Note

Comparison of helium head pressure carbon dioxide and pure carbon dioxide as mobile phases in supercritical fluid chromatography

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In supercritical fluid chromatography (SFC), the solvating power of the mobile phase can be continuously varied and controlled by regulating the density. The density is a function of pressure and temperature and its variations become particularly important as the critical point is approached. The retention decreases sharply when the density of the eluent is decreased.

In these experiments we used two different sources of supercritical carbon dioxide as the mobile phase: helium head pressure carbon dioxide (HHPCD) and pure carbon dioxide. The retention times obtained with HHPCD were much higher than those obtained with pure carbon dioxide at the same temperature, pressure and flow-rate.

The few papers that have reported the use of HHPCD led to some controversy: Porter *et al.*[1] had problems with HHPCD in terms of reproducibility of retention times and peak areas, whereas Schwartz *et al.*[2] and Rosselli *et al.*[3] did not experience severe problems. Porter *et al.*[1] and Rosselli *et al.*[3] reported a slight increase in retention times when HHPCD was used instead of pure carbon dioxide. We observed a considerable increase in retention times with HHPCD.

EXPERIMENTAL

Instrumentation

All experiments were carried out on the device described elsewhere[4].

A Milton Roy Dosapro (Pont St. Pierre, France) minipump was used with a numerical manometer (Touzart & Matignon, Vitry sur Seine, France) of 0–40 MPa, assuming regulation of the pressure. The pump head was cooled to 1°C. To improve the pumping of liquid carbon dioxide, sapphire ball checks and seats were replaced with stainless-steel parts. Carbon dioxide cooled to 1°C in a thermostated bath was pumped without any problems at least up to 32 MPa.

Injections were made with a Rheodyne Model 7413 valve with a 1-μl internal loop (Touzart et Matignon, Vitry sur Seine, France).

A Perkin-Elmer LC-75 spectrophotometric detector (St. Quentin en Yvelines France) with an 8-μl cell was used. Aromatic compounds were detected at 254 nm and fatty acid esters at 212 nm. A Spectra-Physics SP 4270 (Les Ulis, France) integrator was used.

Pressure was measured at the column inlet and outlet with the numerical manometer; column pressure drops were *ca.* 1 MPa. Pressure reduction was done after the detector in two steps by two mechanical pressure regulators, the first being heated to the column temperature to avoid condensation of carbon dioxide.

Materials

Carbon dioxide of N 45 quality (99.995%) from Air Gaz France (Vigy, France) was delivered in cylinders of 5 MPa, liquefied in our laboratory in a cooling bath at 1°C and pumped up to the working pressure.

Helium head pressure carbon dioxide (HHPCD) (Carbolium[5]) of HP quality from Carboxyque Française (Paris-La Défense, France) was delivered in cylinders of 20 MPa with helium as pressuriser gas. HHPCD was used without a pump at the cylinder pressure, or if necessary was pumped up to desired pressure with the above mentioned pump.

Columns

The columns used were reversed-phase Hypersil C_{18}-bonded silica (5 μm) (135 × 4.6 mm I.D.) and polar Hypersil silica (5 μm) (235 × 4.6 mm I.D.), purchased from Chromatofield (Chateauneuf les Martigues, France).

The above-mentioned phenomenon of a weaker elution strength of HHPCD relative to pure carbon dioxide under the same chromatographic conditions was seen on both types of columns and with various solutes. As can be seen from the following examples, at different temperatures different complementary pressures of HHPCD had to be used to obtain the same retention as with pure carbon dioxide.

On the silica column (41.9°C, flow-rate 1.04 l/min) the methyl esters of fatty acids eluted with pure carbon dioxide at 13.0 MPa have retention times of 17.7 and 21.0 min, respectively, if HHPCD is used, a much higher presure of 19.5 MPa must be applied to give the same retention. As illustrated in Fig. 1, pure carbon dioxide at 19.5 MPa gives very short retention times of 8.6 and 9.8 min respectively. The aromatic compounds benzene, naphthalene and dibenzyl were deluted under milder elution conditions (49.4°C, flow-rate 0.85 l/min) with pure carbon dioxide at 13.5 MPa with retention times of 2.0, 2.9 and 4.0 min, respectively. To obtain the same retention with HHPCD a pressure of 18.5 MPa was necessary. On the C_{18}-bonded silica column (54.5°C, flow-rate 0.85 l/min) at 12.9 MPa with pure carbon dioxide the retention times of the fatty acid methyl esters $C_{20:5}$ and $C_{22:6}$ were 5.2 and 6.6 min, respectively. To obtain the same retention with HHPCD a pressure of 16.4 MPa was necessary.

At first we suspected the presence of traces of water in the carbon dioxide cylinder, acting as a polar modifier. To avoid moisture, a 20 × 2 cm drying column filled with glass-wool and phosphorus pentoxide was introduced between the carbon dioxide cylinder and the pump; no changes in chromatographic behaviour were observed, so the hypothesis of water presence was rejected. A systematic study of a test mixture of aromatic solutes on the silica column at different pressures using HHPCD and pure carbon dioxide was undertaken. The test mixture consisted of benzene, which
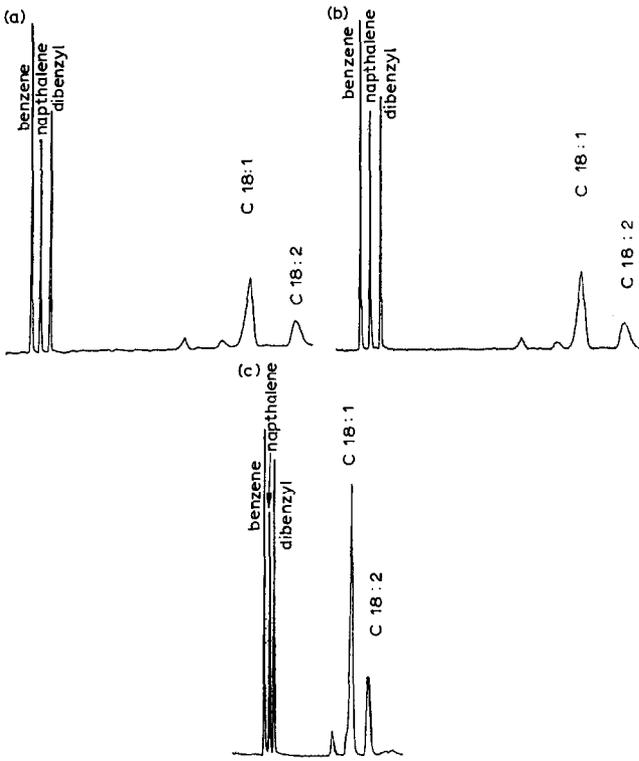
Fig. 1. Methyl esters of fatty acids and aromatic compounds. Silica column, 235 × 4.6 mm I.D., 41.9°C, flow-rate 1.04 l/min. Pressure: (a) 19.5 MPa of HHPCD; (b) 13.0 MPa of pure carbon dioxide; (c) 19.5 MPa of pure carbon dioxide. Retention times: (a) $C_{18:1}$ 17.6 min, $C_{18:2}$ 20.9 min; (b) $C_{18:1}$ 17.7 min, $C_{18:2}$ 21.0 min; (c) $C_{18:1}$ 8.6 min, $C_{18:2}$ 9.8 min.
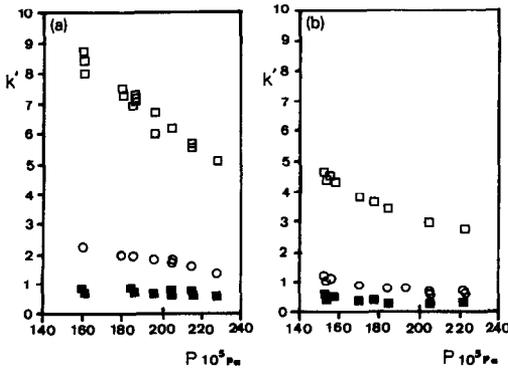

Fig. 2. $k' = f(P)$. Temperature, 39.6°C; flow-rate, 0.85 l/min. Mobile phase: (a) HHPCD; (b) pure carbon dioxide. ■ = Benzene; ○ = dibenzyl; □ = methyl benzoate.

is slightly retained, dibenzyl and methyl benzoate with relatively long retention times of 6–12 min in our conditions (39.6°C; flow-rate 0.85 l/min).

Capacity factors (k') were calculated using the eluent residence time t_0, obtained from the model proposed by Perrut[6] based on Darcy's law.

We expected, and it was confirmed experimentally, that the retention time of benzene (an almost non-retained substance) was hardly influenced by the pressure and nature of the eluent. With dibenzyl and methyl benzoate there are obviously different interactions with the column, resulting in different k' values with the two eluents (Fig. 2).

Determination of density

In the literature we could not find any density values for carbon dioxide mixed with helium under our pressure and temperature conditions, so we estimated the HHPCD and carbon dioxide densities with the same method based on tables of the Lee–Kesler equation of state[7].

Carbon dioxide. The density of pure carbon dioxide, D, was calculated from the equation

$$D = MP/ZRT \tag{1}$$

where M is the molar mass of CO_2, P is pressure, T is temperature, R is the gas constant and Z is the compressibility factor. The compressibility factor Z, correlated with the reduced temperature $T_r = T/T_c$ and reduced pressure $P_r = P/P_c$, were found in the Lee–Kesler tables. The critical temperature T_c and critical pressure P_c are 304.15 K and 7.38 MPa, respectively, and the acentric factor $\omega = 0.239$.

With this method the carbon dioxide densities were found to be higher than those obtained by using the Peng–Robinson equation of state, and closer to the experimentally based values published by IUPAC[13]. The calculated values are plotted in Fig. 3 at different temperatures.

Helium head pressure carbon dioxide. The chromatographic analysis showed that the carbon dioxide leaving the HHPCD cylinder at 20 MPa at 15°C has a helium concentration of 5.8% (v/v), which diminishes linearly to 4.4% as the cylinder pressure decreases to 15 MPa. The real concentration of helium in the HHPCD varies slightly with the cylinder pressure, but we chose a simplified approach, considering a constant concentration (mean value of 5%) over the whole range of 15–20 MPa cylinder pressure in subsequent calculations.

We could not find published liquid–vapour equilibrium data for the He–CO₂ system for our pressure and temperature ranges. However, the data for slightly lower pressures or temperatures[8,9] permitted it to be verified that the above-mentioned concentrations correspond well to the published values, assuming linearity.

To calculate the pseudocritical pressure and temperature of this mixture we followed the recommendations of Solen *et al.*[10]. Subscripts 1 and 2 are used for carbon dioxide and helium, respectively. For quantum gases (helium), slightly temperature-dependent critical constants T_c and P_c must be used.

$$T_{C2} = T_C^0/(1 + C_1/M_2T) \tag{2}$$

$$P_{C2} = P_C^0/(1 + C_2/M_2 T) \tag{3}$$

$$V_{C2} = 0.291 \, RT_C^0/P_C^0 \tag{4}$$

where $T_C^0 = 10.5$ K, $P_C^0 = 0.676$ MPa, $C_1 = 21.8$ K, $C_2 = 44.2$ K, V_{C2} = critical volume of helium = 38.2 cm^3 mol^{-1}, V_{C1} = critical volume of carbon dioxide = 93.9 cm^3 mol^{-1} and M_2 = molar mass of helium.

For a mixture, the interaction constants must be calculated[11]:

$$k_{12} = 1 - \frac{8(V_{C1}V_{C2})^{1/2}}{(V_{C1}^{1/3} + V_{C2}^{1/3})^3} \tag{5}$$

$$V_{C12} = (V_{C1}^{1/3} + V_{C2}^{1/3})^3/8 \tag{6}$$

$$M_{12} = \frac{2}{(1/M_1) + (1/M_2)} \tag{7}$$

where M_1 = molar mass of carbon dioxide.

Whenever either component is a quantum gas, we calculate

$$T_{C12} = \frac{(T_{C1}T_{C2})^{1/2}(1-k_{12})}{1 + (C_1/M_{12}T)} \tag{8}$$

For the utilization of Lee–Kesler tables (as was done with pure carbon dioxide) we applied the laws for mixtures proposed by Plocker et al.[12]. The following laws permit the pseudocritical constants of HHPCD to be calculated for molar fractions y_1 and y_2 of carbon dioxide and helium, respectively:

$$V_{CM} = y_1^2 V_{C1} + 2y_1 y_2 V_{C1} + y_2^2 V_{C2} \tag{9}$$

$$T_{CM} = \frac{1}{V_{CM}^{1/4}} (y_1^2 V_{C1} T_{C1} + 2y_1 y_2 V_{C12}^{1/4} T_{C12} + y_2^2 V_{C2}^{1/4} T_{C2}) \tag{10}$$

$$\omega_M = y_1 \omega_1 + y_2 \omega_2 \qquad (\omega_1 = 0.239; \; \omega_2 = 0.0) \tag{11}$$

$$P_{CM} = (0.2905 - 0.085\omega_M)RT_{CM}/V_{CM} \tag{12}$$

The reduced parameters $T_r = T/T_{CM}$ and $P_r = P/P_{CM}$ were calculated for HHPCD and with the compressibility factor Z from Lee–Kesler tables the density of the mixture was obtained from eqn. 1, where M is replaced by the molar mass of HHPCD.

RESULTS AND DISCUSSION

The pure carbon dioxide (CO_2) and HHPCD densities at different temperatures and pressures are given in Fig. 3. As it can be seen, the corresponding CO_2 densities are
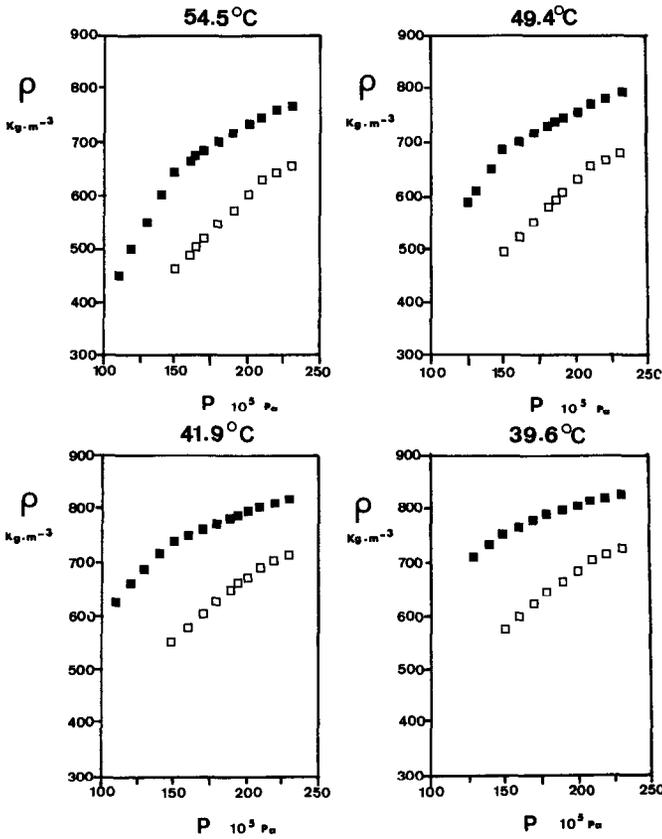
Fig. 3. Densities of (□) HHPCD and (■) pure carbon dioxide at different pressures and temperatures. Concentration of 5% of helium in carbon dioxide.

much higher than those of HHPCD, which explains the higher elution strength of CO_2 under the same conditions.

Table I gives some values referring to our experiments. The pressures and temperatures of the experiments and the calculated densities of HHPCD (D_{HC}) and of CO_2 (D_{CO2}) at a given temperature and pressure are listed in the first four columns. As can be seen the CO_2 values are much higher than those of HHPCD.

TABLE I

COMPARISON OF PRESSURES AND CORRESPONDING DENSITIES OF PURE CARBON DIOXIDE AND HHPCD

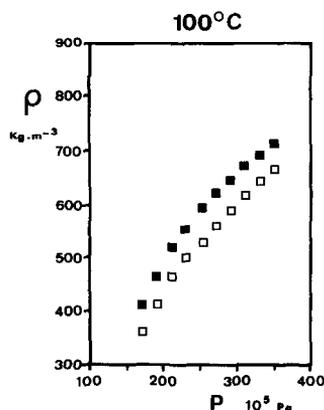| Temperature ($°C$)	Pressure (MPa)	D_{HC} (kg/m^3)	D_{CO_2} (kg/m^3)	$P_{CO_2eqv.}$ exp. (MPa)	D_{CO_2} theor. (kg/m^3)	$P_{CO_2eqv.}$ (MPa)	Δ (%)
41.9	19.5	660	790	13.0	690	12.0	8
54.5	16.4	501	672	12.9	545	12.0	7
49.4	18.5	591	739	13.5	630	12.5	7

Fig. 4. Densities of (□) HHPCD and (■) pure carbon dioxide at 100°C and at different pressures. Concentration of 3% of helium in carbon dioxide.

We calculated the density of CO_2 at the pressures experimentally observed (fifth column) and giving the same retention as HHPCD. The CO_2 densities obtained (sixth column) are very close to those of HHPCD (third column), which explains why a lower CO_2 pressure was needed to obtain the same retention.

We also calculated the theoretical pressures at which CO_2 would have the same density as HHPCD. As can be seen, the calculated pressures (seventh column) are slightly lower, by 7–8% (last column) than those observed experimentally (fifth column).

We can consider that the simplified hypothesis (taking into consideration only density changes as a function of helium concentration) explains very well the higher retention times obtained with the HHPCD, and the first approximation presented here leads to a satisfactory interpretation of the results.

The fact that Rosselli et al.[3] and Porter et al.[1] observed only slightly higher retention times in the case of HHPCD is explained as follows. In both instances the HHPCD was used in cylinders of pressure ca. 10.75 MPa. According to the equilibrium data, at 20°C there is about 3% of helium dissolved in carbon dioxide. We made density calculations assuming 3% of helium CO_2 under the experimental conditions of Rosselli et al.[3] and, as can be seen from Fig. 4, the differences between HHPCD and CO_2 are much smaller than those under our experimental conditions (Fig. 3).

CONCLUSIONS

The lower elution strength of HHPCD compared with CO_2 at the same temperature, pressure and flow-rate is due mainly to its lower density. It is surprising that the presence of a relatively small concentration of helium decreases the elution strength of the HHPCD so much, but the important density variations can explain this phenomenon. This fact must be taken into consideration if different sources of supercritical carbon dioxide are used.

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