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METHODS FOR THE ANALYSIS OF INORGANIC ANIONS

IV*. REVERSED-PHASE HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY WITH AQUEOUS HYDROPHOBIC ION PAIRS AS ELUENTS

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SUMMARY

Inorganic anions can be well resolved on a reversed-phase C_{18} chromatographic column by using a dilute aqueous solution of tetrabutylammonium salicylate as eluent. The retention behaviour of six anions ($H_2PO_4^-$, Cl^-, Br^-, NO_3^-, I^- and SO_4^{2-}) on this column was investigated. The retention is sensitive to pH changes of the eluent which probably forms a double layer on the stationary phase, permitting a dynamic anion-exchange chromatographic separation. This efficient and simple method has been applied to the quantitation of the anion contents of fruit juices.

INTRODUCTION

Inorganic anions have been separated on reversed phases (C_8 and C_{18}), with solutions of tetrabutylammonium phosphate[1,2], tetrabutylammonium phthalate[3] or octylammonium phosphate[4,5], buffered at various pH values, as eluents. Cassidy and Elchuk[6] introduced reversed phases coated with quaternary ammonium salts. More recently, Barber and Carr[7] described a separation system with a solution of (α-naphthylmethyl)tributylammonium acetate–hexanesulphonate at pH 4.75 as the eluent.
They termed this method "reversed-phase ion-interaction chromatography".

This paper deals with a detailed investigation of the separation of inorganic anions on a reversed-phase column (LiChrosorb C_{18}, 10 μm) with simple aqueous mobile phases, containing quaternary ammonium hydroxide and salicylic acid but no organic solvents or buffers. The aim was to devise simple and efficient systems for the analysis of inorganic anions with the aid of conductometric detection similar to those widely used for the reversed-phase chromatography of organic substances.

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EXPERIMENTAL

Equipment for high-performance liquid chromatography (HPLC)

Pump: Knauer HPLC pump, with the flow-rate set at 1.0 ml/min. Injector: Rheodyne Model 7010 with a 20-μl sample loop. Detector: Knauer conductivity detector. Integrator: Spectra-Physics SP 4100 computing integrator. Recorder: chart speed 0.5 cm/min.

Chromatography

The column was operated at ambient temperature and an applied pressure of 50 bar. Stationary phase: LiChrosorb RP-18, 250 × 4.6 mm I.D., particle size 10 μm. A 10^{-4} *M* aqueous solution of methyl green with 50 ml acetic acid per litre, was pumped through the column until the dyestuff appeared at the column exit. The column was then washed with water. Mobile phase: a mixture of $5 \cdot 10^{-4}$ *M* tetrabutylammonium hydroxide and salicylic acid in twice distilled water; pH adjustments were made with salicylic acid.

RESULTS AND DISCUSSION

Two typical chromatograms obtained with the LiChrosorb RP-18 column are shown in Fig. 1a and b. The mobile phase consisted of a hydrophobic cation (tetrabutylammonium hydroxide) and an aromatic hydrophobic anion (salicylic acid), which are assumed to form a double layer, similar to that described by Iskandarani and Pietrzyk[8]. The injected ions interchange with the salicylate ions and migrate differentially along the column. The peaks of six inorganic ions are well resolved.

These chromatograms can be greatly improved by first treating the column with a cationic dyestuff, such as methyl green. Fig. 2a and b shows chromatograms obtained by this means, the six ions analyzed being the same as in Fig. 1. The mobile phase was again tetrabutylammonium hydroxide and salicylic acid. Although these chromatograms cannot be compared quantitatively, because they were not recorded at exactly the same pH, a qualitative comparison of those in Fig. 2 with those in Fig. 1 is nevertheless informative. It is seen that the retention times on the dyestuff-treated column are shorter, the pH effects are stronger and the peaks are much sharper, than those with the untreated column.

pH Effects

In Fig. 1a and b as well as 2a and b important pH effects can be observed. In the lower pH region (pH 4.13 in Fig. 1a, and pH 3.9 in Fig. 2a) the background conductivity is lower, and the peaks are therefore higher, in Figs. 1a and 2a than in Figs. 1b and 2b. Furthermore, and still in the lower pH region, the $H_2PO_4^-$ peak (1) is much higher than at higher pH values. This is because at pH 4 all the phosphate exists as the monovalent anion, whereas at pH 6–7 there is a mixture of monovalent and divalent phosphate anion HPO_4^{2-} does not appear in Figs. 1b and 2b because its retention time is much longer than that of SO_4^{2-}. With an increase in pH, moreover, the untreated column yields steadily increasing retention times (see Table I), whereas on the treated column the retention times of the anions tend to pass through a maximum around pH 4.9 (Table II).

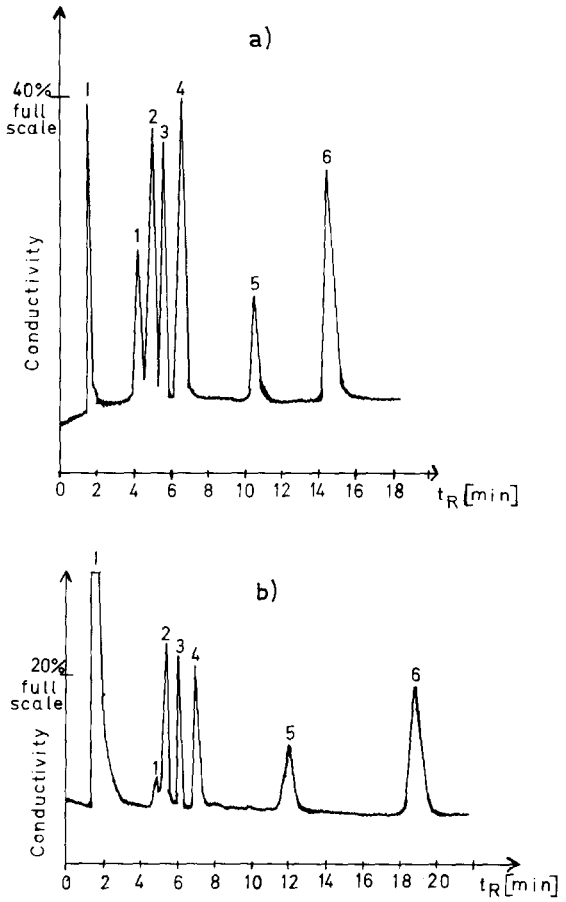

Fig. 1. HPLC separation of anions. Column: 250 × 4.6 mm, packed with LiChrosorb RP-18 (10 μm). Mobile phase: 5×10^{-4} *M* tetrabutylammonium hydroxide, adjusted to pH 4.13 (a) or to pH 7.0 (b) with salicylic acid. Flow-rate: 1 ml/min. Sample size: 20 μl. Conductivity detector: sensitivity 500 (recorder, 100 mV). Peaks: I = injection peak; 1 = $H_2PO_4^-$ (50 ppm); 2 = Cl^- (20 ppm); 3 = Br^- (50 ppm); 4 = NO_3^- (30 ppm); 5 = I^- (50 ppm); 6 = SO_4^{2-} (50 ppm).

Another interesting feature of the chromatograms in Fig. 2a and b is the change in the locations of the iodide and the sulphate ions. At pH 3.9 (Fig. 2a) sulphate emerges after iodide, whereas at pH 6 the opposite is true. Table II, summarizing the retention times as functions of pH, shows that the retention time of sulphate is more sensitive to pH changes than is that of iodide.

Determination of anions in fruit juices

Fruit juices can be analyzed by this method (with a dyestuff-loaded column) without any pretreatment. The juices are diluted 1:10 in water, and 20-μl samples are directly injected into the chromatograph. In our experiments $H_2PO_4^-$, Cl^- and SO_4^{2-} could be quantitatively determined by the standard addition method. The calibration curves show that there is some background conductivity associated with

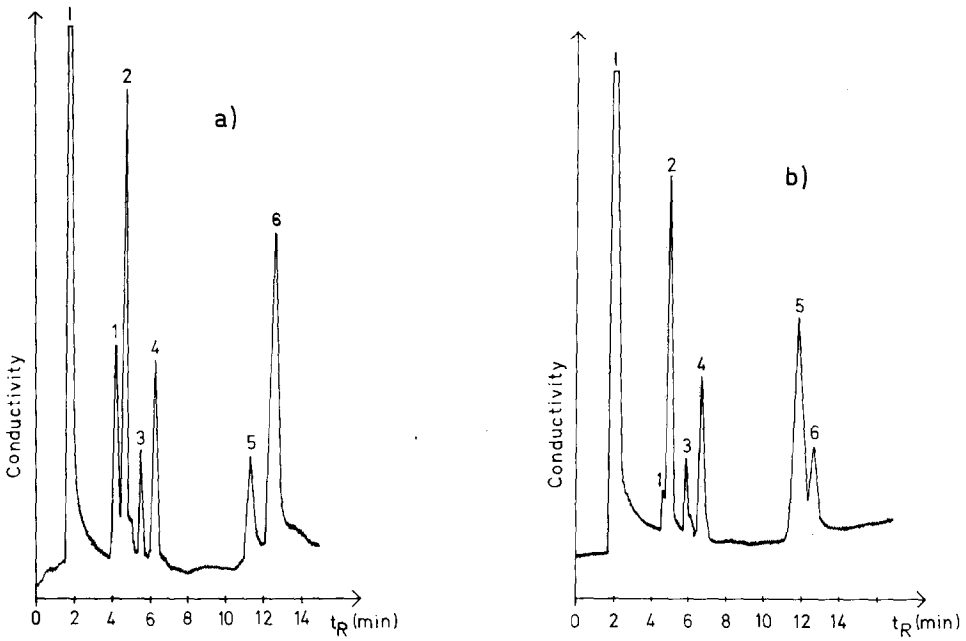

Fig. 2. HPLC separation of anions on a dyestuff-loaded column. Column: see Fig. 1, loaded with methyl green (see Experimental). Mobile phase: see Fig. 1, adjusted to pH 3.9 (a) or to pH 6.0 (b). Other conditions as Fig. 1. Integrator attenuation = 32. Peaks: I = injection peak; 1 = $H_2PO_4^-$ (40 ppm); 2 = Cl^- (10 ppm); 3 = Br^- (10 ppm); 4 = NO_3^- (10 ppm); 5 = I^- (20 ppm) (a), SO_4^{2-} (20 ppm) (b); 6 = SO_4^{2-} (20 ppm) (a), I^- (20 ppm) (b).

these ions in the fruit juices, and accordingly separate linear equations were defined for each ion, as detailed in Table III. Nitrate was present in apple juice in minute quantities only, as indicated by a mere shoulder in Fig. 3a. It was completely absent from orange juice (Fig. 3b). The nitrate concentration in the apple juice is below 1 ppm and cannot be determined by this method. It is, however, determinable by another chromatographic method[9], in which an amino column is used as separator and a sensitive UV detector is capable of indicating the presence of nitrate at that low concentration.

TABLE I

RETENTION TIMES (min) OF SIX ANIONS ON A RP-18 COLUMN, UNTREATED WITH METHYL GREEN, AS A FUNCTION OF pH

pH	$H_2PO_4^-$	Cl^-	Br^-	NO_3^-	I^-	SO_4^{2-}
3.1	3.4	3.8	4.4	5.0	7.8	8.4
3.4	3.8	4.3	5.2	5.8	9.8	11.3
3.6	3.8	4.4	5.3	5.9	9.8	12.0
4.1	4.2	4.9	5.5	6.4	10.2	14.6
5.3	4.6	5.0	5.8	6.8	10.7	16.6
7.0	4.8	5.2	6.1	7.3	11.6	18.6

TABLE II

RETENTION TIMES (min) OF SIX ANIONS ON A RP-18 COLUMN, TREATED WITH METHYL GREEN, AS A FUNCTION OF pH

pH	$H_2PO_4^-$	Cl^-	Br^-	NO_3^-	I^-	SO_4^{2-}
3.05	3.15	3.55	3.83	4.55	6.98	6.31
3.5	3.60	4.06	4.80	5.46	9.49	8.88
3.9	4.15	4.66	5.48	6.23	11.26	12.52
4.5	4.48	4.88	5.79	6.60	12.07	13.77
4.9	4.65	5.02	5.97	6.81	12.44	14.64
5.3	4.55	4.92	5.86	6.69	12.46	13.87
6.0	4.42	4.92	5.84	6.64	12.60	11.83

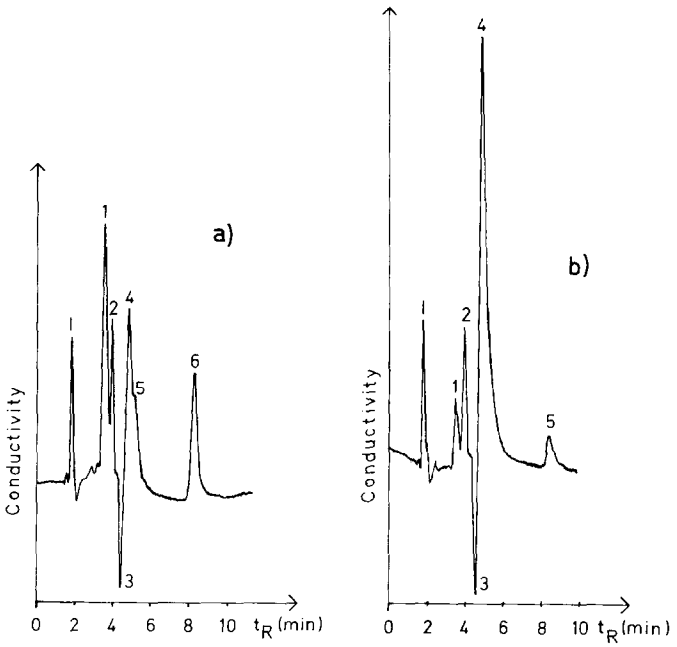

Fig. 3. Anion analysis in fruit juices. For conditions see Fig. 2. Mobile phase adjusted to pH 3.4. Sample size: 20 μl. Integrator AT = 128. a, Apple juice (diluted 1:10). Peaks: I = injection peak; 1 = $H_2PO_4^-$; 2 = Cl^-; 3 = system peak; 4 = unknown; 5 = NO_3^-; 6 = SO_4^{2-} (for amounts see Table III). b, Orange juice. Peaks: I = Injection peak; 1 = $H_2PO_4^-$; 2 = Cl^-; 3 = system peak; 4 = unknown; 5 = SO_4^{2-} (for amounts see Table III). The term "system peak" denotes a peak representing an organic acid forming an integral part of the juice tested.

TABLE III

ANION CONCENTRATIONS (ppm) IN APPLE AND ORANGE JUICES

Evaluations by the standard addition method; results from the chromatograms in Fig. 3a and b. NO_3^-: Not determinable.

	$H_2PO_4^-$	Cl^-	SO_4^{2-}
Apple juice			
Concentration	63.9	5.3	15.7
Standard addition calibration curve*	$y = 0.098x + 6.26$	$y = 0.608x + 3.22$	$y = 0.212x + 3.33$
r	0.96883	0.99862	0.98593
Orange juice			
Concentration	13.5	5.7	2.8
Standard addition calibration curve*	$y = 0.107x + 1.44$	$y = 0.572x + 3.25$	$y = 0.250x + 0.71$
r	0.98997	0.99956	0.99960

* y = Peak height in cm, x = concentration of anions in the sample in ppm.

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