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RETENTION AND SELECTIVITY IN CARBON DIOXIDE SUPERCRITICAL FLUID CHROMATOGRAPHY WITH VARIOUS STATIONARY PHASES

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SUMMARY

A study has been conducted on the influence of various parameters on solute retention in supercritical fluid chromatography (SFC) with carbon dioxide, using columns packed with small particles. The influence of the physical state of carbon dioxide on the solute retention on bare silica has been accurately studied.

The influence of polar modifier addition to carbon dioxide has been studied on three stationary phases: bare silica, octadecyl-bonded silica and "Pirkle's phase" for enantiomeric separation. We found that the retention mechanism in SFC with carbon dioxide is very similar to that observed in high-performance liquid chromatography with an apolar solvent such as hexane.

Enantiomeric separation on a chiral-bonded stationary phase constitutes an interesting application of SFC. Some phosphine oxides have been resolved, with retention times of <90 s.

INTRODUCTION

Supercritical fluid chromatography (SFC) has recently become popular because of the enhancement of column performance in comparison with liquid chromatography (LC) due to relatively fast solute diffusion, and the possibilities of new detection methods such as flame ionization detection (FID) and Fourier transform-infrared (FT-IR). Moreover, supercritical fluids have liquid-like densities, so they can dissolve a great number of solutes at low temperature (*e.g.* 40°C for carbon dioxide). Moreover, it becomes possible to resolve relatively non-volatile, thermally unstable or high-molecular-weight solutes, which cannot be analysed by gas chromatography (GC). The combination of these properties gives a powerful chromatographic technique that is complementary to both GC and high-performance liquid chromatography (HPLC).

Carbon dioxide, with its moderate critical parameters of 31°C and 74 bar, is one of the most frequently used supercritical fluids. Because of its low polarity, similar to that of hexane¹, carbon dioxide is particularly interesting with adsorption chromatography or any other chromatographic technique requiring a low polarity mobile phase. For instance, enantiomeric resolutions with a chiral phase ("Pirkle's phase") can be carried out with carbon dioxide-alcohol mixtures as mobile phase.

With supercritical carbon dioxide, almost any HPLC stationary phase can be used for SFC, but retention mechanisms are still unknown. So it is the purpose of this paper to discuss the retention mechanisms with several stationary phases (bare silica, alkyl-bonded silica, and "Pirkle's phase") for pure carbon dioxide and carbon dioxide-modifier mixtures.

EXPERIMENTAL

Apparatus

The apparatus is described elsewhere¹. The carbon dioxide, which is contained in a standard container with an eductor tube, supplies the pump of a Varian 5500 chromatograph (Varian, Palo Alto, CA, U.S.A.).

The pump head has to be cooled to improve the pump efficiency. So, it is placed in close thermal contact with a clean-on-heat exchanger through which cold ethanol is circulated.

Unfortunately, below 55-bar pressure, it is impossible, at the moment, to mix carbon dioxide and liquid solvents with the proportioning valves of the Varian chromatograph, and the addition of the polar modifier is realized with a Gilson pump (Model 302, Gilson, Villiers-le-Bel, France).

Temperature control was achieved with a constant-temperature water-bath (Tamson, type TX-9, Ets. Bourseuil, Plaisir, France). A Varian UV 200 spectrophotometer was used with a special detection cell that has been modified to withstand pressures up to 350 bar. The pressure was controlled by a manually back-pressure regulator Tescom (Model 26-3220-24004, GEC Composants, Asnières, France) situated downstream of the detector.

Chromatographic columns

The chromatographic columns used are 10–25 cm × 0.46 cm I.D., packed according to the conventional slurry technique. Various stationary phases were used: (i) 7–8 μm Zorbax octadecyl-bonded silica (Du Pont, Wilmington, DE, U.S.A.); (ii) 5-μm experimental octadecyl-bonded silica (Rhône-Poulenc Recherches, France); (iii) 5-μm LiChrosorb Si 60 silica (Merck, Darmstadt, F.R.G.); (iv) 10-μm aminopropyl LiChrosorb NH₂ silica-bonded with (*R*)-N-(3,5-dinitrobenzoyl)phenylglycine. This stationary phase was first used by Pirkle^{2–4} for enantiomeric separation.

Chemicals and reagents

Solvents. Carbon dioxide was of high purity, N45 grade (99.995%) (Air Liquide, France); methanol and acetonitrile were HPLC grade (Prolabo, Paris, France); methyl-*tert.*-butyl ether (MTBE) was Spectrosol grade (SDS, Peypin, France); ethanol and 2-propanol were UV spectroscopic grade (Prolabo); and methoxyethanol was *pro analysi* grade (Merck).

Solutes. Phosphine oxide synthesis has been described elsewhere⁵. The other solutes were all of analytical grade and were purchased from various producers.

RESULTS AND DISCUSSION

Influence of the physical state of carbon dioxide on retention

The physical state of carbon dioxide is characterized by two parameters: pressure and temperature or, more precisely, density and temperature. As already observed on a PRP-1 column by Lauer *et al.*⁶, the phenanthrene capacity factor logarithm on a silica column is a linear function of reciprocal temperature (Fig. 1). This important decrease of retention with temperature, at constant density, can be explained mainly by the increasing solute solubility.

Fig. 1 also shows that retention decreases with density at constant temperature. This tendency is often explained by the solubility variations caused by density variations^{7,8}. In most cases, solute solubility increases with density. However, this explanation is not always sufficient to explain retention phenomena in SFC: Fig. 2 shows the retention and solubility variations with pressure at various temperatures when the solute is phenanthrene. When pressure increases at constant temperature, density increases and, as a consequence, retention decreases. When temperature increases at constant pressure, two cases must be considered: for pressures < 200 bar,

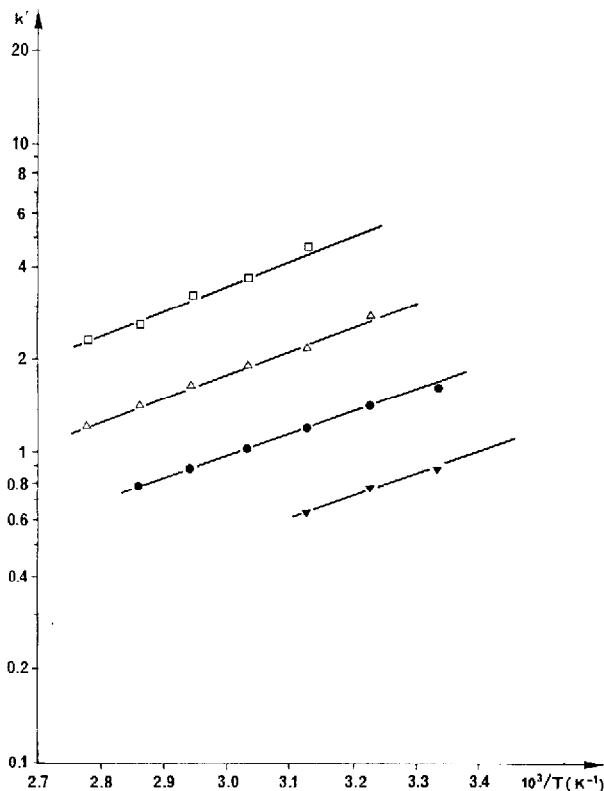


Fig. 1. Logarithm of capacity factor of phenanthrene vs. the reciprocal of the temperature at various densities (ρ) of carbon dioxide: (\blacktriangledown) $\rho = 0.9$ g cm⁻³; (\bullet) $\rho = 0.8$ g cm⁻³; (\triangle) $\rho = 0.7$ g cm⁻³; (\square) $\rho = 0.6$ g cm⁻³. Column, 25 \times 0.46 cm I.D.; detection, UV at 220 nm; stationary phase, 5 μ m LiChrosorb Si 60 silica; flow-rate: 2 ml min⁻¹.

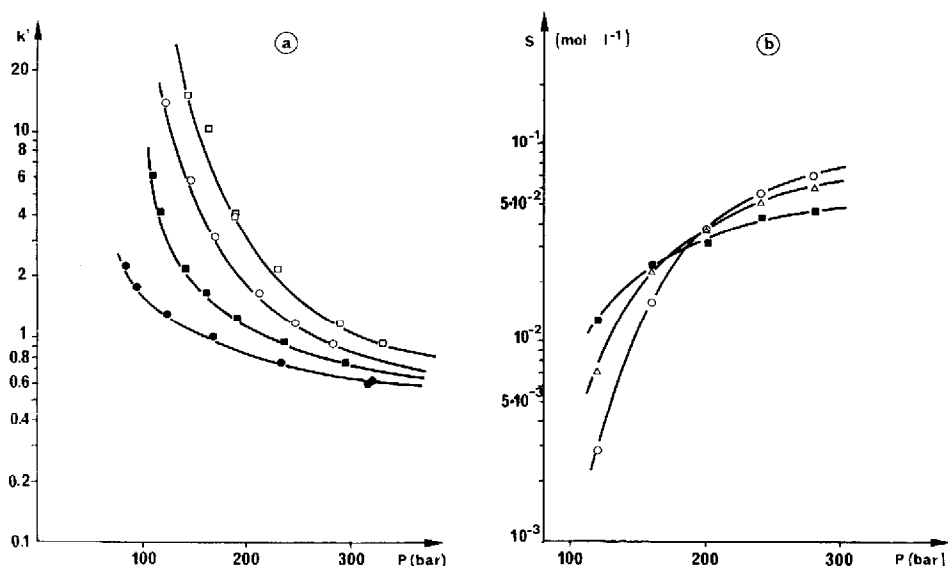


Fig. 2. Logarithm of capacity factor (a) and logarithm of solubility⁹ (b) of phenanthrene in carbon dioxide vs. the pressure at various temperatures. (a): (●) 300 K; (■) 320 K; (○) 340 K; (□) 360 K. (b): (■) 318 K; (△) 328 K; (○) 338 K. Other operating conditions as in Fig. 1.

retention increases and solubility decreases. On the contrary, at high pressure ($P > 200$ bar), Fig. 2 shows that both the capacity factor and the solubility increase with temperature. Thus, there must be an increase in the solute-stationary phase interactions, which can only be explained by a change of the apparent nature of the silica. It should be noted that the same phenomenon appears with naphthalene, whose solubilities in carbon dioxide are well known^{10,11}. A possible explanation may be found in the influence of water (≈ 10 ppm in carbon dioxide) on the stationary phase activity.

In a recent paper on liquid-solid chromatography (LSC), Souteyrand and co-workers^{12,13} showed that the number of free silanol groups covered by one water molecule is linked to the water content in the mobile phase (the higher the water content, the higher the mole fraction of water on free silanol groups). The same authors have shown that the adsorption energy on a free silanol group covered by a water molecule is much lower than that obtained on a free silanol group covered by molecules of an apolar solvent.

In the case of supercritical carbon dioxide, water solubility increases with temperature (at 200 bar, when temperature increases from 25 to 75°C, water solubility increases from 1500 to 4500 ppm^{14,15}). Consequently, when the temperature increases, some water molecules are transferred from the stationary phase to the supercritical phase, and the number of free silanol groups covered by a water molecule decreases for the benefit of free silanol groups covered by molecules of carbon dioxide. As the adsorption energy of the solutes on these latter groups is higher than that obtained on the former, there can be simultaneous increase in the retention and the solubility of the solute.

Influence of the addition of modifier

Another way of modifying the mobile phase polarity and, consequently, the retention, is to add to carbon dioxide a small amount of polar solvent. In SFC, one of the most frequently used polar modifiers is methanol.

However, many other organic solvents with different chemical properties are soluble in supercritical carbon dioxide. Snyder's solvent classification scheme¹⁶⁻¹⁸ has already been successfully applied to HPLC for several separation optimizations. A first attempt to apply this method to SFC was proposed by Randall¹⁹ and here it is applied to a phenol separation with supercritical fluid-solid chromatography (SFSC).

SFSC

Fifteen phenol derivatives were chosen in order to study the influence of modifier nature and concentration of carbon dioxide on retention with LiChrosorb Si 60 silica.

In LSC, optimization of the selectivity is achieved by blending three liquids with various solvating properties. Usually, the three chosen solvents are: MTBE (proton acceptor), methylene chloride (strong dipole-dipole) and chloroform (proton donor). These three modifiers are added to carbon dioxide in such quantities that the separation time should be roughly the same in each case. The best selectivity is obtained with MTBE, *i.e.* with a proton acceptor solvent. With carbon dioxide-methylene chloride and carbon dioxide-chloroform mixtures, the chromatographic peaks are broad (Fig. 3b and c). From this point of view, the same results were obtained by Randall¹⁹ but no satisfying explanation can be found.

More recently, Snyder has developed a retention model in LSC¹⁸, taking into account solvent localization on a silica surface. In this way, the solvent strength for a polar solvent varies according to its content in the mobile phase. Non-polar or intermediate polar solvents are not localized and consequently their solvent strengths (ϵ_0 values) are constant, whatever their content in the apolar solvent.

With this modern theory, another set of solvents is selected: (i) a non-localizing solvent, *e.g.* chloroform or methylene chloride;

(ii) a strong dipolar localizing solvent, *e.g.* acetonitrile;

(iii) a basic localizing solvent, *e.g.* methanol or MTBE.

The selectivity triangle for such systems is limited by methanol, acetonitrile and methylene chloride. The phenol derivative separations with these three modifiers are shown in Fig. 3e. In the case of acetonitrile, very broad chromatographic peaks are obtained and the solutes can hardly be resolved. The best selectivity is obtained with methanol, which is, as MTBE, a proton acceptor solvent. It probably gives hydrogen bonds with phenol derivatives and, thus, higher solute-solvent interactions with greater selectivities in shorter analysis times are obtained.

From Snyder's theory, the solvent strength ϵ_{AB} of a binary mixture AB can be expressed as:

$$\epsilon_{AB} = \frac{\epsilon_A + \log(N_B 10^{\alpha'n(\epsilon_B - \epsilon_A)} + 1 - N_B)}{\alpha'n}$$

where ϵ_A and ϵ_B are the solvent strengths for pure solvents A and B; α' is the adsorbent activity parameter ($\alpha' = 1$ for "standard" activity); n is the cross-sectional area

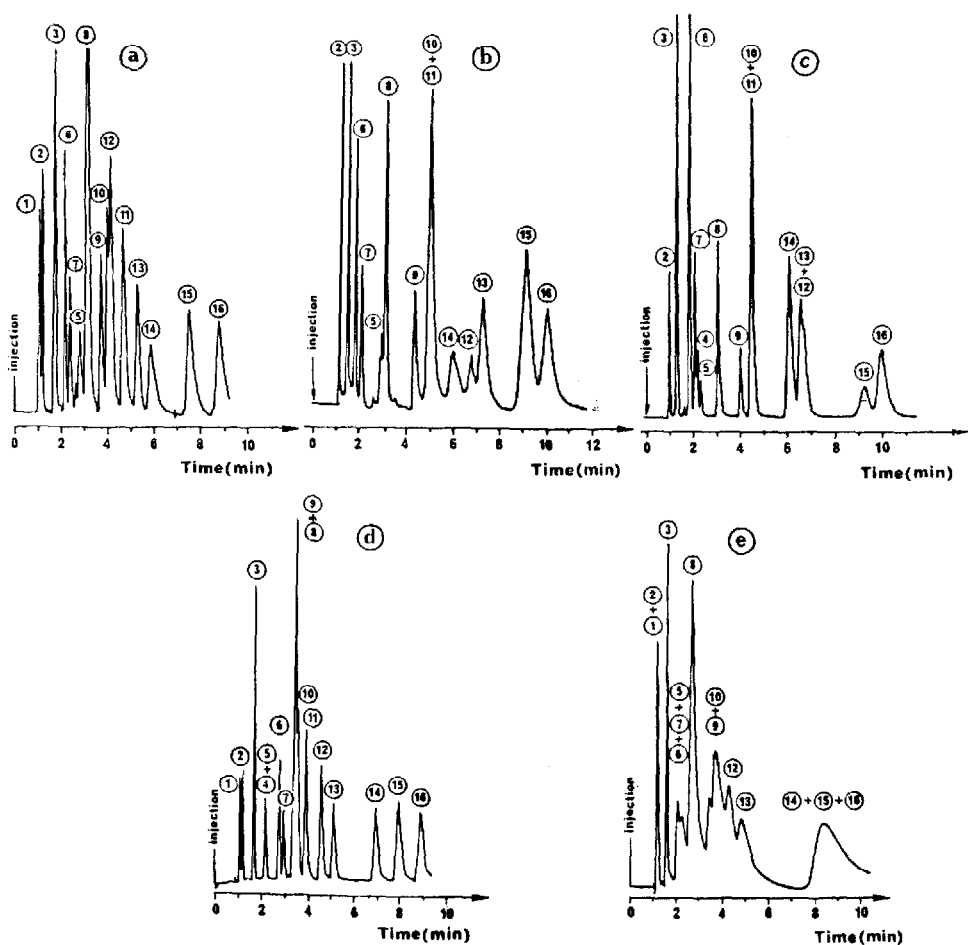


Fig. 3. Separation of phenol derivatives in supercritical carbon dioxide containing a polar modifier at various concentrations: (a) 8.0% (w/w) MTBE, (b) 33% (w/w) methylene chloride, (c) 39% (w/w) chloroform, (d) 1.1% (w/w) methanol, (e) 4.1% (w/w) acetonitrile. Column, 25×0.46 cm I.D.; detection, UV at 220 nm; stationary phase, $5 \mu\text{m}$ LiChrosorb Si 60 silica; average column pressure, 170 bar; temperature, 32°C ; flow-rate, 4 ml min^{-1} . Solutes: 1 = dichloromethane; 2 = 2,6-di-*tert.*-butyl-4-methyl phenol; 3 = 2-*tert.*-butyl-4,6-dimethyl phenol; 4 = 2,6-dimethyl phenol; 5 = 2,4,6-trimethyl phenol; 6 = 2-*tert.*-butyl-5-methyl phenol; 7 = 2-*tert.*-butyl-4-methyl phenol; 8 = 2-*sec.*-butyl phenol; 9 = 2,5-dimethyl phenol; 10 = 2,4-dimethyl phenol; 11 = 4-*tert.*-butyl-2-methyl phenol; 12 = phenol; 13 = 4-*sec.*-butyl phenol; 14 = α -naphthol; 15 = β -naphthol; 16 = 4-phenyl phenol.

(1 unit = 0.085 nm^2) for a molecule of solvent B; and N_B is the mole fraction of solvent component B in the mobile phase.

We can define the silica activity as "standard" ($\alpha' = 1$) and the carbon dioxide solvent strength ε_A is equal to zero. ε_B and n for pure solvents are given in the literature¹⁸. Thus, for each modifier mixture resolving the phenol derivatives in *ca.* 9 min at the same flow-rate, the solvent mixture strength value can be calculated:

carbon dioxide–MTBE	: (92:8, w/w); $N_B = 4.2 \cdot 10^{-2}$; $n = 4.1$; $\epsilon_B = 0.48$; $\epsilon_{AB} \approx 0.17$.
carbon dioxide–methylene chloride:	(67:33, w/w); $N_B = 2 \cdot 10^{-1}$; $n = 4.1$; $\epsilon_B = 0.32$; $\epsilon_{AB} \approx 0.17$.
carbon dioxide–chloroform	: (61:39, w/w); $N_B = 1.9 \cdot 10^{-1}$; $n = 5$; $\epsilon_B = 0.26$; $\epsilon_{AB} \approx 0.13$.
carbon dioxide–methanol	: (98.9:1.1, w/w); $N_B = 1.49 \cdot 10^{-2}$; $n = 3.7$; $\epsilon_B = 0.7$; $\epsilon_{AB} \approx 0.22$.
carbon dioxide–acetonitrile	: (95.9:4.1, w/w); $N_B = 4.2 \cdot 10^{-2}$; $n = 3.1$; $\epsilon_B = 0.52$; $\epsilon_{AB} \approx 0.15$.

All the mixtures (carbon dioxide–modifier) have about the same eluent strength, $\epsilon_{AB} \approx 0.18 \pm 0.05$. So, these modifiers are added in such quantities that their eluting power should be about the same, but their selectivities are different. It is thus possible in SFSC, as in LSC, to obtain important selectivity variations according to the nature of the modifier. The influence of methanol content on the retention of some phenol derivatives is shown in Fig. 4. First, modifier addition can involve great selectivity variations. Secondly, the lower the modifier concentration, the greater its effect on retention. This is a consequence of the interaction of methanol with the free silanol groups on the silica. This phenomenon is well known in LSC and, on this point, water must play an even more important role, as its adsorption energy on silica is greater than that of methanol. Water influence on carbon dioxide has not been studied yet as such small quantities have to be added, which are difficult to control.

Figs. 5–7 show various applications of SFC. The possibility of changing the mobile phase density and the modifier concentration causes large retention changes. In Fig. 5, the physical state of carbon dioxide is near the gas state ($\rho = 0.18 \text{ g/cm}^3$) and it is possible to resolve thirteen aromatic and olefinic hydrocarbons. On the contrary, Figs. 6 and 7 show, respectively, fast separations of polyaromatic hydrocarbons and of aniline derivatives in carbon dioxide mixed with a small quantity of methanol.

Partition chromatography

Reversed-phase chromatography is the most popular chromatographic technique. So, it was very interesting to use it with supercritical carbon dioxide. But, because of its apolar nature, carbon dioxide does not behave like the solvents generally used in reversed-phase liquid chromatography (RPLC) and, consequently, the retention mechanism is different. This difference is pointed out in Fig. 8, where the logarithm of the capacity factor of polar (phenol derivatives) and medium polar (polyaromatic hydrocarbons) solutes are plotted against methanol content.

In pure carbon dioxide, the elution order is: (1) naphthalene, (2) phenol, (3) chrysene, (4) resorcinol. In liquid chromatography with polar solvents (methanol, acetonitrile, water), the elution order, given by Rekker's hydrophobic constant Σf^{20} must be: (1) resorcinol ($\Sigma f = 1$), (2) phenol ($\Sigma f = 1.5$), (3) naphthalene ($\Sigma f = 3$), (4)

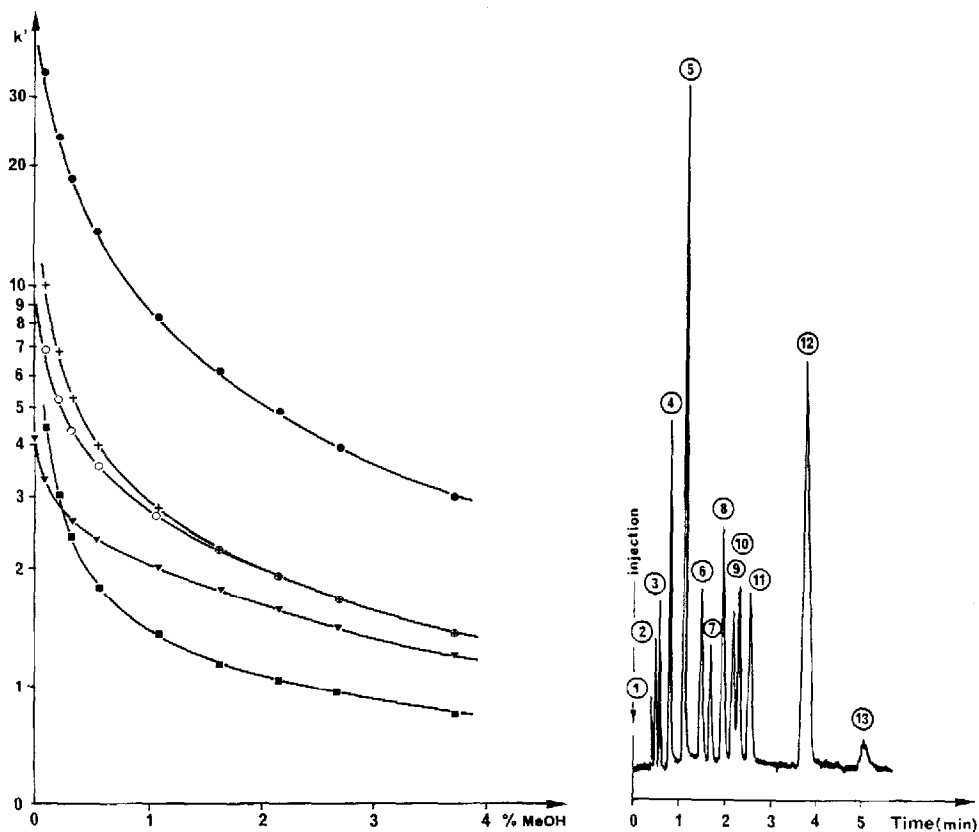


Fig. 4. Logarithm of capacity factor of phenol derivatives vs. methanol (MeOH) percentage (w/w in carbon dioxide). Other operating conditions as in Fig. 3. Solutes: (■) 2,4,6-trimethyl phenol, (▼) 2-*tert.*-butyl-5-methyl phenol, (○) 2,5-dimethyl phenol, (+) 2,4-dimethyl phenol, (●) 4-phenyl phenol.

Fig. 5. Separation of olefinic and aromatic hydrocarbons by carbon dioxide SFSC. Column and stationary phase as in Fig. 1. Inlet pressure, 128 bar; outlet pressure, 70 bar; temperature, 91°C. Detection, UV at 212 nm; flow-rate, 6 ml min⁻¹. Solutes: 1 = 2-methyl-2-butene; 2 = *n*-hexene; 3 = cyclohexene; 4 = benzene; 5 = toluene; 6 = *o*-xylene; 7 = *m*-xylene; 8 = 1,2,3-trimethylbenzene; 9 = 2-ethyltoluene; 10 = *tert.*-butylbenzene; 11 = 1,3,5-trimethylbenzene; 12 = 1,3,5-triethylbenzene; 13 = *p*-di-*tert.*-butylbenzene.

chrysene ($\Sigma f = 5$). So, with carbon dioxide, the retention behaviour is different from that obtained in liquid chromatography where solute retention increases with its hydrophobic factor: it can be compared paradoxically with retention on a polar stationary phase, although some medium polar compounds such as polyaromatic hydrocarbons are retained to a greater extent ($k'_{\text{chrysene}} > k'_{\text{phenol}}$).

Moreover, the addition of a polar modifier (methanol) to carbon dioxide entails a decrease in solute retention, especially with polar derivatives.

The same retention behaviour has already been observed in liquid chromatography by Wu and Deming²¹: they observed that the retention of some polar aromatic solutes on a C₁₈ column decreases as a polar modifier (alcohol) is added to hexane. Thus, when carbon dioxide or hexane, both apolar solvents, are used as the

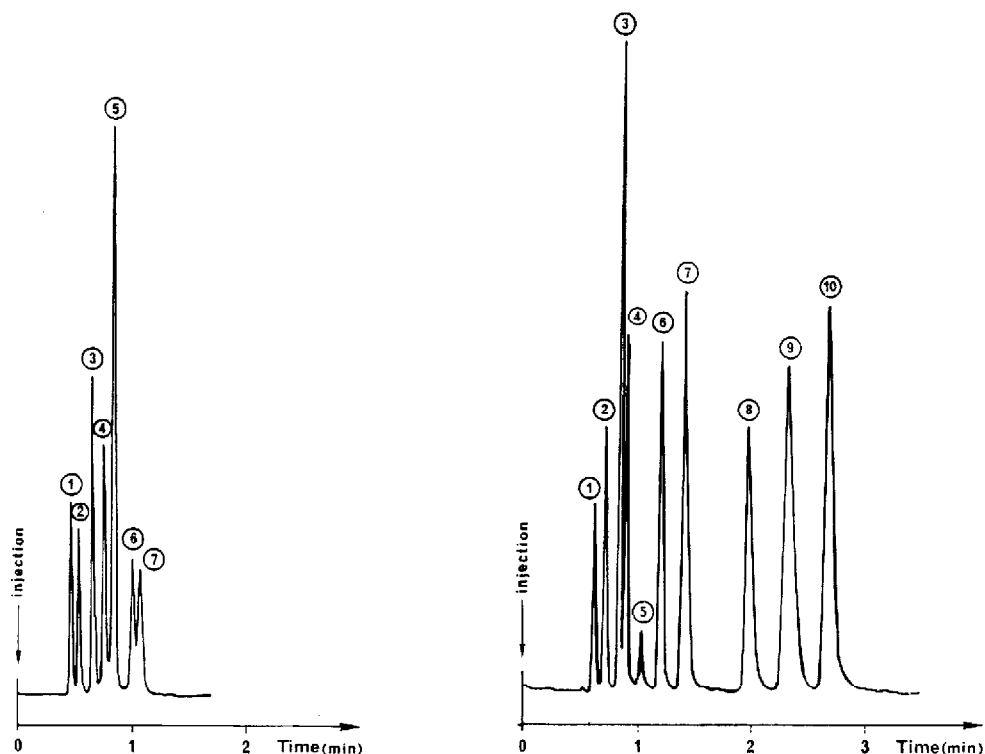


Fig. 6. Separation of aromatic hydrocarbons by SFSC. Mobile phase, carbon dioxide-methanol (98:2, w/w); column and stationary phase as in Fig. 1; inlet pressure, 200 bar; outlet pressure, 130 bar; temperature, 36°C. Detection, UV at 220 nm; total flow-rate, 9.2 ml min⁻¹. Solutes: 1 = benzene; 2 = naphthalene; 3 = phenanthrene; 4 = pyrene; 5 = chrysene; 6 = benzo(a)pyrene; 7 = perylene.

Fig. 7. Separation of aniline derivatives by SFSC. Mobile phase: carbon dioxide-methanol (97.7:2.3, w/w). Column and stationary phase as in Fig. 1. Inlet pressure, 230 bar; outlet pressure, 165 bar; temperature, 36°C; detection, UV at 254 nm; total flow-rate, 9.3 ml min. Solutes: 1 = dimethylaniline; 2 = N-ethyl-aniline; 3 = N-methyl-aniline; 4 = N-benzyl-methyl-aniline; 5 = unidentified impurity; 6 = *o*-toluidine; 7 = aniline; 8 = *p*-chloroaniline; 9 = β -naphthylamine; 10 = *m*-nitroaniline.

mobile phase on reversed-phase columns, an effect called non-polar non-polar chromatography appears.

Three explanations may be proposed. First, the polar modifier may react as a surfactant and, consequently, decrease the interfacial tension between the mobile and the stationary phase. Secondly, there may be an influence of the residual silanol groups remaining on the stationary phase surface. There would be competition between solute and mobile phase modifier for these active sites. Finally, there may be an association of modifier and solute in the mobile phase. The mathematical models accounting for these three explanations lead to identical retention behaviours, and it is not known which of them really dominates²¹.

Chiral separation

The chiral stationary phase obtained by covalent bonding of (*R*)-N-(3,5-dinitrobenzoyl)phenylglycine to a bonded aminopropyl silica gel allows successful en-

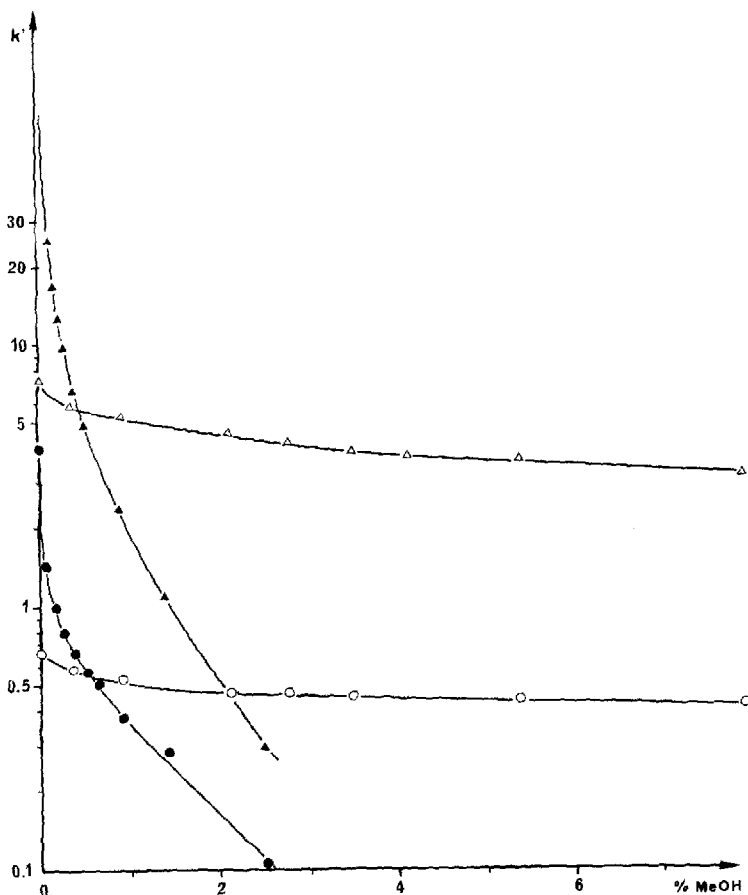


Fig. 8. Logarithm of capacity factor of various solutes vs. methanol (MeOH) percentage in carbon dioxide. Column, 15×0.46 cm I.D.; detection, UV at 254 nm; stationary phase, $7-8 \mu\text{m}$ Zorbax octadecyl-bonded silica; average column pressure, 240 bar; temperature, 40°C ; total flow-rate, 3 ml min^{-1} . Solute: (●) phenol; (▲) resorcinol; (○) naphthalene; (△) chrysene.

antiomeric resolution for various compounds in liquid chromatography. In most of these separations, the mobile phase is a binary mixture: an apolar solvent such as hexane, with a polar modifier such as 2-propanol usually added in amounts of $< 25\%$ (v/v). But, as the analysis time is often long (between 10 min and several hours), it might be very favourable to substitute sub- or supercritical carbon dioxide²² for hexane²⁻⁵.

As an example of enantiomeric separation, we chose phosphine oxides, which are chiral precursors for chiral phosphine ligands used in the synthesis of Wilkinson-type catalysts for the homogeneous-phase catalytic hydrogenation of prochiral substrates.

Here, as in any other SFC separation, some parameters must be optimized: those that determine the physical state of carbon dioxide (*i.e.*, temperature and density) and those that determine the composition of the mobile phase (*i.e.* the nature and concentration of modifier in carbon dioxide).

Fig. 9a and b shows the influence of modifier content on solute retention and on selectivity, respectively, for three different pure alcohols and alcohol-water mixtures dissolved in carbon dioxide. The polarities of these modifiers, according to Rohrschneider^{16,23}, are: water (10.2) > methanol (5.1) > ethanol (4.3) > 2-propanol (3.9). The modifier molecule competes with the solute on the specific sites of the stationary phase. Therefore, the higher the modifier concentration, the lower the retention. In addition, the less polar the alcohol, the greater the capacity factors and the selectivities.

These results are in good agreement with those published by Zief *et al.*²⁴ for classical liquid chromatography. A small addition of water (5% in the modifier, *i.e.* 0.2–0.5% in the mobile phase) greatly decreases solute retention (Fig. 9a). As in adsorption chromatography, this effect is much more significant when the modifier is less polar (*i.e.* when the modifier polarity is much lower than that of water²⁵). The fixation of the modifier, and especially water, on the residual silanol groups decreases

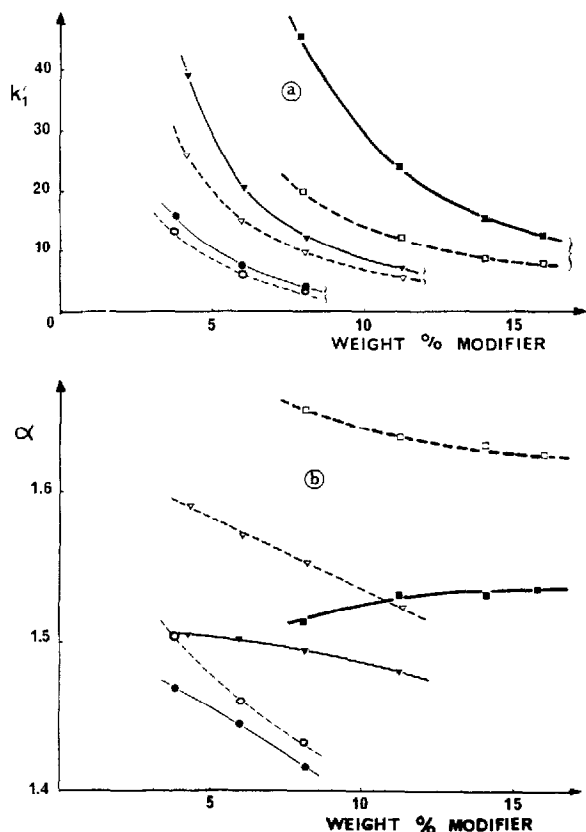


Fig. 9. Influence of modifier concentration on the retention of the first eluted enantiomer of the methyl-1-(4-methyl-naphthyl) phenyl phosphine oxide (a) and on the selectivity (b). Average column pressure, 260 bar; temperature, 20°C; column, 100 × 4.6 mm I.D.; detection, UV at 280 nm; stationary phase, 10 μ m aminopropyl LiChrosorb NH₂ silica-bonded with (*R*)-N-(3,5-dinitrobenzoyl)phenylglycine; carbon dioxide flow-rate, 3.5 ml min⁻¹. Modifier: (●) methanol, (○) methanol-water, (▼) ethanol, (▽) ethanol-water, (■) 2-propanol, (□) 2-propanol-water. Full line, pure alcohol; dashed line, alcohol-water (95:5, v/v).

their adsorption energy^{13,26} and, consequently, the observed retention time. It also results in an enhancement of the chiral recognition of the stationary phase, due to a minimization of the non-specific polar adsorption and, therefore, higher selectivity α (Fig. 9b).

Fig. 10 shows the variation of $\log \alpha$ vs. reciprocal of the temperature (K^{-1}). At a given temperature, the selectivity values remain constant (within 1%) when the carbon dioxide pressure varies between 60 and 270 bar. As, at a given temperature, pressure and density are linked, the influence of carbon dioxide density on the selectivity is weak with regard to that of temperature and modifier nature. As illustrated in Fig. 10, these last two parameters can induce great variations of selectivity: from a thermodynamic point of view, the lower the temperature, the higher the selectivity.

However, resolution takes into account both kinetic and thermodynamic parameters. At high density, efficiency increases with temperature, following the increase in solute diffusion coefficient. As selectivity decreases with temperature, a compromise must be chosen to optimize the resolution, and the optimum chromatographic conditions are found in the subcritical range at a density of *ca.* 1 g/cm³. The variations of the resolution with the nature of the mobile phase are shown in Fig. 11. Generally, addition of water in modifier increases both selectivity and efficiency and, therefore, the same resolution may be obtained in a shorter separation time. The improvement provided by water is more important if the alcohol is less polar; *i.e.* an addition of 5% (v/v) water in 2-propanol gives an efficiency increase of *ca.* 40%. The same addition of water in methanol gives only a 5% increase in efficiency.

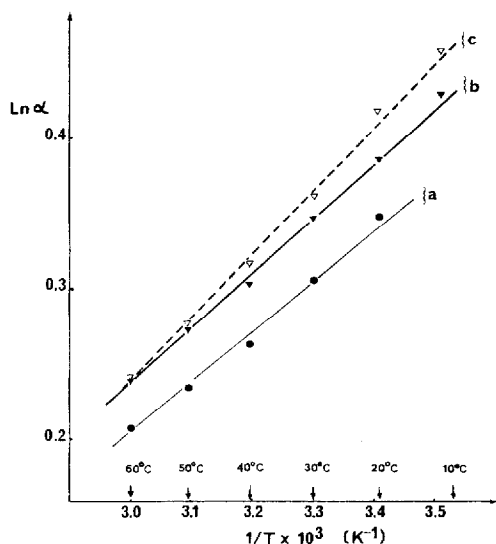


Fig. 10. Effect of temperature on the selectivity between the enantiomers of the methyl-1-(4-methylnaphthyl)phenyl phosphine oxide for various modifiers. Column and stationary phase as in Fig. 9. Mobile phase: (a) carbon dioxide–methanol (91.8:8.2, w/w), (b) carbon dioxide–ethanol (91.9:8.1, w/w), (c) carbon dioxide–ethanol–water (91.9:7.69:0.51, w/w). Flow-rates: (a) carbon dioxide 3.5 ml min⁻¹, methanol 0.35 ml min⁻¹; (b) carbon dioxide 3.5 ml min⁻¹; ethanol 0.35 ml min⁻¹; (c) carbon dioxide 3.5 ml min⁻¹; ethanol–water (95:5, v/v) 0.35 ml min⁻¹. Each selectivity point is a mean of the selectivities at various pressures (60–270 bar).

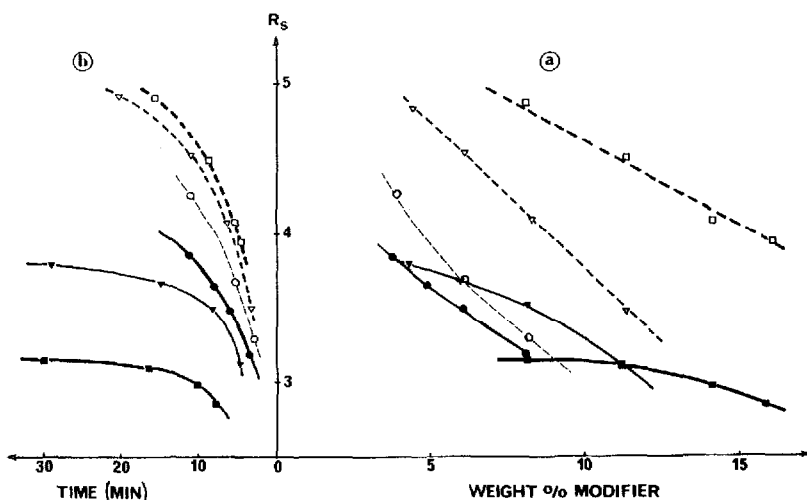


Fig. 11. Variation of resolution R_s between the enantiomers of the methyl-1-(4-methylnaphthyl)phenyl phosphine oxide with (a) concentration of modifier and with (b) separation time. Operating conditions and symbols as in Fig. 9.

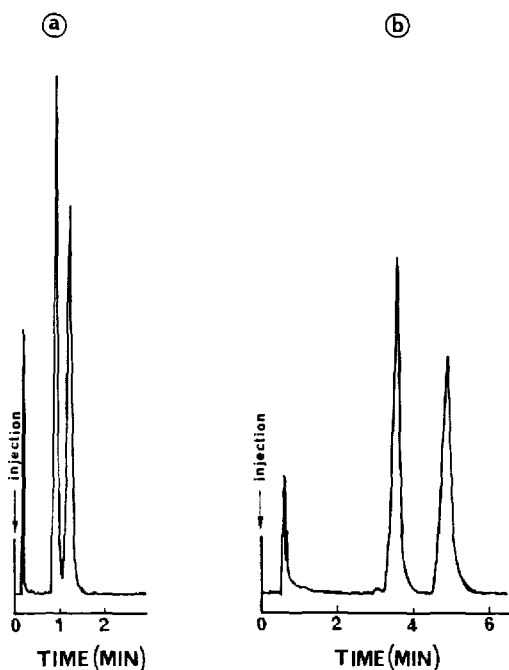


Fig. 12. Chiral separation of methyl-1-(4-methylnaphthyl)phenyl phosphine oxide on Pirkle's phase with (a) high speed or with (b) high resolution. Column and stationary phase as in Fig. 9. (a) Mobile phase, carbon dioxide-methanol-water (91.8:7.69:0.51, w/w); flow-rates: carbon dioxide 10 ml min^{-1} , methanol-water (95:5, v/v) 1.0 ml min^{-1} ; inlet pressure, 275 bar; outlet pressure, 230 bar; temperature, 20°C ; UV detection at 280 nm. (b) Mobile phase, carbon dioxide-methanol-water (94:5.65:0.35, w/w); flow-rates: carbon dioxide 3.5 ml min^{-1} , methanol-water (95:5, v/v) 0.25 ml min^{-1} ; inlet pressure, 270 bar; outlet pressure, 255 bar; temperature, 23°C .

Thus, with 2-propanol, the addition of water leads to an increase in resolution of *ca.* 50%, while the separation time is divided by 2. This gain is clearly illustrated in Fig. 11b.

Fig. 12 shows the SFC enantiomeric separation of methyl-1-(4-methylnaphthyl) phenylphosphine oxide within a very short analysis time (90 s; Fig. 12a) and with a high resolution (≈ 3.5 ; Fig. 12b).

In the case of chiral separations, as in that of adsorption and partition chromatography, carbon dioxide acts like hexane, and for retention, mechanisms in liquid chromatography and SFC are identical.

In conclusion, we have demonstrated that water has a very important role as modifier in enantiomeric separation, probably owing to the presence of some residual silanol groups in the chiral stationary phase.

CONCLUSIONS

The overall results demonstrate that, with carbon dioxide SFC, it is in part possible to combine the advantages of liquid chromatography (variations of selectivity with modifier addition) and those of gas chromatography (high efficiency per unit time).

Moreover, with the three stationary phases studied, the retention mechanism in supercritical carbon dioxide seems to be very similar to that observed in an apolar solvent such as hexane. Consequently, the influence of water in SFC should be, as in liquid-solid chromatography, very important and has still to be studied accurately.

Enantiomeric separations on a chiral-bonded phase constitute an interesting application of SFC. By the choice of proper conditions and suitable modifiers, separation performances are improved in comparison with liquid chromatography, both from a thermodynamic and a kinetic point of view.

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REFERENCES

- 1 P. Mourier, M. Caude and R. Rosset, *Analisis*, 13 (1985) 299.
- 2 W. H. Pirkle, D. W. House and J. M. Finn, *J. Chromatogr.*, 192 (1980) 143.
- 3 W. H. Pirkle, J. M. Finn, B. C. Hamper, J. Schreiner, J. R. Pribish, in E. L. Eliel and S. Otsuka (Editors), *ACS Symp. Ser.*, 185 (1982) 245.
- 4 W. H. Pirkle, M. H. Hyun and B. Bank, *J. Chromatogr.*, 316 (1984) 585.
- 5 P. Pescher, A. Tambute, L. Oliveros, M. Caude and R. Rosset, *Nouv. J. Chim.*, 9 (1985) 621.
- 6 H. H. Lauer, D. McManigill and R. Board, *Anal. Chem.*, 55 (1983) 1370.
- 7 L. G. Randall, *Sep. Sci. Technol.*, 17 (1982) 1.
- 8 P. A. Peadar and M. L. Lee, *J. Liq. Chromatogr.*, 5 (2) (1982) 417.
- 9 R. T. Kurnik, S. J. Holla and R. C. Reid, *J. Chem. Eng. Data*, 26 (1981) 47.
- 10 M. McHugh and M. E. Paulaitis, *J. Chem. Eng. Data*, 25 (1980) 326.
- 11 G. T. Hong, M. Modell and J. W. Tester, in M. E. Paulaitis, J. M. L. Penninger, R. D. Gray, Jr. and P. Davidson (Editors), *Chemical Engineering at Supercritical Fluid Conditions*, Ann Arbor, MI, 1983, p. 263.
- 12 C. Souteyrand, M. Thibert, M. Caude and R. Rosset, *J. Chromatogr.*, 262 (1983) 1.

- 13 C. Souteyrand, M. Thibert, M. Caude and R. Rosset, *J. Chromatogr.*, 316 (1984) 373.
- 14 R. Wiebe and V. L. Gaddy, *J. Am. Chem. Soc.*, 63 (1941) 475.
- 15 C. R. Coan and A. D. King, Jr., *J. Am. Chem. Soc.*, 93 (1971) 1857.
- 16 L. R. Snyder, *J. Chromatogr. Sci.*, 16 (1978) 223.
- 17 L. R. Snyder, *J. Chromatogr.*, 92 (1974) 223.
- 18 L. R. Snyder, *High Performance Liquid Chromatography*, Vol 3, Academic Press, New York, 1983, p. 157.
- 19 L. C. Randall, *ACS Symp. Ser.*, 250 (1984) 135.
- 20 R. F. Rekker, *The Hydrophobic Fragmental Constant*, Elsevier, Amsterdam, 1977.
- 21 C. G. Wu and S. N. Deming, *J. Chromatogr.*, 302 (1984) 79.
- 22 P. Mourier, E. Eliot, M. Caude, R. Rossett and A. Tambute, *Anal. Chem.*, 59 (1985) in press.
- 23 L. Rohrschneider, *Anal. Chem.*, 45 (1973) 1241.
- 24 M. Zief, L. J. Crane and J. Horvath, *J. Liq. Chromatogr.*, 7 (1984) 709.
- 25 J.-P. Thomas, A. Brun and J.-P. Bounine, *J. Chromatogr.*, 172 (1979) 107.
- 26 J. H. Knox, *J. Chromatogr. Sci.*, 15 (1977) 352.