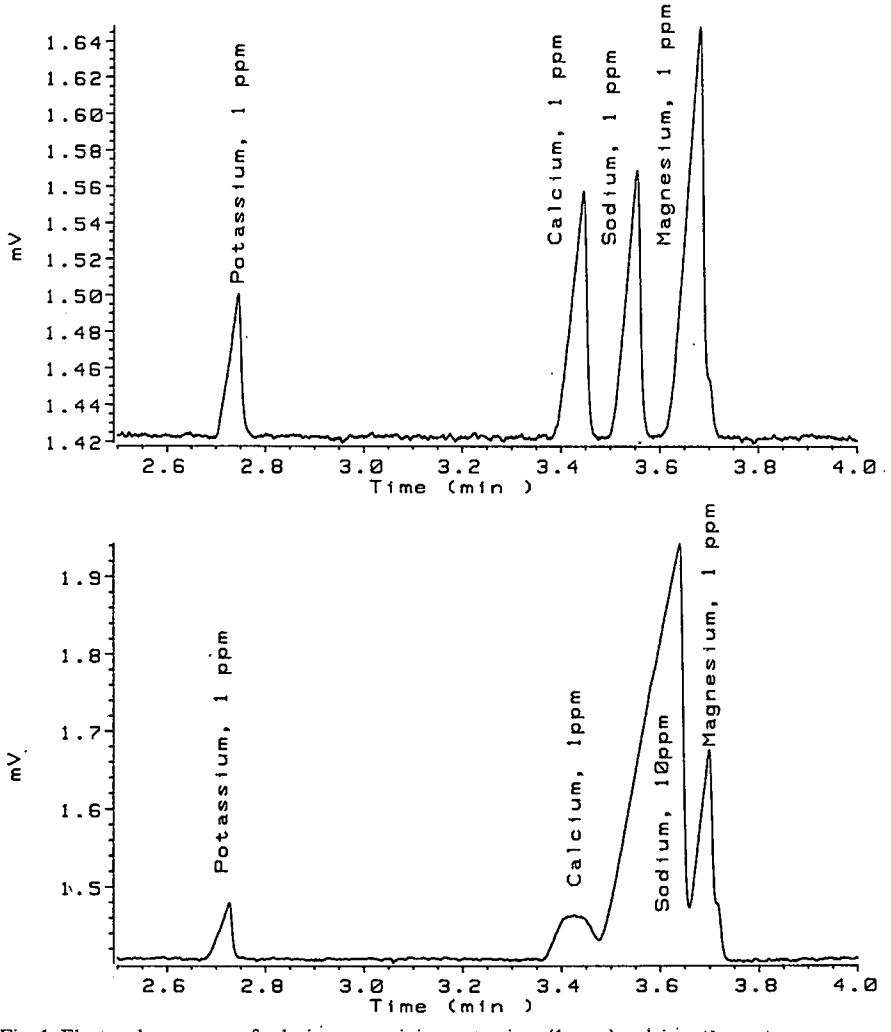


Fig. 1. Electropherograms of solutions containing potassium (1 ppm), calcium (1 ppm), magnesium (1 ppm) and sodium [1 ppm (top) or 10 ppm (bottom)]. Electrolyte: 5 m*M* UV-Cat 1, 6.5 m*M* HIBA, pH 4.4. Hydrostatic injection, 10 cm for 30 s. Time constant: 0.3 s.

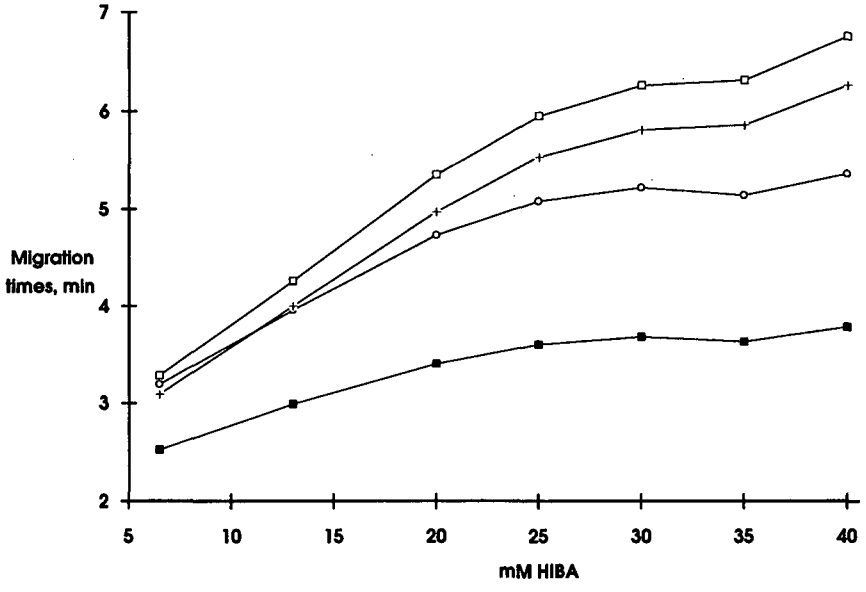


Fig. 2. Cation migration times *versus* HIBA concentration in carrier electrolyte. Other conditions as in Fig. 1. ■ = Potassium; ○ = sodium; + = calcium; □ = magnesium.

cause interference between sodium and other analytes. Our first experiments were designed to maximize resolution between sodium, calcium and magnesium.

Since complexation with HIBA affects the mobilities of alkaline earths over that of the alkali metals, we investigated the effect of increasing its concentration in carrier electrolyte. As the HIBA concentration increases, two changes occur in the separation. The electroosmotic flow decreases [8] resulting in a net increase in the migration times for all the cations, and the mobility of magnesium and calcium decrease due to greater interaction with HIBA. At approximately 13 m*M* HIBA, calcium and sodium co-migrated. Increasing HIBA concentration further changed the order of migration to potassium, sodium, calcium and magnesium (Fig. 2).

Increasing HIBA concentration caused a rise in buffer conductivity, and the running current increased from 5.4 $\mu$A at 6.5 m*M* HIBA to 27 $\mu$A at 40 m*M*. Higher current caused significant increase in baseline noise due to additional Joule heating, and a marked decrease in analyte peak response. Detector time constant was changed from 0.3 to 1 s, which greatly improved baseline stability, but also slightly reduced peak responses. Carrier electrolyte containing 30 m*M* HIBA proved to be the best compromise between peak separation, acceptable baseline noise level and sensitivity (Fig. 3).

*Precision and linearity of response*

With the instrumental conditions established, solutions containing from 5 to 50 ppm of each cation were analyzed. Precision of peak area response was evaluated by computing its percent relative standard deviation for 10 replicate injections. As shown in Fig. 4, sodium, calcium and magnesium behaved similarly, but potassium, which has the lowest peak response of all analytes, was quantitated much less precisely. If a relative standard deviation (R.S.D.) of 2% is chosen as the limit of acceptable precision, sodium, calcium and magnesium can be quantitated at levels as low as approximately 5 ppm, and potassium as low as approximately 20 ppm. With increasing analyte concentrations resolution between peaks deteriorated. At 50 ppm each, resolution between sodium and calcium (USP method [9]) was 1.9 and 1.5 between calcium and magnesium. All peaks retained very sharply defined slopes, so even at the highest concentrations they were essentially baseline resolved. Acceptable linearity of peak response was obtained in the entire concentration

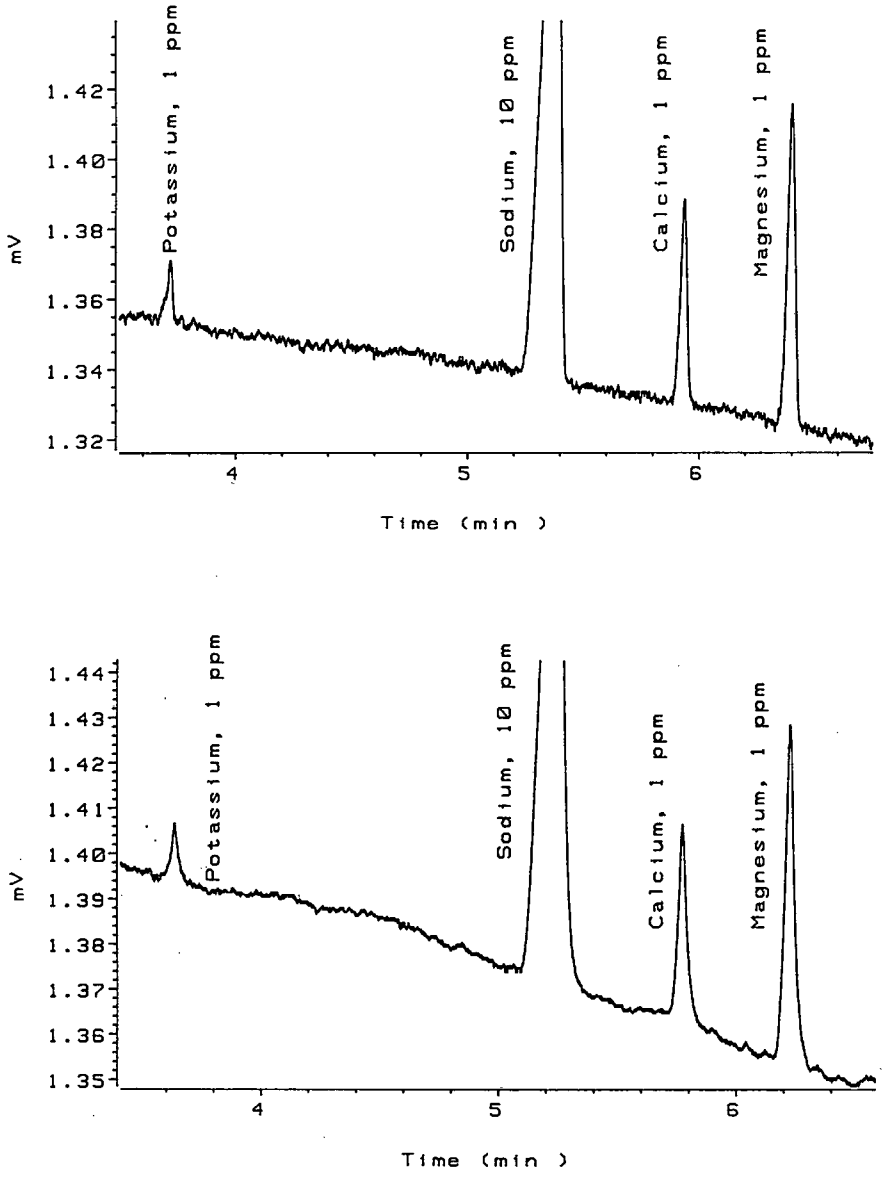


Fig. 3. Separations at 30 m*M* HIBA. Time constant: 0.3 s (top) and 1 s (bottom). Other conditions as in Fig. 1.

range with correlation coefficients ($r^2$) ranging from 0.997 to 0.9997.

*Accuracy assessment*

The solutions with highest ionic strength, usually containing high concentrations of sodium chloride, had seriously distorted potassium peaks, while the shapes of calcium and magnesium peaks were unaffected. An experiment was performed in which CE analysis was done on solutions containing constant amounts of potassium, calcium and magnesium, 40, 10 and 10 ppm, respectively, and from 60 to 400 ppm sodium. As shown in Table I, sodium levels of more than approximately 80 ppm caused decrease in potassium peak response.

Table II lists simulated parenteral solutions chosen

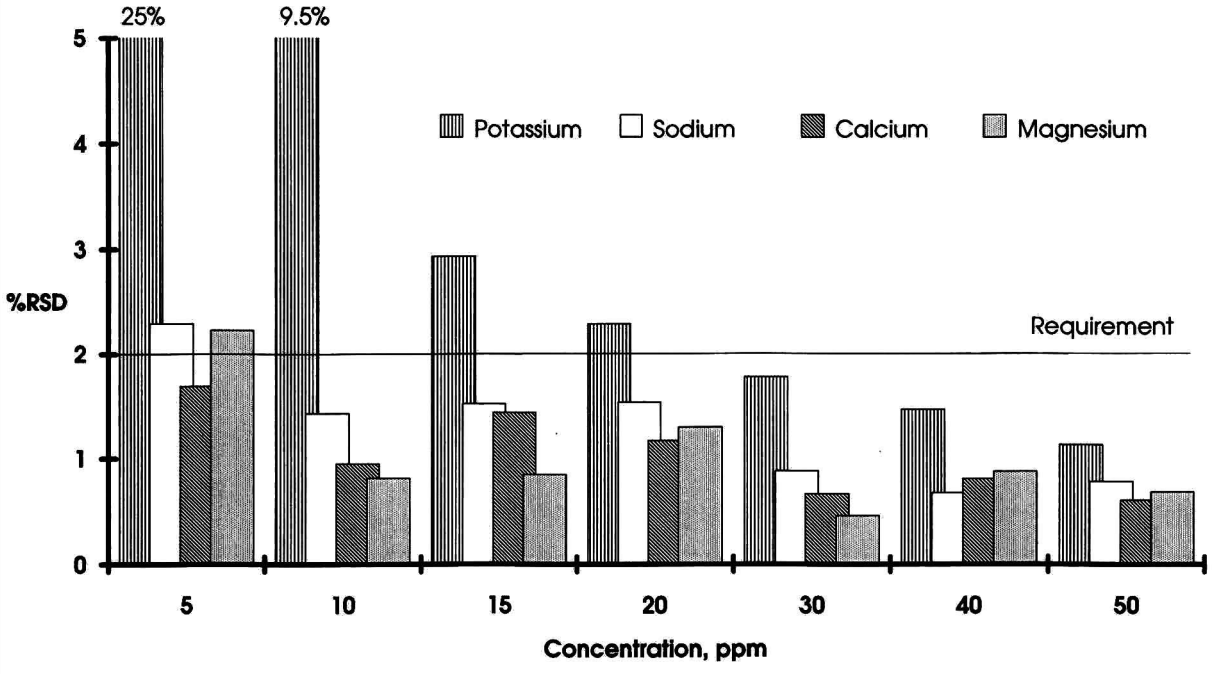


Fig. 4. Peak area response precision for ten replicates *versus* analyte concentration. Electrolyte: 5 m*M* UV-Cat 1, 30 m*M* HIBA, pH 4.4. Hydrostatic injection, 10 cm for 30 s. Time constant: 1 s.

for the study. These represent several worst cases with respect to the concentration ratio of sodium to other cations, as well as the presence of potentially interfering substances. As shown in Table II, sodium could be quantitated accurately in all tested solutions. However, in most cases, the sodium peak overlapped with the calcium and magnesium peaks at maximum practical dilutions, making their quantitation impossible.

TABLE I

QUANTITATION OF POTASSIUM, CALCIUM AND MAGNESIUM IN THE PRESENCE OF EXCESS SODIUM

Percent theoretical recovery.

| | $Na^+$ (ppm) | | | | | |
|---|---|---|---|---|---|---|
| | 60 | 80 | 100 | 200 | 300 | 400 |
| $K^+$ | 97.3 | 97.8 | 91.0 | 85.9 | 77.3 | 71.1 |
| $Ca^{2+}$ | 99.7 | 102.2 | 99.1 | 99.1 | 93.3 | 95.2 |
| $Mg^{2+}$ | 98.3 | 103.1 | 97.4 | 97.0 | 96.9 | 98.4 |

Ammonium ion can co-migrate with potassium under these separation conditions. No solution tested here contained ammonium salts added intentionally, and absence of ammonia was also confirmed analytically. Amino acid blends did not interfere with the analysis.

## CONCLUSIONS

The results of this work demonstrate that sodium, potassium, calcium and magnesium can be quantitated simultaneously as long as the sample can be diluted sufficiently so that the level of sodium is below approximately 80 ppm, while the other analyte concentrations remain above 5 ppm (20 ppm for potassium). However, the method described in this article was not practical for the parenteral solutions evaluated in this article due to very high levels of sodium in most of these solutions. We plan to continue to develop a workable CE method by extending the linear range of the method to accommodate higher levels of sodium. Initially, we will address the following to aid the accommodation of

TABLE II

ANALYTE RECOVERY FOR SIMULATED PARENTERAL SOLUTIONS

NQ = Not quantifiable: potassium, distorted peak; calcium or magnesium, sodium peak overlap.

| No. | Sample components | Sodium | | Potassium | | Calcium | | Magnesium | | Dilution factors |
|---|---|---|---|---|---|---|---|---|---|---|
| | | ppm | Recovery (%) | ppm | Recovery (%) | ppm | Recovery (%) | ppm | Recovery (%) | |
| 1 | Carbonate, citric acid | 1856 | 102.1 | | | | | 2192 | 99.3 | 50 |
| 2 | Chloride, dextrose | 3386 | 100.9 | 163 | NQ | 94 | 93.6 | | | 80 (Na)<br>4 (K)<br>10 (Ca) |
| 3 | Chloride, lactate, dextrose | 2971 | 99.4 | 158 | NQ | 55 | NQ | | | 80 (Na)<br>4 (K) |
| 4 | Chloride, lactate, acetate, dextrose | 931 | 99.8 | 632 | 92.3 | 100 | NQ | 37 | NQ | 22 (Na)<br>17 (K)<br>0 (Mg) |
| 5 | Chloride, dextrose | 1783 | 100.2 | 199 | 90.7 | | | | | 42 (Na)<br>5 (K) |
| 6 | Chloride, phosphate, sulphate | 3169 | 99.2 | 212 | NQ | | | 20 | NQ | 83 (Na)<br>6 (K) |

higher sodium levels in the sample: (1) investigate a stronger complexing agent to replace HIBA that will offer a different, perhaps better, selectivity; and (2) increase the ionic strength of the electrolyte to improve sample stacking.

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CHROMSYMP. 2500

# Explosive residue analysis by capillary electrophoresis and ion chromatography☆

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ABSTRACT

Capillary electrophoresis is investigated for application as a complementary technique to ion chromatography in the analysis of low-explosive residues. Detection limits, interference problems, and matrix effects are examined by comparing the use of ion chromatography and capillary electrophoresis in parallel analyses. The residue from several different types of explosive devices are examined, and the results show capillary electrophoresis to be a useful new technique in explosive analysis yielding good sensitivity, high resolution and short analysis times.

## INTRODUCTION

During the blast of a low explosive, a complex series of chemical reactions takes place. The goal of the forensic chemist is to piece together clues from the residue left behind which can point to the type of explosive material used. For many years, the most powerful tool in these investigations has been ion chromatography (IC) [1]. Parts per million levels of the anions and cations left behind from the blast are easily detected and quantitated using this technique. For example, black powder which consists of charcoal, sulfur and potassium nitrate may produce nitrite, nitrate, sulfate, sulfide, thiocyanate and carbonate anions on analysis of an aqueous extract of its residue. The presence of anions, such as these, is among the most important evidence used to determine the nature and source of the explosive.

Ion chromatographic (IC) analysis suffers from the lack of a good complementary technique for peak confirmation. While X-ray and infrared techniques can be used for residue analysis, these techniques lack the sensitivity and specificity required to verify chromatographic peaks. Instead, the presence of specific anions is commonly confirmed by using a combination of two different IC columns and detection schemes. At the FBI Laboratory two separate systems for this analysis are used: (1) a traditional dual-column ion analysis with suppressed conductivity detection, and (2) a single-column ion analysis with inverse photometric detection [2,3]. Problems with the traditional dual-column IC system include the inability to determine carbonate due to the fact that the eluent is hydrogencarbonate, and the inability to elute certain strongly retained anions, such as perchlorate.

The recent development of capillary electrophoresis (CE) for ion analysis has provided an opportunity to resolve these problems [4,5]. While still providing information in a format similar to IC, the CE system operates using a completely different sep-

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aration mechanism. The result is a nearly orthogonal separation that is an ideal complement to IC. Analyses reported in the literature have revealed rapid and highly efficient separations of both anions and cations [6].

For our method, we have selected an indirect photometric procedure which requires a borate buffer system with a dichromate chromophore and a diethylenetriamine (DETA) electroosmotic flow modifier [7]. In this system the polarity is set to allow detection at the positive electrode. Electroosmotic flow inside the capillary moves the buffer and analyte ions toward the detector, and separation occurs as a result of differences in electromigration of the anions. For our purposes, questions concerning detection limits, system suitability, and potential interferences had to be answered. In this paper we address these concerns and apply the CE system to various explosive residue problems. In order to better illustrate the use of this technique as a complement to our existing IC analyses, we compare results acquired with a single-column IC to those from the CE system.

## EXPERIMENTAL

### *Capillary electrophoresis*

The CE system used was a Dionex CES I (Sunnyvale, CA, USA) equipped with an 65 cm × 75 μm I.D. fused-silica capillary and an ultraviolet detector. In addition, a Spectra-Physics 1000 CE (San Jose, CA, USA) equipped with a similar column and a scanning ultraviolet detector was used to determine the most appropriate wavelength for analysis. The detector was positioned at the positive end of the capillary (reversed polarity), and was operated in the UV mode at a wavelength of 280 nm and a potential of 20 000 V. Some analyses were also carried out at 265 nm or 205 nm. Analyses of results was performed using Laboratory Data Systems (Pittsburgh, PA, USA) LabData 200 software. Potassium dichromate, sodium tetraborate, boric acid, DETA, and sodium hydroxide were used as received. The buffer system was prepared by adding 0.53 g of potassium dichromate, 0.76 g of sodium tetraborate and 2.47 g of boric acid to 1 l of deionized water [7]. The pH was adjusted to 7.8 with DETA, and the solution filtered through a 0.45-μm nylon 66 filter. The resultant buffer solution was 2 m*M* in borate, 40 m*M* in boric acid, 1.8 m*M* in dichromate and 1 m*M* in DETA. The 75-μm fused-silica column was prepared for use by first flushing for 2 min with 100 m*M* NaOH. Approximately 50 nl of sample were injected onto the column using a gravity injection technique.

### *Ion chromatography*

The IC system used was a Waters (Milford, MA, USA) 600E multisolvent delivery system attached to a Kratos Spectra flow 783 variable-wavelength UV detector set at 280 nm and a Waters WISP 710B autosampler. The column used was a Vydac 302IC4.6 (Hesperia, CA, USA) with a flow-rate of 2.5 ml/min and an injection volume of 25 μl [2]. Detector signals sent to the Laboratory Data Systems LabData 200 system. Isophthalic acid (Aldrich) was used as received. The isophthalic acid eluent had to be prepared in a special manner due to the low solubility of the free acid in water. To prepare this eluent, 0.75 g of the isophthalic acid was added to 3 l of boiling water along with approximately 2 ml of 2 *M* KOH. Following dissolution of

TABLE I

RETENTION TIMES OF IONS RELATIVE TO BROMIDE

Results compiled from a series of individual chromatographic runs.

| Ion | Relative retention time | |
|---|---|---|
| | IC | CE |
| Iodate | 0.65 | 2.17 |
| Acetate | 0.65 | 2.17 |
| Fluoride | 0.77 | 1.62 |
| Chloride | 0.85 | 1.05 |
| Nitrite | 0.92 | 1.10 |
| Phosphate | 0.97 | 2.04 |
| Bromide | 1.00 | 1.00 |
| Chlorate | 1.04 | 1.27 |
| Nitrate | 1.18 | 1.08 |
| Formate | 1.20 | 1.47 |
| Azide | 1.25 | 1.19 |
| Sulfate | 1.41 | 1.12 |
| Sulfite | 1.41 | 1.12 |
| Maleate | 1.48 | 1.46 |
| Tartrate | 1.59 | 1.57 |
| Sulfide | 1.76 | – |
| Iodate | 2.39 | 1.04 |
| Thiocyanate | 3.78 | 1.40 |
| Perchlorate | 5.00 | 1.21 |
| Carbonate | 6.87 | 1.79 |

the acid, the solution was cooled and the pH adjusted to 4.6 using additional 2 *M* KOH.

*Sample preparation*

Standards were made up by preparing 100 ppm solutions of the desired components and diluting them as necessary. The materials tested for a response on the two analytical systems are listed in Table I. All solutions and extracts were prepared using 18 MΩ deionized water. Pipe bombs containing a variety of explosive materials were deflagrated by the FBI Explosives Unit in holes dug at the demolition range at the Marine Corps base in Quantico, VA, USA. Fragments of these bombs were collected and brought back to the laboratory for analysis. The residue from the blast was collected by washing the fragments with deionized water and filtering through a prerinsed Gelman (Ann Arbor, MI, USA) 0.2-μm nylon syringe filter. These solutions were spiked with a small amount of KBr standard for use as a retention time marker.

## RESULTS AND DISCUSSION

The requirements for a good chromatographic analysis of explosive residues include reproducible retention times, minimal interferences, and the ability to clearly separate the specific ions present in the blast residue. Among the most important of these ions are nitrite, nitrate, sulfate, chlorate, carbonate and perchlorate. Such ions result from the reaction of oxidizers such as potassium nitrate, potassium chlorate and potassium perchlorate with fuels such as carbon, sulfur and sugar. The ability to clearly distinguish the presence of these major ions as well as other associated ions is the major criterion for an acceptable method. Quantitative analysis of the ions is not generally a practical concern. This is because it is not possible to determine the conditions present during the blast. Varying amounts of burned and unburned material are always present, and reaction conditions will vary based on the type of containment, initiator, and condition of the powder used in the device. Instead, the explosives examiner looks for the presence or absence of certain characteristic ions. Thus an ideal method will clearly show all relevant ions in a single chromatographic run with as good a separation as possible in order to avoid any ambiguities.

With these requirements in mind, we have investigated the CE separation. The ability of this technique to isolate a wide variety of anions offers a

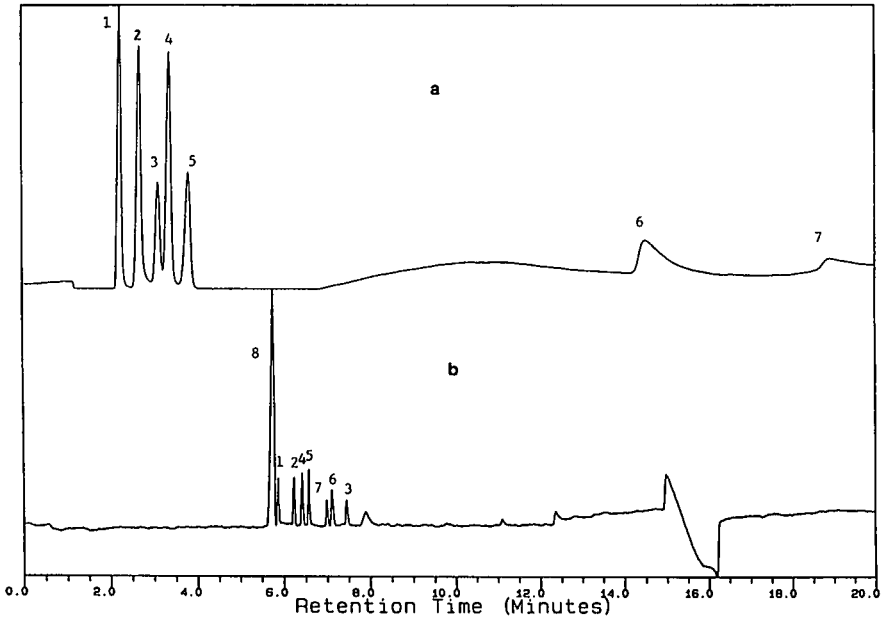


Fig. 1. Analysis of an anion standard using (a) IC and (b) CE. Peaks: 1 = chloride; 2 = nitrate; 3 = chlorate; 4 = nitrate; 5 = sulfate; 6 = thiocyanate, 7 = perchlorate; 8 = bromide.

clear advantage in its favor [8]. Apart from our IC analysis using the Vydac column, most other separations using IC require several different procedures or a complex gradient technique to achieve these results. .CE offers the potential of giving an excellent separation using a simple and rapid analytical procedure [9,10]. To compare the results run using CE, we have used an IC method developed in this laboratory specifically for explosive residue analysis [2]. This is the method using the Vydac 302IC4.6 column with 1.5 m*M* isophthalic acid as the eluent. The low ion-exchange capacity of this column permits a good separation of rapidly eluting ions such as chloride, nitrite and nitrate, as well as late eluting anions such as thiocyanate, perchlorate and carbonate [11].

*Comparison of the elution of standards*

A variety of solutions of ion standards were prepared and analyzed using both CE and IC. Fig. 1 shows an analysis of a 10 ppm standard of anions commonly encountered in explosive residue using both CE and IC. The figure clearly shows the extensive differences between the two techniques. The elution order and the retention times are drastically different. Note that the peaks for the CE separation are sharp but not well separated, while those peaks in the IC are better separated but not as sharp. This observation summarizes the practical difference between the two techniques. The CE separation is achieved by the use of high theoretical plate counts (70 000 or more) at the expense of capacity while the IC separation has greater capacity but is not as efficient.

Part of the reason for these differences in elution order lies in the dissimilar separation modes of the two techniques. The CE separation is based on differences in electrolytic conductivity, allowing the elution order to be accurately predicted by using a table of electrolytic conductivity values [8,12]. This

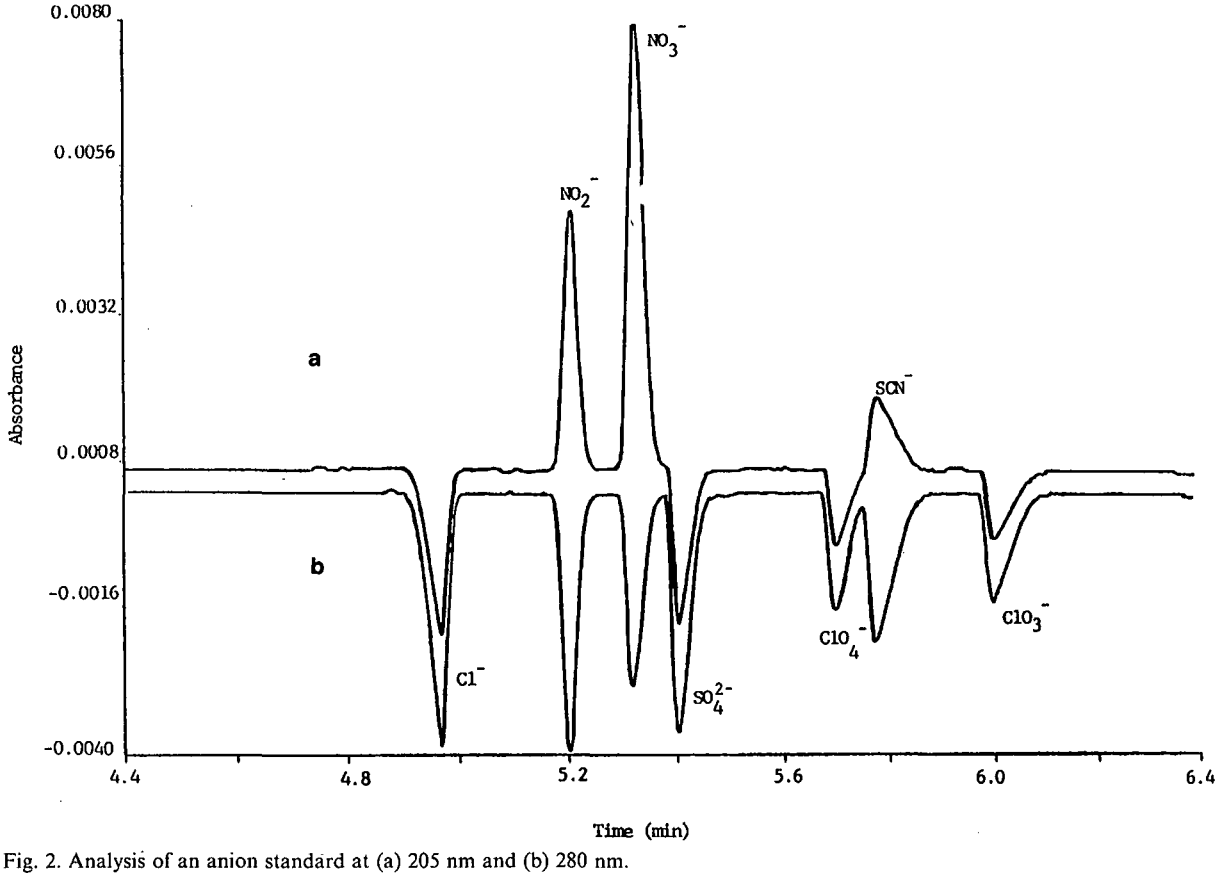


Fig. 2. Analysis of an anion standard at (a) 205 nm and (b) 280 nm.

property can be extremely useful in instances where unknown peaks appear. More subtle forces are at work in the IC separation including equilibria, concentration, and size and density effects [13]. As one would expect, there is little association between the retention times of the two different techniques. This observation is the key to understanding the use of CE as a confirmation technique. Since there is so little relationship between the separation mechanisms, it is highly unlikely that two ions could coelute undiscovered in one technique without being separated using the other. Actual retention times for a variety of anions are given in Table I.

The reproducibility of retention times was checked by running the 10 ppm standard 12 times over a period of 2 months on the same capillary. A relative standard deviation of less than 1% for the retention times of most ions was observed. In order to compensate partially for these slight variations, we have used bromide as a marker for calculating relative retention times. Bromide was selected because it was the earliest eluting ion tested, and because it is not usually found in explosive compositions [14].

It was also found that a useful technique for determining peak identity in CE is to do a second analysis with detection at a lower wavelength. We have determined that at a wavelength of 205 nm, nitrite, nitrate and thiocyanate produce peaks in a positive direction due to their UV absorbance. Anions that do not absorb at this wavelength produce peaks in the negative direction due to displacement of the absorbent buffer. This yields an electropherogram that, while only slightly less sensitive than that at 280 nm, produces a distinctive pattern of positive and negative peaks allowing easy identification of ions. Fig. 2 shows the results of an analysis of our standard recorded at 205 and 280 nm using a CE system with a scanning UV detector (Spectra-Physics 1000 CE).

*Sensitivity and dynamic range*

When analyzing the residues from explosive devices, sensitivity is usually not a problem if a significant portion of the device is recovered. Generally more than adequate amounts of residue can be found in an aqueous extract of the fragments. Situations to arise, however, when samples are either limited in size, or have been washed during efforts to extinguish a subsequent fire. Such problems make it important to determine the minimal detectable concentrations for ions of interest. There are also practical concerns with sensitivity when a comparison is made between two techniques. Injection overload and dynamic range are problems in CE due to the low capacity of the capillary column. Sample concentrations which are ideal for column chromatography may require significant dilution in order to be analyzed by CE. For these reasons detection limits and dynamic range for both the IC and CE techniques were determined. A stock solution containing 1000 ppm each of nitrite, nitrate, chlorate and perchlorate was prepared and serial dilutions were made. These solutions were injected on both systems and limits of detectability were calculated. For the CE system, the minimum detectable concentration calculated as three times the background signal was 0.5 ppm, while that for the IC column was 2 ppm. It should also be noted that the sample volume used in the CE system was 1000 times less than that of the IC.

The dynamic range of the CE system was found to be similar to that suggested in earlier literature or approximately 2–3 orders of magnitude [7,8]. This range was limited however by the requirement that adequate resolution be maintained between the peaks of interest. A more reasonable range of concentrations would be between 1 and 50 ppm. This can be compared to the 5 to 200 ppm range of concentrations applicable to our IC system which utilized a 25-$\mu$l injection loop. The practical result of these studies was that effective analysis of residue by both systems requires that the residue solution analyzed by the IC system had to be diluted 5- to 10-fold in order to achieve the most effective CE separation. Such dilutions become necessary in situations in which there is an abundance of residue found on the bomb fragments at the crime scene. In such circumstances, the maximum effective concentration should be used in both instruments to be certain that important minor components are not missed.

*Analysis of pipe bomb fragments*

To test the applicability of this analysis scheme, four pipe bombs were prepared by the FBI's Explosive Unit, and detonated on the explosives demolition range at the Marine Corps Base in Quantico,

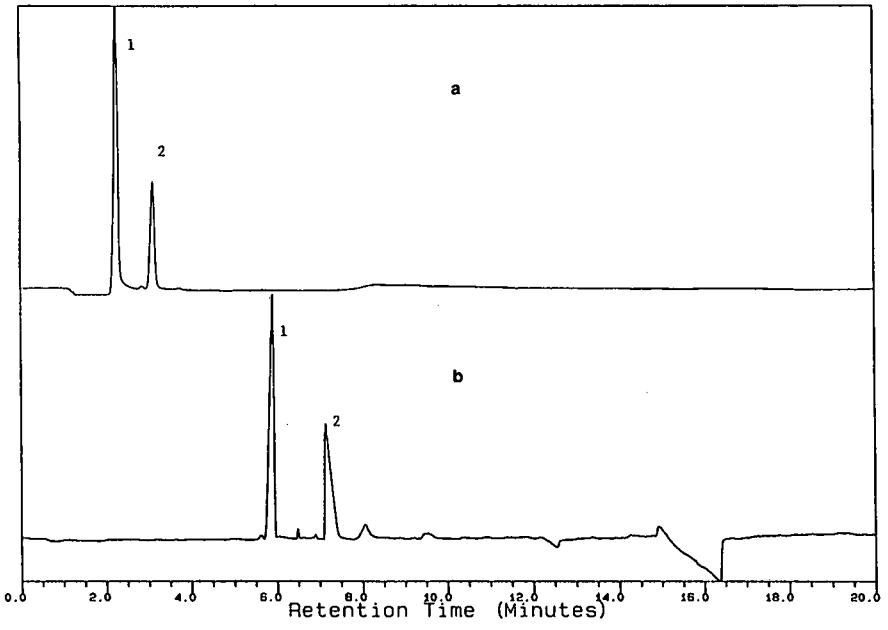


Fig. 3. The analysis of residue taken from a pipe bomb containing a mixture of potassium chlorate and vaseline using (a) IC and (b) CE. Peaks: 1 = chloride; 2 = chlorate.

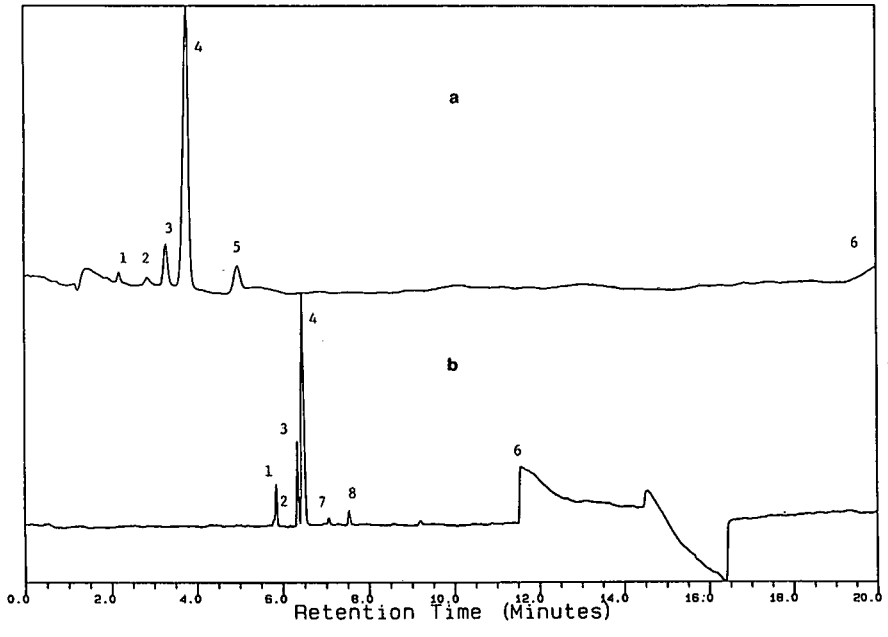


Fig. 4. The analysis of residue taken from a black powder pipe bomb using (a) IC and (b) CE. Peaks: 1 = chloride; 2 = nitrite; 3 = nitrate; 4 = sulfate; 5 = sulfide; 6 = hydrogencarbonate; 7 = thiocyanate; 8 = cyanate.

VA, USA. The bombs were filled with the following explosive mixtures: (1) potassium chlorate–vaseline, (2) black powder, (3) smokeless powder and (4) a mixture of black and smokeless powder. Fragments from each of these bombs were extracted with water, filtered, and run on both systems.

The results of the chlorate–vaseline and black powder bombs are shown in Figs. 3 and 4. The sample runs may be overlaid with their respective standards, allowing unambiguous peak assignments. Dual IC and CE runs are made because establishment of peak identity is crucial in the forensic arena where the type of explosive powder used can be critical in determining the guilt of innocence of a suspect. In actual casework each of these peaks would also be individually spiked to further establish peak confirmation.

As can be seen from these results, both product and reactant ions remain in the residue. In Fig. 3, the residue from the potassium perchlorate–vaseline pipe bomb, the chlorate ion peak is seen along with the chloride ion. Hydrogencarbonate was expected but not seen and was perhaps tied up by the residual vaseline left on the bomb. These ions are the products and reactants of the following equation:

$$KClO_3 + (CH_2)_n \rightarrow K^+ + Cl^- + HCO_3^- + H_2O$$

The black powder residue (Fig. 4) shows the nitrate ion from the unburned potassium nitrate as well as the ions nitrite, sulfate, sulfide and hydrogencarbonate. These aqueous ions are consistent with the presence of a potassium nitrate oxidizer and a carbon and sulfur fuel, and are the products and reactants of this equation:

$$KNO_3 + C + S \rightarrow NO_2^- + HS^- + SO_4^{2-} + HCO_3^- + K^+$$

The key to a successful analysis of explosive residue evidence is to examine the pattern of amount and type of ion and reconstruct the hypothetical explosive mixture. In the future, a variety of such mixtures will be tested in order to obtain detailed information on the types and distributions of such residue.

## CONCLUSIONS

The large differences in separation mode and action of the two techniques give great assistance to the analyst in determining the nature of the sample. Use of IC and CE in tandem greatly reduces problems caused by interfering ions and allows easy peak confirmation. In addition, the inherent sensitivity advantage of CE works in concert with the greater capacity of the IC system allowing a wide variety of concentrations and types of residue to be screened. For these reasons, we have found that the application of these two techniques greatly expands the capability of our laboratory in undertaking explosive residue analyses.

## ACKNOWLEDGEMENTS

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# Effect of electrolyte composition on the separation of inorganic metal cations by capillary ion electrophoresis

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ABSTRACT

Capillary ion electrophoresis (CIE; Waters' tradename Capillary Ion Analysis) is a capillary electrophoresis technique in which the conditions for separation are optimized for the analysis of inorganic or low-molecular-weight ions. Among the variable parameters in CIE, control of the electrolyte composition provides one of the more significant means for manipulating separations. In the separation by CIE of mixtures of various alkali metal, alkaline earth metals and transition metals their relative selectivity could be changed by altering the pH and the concentration of the complexing agent in the electrolyte. To permit the use of indirect photometric detection and to ensure symmetrical peak shape a highly UV absorbing amine, with an electrophoretic mobility similar to those of the analyte cations, was chosen as the major component of the electrolyte. The reproducibility in terms of the migration times was less than 0.5% relative standard deviation and in terms of peak area was better than 2% relative standard deviation. Detection limits ranged from low to mid-parts per billion ($10^9$). Applications of this technique for the analysis of metal cations in tap water, orange juice and in acid-etching baths is demonstrated.

## INTRODUCTION

Cation-exchange chromatography is commonly used to analyze for inorganic metal cations [1,2], and while methodology for these applications is relatively well-developed, additional improvements are still being made [3]. Although a fairly large number of reports have appeared in recent years describing separations of inorganic metal cations by isotachophoresis [4–10], only a few applications by capillary electrophoresis (CE) have been reported [11–16]. Capillary ion electrophoresis (CIE; Waters' tradename Capillary Ion Analysis) is a CE technique in which the conditions for separation are optimized for the analysis of inorganic or low-molecular-weight ions.

There are a number of variables, such as the hardware configuration and the electrolyte composition, which will affect the separation of analytes in CIE. While the mode of detection, the applied voltage and even the length of the capillary may affect the separation, the focus of this paper is the effects of the electrolyte composition on the separation.

Separations by CIE are based on differences in the electrophoretic mobilities of the injected ions [17, 18]. Electrophoretic mobilities are influenced by the structural properties of solutes such as size, shape, Stokes' radius, charge and mass, in addition to the interaction of those solutes with the carrier electrolyte. Each of these variables is affected by properties of the electrolyte such as pH, ionic strength or viscosity. When employing indirect photometric detection, the chemical nature and the concentration of the UV active component are also factors which influence the separation [19].

Reports in recent literature have described methods to improve peak resolution by manipulating the mobilities of the biological analytes through variation of the electrolyte parameters [19,20]. Similar

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principles were applied by Swaile and Sepaniak [21] to the determination of three metal ions, Ca(II), Mg(II) and Zn(II) by capillary electrophoresis, with on-column chelation using 8-hydroxyquinoline-5-sulfonic acid and indirect fluorescent detection.

In this paper, the effect of changing both the pH of the electrolyte and the nature and concentration of the complexing agent in the electrolyte on the separation and detection of a group of alkali, alkaline earth and transition metals by indirect UV detection will be discussed. The applicability of this means of modification of peak selectivity will be demonstrated by the separation of cations in various complex sample matrices.

## EXPERIMENTAL

A Waters (Milford, MA, USA) Quanta 4000 capillary electrophoresis system, equipped with a positive power supply, was used throughout this study. Fused-silica capillaries, 60 cm total length, 75 $\mu$m internal diameter and 52 cm from the point of sample introduction to the detector window, were obtained from Waters (AccuSep capillaries). Indirect UV detection was achieved at 214 nm with a zinc lamp and a 214-nm optical filter, or at 185 nm with a mercury lamp and a 185-nm optical filter. The samples were introduced into the capillary using 30-s hydrostatic injections, from a height of 10 cm. The separation voltage was set at 20.00 kV.

Standard 2-ml polyethylene sample vials (Sun Brokers, Wilmington, NC, USA) were used as containers for the carrier electrolyte and all the standards and samples. A Waters 860 data station and Waters SIM interface were used to record and evaluate the electropherograms at a sample rate of 20 points per second. The subsequent statistical processing was performed using CricketGraph (Cricket Software, Malvern, PA, USA) with a Macintosh SE personal computer (Apple Computers, Cupertino, CA, USA).

All solutions, electrolytes and standards were prepared using 18-M$\Omega$ water generated by Milli-Q laboratory water purification system (Millipore, Bedford, MA, USA). The transition metal standards were prepared by the dilution of standards obtained from Sigma (St. Louis, MO, USA), while the alkali and alkaline earth metal standards were prepared from salts obtained from Aldrich (Milwaukee, WI, USA), as was the analytical reagent grade $\alpha$-hydroxyisobutyric acid (HIBA). The UV background-providing component of the electrolyte, UVCat-1, was obtained from Waters. The concentration of UVCat-1 was held constant at 5 m$M$, while the HIBA concentration was varied as specified in the figures.

## RESULTS AND DISCUSSION

The equivalent ionic conductivities [22] of the cations, $\lambda_i$, are directly related to the electrophoretic mobilities [11]. Theoretical selectivity of cation separations in CIE can be predicted on the basis of the value for each cation. The closer the equivalent ionic conductivities for each cation, the more challenging the separation. The equivalent ionic conductivities of the alkali metals are sufficiently different for their separation to be readily achieved [11]. For the remainder of the metals, however, differences in individual ionic mobilities alone do not provide a separation. To separate cations with similar ionic mobilities, an additional separation mechanism must be introduced. One frequently used mechanism is based on weak complexation [12], where the mobility of each cation is modulated by its degree of complexation with the complexing agent. Since each metal has a unique affinity for the complexing agent, their mobilities are altered to different extents, providing the differences in mobility needed for separation.

The velocity of the electroosmotic flow (EOF), and the electrophoretic mobilities together provide the "apparent" mobility which is measured in CIE

TABLE I

FORMATION CONSTANTS SHOWING THE DIFFERENCE IN AFFINITY FOR VARIOUS COMPLEXING AGENTS WITH DIFFERENT METAL CATIONS

| Cation | Formation constant (log $K$) | | |
|---|---|---|---|
| | Citrate | Oxalate | HIBA |
| Barium | 2.98 | 2.31 | 0.36 |
| Magnesium | 3.29 | 3.43 | 0.81 |
| Calcium | 4.68 | 3.00 | 0.92 |
| Manganese | 3.67 | 3.97 | 0.96 |
| Zinc | 4.71 | 4.89 | 1.71 |
| Copper | 4.35 | 6.16 | 2.74 |

[11]. The "apparent" mobility may be reduced by use of a suitable complexation equilibrium, due to the reduction in the effective charge on the complexed metal. Table I shows the formation constants for three common complexing agents [23], with a variety of metals ions. The larger the formation constant, $K$, the lower the apparent charge on the cation and thus the slower the mobility of the cation would be expected to be. It is apparent from the table, that different complexing agents will provide different degrees of complexation with any given metal ion and that the choice of complexing agent will depend on the application. However, if too strong a complexing agent is used, the advantage of speed, provided by CIE, is compromised. The complexing agent used throughout this work was HIBA, due to its solubility and transparency in the UV-absorbing electrolyte, UVCat-1. It is stable

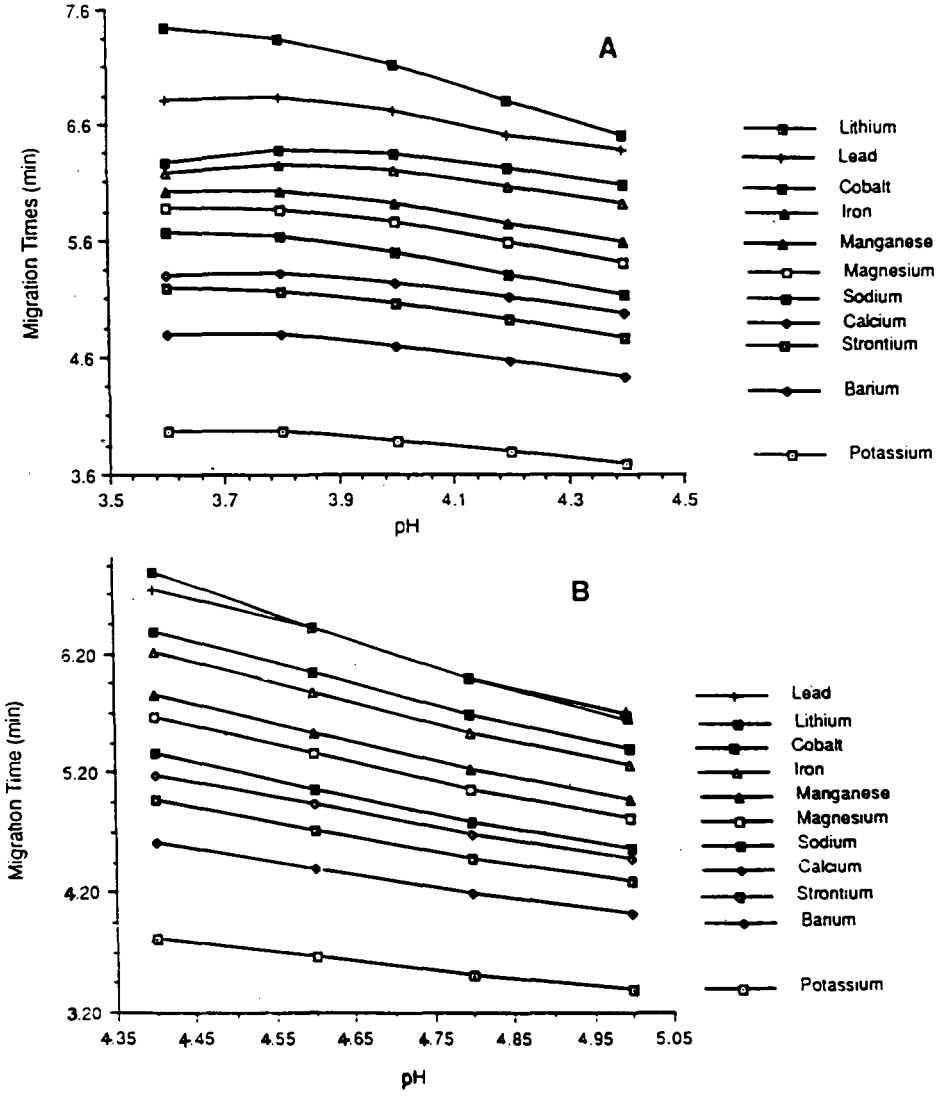


Fig. 1. (A) Graph showing the effect of decreasing the pH on the migration times of various inorganic metal cations. The electrolyte was 5 m$M$ UVCat-1 with 6.5 m$M$ HIBA, the natural pH of which was 4.4, and the pH was decreased using acetic acid. (B) Graph showing the effect of increasing the pH on the migration times of various inorganic metal cations. The electrolyte was the same as described in (A), except that the pH was raised using N,N-diethylaminoethanol.

under normal conditions and rapidly establishes a complexation equilibrium in the pH range of the electrolyte.

The interaction between the metal ion (M) and the complexing agent, as explained by Swaile and Sepaniak [21], can be described by the equilibrium expression

$$K' = \frac{[M(HIBA^-)_n]}{[M][HIBA^-]^n} \quad (1)$$

where $K'$ is the overall conditional formation constant for that metal ion and $n$ is the number of ligands. The reaction occurs in a stepwise fashion with different metal–$HIBA^-$ complexes existing simultaneously. The observed electrophoretic mobility of the metal ion, $\mu_{obs}$, can therefore be assumed to be a combination of the mobilities of the various forms of the metal present. The net mobility of the resulting analyte band, then, is determined by the distribution between the various possible forms of the metal complex.

The effect of the carrier electrolyte pH on the overall conditional formation constant is described by

$$K' = \alpha^n K_f \quad (2)$$

where $K_f$ is the conditional formation constant at infinite dilution and $\alpha$ is the degree of protonation of the complexing agent. Reducing the pH, then, decreases the value of $\alpha$ and the concentration of the complex.

The effect of pH on the migration times of eleven alkali, alkaline earth and transition metals of altering the pH can be seen in Fig. 1. Using 6.5 m*M* HIBA, the natural pH of the electrolyte was pH 4.4. The pH was taken below pH 4.4 with acetic acid (Fig. 1A) and above pH 4.4 with N,N-diethylaminoethanol (Fig. 1B). There are two effects worth noting. Firstly, as the pH is lowered, the migration times increase, due to the decrease in the EOF. Likewise, as the pH is increased, the migration times decrease, due to the increase in the EOF. The velocity of the EOF is primarily a function of the number of dissociated, negatively charged silanol groups on the inside of the capillary wall. The more negatively charged silanol groups that are available, the greater the velocity of the EOF. As the pH is decreased, the number of dissociated silanol groups decreases and thus the velocity of the EOF also decreases.

The second point of interest, is that in addition to the minor change in mobility due to the EOF, the pH is also affecting the complexing equilibrium to a small extent. This becomes apparent by comparing

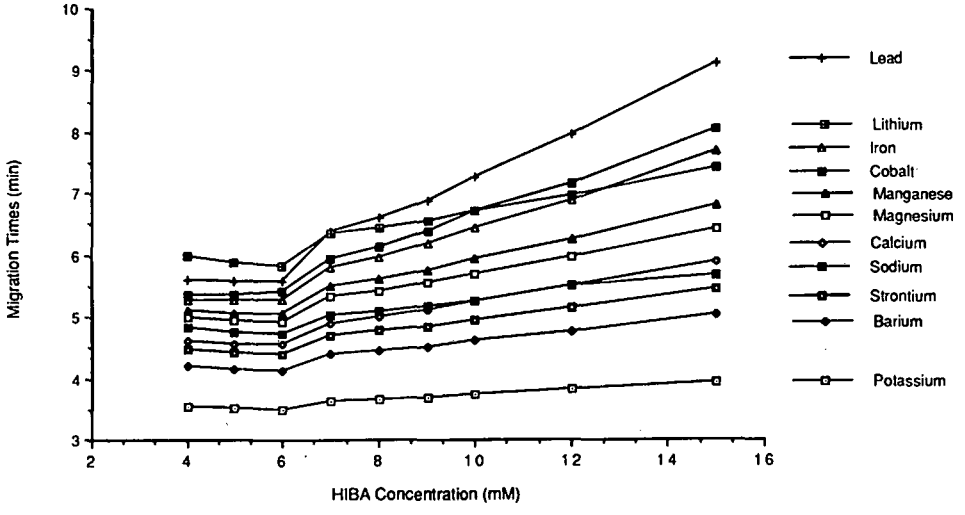


Fig. 2. Graph showing the effect of changing the concentration of HIBA on the migration times of a group of alkali, alkaline earth and transition metals. By altering the concentration of HIBA, the relative migration times of the metals can be changed. Below 6.5 m*M* HIBA, the pH was maintained at 4.4 with acetic acid. Above 6.5 m*M* HIBA, the pH was maintained at 4.4 with N,N-diethylaminoethanol. The background electrolyte was 5 m*M* UVCat-1.

the slopes of the migration times. The alkali metals do not form complexes and therefore their slopes can be expected to accurately reflect the change in mobility of the EOF with pH. However, the slopes of the other metals do not follow those of the alkali metals, indicating a secondary effect, namely the pH effect on the complexation equilibrium.

An even more significant effect on the selectivities of the cations can be seen by altering the concentration of HIBA, whilst maintaining the pH at 4.4. Fig. 2 shows the effect of changing the HIBA concentration on the migration times of the alkali, the alkaline earth and the transition metals. In order to maintain the pH at 4.4, below 6.5 m*M* acetic acid was used, while above pH 4.4 N,N-diethylaminoethanol was added. Since the alkali metals show no significant complexation with HIBA, and since their relative migration times do not change, the increase in migration times as the HIBA concentration is increased most likely reflects the decrease in the EOF due to a decrease in ionic strength with increasing HIBA. The relative migration times of the alkaline earth metals do change a little as the HIBA concentration increases, indicating that in addition to the effect of the EOF, the change in migration times is also due in part to increased complexation in the presence of additional HIBA. The effect of an increased amount of HIBA is even more pronounced with the transition metals where there is a significant increase in resolution between the metals at 15 m*M* HIBA when compared with 6 m*M* HIBA. By plotting all the data on a single graph, it becomes apparent that the elution order of the metals can be manipulated by altering the concentration of the complexing agent. The separation of 15 cations, including 8 transition metals, in less than 8 min using 6.5 m*M* HIBA as the complexing agent has been shown previously [11].

The minimum detectable concentrations for the metals, based on peak height of twice the baseline noise, range from low- to mid-ppb ($10^9$) levels [11] for the electrophoretic buffer containing UVCat-1 and HIBA at pH 4.4. The relative standard deviation (R.S.D.) in migration time for each of the group IA and group IIA cations, shown in Table II, was less than 0.5% ($n = 5$) while the R.S.D in peak area was less than 2%. The linear dynamic range was almost two orders of magnitude above the detection limit, the correlation coefficients ranging from 0.990 for sodium to 1.000 for magnesium. The upper limit of calibration reflected a loss of resolution between the peaks rather than loss of linearity.

TABLE II

DATA FROM FIVE CONSECUTIVE INJECTIONS SHOWING THE REPRODUCIBILITY OF CIE IN MIGRATION TIMES AND PEAK AREA FOR A VARIETY OF ALKALI AND ALKALINE EARTH METALS

The electrolyte was 5 m*M* UVCat-1 with 6.5 m*M* HIBA, natural pH. All other conditions as in Fig. 3.

| Cation | Migration time, R.S.D. (%) | Peak area, R.S.D. (%) |
|---|---|---|
| Lithium | 0.38 | 1.40 |
| Potassium | 0.34 | 1.80 |
| Sodium | 0.37 | 1.22 |
| Calcium | 0.37 | 1.20 |
| Magnesium | 0.83 | 0.80 |
| Barium | 0.37 | 0.97 |
| Strontium | 0.38 | 1.07 |

The potential of the discussed method for manipulating peak selectivity during the analysis of cations in complex matrices is shown in Figs. 3–5. In Fig. 3, which shows the relatively simple sample matrix tap water, the electrolyte employed to effect the separation was 5 m*M* UVCat-1 with 6.5 m*M* HIBA at pH 4.4, using indirect UV detection at 214 nm. The notable feature of this separation is that in addition to the alkali and alkaline earth cations expected in the sample, copper was also detectable in the same 8-min run.

A more complex, acidic matrix is shown in Fig. 4, where a sample of commercial orange juice was diluted 1:100 in Milli-Q water and injected without filtration. When analyzing for small quantities of metal ions, it is often undesirable to filter the sample, since cation contaminants may be introduced by the filters themselves. The fact that samples such as orange juice may be run without filtration may be of use for other samples, such as blood sera. The electrolyte for this separation contained 5 m*M* UVCcat-1, with 8 m*M* HIBA instead of 6.5 m*M* HIBA. The HIBA concentration was increased to improve the resolution between calcium and sodium. The indirect UV detection for this sample was carried out at 185 nm instead of at 214 nm. The

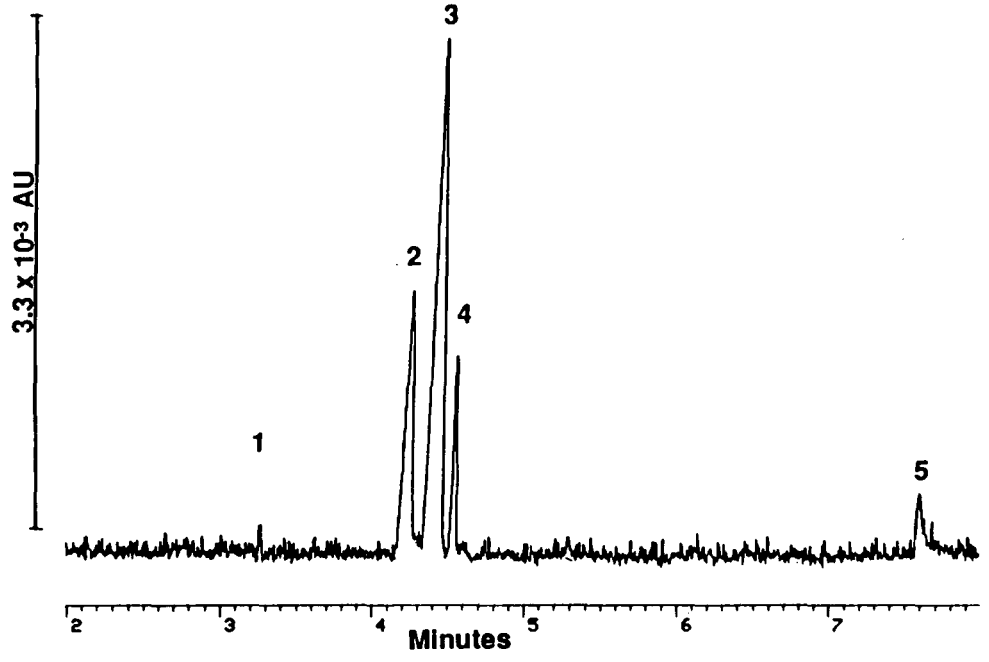


Fig. 3. CIE analysis of a tap water sample. Carrier electrolyte: 5 m*M* UVCat-1, 6.5 m*M* HIBA, pH 4.4; capillary: 60 cm × 75 μm I.D. fused silica; voltage: 20 kV (positive); hydrostatic injection: 30 s from 10 cm height; indirect UV detection at 214 nm. The sample was diluted 1:4 in Milli-Q water before injection. Peaks: 1 = potassium (0.3 ppm); 2 = calcium (4.3 ppm); 3 = sodium (12.5 ppm); 4 = magnesium (1.0 ppm); 5 = copper (1.1 ppm).

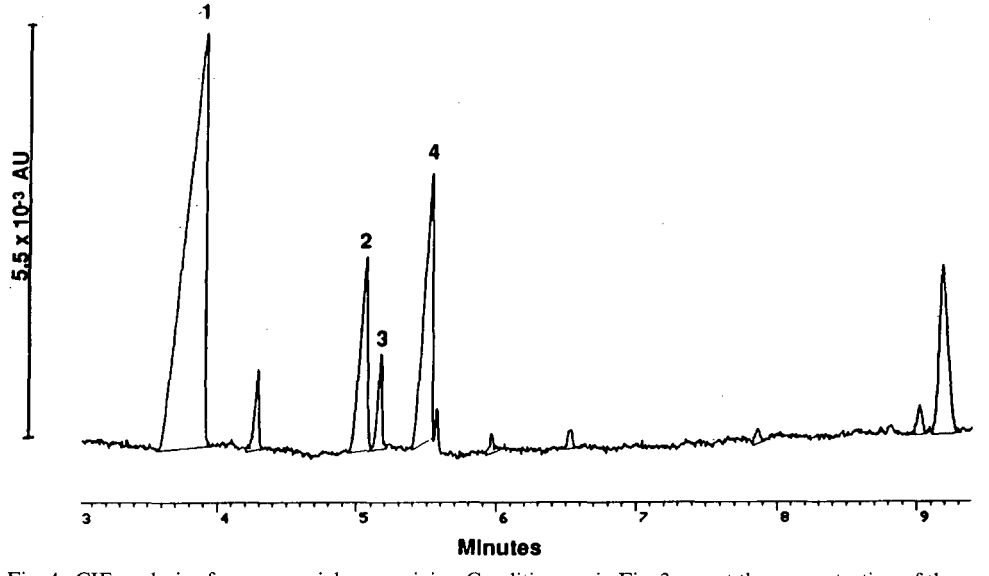


Fig. 4. CIE analysis of a commercial orange juice. Conditions as in Fig. 3 except the concentration of the complexing agent, HIBA is 8 m*M* and the detection system was indirect UV at 185 nm. The sample was diluted 1:100 in Milli-Q water before injection. Peaks: 1 = potassium; 2 = calcium; 3 = sodium; 4 = magnesium. Other peaks are inidentified.

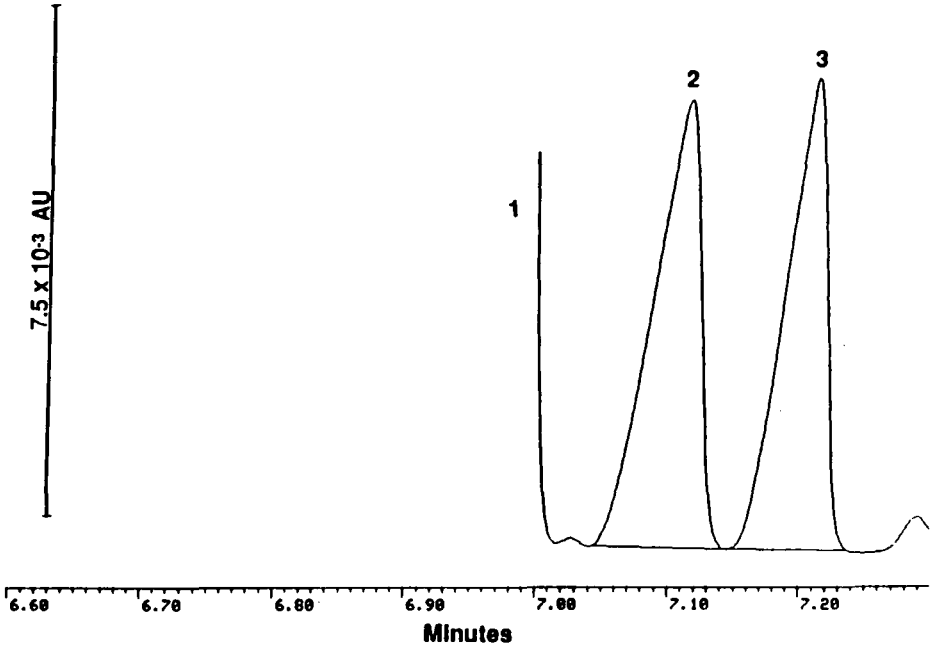


Fig. 5. CIE analysis of trace metals in an acid etching bath. Conditions are as described in Fig. 4. The sample was diluted 1:50 in Milli-Q water before injection. Peaks: 1 = sodium (1000 ppm); 2 = nickel (5 ppm); 3 = zinc (5 ppm).

change in detection wavelength was found to improve the sensitivity by a factor of 2.

The sample depicted in figure 5 was from an acid etching bath containing 5% phosphoric acid, 5% nitric acid and 5% sodium with trace amounts of nickel and zinc. The sample was diluted 1:50 in Milli-Q water, producing a solution containing 1000 ppm sodium. Nickel and zinc were added to the diluted sample to a concentration of 5 ppm and the separation effected with good baseline resolution, using 8 m*M* HIBA to enhance the resolution of the nickel and zinc from the sodium with indirect UV detection at 185 nm. Calculated detection limits for nickel and zinc in the original etching bath correspond to less than 5 ppm.

Electrophoretic buffer parameters which influence the complexation reaction also affect the detectability and efficiency in CE [20]. As the concentration of HIBA is increased, the metal–HIBA equilibrium is shifted towards the formation of complex, resulting in longer analysis times. However, if high HIBA concentrations are used to control the selectivity, large electrophoretic currents are observed which can degrade efficiency and which will affect detectability. In addition, in order to produce sharp peaks, the mobilities of the analyte ions should closely match the mobility of the corresponding ions in the electrolyte. As the concentration of HIBA is increased, the match of the analyte ion mobilities with the electrolyte mobility deteriorates due to the decreased ion mobilities. As a consequence, the efficiency of the separation decreases.

CIE offers the possibility for the rapid, reproducible and sensitive analysis of inorganic ions. With this method, peak selectivity can be manipulate and very low concentrations of cations can be determined in complex matrices.

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# Applications of ion chromatography and capillary ion electrophoresis in the alumina and aluminium industry

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ABSTRACT

Applications of ion chromatography and capillary ion electrophoresis (Waters' trade name: Capillary Ion Analysis) for the determination of anionic solutes in the alumina/aluminium industry are presented. The use of ion chromatography for the determination of chloride and sulfate in alumina refinery liquors and for the analysis of fluoride in hydroxide fused samples from aluminium smelting environs is discussed. The results obtained are compared with traditional wet chemical methods of analysis. Capillary ion electrophoresis, a relatively new separation techniqe which has a different selectivity from ion chromatography, permits very rapid quantitation of oxalate in Bayer liquor, and the results from this methodology are compared to those obtained using capillary gas chromatography. The potential for other applications of these techniques in the alumina/aluminium industry will also be discussed.

## INTRODUCTION

The analysis of ionic species is of great importance in alumina (aluminium hydroxide) production, which involves the extraction of alumina from bauxite via the Bayer process, and also in the subsequent electrolytic reduction of the alumina to form aluminium metal. Solutes of interest in these industries include inorganic anions such as fluoride, chloride, sulfate, phosphate and silicate, as well as organic acids such as oxalate, succinate, malonate, and a variety of short-chained carboxylic acids. These anions are typically analyzed by traditional wet chemical or spectroscopic techniques [1] in matrices ranging from process waters to very complex, high ionic strength solutions such as liquors from the Bayer process. Ion chromatography (IC) is now a well accepted analytical technique which is finding increased usage in industrial production management, process control and environmental monitoring, and offers considerable advantages over classical methods of anion analysis in terms of ease of use, speed, precision and accuracy [2]. In this paper, two complex applications of IC in the alumina and aluminium industry are presented; the analysis of chloride and sulfate in Bayer liquors and the determination of fluoride in environmental samples prepared by hydroxide fusion. The IC results are compared to those obtained by classical wet chemical methods [1,3].

Capillary ion electrophoresis (CIE) (Waters' trade name: Capillary Ion Analysis, CIA) is a recently introduced analytical technique for the determination of inorganic and organic anions which results in a different separation selectivity compared to that obtained by conventional anion exchange in ion chromatography. The different selectivity is particularly useful for the analysis of samples containing short-chained carboxylic acids in the presence of inorganic anions [4,5]. There are a number of additional advantages of this approach for the

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determination of anions, including very rapid analysis times, high separation efficiences (> 250 000 theoretical plates have been demonstrated using this approach) and only nanoliter sample volumes are required. The use of CIE for the determination of oxalate in Bayer liquor, certainly the most critical anion analysis in the alumina industry, is discussed; and the results are compred to those obtained by capillary gas chromatography. The possibility for other applications of both IC and CIE in the alumina and aluminium industry will also considered.

## EXPERIMENTAL

### *Ion chromatographic system*

The ion chromatograph consisted of a Waters Chromatography Division of Millipore (Milford, MA, USA) Action Analyzer, WISP 712 autoinjector, Model 431 conductivity detector and an 820 Maxima data station. A Waters Reagent Delivery Module (RDM) was added to the system for Solid Phase Reagent (SPR) conductivity detection. Three analytical columns from the Waters IC-Pak range were used: an IC-Pak Anion (50 × 4.6 mm I.D.), an IC-Pak Anion HC (150 × 4.6 mm I.D.) and an IC-Pak Ion Exclusion (300 × 7.8 mm I.D.) column.

### *Capillary ion electrophoresis system*

The capillary electrophoresis instrument used was a Waters Quanta 4000 with a Waters 820 data station. Data were collected at 20 points per second with CIE. The separations were carried out using conventional fused-silica capillaries obtained from Waters. Detection was carried out using indirect photometry at 254 nm.

### *Reagents and procedures*

Water (18 MΩ) purified using a Millipore Milli-Q Water Purification System (Bedford, MA, USA) was used for all solutions. Millitrap $H^+$ sample preparation cartridges were obtained from Waters and Maxi-clean IC $H^+$ solid-phase extraction cartridges were obtained from Alltech (Deerfield, IL, USA). Sodium tetraborate [analytical reagent (AR) grade], glycerin [laboratory reagent (LR) grade], tartaric acid (LR), lithium hydroxide (AR) and boric acid (AR) were obtained from Ajax Chemicals (Sydney, Australia), as were the analytical grade sodium salts used for the preparation of all the anion standards. Sodium gluconate (LR) was obtained from Fluka (Buchs, Switzerland). Acetonitrile, methanol and *n*-butanol (HPLC grade) were obtained from Waters. Sodium chromate tetrahydrate was obtained from Aldrich (Milwaukee, WI, USA). Oxalic acid dihydrate [guaranteed reagent (GR) grade] was obtained from Merck (Darmstadt, Germany). The electroosmotic flow modifier, CIA-Pak OFM anion-BT, and Z1-Methyl are proprietary chemicals obtained from Waters. Eluents and electrolytes were prepared daily, filtered and degassed with a Waters solvent clarification kit. Specific operating conditions are provided as captions to the figures.

## RESULTS AND DISCUSSION

### *Analysis of chloride and sulfate in Bayer liquors by ion chromatography*

The Bayer process involves the extraction and precipitation of alumina (aluminium hydroxide) from bauxite. The process uses hot sodium hydroxide extraction and is cyclic; hence, soluble impurities accumulate in the process liquor stream. Chloride and sulfate themselves do not exert any detrimental effects upon the Bayer process unless present at extreme concentrations, however, the sodium associated with each anion can cause significant problems with alumina refining chemistry. Increased sodium levels cause decreased stability of oxalate and increased difficulty of oxalate removal from the liquor, increased liquor viscosity and elevated boiling point [1]. Bayer liquor is a very complex matrix which can contain approximately 3.5 *M* sodium hydroxide, 0.5 *M* sodium carbonate, 1.0 *M* sodium aluminate [$NaAl(OH)_4$], 0.4 *M* sodium chloride, 0.25 *M* sodium sulfate, 2–3.5 g/l sodium oxalate and 25–30 g/l total organic carbon present as organic acid anions. The high ionic strength and pH, coupled with the fact that aluminium hydroxide is insoluble in the pH range of approximately 5–10, make this a challenging sample for analysis by ion chromatography. The poor solubility of aluminium hydroxide restricts the choice of eluents to those which are of very low pH, very high pH or those which keep the alumina soluble through complex formation. The poor solubility of alumina also complicates the use of suppressed ion chromatography [1] for this analysis as the aluminium hydroxide

can precipitate in the suppressor, reducing both its lifetime and efficiency.

Hydroxide was initially chosen as an eluent with a Waters IC-Pak Anion column and conductivity detection for the determination of chloride and sulfate in Bayer liquor. Apparently well resolved peaks for chloride and sulfate resulted when using this eluent, however the results obtained for the quantitation of sulfate by IC were consistently high compared to those by gravimetric analysis. Dilution of the eluent indicated that sulfate was co-eluting with divalent organic acids, such as succinate and oxalate. A weaker, complexing eluent (borate/gluconate) was then used for the analysis of the same sample. This mobile phase allowed resolution of sulfate from both succinate and oxalate; however, the extreme pH of the sample caused a system peak [6,7] which interfered with the quantitation of chloride. The chromatographic run time was also significantly increased. Tartrate/borate eluents are typically operated in the pH range 3–5 [8] and offer a considerable advantage for this analysis as their use strongly discriminates against the retention of organic acid anions. Both mono- and divalent organic acid anions elute at the void volume with tartrate/borate as a result of the low eluent pH and high boric acid concentration. Hence, this eluent is particularly suited to the analysis of chloride and sulfate in Bayer liquors and also for other samples such as soil and plant extracts. Fig. 1 shows a chromatogram of chloride and sulfate in a Bayer liquor sample using an eluent of 3.0 m*M* tartaric acid, 0.6 *M* boric acid and an IC-Pak Anion HC column with conductivity detection. The sample was diluted 1:1000 with 3 m*M* tartaric acid, filtered through 0.45-$\mu$m Millipore Millex durapore filter and passed through a Waters Millitrap cartridge in order to improve the baseline stability [9]. Table I shows comparative data for Bayer liquor analysis by IC and conventional wet chemical methods for 25 replicates of two different samples from each of three refinery sites. The % variation (at 1 $\sigma$) for each of the samples is given in brackets in the Table. The precision of the titrimetric method for chloride analysis was approximately 0.90% and the precision of the gravimetric method for sulfate analysis was approximately 2.55% at 1 $\sigma$. The results obtained using IC were similar to the conventional methods, with IC showing comparable precision for both chloride and sulfate, better accuracy for chloride (the titrimetric method overestimated chloride by approximately 3%) and the added advantage of significantly improved throughput for these analyses.

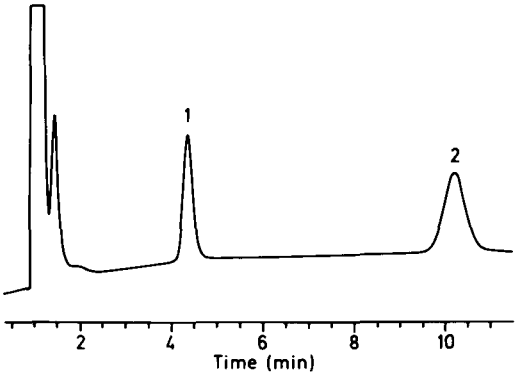


Fig. 1. Chromatogram of a Bayer liquor sample using a tartrate/borate eluent and conductivity detection. Conditions: column, Waters IC-Pak Anion HC; eluent, 3.0 m*M* tartaric acid, 0.6 *M* boric acid adjusted to pH 4.5 with hydroxide; flow-rate, 2.0 ml/min; injection volume, 100 $\mu$l; detection, conductivity; sample preparation, 1:1000 dilution with 3 m*M* tartaric acid, filtration through a Millex 0.45-$\mu$m durapore filter and passage through a Waters Millitrap $H^+$ cartridge. Solutes: 1 = chloride (31 ppm); 2 = sulfate (43 ppm).

*Analysis of fluoride in aluminium refinery environmental samples by ion chromatography*

Fluoride is a component of the electrolyte (cryolite) used in the electrolytic reduction of alumina to aluminium metal and can be released into the atmosphere as a result of the production process. Hence, the determination of fluoride in the environs of aluminium refineries is of great importance, considering the toxicity of this anion to both flora and fauna. Samples from refinery environs (such as water, soil, vegetation and even bones from carrion) are required to be analyzed for fluoride, although as yet no standardized method for sample preparation exists. The two current methods used for sample preparation prior to fluoride analysis are acid leaching or hydroxide fusion. All the samples for this work were prepared by hydroxide fusion prior to analysis by either ion chromatography or an autoanalyzer with on-line distillation and colourimetric determination after reaction with alizarin fluorine blue–lanthanum reagent [3].

Typically, the best ion chromatographic ap-

TABLE I

COMPARATIVE DATA FOR THE ANALYSIS OF CHLORIDE AND SULFATE IN BAYER LIQUORS BY ION CHROMATOGRAPHY AND CONVENTIONAL METHODS

The precision of the titrimetric method for chloride analysis and the gravimetric method for sulfate analysis was approximately 0.90% and 2.55%, respectively.

| Sample | Cl (g/l) (% variation) | | $SO_4$ (g/l) (% variation) | |
|---|---|---|---|---|
| | IC ($n$ = 25) | Titrimetric | IC ($n$ = 25) | Gravimetric |
| Refinery 1-a | 12.9 (1.8) | 13.4 | 23.9 (1.6) | 21.4 |
| Refinery 1-b | 14.6 (2.1) | 15.1 | 25.7 (2.2) | 25.7 |
| Refinery 2-a | 14.8 (1.1) | 14.5 | 30.4 (1.8) | 30.7 |
| Refinery 2-b | 17.2 (2.6) | 17.6 | 35.4 (1.7) | 35.9 |
| Refinery 3-a | 16.7 (2.1) | 17.3 | 14.8 (2.7) | 15.4 |
| Refinery 3-b | 18.4 (1.6) | 18.6 | 16.3 (2.7) | 17.2 |

proach for the analysis of fluoride in high ionic strength samples is based on an ion-exclusion separation [10]. A Waters IC-Pak ion-exclusion column was initially used with a camphorsulfonic acid eluent and conductivity detection for the determination of fluoride in the hydroxide fused samples. Fig. 2 shows a chromatogram of fluoride in a forage vegetation sample after being ashed, fused with 1.5 *M* hydroxide, diluted 5× and passed through a 0.45-$\mu$m Millex durapore filter. The fluoride peak was well resolved from the large void disturbance despite the direct injection of 0.3 *M* hydroxide into the chromatographic system. However, a problem occurred with this analysis as a result of the high silica (up to 40% w/w) levels in the soil and plant fusion samples. After approximately 40 sample injections, the fluoride peak response started to decrease and the calibration curve became non-linear. This decreased response was found to occur as a result of silica build-up on the ion-exclusion column. While the column could be regenerated by washing with strong acid then water overnight to restore the fluoride response to that of a new column, this approach was obviously of limited utility.

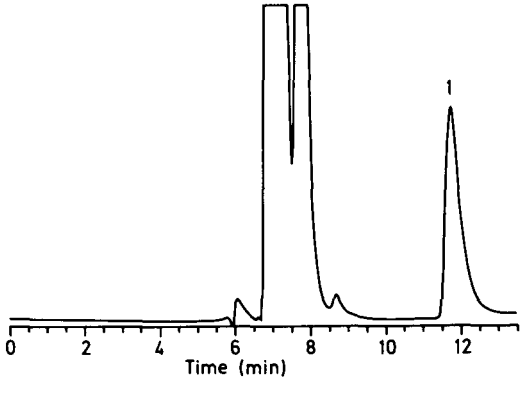


Fig. 2. Chromatogram of a forage vegetation sample using a camphorsulfonic acid eluent and an ion-exclusion separation. Conditions: column, Waters IC-Pak Ion Exclusion; eluent, 2.0 m*M* camphorsulfonic acid; flow-rate, 1.0 ml/min; injection volume, 100 $\mu$l; detection, conductivity; sample preparation, ashing, hydroxide fusion, 1:5 dilution with water: Solute: 1 = fluoride (34 ppm).

The next approach studied was to use an ion-exchange separation with conductivity detection, however the 1.5 *M* hydroxide in the fused sample required much larger sample dilutions than was necessary when using an ion-exclusion separation in order to resolve the weakly retained fluoride peak from the large void disturbance. A borate mobile phase was initially used but the large sample dilution meant that the fluoride concentration in the final sample diluent was frequently below the detection limit of this approach. Solid-phase reagent conductivity detection was then investigated as this detection technique has been shown to permit a high degree of sample matrix independence [11,12]. Hydroxide, borate and carbonate/bicarbonate mobile phases are applicable for use with this detection method, with the latter giving the best separation selectivity for this application. The calibration curve obtained using this eluent and detection method was linear in the range of 0.01 to 5 ppm

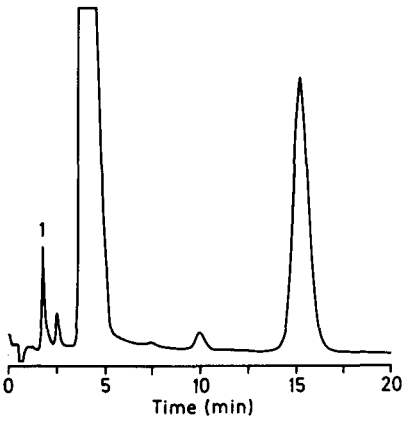


Fig. 3. Chromatogram of a forage vegetation sample using a carbonate/bicarbonate eluent and an ion-exchange separation with solid-phase reagent conductivity detection. Conditions: column, Waters IC-Pak Anion; eluent, 1.2 m*M* carbonate, 1.2 m*M* bicabonate; flow-rate, 1.2 ml/min; injection volume, 100 μl; detection, SPR-conductivity at 0.8 ml/min; sample preparation, ashing, hydroxide fusion, 1:50 dilution with water then 2 ml through a Maxi-clean $H^+$ cartridge with the first 0.5 ml discarded to waste. Solute: 1 = fluoride (0.4 ppm).

fluoride. A Millitrap $H^+$ cartridge was used to neutralize the fused sample in order to further decrease the magnitude of the void peak, however the silica in the samples eventually bound to ion-exchange fiber in the cartridge resulting in decreased fluoride recoveries. Maxi-clean $H^+$ cartridges proved more appropriate for this particular application as they are single use devices, unlike the Millitrap $H^+$ cartridge; consequently, they did not have problems with silica build-up. Fig. 3 shows a chromatogram of fluoride in a fused forage vegetation sample after a 50× dilution and passage through a Maxi-clean $H^+$ cartridge using a carbonate/bicarbonate eluent, a Waters IC-Pak Anion column with solid phase reagent conductivity detection. Quantitative recoveries (90–105%) were obtained for samples spiked with fluoride and Table II shows the reproducibility for three vegetation samples, each passed through three separate cartridges. The agreement between the results obtained by ion chromatography and the autoanalyzer method are shown in Table III. The correlation between the two methods was only fair and this probably occured as a result of the two methods performing slightly different analyses. The autoanalyzer, with on-line distillation, determines the "total" fluoride while ion chromatography only determines "free" fluoride in the fused sample, hence some difference between the results is not surprising. Ion chromatography could be expected to give better correlation with the autoanalyzer method if the samples were prepared using an acid leach rather than hydroxide fusion, as the acid leach only extracts free fluoride. The ion chromatographic and autoanalyzer methods showed very good correla-

TABLE II

REPRODUCIBILITY OF FLUORIDE QUANTITATION WITH MAXI-CLEAN IC $H^+$ SPE CARTRIDGES

| Sample No. | Fluoride concentration (ppm after 50× sample dilution) |
|---|---|
| 42 | 0.211 |
| 42 | 0.213 |
| 42 | 0.239 |
| Average ± R.S.D.[a] | 0.221 ± 7.2% |
| H | 0.196 |
| H | 0.168 |
| H | 0.188 |
| Average ± R.S.D. | 0.182 ± 9.7% |
| 56 | 0.668 |
| 56 | 0.680 |
| 56 | 0.679 |
| Average ± R.S.D. | 0.670 ± 1.0% |

[a] R.S.D. = relative standard deviation.

TABLE III

COMPARATIVE DATA FOR THE ANALYSIS OF FLUORIDE IN VEGETATION AND WATER BY ION CHROMATOGRAPHY AND AUTOANALYZER

| Sample | | Ion chromatography (ppm fluoride) | Autoanalyzer (ppm fluoride) |
|---|---|---|---|
| Vegetation | 1 | 244 | 210 |
| | 2 | 253 | 250 |
| | 3 | 177 | 185 |
| | 4 | 321 | 395 |
| | 5 | 78 | 75 |
| Water | 1 | 0.07 | <0.1 |
| | 2 | 3.5 | 3.6 |
| | 3 | 3.4 | 3.6 |
| | 4 | 0.4 | 0.4 |
| | 5 | 0.9 | 0.8 |

tion for water samples, where the fluoride was only present as the free anion.

*Analysis of oxalate in Bayer liquors by capillary ion electrophoresis*

Capillary ion electrophoresis is a relatively new separation technique which utilizes narrow diameter capillaries (typically polyimide-coated, 25–100 μm fused silica) to separate ionic solutes according to their mobility under the influence of an applied potential (usually 10–30 kV). The separation of inorganic anions and low-molecular-weight organic acids by capillary ion electrophoresis (also termed Capillary Ion Analysis) offers a totally different separation selectivity compared to that obtained using conventional anion exchange in ion chromatography [4]. The selectivity of CIE is particularly advantageous for the determination of oxalate in Bayer liquors, which, as discussed previously, are a strongly alkaline matrix containing very high levels of both inorganic anions and organic acids. The mobility of oxalate is intermediate between the very mobile inorganic anions in the sample, such as chloride and sulfate, and the less mobile organic acids, such as succinate and acetate, hence the peak is well resolved from the other components in Bayer liquor.

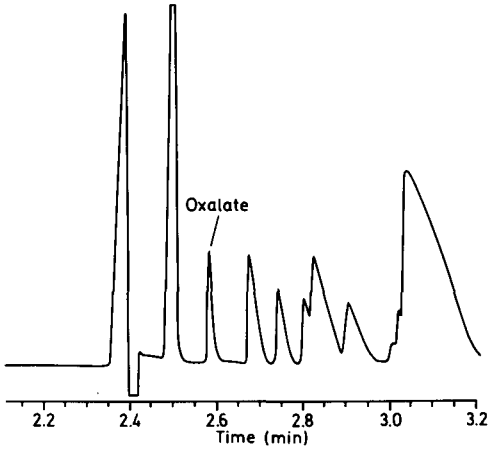


Fig. 4. Electropherogram of oxalate in a Bayer liquor sample obtained by CIE using optimized conditions. Conditions: capillary, 60 cm × 75 μm I.D. fused silica; power supply, negative at 20 kV; electrolyte, 5 m*M* chromate, 2.5 m*M* CIA-Pak OFM anion-BT, 5% (v/v) methanol, 1.0 *M* Z1-Methyl at pH 10.5; injection, hydrostatic for 45 s; detection, indirect UV at 254 nm; sample preparation, 1:200 dilution with water. Solute: oxalate (approximately 10 ppm).

A variety of different conditions, such as capillary diameter and length, electrolyte composition, running voltage, sample dilution and injection time were investigated in order to optimize the analysis of oxalate by CIE. The use of a 60 cm × 50 μm I.D. capillary gave better resolution of oxalate from the other peaks in the sample matrix than did a 60 cm × 75 μm I.D. capillary, however the response for the oxalate peak was less and the area precision was poor compared to that obtained when using the 75-μm capillary. The use of 100 cm x 75 μm I.D. capillary gave improved resolution when compared to a 60-cm capillary, although run times were significantly longer with a 100-cm capillary. The electrolyte used for the analysis of high-mobility anions in CIE typically contains 5 m*M* chromate and 0.5 m*M* CIA-Pak OFM anion-BT at a pH of 8.0 [4], however adjusting the electrolyte pH to 10.5 and adding 5% methanol and 1.0 *M* Z1-Methyl (a zwitterionic reagent used to prevent the adsorption of charged macromolecules to the capillary wall) improved the baseline noise and reproducibility of the oxalate analysis. Fig. 4 shows an electropherogram of oxalate in a Bayer liquor sample obtained by CIE using the optimized conditions. The two large peaks migrating before oxalate are chloride and sulfate, respectively, while the later migrating peaks are organic acids such as tartrate, succinate and acetate. This separation demonstrates several advantages of CIE in comparison to IC. CIE is a very matrix independent technique, *e.g.*, cations do not participate in the separation since they travel in the opposite direction to the anions and neutral solutes are carried along by the electroosmotic flow and have appreciably longer migration times than ionic solutes. The neutral solutes and late migrating anionic species can simply be purged from the capillary once the desired separation is obtained and so no "void" peak appears in the electropherogram. The high pH of the sample did not create any problems as hydroxide is the most mobile anion and migrates well resolved from all other ionic solutes, which is not the case for most ion chromatographic separations. No sample pretreatment other than dilution in water was necessary and the run time was less than 5 min. Between samples, the capillary can be purged with hydroxide, water and then electrolyte to re-

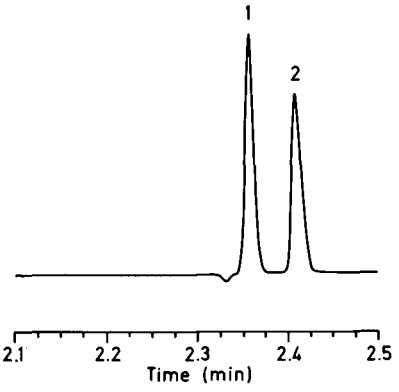


Fig. 5. Electrophetogram of chloride and sulfate in Bayer liquor obtained by CIE using standard conditions. Conditions as for Fig. 4 except: electrolyte, 5 m*M* chromate, 0.5 m*M* CIA-Pak OFM anion-BT; injection, hydrostatic for 30 s; sample preparation, 1:100 dilution with water. Solute: 1 = chloride, 2 = sulfate.

move any solutes which may adhere to the charged capillary walls ensuring a reproducible capillary surface, hence stable migration times.

The best results obtained for the replicate analysis of oxalate in a typical Bayer liquor sample were 2.87 g/l (at 0.38% R.S.D.) and 2.92 g/l (at 0.30% R.S.D.) using external and standard addition calibration respectively; compared to 2.70 g/l (at 0.74% R.S.D.) by capillary gas chromatographic analysis for the same sample. The batch precision is more typically in the order of 1% R.S.D. and present investigations centre upon determining the long term precision of the CIE method for oxalate analysis and the determination of other species in the liquor. Fig. 5 shows an electropherogram, obtained using the standard CIE conditions for the analysis of high mobility anions (chloride and sulfate) in a Bayer liquor sample. The precision for this analysis by CIE is very similar to that obtained by IC, however the run time is less than 3 min compared to 12 min when using ion chromatography.

## CONCLUSIONS

Both ion chromatography and capillary ion electrophoresis offer practical, alternative analytical methods for the analysis of chloride, sulfate and oxalate in alumina refinery liquors. Ion chromatography shows comparable precision (*ca.* 2% for chloride and 3% for sulfate at one $\sigma$) to titrimetric and gravimetric methods for the analysis of chloride and sulfate respectively in Bayer liquors, with better accuracy for chloride and significantly improved throughput for these analyses. The selectivity of capillary ion electrophoresis is particularly appropriate for the determination of oxalate in Bayer liquor. Optimization of conditions such as capillary dimensions, electrolyte composition, running voltage, sample injection and dilution permit a within batch precision of less than 1% R.S.D. for oxalate in Bayer liquor. The results obtained by CIE showed reasonable agreement to those obtained by the significantly more complex and time consuming derivatization capillary gas chromatographic method. The combination of an ion-exchange separation, carbonate/bicarbonate eluent with solid phase reagent conductivity detection and sample clean-up using a Maxi-clean IC $H^+$ cartridge allows the ion chromatographic determination of fluoride in hydroxide fused soil and plant samples from aluminium refinery environs. The results obtained by ion chromatography showed only fair agreement to those from an autoanalyzer, although ion chromatography could be expected to give very similar results to the autoanalyzer method if the samples were prepared using the more conventional approach of acid leaching rather than hydroxide fusion. Both ion chromatography and capillary ion electrophoresis show great potential for further applications in the aluminium/alumina industries and future work involves determining the long term precision of the oxalate analysis and the quantitation of other species, such as chloride, sulfate, fluoride and additional organic acids in Bayer liquors by CIE.

## ACKNOWLEDGEMENTS

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# Author Index

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Journal of Chromatography

# NEWS SECTION

## SHORT CONFERENCE REPORT

### INTERNATIONAL ION CHROMATOGRAPHY SYMPOSIUM 1991, DENVER, CO, USA, OCTOBER 6–9, 1991

The International Ion Chromatography Symposium 1991 was held October 6–9 at the Hyatt Regency Tech Center in Denver, CO, USA. Over 240 scientists from 13 countries and 37 states participated in this technical symposium. John D. Lamb, Associate Professor of Chemistry at Brigham Young University in Provo, UT, USA, served as Program Chairman for the meeting and worked with a Scientific Committee of eleven renowned scientists in the field of ion chromatography (IC) to develop a technical program of impressive depth and diversity.

Purnendu K. Dasgupta of Texas Tech University presented the keynote lecture, "Ion Chromatography: *Quo Vadis Domine?*". The program included 102 technical presentations on IC in a variety of topic areas including: fundamental principles and general aspects of IC; separations using novel stationary and mobile phases; novel applications; sample handling and pretreatment; industrial problem-solving; gradient separations; detection and post-column treatment; and capillary electrophoresis for inorganic ions. The conference was truly international in scope with scientists coming from Australia, Austria, Belgium, Canada, Denmark, Germany, Italy, Japan, Russia, Sweden, Switzerland, the United Kingdom, and the United States.

A highlight of the meeting was the recognition of two scientists for their outstanding contributions in developing the methodology of IC. Award recipients were William Jones of the Waters Chromatography Division of Millipore Corporation and John Riviello of Dionex Corporation. Jones presented an award lecture on "The Scope of Capillary Ion Analysis", and Riviello gave an award lecture on "Conductometric Detection in Ion Chromatography: A Historical Perspective".

The next International Ion Chromatography Symposium will be held at Johannes-Kepler-University in Linz, Austria, September 21–24, 1992. Professor Günther Bonn of the Analytical Chemistry Department at Johannes-Kepler-University will serve as Program Chairman. For more information contact: Century International, P.O. Box 493, Medfield, MA 02052, USA. Tel.: (+1-508) 359-8777; Fax: (+1-508) 359-8778.

Janet R. Strimaitis
Medfield, MA, USA

Fig. 1. John Lamb, Brigham Young University, Program Chairman; William Jones, Waters Division of Millipore, Award Recipient; and John Riviello, Dionex Corporation, Award Recipient.

Fig. 2. The Scientific Committee. Front row (left to right), James Fritz, Iowa State University; John Lamb, Brigham Young University; Petr Jandik, Waters Division of Millipore; Donald Pietrzyk, University of Iowa; back row (left to right), Paul Haddad, University of New South Wales; Richard Cassidy, University of Saskatchewan; Hamish Small, Consultant; Doug Gjerde, Sarasep, Inc; John Stillian, Dionex Corporation.

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# Stationary Phases in Gas Chromatography

*by* **H. Rotzsche**, *VEB Chemiewerk, Nünchritz, Radebeul, Germany*

Journal of Chromatography Library Volume 48

The primary aim of this volume is to make the chemist familiar with the numerous stationary phases and column types, with their advantages and disadvantages, to help in the selection of the most suitable phase for the type of analytes under study. The book also provides detailed information on the chemical structure, physicochemical behaviour, experimental applicability, physical data of liquid and solid stationary phases and solid supports. Such data were previously scattered throughout the literature. To understand the processes occurring in the separation column and to offer a manual both to the beginner and to the experienced chromatographer, one chapter is devoted to the basic theoretical aspects. Further, as the effectiveness of the stationary phase can only be considered in relation to the column type, a chapter on different column types and the arrangement of the stationary phase within the column is included.

The secondary aim of this book is to stimulate the development of new and improved standardized stationary phases and columns, in order to improve the reproducibility of separations, as well as the range of applications.

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*Journal of Chromatography* and *Journal of Chromatography, Biomedical Applications*

| MONTH | O 1991–F 1992 | M | A | M | J | |
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| Journal of Chromatography | Vols. 585–593 | 594/1 + 2<br>595/1 + 2 | 596/1<br>596/2<br>597/1 + 2 | 598/1<br>598/2<br>599/1 + 2<br>600/1<br>600/2 | 602/1 + 2 | The publication schedule for further issues will be published later. |
| Cumulative Indexes, Vols. 551–600 | | | | | | |
| Bibliography Section | | 610/1 | | | 610/2 | |
| Biomedical Applications | Vols. 573 and 574 | 575/1<br>575/2 | 576/1 | 576/2<br>577/1 | 577/2 | |

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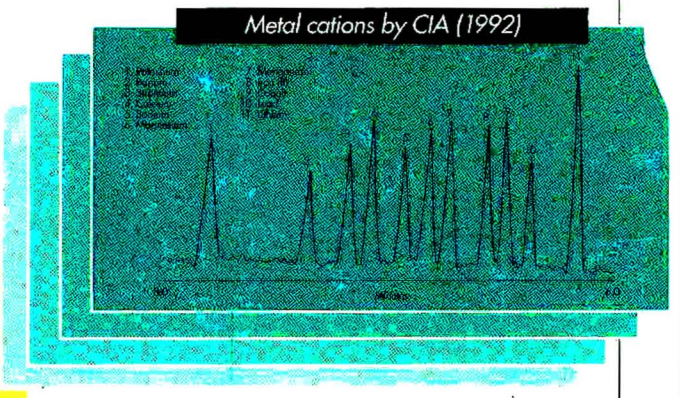


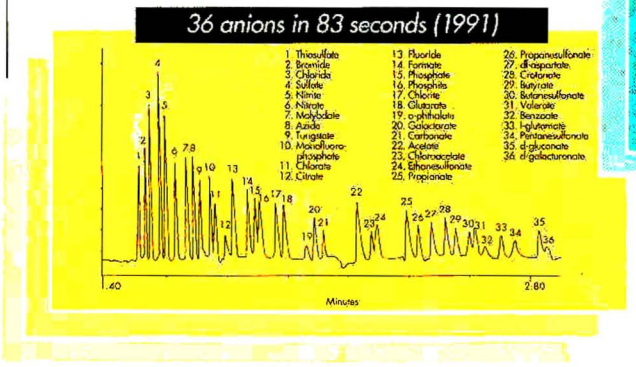


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