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Note

Separation of sulphonic acids by normal-phase liquid chromatography using crown ethers in the mobile phase

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The separation of strongly ionic substances such as sulphonic acids by column liquid chromatography is still difficult. In many instances conventional ion exchange has been used as a separation method¹⁻⁴, but in recent years ion-pair chromatography has become an attractive alternative^{5,6}. Most of these ion-pair separations have been performed with hydrophobic column packings and aqueous eluents⁷⁻⁹, and in a few instances liquid-liquid systems with porous silica have been used¹⁰.

In this paper we report an investigation of the applicability of crown ethers, dissolved in the mobile phase as a complex between the crown ether and a potassium salt, which will be denoted by CK^+A^- . This results in an increased solubility of sulphonates in the organic phase by the formation of (CK^+) sulphonates.

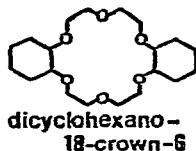
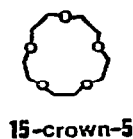
EXPERIMENTAL

Apparatus

High-performance liquid chromatographic (HPLC) experiments were carried out with a high-pressure liquid chromatograph (Hupe & Busch 1010 A, Hewlett-Packard) using UV detection at 254 nm (Zeiss PM2 DLC), a high-pressure sampling valve (Rheodyne, Model 7120) with a 20- μ l loop, a stainless-steel column (150 \times 4.6 mm I.D.) and a linear potentiometric recorder (Servogor 542, Goerz). All connections and couplings were made of stainless steel.

Materials

The three crown ethers (Aldrich, Milwaukee, WI, U.S.A.) used, 15-crown-5-(1,4,7,10,13-pentaoxacyclopentadecane), 18-crown-6-(1,4,7,10,13,16-hexaoxacyclooctadecane) and dicyclohexano-18-crown-6-(2,3,11,12-dicyclohexano-1,4,7,10,13,16-hexaoxacyclooctadecane) can be represented by:



Silica gel Si 60 (Merck, Darmstadt, G.F.R.), classified to a particle size range of 8–10 μm by means of an air classifier (Alpine MZR), was used as adsorbent. Benzene-, 3-nitrobenzene-, 3-aminobenzene- and 4-aminotoluenesulphonic acids as the sodium salts were obtained from Fluka (Buchs, Switzerland). The other sulphonic acids as the potassium salts were kindly donated by Prof. H. Cerfontain, Laboratory for Organic Chemistry, University of Amsterdam. All other chemicals were of analytical reagent grade and used as delivered, except potassium propionate and butyrate, which were prepared from the acids with potassium carbonate.

Procedures

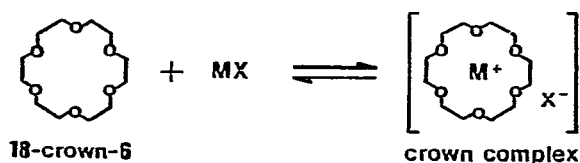
The capacity ratio (k'_i) of a component for a given eluent was derived from its retention time, t_{Ri} , and that of an unretained compound, t_{R0} (toluene). The columns were packed by means of a balanced slurry technique¹¹.

The freshly packed columns were washed successively with 50 ml of acetone, 50 ml of methanol and 100 ml of the selected organic solvent mixture without crown ether and salt and then finally eluted with the eluent until constant retention was achieved. When changing the eluent, at first the column was eluted with 50 ml of a solution of 0.015 *M* 18-crown-6 in dichloromethane–methanol (4:1) in order to remove the excess of salt and then with 50 ml of dichloromethane–methanol (4:1) in order to remove the crown ether.

Solutions of the sulphonates were prepared in the eluent containing an extra amount of crown ether. When sulphonic acids were dissolved an amount of potassium salt had to be added.

RESULTS AND DISCUSSION

The application of crown ethers in synthetic organic chemistry has become increasingly popular since their introduction by Pedersen¹² in 1967. Owing to their extremely electron-rich ring, these crown ethers (macrocyclic polyethers) are able to host a cation of a certain size according to:



The crown ether complex formed is soluble in moderately non-polar organic solvents such as toluene or dichloromethane. For instance, in this way potassium hydroxide can be dissolved in toluene or potassium permanganate in dichloromethane. The application of crown ethers in liquid chromatography was first reported by Blasius and co-workers and Cram and co-workers. These groups prepared crown ether-based resins^{13,14} or bonded chemically crown ethers to a polystyrene divinylbenzene resin¹⁵ or to a silica gel¹⁶. They also used chiral crown ethers and were so able to separate enantiomers of amino acids^{17,18}.

In all of these applications the possibility of a crown ether hosting the cations

to be separated was used to obtain the retention. However, the penetration of cations into and their transport out of the crown ether is a slow process from the chromatographic point of view, which consequently will lead to poor column efficiency. Further, crown ethers have also been applied in the extraction and ion-exchange chromatography of alkali metals¹⁹⁻²¹.

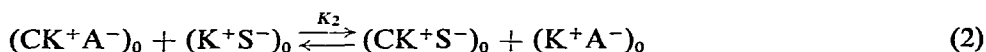
In this investigation, crown ethers (18-crown-6, 15-crown-5 and dicyclohexano-18-crown-6), in which K^+ is hosted, were used to increase the solubility of sulphonates in organic solvents and thus to be able to influence the degree and order of retention of such substances in normal-phase adsorption chromatography.

In order to obtain a general idea of the processes that govern the retention behaviour of sulphonates, chromatographic runs were carried out with three different mobile phases. With a mobile phase of mixtures of dichloromethane and methanol and which did not contain a crown ether, the sulphonates under investigation were strongly retained and showed severe peak tailing. When a crown ether as such was added (*i.e.*, without the incorporation of a potassium ion) to the mobile phase, the sulphonates were almost unretained.

An intermediate retention for the sulphonates was obtained when an alkali metal salt, *e.g.*, potassium acetate, was added to the mobile phase at a concentration higher than that of the crown ether. The retention behaviour of sulphonates with these three different mobile phases lead to the assumption that in the latter instance the retention is governed by two equilibria: (i) adsorption of the sulphonate K^+S^- on to the silica surface according to



where the subscript o represents the organic phase and ads the stationary phase; and (ii) complexation of the sulphonate ion by the crown potassium complex (CK^+A^-) in the organic phase according to



where CK^+S^- is the crown potassium sulphonate complex and K^+A^- is the potassium salt. Assuming these two equilibria, the distribution coefficient, D_i , of a solute i can then be derived from eqns. 1 and 2:

$$D_i = \frac{K_1}{1 + K_2 \cdot \frac{[CKA]_o}{[KA]_o}} \quad (3)$$

Fig. 1a shows the dependence of the capacity ratio (k'_i), which is proportional to D_i , at different $[CKA]_o$ values and at constant $[KA]_o$ (potassium acetate concentration*) in the mobile phase. It can be seen that k'_i decreases with increasing $[CKA]_o$, as predicted by eqn. 3. When plotting $1/D_i$ versus $[CKA]_o$ at constant $[KA]_o$, one would expect a linear dependence on the basis of eqn. 3. However, this was not found,

* In all figures, the potassium concentration given is the free concentration.

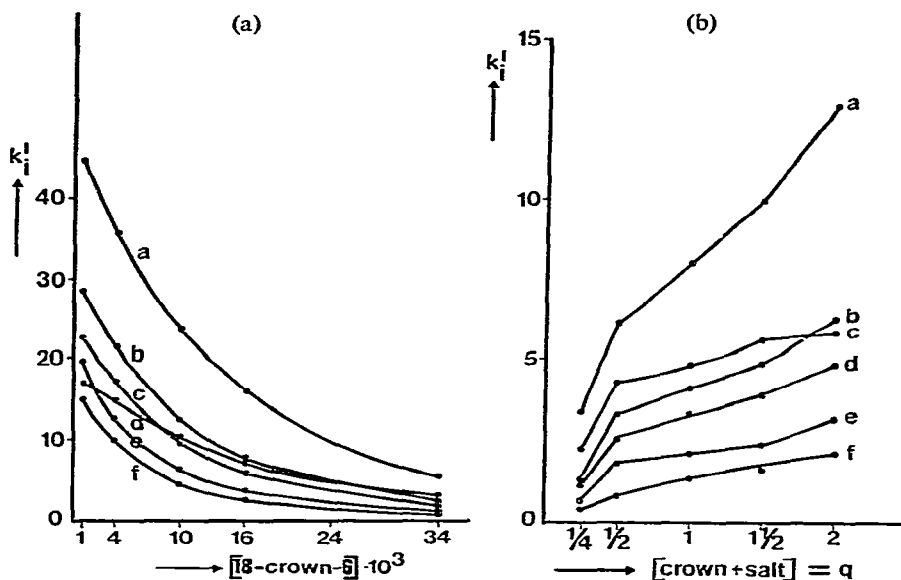


Fig. 1. (a) Dependence of the capacity ratio (k'_i) on the concentration of 18-crown-6 in the mobile phase. Mobile phase composition: 18-crown-6 (CKA), 0.014 M potassium acetate (KA) in CH_2Cl_2 -methanol (9:1). Solutes: (a) 4-aminotoluene-; (b) benzene-; (c) diphenyl-2-; (d) 2-iodobenzene-; (e) 3-iodobenzene-; (f) 9,10-anthraquinone-2-sulphonate. (b) Dependence of the capacity ratio on the CKA + KA concentration with constant $[\text{CKA}]/[\text{KA}]$ ratio. Mobile phase composition: $q = 0.01$ M CKA + 0.014 M KA in CH_2Cl_2 -methanol (9:1). Solutes as in (a).

which indicates that the assumed simple representation of the mechanism is not adequate for describing the retention of sulphonates. Minor changes in the nature of the stationary phase, for instance owing to adsorption of KA, may be responsible for this non-linearity. This could also be concluded from Fig. 1b, showing k'_i as function of $[\text{CKA}]_0$ and $[\text{KA}]_0$ but keeping the ratio $[\text{CKA}]_0/[\text{KA}]_0$ constant. If eqn. 3 is valid, no effect on k'_i should be found. As can be seen from Fig. 1b, the capacity ratios increase gradually with increasing concentration. However, there is a slight tendency for k'_i to level off at higher concentrations.

The effect of the type of crown ether on the retention of sulphonates was investigated with three different crown ethers and is shown in Fig. 2. As can be seen, useful differences in retention and selectivity occur with these different crown ethers.

As has already been mentioned, it is necessary to add an excess of an alkali metal salt to the mobile phase. The effect of the concentration of the potassium salt on the retention was investigated by the addition of different amounts of potassium acetate to a 0.01 M 18-crown-6 eluent. The results are shown in Fig. 3a. The retention of the sulphonates increases almost linearly with increasing salt concentration, but with different slopes. This result suggests that the term $(1 + K_2 \cdot [\text{CKA}]_0/[\text{KA}]_0) \approx K_2 \cdot [\text{CKA}]_0/[\text{KA}]_0$.

The acetate concentration in the mobile phase seems to be a valuable parameter for varying the retention in a more or less predictable way and can be used to produce gradients. The effect of the type of the competitive anion was investigated with potassium acetate, propionate and butyrate and the results are shown in Fig. 3b. At

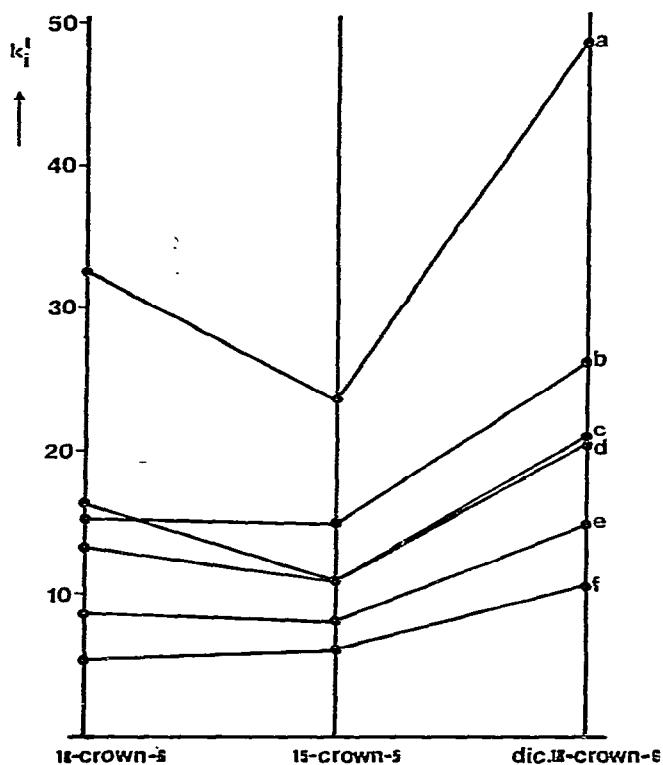


Fig. 2. Effect of the nature of the crown ether in the mobile phase on the capacity ratio. Mobile phase composition: 0.01 *M* CKA, 0.014 *M* KA in CH_2Cl_2 -methanol (9:1). Solutes as in Fig. 1a.

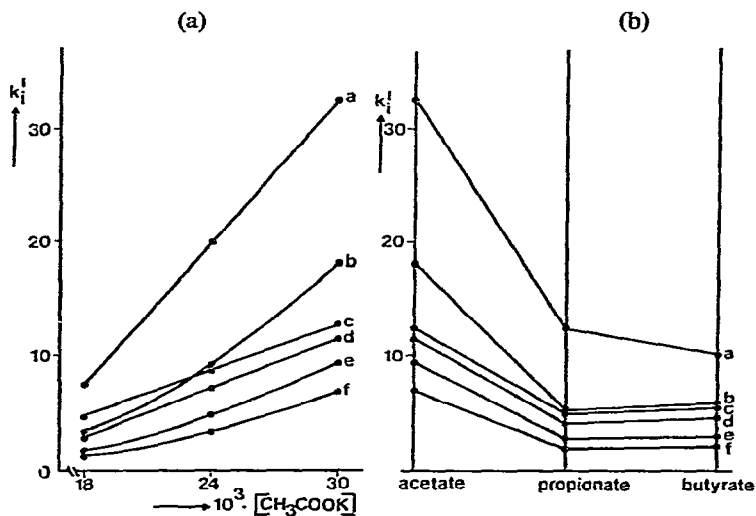


Fig. 3. (a) Dependence of the capacity ratio on the potassium acetate (KA) concentration in the mobile phase. Mobile phase composition: KA, 0.01 *M* CKA in CH_2Cl_2 -methanol (9:1). Solutes as in Fig. 1a. (b) Effect of the nature of the competitive anion (A) on the capacity ratio. Mobile phase composition: 0.01 *M* CKA, 0.02 *M* KA in CH_2Cl_2 -methanol (9:1). Solutes as in Fig. 1a.

similar concentrations of these anions, acetate caused the largest retention for the selected sulphonates and so acted as the strongest competitive anion.

Apart from the above-mentioned anions, the inorganic anions Cl^- , Br^- , NO_3^- , ClO_4^- , H_2PO_4^- , CNS^- , CO_3^{2-} and HSO_4^- were also tested. However, these anions could not be dissolved in the mobile phase at concentrations higher than the crown ether concentration necessary to obtain the required retention, and so are not suitable counter ions.

Fig. 4 shows the k'_i values of various sulphonates as a function of the methanol content of the mobile phase. k'_i decreases with increasing methanol content, as is commonly found in adsorption chromatography. Ethanol and propanol were also tested as polar organic modifier. With 20% of the alcohol in dichloromethane the k'_i values of the sulphonates were about a factor of five larger with ethanol and propanol than with methanol, while no significant selectivity changes were observed. In this respect organic modifiers belonging to different groups such as tetrahydrofuran, dioxane and nitromethane might behave differently, but this was not yet investigated.

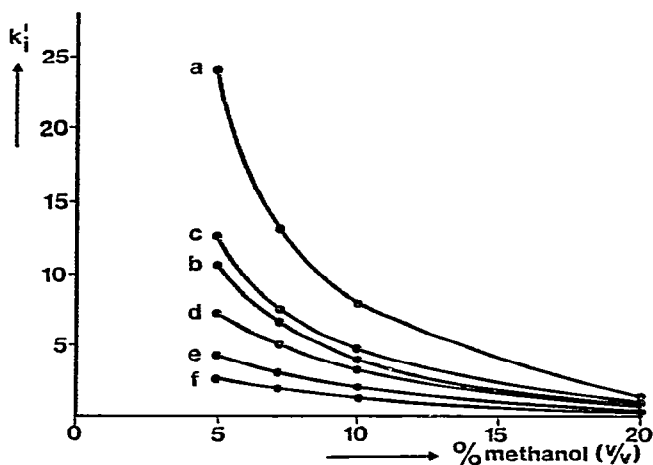


Fig. 4. Dependence of the capacity ratio on the methanol content of the mobile phase. Mobile phase composition: 0.01 M CKA, 0.014 M KA and methanol in CH_2Cl_2 . Solutes as in Fig. 1a.

The applicability of the developed crown ether systems is demonstrated in Figs. 5 and 6, showing the separation of test mixtures of substituted monosulphonic acid salts.

For more complex sulphonates bearing amine groups, such as many food additives (dyes), the developed phase systems are less suitable because of significant peak tailing. For these substances, however, well equilibrated liquid-liquid systems consisting of an aqueous acetate buffer as the stationary phase (coated *in situ*²²) and dichloromethane-methanol mixtures containing a crown ether as the mobile phase were found to be more promising, as is demonstrated in Fig. 7.

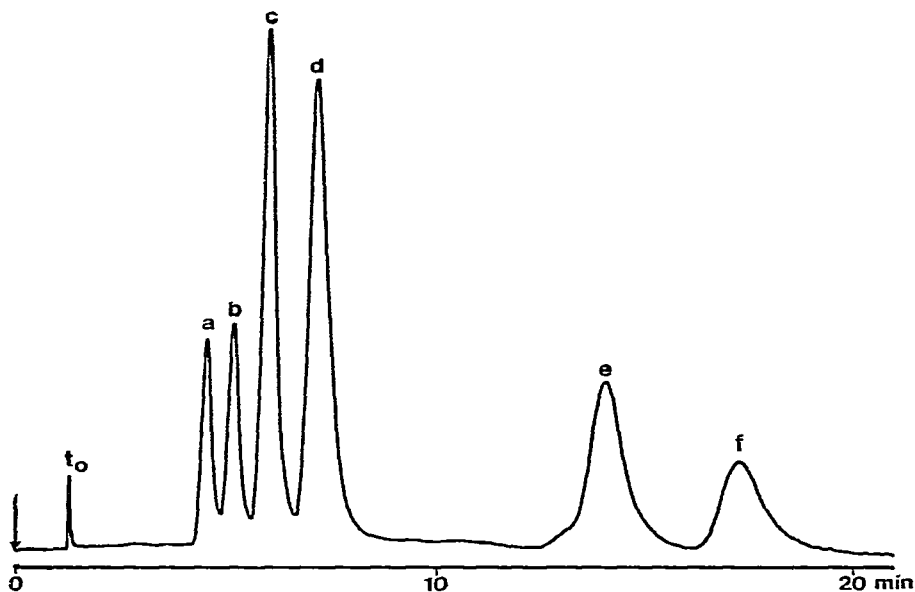


Fig. 5. Separation of a test mixture of sulphonates. Mobile phase composition: 0.01 *M* 15-crown-5 (CKA), 0.014 *M* KA in CH_2Cl_2 -methanol (9:1). Solutes: (a) 9,10-anthraquinone-2-; (b) biphenylene-2-; (c) diphenyl-3-; (d) diphenyl-4-; (e) 4-aminotoluene-; (f) 3-aminobenzenesulphonate. t_0 = unretained (toluene).

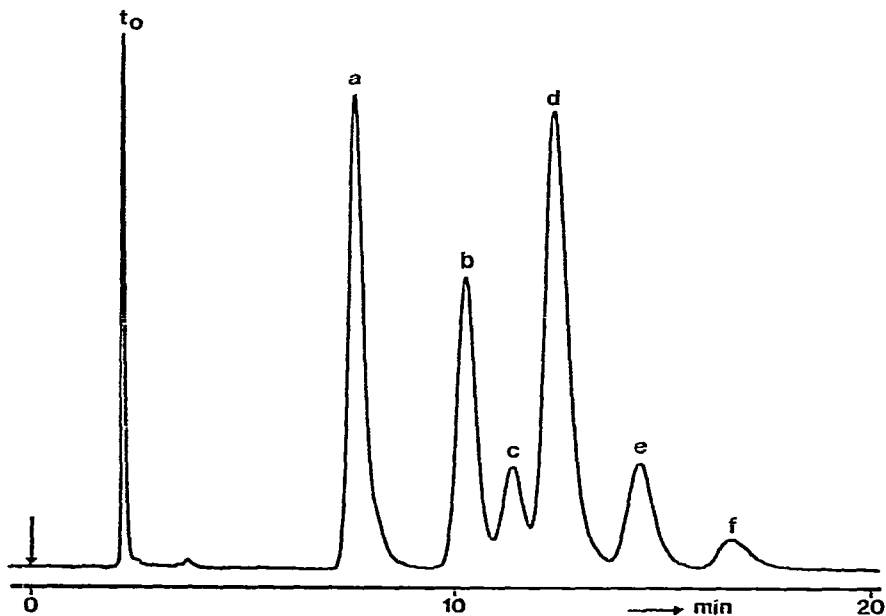


Fig. 6. Separation of a test mixture of halogen-substituted sulphonates. Mobile phase composition: 0.01 *M* 18-crown-6 (CKA), 0.014 *M* KA in CH_2Cl_2 -methanol (9:1). Solutes: (a) 3-nitrobenzene-; (b) 3-iodobenzene-; (c) 3-chlorobenzene-; (d) 4-iodobenzene-; (e) 1-iodobenzene-; (f) 1-chlorobenzenesulphonate. t_0 = unretained (toluene).

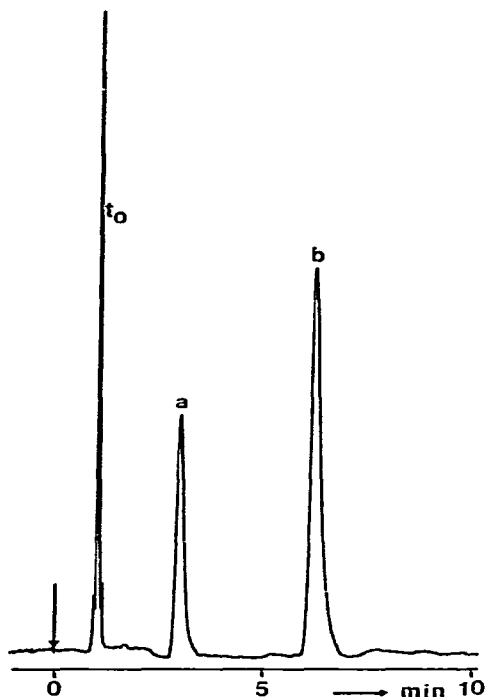


Fig. 7. Separation of two food additives (dyes) by liquid-liquid chromatography. Stationary phase composition: 0.1 M acetic acid, 0.05 M potassium acetate in water on Si 60. Mobile phase composition: 0.0075 M 18-crown-6 in CH_2Cl_2 -methanol (94:6). Solutes: (a) Patent Blue V, C.I. 42051; (b) Lissamine Green B, C.I. 44090.

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