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SAMPLE-INDUCED INTERNAL GRADIENT OF IONIC STRENGTH IN ION-EXCLUSION MICROCOLUMN LIQUID CHROMATOGRAPHY

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SUMMARY

A simple method for the generation of a variable internal gradient of decreasing ionic strength is described. The principle permits the gradient elution of organic ions under the conditions of ion-exclusion chromatography, *i.e.*, with the signs of the solute and sorbent charges being the same. The method allows the gradient to be induced simultaneously with sample introduction on to the separation column. Such conditions are useful for trace analysis based on on-column preconcentration from a large sample volume and subsequent gradient elution. The method was applied to the separation of UV-absorbing organic anions on a microcolumn packed with a reversed-phase sorbent with fixed anionic groups.

INTRODUCTION

Column liquid chromatography (LC) is often used to determine trace concentrations of solutes in complex matrices. The analytical sensitivity can be improved and the “general elution problem” can be solved by the use of a continuous gradient of the mobile phase composition which leads to a continuous decrease in solute retention on the separation column¹. The retention of ionized solutes can be controlled by adjusting the mobile phase pH, ionic strength and contents of organic solvent and ion-interacting compounds. In conventional LC, a mobile phase gradient is generated by time-programmed addition of the streams of components in the solvent delivery device. In microcolumn LC, this method of gradient generation is difficult. Therefore, gradient mixers have been suggested, *e.g.*, tubular mixers^{2,3} can generate a gradient of variable profile for microcolumns of 1 mm I.D..

The ultimate independence of pre-column gradient instrumentation can be achieved if a gradient is generated in the separation column. Internal gradients of pH⁴ and counter-ion⁵ and co-ion⁶ concentration have been suggested for microcolumn LC.

The control of ionic strength is a powerful technique in ionic solute gradient elution⁷. If the signs of the charges of the solute and the stationary phase are opposite, an increase in ionic strength decreases the solute retention. Organic ionic com-

pounds can also be effectively separated on a stationary phase that has a charge of the same sign as the solute; this is the principle of ion-exclusion chromatography⁸. It has been shown both theoretically⁹ and practically^{9,10} that the solute retention decreases with decreasing mobile phase ionic strength in this type of chromatography.

In this work, the generation of an internal gradient of decreasing ionic strength is described. Such a gradient permits the gradient elution of organic anions under the conditions of ion-exclusion chromatography. Aromatic sulphonic and carboxylic acids were chosen as model solutes because of their good detectability with a UV photometric detector. The suitability of the method for solute on-column preconcentration and trace analysis by microcolumn LC is discussed.

EXPERIMENTAL

Apparatus

The chromatograph used included an MC 300 micropump (Mikrotechna, Prague, Czechoslovakia), a laboratory-made six-port valve with external loops (1 or 60 μl), an SF 769 Z UV detector (Kratos, Ramsey, NJ, U.S.A.) equipped with a 0.5- μl flow cell and a CDLC 1 conductivity detector (Laboratory Instruments, Prague, Czechoslovakia) with a 1- μl capillary flow cell. An OP 208/1 pH meter (Radelkis, Budapest, Hungary) equipped with a 100- μl flow electrode was connected to the outlet of the chromatograph to monitor the pH of the column effluent. Chromatograms were recorded on a TZ 4200 dual recorder (Laboratory Instruments). Conductivities of the water used and of the solutions prepared were checked with an OK 102/1 bath conductimeter (Radelkis).

Chemicals

Salts used for the mobile phase preparation were of analytical-reagent grade (Lachema, Brno, Czechoslovakia). Model mixtures of organic anions were prepared from deionized water and free organic acids or their sodium salts and adjusted to pH 7 with ammonia solution. Sodium benzenesulphonate, toluenesulphonic acid, benzoic acid, 1-naphthylacetic acid, 2,4-dichlorophenoxyacetic acid and sodium dodecyl sulphate (SDS) were from Lachema, 2-naphthalenesulphonic acid, toluic acid and 3,4-dimethylbenzoic acid from Aldrich (Milwaukee, WI, U.S.A.), indole-3-acetic acid, indole-3-butyric acid and 2-(2,4-dichlorophenoxy)propanoic acid from Sigma (St. Louis, MO, U.S.A.), 1-naphthalenesulphonic acid from Fluka (Buchs, Switzerland) and 1-naphtoic acid from BDH (Poole, U.K.). Sodium 3,4-dimethylbenzenesulphonate was prepared by sulphonation of *o*-xylene and recrystallized. The deionized water used had a conductivity of 2 $\mu\text{S cm}^{-1}$.

Microcolumn preparation

The microcolumn used was a CGC (150 \times 1 mm I.D.) glass cartridge (Tessek, Prague, Czechoslovakia) packed with Separon SGX C₁₈ (5 μm) reversed-phase silica-based sorbent. The microcolumn was fitted directly to the six-port sampling valve. The packing was dynamically modified with SDS by injection of 5 \times 60 μl of 0.1 mol l⁻¹ SDS solution. The microcolumn was then washed with about 10 ml of deionized water. The retentions of solutes were stable enough to demonstrate the influence of the salt concentration in the mobile phase. The void volume (90 μl) of the micro-

column was determined as the retention volume of glycerine measured as the deflection of the UV detector baseline caused by the change in the refractive index of the mobile phase.

RESULTS AND DISCUSSION

Isocratic elution

An earlier study¹⁰ showed that the nature of the salt dissolved in the mobile phase has only a small influence on the retention of organic anions separated by ion-exclusion chromatography. On the basis of this finding, our preliminary experiments and the UV transparency and corrosivity of the salt, ammonium sulphate was chosen to adjust the mobile phase ionic strength in the isocratic measurements.

In order to investigate the influence of the salt concentration on solute retention, sodium salts of selected sulphonic and carboxylic acids were chromatographed using mobile phases containing several concentrations of ammonium sulphate. The mobile phase pH was adjusted to pH 7 with ammonia solution. The volume of sample solution injected was 3 μl , which was calculated as the sum of the valve internal and external loops. Examples of chromatograms obtained after the injection of some model mixtures are shown in Fig. 1. The conductivity trace is corrected for the volume between the cells of the UV and conductivity detectors. Simultaneous conductivity and UV photometric detection not only permits the detection of organic anions, but also enables the elution of Na^+ ion to be followed.

Fig. 2 shows that the retention of Na^+ ion decreases with increase in the ionic

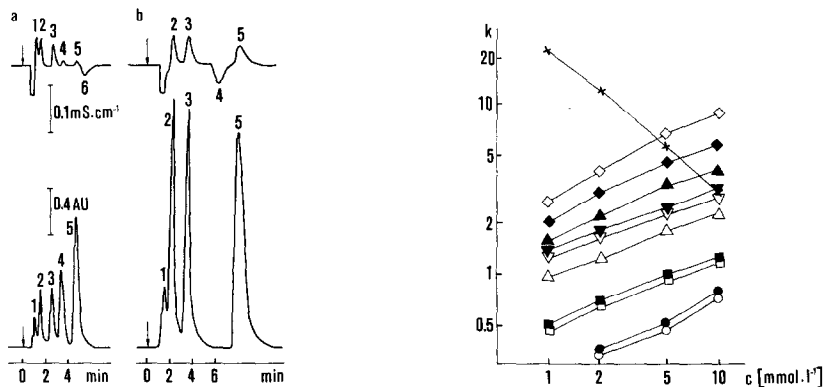


Fig. 1. Chromatograms of organic anions separated by isocratic ion-exclusion chromatography. Microcolumn: CGC (150 \times 1 mm I.D.), packed with Separon SGX C₁₈ (5 μm), dynamically modified with SDS. Mobile phase: 5 mmol l⁻¹ ammonium sulphate in deionized water (pH 7); flow-rate, (a) 2 and (b) 1.3 $\mu\text{l s}^{-1}$. Detection: upper trace, conductivity; lower trace, UV at 260 nm. Peaks: (a) 1 = benzenesulphonate, 2 = toluenesulphonate, 3 = 3,4-dimethylbenzenesulphonate, 4 = 1-naphthalenesulphonate, 5 = 2-naphthalenesulphonate, 6 = sodium ion; (b) 1 = benzoate, 2 = 4-methylbenzoate, 3 = 3,4-dimethylbenzoate, 4 = sodium ion, 5 = 1-naphthylacetate.

Fig. 2. Dependence of the capacity ratio (k) of organic anions on the molarity of ammonium sulphate in the mobile phase (c) at pH 7. Solutes: ●, benzenesulphonate; ■, toluenesulphonate; ▼, 3,4-dimethylbenzenesulphonate; ▲, 1-naphthalenesulphonate; ◆, 2-naphthalenesulphonate; ○, benzoate; □, 4-methylbenzoate; ▽, 3,4-dimethylbenzoate; △, 1 naphthoate; ◇, 1-naphthylacetate; ×, sodium ion. Other conditions as in Fig. 1.

strength of the mobile phase. The negative tangent of this dependence in log-log coordinates is close to unity, which indicates a common ion-exchange mechanism for Na^+ retention. In the system used, the retention of Li^+ ion was found to be identical with that of Na^+ ion, whereas the retention of Cs^+ ion was about 1.3 times that of Na^+ ion. Such a low selectivity for alkali metal ions was also observed earlier for C_{18} silica-based sorbents dynamically modified with alkyl sulphonates¹¹. Assuming the selectivity coefficient for $\text{Na}^+ - \text{NH}_4^+$ ion exchange to be close to unity, the exchange capacity can be calculated as the product of the column dead volume, the concentration of ammonium ions in the mobile phase and the capacity ratio of the Na^+ ion. This gives a value of about $5 \mu\text{equiv.}$ for the microcolumn used.

The retentions of aromatic anions increase with increase in the salt concentration from 1 to 10 mmol l^{-1} (Fig. 2). Similar dependences were found in an earlier study¹⁰ for the same or similar solutes, with the concentration of sodium sulphate varying from 20 to 400 mmol l^{-1} . In the latter instance, the anionic groups of the sorbent originated solely from dissociation of residual silanol groups. Calculations based on previous measurements¹² led to about $0.5 \mu\text{equiv.}$ of dissociated silanols in the volume of C_{18} silica reversed phase, which is equivalent to the volume of the microcolumn packing used in this study.

The isocratic elution of aromatic sulphonic acids also demonstrates the need for solving the "general solution problem". For example for a concentration of ammonium sulphate in the mobile phase of 1 mmol l^{-1} , the capacity ratio (k) for benzenesulphonate is near to zero and k for anthraquinone-2-sulphonate is 40.

Previous paragraphs lead to the following conclusions essential for the study of the gradient elution of organic anions: (1) dynamic modification of the C_{18} silica support with dodecylsulphate increases substantially the number of fixed anionic groups on the stationary phase surface; and (2) the retentions of organic anions on the sorbent used increase with increase in the salt concentration at both low and high ionic strengths.

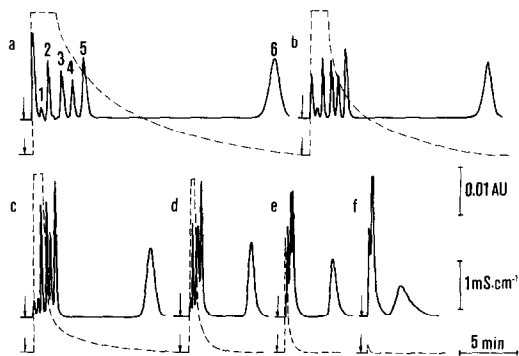


Fig. 3. Dependence of internal gradient profile on the concentration of ammonium sulphate in the injected solution. Mobile phase delivered by pump: deionized water adjusted to pH 7 with ammonia solution, conductivity $12 \mu\text{S cm}^{-1}$. Full-line peaks and concentrations in the injected solution (mmol l^{-1}): 1 = benzenesulphonate (1); 2 = 4-methylbenzenesulphonate (1); 3 = 3,4-dimethylbenzenesulphonate (1); 4 = 1-naphthalenesulphonate (0.2); 5 = 2-naphthalenesulphonate (0.2); 6 = 2-anthraquinonesulphonate (0.02). Broken line: conductivity of the column effluent. Concentration of ammonium sulphate in the injected solution: (a) 3; (b) 1; (c) 0.3; (d) 0.1; (e) 0.03; (f) 0 mol l^{-1} . Other conditions as in Fig. 1a.

Internal gradient

An internal gradient of decreasing ionic strength was obtained by injection of ammonium sulphate solution (pH 7) on to the microcolumn. The mobile phase delivered by the pump was deionized water adjusted to pH 7 with ammonia solution. The conductivity of this mobile phase was $12 \mu\text{S cm}^{-1}$. Keeping the volume injected at $3 \mu\text{l}$, the variation of the salt concentration in the solution introduced influences the profile of internal gradients of decreasing ionic strength obtained (Fig. 3). Here, the gradient profile was monitored with the conductivity detector (broken line). The solution conductivity can be converted into an approximate salt concentration if the conductivity of a 1 mmol l^{-1} solution of ammonium sulphate is considered to be $234 \mu\text{S cm}^{-1}$.

The observed variability of the salt elution profile with salt concentration in the solution injected cannot be explained only by overloading of column ion-exchange capacity (*e.g.*, ref. 13). In the present instance, the column behaves as a mixer in which washing out of salt corresponds to slower kinetics than that of solute equilibration between the mobile and stationary phases. The influence of precolumn volumes can be eliminated as the microcolumn is fitted directly to the sampling valve (see Experimental). Further, if a microcolumn of the same dimensions packed with non-porous glass beads $6 \mu\text{m}$ in diameter was fitted instead of the separation microcolumn, the salt concentration dropped to the steady-state level within about one dead volume even when the highest concentrations were injected. A rigorous explanation of the observed salt elution profile would require further investigations beyond the scope of this work.

Nevertheless, the variable internal gradient of decreasing ionic strength obtained has a practical value. The injected solution contained also aromatic sulphonates detectable by the UV detector (Fig. 3, full line). An increase in the salt concentration in the sample solution leads to an increase in gradient time and, consequently, to increased retention times of solutes. Simultaneously, the average steepness of the gradient decreases with increasing salt concentration. In agreement with the theory of gradient elution¹, a lower steepness is reflected by an increase in the peak widths.

The gradient profiles in Fig. 3 indicate that for all the concentrations of the salt in the sample, the lowest elution strength occurs at the very beginning of the chromatogram. In other words, the solute is most strongly retained at the moment of injection at the top of the column. These conditions are favourable for on-column solute preconcentration. Fig. 4 shows examples of chromatograms obtained with a sample volume of $60 \mu\text{l}$ (*i.e.*, two thirds of the microcolumn void volume). Fig. 4a represents the chromatogram of aromatic sulphonates under conditions similar to those for Fig. 3a. It shows good solute focusing even for the first eluted compounds. The solutes are eluted in the peaks which have volumes less than that of the sample. Hence the conditions are advantageous for trace analysis. Chromatograms similar to Fig. 4a were also obtained when aromatic sulphonates were injected in concentrated solutions of NaCl, KCl, NH_4Cl , KNO_3 , NaNO_3 , Na_2SO_4 , NaClO_4 and CH_3COONa .

UV-absorbing organic anions comprise a number of analytically important groups of compounds, *e.g.*, sulphonated dyes and their intermediates, nucleotides and pesticides. Fig. 4b shows the on-column focusing and gradient elution of a model mixture of some plant growth regulators (derivatives of acetic acid). Under the condi-

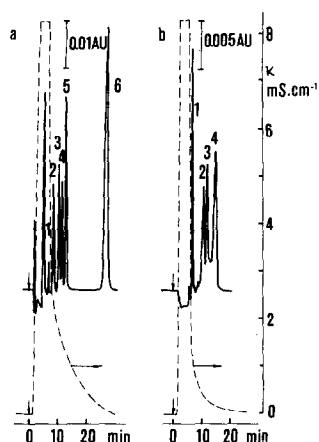


Fig. 4. On-column preconcentration and gradient elution of organic anions. Sample volume, $60\mu\text{l}$. (a) Ammonium sulphate concentration in the injected solution, 3 mol l^{-1} . Peaks and concentrations in the injected solution ($\mu\text{mol l}^{-1}$): 1 = benzenesulphonate (100), 2 = 4-methylbenzenesulphonate (100), 3 = 3,4-dimethylbenzenesulphonate (100), 4 = 1-naphthalenesulphonate (20), 5 = 2-naphthalenesulphonate (20), 6 = 2-antraquinonesulphonate (2). (b) Ammonium sulphate concentration in the injected solution, 0.1 mol l^{-1} . Detector wavelength, 280 nm. Peaks and concentrations in the injected solution (mg l^{-1}): 1 = indole-3-acetate (0.5), 2 = 2,4-dichlorophenoxyacetate (1), 3 = 1-naphthoxyacetate (2), 4 = 2-(2,4-dichlorophenoxy)propionate (2). Other conditions as in Fig. 3.

tions used, the 3-indolylbutyrate is coeluted with 2,4-dichlorophenoxyacetate. All the compounds shown were injected at concentrations of about 1 mg l^{-1} . The chromatogram shows that the detection limit is by about 1–2 orders of magnitude lower than the concentrations injected. Dilution of the sample solution by adding salt solution is not significant as it represents as little as *ca.* 3% of the sample volume if saturated salt solution is used.

CONCLUSIONS

The experiments described provide evidence that a variable internal gradient of decreasing mobile phase ionic strength can be generated by injecting concentrated salt solution on to a column packed with a suitably modified sorbent provided that the isocratic pump delivers dilute salt solution. In agreement with the isocratic measurements, such a gradient leads to a continuous decrease in the retention of organic ions on a sorbent having both hydrophobic sites and ionic groups of charge sign similar to that of the solute.

The principle was verified with a number of anionic solutes employing several salts in the injected sample solution. According to the theory developed for isocratic conditions⁹, it should also be applicable to cationic solutes provided that the sorbent in the column contains positively charged groups.

This new form of internal gradient offers great flexibility of the control of gradient time and steepness by the adjustment of the salt concentration in the injected sample solution. The widest applicability of the method can be seen in the gradient separation and trace analysis of organic ions by microcolumn LC.

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