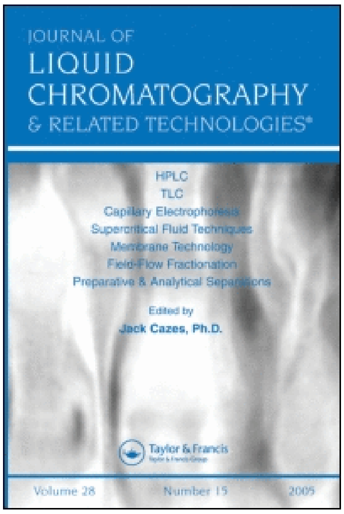

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APPLICATION OF SMALL CAPACITY CATION EXCHANGERS IN ION-EXCLUSION CHROMATOGRAPHY

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ABSTRACT

Different attempts to increase retention of strong acids in ion exclusion chromatography are presented. A decrease of the dissociated forms of the sorbent functional groups which increases the retention of strong acids can be achieved either by decreasing the effective charge of the support, or by using a week exchanger as silica, instead of conventionally sulfonated resins.

Another suggested way is the addition of ion pairing reagents to the mobile phase. All these attempts, which improve the separations, fit with a proposed theoretical model.

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INTRODUCTION

Ion exclusion chromatography (IEC) is a technique which allows the separation of acids (or bases) of medium strength.[1] The characteristic feature of the IEC technique is the electric charge sign of dissociated ion-exchange resin functional groups, which is the same as the electric charge sign of the analysed ionic compound. It follows that samples of negatively charged ions, e.g., dissociated acidic compounds, are separated on cation exchange resins with anionic functional groups. Usually these are sulfonic acid groups.

Since the separation mechanism is described in detail elsewhere,[1,2] just a brief review will be given. Neutral species are allowed to penetrate into the stationary phase constituted by water molecules immobilised in the pores of the support (a large ion exchange capacity resin) and water molecules bound to their functional groups. Conversely, charged ions with identical signs are repulsed by these groups. As a consequence, the retention of a single compound will depend on the ratio between the concentrations of its ionised and neutral forms. Owing to the fact that strong acids (as well as strong bases) are completely dissociated, they are eluted, unseparated, with the solvent front.

To overcome such limitation, two approaches my be used. The first one consists in reducing the concentration of solute dissociated species by eluting with strong concentrated acids. Such an idea, even if theoretically right, encounters practical difficulties, when acids with high dissociation constants are involved. More intriguing is the attempt to reduce the repulsion between the stationary phase functional groups and the solute ions, by decreasing the acidity of the former. This can be achieved by decreasing the concentration of the functional groups, or the magnitude of their negative charge, by using weak acidic solids instead of polysulfonated resins.[3-5]

The aim of the present paper is to illustrate how these techniques work with ion exclusion chromatographic separations.

EXPERIMENTAL

Apparatus

The chromatographic measurements were performed with a Beckman chromatographic system consisting of a Model 110B solvent delivery module, Model 340 organizer, and Model 160 absorbance detector. The retention time, detected at 210 nm, was measured using a Hewlett-Packard HP 3394A Integrator.

The Bio-Rad (Richmond, USA) Aminex ion-exclusion HPX-87H organic acid analysis column (300 x 7.8 mm I.D., hydrogen form, 8% cross-linked cation exchanger, 9 μm particle diameter) was used. The silica column was packed with a slurry packing apparatus (IChF-PAN, Warsaw, Poland).

Reagents

All the products, analytical grade reagents (ZOCh, Lublin, Poland; Sigma, St. Louis, MO USA; BDH, Port Fairy, UK; Fluka, Buchs, Switzerland; Carlo Erba, Milan, Italy), were used without further purification. LiChrosorb Si 100, 5 μm packing material (Merck, Darmstadt, FRG), was used to prepare the silica column, using water as a dispersing, as well as, a pressing phase.

Procedure

The mobile phase, prepared from triply distilled water passed through a Millipore (Bedford, MA USA) Milli-Q water purification system, was filtered through a Millipore 0.22 mm membrane filter, and degassed in an ultrasonic bath before using. The columns were equilibrated for at least 1 hour before use at room temperature. Stock solutions, 10 mM of solute acids, were diluted to make the selected concentrations. Every sample was injected six times and the average was taken for further elaboration.

RESULTS AND DISCUSSION

Recently[6] an equation describing the influence of some physicochemical and chromatographic parameters on the solute retention expressed as the distribution coefficient, K_d , has been developed:

$$K_d = \frac{1 + {2K_a^S \gamma_{H^+}^S \gamma_{F^-}^S} \Big/ {\sqrt{K_f^2 + 4K_f c_f \gamma_{H^+}^S \gamma_{F^-}^S} - K_f}}{1 + {2K_a^M} \Big/ {\sqrt{K_b^2 + 4K_b c_b} - K_b}} K_P, \qquad (1)$$

where K_a^S and K_a^M denote the solute acidic dissociation constants in the stationary and mobile phases, respectively, K_b and K_f are the buffer and the functional groups dissociation constants, c_b and c_f refer to the buffer and functional group concentrations, $g^{S}_{H^+}$ and $g^{S}_{F^-}$ are the activity coefficients of the hydrogen ions and dissociated functional groups in the stationary phase, and K_P is the distribution coefficient of the un-dissociated form of the analysed acid.

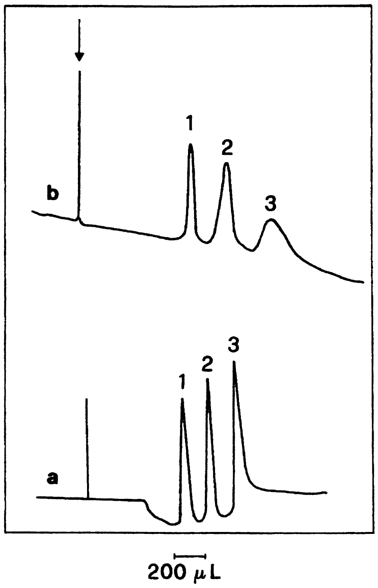

Figure 1. Separation of aliphatic fatty acids on the silica column. Analysed acids: 1 - formic, 2 - propionic and 3 - valeric, solute concentration - 1 mM, volumetric flow rate - 0,5 mL/min., column: LiChrosorb Si 100, 5 μm, 150 x 4 mm I.D., detector - UV-210 nm, mobile phase: (a) - water, (b) - 0,1 mM H_2SO_4.

After simplification assumptions that buffer and functional groups are strong acids and that their concentrations are much higher than that of the solute, the above equation can be shortened to the form:

$$K_d = \frac{1 + K_a^S / c_f}{1 + K_a^M / c_b} K_P. \tag{2}$$

From the analysis of above equations, we note that a decrease of the dissociated form of the resin functional groups should increase the solute retention. This can be achieved by decreasing the functional group concentrations or by decreasing their dissociation constants.

Another possibility to increase retention and improve separation involves decreasing the functional group concentrations by an ion interaction reagent added to the mobile phase. In the last case, additionally, changing of the hydrophobic adsorption constant is observed.

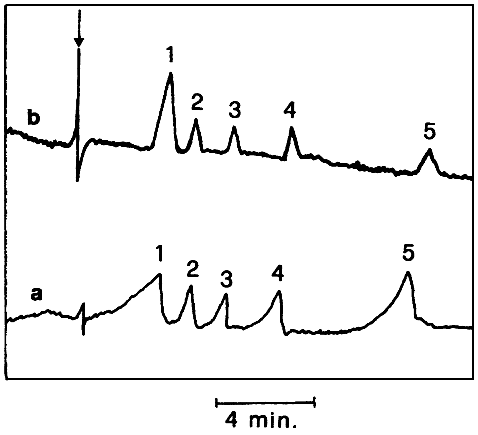

Figure 2. Chromatograms of the following acids: 1 - formic, 2 - acetic, 3 - propionic, 4 - butyric and 5 - valeric. Mobile phase: (a) - water and (b) - 0.5 mM TBABr. Flow rate - 1 mL/min. Column: BioRad HPX-87H.

Silica was selected as a testing model since its weak cation exchange properties are known[7-10] and its free silanol groups have been seen to influence retention even in reversed phase chromatography.[11] Its pK_a value is *7.1* (in water it gives $pH \approx 5$), and its isoelectric point is about *2*.[9,12]. Aliphatic acids, taken here as an example, separated on the silica column are presented in Figure 1. The order of their retention is similar to the commercially available ion exclusion columns.[1] This suggests the hydrophobic adsorption influences their retention. It can be explained by lower silica polarity in comparison to water.[13]

Another possibility to decrease the negative charge of the resin is the addition of an ion-pairing reagent to the mobile phase. In this case, it is possible to use a conventional ion-exclusion column.

It is worth noting that the presence of ion-pairing reagents also influences the activity coefficients dielectric constant of the stationary phase.[6,14] According to equations (1) and (2), these two effects additionally influence the solute retention (a decrease of the dielectric constant causes a decrease the dissociation constant).

The separation of some aliphatic in pure water and 0.5 mM solution of tetrabutylammonium bromide are presented in Fig. 2. As in the case of nitrobenzoic[15] acids, there is an improvement of the peaks symmetry, an increase of retention and of their independence on the solutes concentration.

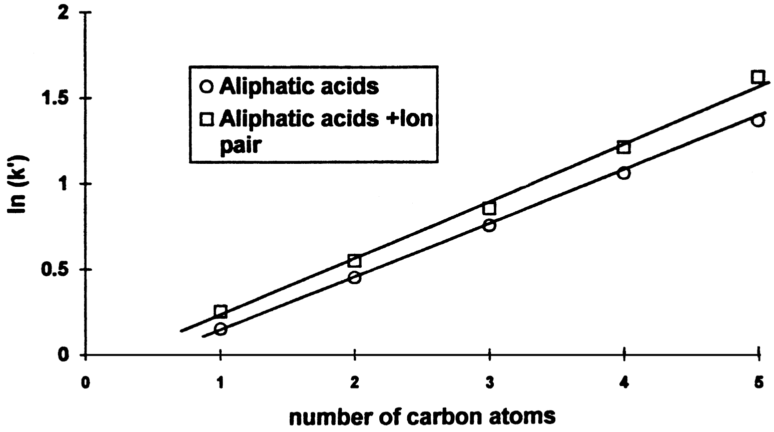

Figure 3. The dependence of the logarithm of the capacity coefficient, k', on the number of carbon atoms in aliphatic acids and aliphatic acids separated as ion pairs. Conditions as in Fig. 2.

From Figures 1 and 2, it can be observed that, in all cases, retention increases proportionally to the molecular dimensions (molecular surface area) of the analysed compounds. This suggests the influence of hydrophobic adsorption on the retention of analysed acids. To confirm this, the dependence of the logarithm of the capacity coefficient on the number of carbon atoms in the molecule is presented in Fig. 3.

CONCLUSIONS

In conclusion, by IEC, even the separation of strong acids can be achieved provided that the charge of the resin is small or a weak acid is used as a support. The use of silica, a very cheap support, can open, therefore, new perspectives to this technique. Another way to obtain decrease of the functional groups concentration is the addition of an ion interaction reagent to the mobile phase, which decreases the effective charge of the resin, allowing the solute to penetrate the resin. The strong influence of the ion pairing reagent on the hydrophobic adsorption was actually observed. Furthermore, the use of ion-pairing reagents, even in small concentrations, improves the separation of compounds which are generally eluted very close to each other. It is worth to note that, ion-pairing reagents, when used without additional buffer, offer excellent conditions for detectors such as conductometric, potentiometric, or electrokinetic, whose sensitivity decreases by a short circuit effect when ions are present in the mobile phase.

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