

Packed column supercritical fluid chromatography with carbon dioxide–polar modifiers

Influence of carbon dioxide density on retention

A. VILLERMET, D. THIÉBAUT*, M. CAUDE and R. ROSSET

Laboratoire de Chimie Analytique de l'ESPCI, 10 Rue Vauquelin, 75231 Paris Cedex 05 (France)

ABSTRACT

The influence of the CO₂ density in mixed mobile phases containing methanol, acetonitrile or carbon tetrachloride was investigated by packed column supercritical fluid chromatography. With bare silica and polystyrene–divinylbenzene (PRP1), the retention of model solutes was found to decrease with increase in the CO₂ density by the same order of magnitude as with neat CO₂ on bare silica. This is thought to be the result of variation in the solvation of the solutes in the mobile phase whereas the solvation of the stationary phase is governed by the amount of modifier in the mobile phase. The same relationship as with pure CO₂ was also found between retention and temperature on bare silica but the maximum retention depended on the boiling point of the solute. Liquid and gas chromatographic like behaviour occurred with silica and PRP1, respectively. Examples of density gradients for improving the separation time and selectivity are given.

INTRODUCTION

Since its revival in the 1980s, supercritical fluid chromatography (SFC) has been extensively studied and much progress has been made concerning the understanding of fundamentals and apparatus has been developed. However, only a few routine industrial applications have been published, despite the advantages of SFC over liquid chromatography (LC) (detection, faster separations) and gas chromatography (GC) (thermolabile compounds), because both LC and GC have reached a high level of sophistication.

With packed columns, SFC has been found to compete with LC for the separation of solutes in a wide polarity range by adding polar modifiers to the supercritical fluid. For example, carbohydrates [1], alkaloids [2], enantiomers [3] and polymers [4] have been successfully separated, generally under isocratic conditions. In contrast, with capillary columns, separations are often carried out with a CO₂ density gradient, because when GC detectors are used, polar modifiers are proscribed.

From the experience gained in our laboratory, we are convinced that SFC is easier to perform with conventional packed columns than capillary columns, essen-

tially because more reliable devices exist for pressure control and LC injection valves can be used without modification. Unfortunately, the potential of SFC with packed columns will remain unrealized if one cannot take advantage of density programming in combination with complex mobile phases [5].

This paper demonstrates the usefulness of density gradients with carbon dioxide-polar modifier mixtures on packed columns. For complex mixtures, density and polar modifier gradients can be combined to enhance the selectivity.

EXPERIMENTAL

Apparatus

The SFC instrument consisted of a liquid chromatograph modified and commercialized for packed column SFC (Model 2500 M; Varian, Les Ulis, France). It consisted of two piston pumps (Model 2510), one with its head cooled to 0°C to deliver CO₂ and the other delivering modifiers without modification, a mixing chamber, a Valco injection valve with a 10- μ l loop, a column oven, UV detector (Model 2550) and a Tescom back-pressure regulator thermoregulated at 30°C in order to avoid freezing during the expansion of the mobile phase. The pressure regulator was modified in the laboratory in order to apply pressure gradients. The knob of the Tescom valve was monitored by an electric motor taken from a Varian Model 4100 liquid chromatograph. The gradient controller of the Model 4100 chromatograph allowed monitoring of the rotation speed of the valve.

Columns

Two types of stationary phase were used: bare silica either LiChrosorb Si 60, 5 μ m (15 \times 0.46 cm I.D.) or Pecosphere 5C SI, 5 μ m (15 \times 0.41 cm I.D.) (Perkin-Elmer), and Polystyrene-divinylbenzene, PRP1, 10 μ m (25 \times 0.41 cm I.D.) (Touzart et Matignon, Vitry-sur-Seine, France). PRP1 was preferred over ODS silica because the retention mechanism has not been clearly elucidated on ODS silica and it has been shown that strong interactions occur between residual silanols and polar solutes. On PRP1, there is no silanol group and apolar partition and according to the solute structure, π - π interactions are responsible for the retention.

Mobile phases

Industrial carbon dioxide was purchased from L'Air Liquide (Paris, France). An increase in CO₂ density was obtained by closing the back-pressure regulator while the CO₂ was delivered at constant flow-rate by the pump. This means that the volumetric CO₂ flow-rate and the linear velocity of the mobile phase decreased slightly as the density increased.

Methanol (Prolabo, Paris, France), acetonitrile (Rathburn, obtained from Chromoptic, Montpellier, France) and carbon tetrachloride (Rathburn, obtained from Chromoptic) were used as modifiers in the mobile phase and were of HPLC grade.

It has been stated that one mechanism by which a polar modifier could influence retention is the density variation due to the amount added to the CO₂ [6]. In order to express the density of the mobile phase simply, in this paper only the CO₂ density will be considered because the percentage of polar modifiers in the mobile phase remained constant during the experiments.

Test solutes

Diphenyl phthalate and substituted polynuclear aromatic hydrocarbons (PAHs) were used as test solutes (Table I).

RESULTS AND DISCUSSION

Influence of density on retention

Bare silica. Fig. 1a and b shows the variation of the test solute capacity factors as a function of the CO₂ density for CO₂-methanol (94.4:5.6, mol/mol) and CO₂-acetonitrile (93.9:6.1, mol/mol) mixtures, respectively. A strong dependence of density on the retention is observed. Fig. 2a and b shows that $\log k'$ is a linear function of the density within the range investigated, as already reported for pure CO₂ [7-10]. Comparison of the present data with those reported by Mourier *et al.* [8] demonstrates that the retention decreases with increase in density by the same order of magnitude on bare silica with or without modifiers in the mobile phase. The k' value can be divided by a factor of *ca.* 2 by increasing the density from 0.56 to 0.77 g/cm³.

These important results emphasize that the CO₂ density is a major parameter affecting retention in SFC with packed columns both with pure CO₂ and with CO₂-polar modifier mixtures. Hence, the influence of density, often neglected with packed columns, must be taken into account for monitoring solute retentions.

Polystyrene-divinylbenzene (PRPI). The variation of k' as a function of the CO₂ density in the mobile phase is shown with methanol (Fig. 3a), acetonitrile (Fig. 3b) and carbon tetrachloride (Fig. 3c) as modifiers.

Again, the solute retentions are strongly dependent on density and the higher the k' the greater is the slope of the line. This effect is much more important for diphenylphthalate, probably owing to the high density of π electrons in this solute. The extent of the variation of k' is of the same order of magnitude as with bare silica with methanol or acetonitrile as modifier. This suggests that the density of CO₂ governs the solute retention by modification of the solubility of the solute in the mobile phase [11]. In contrast, the stationary phase solvation would mostly depend on the modifier used with the CO₂. With carbon tetrachloride as modifier, the effect of CO₂ density is less pronounced.

TABLE I
TEST SOLUTES

Solute	p <i>K</i> _a	Boiling point (°C)
α -Naphthol	9.3	288
β -Naphthol	9.5	289
α -Naphthylamine	10.1	301
α -Naphthaldehyde		291
Benzoic acid	4.2	249
Indeno[1,2,3- <i>cd</i>]pyrene	—	—
Diphenylphthalate	—	—
Dodecylbenzene	—	331
Nonadecylbenzene	—	—

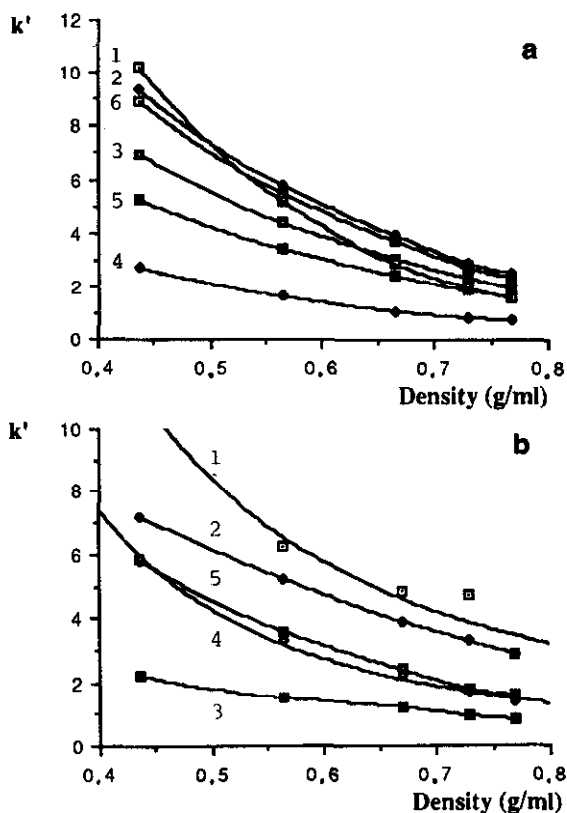


Fig. 1. Variation of solute capacity factors measured on bare silica with the CO₂ density in the mobile phase. Column, 150 × 4.6 mm I.D.; Stationary phase, 5- μ m LiChrosorb Si 60; mobile phase, (a) CO₂-methanol (94.4:5.6, mol/mol) and (b) CO₂-acetonitrile (93.9:6.1, mol/mol); flow-rate (0°C), 5 ml/min; detection, UV at 254 nm. Solutes: (a) 1 = indeno[1,2,3-*cd*]pyrene/2 = α -naphthol; 3 = α -naphthylamine; 4 = α -naphthaldehyde; 5 = benzoic acid; 6 = β -naphthol; (b) 1 = β -naphthol; 2 = α -naphthylamine; 3 = α -naphthaldehyde; 4 = indeno[1,2,3-*cd*]pyrene; 5 = diphenyl phthalate.

In the investigated range of density, the retention on PRP1 can be expressed by

$$k' = a - b\rho \quad (1)$$

where a and b are constants. For the solutes studied, except diphenyl phthalate, a and b were found to be similar; consequently, for these solutes eqn. 1 becomes

$$k' = a(1 - \rho) \quad (2)$$

This means that the selectivity should remain constant with variation in density (except when diphenyl phthalate is considered).

One must point out that the retentions were higher on PRP1 than silica because the test solutes contained aromatic rings, which strongly interact with those of the stationary phase to give π - π complexes.

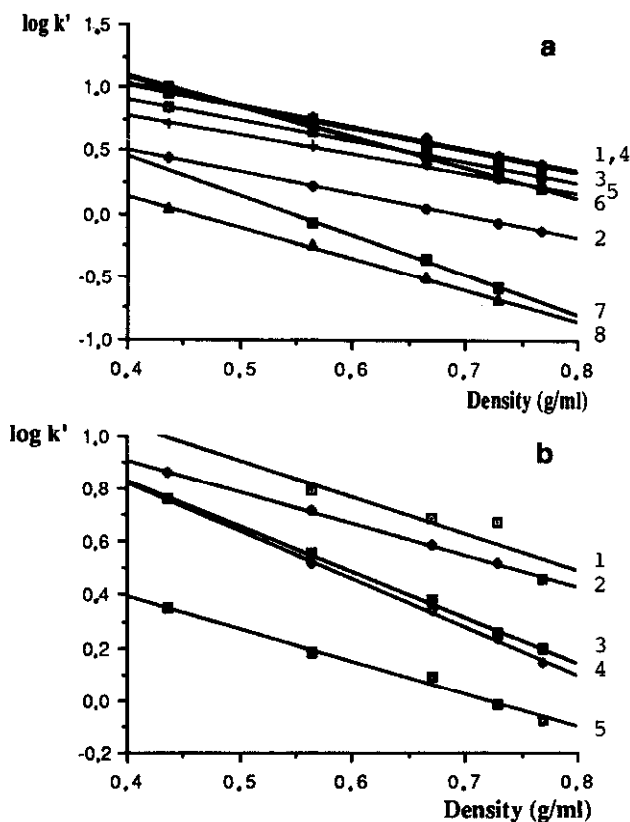


Fig. 2. Variation of logarithm of solute capacity factors on bare silica with the CO₂ density in the mobile phase. Conditions as in Figure 1. Solutes: (a) 1 = α -naphthol; 2 = α -naphthaldehyde; 3 = α -naphthylamine; 4 = β -naphthol; 5 = benzoic acid; 6 = indeno [1,2,3-*cd*]pyrene; 7 = nonadecylbenzene; 8 = dodecylbenzene; (b) 1 = β -naphthol; 2 = α -naphthylamine; 3 = α -naphthaldehyde; 4 = indeno[1,2,3-*cd*]pyrene; 5 = diphenyl phthalate.

Influence of density on selectivity

Bare silica. The plot of selectivity (α) versus CO₂ density in CO₂-methanol and CO₂-acetonitrile mobile phases is shown in Fig. 4. Straight lines are obtained but their slope is positive, negative or zero, meaning that the selectivity can increase, decrease or remain constant when the density increases. When α becomes lower than 1, the elution order of the solutes is reversed. These results are in good agreement with the behavior observed with neat CO₂ and Janicot's data [12] showing that the variation in selectivity occurred only for non-homologous families of compounds when the CO₂ density was increased.

Polystyrene-divinylbenzene. As one can deduce from the retention data shown in Fig. 3, on PRP1 the selectivity calculated for adjacent peaks varies with CO₂ density only when diphenyl phthalate is considered because its retention is strongly dependent on density. Otherwise, α can be considered as a constant for a given mobile phase (Fig. 5).

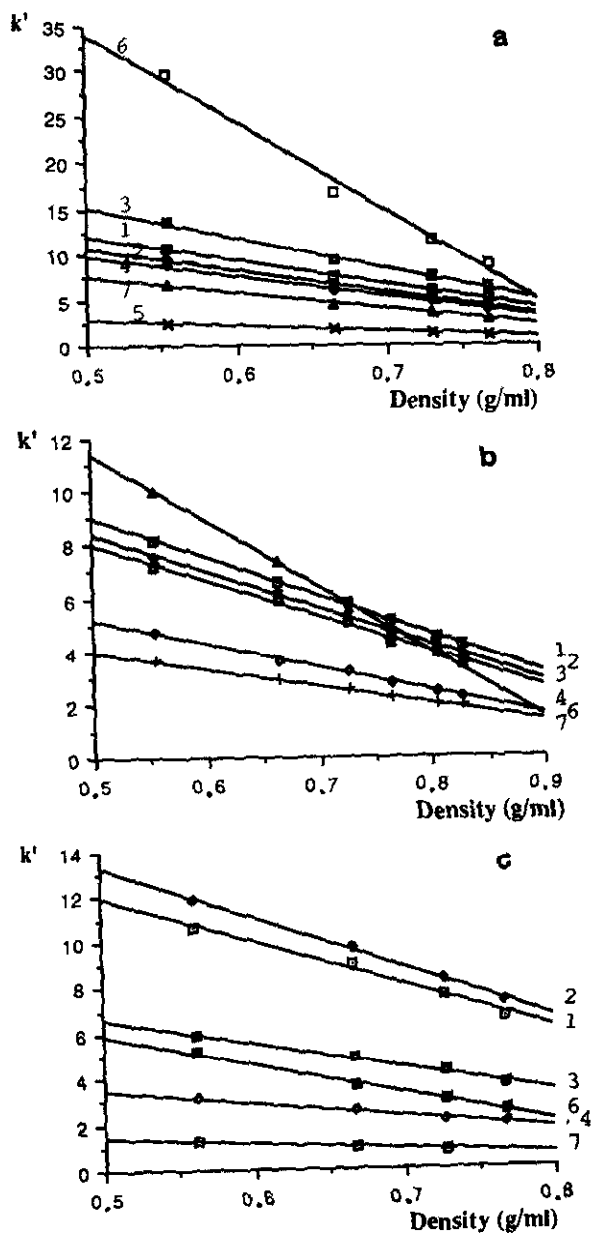


Fig. 3. Variation of solute capacity factors on PRPI as a function of CO_2 density in the mobile phase. Column, 250×4.1 mm I.D.; stationary phase, $10\text{-}\mu\text{m}$; mobile phase, (a) CO_2 -methanol (94.4:5.6, mol/mol), (b) CO_2 -acetonitrile (93.9:6.1, mol/mol) and (c) CO_2 -carbon tetrachloride (94.4:5.6, mol/mol); Flow-rate (0°C), 5 ml/min; detection, UV at 254 nm. Solutes: 1 = α -naphthol; 2 = β -naphthol; 3 = α -naphthylamine; 4 = α -naphthaldehyde; 5 = benzoic acid; 6 = diphenyl phthalate; 7 = dodecylbenzene.

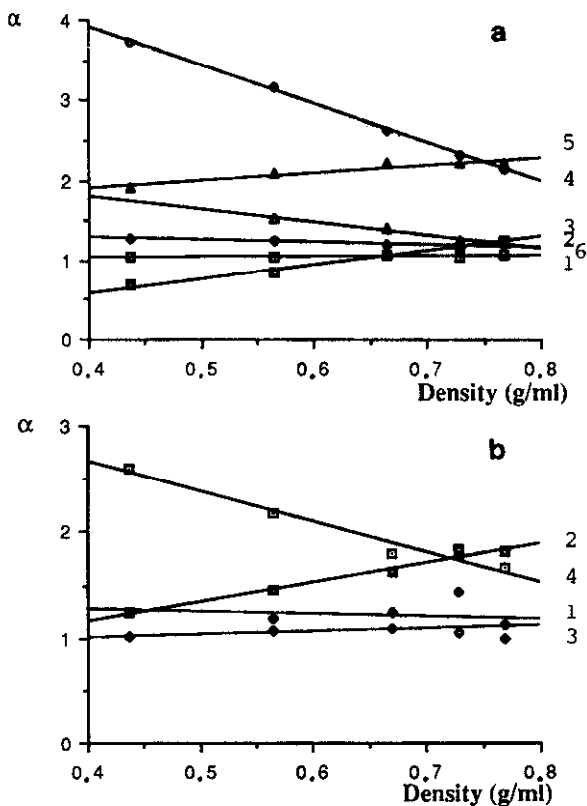


Fig. 4. Variation of solute selectivity on bare silica as a function of CO₂ density in the mobile phase. Conditions as in Figure 1. Solutes: (a) 1 = β -naphthol- α -naphthol; 2 = α -naphthol- α -naphthylamine; 3 = α -naphthylamine-indeno[1,2,3-*cd*]pyrene; 4 = indeno[1,2,3-*cd*]pyrene- α -naphthaldehyde; 5 = benzoic acid- α -naphthaldehyde; 6 = nonadecylbenzene-dodecylbenzene; (b) 1 = β -naphthol- α -naphthylamine; 2 = α -naphthylamine-diphenylphthalate; 3 = diphenylphthalate-indeno[1,2,3-*cd*]pyrene; 4 = indeno[1,2,3-*cd*]pyrene-dodecylbenzene.

Consequently, eqn. 2 cannot be valid for diphenyl phthalate. For the other solutes α can be expressed as follows:

$$\alpha = \frac{a(1 - \rho)}{a'(1 - \rho)} \quad (3)$$

meaning that α is a constant for pairs of solutes whose retention *versus* density can be expressed by eqn. 2.

Even when α does not vary with changes in CO₂ density, the use of CO₂ density programming can be very useful for decreasing the duration of analysis without a significant decrease in resolution (except the influence of k' on the resolution).

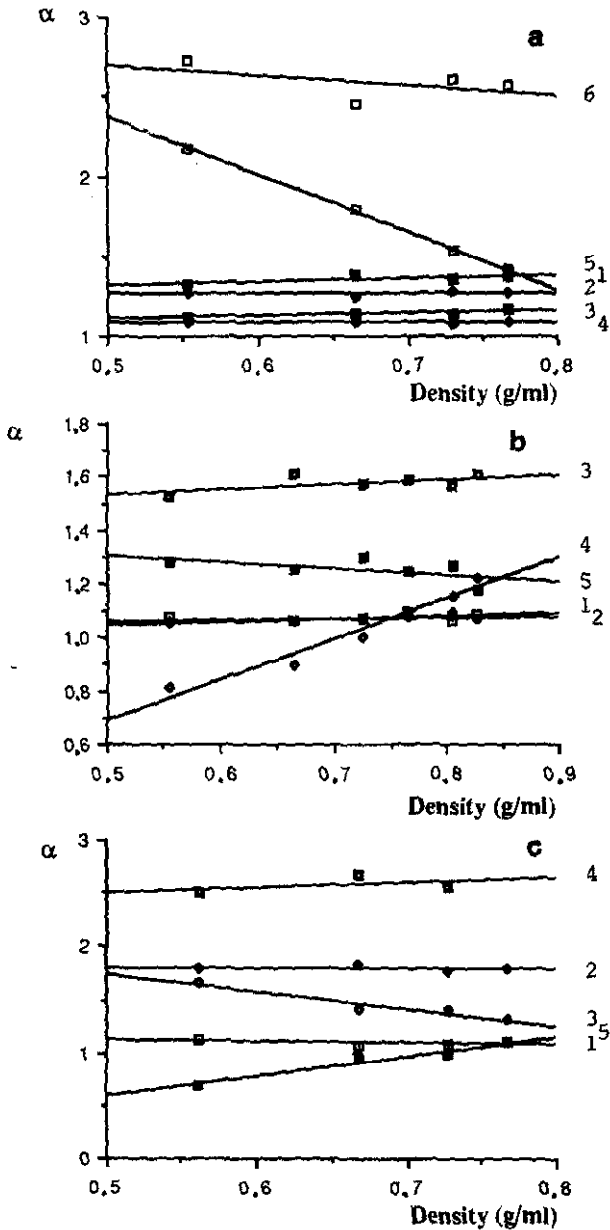


Fig. 5. Variation of solute selectivity on PRP1 as a function of CO_2 density in the mobile phase. Conditions as in Figure 2. Solutes: (a) 1 = diphenylphthalate- α -naphthylamine; 2 = α -naphthylamine- α -naphthol; 3 = α -naphthol- β -naphthol; 4 = β -naphthol-naphthaldehyde; 5 = α -naphthaldehyde-dodecylbenzene; 6 = dodecylbenzene-benzoic acid; (b) 1 = α -naphthol- β -naphthol; 2 = β -naphthol- α -naphthylamine; 3 = α -naphthylamine-naphthaldehyde; 4 = α -naphthol-diphenyl phthalate; 5 = α -naphthaldehyde-dodecylbenzene; (c) 1 = β -naphthol- α -naphthol; 2 = α -naphthol- α -naphthylamine; 3 = α -naphthylamine-naphthaldehyde; 4 = α -naphthaldehyde-dodecylbenzene; 5 = α -naphthylamine-diphenyl phthalate.

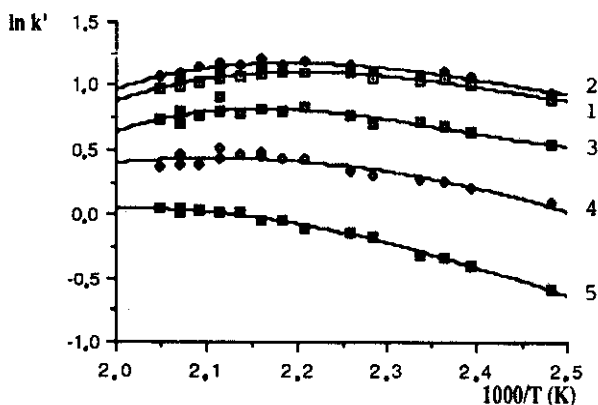


Fig. 6. Variation of logarithm of solute capacity factors on bare silica with reciprocal of mobile phase temperature. Conditions as in Fig. 1a. Solutes: 1 = α -naphthol; 2 = β -naphthol; 3 = α -naphthylamine; 4 = α -naphthaldehyde; 5 = dodecylbenzene.

Influence of temperature on retention

The influence of temperature on retention was studied on silica and PRP1. Fig. 6 shows the plot of the logarithm of the capacity factor *versus* the reciprocal of temperature for the test solutes on silica. The shape of the curve shows LC-like behaviour (increase in k' with increase in temperature) and a GC-like behaviour (decrease in k' with increase in temperature). It has been stated that the maximum k' is independent of the nature of the solute [13]. Fig. 6 shows that the position of the maximum (T_f) depends on the solute boiling point (b.p.) (Table I). For example, T_f for dodecylbenzene (b.p. = 331°C) is higher than that of α -naphthylamine and α -naphthaldehyde (b.p. = 301 and 291°C, respectively).

At a given temperature, the distance between the curves corresponds to the selectivity. From Fig. 6, selectivity variations can be obtained by changing the temperature.

Using the equation [14]

$$\ln k' = \frac{-\Delta H_T^0}{RT} + \frac{\Delta S_T^0}{R} - \ln \beta \quad (4)$$

where ΔH_T^0 is the change in enthalpy of solute transfer, ΔS_T^0 is the change in entropy of solute transfer, β is the phase ratio of the column, T is temperature (K) and R is the gas constant, ΔH_T^0 can be deduced from the slope of the straight portion of the curves plotted in Fig. 6, assuming that the variation in density with temperature can be neglected within the studied range.

Because the slopes of the straight lines are negative, positive values of ΔH_T^0 are obtained (Table II). These values are intermediate between GC and LC. Similar results and T_f values reported by Chester and Innis [15] with neat CO₂ for derivatized oligomers of polysaccharides separated on a capillary column indicated that the affinity of the solutes for the mobile phase was high. Consequently, the mobile phase solvation was the main partitioning mechanism.

TABLE II

VARIATION OF TEST SOLUTE TRANSFER ENTHALPIES (ΔH_T^0) AND ENTROPIES (ΔS_T^0) DETERMINED ON SILICA

Solute	ΔH_T^0 (kJ mol ⁻¹)	ΔS_T^0 (J mol ⁻¹ K ⁻¹)
α -Naphthol	3.81	8.43
β -Naphthol	4.67	10.98
α -Naphthylamine	6.66	12.29
α -Naphthaldehyde	8.94	14.12
Dodecylbenzene	13.51	20.06

In contrast, on PRP1, solute retentions except for diphenyl phthalate were found to decrease with increase in temperature (between 20 and 70°C) for a constant pressure of 232 bar. No maximum occurred, indicating the strong influence of the stationary phase on the partition mechanism. Our results obtained with a modified mobile phase are in good agreement with previous results at constant density for model compounds on PRP1 with neat CO₂ and N₂O over the temperature range 20–60°C [16].

Use of simultaneous modifier and density gradients

The pressure programming device monitoring the pressure regulator allowed density gradients over a wide range from 5 to more than 100 bar/min. Such a high ramp is necessary because very fast separations can be done on packed columns, as shown in Fig. 7: the separation of twelve PAHs on bare silica was achieved in less than 3 min. It must be pointed out that the device used allows density gradients without an increase in the mobile phase velocity (in contrast, a slight decrease in the linear velocity of CO₂ occurs during the increase in the CO₂ density because the CO₂ is pumped at a constant mass flow-rate). Thus, to a first approximation, the operating conditions are maintained in the column while the column pressure drop will only depend on the changes in the mobile phase viscosity during density gradients. This increase in mobile phase viscosity can entail a mobile phase density gradient in the column and, therefore, peak broadening [17]. From a practical point of view, this negative effect has not often been observed [18,19].

Fig. 8 shows the separation of phenol derivatives. Addition of a modifier to the mobile phase is required to elute the more polar solutes, but the separation of *o*-cresol and 2,3-dimethylphenol needs simultaneously a low methanol content in the mobile phase (less than 2%) and a low CO₂ density (0.35 g/cm³). In contrast, the density must be raised to 0.63 g/cm³ and the methanol content to 3.3% in order to separate methylhydroquinone and resorcinol. Owing to the high polarity of phloroglucinol, its elution requires both a high CO₂ density and a high methanol content (8%). Consequently, the separation involved a methanol gradient (from 2 to 8%) and two pressure ramps, the first to elute compounds having two hydroxyl groups and the second one to elute phloroglucinol.

During high-ramp pressure gradients, a negative baseline drift occurred because the physical properties of the detection medium changed [20]. This effect can be attenuated by cooling (at 0°C) the connection tubing between the column and the

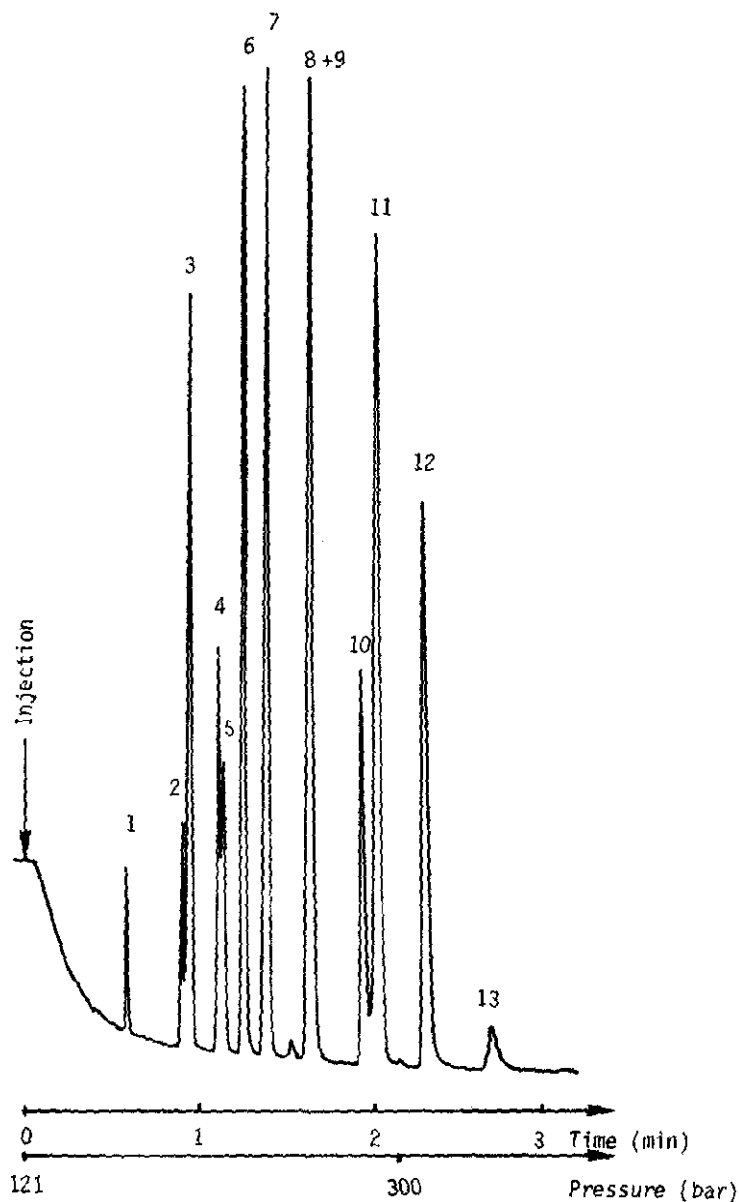


Fig. 7. Packed column SFC separation of PAHs using a fast pressure gradient. Column, 150 × 4.6 mm I.D.; stationary phase, 5- μ m Pecosphere bare silica; mobile phase, CO₂; flow-rate (0°C), 4 ml/min; temperature, 85°C; detection, UV at 254 nm. Solutes: 1 = toluene; 2 = naphthalene; 3 = chloronaphthalene; 4 = acenaphthene; 5 = acenaphthylene; 6 = fluorene; 7 = phenanthrene; 8 = fluoranthene; 9 = pyrene; 10 = benzantracene; 11 = triphenylene; 12 = benzopyrene; 13 = benzoperylene.

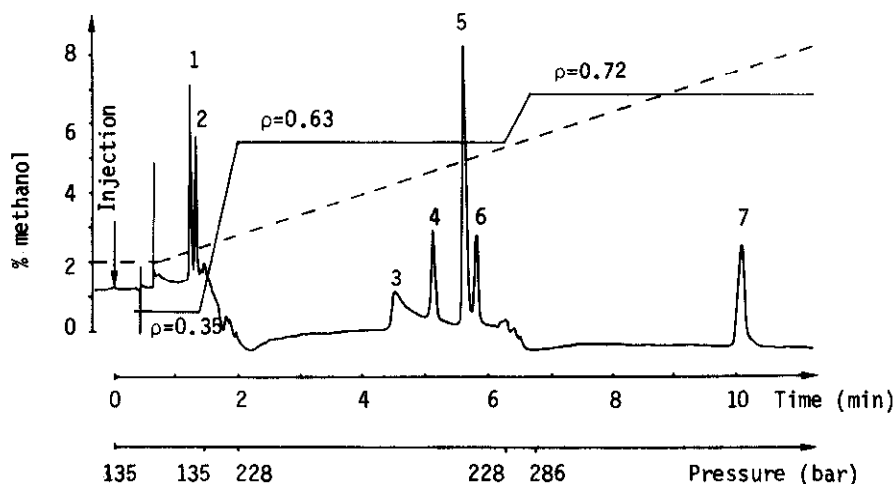


Fig. 8. Packed column SFC separation of phenol derivatives using pressure and modifier gradients. Column, 150×4.6 mm I.D.; stationary phase, $5\text{-}\mu\text{m}$ Pecosphere bare silica; mobile phase, CO_2 -methanol; flow-rate (0°C) 4 ml/min; temperature, 85°C ; detection, UV at 254 nm. Solutes: 1 = *o*-cresol; 2 = 2,3-dimethylphenol; 3 = resorcinol; 4 = methylhydroquinone; 5 = orcinol; 6 = hydroquinone; 7 = phloroglucinol.

detector. In that event, the CO_2 density does not vary greatly with the pressure and an LC-like behaviour is observed.

CONCLUSIONS

Mobile phase density programming in CO_2 -polar modifier binary mixtures has often been a neglected parameter, particularly with packed columns. However, its influence on the solute chromatographic parameters is of the same order of magnitude as that with pure CO_2 . Consequently, the physical parameters, temperature, CO_2 density and eluent composition (nature of the polar modifier), associated with the choice of the stationary phase (Table III) give general possibilities for improving the separation with respect to analysis time or resolution with multiple gradients. Among these, changes in the CO_2 density and polar modifier gradient in large variations in the chromatographic parameters and must be taken into consideration in the future.

TABLE III

FUNDAMENTAL PARAMETERS AFFECTING SEPARATION IN GC, LC AND SFC

Parameter	GC	LC	SFC
Stationary phase	+	+	+
Mobile phase composition	-	+	+
Pressure	-	-	+
Temperature	+	\pm	+

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