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STUDIES ON SAMPLE PRECONCENTRATION IN ION CHROMATOGRAPHY

VIII. PRECONCENTRATION OF CARBOXYLIC ACIDS PRIOR TO ION-EXCLUSION SEPARATION

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SUMMARY

A procedure for the preconcentration of low-molecular-weight carboxylic acids is reported. Up to 50 ml of a sample containing trace levels of carboxylic acids was loaded onto a low-capacity anion-exchange precolumn conditioned with an eluent of methanesulphonate at pH 9.0. The carboxylate anions were found to bind quantitatively to the precolumn even when the sample contained predominantly neutral, undissociated acids. The bound ions were then transferred to an ion-exclusion analytical column using methanesulphonic acid at pH 2.7 and eluted from the analytical column with the same eluent. UV detection at 200 nm was used. The method was used for the simultaneous analysis of formic, acetic, propionic and butyric acids, giving detection limits of 5.9, 9.4, 5.6 and 9.2 ppb, respectively, for a 10-ml sample volume, and a precision of 1.85% relative standard deviation. The procedure was successfully applied to the determination of carboxylic acids in Antarctic ice.

INTRODUCTION

Ion-exclusion chromatography is widely used for the determination of carboxylic acids. This technique, first described by Wheaton and Bauman in 1953[1], utilises a cross-linked, resin-based, cation-exchange column to effect the separation of weak acids (both organic and inorganic) and even small neutral species. Strong acid anions are excluded from the resin according to the Donnan principle and elute at the void volume of the column[2]. Weaker acid anions existing largely in the molecular form are retained on the stationary phase by a combination of ion exclusion, size exclusion and hydrophobic interactions. For low-molecular-weight acids, ion exclusion is the dominant retention mechanism and the retention times are strongly correlated with the pK_a of the acid[3]. Mineral acid solutions are typically used as eluents in ion-exclusion chromatography to ensure that the organic acids are predominantly in the molecular form, resulting in increased retention times. However, water has also been used as an eluent[3].

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Detection of the eluted weak acid species has conventionally been carried out by spectrophotometric methods, although recently other detection methods have also been used. Most aliphatic carboxylic acids absorb only weakly, hence the sensitivity obtained when using direct UV detection is poor. Detection limits for these solutes are typically of the order of 1 ppm or greater with spectrophotometric detection[1]. Lower detection limits have been reported for other solutes, such as 10 ppb* for dimethyl sulphoxide after separation on an ion-exclusion column using direct UV detection at 195 nm (ref. 4).

Conductimetric detection may be used with a hydrochloric acid eluent after suppression of the background conductivity with a cation-exchanger in the silver form[5], although the suppressor column ultimately becomes blocked with precipitated silver chloride. Itoh and Shinbori[6] found that non-suppressed conductimetric detection was possible in ion-exclusion chromatography when weakly conducting carbonic acid was used as the eluent. Tanaka and Fritz[7] have reported a similar approach using a benzoic acid eluent and they achieved detection limits from 60 ppb to 1.4 ppm for the C_1–C_4 aliphatic carboxylic acids. The same authors[8] have recently reported the use of post-separation "enhancement" columns for conversion of carbonic acid to the more conductive anionic form to improve detection sensitivity. A detection limit of 90 ppb was achieved using two enhancement columns in series. A similar approach has been presented by Murayama *et al.*,[9] who used a suppressor column which exchanged H^+ for K^+ to achieve conversion of the weak acid solute to the more conductive anionic form and the background eluent (sulphuric acid) to the less conductive potassium sulphate. Sensitivity for acetic acid was increased 33 fold using the suppressor column.

Electrochemical detection[10] has also been used in conjunction with ion-exclusion chromatography for the determination of sulphite. Sulphuric acid was used as the eluent and a detection limit of 100 ppb was obtained; however, this approach is of limited utility in ion-exclusion chromatography as most of the solutes amenable to this separation mode are not electroactive.

The above discussion illustrates that detection limits for most weak acid species, even with the aid of suppressor and enhancement columns, are generally in the order of 100 ppb or greater and these are similar to detection limits obtained for anions by conventional ion chromatography. In ion chromatography the most common way to lower detection limits is to preconcentrate the solute ions from the sample onto a small ion-exchange precolumn. The trapped solute ions are then eluted to the analytical column for separation and quantitation[11,12]. However, the analogous approach is not viable for ion-exclusion chromatography because water acts an eluent and can elute the weak acid solutes from an ion-exclusion precolumn during sample loading.

In this paper we present an ion chromatographic preconcentration method for the analysis of trace levels of carboxylic acids. The solutes are trapped as anionic species on an anion-exchange concentrator column and eluted from this column as the neutral acid species, to be separated on an ion-exclusion analytical column, with subsequent detection by direct UV absorption.

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* Throughout this article the American billion (10^9) is meant.

EXPERIMENTAL

Instrumentation

The liquid chromatograph used consisted of a Millipore Waters (Milford, MA, U.S.A.) Model M590 programmable pump and events unit, Model M481 variable-wavelength UV detector operated at 200 nm, Model M730 data module, two pneumatically controlled high-pressure, six-port, switching-valves and two low-pressure, solvent select valves. All the valves were combined in a Waters automated valve switching (WAVS) unit. A Model U6K injector was incorporated into the liquid chromatograph when manual injection was required.

A Bio-Rad (Richmond, CA, U.S.A.) HPX-87H organic acid analysis column (300 × 7.8 mm I.D.) was used as the analytical column. A Waters IC anion concentrator (5.0 × 6.0 mm I.D.) packed with methacrylate resin to give a total ion-exchange capacity of 2.15 μequiv. was used as the concentrator column. This column was housed in a Waters Guard Pak precolumn module. A schematic diagram of the apparatus is illustrated in Fig. 1 and Fig. 2 shows details of the interconnections of the valves.

Reagents

All water was doubly distilled and passed through a Millipore (Bedford, MA, U.S.A.) Milli-Q water purification apparatus. Standard solutions (1000 ppm) of formic, acetic, propionic and *n*-butyric acids were prepared by dissolving the appropriate amounts of the analytical-grade acids in pure water. These solutions were diluted daily with the aid of Gilson (Villiers, France) Pipetman autopipettes to give the required trace solutions.

Methanesulphonic acid (5 m*M*) operated at a flow-rate of 0.8 ml/min was used as the eluent for the separation of the organic acids, and methanesulphonate (5 m*M*)

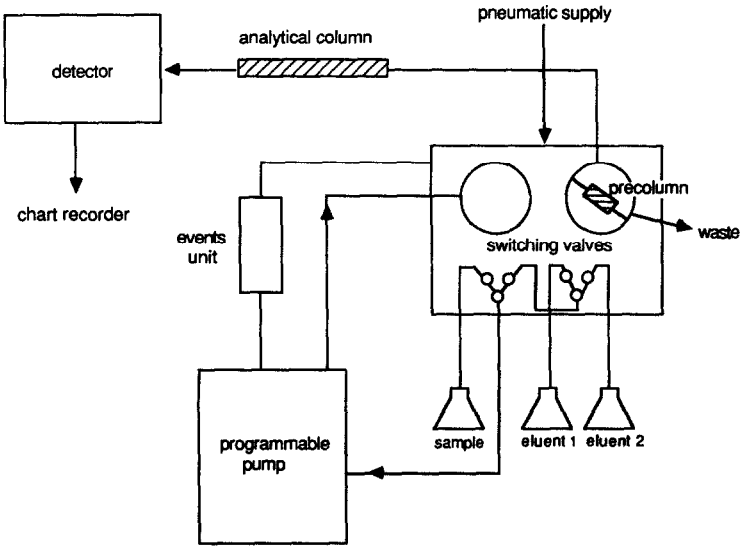

Fig. 1. Schematic diagram of the preconcentration apparatus employed in this study.

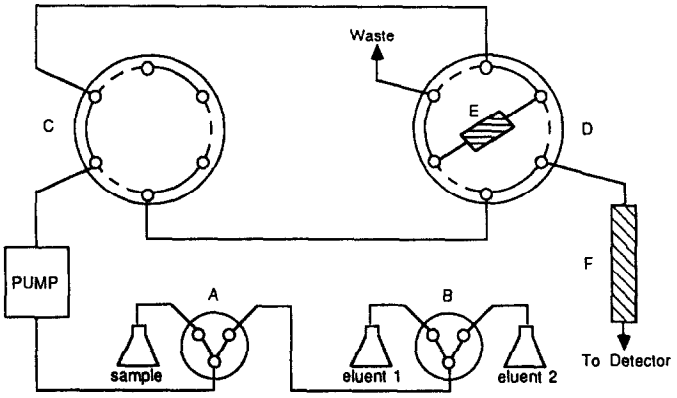

Fig. 2. Details of the interconnections of the valves. A, B = Low-pressure solvent select valves; C, D = high-pressure column switching valves; E = anion-exchange concentrator column; F = ion-exclusion column.

adjusted to a pH of 9.0 was used as the eluent to condition the concentrator column prior to sample loading. Eluents were diluted daily from stock solutions which were prepared by dissolving weighed amounts of analytical grade methanesulphonic acid in approximately 800 ml of water, after which the pH of the solution was adjusted (if required) by dropwise addition of 1.0 *M* lithium hydroxide and the solution diluted to 1 l. Each eluent was filtered through a 0.45-μm membrane filter and degassed in an ultrasonic bath before use.

Procedures

The preconcentration of samples was carried out by varying the flow-paths in a timed sequence, using a similar procedure to that previously reported by us for the preconcentration of anionic solutes[13]. This procedure was modified to include the use of two eluents and an outline of the basic program required for the preconcentration of organic acids is given in Table I.

RESULTS AND DISCUSSION

Selection of chromatographic approach

On-line preconcentration with ion-exchange concentrator columns is routinely used in ion chromatography for the determination of ionic solutes at the ppb level. Quantitative recovery of solute anions can be achieved for sample volumes up to 100 ml[14], provided appropriate conditions are chosen. The extension of this approach to the preconcentration of carboxylic acids using ion-exclusion concentrator and analytical columns is not practical because water acts as an eluent in ion-exclusion chromatography[3] and therefore the acids will not bind quantitatively to the concentrator column during the sample loading step.

Carboxylic acids may be preconcentrated in their anionic forms on an anion-exchange precolumn[15] but their separation by ion exchange is difficult because analytical ion exchangers exhibit very poor selectivity for carboxylate anions. In addition, it is likely that interference from inorganic anions will be encountered.

TABLE I

BASIC OUTLINE OF A TYPICAL PROGRAM FOR THE PRECONCENTRATION OF ORGANIC ACIDS

Step No.	*Solution delivered*	*Flow-rate (ml/min)*	*Volume delivered (ml)*	*Purpose of step*
1	Eluent*	1.0	20.0	Equilibratio of ion-exchange precolumn
2	Sample	5.0	15.0	Flush pump and tubing with sample
3	Sample	1.0	10.0	Load sample onto pre-column
4	Eluent**	5.0	15.0	Flush pump and tubing with eluent 2
5	Eluent 2	0.5	1.0	Quantitatively strip acids from concentrator column
6	Eluent 2	0.8	16.0	Separate acids on analytical column***

* 5 m*M* methanesulphonate, pH 9.0.
** 5 m*M* methanesulphonic acid, pH 2.7.
*** The precolumn is removed from the flow-path for the separation step in order to minimise baseline drift. On completion of step 6, the program returns to step 1.

Alternatively, carboxylic acids could be preconcentrated in their anionic form on an ion-exchange precolumn, followed by elution of the trapped anions to an ion-exclusion analytical column, where they would be separated as the acid species. The major difficulty of this approach is to achieve reproducible, quantitative binding of the acidic solutes to the anion-exchange precolumn.

The form in which the carboxylic acid is present in the sample has an obvious influence of the degree of binding onto the concentrator column. If the ion-exchange selectivity coefficient for the carboxylate anion is sufficiently high, then this ion can be expected to be quantitatively bound provided that the sample does not contain significant concentrations of ionic species with greater selectivity coefficients. This binding will push the dissociation equilibrium of the acid towards increased ionisation, so that quantitative binding can be anticipated even for samples in which the acid is present predominantly in the neutral protonated form. Alternatively, the pH of the sample solution can be adjusted to a point where the carboxylic acids in the sample are present predominantly in their anionic forms and can therefore bind to the concentrator column.

Methanesulphonic acid, used at pH values of 2.7 or 9.0, was chosen as the eluent for two reasons. First, methanesulphonate at pH 9.0 was appropriate for conditioning of the concentrator column prior to sample loading since this eluent had been shown previously to permit quantitative binding of weakly retained anions during sample loading[16]. The pH of this eluent was high in order to encourage dissociation of the carboxylic acids. Second, methanesulphonic acid at pH 2.7 was suitable for the transfer of solutes to the ion-exclusion analytical column and for their subsequent separation since this eluent is known to give an excellent separation of car-

boxylic acids on the HPX-87H column[17]. Use of different eluents to condition the concentrator column and to separate the solutes is inadvisable because of the initial baseline disturbance created in the final chromatogram, even when direct UV detection was used. In fact, the greatest practical difficulty encountered when attempting to implement the proposed preconcentration method was the large disequilibration in the final chromatogram resulting when the ion-exchange precolumn was placed in-line with the ion-exclusion analytical column for transfer of the bound solutes. The use of the same concentration of eluent ion, *i.e.* 5 m*M* methanesulphonate, for the different stages of the preconcentration procedure minimised this disturbance to some extent, although a very large void peak still occurred.

Methanesulphonic acid, even at 5 m*M*, can also be used as an eluent with non-suppressed conductivity detection in ion-exclusion chromatography[17]. However, it was not possible to use conductivity detection with the precocentration approach described as the baseline disturbance at the start of the chromatogram was too great to allow quantitation of early eluting solutes.

Preconcentration of carboxylic acids

Fig. 3 shows the chromatogram obtained after preconcentrating a 10-ml mixture of 200–400 ppb C_1–C_4 aliphatic carboxylic acids on an anion-exchange precolumn, followed by ion-exclusion separation. Quantitative recoveries were obtained for all peaks since the peak areas agreed to within 1% with those obtained by direct injection of an equivalent amount (10 μl of 200–400 ppm) of the acids. The chromatogram also shows two additional peaks: a large solvent peak as previously dis-

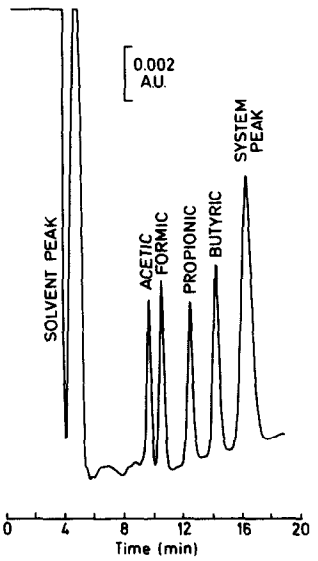

Fig. 3. Preconcentration of a standard solution of carboxylic acids. Conditions: concentrator column, Waters IC anion concentrator; analytical column, Bio-Rad HPX-87H organic acid analysis column. Eluents: 5 m*M* methanesulphonate at pH 9.0 (eluent 1) or pH 2.7 (eluent 2). Sample: 10 ml of 200 ppb formic, 400 ppb acetic, 200 ppb propionic and 400 ppb butyric acids loaded at a flow-rate of 1.0 ml/min. Strip volume: 700 μl. Detection: UV absorption at 200 nm, operated at 0.02 a.u.f.s.

cussed and a late eluting system peak. The system peak appeared when any preconcentration run was made and a small peak was also present when samples were injected directly without preconcentration. One possibility was that this peak was due to carbonate present in the samples, however no correlation could be found between the amount of carbonate or bicarbonate injected and the height of the system peak. The system peak may be attributable to an eluent impurity. The retention time of the system peak could be manipulated by changes in the eluent pH, so conditions could be found under which interference with carboxylate solutes was eliminated.

In the initial stages of this work, the sample solutions to be concentrated were neutralised with 0.01 *M* lithium hydroxide to ionise the acidic solutes and so encourage quantitative binding of the solutes on the precolumn. However, it was found that this step was unnecessary since the recoveries obtained for the preconcentration of untreated sample solutions were identical to those obtained when the acids were neutralised. It appears therefore that the carboxylate anions bind very readily to the concentrator column, and this promotes further dissociation of the acid. For very weak acids such as hydrogen cyanide, it would perhaps be necessary to neutralise the solution, although excess hydroxide in the sample should be avoided as it will compete with the acid anion for exchange sites on the precolumn and so decrease the range of linear sample loading.

Fig. 4 shows the calibration plots obtained when preconcentrating 10-ml volumes of formic, acetic and propionic acids (loaded individually). Detection limits, determined as the concentration producing a signal-to-noise ratio of 3, were 6, 9, 6 and 9 ppb for these acids and butyric acid, respectively, when using a 10-ml sample volume. Larger sample volumes also gave quantitative binding of the organic acid anions, just as in conventional ion chromatography[14]. The preconcentration of 50 ml of 50 ppb formic acid gave an identical peak to that obtained when preconcentrating 10 ml of 250 ppb formic acid. As the preconcentration apparatus used a single, high-precision pump to deliver the sample and eluents, excellent precision was obtained for the preconcentration procedure. A precision of 1.85% R.S.D. was obtained on ten preconcentration runs of 10 ml of 200 ppb formic acid.

The onset of breakthrough of formic acid (a relatively weakly retained anion) on the anion-exchange concentrator was investigated. Increasing concentrations of formic acid were loaded and the recoveries plotted against μequiv. of the solute loaded. The results (Fig. 5) show that breakthrough occurred at 0.34 μequiv., which corresponds to approximately 10 ml of 1.5 ppm formic acid. The binding affinity of formate on this type of concentrator column was similar to that found for chloride in a previous study (0.33 μequiv.)[18] and this reflects the similarity of the ion-exchange selectivity coefficients for these ions. The other carboxylic acids in the series gave slightly extended linear binding ranges.

This method has an obvious drawback in that organic acids could not be preconcentrated successfully in sample solutions containing even moderately high levels of other ionic species, particularly inorganic anions. In these cases, competition for binding sites could be expected from more strongly retained inorganic anions. However, this same limitation exists for any precolumn concentration method. A practical application of the proposed procedure is illustrated in Fig. 6, which shows the chromatogram obtained by preconcentrating 7 ml of a melted Antarctic ice sample. No sample pretreatment was used and the sample was found to contain 220 ppb

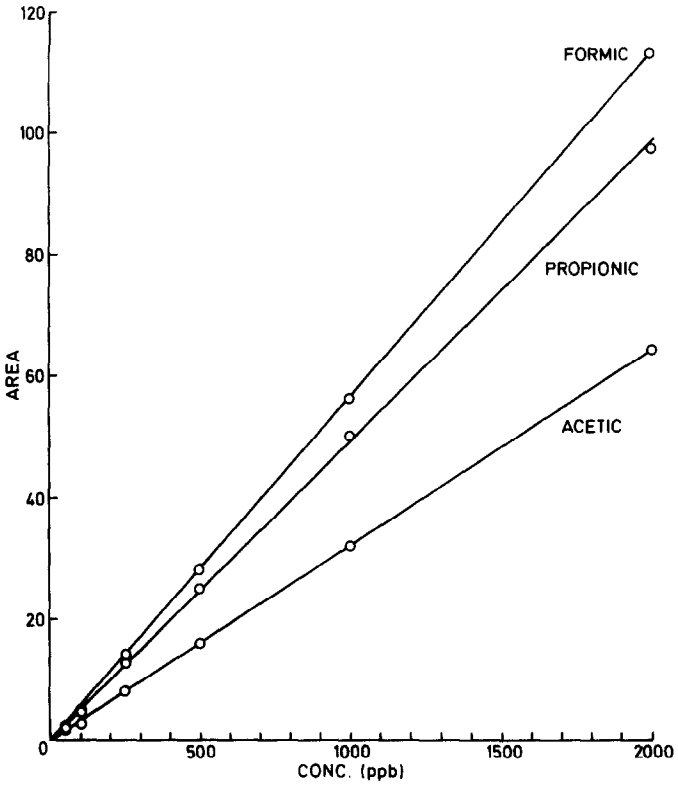

Fig. 4. Calibration plots obtained when loading 10-ml volumes of formic, acetic and propionic acids individually. Conditions as for Fig. 3, except that the sample concentrations varied as shown and a strip volume of 1.0 ml was used.

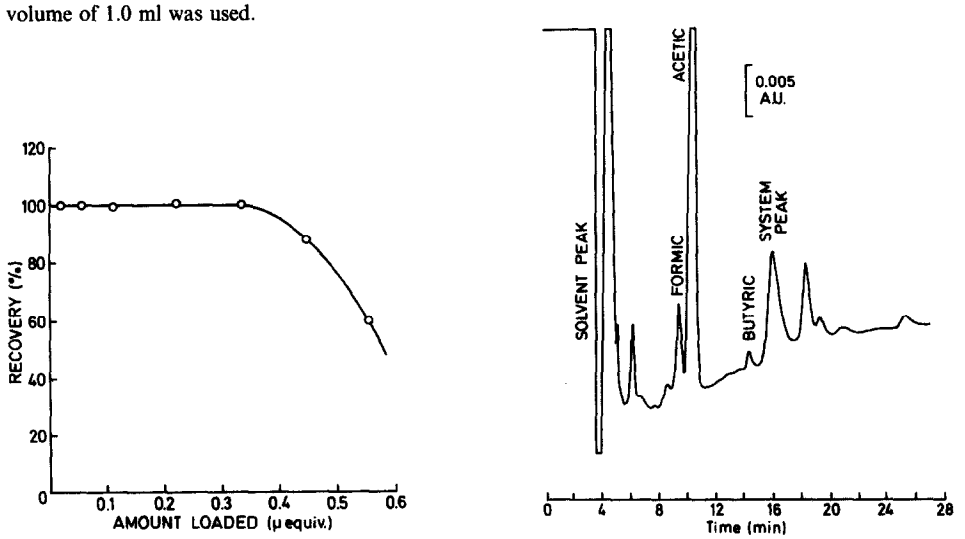

Fig. 5. Effect of the amount of sample loaded on the recovery of formic acid. Conditions as for Fig. 3, except a strip volume of 1.4 ml was used.

Fig. 6. Determination of carboxylic acids in Antarctic ice. Conditions as for Fig. 3, except that a sample volume of 7.0 ml and a strip volume of 1.0 ml were used and the detector sensitivity was 0.05 a.u.f.s.

butyric acid, 510 ppb formic acid, 6 ppm acetic (determined by direct injection) and several other unidentified species. Quantitative recoveries were obtained for all peaks as evidenced by the fact that loading different sample volumes gave linear plots for peak area *versus* sample volume.

CONCLUSIONS

This study has shown that quantitative preconcentration of carboxylic acids is possible using an ion chromatographic system employing an anion-exchange precolumn and an ion-exclusion analytical column. The preconcentration of the carboxylic acids on the precolumn followed the same trends noted previously for the preconcentration of inorganic anions in ion chromatography[14–16,18,19]. Detection limits in the low ppb range were obtained for aliphatic carboxylic acids using direct UV detection. It is envisaged that the method will be applicable to the determination of a large number of organic acid species in sample matrices of low ionic strength, provided that the pK_a values of the acids determined are not high and suitable resolution of these solutes can be attained.

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