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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 mM 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 mM sulphuric acid was adequate, resulting in a significant increase in the sensitivity, but no advantage was found in using the low-conductance eluent.

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## 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<sup>+</sup>-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<sub>a</sub> 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<sup>+</sup>-form removes H<sup>+</sup> by exchange with Ag<sup>+</sup> and Cl<sup>-</sup> 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

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  $\times$  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 mM 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 mM 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 mM 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 ( $\text{NBU}_4^+$ ) when an AMMS-ICE suppressor membrane is used. The limiting equivalent conductivity [5] of the hydronium ion is 350  $\mu\text{S}/\text{cm}^2$  equiv., whereas that of  $\text{NBU}_4^+$  is 19  $\mu\text{S}/\text{cm}^2$  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  $\mu\text{S}/\text{cm}^2$  equiv., respectively. Big, bulky ions have a lower conductivity than small ions, as clearly shown by the  $\text{H}^+/\text{NBU}_4^+$  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 <sup>a</sup> (DM)	Concentration (mM)	Conductance (un-suppressed) ( $\mu$ S)	Conductance (suppressed) ( $\mu$ S)	Conductivity (suppressed) ( $\mu$ S/cm)
Hydrochloric	—	—	—	1.0	—	—	100 <sup>b</sup>
Perfluoroheptanoic	Acid; Riedel-de Haën (61033)	95	11.38	1.0	—	—	40 <sup>b</sup>
1-Octanesulphonic	Sodium salt; Aldrich (22,156-2)	98	11.25	1.0	320-330 <sup>c,e</sup>	—	45 <sup>b</sup>
Sulphuric	Acid; Aldrich (25,810-5)	95-96	0.02	0.5	368 <sup>d,e</sup>	100 <sup>d,e</sup>	—
5-Sulphosalicylic	Acid Riedel-de Haën (33619)	Min. 99.5	0.17	0.5	290 <sup>d,e</sup>	72 <sup>d,e</sup>	—
Perfluorobutyric	Anhydride; Riedel-de Haën (61444)	99	9.17	—	—	—	—
Tridecafluoroheptanoic	Acid; Aldrich (34,204-1)	99	16.63	—	—	—	—

<sup>a</sup> Calculated for 10 l of the eluent containing 1 mM hydronium ions, the price is exclusive of ion-exchange materials, preparation time, etc.

<sup>b</sup> From ref. 2, eluent flow-rate 0.8 ml/min.

<sup>c</sup> From ref. 6.

<sup>d</sup> From this work, eluent flow-rate 0.8 ml/min, column temperature 30°C.

<sup>e</sup> 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<sup>-1</sup>. 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 ob-

tained 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 un-suppressed conductance of 0.5 mM sulphosalicylic acid was found to be lower than the un-suppressed conductance of 1-octanesulphonic acid [6]. The values

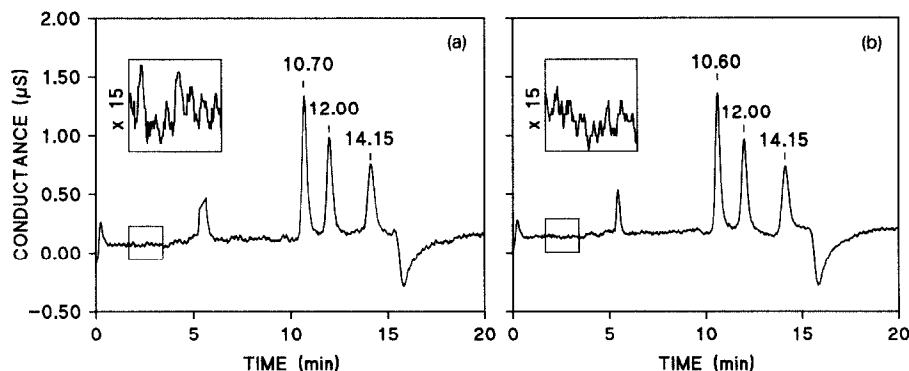


Fig. 1. Ion-exclusion chromatograms of a  $10 \mu\text{M}$  standard solution of small fatty acids obtained with (a)  $1.0 \text{ mM}$  sulphuric acid and (b)  $1.0 \text{ mM}$  5-sulphosalicylic acid. The retention times for formate, acetate and propionate were 10.7 (10.6 in Fig. 1b), 12.0 and 14.15 min, respectively. The insets show the baseline noise magnified 15 times. The suppressed background conductances were (a) 200 and (b) 144  $\mu\text{S}$ . The sample loop was  $20 \mu\text{l}$ . The flow-rate of the eluent was  $0.8 \text{ ml/min}$  and the column temperature was  $30^\circ\text{C}$ . The range settings of the conductimetric detector were  $200 \mu\text{S}$ . 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  $100 \mu\text{S}$  for the  $0.5 \text{ mM}$  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  $2 \mu\text{M}$  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.

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  $0.5 \text{ mM}$  5-sulphosalicylic acid in this work was less than that given for 1-octanesulphonic acid with the same concentration of hydronium ions. 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  $0.2 \text{ mM}$  hydronium ions. Fig. 3 shows a chromatogram obtained when  $0.1 \text{ mM}$  5-sulphosalicylic acid was used. The background conductances of the sulphuric and 5-sulphosalicylic acids containing  $0.2 \text{ mM}$  hydronium ions were 27 and  $23 \mu\text{S}$ , 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  $0.2 \text{ mM}$  hydronium ions compared to those containing  $2.0 \text{ mM}$ . 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-

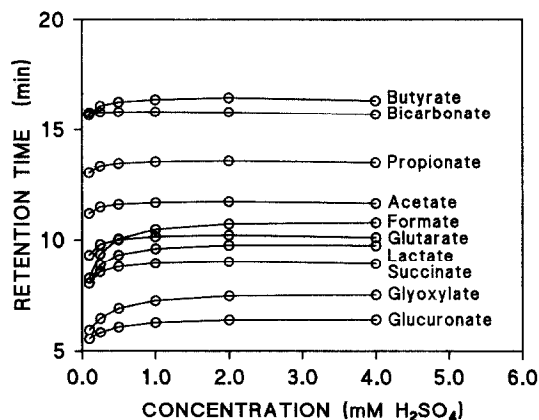


Fig. 2. Retention time of weak organic acids on an ion-exclusion column as a function of the concentration of the eluent. Sulphuric acid was used. Mixtures of weak organic acids with concentrations of 100 or  $500 \mu\text{M}$  were injected. The flow-rate of the eluent was  $0.8 \text{ ml/min}$ . The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

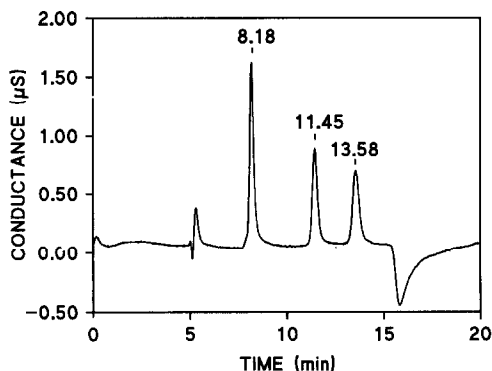


Fig. 3. Ion-exclusion chromatogram of a  $10 \mu\text{M}$  standard solution of formate, acetate and propionate obtained with  $0.1 \text{ mM}$  5-sulphosalicylic acid as the eluent. The retention times were 8.18, 11.45 and 13.58 min, respectively. The suppressed background conductance was  $23 \mu\text{S}$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

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

5-Sulphosalicylic acid is able to form complexes with many cations. The  $\text{Ca}^{2+}$  present was not precipitated by 5-sulphosalicylate. Therefore this eluent is appropriate for analyses of samples with a high concentration of the cation because  $\text{CaSO}_4$  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  $\text{H}^+$ -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  $30^\circ\text{C}$  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 sig-

nal-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  $5 \mu\text{M}$  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  $0.2 \text{ mM}$  hydronium ions and the low baseline noise decreased the detection limits to less than  $1 \mu\text{M}$  for formate. The  $0.1 \text{ mM}$  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  $\text{Ca}^{2+}$  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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