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HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY OF IONS

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SUMMARY

High-performance liquid chromatography of ions (ion-HPLC) can be performed with columns packed with non-polar (or solvophobic) stationary phases often at much higher efficiencies than with ion-exchange resins. The use of suppressor columns which decrease the conductivity of the eluent can increase sensitivity of conductivity detection. Ion-HPLC is possible also without a suppressor column, if specially designed, sensitive conductivity detectors are used.

Reversed-phase chromatography using secondary equilibria in the mobile phase and conductivity detectors offers a new way of investigating the properties of ionic species in aqueous and organic solvents. The separation of Na^+, K^+, NH_4^+ and Ca^{2+}, and of F^-, Cl^-, SO_4^{2-}, NO_2^-, Br^-, $Cr_2O_7^{2-}$, NO_3^-, I^-, etc., is demonstrated on RP-18 columns.

Conductivity detectors can be of assistance in HPLC to characterize and determine ionic equilibria in chemistry, the life sciences and in qualitative and quantitative ion analysis.

INTRODUCTION

In the historical development of high-performance liquid chromatography (HPLC), ion-exchange resins have played a prominent role[1–3]. The more recent development of non-polar stationary phases has led to a rapid increase in the number of applications of reversed-phase chromatography (RPC), especially in the life sciences[4–9]. In RPC, "solvophobic" interactions govern retention[6,7]. Closely related compounds can often be separated more effectively on non-polar stationary phases than in ion-exchange systems (Fig. 1). However, the separation of alkali- and alkaline-earth-metal cations and of several inorganic anions in aqueous systems could not be done effectively until 1975[10]. "Ion chromatography" then became the method of choice in the pharmaceutical industry, in environmental studies, in the control of water quality, etc.[11–14].

Despite of its many advantages, ion chromatography has some drawbacks:

Organic supports are employed which are not pressure resistant and which swell in certain organic eluents. Because of the large particle size, column efficiencies are low (500–1000 plates per metre). Broadened peak shape causes decreased sensitivity of detection.

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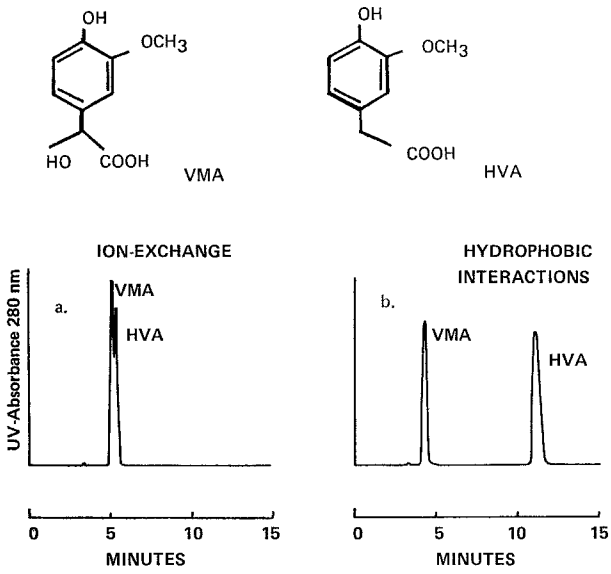

Fig. 1. Comparison of the separation of two closely related organic acids on ion-exchange and on reversed-phase columns. a, Column: Partisil-10 SCX (250 × 4.6 mm); eluent: 0.05 *M* NaH_2PO_4, pH 4.0; flow-rate 0.5 ml/min; 25°C. b, Column: 10 μm LiChrosorb RP-18 (103.07.23.010, Knauer); eluent: 0.1 *M* phosphate buffer (pH 2.1)–acetonitrile (90:10); flow-rate 1.0 ml/min; 25°C.

Only a few eluents can be used, and must be capable of being converted into a non-conducting form in the suppressor column (*e.g.*, HCO_3^- or H^+).

Some investigations have been undertaken in an attempt to avoid these disadvantages. In particular, Fritz and co-workers[15,16] demonstrated the use of anion chromatography with low-capacity organic anion exchangers and low-conductivity eluents. With such eluents no suppressor column is needed for the detection of nanogram amounts of important inorganic anions. Harrison and Burge[17] tested an inorganic, chemically bonded anion-exchange material for the separation of NO_2^-, Cl^-, Br^-, NO_3^- and SO_4^{2-} in formic acid–ammonium formate and in acetic acid–acetate buffers. Although working without a suppressor column offered many advantages, the column performance still remained rather poor. Thus the theoretical plates for SO_4^{2-} (about 200 per metre)[15] and for Cl^- (about 3200 per metre)[17] are far inferior to today's column performances with modern silicious materials (30,000 plates per metre). Recently, Reeve[18] reported the use of a spectrophotometer in the detection at 210–230 nm for inorganic anions of mixed oxidation states on a cyano-bonded silica column with aqueous phosphate buffers and methanol mixtures with cetrimid in the aqueous component.

The aims of the present investigations were:

to increase the column efficiency in ion-HPLC;

to find a silica-based weak cation exchanger of small particle size;

to develop a new type of conductivity detector with an extremely stable baseline at different conductivity levels;

to find other simple non-polar stationary phase–aqueous mobile phase combinations to permit cation and anion analysis with modern HPLC technology.

EXPERIMENTAL

Ion-HPLC with eluent suppression

The chromatographic system used was built up of modular units as shown in Fig. 2. A short-stroke, twin-headed, reciprocating pump (Type 52.00; Knauer, Oberursel, G.F.R.) delivered the eluent (0.0075 *N* HNO_3) at a flow-rate of 3 ml/min. The eluent entered valve 2 (Type 7010; Rheodyne, Berkeley, CA, U.S.A.) and flowed through a precolumn (40 × 4.6 mm) (103.13.52, Knauer). The sample was introduced with valve 1 (Type 7125, Rheodyne) using a 100-μl syringe (101.00.43, Knauer). The separator column was packed with a weak silicious cation exchanger of 5 μm particle size (120 × 4.6 mm) (103.03.51, Knauer). The suppressor column was packed with a strong anion exchanger (250 × 4.6 mm) (103.07.53, Knauer) and had to be regenerated after 900–1000 ml of the eluent had flowed through it (12–14 injections). After leaving the suppressor column the eluent entered a conductivity detector (Type 74.00, Knauer). During the regeneration process the separator column was disconnected by turning valve 2 by 60°, to avoid any damage to the separator due to contact with the regenerating eluent (0.5 *N* NaOH) which was delivered at 10 ml/min. Prior to the regeneration process, eluent selection was done by switching low-pressure magnetic valves. Regeneration took about 15 min. Valve 2 was then returned to its original position and distilled water was delivered until the baseline conductivity reached normal levels. Analysis of samples was then continued.

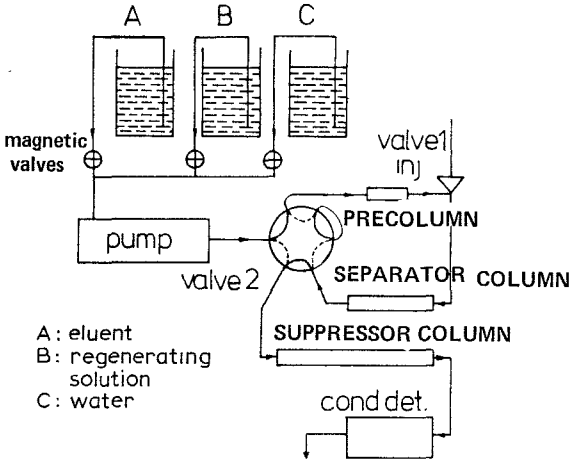

Fig. 2. Ion-HPLC system with suppressor column.

Ion-HPLC on non-polar stationary phases

The system is built up of a pump (Knauer), valve injector (Rheodyne), prepacked column with 10-μm LiChrosorb RP-18 (250 × 4.6 mm) (103.07.23.010, Knauer) and conductivity detector (Type 74.00, Knauer). Heptyl sulphate and tetrabutylammonium hydroxide were used as mobile phases in different concentrations. UV measurements with I^- were carried out at 235 nm using a spectrophotometer (Type 87.00, Knauer). Chromatograms were recorded using a double-channel recorder (Type 42.00, Knauer) or a Hewlett-Packard 3380A printer-plotter integrator.

Materials

All chemicals used in this study were of analytical grade and were purchased from Ferak (Berlin, G.F.R.) or Merck-Schuchardt (Hohenbrunn, G.F.R.). Ion-interacting agents were from Knauer.

Conductivity detection

Most conductivity detectors function according to the Wheatstone bridge principle. Here, even in the compensated state, the alternating voltage applied to the cell produces a considerable current through the cell. This current leads to heat dissipation which causes changes in the resistance of the electrolyte solution. The result is a noisy, drifting baseline. The conductivity detector, developed for the present investigations and for general HPLC use, was designed especially to reduce the current in the measuring cell so that it falls within the micro- to nano-ampere range, thus reducing heat dissipation down to the microwatt range.

The flow cell consisted of a PTFE tube with an inner diameter of 0.5 mm. The two electrodes were tubes made of platinum or of a special stainless steel and fitted in the two ends of the PTFE tube. The distance between the two electrodes in the PTFE tube was usually 5 mm. The cell volume is negligible, about 3 μl. If a solution of high electrolytic conductivity is to be studied, a longer PTFE tube may be used to achieve higher resistance in the cell. The flow cells were designed for flow-rates from 0.1 to 10.0 ml/min. At higher flow-rates the inner diameter of the electrodes and of the PTFE tube can easily be increased.

With 1000 Hz a.c. no polarization problems were observed at the electrodes. Because of the extremely low currents, the surface of the electrodes can also be very small. The construction of the cell results in very low capacity values between the electrodes, making capacity compensation unnecessary; the error is negligible, remains constant and does not influence the measurements. In accordance with Ohm's law:

$$R\,(\text{resistance}) = E\,(\text{alternating voltage})/I\,(\text{current})$$

At constant voltage applied to the cell the current will be proportional to the conductivity. This current was rectified, amplified and registered by a recorder. The baseline can be compensated by a compensating current adjusted by use of a ten-turn helical potentiometer.

For differential measurements, sample and reference cells can be used together. In this manner the temperature coefficient of the electrolytic conductivity is compensated. Owing to the extremely low currents, baseline stability is considerably improved in comparison with other types of conductivity detectors. In low-conductivity eluents extremely sensitive detection of ions in the picogram range is possible. Even without a suppressor column we are still able to detect, *e.g.*, 80 ng Cl^-, as shown in Fig. 6.

RESULTS AND DISCUSSION

Ion exchange

Cation separation using 5-μm silicious cation exchanger. It has been 30 years since the discovery that lithium salts can be used to control the violent swings in

mood of manic depressive patients. Lithium carbonate is now the drug of choice for many manic depressives. Lithium reduces the turnover of choline in the brain. It also seems to interact with the transport system in cellular membranes, a system that controls the organism's natural levels of Na^+, K^+, Ca^{2+} and Mg^{2+}. The measurement of the concentrations of such ions is important in physiology and in the clinical laboratory. Atomic absorption spectroscopy (AAS) is the most widely used method to monitor lithium levels in blood. AAS is, however, expensive and time consuming. It is therefore reasonable to use HPLC for these measurements, especially in the case of availability of a conductivity detector.

The separation of other cations can be improved with the introduction of new, specially designed stationary phases for certain metals, as shown by Fritz and co-workers[13,19]. A review by Swedt[20] of the recent results contains many ways of improving inorganic analysis using HPLC.

The separation of alkali-metal ions was reported by Small *et al.*[10]. Replacing the organic stationary phase of the separator column by an inorganic, surface-modified silica gel we obtain the chromatogram shown in Fig. 3a. A comparison of the two chromatograms shows the efficiency increase due to use of the small particle size silica gel (5 μm). The retention time of the cations is a function of the concentration of the eluent. Increasing concentration of the acid in the mobile phase results in decreasing retention times of the cations. However, the capacity of the suppressor column also decreases with increasing acidity of the eluent mobile phase. In our work, 12–14 consecutive analyses could be completed between two regeneration steps of the suppressor column. The regeneration step was carried out using 0.5 *N* NaOH solution.

The detection limits for all ions are excellent with the conductivity detector: Li^+, 800 pg; Na^+, 2 ng; NH^+, 2 ng; K^+, 4 ng; Rb^+, 8 ng (see also Fig. 3b).

The quality of separation of the cations in Fig. 3a is similar to the results we have achieved without a suppressor column. Conductivity detection becomes, however, more difficult as the baseline stability is decreased. At higher conductivity levels the influence of changes in temperature becomes more significant as the mobility of ions (especially of H^+ or OH^-) is strongly temperature dependent. The eluent leaving the column must therefore be carried through a heat exchanger (low dead volume capillary tubing, 300 × 1.6 × 0.3 mm) in a thermostated medium.

The difference between working with or without a suppressor column depends on experimental factors. With suppressor columns the baseline of the detector is much more stable, as at low ionic strengths, temperature changes do not influence baseline stability. At low noise and no drift, sensitivity of ion detection is improved by one or two orders of magnitude in comparison with the separator column alone. However, with efficient thermostating of the eluent and the conductivity, cell noise and drift can be considerably reduced, so that the sensitivity ratio, depending on the eluent ionic strength, might be as low as a factor of 5–10 between the two types of system.

Another interesting aspect of using the new conductivity detector is the possible detection of underivatized amino acids in the nanomol range. Obviously amino acids can be detected by conductivity as a result of their ionic character. In Fig. 4 the sensitive detection of glycine is shown.

Anion separation with suppressor column. As the concentration of anions is

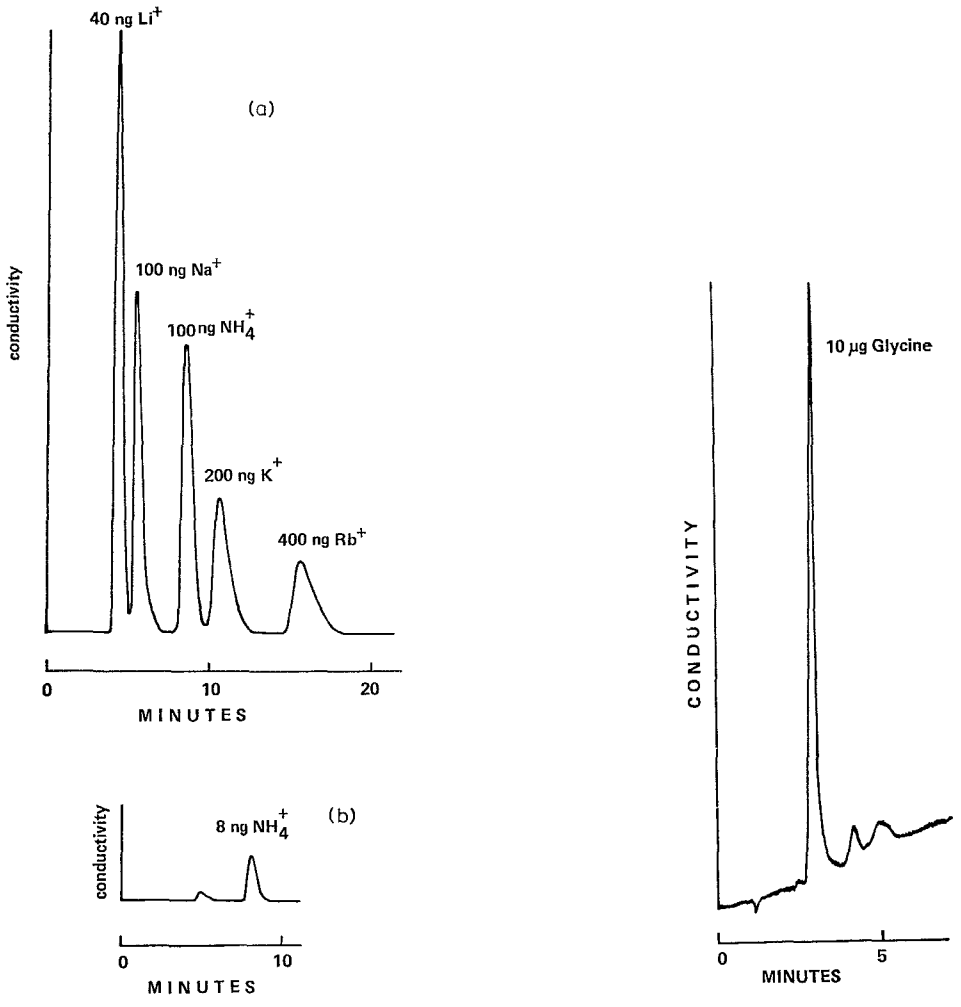

Fig. 3. Cation separation: a, on silicious weak cation exchanger (5 μm particle size); b, detection of NH_4^+ in the low nanogram range. Columns as described under Experimental. Mobile phase: 0.0075 *N* HNO_3; flow-rate, 3.0 ml/min. Temperature: 25°C.

Fig. 4. Detection of amino acids in the low nanomol range using conductivity detection. Column: 5 μm silicious weak cation exchanger (103.03.51, Knauer); suppressor, strong anion exchanger (103.07.53, Knauer). Mobile phase: 0.015 *N* HNO_3; flow-rate, 3.0 ml/min. Pressure: 320 bar. Temperature: 25°C.

often relatively high and there is plenty of sample available, one does not generally use a suppressor column, although it can easily be assembled from an empty column (250 × 4.6 mm) and packing it with a strong cation-exchange resin of the smallest available particle size.

The separator column presents a problem in many applications as eluent pH values above 9 limit the use of silicious anion-exchange resins. Above this pH the silica is hydrolysed and the packing collapses. Therefore we have tried to find a commercially available organic anion exchanger. However, most of those are much

too strong and are not available in small particle sizes, so we directed our efforts towards other possibilities such as using secondary equilibria in connection with non-polar stationary phases to find sufficient retention for most of the aqueous ions of common interest.

Separation of ions on non-polar stationary phases with ion-interacting agents added to the eluent mobile phases

RPC revolutionized liquid chromatography and contributed a great deal to scientific problem solving. It facilitated routine chemical analysis and speeded up recent developments in the life sciences. Water as mobile phase without organic component was shown to have the strongest retentive force. But water is also miscible with aqueous samples, so sample preparation has been simplified. Further, the use of secondary equilibria led to new and unexpected separations based on difficult energetic interactions between the sample and some additives in the mobile phase[21].

However, even under conditions of strongest retention, *i.e.*, on octadecylsilica with decyl sulphate in plain aqueous eluents, some hydrophilic amino acids were not retarded[22]. The separation and detection of strongly hydrated ions, such as the alkali metals, NH_4^+, Ca^{2+}, etc., and anions of non-metals and of their acids, *e.g.*, F^-, Cl^-, Br^-, SO_4^{2-}, HPO_4^{2-}, I^-, NO_2^-, NO_3^-, etc., on non-polar stationary phases using ion interactions have not yet been tried for the simple reasons:

most of these ions do not absorb UV light;

conductivity detection was not considered due to the assumption that ion-pairs would not have measurable conductivity;

ion-pair formation would not lead to retention of completely hydrated ions as mentioned above.

Recent measurements of Scott and Kucera[23] and Bidlingmeyer *et al.*[24], have, however, clearly shown that, although ion-pair formation can be assumed, conductivity measurements do not indicate the presence of undissociated ion-pairs. The opposite is the case: the conductivity of a mixture of oppositely charged hydrophobic ions is the sum of the conductivities of the individual ions, even in a wide range of water–methanol mixtures. We therefore directed our interest to the use of a sensitive conductivity detector specially designed for HPLC for separation of the above ions and for the study of their physicochemical properties in aqueous solutions.

Separation of cations. We have used a LiChrosorb RP-18 prepacked column with a heptyl sulphate solution as eluent. To obtain a good separation an extension of the column length was necessary. Using three columns in series, the separation shown in Fig. 5 could be achieved. The disadvantage of this technique is that Li^+ overlaps with Na^+ and NH_4^+ with Rb^+. However, with this technique, environmental or industrial water samples which do not contain Li^+ and Rb^+ can be analysed for Na^+, K^+ and Ca^{2+}, simply and in a reasonable time. Using 10–20% methanol in the eluent, the retention time could be considerably reduced. Under the above conditions excellent detection limits could also be obtained with the conductivity detector: Li^+, 5 ng; NH_4^+, 25 ng and Na^+, 8 ng.

Obviously there is a need for more selective ion-pair-forming substances. With bi- or tri-dentate ion-pair reagents the selectivity of the phase system could be increased. We are continuing our efforts to find more specific agents for the separation of the alkali- and alkaline-earth metal cations by reversed-phase HPLC.

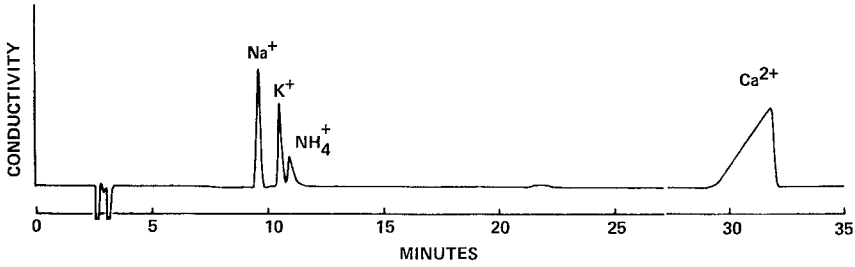

Fig. 5. Cation separation on reversed phase with ion interactions. Column: 10 μm LiChrosorb RP-18 (3 × 250 × 4.6 mm) (103.07.23.010, Knauer). Mobile phase: 0.005 *M* *n*-heptylsulphonate solution, pH 2 (K_2 solution, 102.28, Knauer); flow-rate, 2.0 ml/min. Pressure: 220 bar. Temperature: 25°C.

Under the above conditions (Fig. 5) column stability is excellent due to the non-polar surface of the octadecylsilica. Column lifetime is also increased considerably since hydrolysis is hindered by the surface.

Separation of anions

Conductivity detection. To obtain maximum retention of hydrophilic anions we chose octadecylsilica (C-18) as stationary phase. The separation of eight important anions is shown in Fig. 6. The first peak is at 1.30 min, corresponding to an elution volume of 2.60 ml. This value is higher than the upper exclusion limit at 1.6–1.7 ml but lower than the retention volume of the unretarded eluent molecule (V_0) of *ca.* 3.1–3.3 ml; so we assume that this peak represents some unretarded, excluded ionic species. However, care must be taken in interpreting these results. As the peaks represent increases in electrolyte conductivity, the species eluted here have higher conductivities than the mobile phase. The injection of a salt solution, *e.g.*, $K^+NO_2^-$ (Fig. 7), would result in an exchange of ions of the salt with the ions of the ion-pair reagent $N(C_4H_9)_4^+OH^-$ (TBA-OH) and the buffer ions $HPO_4^{2-}/H_2PO_4^-$ respectively. As we used 0.002 mol/l TBA-OH in 0.025 mol/l Na_2HPO_4 and 0.25 mol/l NaH_2PO_4, pH 6.7, the concentration of OH^- at this pH is negligible. The injection of 20 μg of the salts (all in the potassium form) in Fig. 6 increases the ionic strength of the buffer in 12 ml eluent by only 0.001 %. Assuming an average peak volume of 1 ml, the buffer:sample ion concentration ratio is still 1000:4. The overall ionic strength is therefore determined by the buffer concentration. On injecting $K^+NO_2^-$ the anion NO_2^- would replace $H_2PO_4^-/HPO_4^{2-}$, which would be eluted with K^+ in the eluent front (Fig. 7).

The injection of HCl, H_2SO_4 and HNO_3 results in two major peaks (Fig. 8). The first is at about 1.91 min and is negative, *i.e.*, the eluent conductivity is decreased. A possible explanation would be that H_3O^+ reacts with the buffer as

$$H_3O^+ + HPO_4^{2-} \rightleftharpoons H_2O + H_2PO_4^-$$

decreasing the overall conductivity. The second peak appears at the same retention time as with KCl, K_2SO_4 and KNO_3, so we assume it corresponds to the anion (Fig. 9).

Water gives a negative peak at 1.3 min as its conductivity is lower than the conductivity of the eluent. If its pH is slightly higher than the pH of the eluent, it also gives a positive peak at about 1.9 min (Fig. 10).

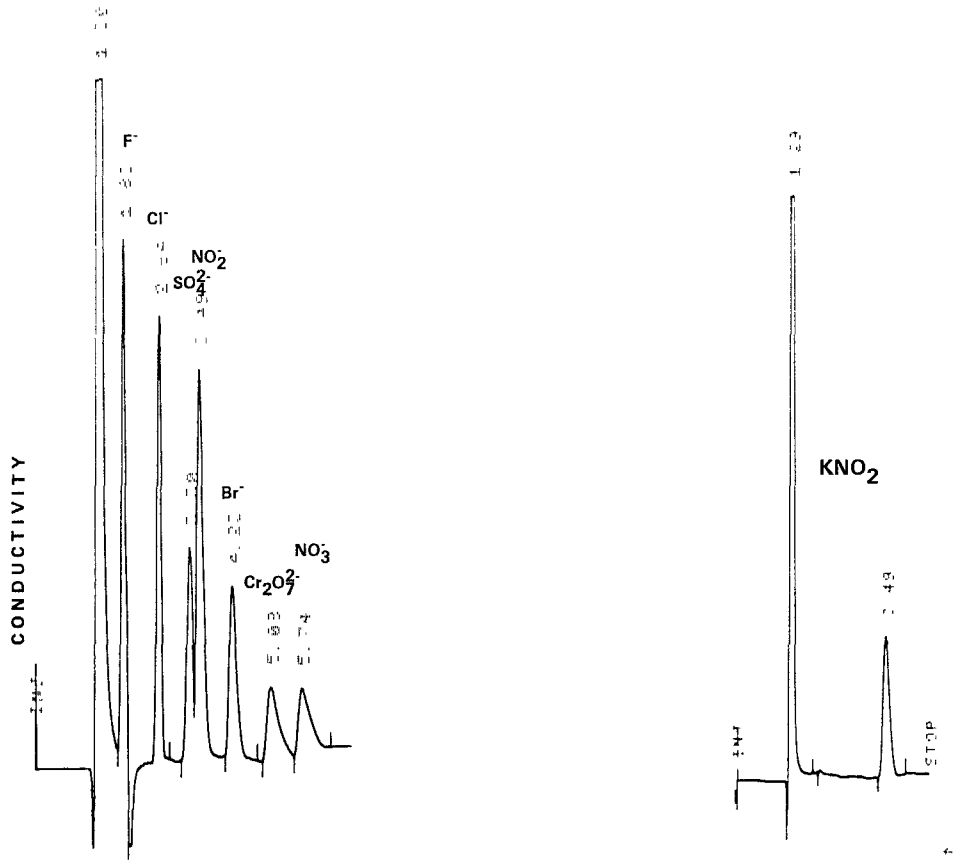

Fig. 6. Separation of some anions with reversed-phase chromatography coupled with conductivity detection. Column: 10 μm LiChrosorb RP-18 (103.07.23.010, Knauer). Mobile phase: 0.002 mol/l tetrabutylammonium hydroxide and 0.05 mol/l phosphate buffer, pH 6.7; flow-rate, 2.0 ml/min. Pressure: 80 bar. Temperature: 25°C. Conductivity detector (74.00, Knauer). Injected samples were dissolved in 0.05 mol/l phosphate buffer, pH 6.7, to give a concentration of 1 mg/ml. Unless otherwise noted, chromatograms correspond to 20-μl injection volume.

Fig. 7. Chromatogram of KNO_2. Conditions as in Fig. 6.

Since bases, such as NaOH, KOH or even $N(C_4H_9)_4OH$, also give a positive peak we believe that this peak corresponds to OH^-, which has a high mobility, higher than those of the buffer ions (Fig. 11). The injection of HCO_3^- also gives a peak at this position corresponding to the equation:

$$HCO_3^- + HOH \rightleftharpoons OH^- + H_2CO_3$$

The dependence of the retention of the anions investigated on the concentration of the ion-interacting agent TBA-OH is shown in Fig. 12. It should be noted that at above 0.005 mol/l TBA-OH there is a small dependence of the retention on the TBA-OH concentration. Anions with two negative charges (SO_4^{2-} and $Cr_2O_7^{2-}$) show a retention maximum at 0.004 mol/l TBA-OH and then a slow decrease. At

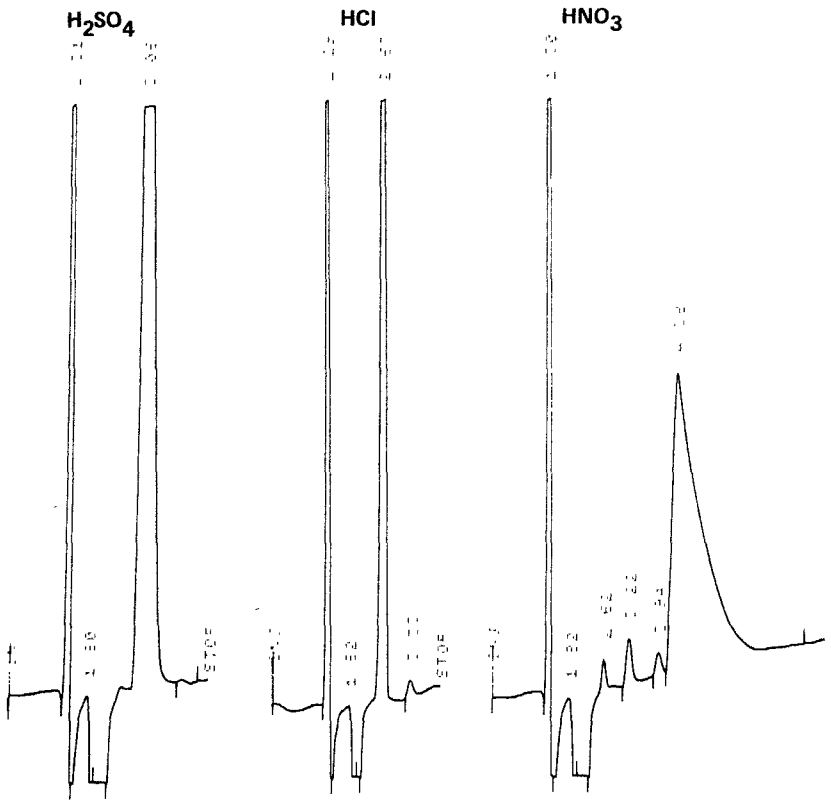

Fig. 8. Chromatograms of H_2SO_4, HCl and HNO_3. Conditions as in Fig. 6.

pH 6.7, $Cr_2O_7^{2-}$ is in a protic equilibrium with CrO_4^{2-}:

$$2\,CrO_4^{2-} + 2\,H^+ \rightleftharpoons Cr_2O_7^{2-} + H_2O$$

The ratio $C_{Cr_2O_7^{2-}}:C_{CrO_4^{2-}}$ is roughly 1:1 at this pH.

The function $k'_{Anion} = f(C_{TBA-OH})$ can be described mathematically[21] and ion-association constants can be calculated. Horváth *et al.*[21] found ion-association constants of 48–125 l/mol for catecholamine–alkyl sulphate systems. Riley *et al.*[25] measured ion-association constants between derivatives of benzoic acid (–OH, $-NH_2$, $-NO_2$, –Cl, $-CH_3$, –H, etc.) and terdecylbenzyldimethylammonium chloride in the range of 346–3560 l/mol. The stationary phases used were octadecylsilica. The calculation of the ion-association constant of TBA-Br corresponding to

$$N(C_4H_9)_4^+ + Br^- \rightleftharpoons N(C_4H_9)_4Br$$

results in:

$$K_{N(C_4H_9)_4^+Br} = \frac{C_{N(C_4H_9)_4^+Br}}{C_{N(C_4H_9)_4^+} \cdot C_{Br^-}} = 8.8 \cdot 10^2 \text{ l/mol}$$

For comparison, the ion-association constant of HBr, which is completely dissociated in aqueous solutions, is 10^{-6} l/mol. The ion-association constant of AgBr, which can

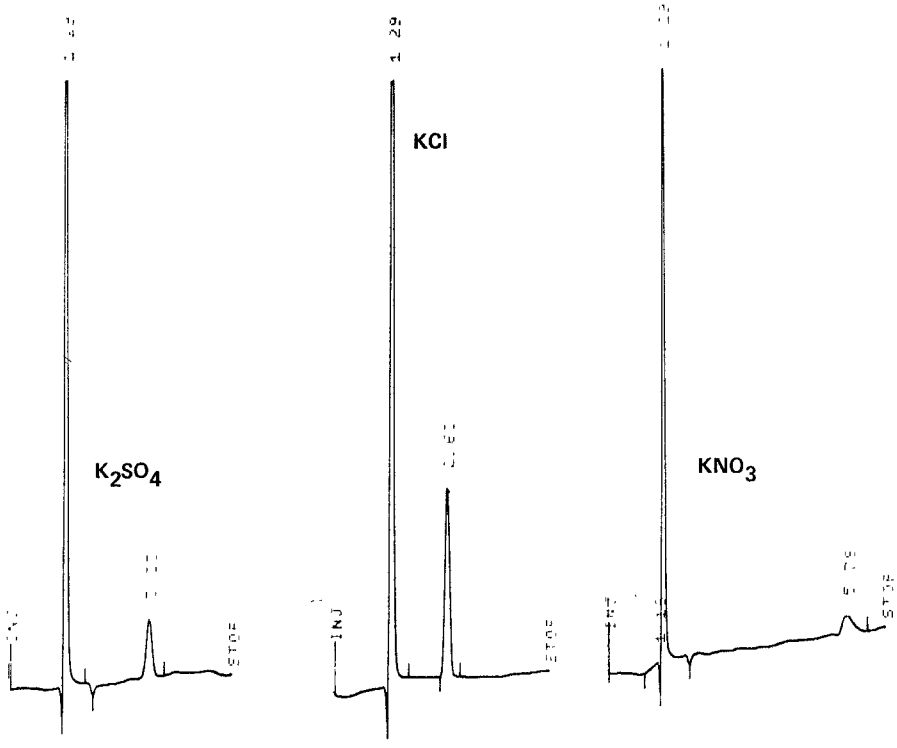

Fig. 9. Chromatograms of K_2SO_4, KCl and KNO_3. Conditions as in Fig. 6.

be treated as completely associated, is $10^{12.4}$ l/mol[26]. The range of ion-association constants obtained by HPLC is so far just a narrow "window" between these two extreme values. Ion-association constants of tetraalkylammonium salts with inorganic anions, estimated from conductivity and vapour pressure measurements, are comparable with the data from HPLC measurements. In aqueous solvents the lifetime of most ion-pairs is less than 10^{-9} sec, and the individual ions will keep their original character[27].

The ionic strength of the eluent also influences the retention due to competition of buffer anions with the sample anions in ion-pair equilibria. For halide anions this dependence is shown in Fig. 13.

With the HPLC conductivity detector, our results support the hypothesis that ion-pair formation under the above conditions is an extremely fast dynamic equilibrium process, where the time periods for the separation of the oppositely charged ions are long enough for conductivity processes.

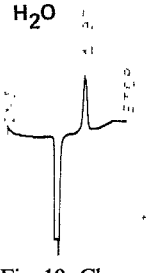

Fig. 10. Chromatogram of H_2O. Conditions as in Fig. 6.

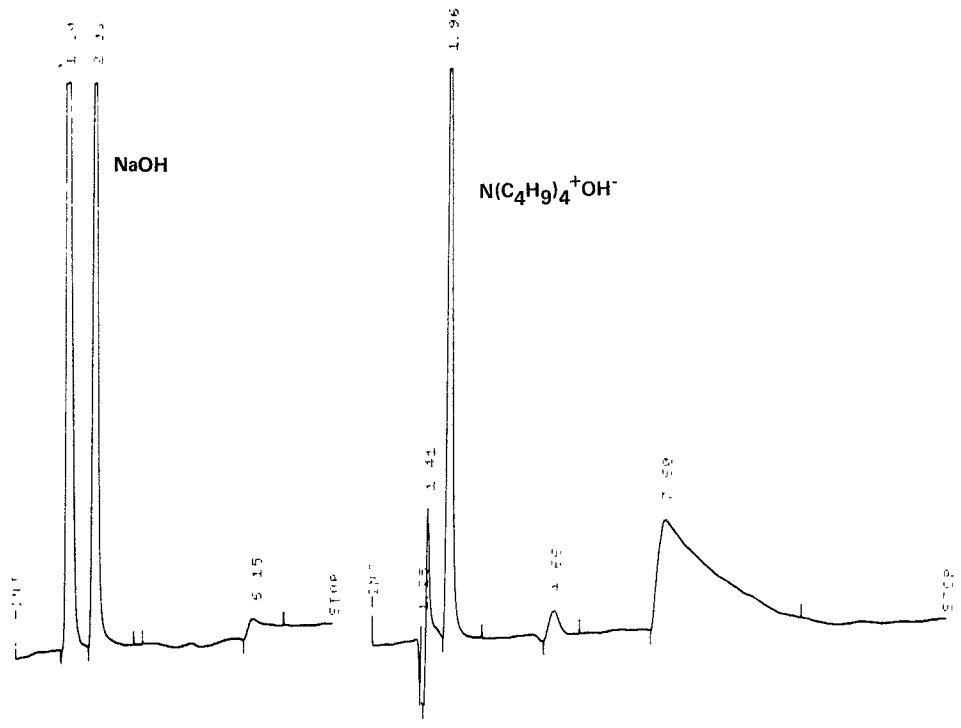

Fig. 11. Chromatogram of NaOH and $N(C_4H_9)_4^+ OH^-$. Conditions as in Fig. 6.

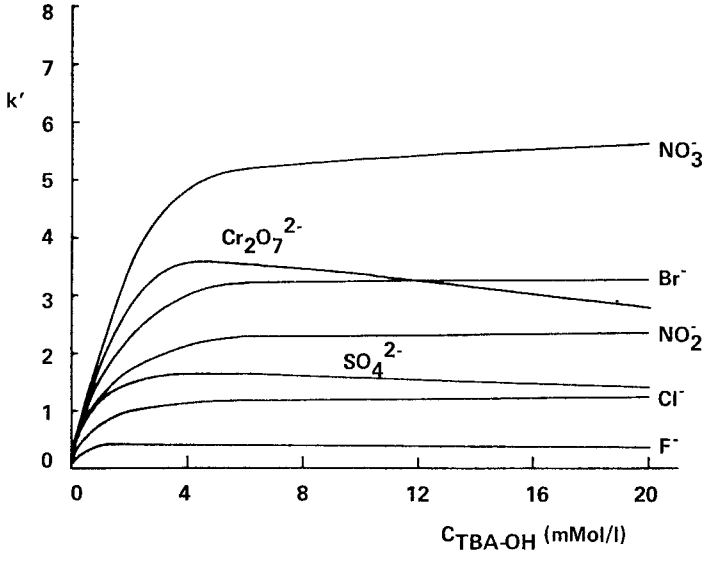

Fig. 12. Dependence of the capacity factor (k') of some anions on the TBA-OH concentration. Column: 10 μm LiChrosorb RP-18 (250 × 4.6 mm) (103.07.23.010, Knauer). Mobile phase: 0.05 mol/l phosphate buffer, pH 6.7 (+ different concentrations of TBA-OH); flow-rate, 2.0 ml/min. Pressure: 80 bar. Temperature: 25°C. Conductivity detector (74.00, Knauer).

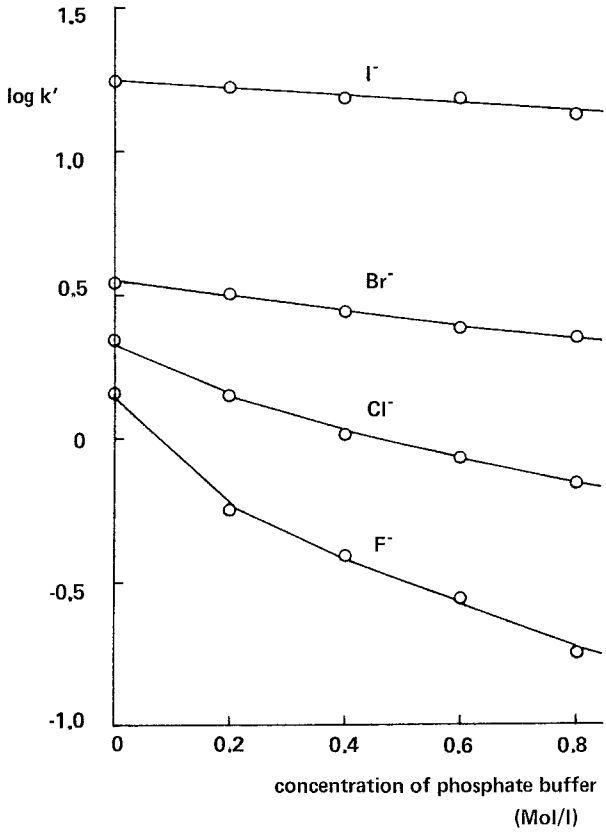

Fig. 13. Dependence of logarithm of the capacity factor (log k') of F^-, Cl^-, Br^- and I^- on the ionic strength of the buffer. Column: 10 μm LiChrosorb RP-18 (250 × 4.6 mm) (103.07.23.010, Knauer). Mobile phase: 0.005 mol/l TBA-OH (+ different concentrations of buffer); flow-rate, 2.0 ml/min. Other conditions as in Fig. 12.

Scott and Kucera[23] and Bidlingmeyer *et al.*[24] stated that a layer of the ion-pair reagent is formed on the surface. The formation of this layer would certainly be based on solvophobic interactions[6]. The reduction of the overall solvophobic surface area of the solvophobic solute–ligand complex, which is an energetically favourable process, is accompanied by the release of surface energy. These authors also assumed that a secondary layer of oppositely charged ions is formed. These findings indicate that the mechanism of the process is a dynamic equilibrium and is not based upon classical ion exchange, where the stationary phase is covalently bonded to the support.

UV detection. In classical wet analysis, redox titrations were carried out with I^- (ref. 28). The reversible reaction of the method called "iodometry" can be written as:

$$I^- \rightleftharpoons 1/2\, I_2 + e^-$$

The end of this reaction was indicated by the appearance of elemental I_2.

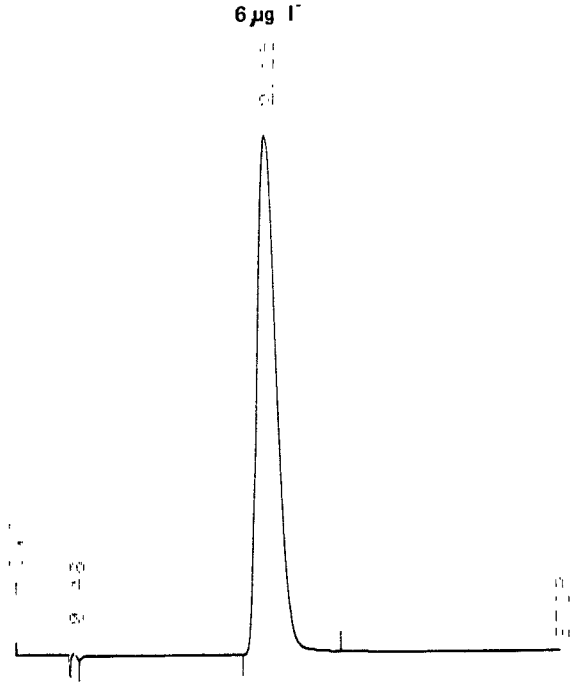

Fig. 14. Chromatogram of I^- with detection at 235 nm. Column: 10 μm LiChrosorb RP-18 (250 × 4.6 mm) (103.07.23.010, Knauer). Mobile phase: 0.0002 mol/l TBA-OH and 0.05 mol/l phosphate buffer, pH 6; flow-rate: 5.0 ml/min. Pressure: 200 bar. Temperature: 25°C. Sample: 6 μg I^- dissolved in the eluent. Detector: spectrophotometer (87.00, Knauer) at 1 a.u.f.s. and 235 nm.

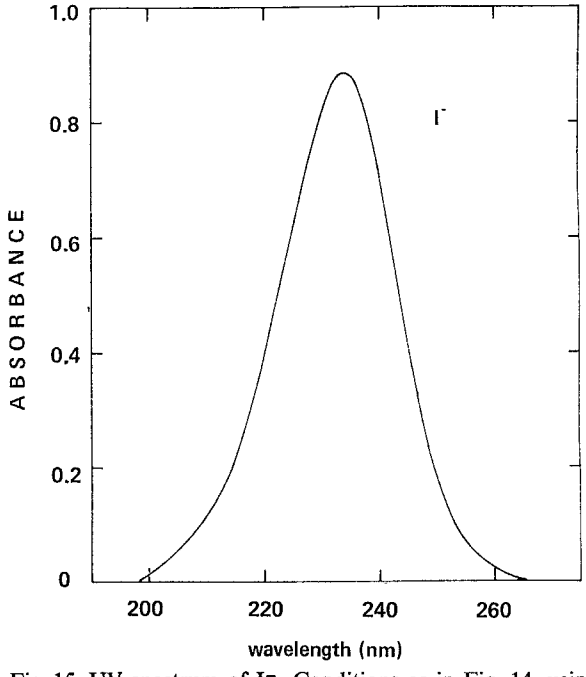

Fig. 15. UV spectrum of I^-. Conditions as in Fig. 14, using stop-flow scanning.

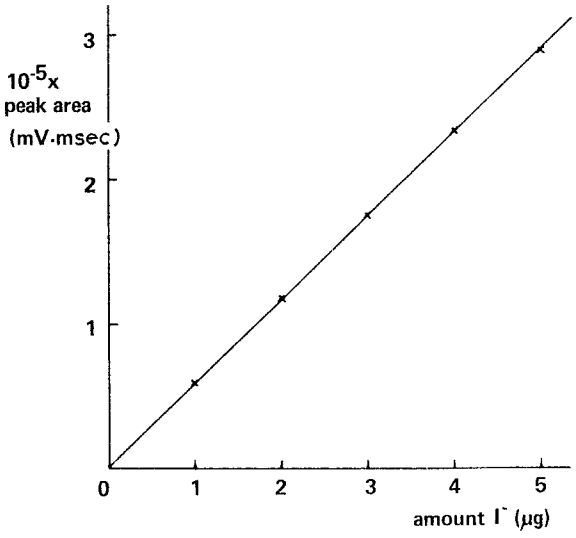

Fig. 16. Linearity of the I^- determination. Conditions as in Fig. 14.

Much easier is, however, the determination of I^- by RPC using mobile phases containing TBA-OH (Fig. 14). The I^- peak is easily detectable at 235 nm with a spectrophotometer (Fig. 15). The linearity of the detection is excellent and the detection limit is less than 10 ng (Fig. 16). As shown by Reeve[18], a number of other anions can be sensitively detected at 200–220 nm.

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