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Effects of modifiers in packed and open-tubular supercritical fluid chromatography

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ABSTRACT

The applicability of packed and open columns for supercritical fluid chromatography using pure carbon dioxide for the elution of a number of selected test components was investigated. It is shown that the number of solutes that can be eluted as symmetrical peaks is much larger in open-tubular capillary supercritical fluid chromatography. For strong hydrogen donors and acceptors, however, poor peak shapes were observed on both types of columns. In both cases, modifiers are needed. Modifiers may effect retention by increasing the mobile phase polarity and density, and by deactivating active sites. The relative influence of these processes was studied in packed and open columns. The effects of various modifiers in packed and open columns were compared under identical operating conditions. The influence of temperature on retention and peak shape was studied for pure carbon dioxide and carbon dioxide–modifier mixtures. The influence of modifiers on retention was found to be larger at lower operating temperatures. For components with high affinities for silanol groups the influence of polar modifiers on the capacity factor and the peak shape was found to be of comparable magnitude in packed and open columns. Columns packed with alkyl-modified poly(styrene–divinylbenzene) and with porous carbon coated with poly(ethylene glycol) were used to study the influence of modifiers without interference from silanol groups. Polar and non-polar modifiers were used to study the relative influence of the polarity and density effects of the modifiers.

In supercritical fluid chromatography (SFC) both packed and open-tubular columns are routinely used. Packed columns generally offer an increased speed of analysis in comparison with open-tubular columns. However, when high plate numbers are necessary, open columns are preferable [1,2]. Plate numbers in excess of approximately 20 000 are not readily attainable in packed-column SFC, because the maximum length of the column is limited by the maximum allowable pressure drop. With open-tubular columns much higher plate numbers can be obtained. Here the pressure drop is lower owing to the higher permeability of the column.

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A dinstinct disadvantage associated with the use of packed columns in SFC with carbon dioxide is the necessity to add modifiers for the elution of even slightly polar solutes. This largely cancels one of the advantages of SFC over liquid chromatography (LC), *i.e.* the improved detector compatibility. The use of modifiers in packed-column SFC has been more extensive than in open-tubular columns. In packed columns low concentrations of modifiers ($< 1\%$) can drastically reduce retention and drastically improve peak shapes [3–5]. In a recent study we observed a 50-fold reduction in the capacity factor of 2-hydroxyethyl methacrylate on a C_{18} packed column upon adding 0.5% ethanol to the carbon dioxide [6]. In comparison, Fields *et al.* [7] observed an approximately ten-fold reduction of the capacity factor of coronene upon adding 9% 2-propanol to the mobile phase in an open-tubular column coated with a 30% biphenyl methyl polysiloxane stationary phase. The different magnitudes of the effects of modifiers on retention in packed and open columns can be explained by the presence of active sites on the surface of the packing material. The effects of modifiers on retention and peak shape in packed-column SFC have been the subject of a large number of investigations (*e.g.* refs. 3–6 and 8–13). In contrast, few studies on the effects of modifiers in open-tubular SFC have been described [14–18]. A direct comparison of literature data on the effects of modifiers in packed and open columns cannot be made easily, because different solutes and different operating conditions have been used.

We recently identified four different ways in which modifiers might influence retention in packed-column SFC [19]. Mobile phase properties affected by the addition of a modifier include the density and the polarity. Because the mobile phase in packed and open columns is identical, these effects of the modifier are the same. By adsorption onto or partitioning into the stationary phase, modifier molecules can deactivate active sites on the packing material or on the column wall. In addition, increased solvation or swelling of the stationary phase can occur. In previous work we correlated the retention behaviour of solutes with the adsorption isotherms of modifiers in packed-column SFC [6]. The variation in the observed capacity factor with the modifier concentration could be described accurately by a mixed-retention model, in which silanol groups and chemically bonded groups were assumed to contribute independently to overall retention. This suggests that the effects of low concentrations of modifiers in packed-column SFC are primarily the result of stationary phase deactivation.

In this paper we will study the applicability of packed and open-tubular columns using pure carbon dioxide for the elution of a variety of components. The effects of modifiers in packed and open columns will be compared using similar stationary phases under identical operating conditions. The effects of temperature on retention and peak shape will be described. Furthermore, the effects of modifiers in packed and open columns will be investigated at various temperatures. Non-silica-based stationary phases for SFC are used to study the effects of modifiers in a system without interfering silanol groups.

EXPERIMENTAL

All experiments with packed columns were performed on a home-built SFC instrument. The mobile phase delivery system consisted of two reciprocating piston

pumps to allow operation with mixed mobile phases. A detailed description of this system has been published previously [20]. UV detection was used exclusively. The carbon dioxide (Air-liquide, Amsterdam, The Netherlands) had a purity of 99.8%. Ethanol and hexane (both p.a. grade) were used as modifiers and were obtained from Merck (Darmstadt, Germany). Several columns were used during the experimental work. A column packed with polydimethylsiloxane-encapsulated silica (Db-C_1) was obtained from Keystone (Bellefonte, PA, U.S.A.). An alkyl-modified polystyrene–divinylbenzene (PS–DVB) column (ACT-1) was purchased from Bètron (Rotterdam, The Netherlands). A column packed with porous carbon coated *in situ* with polyethylene glycol, resulting in what we describe as a 'carbonwax' column, was prepared in house [21]. The totally porous carbon was obtained from Professor J. Knox (University of Edinburgh, Edinburgh, U.K.). All packing materials had a nominal particle size of 5μm. The columns had an inner diameter of 4.6 mm and a length of 150 mm, except the carbonwax column, which had a length of 100 mm.

The experiments with open-tubular columns were carried out on a Carlo Erba 3000 Series SFC system (Carlo Erba, Milan, Italy). The carbon dioxide was obtained from Hoekloos (Schiedam, The Netherlands) and had a purity of 99.8%. Cylinders with carbon dioxide–ethanol mixtures were prepared in house. After filling the pump the composition of the carbon dioxide–modifier mixture in the pump was analyzed. For this analysis, a gaseous sample of the mixed mobile phase was collected in a gas bulb and subsequently analyzed by gas chromatography (GC). In this way uncertainties with regard to the composition of the mixed mobile phase, which might arise from a gradual change in composition of the mixture in a cylinder during usage, were avoided [20]. Several detectors were used during the experiments. A flame ionization detector (FID) and a nitrogen–phosphorous detector (NPD) were obtained from Carlo Erba. A photo ionization detector (PID) was obtained from HNU (Model PI-52-02, HNU, Newton, MA, U.S.A.). All three detectors gave good results with pure carbon dioxide. With mixed mobile phases only the NPD and the PID could be used. Both detectors showed decreasing performance (*i.e.* increased noise and reduced sensitivity and stability) at increasing modifier concentrations. Two open-tubular columns were used. The first one was a DB-1 column (J&W, Folson, CA, U.S.A.) with a length of 10 m, an inner diameter of 100 μm and a film thickness of 0.4 μm. The second column was a CP-Sil 5 CB column (Chrompack, Middelburg, The Netherlands). This column had a length of 10 m, an inner diameter of 50 μm and a film thickness of 0.2 μm. Both stationary phases are poly(dimethylsiloxane) polymers. The chemical nature of these phases is thus comparable with that of the Db-C_1 packed column.

Solutes were obtained from various sources and were all of the highest purity available. The sample concentrations were approximately 1 mg/ml for aromatic and 10 mg/ml for aliphatic solutes. Dichloromethane, which was assumed to be unretained, was used to measure the dead times.

RESULTS AND DISCUSSION

Retention in SFC is determined by the properties of the solute in relation to those of the mobile and the stationary phase. Because the solvent strength of a supercritical fluid very much depends on the operating pressure and temperature, a direct

comparison of the capacity factors and peak shapes in packed and open columns should be made under identical operating conditions. In that case all differences in the elution behaviour can be ascribed to the stationary phase. If the chemical nature of the stationary phases in the two columns is also identical, then different capacity factors or peak shapes must be the result of either different phase ratios or a more pronounced contribution of active sites to retention in one of the columns.

Table I provides a summary of the peak shapes and the capacity factors observed for 22 solutes on the Db-C_1 packed column and the CP-Sil 5 open column. Both stationary phases are poly(dimethylsiloxanes) and in each case pure carbon dioxide was used as the eluent. The peaks are classified into four categories: sharp, symmetrical peaks (+); broadened, possibly asymmetrical peaks (●); very broad,

TABLE I

COMPARISON OF THE ELUTION CHARACTERISTICS OF PACKED AND OPEN COLUMNS WITH PURE CARBON DIOXIDE

Conditions: temperature = 50°C; (average) pressure = 105 bar. Packed column: Db-C_1. Open column: CP-Sil 5 CB. The peak shapes are identified as sharp (+), broadened (●) and very broad (−). Components that are not eluted with a capacity factor of 30 or less are identified by ×. Capacity factors were calculated from the peak maxima. Values obtained from distorted peaks are given in parentheses.

Solute	Peak shape		Capacity factor	
	Packed	Open	Packed	Open
Benzene	+	+	0.12	0.045
Toluene	+	+	0.20	0.046
n-Propylbenzene	+	+	0.40	0.094
sec.-Butylbenzene	+	+	0.48	0.12
Phenol	●	●	(1.43)	(0.13)
4-Nitrophenol	×	−	−	(0.98)
2-Nitrophenol	●	+	0.92	0.15
2,4-Dinitrophenol	×	●	−	(0.28)
Bromobenzene	+	+	0.49	0.11
N,N-Dimethylaniline	−	●	(1.27)	(0.14)
2-Nitrotoluene	+	+	0.81	0.15
Benzyl alcohol	−	●	(2.53)	(0.11)
Methyl benzoate	●	+	(0.83)	0.11
Nitrobenzene	+	+	0.78	0.13
Dimethyl phthalate	●	+	(2.79)	0.20
Naphthalene	+	+	0.70	0.21
Biphenyl	+	+	1.40	0.32
2-HEMA[a]	−	●	(3.85)	(0.066)
Diheptyl ether	+	+	0.71	0.31
2-Tridecanone	●	+	(1.68)	0.27
Cyclohexanone	−	+	(1.00)	0.060
Quinoline	×	−	−	(0.31)
Total score:				
+	10	15		
●	5	5		
−	4	2		
×	3	0		

[a] 2-HEMA = 2-hydroxyethyl methacrylate.

asymmetrical peaks (−); and components that were not eluted with a capacity factor of 30 or less (×). All solutes are of fairly low molecular weight and are readily amenable to GC. The selected components, however, represent a wide variety of chemical functionalities and provide an indication of the applicability of packed and open columns with pure carbon dioxide as the mobile phase.

As can be seen in the table, the capacity factors on the packed column are larger than those on the open column. The components that are eluted as symmetrical peaks from both columns have a 2.5–6 times higher capacity factor on the packed column. A constant factor can be attributed to the larger phase ratio of the packed column. It might be possible that, although both phases are (polydimethylsiloxanes), slight differences exist in the chemical properties of the stationary phases in the packed and the open column. Furthermore, the pressure drop over the packed column was significantly greater than that over the open column, which might influence retention measurements. For the solutes that give poor peak shapes on the packed column, the ratio of the capacity factors is much larger than the factors between 2.5 and 6 mentioned above. These solutes are retained to a much larger extent on the packed column, most likely because of a contribution of active sites to retention. As can be seen in the table, the open column gives better peak shapes than the packed column. From the open column 15 out of the 22 solutes are eluted as sharp, symmetrical peaks. On the packed column only ten solutes fall into this category; the remaining twelve solutes are either eluted as (very) broad peaks or are not eluted at all. This indicates that the problems experienced when trying to elute these solutes from the packed column must be caused at least partly by active sites in the column and not only by a poor solubility in the mobile phase. On this column at least seven components require modifiers (categories − and × in the table). On the open column only the highly polar solutes quinoline and 4-nitrophenol require modified mobile phases.

In Fig. 1 the effects of low modifier concentrations are compared for packed and open columns. Fig. 1A and B shows the effects of the ethanol concentration on the capacity factors of quinoline and 4-nitrophenol, respectively. The capacity factors are normalized to 1 at zero modifier concentration. For quinoline the effect of the modifier is more pronounced in the packed column, although a significant decrease in

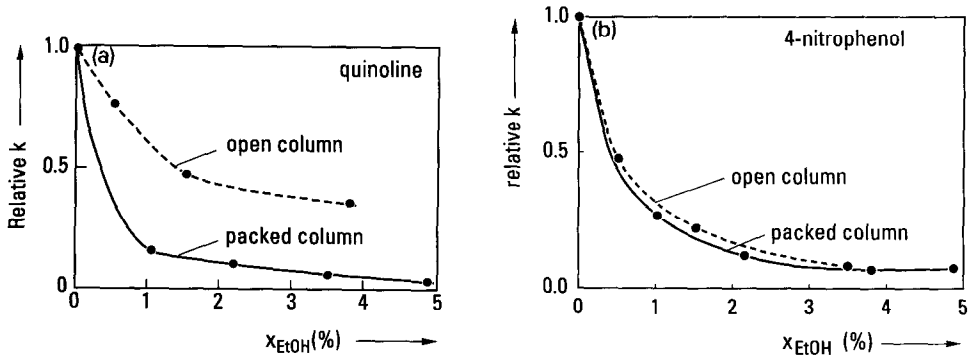

Fig. 1. Variation of normalized capacity factors with modifier concentration in packed and open columns. Open column: DB-1. Packed column: Db-C_1. Conditions: temperature 55°C; (average) pressure, 110 bar. Solutes: A, quinoline; B, 4-nitrophenol. EtOH = Ethanol.

retention also occurs in the open column. As can be seen in Fig. 1B, the effects of modifiers in both columns are very similar for certain solutes when compared on a normalized scale. Fig. 1 clearly illustrates that drastic effects of modifiers on retention are not limited to packed columns but can also occur in open-tubular columns. The fact that very small amounts of modifiers have a dramatic effect on retention in open columns suggests that a significant number of active sites are also present there. Fig. 2 shows the effect of a polar modifier on the peak shape observed for 4-nitrophenol on the open column. A drastic improvement is observed upon the addition of just 0.5% ethanol.

In Table I and Fig. 1 the performance of packed and open columns is compared at identical (average) pressure and temperature. Under these conditions the solvent strengths of the mobile phases are identical and differences in the elution behaviour should be attributed to the stationary phase. In daily practice, however, capillary columns are generally operated at higher temperatures. Two reasons for this can be identified. Firstly, the phase ratio of the open column is much lower than that of the packed column, which implies that a mobile phase with a lower solvent strength must be used to obtain reasonable capacity factors. Operating open columns at higher temperatures is also beneficial with regard to solute diffusivity. Diffusion is enhanced at higher temperatures. This increases the optimum velocity or, at constant velocity, it increases the plate number of the open column. In columns packed with small particles, the interparticle distances are small, so that the speed of diffusion is less critical. Table II gives a summary of the physical properties of carbon dioxide at typical operating temperatures for packed and open columns. The table clearly illustrates the lower solvent strength (or density) and the higher diffusivity under typical conditions for open-tubular SFC.

Because open and packed columns are generally operated at different temperatures, a more detailed study of the effects of temperature on retention and peak shape is required. Fig. 3 illustrates the influence of temperature on retention for the Db-C_1 packed column. In the figure three different zones can be dinstinghuished. The occurrence of these three zones can be understood by carefully evaluating the contri-

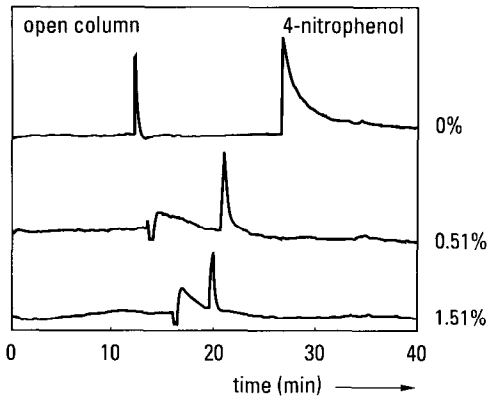

Fig. 2. Effect of modifiers on the peak shape. Open column: DB-1. Solute: 4-nitrophenol. Modifier: ethanol. Further conditions as in Fig. 1.

TABLE II

PHYSICAL PROPERTIES OF CARBON DIOXIDE AT 200 BAR AND DIFFERENT TEMPERATURES

The density values (ρ) were calculated using the IUPAC equation of state [22]. Mobile phase viscosities (η) were obtained from ref. 23. The binary diffusion coefficients (D_m) were estimated for n-C_{30} using the Wilke–Chang equation [24].

Column	Temperature (°C)	ρ (g/ml)	η (10^{-6} Ns/m^2)	D_m (10^{-5} cm^2/s)
Packed	35	0.866	81.3	3.5
	55	0.755	64.3	5.1
Open	80	0.595	45.8	7.7
	150	0.327	30.3	13.9

butions of the mobile and the stationary phase to retention. The contribution of the stationary phase to retention is relatively straightforward. At a constant solvent strength of the mobile phase, adsorption onto or partitioning into the stationary phase decreases constantly with increasing temperature. The contribution of the mobile phase is more complex. In the LC branch at low temperatures the mobile phase is a liquid with a solvent strength that is only slightly affected by the temperature. In this region retention decreases with increasing temperature because of a reduced interaction of the solute with the stationary phase and, eventually, with active sites. In the SFC part of the curve the decrease in the mobile phase density (thermal expansion) causes retention to increase with temperature. At even higher temperatures solute volatility becomes significant and retention will decrease again with increasing temperature. It can be seen that quinoline may be eluted from this column with the same capacity factors (between 3.8 and 5) under LC, SFC and GC conditions. In Fig. 4 the peak shapes obtained on the packed column at three different temperatures are com-

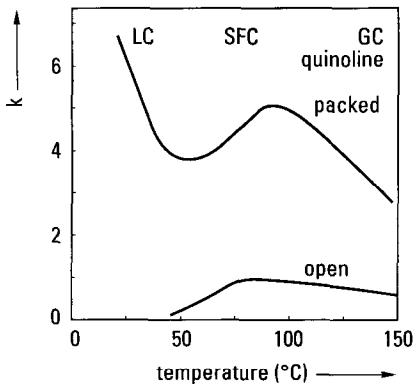

Fig. 3. Effect of temperature on retention in packed and open columns. Solute: quinoline. Open column: CP-Sil 5. Packed column: Db-C_1. The experiments with the open column were performed at a constant pressure of 120 bar. For the packed column constant inlet and outlet pressures of 179 and 162 bar, respectively, were used.

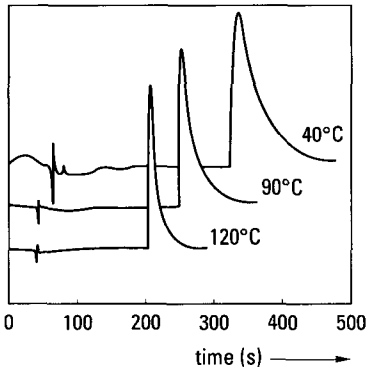

Fig. 4. Effect of temperature on the peak shape. Packed column: Db-C_1. Inlet pressure: 179 bar; Outlet pressure, 162 bar. Solute: quinoline. Mobile phase: pure carbon dioxide.

pared. The figure clearly shows an improved peak shape at higher temperatures, which is an additional reason for performing SFC separations at the maximum temperature allowed by the solute and the stationary phase.

Fig. 5A and B illustrates the influence of ethanol on retention as a function of the temperature. Fig. 5A gives the results on the Db-C_1 packed column. Fig. 5B gives those on the CP-Sil 5 open column. The general trend in the two figures is similar. The effect of the modifier is most pronounced at low temperatures. As an illustration, 4% ethanol gives a sixteen-fold reduction of the capacity factor of quinoline on the packed column at 25°C. At 150°C the same concentration only reduces retention by a factor of 1.7. When 0.5% ethanol was added to the carbon dioxide, the descending branch of the curve in the low-temperature region disappeared. The addition of the

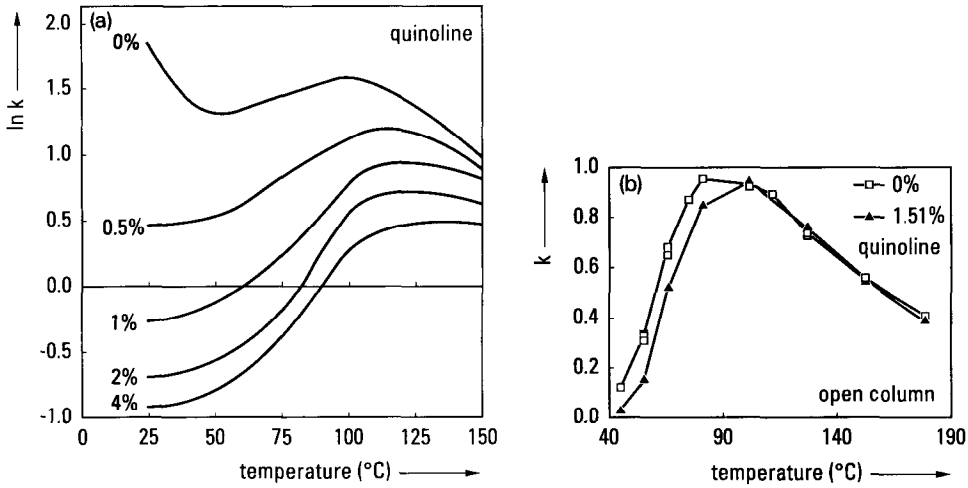

Fig. 5. Effects of ethanol on retention as a function of temperature. Solute: quinoline. (A) Packed column: Db-C_1; inlet pressure: 179 bar; outlet pressure: 162 bar. (B) Open column: CP-Sil 5; pressure: 120 bar.

modifier apparently effectively suppresses the contribution of adsorption to retention. As the contribution of adsorption is largest at low temperatures, the modifier effects are most pronounced in the low-temperature region. This also holds for the open column, as can be seen in Fig. 5B. At temperatures below 90°C, the modifier has a considerable influence on retention. Above 90°C the effect is negligible. The large effects of low modifier concentrations again indicate that adsorption on active sites may also play an important role in open columns.

From the results presented above it can be concluded that packed and open columns may differ in the degree of interaction. The kinds of interaction, however, remain the same. Owing to the interfering effects of silanol groups, no conclusions can be drawn regarding the relative magnitude of mobile and stationary phase effects of the modifier. On both the packed and the open column the mobile phase effects of the modifier are overshadowed by the deactivation effect. To establish the magnitude of the density and the polarity effect of the modifier in the mobile phase, a system free of interfering active sites is necessary. All silica-based stationary phases for packed-column SFC have previously been shown to exhibit some degree of surface activity [19,21]. Non-silica-based phases are necessary to circumvent the contribution of silanol groups to retention. When the stationary phase effects of the modifier can be neglected, the relative magnitude of the polarity and the density effect in the mobile phase can be estimated from a plot of the capacity factor of a test solute *versus* the fluid density. In Fig. 6A and B the effects of the polar modifier ethanol and the non-polar modifier hexane on two columns packed with non-silica-based stationary phases are illustrated. Fig. 6A shows the results obtained on a PS–DVB column, and Fig. 6B those of the carbonwax column. The experiments with modifiers on the PS–DVB column were performed at 55°C and at constant inlet and outlet pressures of 179 and 162 bar, respectively. The experiments on the carbonwax column were performed at 60°C and at inlet and outlet pressures of 179 and 163 bar, respectively. The density of the fluid increased when the modifier was added. Densities of mixed fluids were estimated using the Lee and Kesler method [25], which was previously shown to

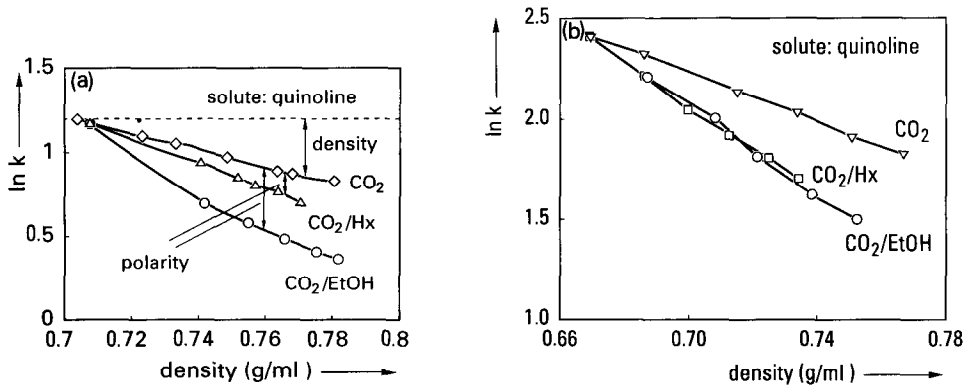

Fig. 6. Effects of polar and non-polar modifiers on retention on non-silica-based materials. Solute: quinoline. Modifers: EtOH = ethanol; Hx = hexane. (A) Packed column: PS-DVB; temperature: 55°C; inlet pressure: 179 bar; outlet pressure: 162 bar. (B) Packed column: carbonwax; temperature: 60°C; inlet pressure: 179 bar; outlet pressure: 163 bar.

yield accurate estimates [19]. The pseudocritical parameters which are required as input data for the density calculations were estimated from the mixing rules described by Lee and Kesler [25]. The density of pure carbon dioxide was varied by varying the pressure. In the figure the distance between the dashed horizontal line and the line for carbon dioxide represents the effect of density on retention. The differences between the curve of pure carbon dioxide and the curves for the mixed mobile phases represent the effect of the mobile phase polarity on retention. Although hexane is essentially a non-polar solute, it still shows a considerable polarity effect, *i.e.* there appear to be significant molecular interactions between the quinoline molecules and the hexane-containing mobile phase. Significant interactions of solute molecules with a hexane mobile phase were also observed by Phillips and Robey [26] in a comparative study on the properties of liquid hexane and carbon dioxide. The interactions between ethanol as a modifier and the solute molecules are more pronounced, giving rise to a larger polarity effect. The approach to separate the density and polarity effect of the modifier as described above is only valid when interactions between the modifier and the stationary phase can be neglected.

In Fig. 6B a plot similar to that of Fig. 6A is given, but in this case for the carbonwax column. The addition of hexane and ethanol reduces retention when compared with pure carbon dioxide at equal densities. However, in contrast to what is observed in Fig. 6A, the effects of both modifiers are identical. Apparently, modifier interaction with the stationary phase or the support material cannot be neglected. Preferential adsorption of hexane on the surface of the carbonaceous material may possibly occur, as indicated by the results of Engel and Olesik [27], who studied porous glassy carbon (PGC) as a stationary phase in SFC. In their study it was found that PGC tends preferentially to adsorb large molecules. The adsorbed molecules may effectively compete with solutes for the carbon surface, thereby reducing solute retention.

CONCLUSIONS

From open columns more solutes can be eluted with pure carbon dioxide as the mobile phase than from packed columns. However, the elution of highly polar solutes also requires modifiers on open columns. The effects of modifiers in packed and open columns can be of comparable magnitude when compared on a normalized scale under identical operating conditions. This suggests that blocking of active sites by the modifier may also play an important role in open columns. Apparently, a considerable number of active sites is also present on open columns. The contributions of these sites to the chromatographic process increases drastically at lower temperatures, so that modifiers become both more necessary and more effective at low temperatures. By performing a separation at the maximum temperature allowed by the solute and the stationary phase, improved peak shapes can often be obtained without modifiers.

Non-silica-based stationary phases are necessary to study the different effects of modifiers in the mobile phase. By plotting the capacity factor of the test solute as a function of the density of the mobile phase, the density and polarity effects of the modifier can be separated.

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