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Evaluation of mixed-mode stationary phases in liquid chromatography for the separation of charged and uncharged oligomer-like model compounds

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Abstract

The possibility to separate charged and uncharged oligomer-like test compounds by high-performance liquid chromatography was investigated, using mixed-mode (reversed-phase/anion-exchange) stationary phases. In order to examine this possibility, several columns were evaluated for their reversed-phase, anion-exchange and mixed-mode properties using neutral and charged model compounds. The OmniPac PAX-500, the PRP-X100 and the mixed-phase PLRP-S/PL-SAX columns showed promising results for the separation of real samples.

1. Introduction

A lot of kinetic–mechanistic aspects of the emulsion polymerization process are still unexplained. Oligomers play a particularly important role during the initiation of the latex particles. One of the questions unanswered concerns the length of the oligomer radicals when they enter the latex particles [1]. Besides that, it is also important to know what kind of termination takes place in the water phase. In many emulsion polymerization processes a charged initiator, like persulfate for instance, is used. Consequently, charged oligomers are formed with one or two sulfate groups. Subsequently, these sulfate groups may hydrolyse to alcohol functions.

To get more insight into the role of oligomers in emulsion polymerization processes, it is necessary to collect qualitative and quantitative information about these compounds. The separation procedures to be developed for oligomers should preferably be able to separate charged and uncharged oligomers both in one analysis procedure. In a consecutive part of this study we intend to scale-up these (analytical) separations to preparative liquid chromatography, to allow further research on the structure of the isolated compounds. This latter consideration puts specific demands on the composition of the applied eluents, with respect to removal after having finished the preparative separation. In this paper the separation of charged and neutral oligomer-like test substances is described, showing ways to analyze real oligomer samples.

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Charged oligomers are especially difficult to separate through reversed-phase or anion-exchange chromatography. Therefore, in this study the use of so-called mixed-mode stationary phases for the separations of charged and neutral oligomers has been investigated. In mixed mode, stationary phases with two different retention processes on one stationary phase are intentionally prepared by the manufacturer. The columns selected for this study showed reversed-phase and anion-exchange properties by [2]: (i) interaction of the non-polar part of the sample molecules with the non-polar backbone of the stationary phase and (ii) anion-exchange interactions of the ionic part of the sample molecules with the cationic groups on the support. In this paper mixed mode reversed-phase/anion-exchange will further be indicated as mixed-mode.

In the literature a number of examples of mixed-mode stationary phases have been reported. Some early examples of mixed-mode columns are soft-gel anion-exchange resins, like DEAE-Sephadex or -cellulose [3,4]. A disadvantage of these columns is that only low pressure is allowed across these columns. Another example is the RPC-5 column. This type of column consists of a non-porous spherical polymer support (polychlorotrifluoroethylene, Plaskon 2300), which is coated with trioctylmethyl ammonium chloride [3–5]. General drawbacks of these columns are bleeding of the ammonium groups from the column, and the non-porous support which is not of a constant quality.

Several authors prepared mixed-mode columns based on silica gel supports, which already showed one of the two retention mechanisms. Two methods were used by McLaughlin and Bischoff [6–9] to prepare these mixed-mode stationary phases. The first method was by modifying an anion-exchange matrix with alkyl chains. A commercially available aminopropylsilyl bonded-phase silica (APS-Hypersil) was reacted with several organic acids that contained hydrophobic sites as well as sites for ionic interactions [6–8]. Other authors also used this first method to prepare mixed-mode silica gel supports, by reacting an anion-exchange silica support with octadecyltrichlorosilane [10]. The second method was to modify a reversed-phase matrix, by attaching anionic groups to the support. For this purpose ODS-Hypersil was coated with trioctylmethyl ammonium chloride [8,9].

Other authors used bare silica gels to prepare mixed-mode stationary phases. Crowther and coworkers [11,12] prepared mixed-mode stationary phases containing C_8 groups and quaternary ammonium groups by a two-step procedure. Kopaciewicz et al. [13] coated silica with polyethyleneimine. After cross-linking with diepoxides, the coating was derivatized with monoepoxides to control the hydrophobicity of the coating. El Rassi and Horváth [14] prepared mixed-mode phases with strong anion-exchange/weak hydrophobic and weak anion-exchange/weak hydrophobic properties on silica supports, by initially attaching dimethylpropylsilane groups to the surface and, subsequently, binding polar moieties to the carbon chains.

The columns described above were mainly used for the separation of (oligo)nucleotides, RNA, DNA and peptides.

Another group of columns also shows, beside their main retention activity, some secondary selectivity not intentionally introduced by the manufacturer (e.g. low-capacity anion-exchange columns also show some reversed-phase properties in many cases). The Hamilton PRP-X100 is an example of this kind of columns. Basically, it is a low-capacity anion-exchange column. However, it also shows reversed-phase properties, because the sample solutes may undergo interactions with its hydrophobic matrix, made up of polystyrene cross-linked with divinylbenzene. These reversed-phase properties were demonstrated by several groups [15–19].

Mixing a reversed phase with an anion-exchange stationary phase (mixed-phase), or connecting an anion-exchange and a reversed-phase column in series, are other possibilities to achieve mixed-mode properties. Comparing a mixed-mode reversed-phase/cation-exchange with a mixed-phase (reversed-phase/cation-exchange) and with a cation-exchange including a reversed-phase column in series, Issaq and Gutierrez [20] observed the best results for the mixed-mode column, using anti-depressants as the model compounds. The performance of the mixed-phase column was less compared to that

of the mixed-mode column, but the retention times were considerably shorter compared to the two columns in series. Crowther et al. [12] also observed that the mixed-phase approach showed inferior results, compared to mixed-mode stationary phases.

A number of commercial mixed-mode columns have been coming onto the market over the last few years. Examples are the ABx columns of J.T. Baker [21,22] and the mixed-mode RP C_4, C_8, C_{18}/Anion columns of Alltech [23,24]. These columns contain silica-based stationary phases. Another example is the OmniPac PAX-500 of Dionex, which is a polymer-based mixed-mode column. This column has a macroporous core of ethylvinylbenzene cross-linked with divinylbenzene showing reversed-phase properties. A latex with quaternary ammonium groups is attached to this macroporous core, giving the stationary phase its anion-exchange properties [25–27].

In this research a number of columns were investigated as to their mixed-mode properties, being in this case reversed-phase/anion exchange, and their suitability to separate charged and neutral oligomers. Polymer-based columns were mainly investigated, because of the relatively high pH of the eluent used in this study. The columns were investigated as to their hydrophobic, anion-exchange and their mixed-mode properties, respectively, by using three different test mixtures consisting of (i) alkylbenzenes, alkylpyridines and alkylhydroxybenzoates, (ii) a number of inorganic anions and (iii) a number of alkane sulfonic acids and styrene sulfates. Finally, the results obtained on the columns investigated are discussed.

2. Experimental

2.1. Instrumentation

The HPLC system consisted of a Bischoff HPLC pump (Bischoff, Leonberg, Germany), a Metrohm 690 ion chromatograph (Metrohm, Herisau, Switzerland), a Bischoff Lambda 1000 UV detector and two recorders (Kipp and Zonen, Delft, Netherlands). The ion chromatograph consisted of a Valco injector (VICI Valco-Europe, Schenkon, Switzerland) and a Metrohm conductivity detector.

2.2. Columns

A number of columns were investigated concerning their hydrophobic, anionic and mixed-mode properties. The OmniPac PAX-500 (250 × 4 mm I.D.) column was from Dionex (Sunnyvale, CA, USA). Two specially prepared mixed-phase columns were from Polymer Labs. (Shropshire, UK); the columns (150 × 4.6 mm I.D.) contained a mixture of 50% of PLRP-S, 8 μm, 1000 Å particles and 50% of PL-SAX, 8 μm, 1000 Å particles as the stationary phase. The columns were slurry packed. Column 1 (PL1) was packed as reversed-phase PLRP-S columns are usually packed, using acetonitrile–water (7:1, w/w) as the packing solvent. Column 2 (PL2) was packed as ion-exchange PL-SAX columns are usually packed, using a buffer as the solvent. Also, a number of low-capacity anion-exchange columns were tested: A Hamilton PRP-X100 (10 μm, 125 × 4 mm I.D.), (Hamilton, Reno, NV, USA); a HEMA-S 1000 Q-L, 7 and 10 μm, and a HEMA-BIO 1000 NH_2, 7 μm (150 × 3 mm I.D.) (Tessek, Prague, Czech Republic).

In addition, two non-polymer-based columns were investigated as well: a Zorbax Bioseries Oligo (5 μm, 150 Å, 80 × 6.2 mm I.D.) from Rockland Technologies (Newport, DE, USA), and an Aluspher RP-Select B (5 μm, 100 Å, 119 × 4.6 mm I.D.) from Merck (Darmstadt, Germany). The Zorbax Bioseries Oligo has a silica-based support of which the silica has a zirconia-treated surface, which is stable up to a pH of 8.5. The Aluspher RP-Select B is prepared from an alumina based material, which can be used in a pH range of 2–12.

2.3. Chemicals

Methanol and acetonitrile, HPLC grade, were purchased from Merck. Tetrahydrofuran was purchased from Westburg (Leusden, Netherlands). Water was purified using a Milli-Q water-

purification system (Millipore, Milford, MA, USA). The aqueous buffers used consisted of solutions of ammonium carbonate (Merck), pH ≈ 9. This type of buffer can be readily removed after preparative-scale separations. Before use, the eluents were filtered and degassed ultrasonically.

Benzene, toluene, ethylbenzene, propylbenzene, butylbenzene and *p*-styrene sulfate (styrene sulfate 1) were from Aldrich (Steinheim, Germany); methyl *p*-hydroxybenzoate and propyl *p*-hydroxybenzoate were from Sigma (St. Louis, MO, USA); butanesulfonic acid was from Eastman Kodak (Rochester, NY, USA); pentanesulfonic acid and octanesulfonic acid were from FSA Laboratory Supplies (Loughborough, UK); dodecanesulfonic acid, sodium fluoride, sodium chloride, sodium nitrite, sodium nitrate, sodium sulfite and sodium sulfate (analytical-reagent grade) were from Merck; *o*-pentylpyridine, *o*-hexylpyridine and 2-phenylethyl sulfate (styrene sulfate 2) were synthesised in our department.

2.4. Methods

The columns were tested in three modes: (i) reversed phase, (ii) anion exchange and (iii) mixed-mode.

The reversed-phase properties of the columns were tested using mixtures of an organic modifier (methanol, acetonitrile or tetrahydrofuran) and water as the eluents. The alkylbenzenes, the alkylhydroxybenzoates and the alkylpyridines were used as test compounds. The compounds were detected by UV detection at 254 nm. The column performance and the capacity factors of the compounds were measured at several eluent compositions. The hold-up time of the column, t_0, was determined by the injection of methanol.

The anion-exchange properties of the columns were tested, using aqueous solutions of ammonium carbonate as the eluents. For the Polymer Labs. and the OmniPac columns, 1% of organic modifier was added to the eluent, to ensure sufficient wetting of the columns. The inorganic anions were used as the test compounds. The compounds were detected using conductivity detection. The capacity factors of the compounds were measured for a range of ammonium carbonate concentrations. The t_0 was determined by the injection of water.

The mixed-mode properties of the columns were tested, using mixtures of an organic modifier and an aqueous solution of a specific concentration of ammonium carbonate as the eluents. The test compounds were the alkanesulfonic acids and the styrene sulfates. The alkanesulfonic acids were used as model compounds for butadiene oligomers and the styrene sulfates as model compounds for styrene oligomers. The detection of the alkanesulfonic acids was performed by conductivity detection, while the styrene sulfates were detected by UV detection at 254 nm. The capacity factors of the test compounds were measured for concentrations of 1 to 90% of the organic modifiers in aqueous ammonium carbonate solutions of two concentrations, $5 \cdot 10^{-3}$ and 10^{-2} *M*. The column performance under mixed-mode conditions was measured for the styrene sulfates. The t_0 was determined by the injection of water.

With exception of the HEMA columns, the flow used in the experiments described above was 1 ml/min. In the case of the HEMA columns a flow of 0.5 ml/min was used.

3. Results and discussion

Since the PRP-X100, the OmniPac PAX-500 and the Polymer Labs. columns (PL1 and PL2) were the only ones to initially show promising results for the separation of charged and uncharged oligomers, the results of the other columns used in this work will not be discussed in detail. The HEMA columns showed only few reversed-phase properties and it was impossible to obtain baseline separation for the alkylbenzenes. The plate numbers for the alkylbenzenes were ca. 2000–3000 plates/m, assuming Gaussian peak shapes, and considerable fronting of the peaks occurred. It was possible to achieve some separation between the alkanesulfonic acids, but the peaks were very broad and the reproducibility was bad. Also, the column per-

formance decreased rapidly after only a few weeks of use, probably due to the fact that these columns were not suitable for the eluents applied [28]. Due to the polarity of the Zorbax Oligo column, it was only possible to obtain separation between the alkylbenzenes at very low organic modifier concentrations. This column showed almost no separation of the inorganic ions. Using this column in reversed-phase and anion-exchange mode these results could be expected because the column consists of a normal silica-based stationary phase. In the mixed-mode test of this column the alkanesulfonic acids and the styrene sulfates also showed low capacity factors, so it was not possible to achieve a separation between these compounds. Finally, the Aluspher Select-B also showed low capacity factors only for the alkanesulfonic acids and styrene sulfates and, therefore, this column was not suitable for these separations either.

3.1. Reversed-phase mode

To test the columns in the reversed-phase mode, useful performance criteria were first defined using the retention times and the resolution of the alkylbenzenes as parameters. The criteria were to obtain a resolution as high as possible, but larger than at least 1 with a retention time below 25 min for butylbenzene. In some cases, where it was not possible to obtain a resolution higher than 1 with these restrictions, a longer retention time was accepted.

The plate number was calculated from [29]:

$$N = 5.54\left(\frac{t_R}{w_h}\right)^2 \quad (1)$$

where t_R is the retention time and w_h is the peak width at half height. In case of increased peak asymmetry values (>1.25) the accuracy of the calculated plate numbers will rapidly decrease [30]. Also from our data the asymmetry factors, *asf*, were calculated at 10% of the peak heights [30].

The resolution was calculated using the following formula [29]:

$$R_s = \frac{t_{R2} - t_{R1}}{(w_{h1} + w_{h2})/1.18} \quad (2)$$

where t_{R1} and t_{R2} are the retention times and w_{h1} and w_{h2} are the peak widths at half height for compounds 1 and 2, respectively.

Under these conditions, the plate number N, the resolution R_s and the asymmetry factor *asf* of the test compounds for the OmniPac, the PRP-X100 and the Polymer Labs. (PL1 and PL2) columns, were measured and are given in the Figs. 1, 2 and 3, respectively, for the three different organic modifiers. The highest plate numbers were observed using acetonitrile as the organic modifier (Fig. 1). The plate numbers for methanol were very low, especially for the Polymer Labs. columns. For the PL1 column it was impossible to obtain a resolution of 1 under these conditions. It is known from literature that polymer-based stationary phases show a poor performance when methanol is used as the organic modifier [31]. The plate numbers were also low for all columns using tetrahydrofuran as the organic modifier. This is in contrast with results published earlier [31]. No results are provided in this study for the OmniPac column using tetrahydrofuran because these columns are not compatible with eluent concentrations containing more than 10% tetrahydrofuran. The best resolution (Fig. 2) of the test compounds was also observed using acetonitrile as the organic modifier. Better results, in this respect, were obtained for methanol compared with tetrahydrofuran. In almost all cases peak tailing was observed on the investigated columns, *asf* > 1 (Fig. 3). Comparing the two Polymer Labs. columns, PL2 showed higher plate numbers, but the *asf* value was higher too.

As mentioned, the reversed-phase performance of the columns was determined for the alkylbenzenes. With the alkylhydroxybenzoates and the alkylpyridines as the test compounds, the behaviour of the columns for other types of compounds was investigated under the same conditions. As an example, the retention times of the alkylbenzenes, the hydroxybenzoates and the pyridines for methanol as the organic modifier in the eluent are given in Table 1 under the conditions used in Figs. 1–3. The retention times on the Polymer Labs. and the OmniPac columns were about the same as for benzene, for both the

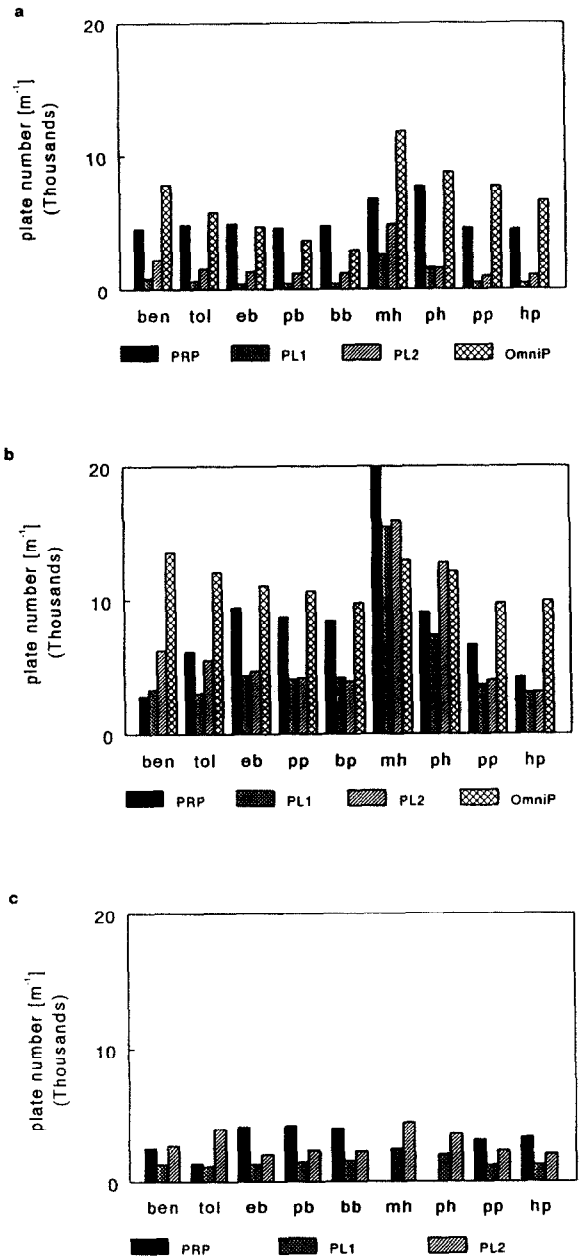

Fig. 1. Plate numbers N for benzene (ben), toluene (tol), ethylbenzene (eb), propylbenzene (pb), butylbenzene (bb), methyl p-hydroxybenzoate (mh), propyl p-hydroxybenzoate (ph), o-pentylpyridine (pp) and o-hexylpyridine (hp) in the reversed-phase mode. Eluents: (a) methanol–water; PL1, PL2: (80:20); PRP-X100: (90:10); OmniPac PAX-500: (95:5, v/v). (b) Acetonitrile–water; PL1, PL2: (45:55); PRP-X100: (73:27); OmniPac PAX-500: (77:23, v/v). (c) Tetrahydrofuran–water; PL1, PL2: (30:70); PRP-X100: (38:62, v/v).

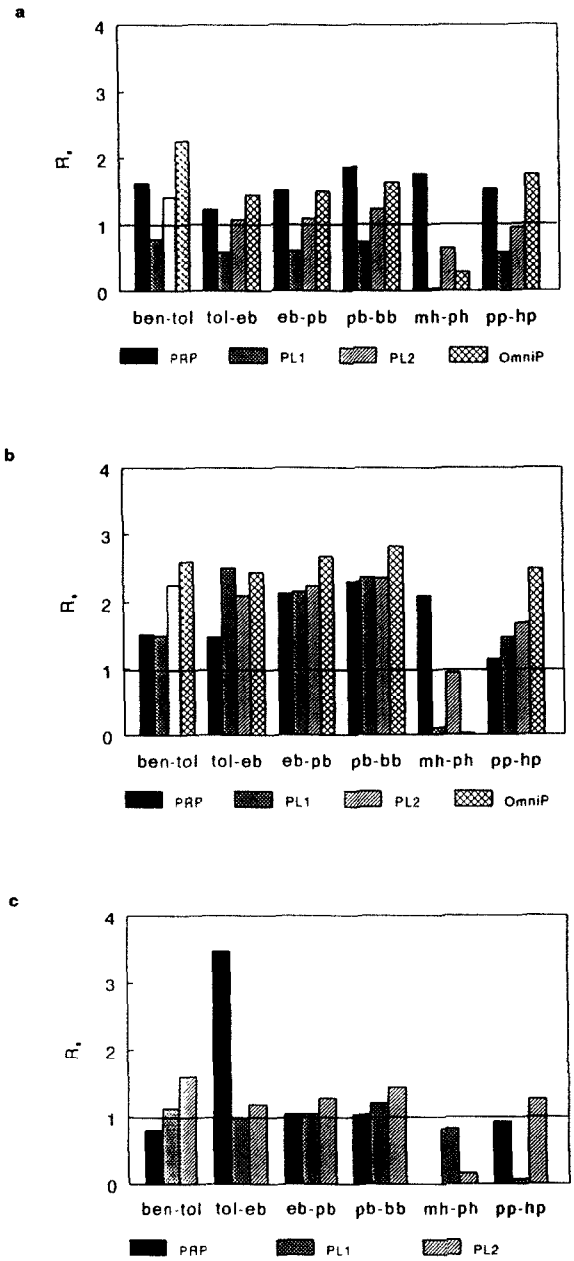

Fig. 2. Resolution R_s between the solute pairs benzene–toluene (ben-tol), toluene–ethylbenzene (tol-eb), ethylbenzene–propylbenzene (eb-pb), propylbenzene–butylbenzene (pb-bb), methyl p-hydroxybenzoate–propyl p-hydroxybenzoate (mh-ph) and o-pentylpyridine–o-hexylpyridine (pp-hp) in the reversed-phase mode. Eluents: (a) methanol–water, (b) acetonitrile–water, (c) tetrahydrofuran–water. Columns: PL1, PL2, PRP-X100 and OmniPac PAX-500. For experimental conditions see Fig. 1.

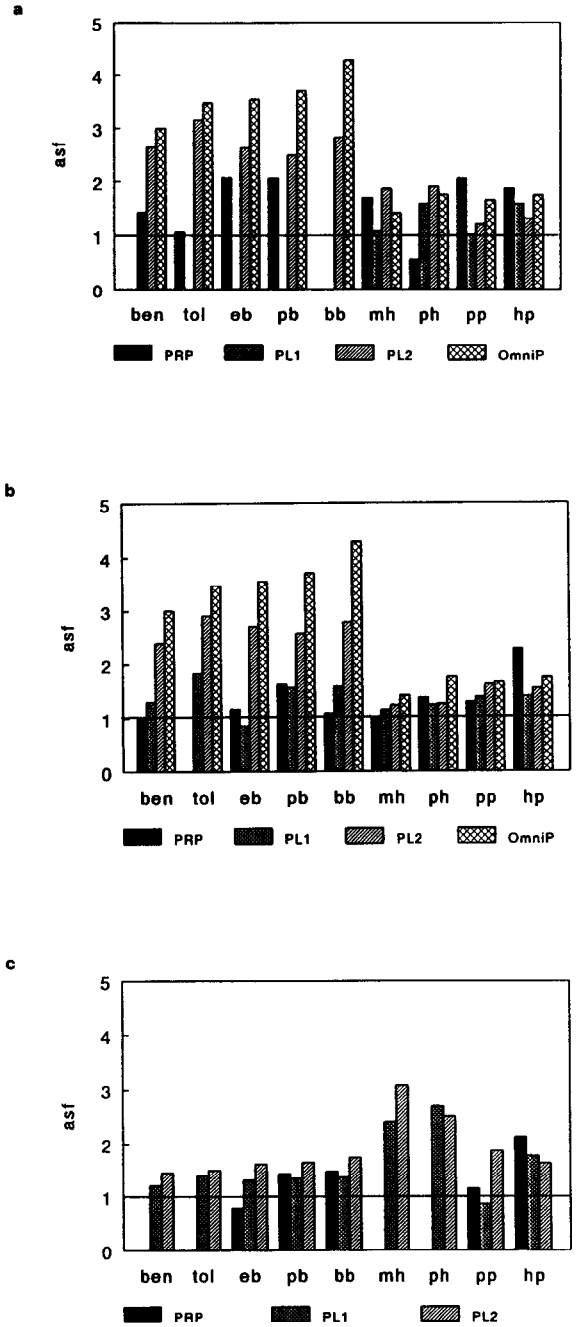

Fig. 3. Asymmetry factors *asf* for benzene, toluene, ethylbenzene, propylbenzene, butylbenzene, methyl *p*-hydroxybenzoate, propyl *p*-hydroxybenzoate, *o*-pentylpyridine and *o*-hexylpyridine in the reversed-phase mode. Eluents: (a) methanol–water, (b) acetonitrile–water, (c) tetrahydrofuran–water. Columns: PL1, PL2, PRP-X100 and OmniPac PAX-500. For experimental conditions see Fig. 1.

hydroxybenzoates and the pyridines, as could be expected considering the polarity of the compounds. However, the retention times on the PRP-X100 column were considerably larger for the alkylhydroxybenzoates than for the pyridines and were in the same range as for butylbenzene. This suggests a strong specific interaction of these esters to the PRP-X100 column.

On the PL1 column the propyl ester of the alkylhydroxybenzoates eluted before the methyl ester (Table 1). For all the columns investigated, it was observed that when the amount of organic modifier in the eluent was increased, at a certain organic modifier concentration the propyl ester eluted before the methyl ester. These observations are in contrast to the general assumption that the log k' vs. the percentage of the organic modifier of members of a specific homologous series results in more or less parallel relationships. This shows the limitations of that model, which is only valid over a limited range of organic modifier concentrations.

On these polymeric columns, the hydroxybenzoates and the pyridines showed about the same performance compared with the alkylbenzenes, as can be seen in Figs. 1–3. The columns did not show a poor performance for basic compounds, in comparison with silica-based reversed-phase columns. This is a result of the lack of interfering interactions by the basic pyridines with the free silanol groups present on silica surfaces [32]. The hydrophobicity of the columns increases in the range PL1, PL2, PRP-X100 and OmniPac PAX-500, taking the percentage of organic modifier necessary to elute the alkylbenzenes from a column into account.

As already mentioned the following formula applies for the capacity factor, as a function of the concentration of organic modifier in the eluent, for reversed-phase stationary phases (over a limited concentration range of organic modifier):

$$\log k' = a - mx \quad (3)$$

where x is the concentration of the organic modifier in the eluent, k' is the capacity factor, and a and m are constants. Bowers and Pedigo [31] showed that this formula also applies using

Table 1
Retention times t_R and capacity factor k' of the alkylbenzenes, the hydroxybenzoates and the pyridines

	PRP-X100		PL1		PL2		Omnipac PAX-500	
	t_R (min)	k'	t_R (min)	k'	t_R (min)	k'	t_R (min)	k'
Benzene	5.02	4.28	4.29	1.66	5.58	2.23	7.67	2.56
Toluene	7.29	6.67	6.53	3.04	4.19	4.19	10.42	3.84
Ethylbenzene	9.59	9.09	4.86	4.86	13.51	6.81	13.06	5.06
Propylbenzene	13.59	13.31	14.35	7.89	20.82	11.04	16.94	6.96
Butylbenzene	20.89	20.99	23.92	13.81	34.69	19.06	23.34	9.83
Methyl *p*-hydroxybenzoate	21.13	21.13	5.78	2.58	3.31	0.92	6.00	1.78
Propyl *p*-hydroxybenzoate	27.35	27.79	2.48	2.48	3.99	1.31	6.19	1.87
Pentylpyridine	4.96	4.22	4.25	1.63	5.59	2.23	7.88	2.66
Hexylpyridine	7.11	6.48	6.20	2.84	8.48	3.91	9.92	3.60

Eluent: methanol–water: PRP-X100: (90:10, v/v); PL1, PL2: (80:20, v/v); OmniPac PAX-500: (95:5, v/v).

polystyrene-based reversed-phase columns. This equation also showed to be valid for the data measured on the PL1, PL2 and the OmniPac PAX-500 columns for all three organic modifiers used, as is shown in Fig. 4.

Generally, the logarithm of the capacity factors of homologous series are linearly dependent on the length of the alkyl chain for reversed-phase columns [33]. This relationship also showed to be valid for the columns used in this study, using alkylbenzenes as the homologous series (Fig. 5).

From the data it can be concluded that the PRP-X100, the OmniPac PAX-500 and both the Polymer Labs. columns showed typical reversed-phase behaviour, when tested in the reversed-phase mode. In turn, this implicates the suitability of these columns for the separations of neutral oligomers.

3.2. Ion-exchange mode

Eq. 4 holds for ions eluting from an ion-exchange column [34].

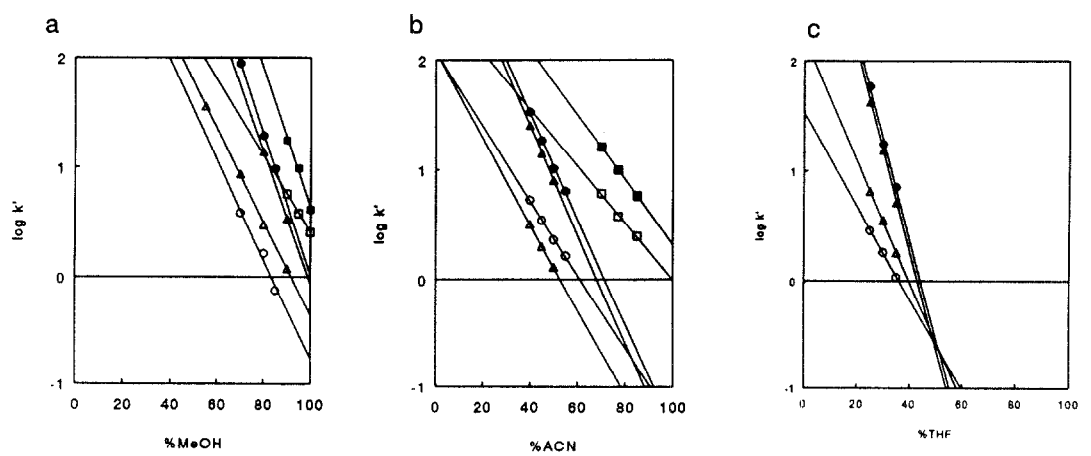

Fig. 4. Logarithm of the capacity factor k' versus the percentage organic modifier (MeOH = methanol; ACN = acetonitrile; THF = tetrahydrofuran) in the eluent. Compounds: toluene and butylbenzene. Columns: PL1, PL2 and OmniPac PAX-500. △ = toluene on PL1; ○ = toluene on PL2; □ = toluene on OmniPac; ▲ = butylbenzene on PL1; ● = butylbenzene on PL2; ■ = butylbenzene on OmniPac.

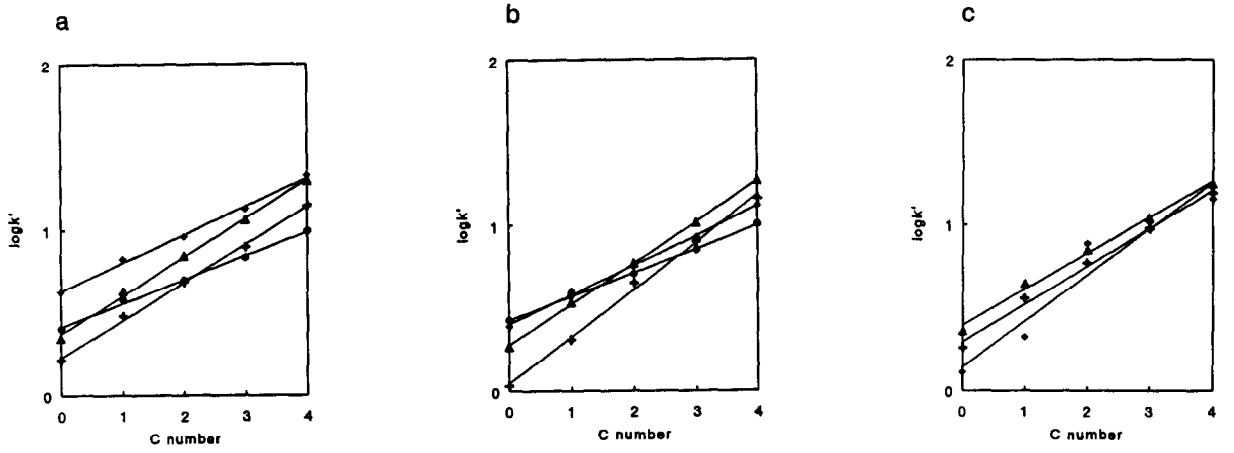

Fig. 5. Logarithm of the capacity factor k' versus the length of the alkyl chain of a homologous series of alkylbenzenes. Compounds: benzene, toluene, ethylbenzene, propylbenzene and butylbenzene. Columns: PL1, PL2, PRP-X100 and OmniPac PAX-500. Eluents: (a) methanol–water; PL1 (+), PL2 (▲): (80:20, v/v); PRP-X100 (◆): (90:10); OmniPac PAX-500 (●): (95:5, v/v). (b) Acetonitrile–water; PL1 (+), PL2 (▲): (45:55, v/v); PRP-X100 (◆): (73:27, v/v); OmniPac PAX-500 (●): (77:23, v/v). (c) Tetrahydrofuran–water; PL1 (+), PL2 (▲): (30:70, v/v); PRP-X100 (◆): (38:62, v/v).

$$\log k' = \frac{c}{b} \log C - \frac{c}{b} \log [E^-] + K \quad (4)$$

where C is the resin capacity, $[E^-]$ the ion concentration in the eluent, c the charge of the solute ion, b the charge of the eluent ion and K a constant.

This relation also showed to be valid for the columns investigated (Fig. 6). This result could be expected for the PRP-X100 column, because it was specifically developed for anion-exchange separations. The anion-exchange behaviour of the OmniPac PAX-500 was already described previously [25]. The slopes and the correlation coefficients (R values) of the lines in Fig. 6, calculated by using linear regression, are presented in Table 2. The low value for R in the case of the fluoride ion regarding the OmniPac column is caused by the low retention times. So interaction occurs between the fluoride peak and the system peaks, yielding inaccurate results for the retention times. The slope for the OmniPac and the PRP-X100 was ca. −1, for the monovalent solute anions. For the Polymer Labs. columns the slope was higher than −1, probably due to the reversed-phase stationary phase present in these columns. Looking to Eq. 4 this means that the ammonium carbonate in the eluent is monovalent, thus in the hydrogencarbonate form, as can be expected at a pH of ca. 9. When divalent anions are used as test compounds, the slope was smaller than −1, but not −2 as was to be expected in combination with monovalent eluent ions. This implies that the divalent anions are not retained as divalent anions. To be retained as a divalent anion, it must face two adjacent anion-exchange sites on a stationary phase that are spaced appropriately [25]. This is apparently not the case.

In Table 3 capacity factors and plate numbers of the columns for nitrate are given. The poorest anion-exchange performance using these eluent mixtures was observed for the OmniPac column. However, it should be emphasized that the OmniPac column is specifically designed for hydroxide as the eluent anion [25]. Better results were observed for the Polymer Labs. columns, especially when higher buffer concentrations were used. In this case short retention times were observed, indicating that the reversed-phase stationary phase has a negative influence on the performance in the anionic mode. Similar effects of the hydrophobicity of anion-exchange columns on the performance of i.e. the nitrate ion were also observed by Weiss [35]. The results for the PRP-X100 column were constant over the

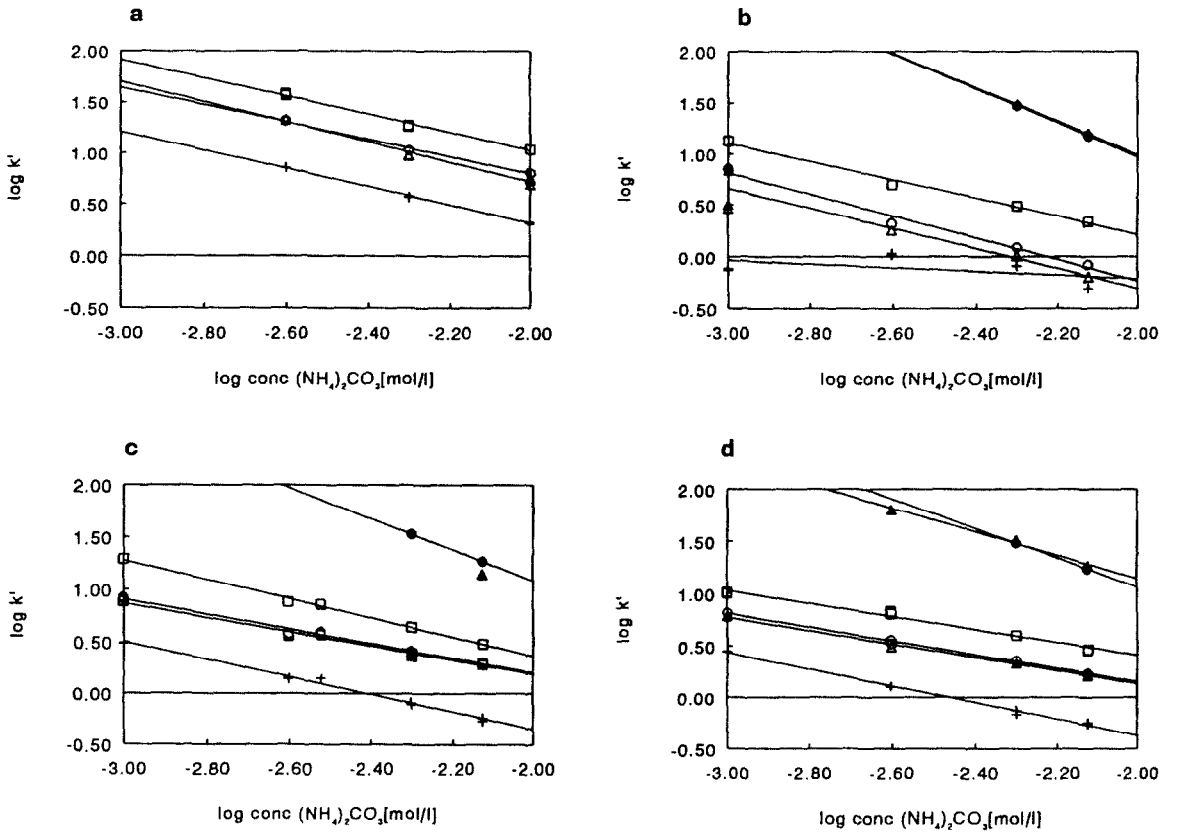

Fig. 6. Logarithm of the capacity factor versus the logarithm of the carbonate concentration in the eluent, the column is in the anion-exchange mode. Eluent: ammonium carbonate, for PL1, PL2 and OmniPac PAX-500 1% methanol was added. Compounds: fluoride (+), chloride (△), nitrite (○), nitrate (□), sulfite (▲) and sulfate (●). Columns: (a) PRP-X100, (b) OmniPac PAX-500, (c) PL1, (d) PL2.

ionic strength range and were, therefore, better at lower buffer concentrations and worse at the higher buffer concentrations.

To find out whether there is a difference between the nature of the organic modifier, used for wetting the OmniPac and the Polymer Labs.

Table 2
Slopes and R values for the lines shown in Fig. 6

	PRP-X100		OmniPac		PL1		PL2	
	Slope	R	Slope	R	Slope	R	Slope	R
F^-	−0.90	0.990	−0.17	0.485	−0.85	0.995	−0.81	0.998
Cl^-	−0.99	0.994	−0.96	0.948	−0.69	0.994	−0.64	0.998
NO_2^-	−0.85	0.998	−1.04	0.992	−0.70	0.995	−0.66	0.999
NO_3^-	−0.88	0.996	−0.89	0.995	−0.92	0.998	−0.62	0.992
SO_3^{2-}			−1.65				−1.12	0.995
SO_4^{2-}			−1.08	0.999	−1.47		−1.41	

For experimental conditions see Fig. 6.

Table 3
Plate numbers N and capacity factors k' of nitrate

$(NH_4)_2CO_3$ concentration concentration (M)	OmniPac		PL1		PL2		PRP-X100, N (plates/m)
	N (plates/m)	k'	N (plates/m)	k'	N (plates/m)	k'	
$1 \cdot 10^{-3}$	2286		–		953		–
$2.5 \cdot 10^{-3}$	1352		1347		1168		2172
$3 \cdot 10^{-3}$	–		2453		–		–
$5 \cdot 10^{-3}$	1566		5612		3845		2178
$7.5 \cdot 10^{-3}$	1621	2.17	14 959	3.01	7447	2.92	2166
$7.5 \cdot 10^{-3}$ (1% ACN)	1818	2.10	10 218	3.23	7239	2.89	

Eluent: ammonium carbonate in water; for the OmniPac PAX-500 and the Polymer Labs. columns 1% organic modifier was added to the eluent. Unless otherwise noted methanol was used as the organic modifier.

columns, the plate numbers and the retention times for nitrate were measured for 1% methanol and for 1% acetonitrile added to the eluent (see Table 3). The difference in retention time using methanol or acetonitrile was small. Somewhat higher plate numbers were observed for the OmniPac column when this column was wetted with acetonitrile. The plate numbers for the Polymer Labs. columns were higher using 1% methanol instead of 1% acetonitrile, especially for the PL1. This is in contrast with the results found for the reversed-phase behaviour of these columns found in this study and in literature [31]. This implies that the effect of the organic modifier on inorganic ions is different from the effect they have on neutral compounds [25].

3.3. Mixed-mode

As indicated above, the logarithm of the capacity factors of the test compounds versus the percentage organic modifier in the eluent provides a straight line in reversed-phase chromatography. In anion-exchange chromatography, the logarithm of the capacity factor is linearly dependent on the logarithm of the anion concentration in the eluent. These linear dependencies were not observed for the PL1 column, when it was used under mixed-mode conditions (see Fig. 7a and b). The other columns also exhibited typical mixed-mode behaviour, as is shown in Fig. 7c, d and e. At lower organic modifier concentrations the sulfonic acids and the styrene sulfates exhibit "reversed-phase" properties: the capacity factor decreases with an increasing organic modifier concentration. Here, the limiting factor for elution is the organic modifier concentration. At higher concentrations of organic modifier, the capacity factor increases rapidly for all components. In this case, the anion concentration in the eluent is the limiting factor for elution.

The same dependency of the retention mechanism with the organic modifier concentration was also observed, for instance, by Grego and Hearn [36]. On a reversed-phase column, they observed reversed-phase behaviour of the column for polypeptide hormones at low concentrations of the organic modifier, while at higher concentrations the ionogenic polypeptide hormones showed polar interactions with the column.

The fluoride ion also deviates from the usual ion-exchange behaviour, using a combination of buffer and an organic modifier as the eluent, is shown in Fig. 7b. This is caused by the f that the organic modifier present in the eluen will induce a decreased hydratation of the solute anion, which results in an increase in the retention time [37]. The change of the capacity factors of the alkanesulfonic acids, as a function of the shorter alkyl chains with the concentration of the organic modifier, in some cases shows similar behaviour to that of the fluoride ion. In these cases the alkyl chain is probably too short to have a substantial interaction with the hydrophobic backbone of the stationary phase.

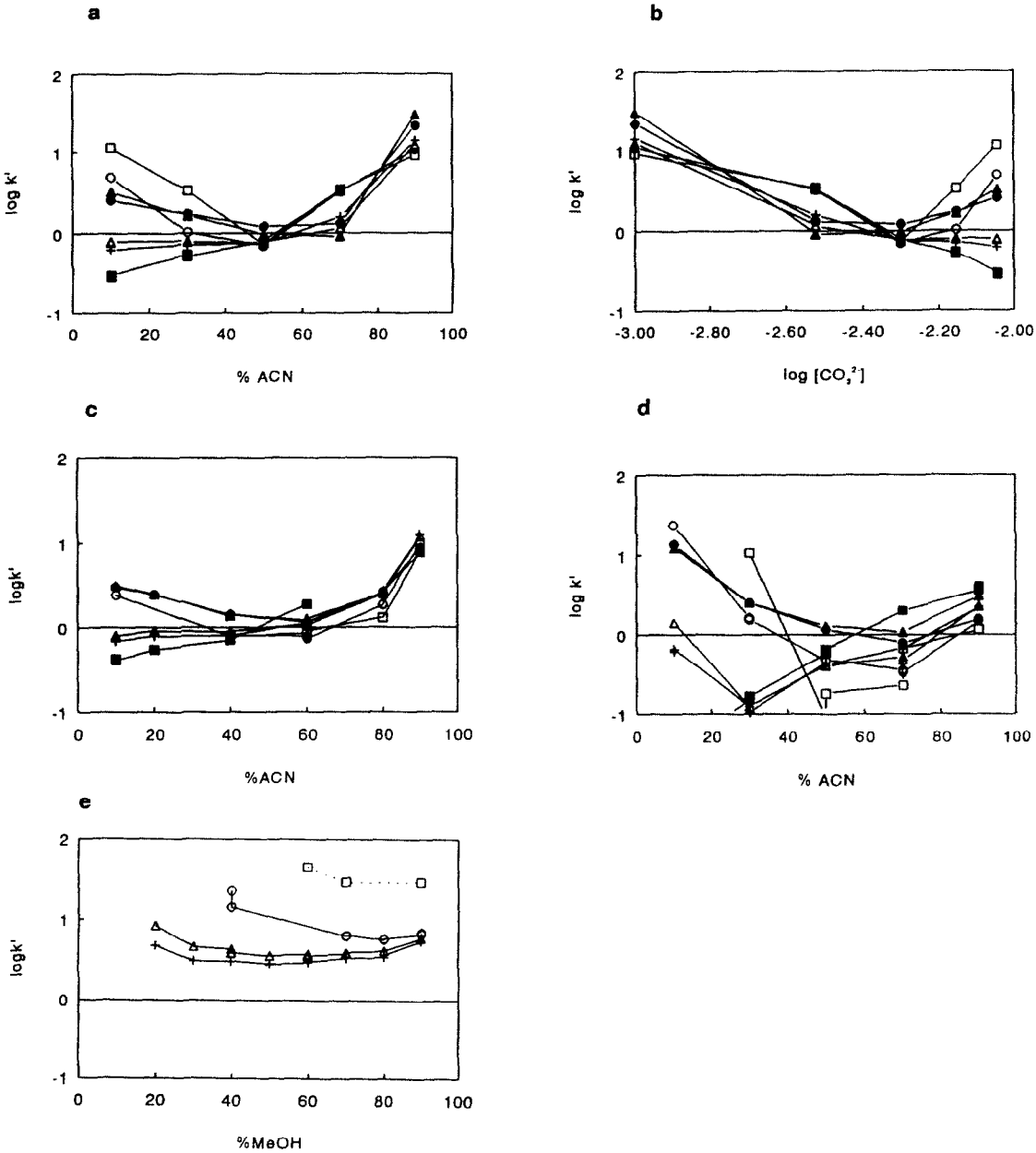

Fig. 7. Influence of the concentrations of the organic modifier and ammonium carbonate in the eluent under mixed-mode conditions. (a) PL2, percentage organic modifier versus the logarithm of the capacity factor; organic modifier: acetonitrile; concentration of $(NH_4)_2CO_3$ in water: 10^{-2} *M*; diluted with organic modifier to the concentration indicated. (b) PL2, logarithm of the concentration of $(NH_4)_2CO_3$ versus the logarithm of the capacity factor; organic modifier: acetonitrile; concentration of $(NH_4)_2CO_3$ in water: 10^{-2} *M*. (c) PL1, percentage organic modifier versus the logarithm of the capacity factor; organic modifier: acetonitrile; concentration of $(NH_4)_2CO_3$ in water: 10^{-2} *M*. (d) OmniPac PAX-500, percentage organic modifier versus the logarithm of the capacity factor; organic modifier: acetonitrile; concentration of $(NH_4)_2CO_3$ in water: 10^{-2} *M*. (e) PRP-X100, percentage organic modifier versus the logarithm of the capacity factor; organic modifier: methanol; concentration of $(NH_4)_2CO_3$ in water: 10^{-2} *M*. + = Butanesulfonic acid; △ = pentanesulfonic acid; ○ = octanesulfonic acid; □ = dodecanesulfonic acid; ▲ = styrene sulfate 1; ● = styrene sulfate 2; ■ = fluoride.

The separation factor α for the solute pairs butane-/pentanesulfonic acid and butane-/octanesulfonic acid, using as the eluents two aqueous ammonium carbonate solutions, of concentrations of respectively $5 \cdot 10^{-3}$ and 10^{-2} M, with methanol or acetonitrile as the organic modifier, is presented in Figs. 8 and 9. In all cases the separation factor was higher for methanol compared to acetonitrile. In addition, α was higher using a higher anion concentration in the eluent. This is another demonstration of the mixed-mode behaviour of the columns; no difference in α would be obtained if anion exchange as the only retention mode was active. This can be explained as follows. Combining Eqs. 4 and 5 for α, with k_1' and k_2' as the capacity factors for components 1 and 2, respectively:

$$\alpha = k_1'/k_2' \tag{5}$$

gives:

$$\alpha = e^{K_2/K_1} \tag{6}$$

where K_1 and K_2 are the constants from Eq. 4 for the compounds 1 and 2, respectively. From the resulting Eq. 6 it is obvious that α is independent of the eluent ion concentration. In addition, no difference in α would be observed if

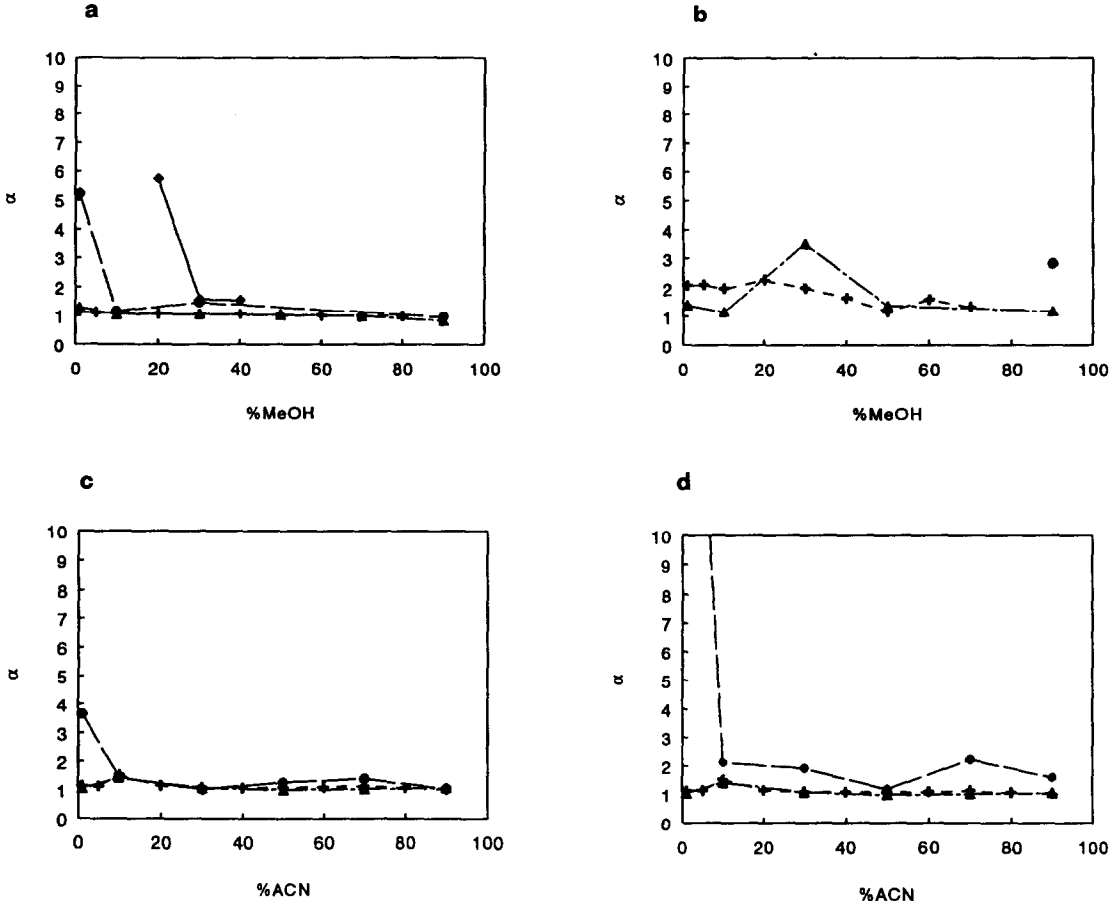

Fig. 8. Separation factor α as function of the organic modifier concentration in the eluent. Ammonium carbonate concentration in water was $5 \cdot 10^{-3}$ M. Columns: PRP-X100 (◆), PL1 (+), PL2 (▲) and OmniPac PAX-500 (●). (a) Methanol, α (butanesulfonic acid–pentanesulfonic acid); (b) methanol, α (butanesulfonic acid–octanesulfonic acid); (c) acetonitrile, α (butanesulfonic acid–pentanesulfonic acid); (d) acetonitrile, α (butanesulfonic acid–octanesulfonic acid).

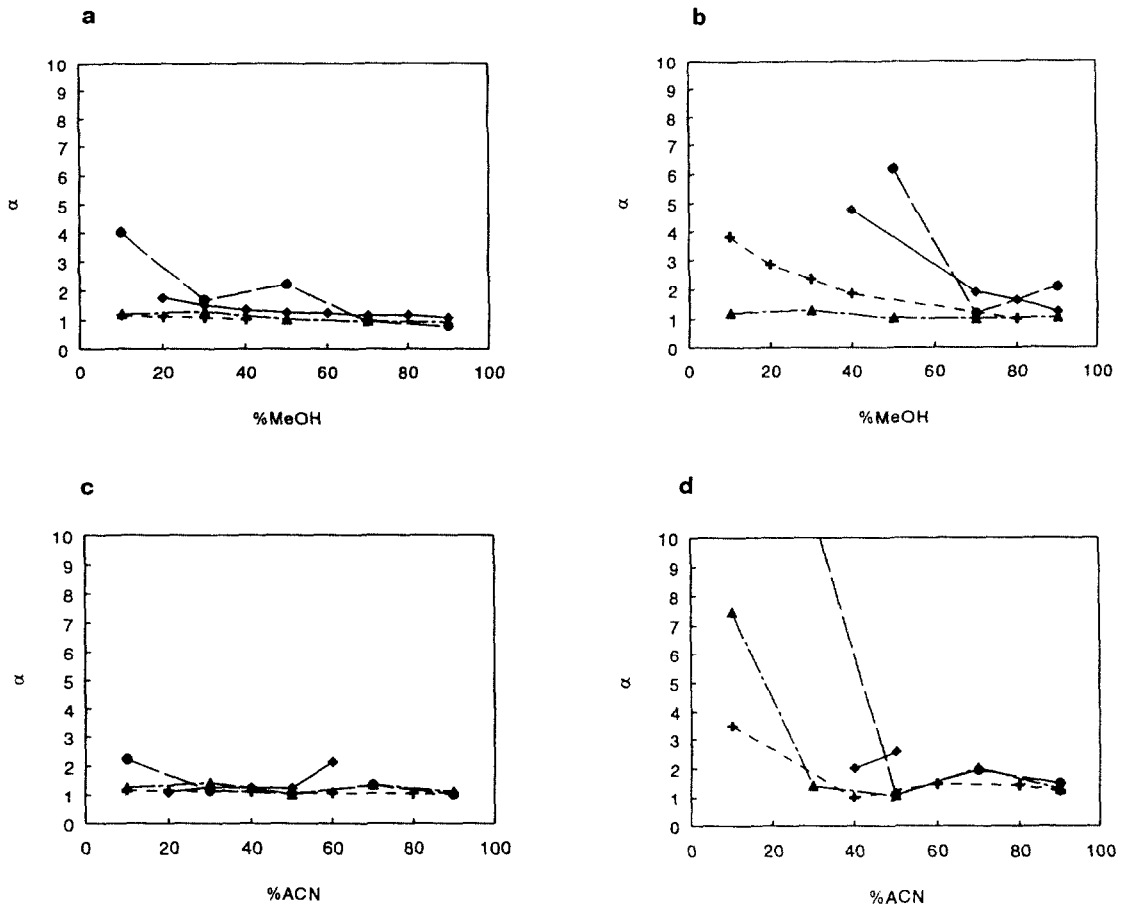

Fig. 9. Separation factor α as function of the organic modifier concentration in the eluent. Ammonium carbonate concentration in water was 10^{-2} *M*. Columns: PRP-X100 (◆), PL1 (+), PL2 (▲) and OmniPac PAX-500 (●). (a) Methanol, α (butanesulfonic acid–pentanesulfonic acid); (b) methanol, α (butanesulfonic acid–octanesulfonic acid); (c) acetonitrile, α (butanesulfonic acid–pentanesulfonic acid); (d) acetonitrile, α (butanesulfonic acid–octanesulfonic acid).

reversed-phase behaviour only took place. No difference in the capacity factors, between the two ion concentrations in the eluent, would be observed if the same concentration of organic modifier was used. The increase in α could be explained with a combination of these two retention modes. Considering the solute pair butane- and octanesulfonic acid, the octanesulfonic acid shows more reversed-phase behaviour and less anion-exchange behaviour. The influence of increasing the anion concentration in the eluent, will decrease the capacity factor less compared to the butanesulfonic acid, which shows less reversed-phase and more anion-exchange behaviour. Besides the higher α values, another advantage of higher anion concentrations in the eluent are the lower retention times of the test compounds.

Next, the efficiency of the columns for styrene sulfates 1 and 2 was investigated. The plate numbers and the asymmetry factors for the styrene sulfates are presented in Table 4 for the OmniPac PAX-500 and the PL2 columns, using methanol and acetonitrile as the organic modifiers in the eluent. A higher plate number was observed in almost all cases for styrene sulfate 1 compared to styrene sulfate 2, which is probably caused by the differences of the sulfate group's

Table 4
Plate numbers N and asymmetry factors *asf* of styrene sulfates 1 and 2 on the Omnipac PAX-500 and the PL2 columns

Organic modifier (%)	Solute	Omnipac				PL2			
		Methanol		Acetonitrile		Methanol		Acetonitrile	
		N (plates/m)	*asf*	N (plates/m)	*asf*	N (plates/m)	*asf*	N (plates/m)	*asf*
10	Styrene sulfate 1	–	–	16 824	1.38	8 964	3.16	8 466	1.76
	Styrene sulfate 2	–	–	1 873	10.70	1 043	9.45	1 887	3.73
30	Styrene sulfate 1	13 348	3.19	16 144	1.93	8 316	1.25	8 609	1.71
	Styrene sulfate 2	1 621	6.25	3 999	5.02	6 328	2.20	3 927	1.01
50	Styrene sulfate 1	2 728	2.50	10 066	2.43	7 211	1.31	8 455	1.64
	Styrene sulfate 2	2 270	5.40	4 608	0.56	6 461	1.39	1 190	0.28
70	Styrene sulfate 1	16 538	2.11	10 181	0.89	6 943	1.29	14 353	1.22
	Styrene sulfate 2	3 478	3.26	960	1.33	7 605	–	349	0.24
90	Styrene sulfate 1	13 345	1.16	18 584	0.50	7 588	1.18	21 288	0.76
	Styrene sulfate 2	998	3.63	246	–	984	3.26	455	0.18

Eluent: mixture of methanol or acetonitrile as the organic modifier and an aqueous ammonium carbonate solution; the ammonium carbonate concentration in aqueous buffer was 10^{-2} M.

location on the styrene molecule. The *asf* was also better for styrene sulfate 1, for high or low concentrations of an organic modifier. For styrene sulfate 2, high asymmetry factors were observed using low organic modifier concentrations, while at high organic modifier concentrations asymmetry factors below 1 were observed for this compound. No significant difference in performance was observed between methanol and acetonitrile when they were used as the organic modifier in the eluent. This contrasts with the reversed-phase and the anion-exchange mode. The retention times between the two styrene sulfates differed no more than 10% in most cases (see Fig. 7). The plate numbers for the alkanesulfonic acids were not measured because of the interference of system peaks which occur when a conductivity detector is used.

In conclusion, all the columns were able to separate butane- from octanesulfonic acid. A separation factor higher than 1 could be obtained using low concentrations of an organic modifier (see Figs. 8 and 9). Therefore, it is to be expected that sulfate monomers can be separated from sulfate dimers of butadiene. More general, in our opinion this approach has a potential for the separation of oligomers in real samples.

For the PRP-X100 column, the retention times for dodecanesulfonic acid and the styrene sulfates were very high for all eluent mixtures used (see Fig. 7e). For butadiene oligomers, it is known that oligomers at least up to $n = 4$, of both monosulfates and disulfates, are formed [1,38,39]. For styrene sulfate, oligomers up to $n = 2$ or 3 are formed [1,40]. This implies that with the PRP-X100 columns, the separation of these oligomers would be very time consuming. One way to decrease the retention times of these compounds is to increase the ammonium carbonate concentration in the eluent. There are two problems concerning this solution. With the increase of the ammonium carbonate concentration conductivity detection becomes difficult, as the conductivity of the eluent may exceed the range of the conductivity detector. Secondly, using a higher ammonium carbonate concentration yielded a more instable baseline, due to a

larger decrease in conductivity by the decomposition of the eluent. This also caused a decrease in the reproducibility of the retention times.

The Polymer Labs. columns showed reasonable retention times for the alkanesulfonic acids and, consequently, are more useful for this separation. However, the selectivity between the butane- and the pentanesulfonic acid is low, $\alpha \approx 1$ (see Figs. 8 and 9), so it probably is a problem to separate isomers formed during the polymerization. In this study the mixed-phase approach, using the Polymer Labs. columns, showed similar results to the mixed-mode approach. The difference in retention times between the PRP-X100 and the Polymer Labs. columns could be explained by the resin capacity of the columns. The resin capacity of the PRP-X100 is ca. 290 μequiv./ml [25]. The resin capacity of PL-SAX is >200 μequiv./ml [41], resulting in a resin capacity of >100 μequiv./ml for the Polymer Labs. columns. From Eq. 4 it is clear that increasing the resin capacity of a column increases the retention times of alkanesulfonic acids, as was shown by Pietrzyk et al., too [42].

With the OmniPac column it is possible to separate butane- from pentanesulfonic acid and to obtain reasonable retention times for the larger alkanesulfonic acids (see Fig. 7d). Here, a disadvantage is that to perform the separation of the smaller and the larger alkanesulfonic acids in one analysis, it is necessary to use a gradient.

4. Conclusions

A number of columns were investigated with respect to their ability to separate model compounds for charged and uncharged oligomers, formed during emulsion polymerization processes. The PRP-X100, the OmniPac and the Polymer Labs. columns showed promising results with respect to the separation of charged and uncharged oligomers of butadiene and styrene. The columns displayed typical reversed-phase, anion-exchange or mixed-mode behaviour when tested under reversed-phase, anion-exchange or mixed-mode conditions, respectively. As expected, the reversed-phase quality of the columns was not comparable to silica-based reversed-phase materials. Under mixed-mode conditions it was shown that manipulation of the eluent, i.e. the ionic strength and the nature and the concentration of the organic modifier therein, provides an good way to separate charged oligomers. It was possible to obtain a separation factor large enough to allow for the separation of charged oligomers. The other columns investigated, being Tessek HEMA, Aluspher RP-Select B and Zorbax Bioseries Oligo, were less useful for the separations of oligomers under the conditions applied in this study.

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