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Retention behavior of carboxylic acids on highly cross-linked poly(styrene–divinylbenzene)-based and silica-based cation exchangers

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Abstract

A highly cross-linked porous poly(styrene–divinylbenzene) resin was reacted with sulfuric acid to give strong cation-exchange resins with capacities from 0.2 to 1.9 mequiv./g. The retention behavior of mono- and dicarboxylic acids on these resins was compared with results obtained from silica-based cation exchangers functionalized with alkylsulfonic acid or phenylsulfonic acid groups. The contribution of different retention mechanisms like ion exclusion, hydrophobic interaction and adsorption to the separation of the analytes on these chromatographic supports was discussed. Additionally the applicability of silica-based cation exchangers was tested in the field of silage analysis.

Keywords: Stationary phases, LC; Carboxylic acids

1. Introduction

Since ion-exclusion chromatography has been introduced by Wheaton and Baumann [1] it has become an important technique for the analysis of carboxylic acids. Usually a strong cation-exchange resin is used as stationary phase which separates these analytes according to their degree of ionization. Strong acids like mineral acids are completely excluded from the pores of the packing material by electrostatic repulsion, whereas neutral compounds e.g., methanol penetrate into the pores of the resin. Between these two extreme cases, partially ionized molecules are retained corresponding to their degree of ionization. Under the conditions commonly used in ion-exclusion chromatography, carboxylic acids are not fully dissociated so that the retention order depends on their pK_a values [2,3].

Most ion-exclusion separations described in the literature for carboxylic acids have been performed with highly sulfonated poly(styrene–divinylbenzene) (PS–DVB) resins of low cross-linkage. It is known that these resins have in addition to actual ion-exclusion effect, additional mechanisms, like size exclusion and hydrophobic interactions involved in the retention of analytes [4]. The inherent reversed-phase character of the separation system can be a useful variable in the optimization of the separation if mobile phases containing organic solvents are employed [5–7]. Unfortunately, due to an unfavorable swelling behavior the compatibility of low cross-linked PS–DVB resins with organic solvents is limited to approximately 20%. Taking full advantage of the reversed-phase characteristics in addition to the ion-exclusion properties requires the use of an highly cross-linked cation-exchange resin. So far only a few papers have reported separations of carboxylic acids on fully solvent-compatible ion-

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exclusion columns [8–10]. The range of applications seems to be not yet fully exploited so that further investigations should be justified, especially as the availability of highly cross-linked monodisperse PS–DVB particles has increased during recent years [11,12]. In this context it was also interesting to investigate as to how far this packing material would be useful in narrow-bore columns. This type of columns offers an enhanced detection limit and an improved compatibility with mass spectrometric detection, which is becoming increasingly important when structure-selective information is required.

An estimation of the extent of reversed-phase mechanisms in ion-exclusion chromatography looks possible if the hydrophobic PS–DVB base material is replaced by a hydrophilic material. For this reason silica modified with sulfonic acids via aliphatic as well as aromatic spacers has been investigated in this work. Silica-based ion-exclusion columns have been rarely used for the separation of carboxylic acids [13], although most recently Ohta et al. [14] have reported the use of unmodified silica for the separation of organic acids. The present work aims at a direct comparison of solvent-compatible PS–DVB and sulfonated silica and at the elucidation of the parameters of the mobile phase that are crucial for the separation. Finally, the applicability of these new stationary phases are demonstrated for real samples in the field of silage analysis.

2. Experimental

2.1. Instrumentation

The chromatographic system consisted of a Waters 625 LC-system equipped with a Waters 600E system controller, a Waters 717 autosampler (Waters, Mil-

ford, MA, USA), an ABI 785A programmable absorbance detector (Applied Biosystems, San Jose, CA, USA) and an HP 3359A chromatographic data acquisition system (Hewlett-Packard, Palo Alto, CA, USA). For column packing a Knauer pneumatic HPLC pump (Knauer, Berlin, Germany) was used.

2.2. Columns

PS–DVB beads were prepared by a two-step microsuspension method similar to the procedure described elsewhere [11,12]. The resulting particles had a mean diameter of 4 μm ($\pm 0.3 \mu\text{m}$), a specific surface area between 92 and 94 m^2/g and a cross-linking degree of 50%. For the sulfonation of the material and the determination of the exchange capacity a procedure similar to the one described by Dumont and Fritz was used [15]. General reaction conditions for the functionalization of 5 g of the resin are given in Table 1. All polymer resins were suspended in acetonitrile–water (1:1), sonicated and packed into 100 \times 2 mm I.D. stainless-steel columns for 30 min at 35 MPa.

Exsil 100/5 SCX was purchased from Alltech (Lancs., UK). The material was suspended in methanol, sonicated and packed into 250 \times 4 mm I.D. stainless-steel columns for 30 min at 40 MPa. A Nucleosil 100-5 SA 250 \times 4 mm I.D. column was purchased from Machery–Nagel (Düren, Germany) and a 250 \times 4 mm I.D. column filled with Exsil 100/5 from SRD (Vienna, Austria).

2.3. Reagents and chemicals

All chemicals, except those used for the synthesis of the polymeric resin, were of analytical-reagent grade. Gradient grade acetonitrile was purchased

Table 1
Reaction conditions for resin sulfonation

Capacity (mequiv./g)	H ₂ SO ₄ (ml)	Acetic acid (ml)	Reaction time (min)	Temperature (°C)
0.0				
0.2	30	30	4	0
0.6	15	50	60	0
1.2	15	50	90	75
1.9	15	50	120	80

from J.T. Baker (Deventer, Netherlands). Doubly distilled water was used.

Sulfuric acid eluents were prepared by dilution of 0.05 M sulfuric acid with doubly distilled water or organic solvent–water. Buffer solutions were obtained by dissolving NaH_2PO_4 in doubly distilled water and adjusting the pH value by addition of phosphoric acid.

Press-liquors of silage samples were obtained by passing the silage through a hand-driven press. Prior to the analysis the liquid was diluted with water by a factor of 500 and passed through a solid-phase extraction cartridge filled with 100 mg of unmodified PS–DVB resin.

3. Results and discussion

3.1. Chromatographic behavior of highly cross-linked PS–DVB-based cation exchangers

As can be seen in Table 1, a highly cross-linked PS–DVB resin has been functionalized by introduction of sulfonic acid groups to give a series of cation-exchange resins with different exchange capacities. Sulfonation of this material led to a maximum exchange capacity of 1.9 mequiv./g, which is less than 50% of the value specified for low cross-linked PS–DVB resins, commonly used in ion-exclusion chromatography. A further difference was the dimension of the columns used for separation. Conventional ion-exclusion columns are filled with nearly the forty fold amount of resin than the 100×2 mm I.D. column used in this work. Fig. 1 shows the influence of the degree of sulfonation on the retention behavior of a selection of carboxylic acids. For most of the investigated solutes a maximum of retention was obtained on a resin with an exchange capacity between 0.2 and 0.7 mequiv./g. These data are similar to some results obtained on a sulfonated PRP-1 resin published earlier by Lee and Lord [8]. As can be seen from this plot the two structural similar acids, maleic acid and fumaric acid, can not be separated on unmodified PS–DVB. By introduction of a small amount of sulfonic acid groups on the surface of the resin both analytes show an increase in retention. This may be explained by the fact that the higher polarity of the sulfonated PS–

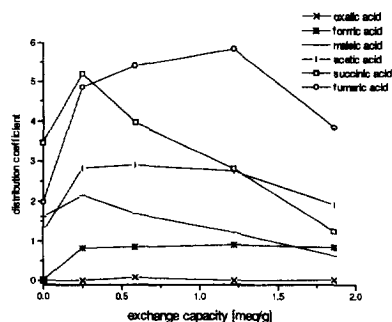


Fig. 1. Influence of the exchange capacity of the resin on the distribution coefficient of selected acids on sulfonated PS–DVB. Chromatographic conditions: eluent: 5 mM H_2SO_4 ; flow-rate: 0.2 ml/min.

DVB particles enhances the ability of the eluent to wet the resin. By a further increase of sulfonation maleic acid and fumaric acid are separated corresponding to their degree of ionization. The distribution coefficient obtained for maleic acid, a strong acid ($\text{p}K_a$ 1.83), decreases if the exchange capacity of the resin and with it the ionic repulsion is increased. On the other hand more than 90% of the fumaric acid molecules ($\text{p}K_a$ 3.03) are protonated under the selected conditions and therefore may penetrate into the pores of the beads, leading to increased hydrophobic interaction between the solute and the PS–DVB resin. This different behavior of a strong and a weak carboxylic acid indicates that an ion-exclusion mechanism is present despite of the low exchange capacity of the column.

Regarding the distribution coefficient for all investigated mono- and dicarboxylic it turns out that only formic acid, oxalic acid and malonic acid show distribution coefficients in the range of 0 and 1. The retention of all higher carboxylic acids is predominantly affected by hydrophobic interaction between the analytes and the resin. Because of the solvent compatibility of these columns this effect can be employed for the separation of higher monocarboxylic acids by gradient elution with up to 70% acetonitrile in the eluent. Under these conditions acids with a carbon chain length up to twelve could be separated within less than 20 min; the separation of acids with even longer chain length might be successful but has not been tried in this work.

3.2. Chromatographic behavior of silica-based cation exchangers

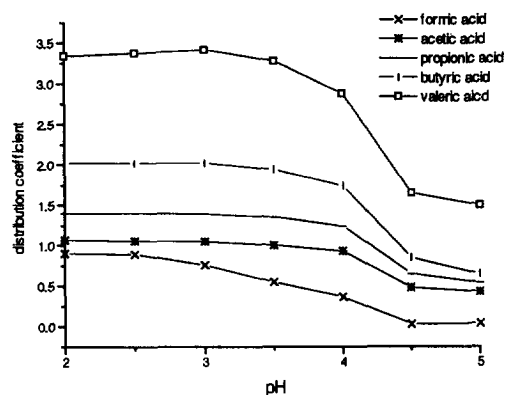
3.2.1. Cation exchangers with phenylsulfonic acid groups

The chromatographic behavior of mono- and dicarboxylic acids has been investigated using a 250×4 mm I.D. column filled with Nucleosil 100-5 SA, a silica-based cation exchanger functionalized with phenylsulfonic acid groups and an exchange capacity of 1.0 mequiv./g. Compared to the PS-DVB-based resin mentioned above this cation exchanger is based on a hydrophilic base material, though to some extent still hydrophobic interactions between the analytes and the phenyl groups of this material should be possible.

Fig. 2 shows the distribution coefficients obtained for the investigated solutes. Similar to the results obtained on PS-DVB-based columns, this Fig. demonstrates that between pH 5 and pH 3 the distribution coefficients of the analytes increase in a way that corresponds to the pK_a values of the solutes. Below pH 3, especially in the case of dicarboxylic acids the plots for strong acids e.g., oxalic acid and malonic acid significantly differ from those obtained for weaker acids like adipic acid and pimelic acid. The increasing distribution coefficients for the stronger acids can be explained by their high degree of ionization at pH 3 which is slightly decreased by lowering the pH to 2. For weaker acids an effect running in opposite direction can be observed. Experiments using NaH_2PO_4 buffers (pH 3) with different molarity showed that increasing ionic strength of the eluent from 5 mM to 50 mM led to a decrease of the distribution coefficient of about 0.1 to 0.15 units. By adjusting the pH values of the eluents with phosphoric acid, the ionic strength of the buffer solutions increased with decreasing pH. At pH 3 already less than 10% of the weaker acids are ionized. Therefore the effect of lower distribution coefficients caused by increasing ionic strength gains more influence on the retention behavior of these solutes. This leads to slightly dropping graphs for these analytes below pH 3.

Regarding the retention volumes obtained for the selected analytes only formic acid, oxalic acid, malonic acid and succinic acid elute within the confines of the interstitial volume of the eluent and

A



B

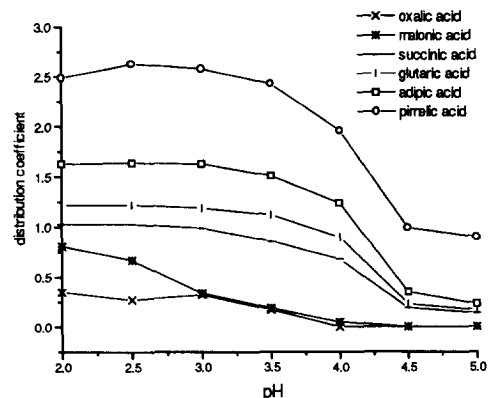


Fig. 2. Influence of the pH of the eluent on the distribution coefficient on Nucleosil 100-5 SA. Chromatographic conditions: eluent: 5 mM NaH_2PO_4 , pH adjusted with H_3PO_4 . (A) Monocarboxylic acids; (B) dicarboxylic acids.

the retention volume obtained for a neutral compound like methanol. This behavior may be explained partially by hydrophobic interaction between the phenyl groups of the spacers and the analytes. However, also adsorption phenomena between the silica gel and the solutes as reported by Ohta et al. [14] should be considered.

3.2.2. Cation exchangers with alkylsulfonic acid groups

Using the Exsil 100/5 SCX column, an ion-exchange column packed with propylsulfonic acid

functionalized silica gel and an exchange capacity of 0.45 mequiv./g, a chromatographic behavior similar to the one on the phenylsulfonate silica could be observed. It should be mentioned, that compared to the Nucleosil material much lower distribution coefficients were obtained on this column. Nevertheless all monocarboxylic acids with more than two carbon atoms and all dicarboxylic acids with more than five carbon atoms show longer retention times than methanol. This effect may be attributed exclusively to adsorption phenomena between the silica gel and the analytes, because of the lack of hydrophobic sites on this chromatographic support. In each of the series of mono- and dicarboxylic acids with increasing carbon chain length, the number of acids eluting in front of methanol is increased by one.

In Fig. 3 a separation of a standard mixture of mono- and dicarboxylic acids on Exsil SCX 100/5 as well as Nucleosil 100-5 SA is depicted. It can be seen that a change in selectivity occurs concerning the elution order of butyric acid and pimelic acid.

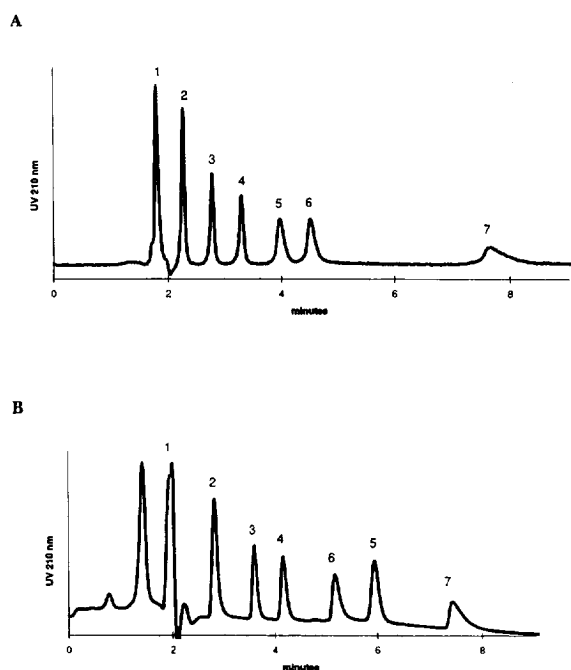


Fig. 3. Separation of carboxylic acids on Exsil SCX 100/5 (A) and Nucleosil 100-5 SA (B). Peaks: 1=malonic acid; 2=formic acid; 3=acetic acid; 4=propionic acid; 5=pimelic acid; 6=butyric acid; 7=valeric acid. Eluent: 5 mM NaH_2PO_4 , pH 3.5; flow-rate: 0.8 ml/min. Detection: UV 210 nm.

Regarding the influence of the pH on the distribution coefficients in the case of the Nucleosil column both carboxylic acids only show a slight decrease in retention going from pH 2.5 to 3.5. In contrast to this behavior the distribution coefficient obtained for butyric acid is nearly constant from pH 2.5 to pH 4, whereas pimelic acid shows a maximum at pH 2.5 and a steady drop till pH 5 on the Exsil column. Therefore at pH 3.5 the elution order of butyric acid and pimelic acid is reversed when switching from the Nucleosil column to the Exsil column. Additionally it should be mentioned that the separations achieved on sulfonated silica-based cation exchangers could not be performed on a Exsil 100/5 column packed with unmodified silica.

3.3. Analysis of fermenting acids in silage samples

To demonstrate the suitability of silica-based cation-exchange columns for the analysis of real samples the amount of acetic acid, lactic acid, propionic acid and butyric acid in a press-liquor of a silage sample has been determined. From the different contents of these fermenting acids conclusions on the quality of the silage sample can be drawn. Fig. 4A shows the separation of a standard mixture of the investigated solutes at a concentration of 25 ppm each in less than 5 min. Linearity of the calibration plots prepared for these solutes was checked from 5 ppm to 100 ppm. A chromatogram of a diluted press-liquor sample containing lactic acid and acetic acid is depicted in Fig. 4B. The investigated sample contained 2.4% lactic acid and 0.5% acetic acid.

4. Conclusion

The investigations described in this paper on the chromatographic behavior of carboxylic acids indicate that various retention mechanisms are involved in the separation of these solutes on cation-exchange columns. As can be seen from the literature even in the case of low cross-linked highly sulfonated PS-DVB resins hydrophobic interactions between the analytes and the resin cannot be neglected besides ion exclusion. Going to solvent compatible highly cross-linked PS-DVB supports with low exchange capacities a distinctly increasing influence of an

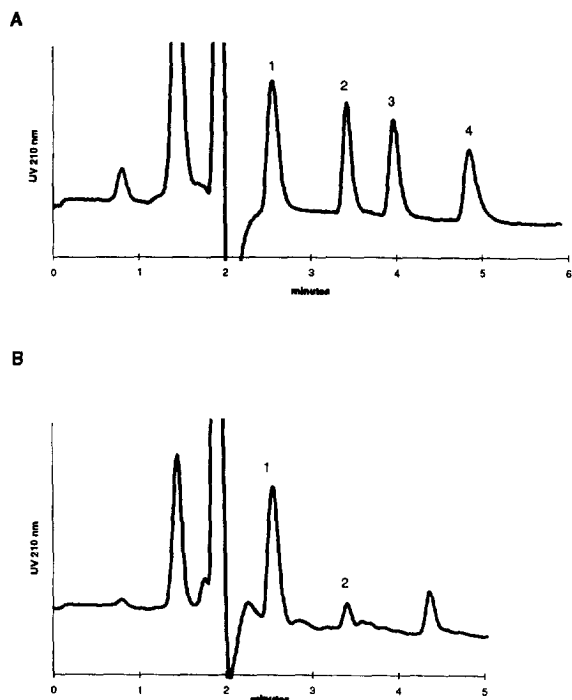


Fig. 4. Separation of fermenting acids on Nucleosil 100-5 SA. (A): Standard mixture containing 25 ppm of each acid; (B): silage press-liquor (diluted 1:500) containing 2.4% lactic acid and 0.5% acetic acid. Peaks: 1=lactic acid; 2=acetic acid; 3=propionic acid; 4=butyric acid. Eluent: 5 mM NaH_2PO_4 , pH 4.0; flow-rate: 0.8 ml/min. Detection: UV 210 nm.

hydrophobic interaction mechanism can be observed. Even by a change in the base material of the cation exchanger from PS-DVB to the more hydrophilic silica gel does not lead to an exclusive contribution of ion exclusion in the retention of carboxylic acids. Besides adsorption phenomena on the silica gel also hydrophobic interactions may occur to some extent if aromatic spacers are used to fix the sulfonic acid groups on the silica support. The results obtained in this work demonstrated that a careful selection of the

base material, the degree of sulfonation as well as the type of spacer used in the case of silica gels leads to additional selectivity in the separation of carboxylic acids. As an example for the suitability of the chromatographic supports discussed in this work, the content of fermenting acids in a silage press-liquor sample was determined within 5 min using a silica-based cation exchanger functionalized with phenylsulfonic acid groups.

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