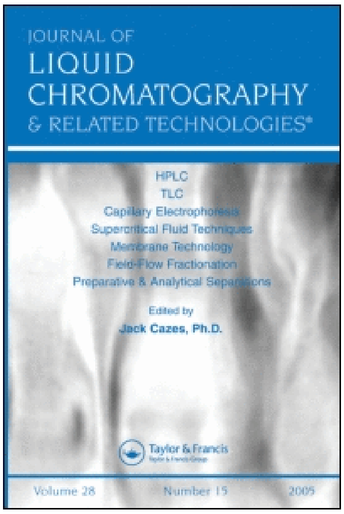

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INVESTIGATIONS ON THE CHROMATOGRAPHIC BEHAVIOR OF CARBOXYLIC ACIDS ON SILICA-BASED CATION EXCHANGERS

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ABSTRACT

Aliphatic mono and dicarboxylic acids have been separated on silica-based ion-exchangers under conditions commonly used in ion-exclusion chromatography. Various retention mechanisms including ion-exclusion, hydrophobic interaction, and adsorption are involved in the separation of these analytes. The contribution of each of these mechanisms can be manipulated by varying the characteristics of the chromatographic support like exchange capacity or type of the spacer molecule used for the binding of the sulfonate groups to the silica. Thereby, fine-tuning of the separation selectivity is possible.

INTRODUCTION

Since ion-exclusion chromatography has been introduced by Wheaton and Baumann,[1] it has become an important technique for the analysis of ionic compounds like carboxylic acids. In the case of acidic analytes strong cation exchange resins are commonly used as stationary phase for the separation of these compounds according to their degree of dissociation. Completely ionized species like mineral acids are totally excluded from the pores of the packing material by electrostatic repulsion and elute at the dead volume V_0 of the column, whereas neutral compounds e.g. methanol can penetrate into the pores of the chromatographic support, showing a retention volume composed of V_0 and V_i, the inner volume of the chromatographic column.[2] Between these two limiting cases, partially ionized analytes are retained corresponding to their degree of ionization.

In general, carboxylic acids are not fully dissociated under the conditions used in ion-exclusion chromatography so that the retention order depends on their pKa values.[3,4] A large number of ion-exclusion chromatographic separations of carboxylic acids performed on highly sulfonated poly(styrene/divinylbenzene) (PS/DVB)-based resins of low cross-linkage can be found in the literature. A well known property of these resins is the fact that the retention of the analytes is strongly influenced by the inherent reversed-phase character of these stationary phases in addition to the actual ion-exclusion effect.[5] Hydrophobic analytes lead to increased retention volumes V_R, exceeding the value $V_0 + V_i$ obtained for small neutral compounds, giving a distribution coefficient K_D which is larger than 1. Beside these effects, size-exclusion plays a certain role in the case of bulky molecules. A number of papers have been published dealing with the contribution of these different mechanisms to the retention of ionic solutes like carboxylic acids in ion-exclusion chromatography.[5-8]

Actually there are only a few reports describing the separation of carboxylic acids on silica-based chromatographic columns under conditions commonly used in ion-exclusion chromatography.[9-11] Unmodified silica gels as reported by Ohta et al.[9] as well as, strongly acidic silica-based ion-exchangers,[10,11] have been employed for this purpose. Nevertheless, no paper has been published including systematic investigations on the retention behavior of carboxylic acids on silica-based ion-exchangers functionalized with different types of sulfonic acids. Similar as in the case of resin-based columns, a number of different mechanisms contribute to the retention of carboxylic acids using these chromatographic supports. Ion-exclusion effects may originate not only from strongly acidic functional groups e.g., sulfonate, but also from the silanol groups of the silica gel, if silica based ion-exchangers are used. This could be demonstrated by Ohta et al.[9] who performed ion-exclusion chromatographic separations of aliphatic carboxylic acids using a column filled with unmodified silica. Whereas reversed-phase interactions play an important role in the case of resin-based ion-exclusion columns, adsorption phenomena between the analytes and the hydrophilic base material can contribute to the retention of the solutes leading to V_R values larger than those obtained for small neutral compounds in the case of silica-based columns. These effects are mainly depending on the surface characteristics of the base material.

Regarding silica gels functionalized with sulfonic acid groups additional hydrophobic interactions have to be taken into account as part of the retention mechanism involved in the separation of carboxylic acids. These effects can be mainly assigned to the spacer molecules connecting the sulfonic acid groups to the surface of the silica gel. Aliphatic spacers e.g. propyl, aromatic spacer e.g. phenyl, as well as spacers combining aromatic and aliphatic regions e.g. phenethyl, are commonly used for this purpose. Therefore, hydrophobic interactions between the analytes and the stationary phase strongly depend on the chemical nature of these spacer-molecules.

In a previously published paper[11] we investigated the chromatographic behavior of organic acids on PS/DVB-based resins showing various degrees of sulfonation, as well as different silica-based cation exchangers. The experiments carried out with silica-based chromatographic supports revealed that the type of sulfonic acid used for functionalization influences the retention of the analytes under investigation. Nevertheless, detailed comparisons of different silica-based cation exchangers are still missing. Therefore, the work presented in this paper intends to elucidate the retention behavior of a series of mono- and dicarboxylic acids on one and the same silica material in its bare form or functionalized with propylsulfonic acid or phenylethylsulfonic acid. Thereby, an estimation of the extent of the different retention mechanisms involved in separations of the selected analytes under conditions commonly used in ion-exclusion chromatographic looks possible, allowing to control separation selectivity by selection of the appropriate stationary phase.

EXPERIMENTAL

Instrumentation

The chromatographic system consisted of a Waters 625 LC-system equipped with a Waters 600E system controller, a Waters 717 autosampler (Waters, Milford, MA, U.S.A.), an ABI 785A programmable absorbance

Table 1

Silica-Based Stationary Phases Investigated in this Work

Stationary Phase	Exchange Capacity	Specific Surface	Remarks
Platinum 100-Phenethyl	0.43 meq/g	210 m^2/g	Silica gel with Ethylphenyl-sulfonate groups
Platinum 100-SCX	0.37 meq/g	210 m^2/g	Silica gel with Propylsulfonate groups
Platinum 100A		210 m^2/g	Unmodified silica gel

detector (Applied Biosystems, San Jose, CA, U.S.A) and an HP 3359A chromatographic data acquisition system (Hewlett-Packard, Palo Alto, CA, U.S.A). For column packing a Knauer pneumatic HPLC pump (Knauer, Berlin, Germany) was used.

Materials

Platinum 100A 5 μm silica bonded with propylsulfonic acid (SCX) and phenylethyl groups (Phenethyl) were gifts from Alltech (Lancs, Great Britain). The Phenethyl material, was reacted with chlorsulfonic acid according to a method published by Cox et al.[12] to give the corresponding cation exchanger. All stationary phases were suspended in methanol, sonicated and packed into 250 x 4 mm I.D. stainless-steel columns for 30 minutes at 40 MPa. A 250 x 4 mm I.D. column filled with Platinum 100A 5 μm was purchased from SRD (Vienna, Austria). Details concerning physical properties of these stationary phases can be found in Table 1.

Reagents and Chemicals

All chemicals, were of analytical-reagent grade. Doubly distilled water was used throughout this work. Buffer solutions were obtained by dissolving NaH_2PO_4 in doubly distilled water and adjusting the pH value by addition of phosphoric acid.

RESULTS AND DISCUSSION

The retention behavior of a series of monocarboxylic acids from formic acid up to valeric acid, as well as dicarboxylic acids from oxalic acid to pimelic acid, have been investigated using the three different separation media described in Table 1.

The distribution coefficients K_D for the selected analytes have been determined from the chromatographic data according to the following equation derived from a formula published elsewhere[3]:

$$K_D = \frac{V_R - V_0}{V_I}$$

where V_R is the retention volume obtained for the selected analyte, V_0 the dead volume, determined by injection of nitric acid, and V_I the inner volume of the column, determined from the retention volume $V_0 + V_I$ of a small neutral compound like methanol. Figure 1 shows the plots, distribution coefficient vs. pH, obtained for monocarboxylic acids with one to five carbon atoms. The curves obtained on all three chromatographic supports appear similar to those typical for ion-exclusion columns. Nevertheless significant differences can be observed, as a result of the chemical properties of the different stationary phases.

The curves expressing the pH dependence of K_D for a series of monocarboxylic acids on the Phenethyl- (Fig. 1A) and the SCX material (Fig. 1B) show a shape comparable to those obtained on PS/DVB-based ion-exclusion columns. Regarding the distribution coefficients, it can be seen that all analytes show higher values on the Phenethyl stationary phase than on the SCX phase. Within a pH range from 2 – 3.5, all investigated monocarboxylic acids (except formic acid) give a K_D higher than 1 (the value normally achieved for a small neutral compound like methanol) in the case of the particles functionalized with phenylethanesulfonic acid; distribution coefficients higher than 1 are only obtained for butyric acid and valeric acid if the SCX stationary phase is used.

Regarding the fact that the surface characteristics, as well as the exchange capacities of these two chromatographic materials, are practically identical, differences in the retention behavior of carboxylic acids may be mainly attributed to the chemical nature of the spacer molecules used to attach the sulfonic acid groups to the silica base material.

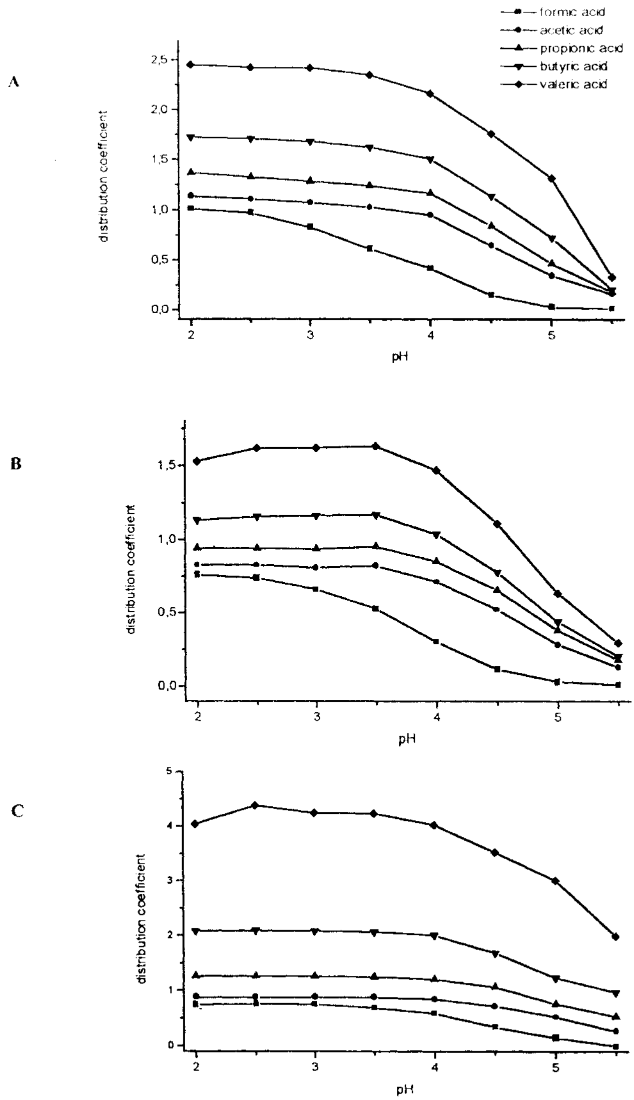

Figure 1. Distribution coefficients K_D for a series of monocarboxylic acids as function of the pH of the eluent. Mobile phase: 5mM NaH_2PO_4, pH adjusted with phosphoric acid. Column dimensions: 250 x 4 mm I.D. A) Platinum 100-Phenethyl; B) Platinum 100-SCX; C) Platinum SiOH 100.

Beside the actual ion-exclusion effect the Phenethyl material obviously provides stronger additional retention effects than the SCX stationary phase. This may be explained by the higher hydrophobicity of the phenethyl groups in comparison with the propyl spacers of the SCX material, leading to the differences in retention between the two stationary phases.

Regarding the distribution coefficients for a series of monocarboxylic acids obtained on a column filled with underivatized silica (shown in Fig. 1C), a slightly different behavior occurs in comparison with the sulfonated chromatographic supports mentioned above. K_D values even higher than those achieved on the Phenethyl column could be observed for valeric as well as butyric acid. These analytes seem to show strong interactions with the bare silica gel caused by increased adsorption on the surface of this chromatographic phase. In the case of the sulfonated stationary phases the surface of the particles is covered to some extent with ethylphenylsulfonic acid groups or propylsulfonic acid groups respectively, reducing adsorption phenomena between these analytes and the surface of the silica gel. Obviously these adsorption effects occurring on the underivatized silica column cause stronger retention (in the case of longer chain fatty acids) than hydrophobic interaction effects in the case of the sulfonated stationary phases. The K_D values for formic acid, acetic acid, and propionic acid obtained on the Platinum 100A column are situated between those for the Phenethyl and the SCX stationary phase. Another difference between the underivatized and the sulfonated chromatographic supports is the elution behavior of the acids between pH 4 and pH 5.5. Whereas, in the case of the Phenethyl as well as the SCX columns all curves expressing the change in K_D of the analytes show a distinct drop within this pH range, this behavior is much less pronounced on the Platinum 100A column. Hence it may be concluded, that the contribution of ion-exclusion to the retention of the selected solutes is less important on the underivatized stationary phase compared to the sulfonated ones. Whereas, in case of the two functionalized chromatographic supports the retention of the analytes was mainly influenced by ion-exclusion as well as hydrophobic interaction mechanisms, interactions between the solutes and the silanol groups of the silica surface (leading to an increased influence of adsorption phenomena) seems to play the most important role on the underivatized material.

Plotting log k′ values versus number of carbon atoms or methylene groups for a series of e.g. alkanes, carboxylic acids, or alkylbenzenes is a common process mainly in reversed-phase chromatography. It demonstrates the linear relationship between log k′ and the increase of surface and with it increasing possibilities of interaction between the stationary phase and the analytes implied by the addition of a methylene group[13] to the base molecule. Figure 2 shows a plot log(k'_1 - k'_2), obtained with 5 mM NaH_2PO_4 (pH 3.5) as eluent,

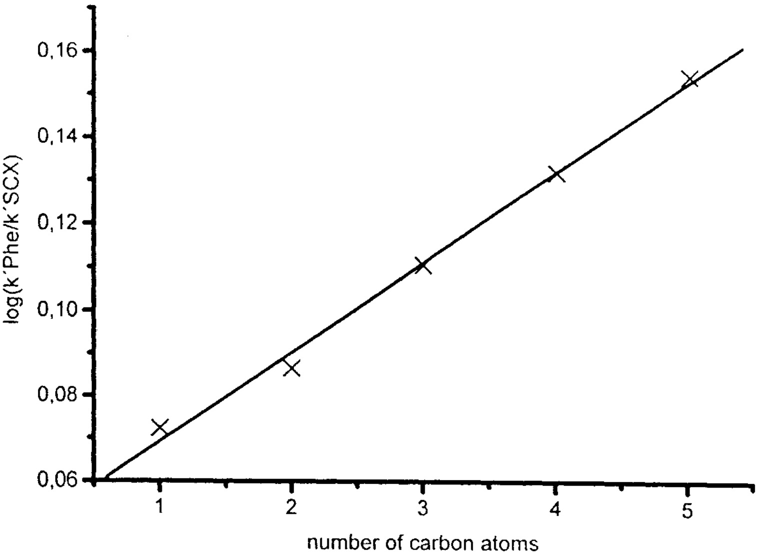

Figure 2. Logarithm of the difference of the k' values obtained on a Platinum SCX and a sulfonated Platinum Phenethyl column versus the number of carbon atoms of a series of monocarboxylic acids. Mobile phase: 5mM NaH_2PO_4, pH adjusted to 2.0 with phosphoric acid. Column dimensions: 250 x 4 mm I.D.

where k'_1 is the capacity factor on the SCX stationary phase and k'_2 is the one related to the Phenethyl material vs. the number of carbon atoms for a series of monocarboxylic acids. A straight line with a correlation coefficient of 0.996 could be obtained. Therefore it can be presumed, that the relationship between the number of carbon atoms and $\log(k'_1 - k'_2)$ is comparable to the one in reversed-phase chromatography. Regarding the similar characteristics of the two ion-exchange stationary phases (same silica base material, similar exchange capacity), the difference in k', leading to an ascending line in Fig. 2 can be assigned mainly to dissimilarities in the interaction between the analytes and the spacer molecules connecting the sulfonic acid groups to the silica. These interactions should be comparable to those obtained in reversed-phase chromatography giving an explanation for the linear relationship depicted in this plot.

Regarding the separation efficiency of the three different chromatographic supports for several mono- and dicarboxylic acids, the Phenethyl material provided the best chromatogram using a 5 mM NaH_2PO_4 buffer (pH 3.5) for elution. This eluent proved to be the most suitable in combination with the

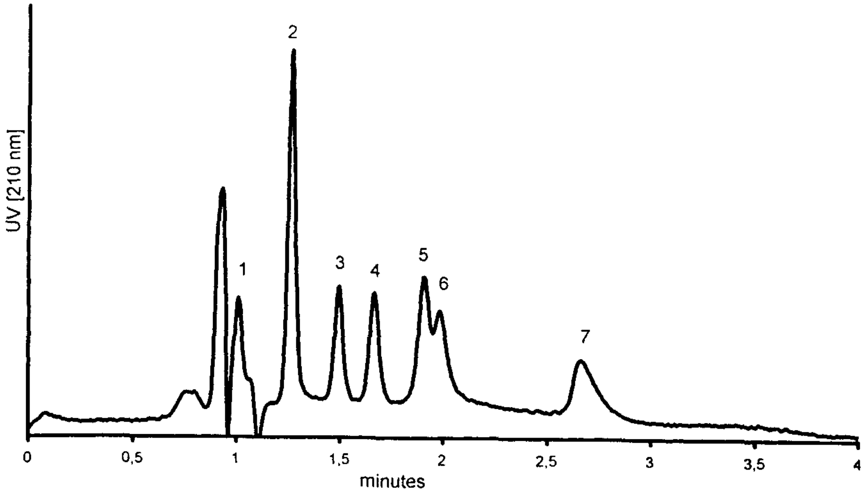

Figure 3. Separation of a mixture of mono- and dicarboxylic acids on a sulfonated Platinum Phenethyl column. Mobile phase: 5mM NaH_2PO_4, pH adjusted to 3.5 with phosphoric acid. Flow rate: 0.8 ml/minute. Column dimensions: 250 x 4 mm I.D. Peaks: 1. Malonic acid; 2. Formic acid; 3. Acetic acid; 4. Propionic acid; 5. Pimelic acid; 6. Butyric acid; 7. Valeric acid.

stationary phases under investigation. As can be seen in Figure 3 all peaks except the pair pimelic acid/butyric acid could be baseline resolved within less than 6 minutes using this stationary phase. Changing to the SCX material, acetic acid, pimelic acid, and propionic acid merge to one peak showing only poor resolution. This is mainly caused by a decrease in retention for pimelic acid on this stationary phase, leading to a change in elution order.

In contrast to the Phenethyl material, pimelic acid elutes between acetic acid and propionic acid, overlapping with both of them. In the case of the underivatized silica column, resolution decreases once again. Only two peaks can be achieved for the pair malonic acid/formic acid and the pair pimelic acid/butyric acid.

Additionally, the peak assigned to the pair malonic acid/formic acid could not be sufficiently resolved from the one obtained for acetic acid. Regarding the elution order obtained on this column, an identical sequence, as obtained on the Phenethyl material, can be found.

CONCLUSIONS

The investigations described in this paper on the chromatographic behavior of carboxylic acids demonstrate that various retention mechanisms are involved in the separation of these solutes on silica-based materials under conditions commonly used in ion-exclusion chromatography. Up to a certain extent these analytes may be separated on unmodified silica-gel columns, but the use of chromatographic materials modified with strongly acidic groups e.g. sulfonic acids leads to enhanced separation efficiency. A comparison between cation exchangers of similar capacity and based on the same silica material, functionalized with aliphatic- or aromatic sulfonic acids revealed that the retention behavior of the selected analytes is strongly affected by the type of spacer molecule connecting the sulfonate group to the silica. Therefore, the contribution of hydrophobic interactions to the retention of the carboxylic acids can be manipulated, allowing the increase of separation efficiency as well as selectivity for a particular analytical problem.

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