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HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC COLUMNS AND STATIONARY PHASES IN SUPERCRITICAL-FLUID CHROMATOGRAPHY

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SUMMARY

Packed columns show identical efficiencies when used in high-performance liquid chromatography (HPLC) or with fluid carbon dioxide in supercritical-fluid chromatography (SFC). Maximum plate numbers, however, are achieved at linear velocities 5–10 times higher in SFC than in HPLC. The advantage of SFC is the possibility of high speeds of analysis.

Problems with HPLC stationary phases arise from the ever present unshielded surface silanols. The use of columns specially prepared for HPLC separations of basic solutes is advantageous. Polymer-encapsulated stationary phases with aliphatic groups seem to have advantages over polystyrene resins. On the other hand, modifiers can be used to reduce silanophilic interactions. Water as a modifier is compatible with flame ionization detection. Equilibrium in modified systems is achieved very rapidly. In combination with fluid density (pressure and/or temperature) variations, system optimization in SFC seems to be less time consuming than in HPLC.

INTRODUCTION

Supercritical-fluid chromatography (SFC) with open-tubular (capillary) columns has increasingly been reconsidered in recent years[1–3]. The high efficiency and speed of analysis that these columns allow in gas chromatography (GC) can only be achieved in SFC when working with fluids at low densities. The diffusion coefficients are then closer to those achieved in gases. For the elution of solutes a higher solvation power of the fluid is required and this may be achieved by increasing the density of the fluid to values around 0.5 g/cm^3 or even higher. The diffusion coefficients of the solutes in the fluids are increasing and approaching values close to those in liquids[4]. This causes, of course, a decrease in efficiency because the radial diffusion is decreasing. To circumvent these problems, the introduction of secondary mass transfer by tight coiling of the column has been recommended[5]. These problems can be avoided either by using narrow-bore capillary columns with inner diameters less than 50 μm or packed columns with particle sizes around 5–10 μm. Another problem with capillary columns stems from the equipment. It is extremely difficult to keep the flow-rate constant during density and pressure programming. Consequently, an (undesirable)

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flow programme is superimposed on the (desired) density programme, leading to additional peak broadening due to the increasing mass-transfer term. With the high flow-rates applicable with packed columns, pressure and density modifications while keeping the volume flow-rate constant are less problematic[6,7].

Another advantage of the use of packed columns in SFC is the variety of stationary phases available and the knowledge of the influence of surface properties and modifications on solute retention accumulated in high-performance liquid chromatography (HPLC).

In this paper, the application of packed columns in SFC and their advantages and problems are discussed.

EXPERIMENTAL

Equipment

The equipment used was a modified version of that described recently[7], consisting of a reciprocating HPLC pump displacing liquid carbon dioxide and flame ionization detection. After passing into the fluid state, the flow-rate was measured via the pressure drop over a packed column. This was used to regulate the flow-rate of the pump resulting, under isothermal and isobaric conditions, in a constant volume flow-rate and hence constant linear velocity. The pressure at the column outlet was kept constant by an adjustable valve. The decoupling of pressure and flow control permitted the measurements of the *H vs. u* curve at different adjustable densities.

An additional regulating circuit was introduced to keep the linear velocity constant during density programming. When the volume flow-rate of the pump is kept constant while the density of the fluid is increased (increasing the pressure at constant temperature), the linear velocity of the fluid decreases. This can easily be measured by the increase in the dead time with increasing system pressure, as shown in Fig. 1, where the volume flow-rate delivered by the pump was kept constant at 3 cm^3/min. To compensate for increasing density and viscosity during pressure and density programming, the volume flow-rate of liquid carbon dioxide has to be increased to keep the dead time constant. The dependence of dead time is independent of the stationary phase and is only a function of the column dimensions and porosity. For each density a volume flow-rate exists where the dead time with the chromatographic column is constant. The experimentally measured values are stored in a computer and fed to the

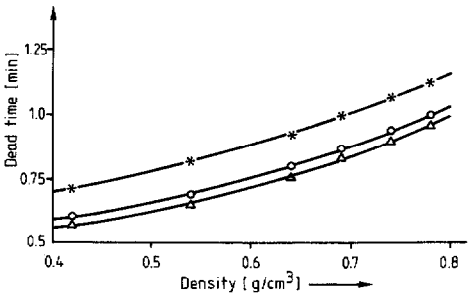

Fig. 1. Dead time as a function of fluid density. Columns, 250 mm × 4.1 mm I.D., packed with silica (LiChrosorb Si 100) and RP-8 and RP-18 derived from it. Inert solute, methane; fluid, carbon dioxide. (✱) Silica; (○) RP-8; (△) RP-18.

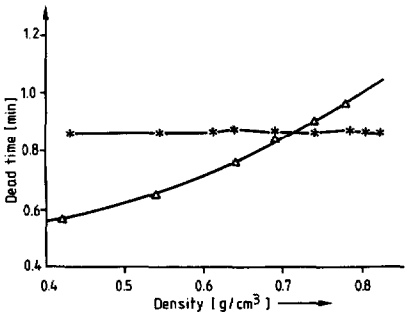

Fig. 2. Dead time as a function of density. Conditions as in Fig. 1. (△) Without flow compensation; (✳) flow compensation for increasing density. For details, see Experimental.

flow-regulating system, increasing the volume flow-rate corresponding to the density programme. As can be seen in Fig. 2, by this means the dead time of the system and hence the linear velocity in the column can be kept constant, and is independent of fluid density.

In pressure- and density-programmed SFC, the reproducibility of the retention time depends strongly on the constancy of the system pressure and the linear velocity. The advantage of the system described here can be seen in Fig. 3, where the same pressure programme was applied without (lower chromatogram) and with (upper chromatogram) flow compensation and hence a constant linear velocity. The relative standard deviation of retention times with this system in the density-programmed mode is 0.25%.

The mobile phase is expanded in the equipment used via a 5-μm orifice directly into the flame[7]. During density programming the volume flow-rate in the gaseous state increases. To keep the flow-rate through the orifice constant, which is necessary for constant response of the detector, a Tescom flow regulator was inserted parallel to the detector, which kept the flow-rate through the orifice constant.

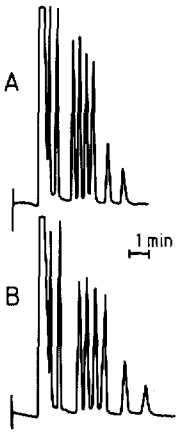

Fig. 3. Demonstration of flow compensation. Separation of C_{10}–C_{22} *n*-alkanes. Column, 250 mm × 4.1 mm I.D., packed with RP-18, 10 μm. Pressure programme, 92–184 bar, linear in 2.5 min. Temperature, 40°C. A, With flow compensation; B, without flow compensation.

Columns and stationary phases

Standard HPLC columns were used. The liquid was first displaced slowly with nitrogen at room temperature. In addition to the commercially available columns PLRP-S (a polystyrene–divinylbenzene resin from Alltech, Belgium, d_p 10 μm) and MH-1 (a polysiloxane-coated silica according to Schomburg *et al.*[8], based on Nucleosil 100, d_p 5 μm, purchased from Gynkothek, Munich, F.R.G.), various columns packed with silica (*e.g.*, LiChrosorb Si 100, d_p 5 and 10 μm, Merck) and chemically modified phases (based on silicas from Merck, Macherey, Nagel & Co. and Grace derivatized in our laboratory by silanization with octadecyl, octyl[9], triamine (γ-diethylenediaminopropyldimethoxy)[10] silanes or encapsulated with polyacrylates[11]) were used. The column length varied between 10 and 25 cm.

Test solutes were purchased from various distributors.

RESULTS AND DISCUSSION

Efficiency with packed columns

In all discussions on SFC, it has been stated that it combines the advantages of GC with respect of high efficiency and speed of analysis with the advantages of LC with respect to high selectivity and ease of optimization by varying the properties of mobile phase. The latter can be easily achieved by varying the density of the mobile phase and/or the temperature. The density increases with increasing pressure, a very large increase in density being obtained close to the critical conditions[12]. Simultaneously with the increase in density, the diffusion coefficients decrease[4]. In fluid carbon dioxide, the diffusion coefficients are in the 10^{-4} cm^2/s range[13]. A value of $7 \cdot 10^{-4}$ cm^2/s was measured for *n*-nonane at a carbon dioxide density of 0.69 g/cm^3, decreasing to $4.5 \cdot 10^{-4}$ cm^2/s at 0.45 g/cm^3 density.

With diffusion coefficients of *ca.* 10^{-5} cm^2/s for low-molecular-weight solutes in liquids, SFC conditions resemble more closely those in liquids than in gases, with diffusion coefficients of *ca.* 10^{-1} cm^2/s. This is obvious when one compares the *H vs. u* curves obtained for non-retarded solutes on packed columns in LC, SFC and GC, as shown in Fig. 4. The efficiency at the minimum of the *H vs. u* curve in LC is comparable to that in SFC. The most important difference, however, is that the minimum in SFC is achieved at linear velocities 5–10 times larger than in LC, thus permitting higher speeds of analysis. The smaller mass-transfer term (*C* term) in SFC (*ca.* 3 ms

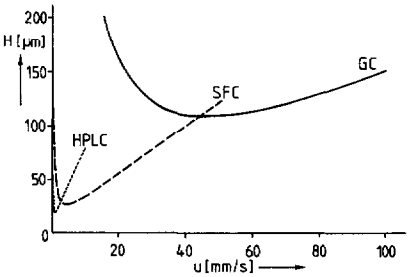

Fig. 4. Efficiency of packed columns in LC, SFC and GC. Column, 250 mm × 4.1 mm I.D., packed with RP-18, 10 μm. HPLC: mobile phase, methanol; solute, benzene. SFC: mobile phase, carbon dioxide; solute, methane. GC: mobile phase, nitrogen; solute, methane.

for 10-μm particles) allows a further increase in the speed of analysis without a significant decrease in efficiency. For comparison, the data obtained with a packed small-particle column in GC[14] have also been included in this plot. The H values in GC are due to the B term in the Van Deemter plot being a factor of 3 higher than in SFC and the maximum plate number (minimum of the H *vs.* u curve) is achieved at linear velocities a factor of 40 larger than in LC and a factor of 10 larger than in SFC. This demonstrates the advantage in GC of the high speed of analysis that is achievable.

In Fig. 5, the H *vs.* u curves in LC and SFC for a 5-μm packed column are compared. The H values under optimum conditions are identical. Reduced H values of *ca.* 3 are obtained. In the LC mode, the minimum of the H *vs.* u curve is *ca.* 0.5–1 mm/s linear velocity. In SFC at low pressure (density 0.64 g/cm^3), the minimum of the H *vs.* u curve is *ca.* 5 mm/s. At higher pressure (density 0.79 g/cm^3), the minimum is shifted to lower linear velocities, because the increasing viscosity causes a decrease in the diffusion coefficient. As can also be seen, the C term is also affected by the density, as expected from theory.

In liquids, the diffusion coefficient decreases with increasing temperature, resulting in higher efficiencies at higher temperatures. Fluids also behave like liquids in this respect. In Fig. 6 the H *vs.* u curves for two different columns measured in each instance at two different temperatures but at an identical density of 0.4 g/cm^3 are shown. The plate heights are reduced at higher temperatures, as expected from theory. Consequently, the efficiency can be increased by increasing the temperature as in LC.

Pressure drop and k'

The most important parameter for adjusting retention in SFC is the fluid density. Increasing the density (at constant temperature) always leads to a decrease in

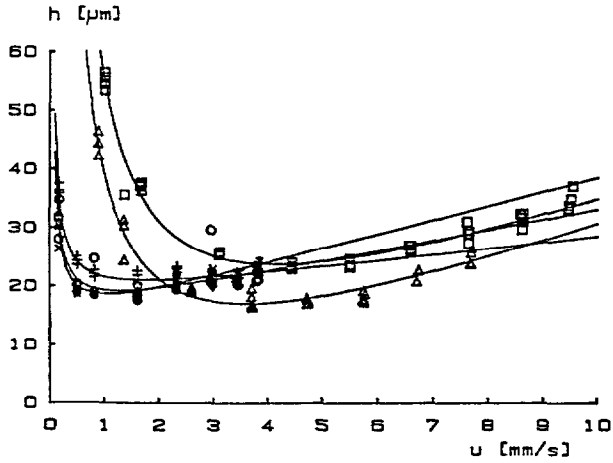

Fig. 5. Efficiency of packed columns in HPLC and SFC. Column, 250 mm × 4.1 mm I.D., packed with RP-18, 5 μm. HPLC: mobile phase, methanol; solutes, (+) nitromethane, (○) anthracene (k' = 0.7) and (×) pyrene (k' = 1.0). SFC: mobile phase, carbon dioxide; solute, methane; temperature, 40°C; pressure, (□) 109 and (△) 140 bar.

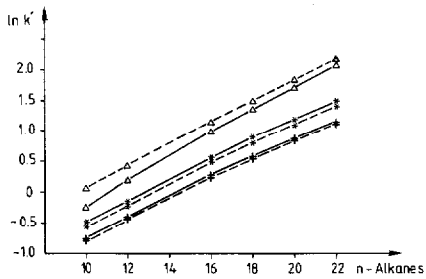

Fig. 6. Influence of column pressure drop on capacity factors. Column, 250 mm × 4.1 mm I.D., packed with RP-18, 10 μm; broken lines, single column; solid lines, two identical columns coupled in series. Temperature, 40°C; pressure, (△) 90, (✳) 105, (+) 120 bar.

retention. Pressure and density programming in SFC corresponds to temperature programming in GC and eluent programming, *i.e.*, gradient elution, in LC. The strong dependence of retention time (capacity ratio, k') on density leads to the argument that in packed columns, owing to the lower permeability by a factor of at least 30 compared with open-tubular columns, the velocity of retarded peaks decreases within the column, leading to reduced resolution and additional peak broadening. Also, the permeability of packed columns is a function of the packing procedure. Consequently, another argument against the use of packed columns in SFC is the difficulty involved in transfering a separation from one column to the other with different permeabilities and hence a different pressure drop.

The influence of pressure drop on k' can be seen in Fig. 6. The k' values were measured at three different end pressures (90, 105 and 120 bar) on one column and with two identical columns coupled in series. As can be seen, at low system pressure (90 bar) the k' values are lower with the two-column system compared with the single column. The pressure drop (at 90 bar end pressure) for the single column is 3 bar and that for the two-column system 6.9 bar. The difference in k' values is in the range of 5–8% between the single- and double-column systems. At an end pressure above 100 bar the differences in k' values are less significant and at pressures above 120 bar they are below 2%, despite the higher pressure drop of 5 bar compared with 10 bar.

The pressure dependence of k' values on doubling the pressure drop has only to be considered for systems with low density, where the fluid compressibility is high and the density shows a strong dependence on pressure. However, it is not advisable to work with systems too close to the critical conditions. At low density the solvation power of the fluids is weak, causing peak distortion and asymmetry (fronting).

The pressure drop with packed columns (d_p = 10 μm) with a length of 25 cm in SFC is in the range 2–5 bar. Consequently, it is possible to work reproducibly with packed columns in SFC when the system pressure is at least 20% above the critical pressure. It should be mentioned that with 10-m long open tubes of 50 μm diameter the pressure drop is also in the range 1–2 bar.

Selectivity with packed columns

Fluid carbon dioxide is a non-polar liquid that can be used to dissolve and extract non-polar and slightly polar solutes[15] such as low-molecular-weight hydrocarbons, alcohols and fatty acid esters. The solubility in carbon dioxide decreases

with increasing molecular weight and polarity, *e.g.*, number of hydroxyl groups in a molecule. The dielectric constant of fluid carbon dioxide increases with increasing density and approaches values in the liquid phase similar to that of *n*-pentane[4]. Consequently, in a first approach the retention behaviour of non-polar solutes was studied with HPLC stationary phases.

It has been shown[7] that hydrocarbons can be separated with both silica and reversed-phase columns. With the latter column type, higher retentions have been observed. Much more surprising is the fact that the influence of fluid density on retention is similar with both types of stationary phases. In Fig. 7, the dependence of retention on fluid density is shown for decylbenzene and fluoranthene with silica and RP-8 and RP-18 columns. Density is usually chosen, because linear plots are obtained only if ln k' is plotted against density. However, the equation of state used for the calculation of fluid density has to be valid in the range used. It has been recommended[15] that pressure and temperature be reported instead of densities, because these are the directly measured values. In this paper densities are used, but when necessary the column end pressure and temperatures are also given. As can be seen, with all stationary phases the influence of density on solute retention is identical (at constant temperature). The less polar the stationary phase, the higher is the retention at constant density (isopycnic conditions). Here carbon dioxide exhibits typical polar eluent properties like methanol in RP chromatography. With silica alkanes are eluted almost inert and the aromatic hydrocarbon fluoranthene is much more strongly retarded than decylbenzene. Here additional polar interactions certainly contribute to retention and carbon dioxide acts like a non-polar eluent, *e.g.*, hexane in normal-phase chromatography. Alkanes inert with silica columns are (with identical carbon number) much more strongly retarded than the phenylalkanes with RP columns. Here, hydrophobic interactions contribute to retention in RP and again carbon dioxide can be considered as a reversed-phase eluent. Depending on the properties of the stationary phase, either polar (silanophilic) or non-polar interactions govern solute retention in SFC. The silanophilic interactions are clearly recognizable with more polar solutes as will be discussed later, and have to be minimized in order to be able to separate solutes other than hydrocarbons in SFC. Most of the problems with packed columns stem from the silanophilic interactions with residual silanol groups on the chemically modified silica. As in LC, the silanophilic interactions can be masked by

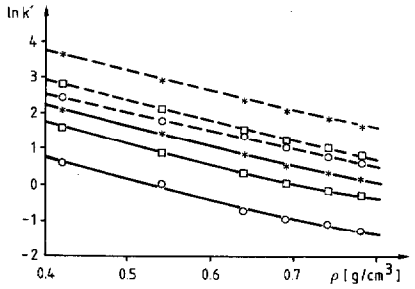

Fig. 7. Retention and fluid density. Columns, 250 mm × 4.1 mm I.D., packed with (○) SiO_2, (□) RP-8 and (✳) RP-18. Mobile phase, carbon dioxide; temperature, 40°C. Solutes: decylbenzene (solid lines) and fluoranthene (dashed lines).

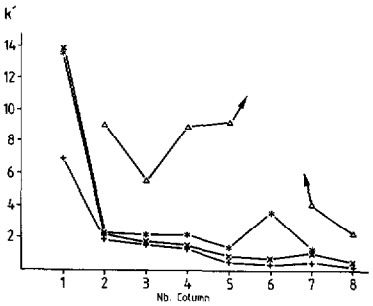

Fig. 8. Selectivity of packed columns. Columns: 1, PLRP-S, 10 μm, 150 mm × 4.6 mm I.D. (Alltech); 2, MH-1, 5 μm, 250 mm × 4.6 mm I.D. (Gynkotech); 3, Si 100 RP-18, 10 μm, 100 mm × 4.1 mm I.D.; 4, Si 80 RP-18, 10 μm; 5, poly(butyl acrylate) on Si 60, 5 μm; 6, polyacrylamide on Si 100, 10 μm; 7, triamine on Si 100, 10 μm; 8, LiChrosorb Si 100, 10 μm. Columns 3–8 were experimental products of our laboratory; dimensions of columns 4–8, 250 mm × 4.1 mm I.D. Mobile phase, carbon dioxide; temperature, 313 K; pressure, 120 bar. Solutes: (+) hexadecane; (×) decylbenzene; (∗) ethyl palmitate; (△) fluoranthene.

coverage with strong bases. In SFC this has been done by several injections of triethylamine before analysis of polar solutes[7]. Another possibility of circumvent these problems is to use polymer-coated stationary phases[8,11], which exhibit excellent properties in LC separations of strongly basic solutes, or polymeric stationary phases such as cross-linked polystyrenes[16].

In Fig. 8 the retentions of test solutes (16 carbon atoms) are plotted for eight different stationary phases. The highest retention was obtained with the polystyrene resin. Fluoranthene could not be eluted with this column, probably owing to strong aromatic–aromatic interactions. The smallest retentions were measured with the silica column. Symmetric peaks were obtained especially with polymer-coated columns (2, 5, 6) and with the triamine-modified stationary phases. These stationary phases are used with advantage in LC for the separation of basic solutes, where symmetric peak shapes are obtained and retention is independent of sample size up to 10^{-4} g of sample per gram of stationary phase. With these stationary phases the surface silanols are totally shielded. For use in SFC, consequently, stationary phases in which the surface silanols are completely covered and do not contribute to retention are recommended. With the totally polymeric stationary phases, the strong solute–surface interactions lead to too high retentions. The same has been found in LC with these columns. Comparing the selectivity of the polymer-coated stationary phases with that of a standard reversed-phase, it can be seen in Fig. 9 much more clearly that the selectivity is independent of pressure or density. It is striking that the polysiloxane-coated phase (where C_{18} groups are in the polysiloxane chains) shows similar selectivity to a standard reversed-phase (RP-18), whereas the selectivity is much higher with a polymeric phase based on butyl acrylate.

The ester functionality shows similar selectivity to the medium-polarity ester phases in GC. In Fig. 10 the separations of the test mixture with RP-18 and the acrylic phase are compared. Those solutes containing an aromatic ring are retarded more strongly with the acrylic ester phase, whereas the aliphatic solutes are eluted earlier compared with RP-18. This shows that (as in GC) it seems possible to adjust the selectivity of a polymer-coated stationary phase by varying the functional groups in the polymeric film.

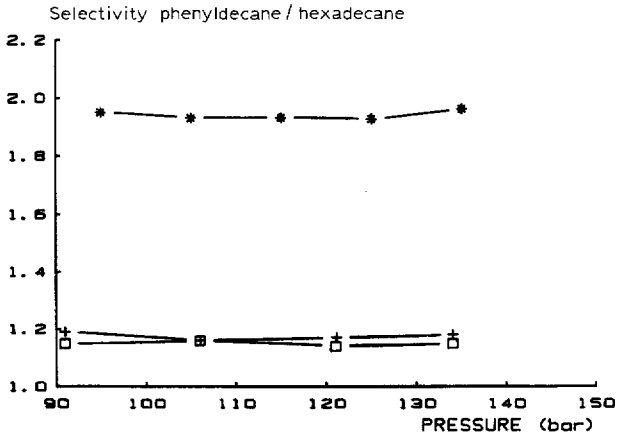

Fig. 9. Selectivity and pressure. Columns, temperature and solutes as in Fig. 8; pressure varied between 90 and 135 bar.

Adjustment of retention

Pressure programming. As discussed above, the retention decreases with increasing density or pressure at constant temperature. The selectivity is not influenced by the density, the slopes of the curves in Fig. 7 being almost parallel. It is therefore possible to reduce analysis time by pressure and density programming. Working at low temperatures, in the range 90–180 bar the density increases from 0.5 to 0.8 g/cm^3. Even in this range the retention decreases only by a factor of 3–5 compared with a factor up to 10 000 in RP chromatography with a gradient from water to methanol. At higher temperatures the change in density with pressure is less pronounced, and consequently pressure programming is less effective. The advantage of pressure programming is, however, that after returning to the initial pressure the column is immediately in its initial conditions and no additional regeneration time is required. It should be mentioned that in this instance the flow-rate has been increased during pressure programming, as discussed above, to keep the linear velocity constant.

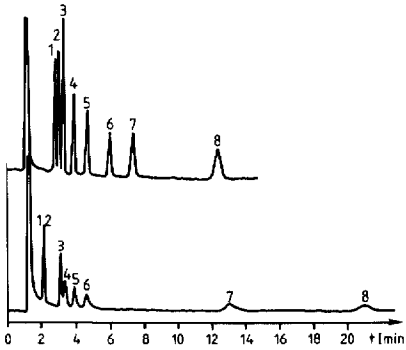

Fig. 10. Separation of test mixture on alkyl and ester phases. Upper chromatogram, RP-18 (column 4 in Fig. 8); lower chromatogram, acrylic ester (column 5 in Fig. 8). Mobile phase, carbon dioxide; linear velocity, 5 mm/s; density, 0.54 g/cm^3; temperature, 40°C. Samples: 1 = 1-hexadecene; 2 = hexadecane; 3 = decylbenzene; 4 = 1-chlorohexadecane; 5 = 1-bromohexadecane; 6 = 1-iodohexadecane; 7 = 2-phenylnaphthalene; 8 = fluoranthene.

Influence of temperature. Increasing the temperature at constant pressure decreases the density, resulting in an increase in retention time. Consequently, to enlarge the scope of density programming, a negative temperature programme (reduction of column temperature) is required. On the other hand, for volatile components it can be shown that increasing temperature can also reduce the retention time. It is questionable whether this group of solutes are not better separated by GC. In Fig. 11 the influence of density and temperature on the separation of polar volatile components is demonstrated. At a constant density (0.57 g/cm^3) the analysis time is reduced and the peak shape improved when the temperature is increased from 40 to 60°C. To keep the density constant, the pressure at column outlet has to be increased from 95 to 140 bar. The influence of density on separation at the lower temperature is also demonstrated in Fig. 11. An increase in density from 0.57 to 0.75 g/cm^3 also improves the peak shape, but the decrease in retention time reduces the resolution. With less volatile solutes, where the vapour pressure is negligible, an increase in temperature always leads to an increase in retention.

Addition of modifier. In LC, where silanophilic interactions also play an important role, these interactions can be minimized in RP chromatography by the addition of modifiers, such as triethylamine and other strong organic bases. In normal-phase chromatography with non-polar eluents, the strong interactions of solutes with the stationary phase are often modified by the addition of small amounts (less than 1%) of polar components such as methanol or water. The problems with water, always present in the eluents, and the extremely long time required to achieve equilibrium, have hindered the application of normal-phase chromatography. The use of modifiers has also been advocated in SFC. However, with organic modifiers, flame ionization detection (FID) cannot be used. Water is only partially soluble in fluid carbon dioxide (less than 0.3%)[17], but is fully compatible with FID when used as a modifier. The

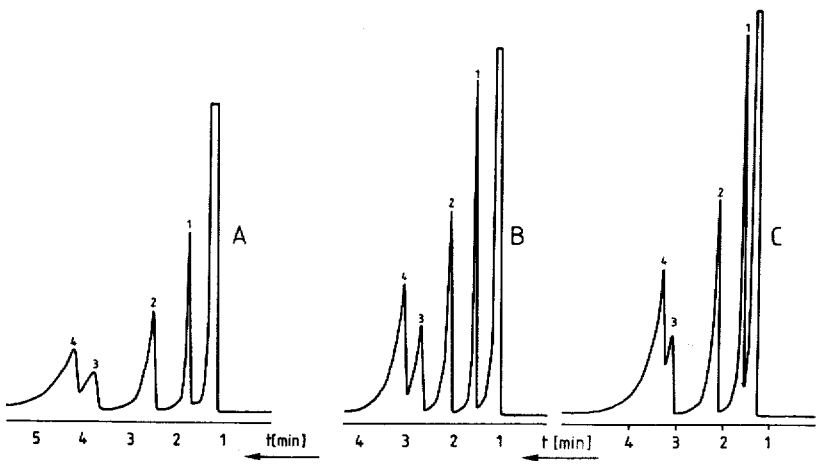

Fig. 11. Influence of density and temperature: isopycnic and isobaric conditions. Column, 250 mm × 4.6 mm I.D., packed with MH-1, 5 μm. Mobile phase, carbon dioxide; linear velocity, 8 mm/s. (A) Density 0.57 g/cm^3, pressure 95 bar, temperature 313 K; (B) density 0.57 g/cm^3, pressure 140 bar, temperature 333 K; (C) density 0.75 g/cm^3, pressure 140 bar, temperature 313 K. Solutes: 1 = phenyl acetate; 2 = acetophenone; 3 = phenol; 4 = 2,6-dimethylaniline.

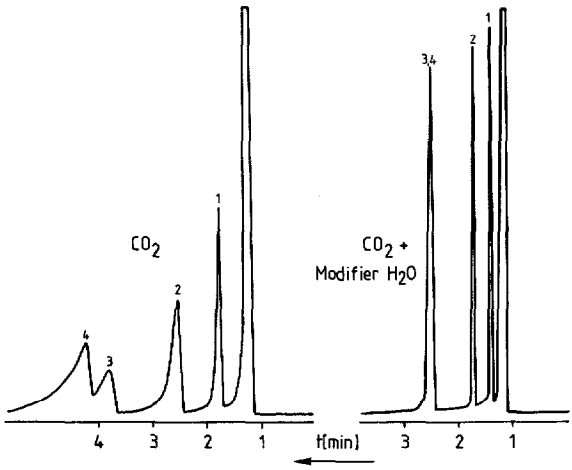

Fig. 12. Water as modifier. Column and solutes as in Fig. 11. Conditions: pressure, 95 bar; temperature, 313 K. Dry and water-saturated carbon dioxide used as mobile phase.

striking influence of water (added via a saturator[18] to the fluid carbon dioxide) can be seen in Fig. 12. The retention decreases, as expected, but much more striking is the improvement in peak shape. This indicates that most of the silanophilic interaction is blocked by water[19].

The influence of water on solute retention can be seen much more clearly in Fig. 13, where the dependence of retention on density is compared for dry and water-saturated carbon dioxide. With the unmodified eluent the k' values decrease by a factor of 11 when the fluid density is increased from 0.5 to 0.8 g/cm^3. Because the vapour pressure of the solutes is negligible in the temperature range used here, the k'

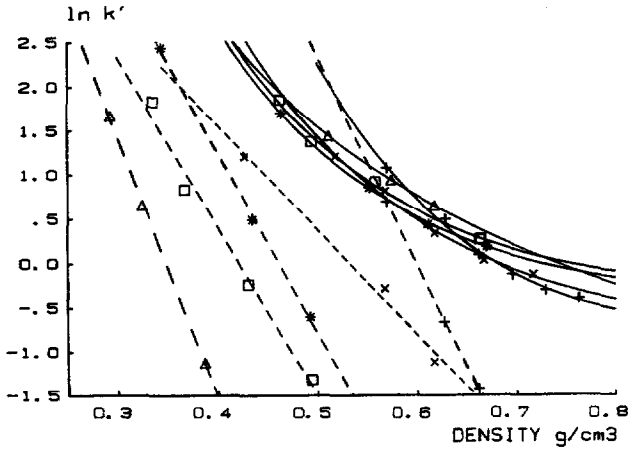

Fig. 13. Influence of temperature and density on retention with water-modified carbon dioxide. Column, 250 mm × 4.1 mm I.D. Solid lines, dry carbon dioxide; dashed lines, water-saturated carbon dioxide. Temperature: (+) 313 K; (×) 318 K; (*) 323 K; (□) 328 K; (△) 333 K. Solute: C_{22} *n*-alkane.

values are hardly affected by temperature variations. The opposite is true when water-saturated carbon dioxide is used. The k' values depend very strongly on density. With a density variation of only 0.1 g/cm^3 the k' values vary by a factor of 11. Much more striking here is the influence of temperature. On increasing the temperature by 20°C the same k' values are obtained at densities of 0.6 and 0.33 g/cm^3 with water-saturated carbon dioxide. This, of course, is a function of the solubility of water in carbon dioxide, which increases in the fluid range with increasing temperature.

The modifier water is prefentially adsorbed within the pores of the stationary phase. A similar behaviour has been found in normal-phase chromatography when *in situ* partitioning systems have been generated by using non-polar eluents saturated with water or other polar modifiers[20]. The loading of the stationary phase with polar modifier, *e.g.*, the decrease in pore volume, can be determined experimentally by measuring the variation of dead time with a marker that does not interact with the water layer within the pores. As can be seen in Fig. 14, the pore volume of the silica-based stationary phase decreases with increasing water content of the mobile phase, which is a function of pressure/density and temperature. It should be noted that the increase in water solubility[17] in fluid carbon dioxide is most noticeable in the pressure range 90–120 bar at temperatures around 50°C. It is worth mentioning that with the same stationary phase in the system acetonitrile–water (95:5, v/v) the same part of the pore volume is filled with water[10].

One great disadvantage in the use of water-modified systems in normal-phase liquid chromatography is the extremely long time required to achieve constant retention times and to obtain phase equilibrium[21]. Usually it takes 12–20 h to achieve equilibrium in the hexane–water–silica system. The advantage of modified fluid chromatography is that equilibrium conditions are approached much faster. In Fig. 15 the loading of the stationary phase with water (decrease in pore volume) is shown as a function of time. In the first 5–10 min most of the water is already deposited in the pores and equilibrium conditions are approached in about 20 min. The optimization of the separation conditions by variation of temperature and pressure and hence modifier loading of the stationary phase is therefore much faster than in LC.

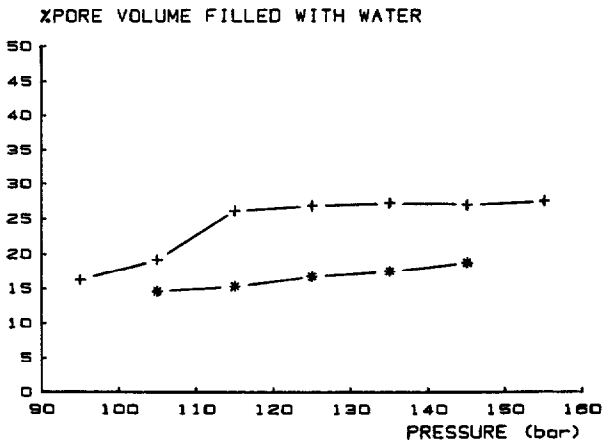

Fig. 14. Coating of stationary phase with water. Column, 250 mm × 4.1 mm I.D., packed with triamine on Si 100. Water-saturated carbon dioxide. Solute, methane; temperature, (*) 318 K; (+) 328 K.

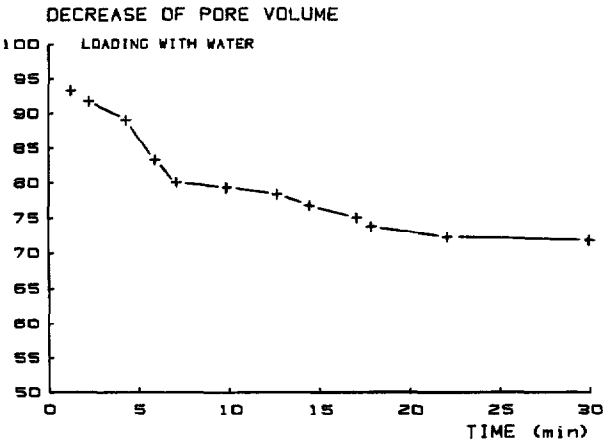

Fig. 15. Kinetics of water coating. Conditions as in Fig. 14 with temperature 328 K.

Sub- versus supercritical chromatography

As density is the main parameter affecting retention and analysis time in SFC, the question arises of whether the densest form of carbon dioxide, the liquid phase, can be used as mobile phase. Of course, to be able to work with liquid carbon dioxide, SFC equipment has to be used also. It seems that the selectivities with the same

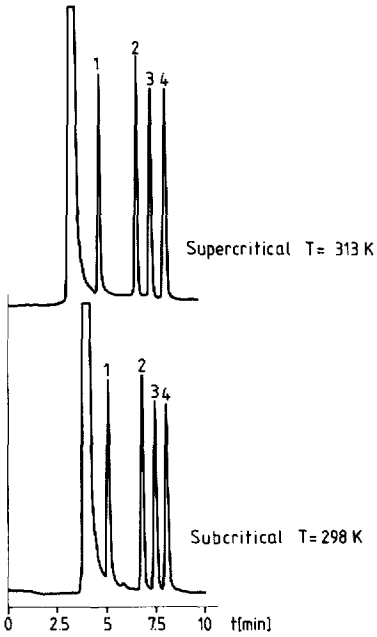

Fig. 16. Selectivity with liquid and fluid carbon dioxide. Column, 250 mm × 4.1 mm I.D., packed with PLRP-S, 10 μm. Pressure, 120 bar; temperature, 298 K (liquid carbon dioxide) and 313 K (fluid carbon dioxide). Solutes: 1 = ethyl benzoate; 2 = ethyl myristate; 3 = methyl pentadecanoate; 4 = ethyl palmitate.

stationary phase are almost identical whether fluid or liquid carbon dioxide is used. In Fig. 16, the separations of fatty acid esters with sub- and supercritical carbon dioxide are compared. The pressure was 120 bar in both instances. As liquids are almost incompressible, the retention is not affected by pressure. The liquid chromatogram was taken at 25°C and the supercritical fluid chromatogram at 40°C. As vapour pressure does not play a role with these solutes, the influence of temperature is not noticeable. The use of liquid carbon dioxide as the mobile phase in chromatography is worthwhile for future consideration, because it seems that some separations, where extremely specific solute–stationary phase interactions play a role, as in enantiomeric separations, can be achieved with improved selectivity when working with liquid (subcritical) carbon dioxide compared with supercritical conditions[22].

CONCLUSIONS

The use of packed columns in SFC results in highly efficient separations with plate heights comparable to those in LC when identical stationary phases are used. The maximum plate number (minimum of the Van Deemter plot) is approached at linear velocities that are a factor of 5–10 higher than with the same column under LC conditions. Consequently, a higher speed of analysis is achievable in SFC.

The use of packed columns increases the potential of SFC, because a variety of stationary phases with distinct and often well understood selectivities are available. This enlarges the scope of the potential applications of SFC, because the selectivity and number of potential fluids are very limited, if not restricted to carbon dioxide alone. The polarity of carbon dioxide corresponds to that of hydrocarbons and consequently the silanophilic interactions can exhibit a disturbing influence, especially if polar solutes are to be separated. The use of modified systems, with water as modifier, can minimize this influence and FID can still be used. The achievement of equilibrium conditions even in modified systems is much faster than in LC, facilitating system optimization where many changes of the operating conditions are necessary[23].

The only stationary phase that can be used in SFC for the separation of more polar phases are those in which the surface silanols of the base material are totally shielded. Preferably stationary phases developed for the LC of basic solutes should be used. New types of stationary phases, either with tentacle-type bonded groups[24] or where the surface is coated with a homogeneous polymer film, have been used in SFC. Polymer-encapsulated silica, where functional groups in the polymer film can be varied, appears promising for future developments in SFC.

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