



Unexpected retention behavior of supercritical fluid chromatography at the low density near critical region of carbon dioxide

Abhijit Tarafder, Georges Guiochon*

Department of Chemistry, University of Tennessee, Knoxville, TN, USA

ARTICLE INFO

Article history:

Received 22 September 2011

Received in revised form

16 December 2011

Accepted 30 December 2011

Available online 12 January 2012

Keywords:

Isopycnic

SFC

Supercritical fluid chromatography

Operating conditions

Retention mechanism

Near critical

ABSTRACT

The retention factors of octylbenzene, octadecene, anthracene, and pyrene eluted from columns packed with neat silica and C₁₈-bonded silica by pure carbon dioxide near its critical region increase with increasing temperature along low-density isopycnic lines. This behavior is markedly different from the one observed in nearby regions of the pressure–temperature diagram of CO₂, where the retention factors of these compounds decrease with increasing temperature along high-density isopycnic lines. Several possible reasons that could explain this reversal in the behavior of the retention factors are discussed. The most plausible is related to the formation of multilayer adsorption of CO₂ on the stationary phases in a density range slightly lower than the critical density.

© 2012 Elsevier B.V. All rights reserved.

1. Introduction

Retention mechanisms in SFC seem to be hybrids between the mechanisms encountered in gas (GC) and in liquid chromatography (LC). In GC, solute–stationary phase interactions are the essential if not the only ones controlling retention. The role of the carrier gas is limited to transporting solute vapors along the column. Only with highly efficient open tubular columns had it been reported that interactions between the carrier gas and the solute vapors interfered with the interactions between the stationary phase and the sample molecules and affected to even a small extent the retention mechanism of analytes [1]. In LC, on the other hand, both solute–mobile phase and solute–stationary phase interactions control retention. The solvation power of the mobile phase in LC, one of the main physical properties representing solute–mobile phase interactions, can strongly affect solute retention.

In SFC, either a GC-like or a LC-like retention mechanism is possible, depending on the region of the pressure–temperature diagram in which the analyses are carried out [2], because SFC analyses can be carried out over a wide range of CO₂ density [3]. At high densities, which are typically achieved under high pressures and at low temperatures, the solvation power of CO₂ is high and a LC-like mechanism controls the retention. At low densities, i.e.,

under low pressures and at high temperatures, on the other hand, the solvation power of CO₂ drastically reduces and the retention mechanism closely resembles the one observed in GC, where the solute volatility and the solute–stationary phase interactions are the main controlling factors. In the intermediate regions, competing retention mechanisms, involving both solute–stationary phase interactions and solute–mobile phase interactions, tend to take place, to a degree depending on the compounds involved. A survey of the literature found several reports in which the dependence of retention factors on the temperature, the pressure, or the density were investigated in this intermediate region. A brief review of these studies is provided in the following subsections, which will be useful in the analysis presented in the subsequent sections.

1.1. Variation of the retention factors with temperature at constant pressure

The dependence of the retention factors, *k*, of different compounds on the temperature at constant pressure was studied and reported by various authors [2,4–6]. In the supercritical region, *k* first increases with increasing temperature, starting from the critical temperature. It reaches a maximum and then decreases. The physical reason for this behavior was explained by a change in the dominant retention mechanism. First, we have a LC-like mechanism and *k* increases with increasing temperature because the mobile phase density decreases, which reduces its solvating power and, in turn, increases the adsorption of the analytes and their

* Corresponding author. Tel.: +1 8659740733; fax: +1 8659742667.

E-mail address: guiochon@ion.chem.utk.edu (G. Guiochon).

relative residence times on the stationary phase. When the temperature increases further, the system progressively shifts toward a GC-like mechanism; the analyte vapor pressure increases with increasing temperature, outweighing the former effect [2,4–6]. Obviously, both mechanisms coexist and, depending on the temperature the influence of one or the other dominates.

There is, however, a serious problem in using this method to understand the mode of the controlling retention mechanism (LC or GC) because (1) the solvation power of CO₂ varies widely from one analyte to the other, depending on their physico-chemical properties, and more importantly (2) the solubility of analytes does not always decrease with the temperature under isobaric conditions; rather, there is a crossover pressure, the value of which varies from solute to solute, above which the solubility increases with increasing temperature under isobaric conditions. So, as pointed out by Chester and Innis [2], the retention factor can either decrease or increase with increasing temperature, depending on the individual properties of these compounds and on the constant pressure considered.

1.2. Variation of the retention factors with the pressure at constant temperature

There is no dramatic variation of the retention factors with the pressure at constant temperature, in contrast with the previous case. The retention factors constantly decrease with increasing pressure at constant temperature, due to the correlative monotonic increment of the mobile phase density. The rate of change of the retention factors per unit change in the pressure, however, greatly varies with the temperature at which the experiments are conducted. At temperatures greater than but close to the critical point, the retention factors vary rapidly with the pressure, due to the strong compressibility of the mobile phase, hence to the rapid variations of its density in this region. At temperatures farther from the critical temperature, the effect of an increasing pressure on the retention becomes less important.

1.3. Variation of the retention factors with the temperature at constant mobile phase density

In contrast to the dependence of the retention factors with the temperature at constant pressure, which gives complex, even puzzling results (see Section 1.1), the variation of the retention factors against the inverse of the temperature at constant density is almost always linear and monotonic. The retention factors decrease constantly with increasing temperature along the isopycnic lines. As pointed out by Chester and Innis [2] there is no difference between LC-like and GC-like operation along the constant density lines. Roth went so far as qualifying such monotonic variations as useless [7] because they do not provide any specific information regarding the retention behavior or the retention mechanism.

In a recent publication, we showed that the iso-density or isopycnic lines can be useful in method development and in developing insights into a separation process [3]. In the same paper we reported an unexpected retention behavior in the low pressure range, near the critical region of CO₂ [3]. It was shown that at densities of 0.65 g/mL or higher, the retention factors of anthracene and pyrene decrease constantly with increasing temperature along the isopycnic lines. Such behavior is expected, because the analyte solubility increases constantly with increasing temperature. However, it was also observed that at densities of 0.55 g/mL or lower, this general trend was no longer valid. Along the 0.55 g/mL isopycnic line, the retention factor of anthracene actually increases slightly. Along the 0.45 g/mL isopycnic line, the retention factors of both pyrene and anthracene increase with increasing temperature up to a certain temperature, beyond which they decrease. Along the 0.35 g/mL

isopycnic line, the retention factors increase constantly up to 360 K, the highest temperature at which the measurements were carried out. As was noted then, this type of variation of the retention factors cannot be controlled only by variations of the solubilities of the test compounds in CO₂, because the solubility of all compounds increases with increasing temperature at constant density, which would be expected to lead to a decrease of these retention factors. Besides, the experimental data regarding the solubility of several compounds show that, below the critical density of CO₂, these solubilities remain nearly constant when the temperature increases under isopycnic conditions [8]. So the role of the solubility in the reversal of the retention behavior was ruled out, but no alternative explanation was provided to understand this behavior [3].

Interestingly, most published reports dealing with the temperature dependence of retention factors along isopycnic lines do not contain data in this low-density zone, so possible similar effects remained largely unreported, hence unexplained. We could find only two instances, illustrated in Fig. 1, in which clear indications of a significant shift in the trend of the variations of retention factors with temperature had been reported, but without any further explanations. Fig. 1a shows that the higher abscissa ticked, $1/T = 3.0 \times 10^{-3}$, corresponds to $T \approx 60^\circ\text{C}$, the higher temperature boundary of the region in which Berger [6] reported serious difficulties in obtaining satisfactory chromatograms. Roth [9] calculated the retention factor of naphthalene in a PDMS–CO₂ system and plotted the data versus the inverse of the absolute temperature at several densities. In Fig. 1b, the trends of these data are shown, at temperatures between approximately 30 and 72 °C, a temperature range that includes the critical temperature. These plots show that the retention factor of naphthalene increases constantly with increasing temperature at densities above about 0.6 g/mL while at lower densities the reversed trend is observed. These theoretical results are surprising because, according to our experimental results, the retention factors decrease with increasing temperature at high densities, in agreement with Roth's prediction, while at low densities, they follow the opposite trend only up to a certain temperature, a reversal which is in contrast with Roth's results. This suggests that the model used by Roth does not include some important phenomenon, the existence of which would explain the unexpected reversal observed.

The goal of this work was to investigate more thoroughly whether a reversal in this trend of retention factor variation takes place in the near critical region, as observed earlier [3], or was it an artifact of the experimental conditions used in that study. The additional motivation was to attempt to understand it, or at least to narrow down to the plausible physical phenomena controlling this trend reversal.

2. Theory

The average mobile phase density inside a column is controlled by the average temperature and by the pressure, which in turn is controlled by the inlet/outlet pressure and the pressure drop. If the operating flow rate is increased but the inlet pressure is kept constant, the outlet pressure must be decreased and, as a consequence, the average mobile phase density in the column decreases. This causes an increase in the average retention factors of all analytes and often a larger increase in their separation factors [10]. To keep constant the retention factors or the separation factors of certain compounds, the average density of the mobile phase in the column must be kept constant, hence the mobile phase density at the column inlet must be increased to compensate for its decrease at the column outlet. The intensity of these effects depends on the mobile phase viscosity and on the column permeability.

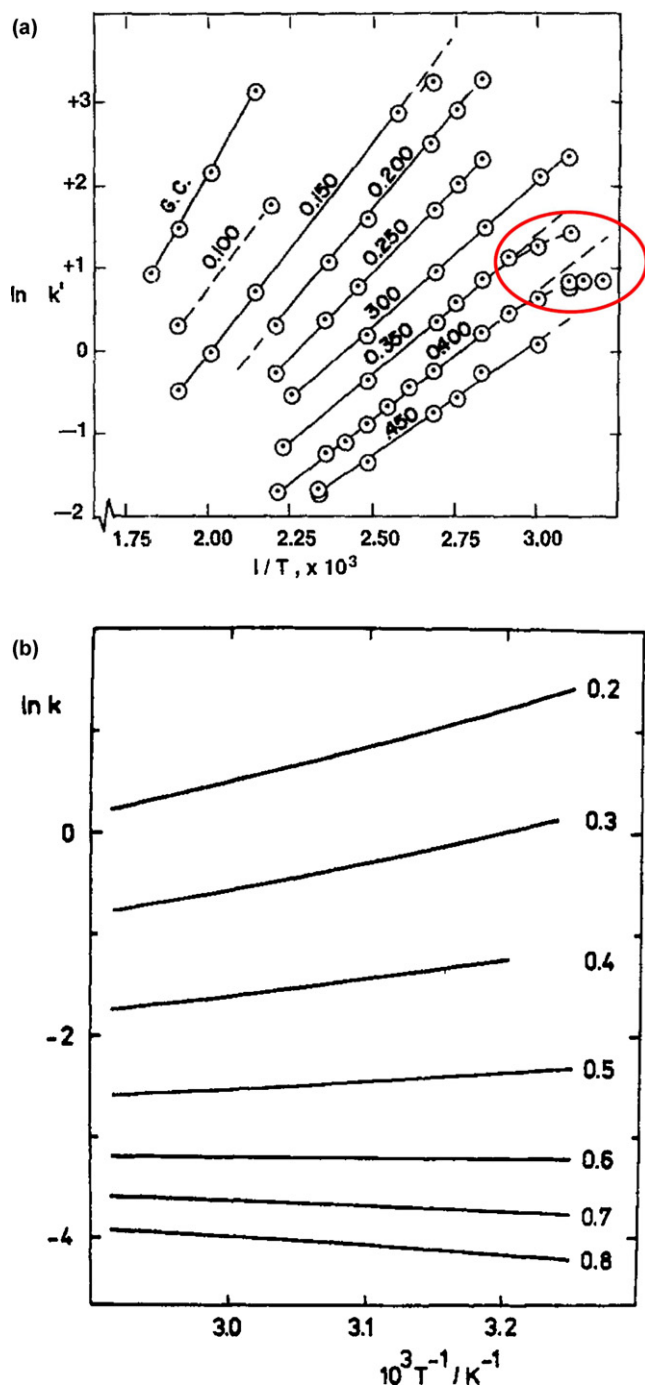


Fig. 1. (a) The variation of the logarithm of the retention factors against the inverse of the absolute temperature along different isopycnic lines. The deviations from the trends along the 0.35 and 0.4 g/mL isopycnic lines are highlighted. (b) The variation of the logarithm of the retention factors with the inverse of the absolute temperature along various isopycnic lines. The figure shows a reversal in the trend of the retention factor variations from 0.6 g/mL.

(a) Reproduced with permission from Ref. [6]. (b) Reproduced with permission from Ref. [9].

The influence of the pressure or of the density of the mobile phase on the chromatographic behavior has been abundantly studied in the literature during the last fifty years [11–15] but most authors have avoided making measurements of retention factors in the critical region of the mobile phase because these factors vary rapidly with the mobile phase density [11]. Fig. 2 illustrates the

dramatic changes in $\log k$ with increasing pressure across the critical domain [16].

Perrut and Dellacherie [17] reported that the retention factor of naphthalene, 1- and 2-methylnaphthalene, and 2,6-dimethylnaphthalene in pure CO_2 are simply related to the temperature through the relationship

$$k_i = e^{a_i - bT} \rho_{av}^{-n} \quad (1)$$

where ρ_{av} is the average density of the mobile phase, T the temperature, a_i , b , and n are numerical parameters, the former specific of the compound, the latter one constant, 3.42. The coefficient b seems to depend on the chemical structure of the compounds. Differentiation of Eq. (1) gives:

$$\left(\frac{\partial \log k_i}{\partial P_{av}} \right)_T = -n \left(\frac{\partial \log \rho_{av}}{\partial P_{av}} \right)_T \quad (2)$$

where P_{av} is the average pressure in the column. Since $\partial \log \rho / \partial P$ is the isothermal compressibility, κ , of the mobile phase, which can easily be derived from an equation of state [18], the author concluded that

$$\left(\frac{\partial \log k_i}{\partial P_{av}} \right)_T = -n\kappa \quad (3)$$

This equation shows that retention should decrease with increasing average column pressure hence with increasing average density of the mobile phase at constant temperature

2.1. Partial molar volumes of solutes

Van Wasen et al. [13] derived the following relationship between the retention factor and the partial molar volumes of the solute i in the adsorbed phase and in the solution, $V_{i,ads}$ and $V_{i,sol}$, respectively

$$\left(\frac{\partial \log \rho k_i}{\partial P} \right)_T = \frac{V_{i,ads} - V_{i,sol}}{RT} \quad (4)$$

They measured the partial molar volumes of naphthalene and fluorene in carbon dioxide in the critical region [19]. In the gas phase, the partial molar volume of compounds decreases with increasing pressure until the pressure nears the critical pressure. At about 10% below the critical pressure of CO_2 , the partial molar volume begins to increase more and more rapidly with increasing pressure, then decreases when the pressure exceeds the critical pressure. The amplitude of this spike increases rapidly with decreasing difference between the temperature at which the measurements are made and the critical temperature. The amplitude of this spike corresponds to an approximately six times higher partial molar volume at the critical pressure than under 60 bar at 35 °C, three times higher at 40 °C, and twice at 50 °C (see Fig. 3, top right). So, around the critical pressure, $V_{i,sol}$ increases considerably, making the product ρk_i very sensitive to small pressure fluctuations. The dependence of the retention factor of naphthalene on the pressure were shown earlier in [20], Fig. 8 in [16], consistent with the results of van Wasen and Schneider. All this suggests that clustering of a large number of solvent molecules takes place around each solute molecule.

Similar results have been reported by numerous other authors for several different fluids [21,24–27]. Fig. 3 compares values of the partial molar volume of solutes in carbon dioxide measured and calculated using different models of statistical thermodynamics [21,24]. These results clearly demonstrate that, close to the critical point, the molecules of the solvent tend to congregate around the molecules of larger, more polarizable solute molecules.

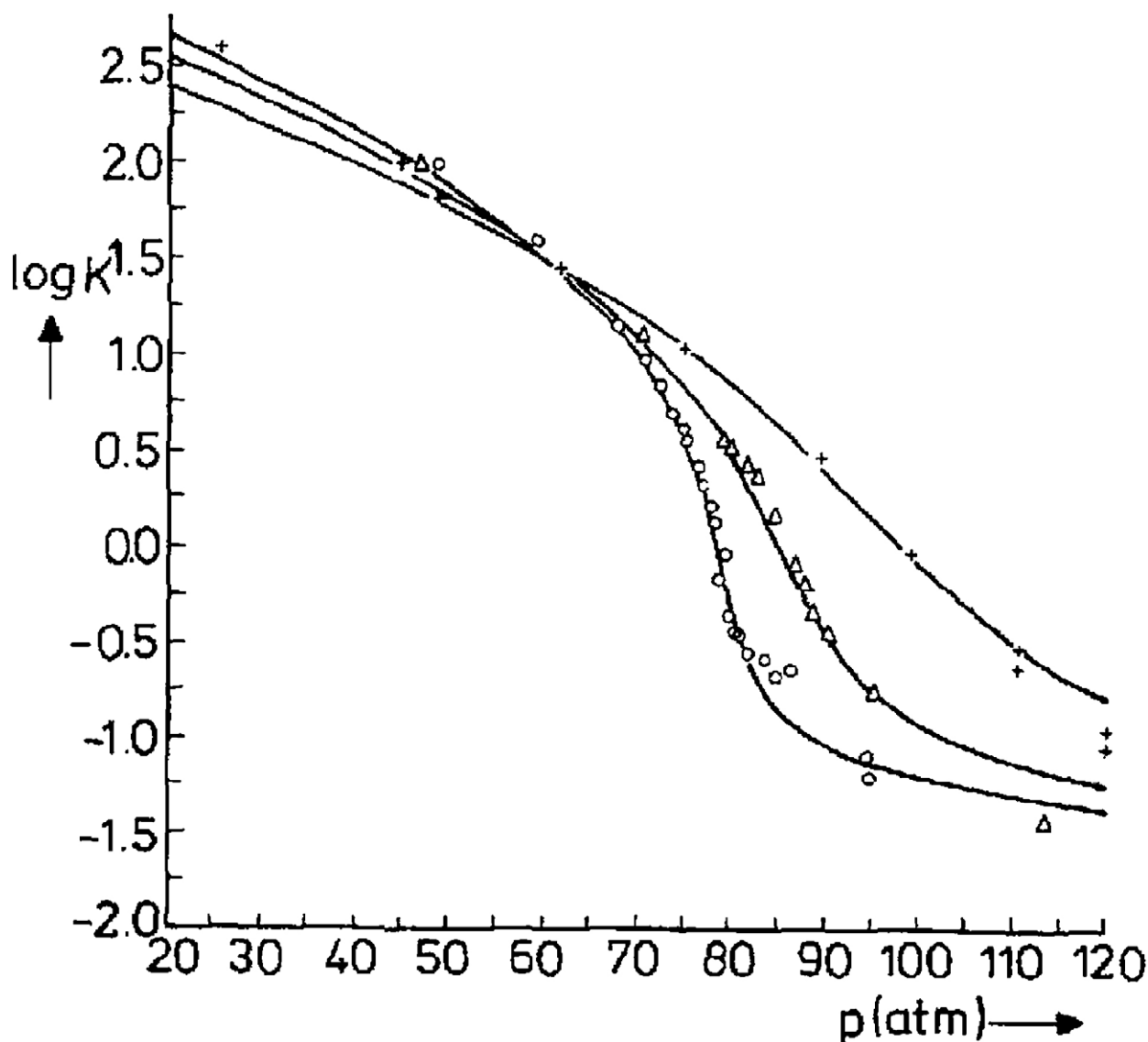


Fig. 2. Retention factor of naphthalene in carbon dioxide as a function of the pressure at different temperatures: (○) 35 °C; (△) 40 °C; (+) 50 °C [16].

2.2. Adsorption of carbon dioxide on solid surfaces

There is a considerable possibility that the same effect should lead to the clustering of CO₂ molecules against the surface of adsorbents and the formation of several adsorbed layers. Several authors have measured the adsorption of carbon dioxide on the surface of adsorbents [28,29]. Giovanni et al. measured the adsorption isotherms of carbon dioxide on silica as a function of the density determined simultaneously by gravimetry [29]. This excellent procedure avoids the use of an equation of state, which lacks accuracy in the neighborhood of the critical point due to the rapid variation of the density with the temperature and pressure in this region. It provides directly the excess volume of adsorption. The data plotted versus the density between 312.0 and 465.9 K are shown in Fig. 4. The adsorption maximum takes place at almost the same density in the whole temperature range. This density (ca. 0.30 g/L) is significantly less than the critical density (ca. 0.47 g/L). These isotherms are consistent with those reported by Strubinger et al. [30], which were measured using a combination of a tracer pulse chromatographic method and mass spectrometric detection (Fig. 4). If the excess adsorption data are plotted versus the pressure, all the isotherms intersect beyond their maximum [29]. The

pressure at which the adsorption maximum takes place increases with increasing temperature while the corresponding loading effect which occurs with solubility data [20].

Thus, the surface of adsorbents is covered by a thin layer of carbon dioxide when the density of the mobile phase is close to the critical density. The thickness of this layer decreases when the density becomes markedly higher.

3. Experimental

The experimental study was carried out with octylbenzene (99%, Aldrich), octadecene (95%, Fluka) and two PNAs – anthracene (99.9%, Sigma) and pyrene (99%, Aldrich). Benzene was used as the tracer to measure the column void volume. The PNAs were co-injected while the other two compounds were injected separately. The concentrations of all these samples were around 1 g/mL.

The instrument used in all the experiments was a TharSFC system (Waters, Mildford, MA, USA). This instrument includes a fluid delivery module with three parallel reciprocating pumps with heads maintained at 4 °C, an autosampler, an oven, a photo-diode array detector (Waters 2998), and an automated back pressure

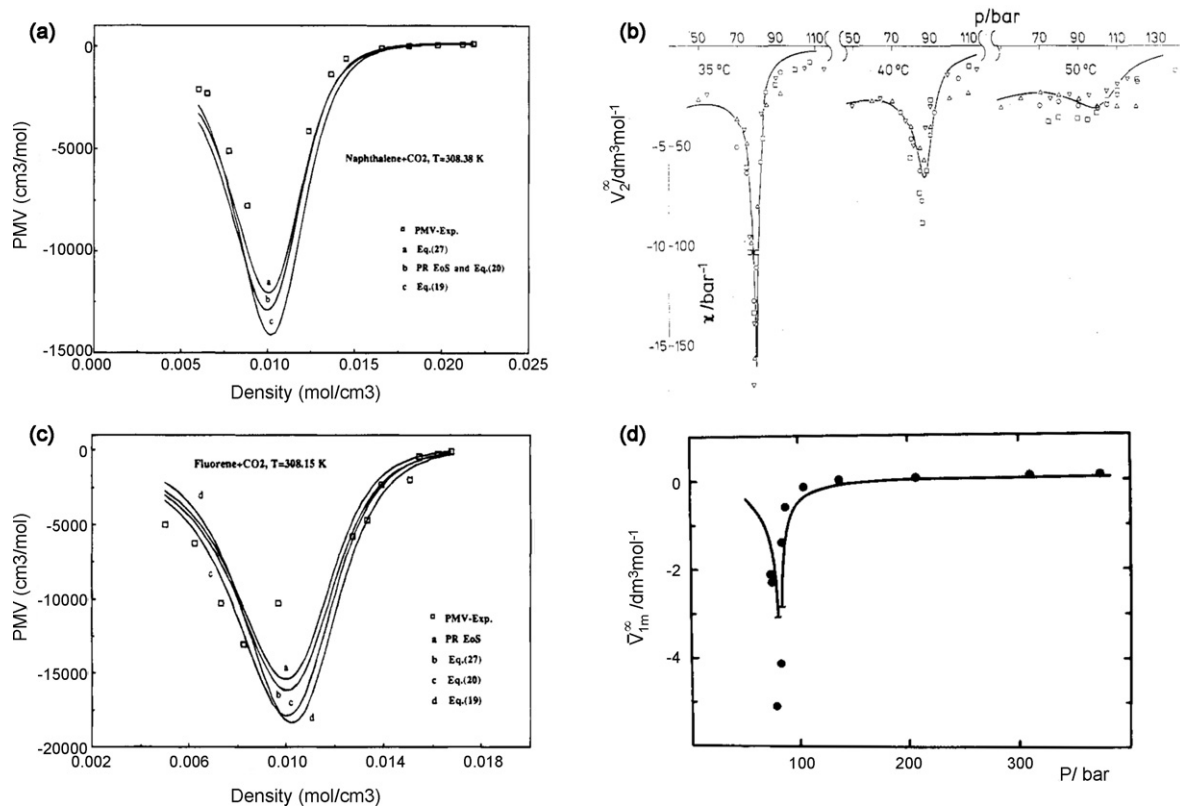


Fig. 3. Left: partial molar volumes of naphthalene (top) and fluorene (bottom) in CO₂ calculated using different models [21]. Right, top: the partial molar volumes of naphthalene and fluorene in carbon dioxide at different temperatures. Experimental data [19] and calculated curve [22]. (▼) Naphthalene on PerisorbA; (▲) naphthalene on PerisorbRP-8; (□) fluorene on PerisorbA; (○) fluorene on Perisorb RP-8. Right, bottom: experimental [23] and calculated [24] partial molar volumes of naphthalene at 35.23 °C as a function of pressure.

regulator (ABPR). In contrast to HPLC, the operating pressures mentioned in SFC are the outlet pressures, which are the values set for the ABPR, not the inlet pressures.

Two different columns were used for this study. A 0.46 cm × 15 cm Prototype XBridge C18 column packed with 10 μm particles and a 0.46 cm × 15 cm Prototype column packed with 10 μm silica particles. Both the columns were from Waters (Mildford, MA, USA) and will be referred to as the C18 column and the neat silica column in the rest of this report. Particles of a large size were purposefully chosen to maximize the column permeability and minimize the variation of the local pressure along the column, hence of the local retention factor. The mobile phase was neat CO₂ from Airgas (Knoxville, TN, USA). In all the experiments the flow rate was kept constant at 2 mL/min, the lowest flow rate at which the ABPR can operate properly. According to the manufacturer of the TharSFC, in order to ensure that the flow rate stays constant at the set value, at the specified temperature and pressure, the frequency of the piston strokes in the pumps is controlled based on the data set within the software settings of the instrument, which account for the density and compressibility of the mobile phase.

4. Results and discussion

Several factors may cause a reversal in the trend of the temperature dependence of the retention factors along isopycnic lines. Fig. 5 shows plots of the retention factors of anthracene and pyrene versus the temperature under different isopycnic conditions. The data used in this plot were taken from [3], in which this phenomenon was first discussed. At densities larger than 0.65 g/mL the retention factors decrease constantly with increasing

temperature, as should be reasonably expected, based on previously published results and on the explanations provided [6,31]. Below this density, the plot becomes more complex. First, along the isopycnic line at $\rho = 0.55$ g/mL, the retention factor remains almost constant in a certain temperature range while at lower densities, it increases with increasing temperature in a limited range of temperature, before seemingly returning to the general trend of decreasing with increasing temperature. This maximum temperature increases with decreasing density. The dashed lines in Fig. 5 represent trends expected at densities of 0.55 g/mL and lower, had the data points followed the same trend as the higher density points. Although there is no physical basis behind the positions of the dashed lines, they illustrate the trend reversal and suggest a different interpretation. Instead of considering that the retention factors increase along the isopycnic lines is a reversal to the standard trend, this variation could be understood as the result of the compounds eluting earlier than expected at the temperature and density considered.

This earlier than expected elution can result from several factors involving the interactions of the three main agents which control retention in chromatography, the stationary phase, the mobile phase and the solutes. The first set of experiments that we carried out were set to separately understand the roles played by these agents. In the earlier study [3], C₁₈-bonded silica was used as the stationary phase while two PNAs, anthracene and pyrene, were used as the solutes. To check whether the observed phenomenon of trend reversal is stationary-phase specific we carried out further experiments with two different stationary phases, C₁₈-bonded silica and neat silica. These phases were chosen as they represent opposite polarities and hence very different natures of the solute-stationary phase interactions. The idea was that, if

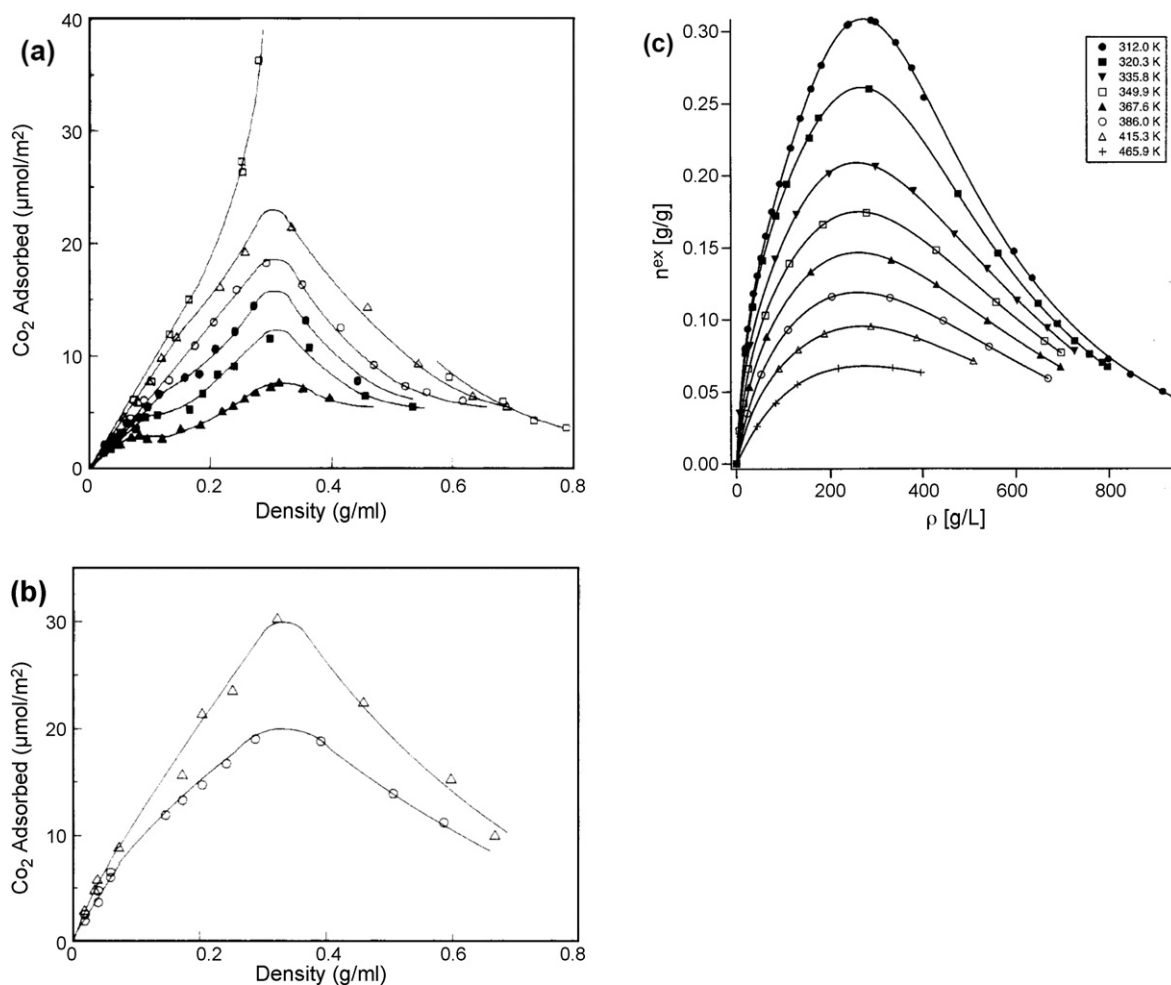


Fig. 4. Left, top: excess adsorption isotherm of CO₂ on ODS. Bottom: excess adsorption isotherm of CO₂ on silica surface [28,30]. Right: excess amount of CO₂ adsorbed on silica gel [29].

experiments with both stationary phases lead to the trend reversal, it may be concluded that the role of the stationary phase is irrelevant and the focus can be shifted to the roles of the solutes and the mobile phase.

4.1. Possible role of the stationary phase

Detailed experimental conditions for this series of experiments are given in the experimental section (Section 3). The neat

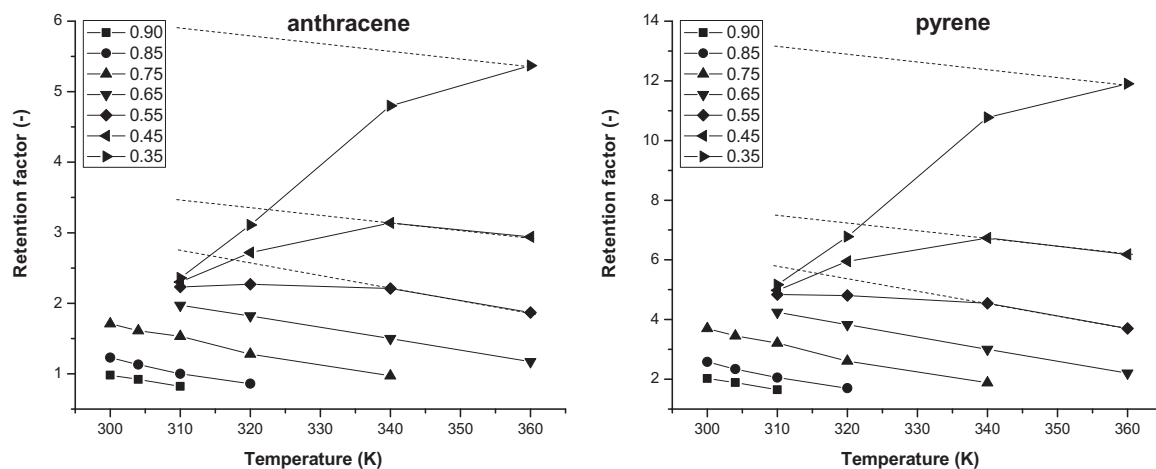


Fig. 5. Retention factors of anthracene and pyrene plotted against the temperature under isopycnic conditions between 0.35 and 0.90 g/mL (data from Ref. [3]). The solid lines joined the data points to guide the eyes; the dashed lines illustrate a possible general trend of the retention factor variations at densities of 0.35, 0.45 and 0.55 g/mL, if it were the same for all isopycnic conditions.

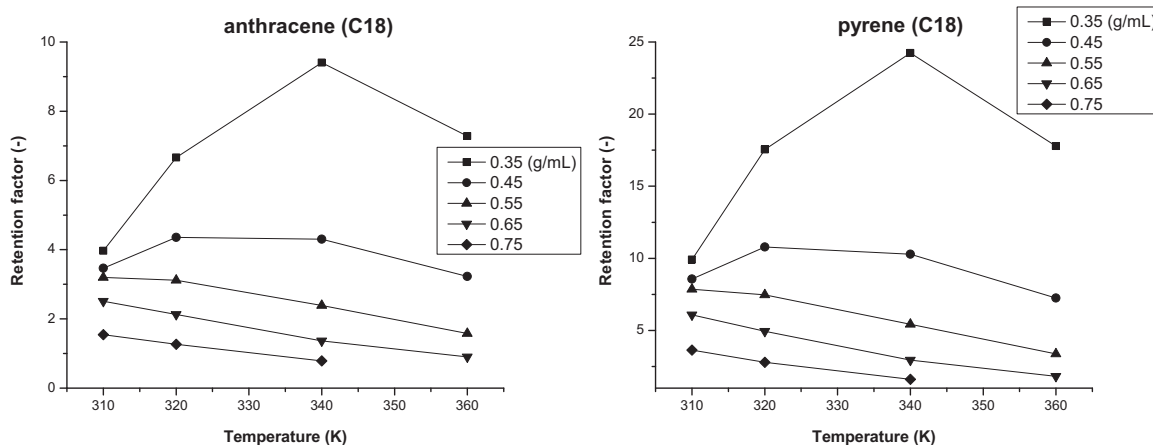


Fig. 6. Plots of the retention factors of anthracene and pyrene versus the temperature along isopycnic lines on a C18 column.

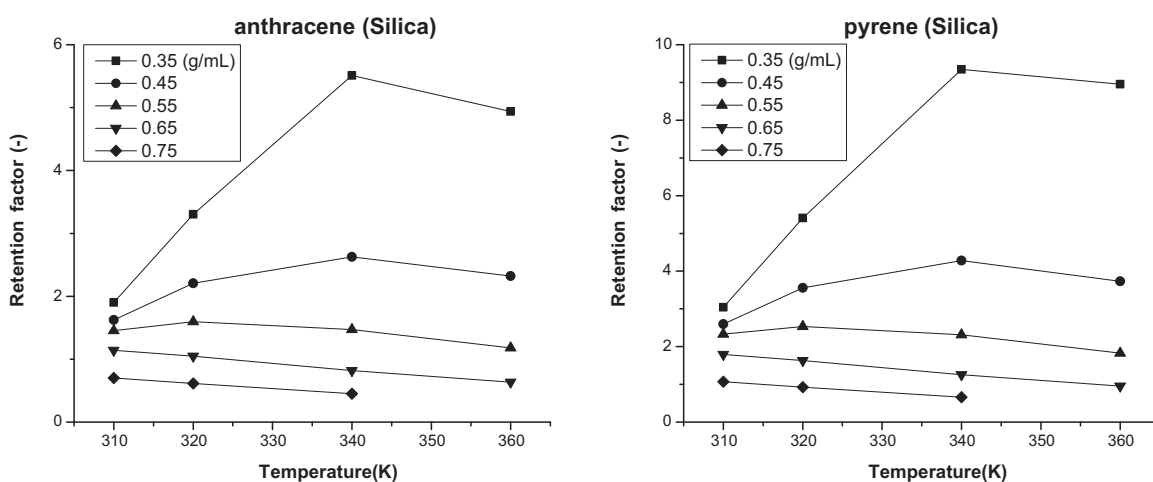


Fig. 7. Plots of the retention factors of anthracene and pyrene versus the temperature along isopycnic lines on the neat silica column.

silica and C18 columns used were both from Waters, the solutes were anthracene and pyrene. Their retention factors are plotted in Figs. 6 and 7. The detailed operating conditions are supplied in Tables 1 and 2 along with the retention factors.

As shown by Figs. 6 and 7, the trend reversal or early elution phenomenon is not specific to a particular column nor to a

certain stationary phase. In fact, in spite of the large differences in the numerical values of the retention factors, the plots for both columns show very similar patterns. They demonstrate very similar trend reversals, beginning for both columns for the same isopycnic line (0.55 g/mL). The clear conclusion to draw from these results is that the stationary phase plays an insignificant role in the trend

Table 1
Retention factors of anthracene and pyrene on the C18 column.

Temperature (K)	Pressure (bar)	Density (g/mL)	k_1	k_2
310.0	81.0	0.35	3.97	9.91
320.0	93.0	0.35	6.67	17.55
340.0	117.0	0.35	9.40	24.23
360.0	141.0	0.35	7.28	17.79
310.0	84.0	0.45	3.46	8.56
320.0	100.0	0.45	4.36	10.79
340.0	134.0	0.45	4.30	10.29
360.0	167.0	0.45	3.23	7.25
310.0	86.0	0.55	3.20	7.86
320.0	108.0	0.55	3.12	7.47
340.0	154.0	0.55	2.39	5.42
360.0	200.0	0.55	1.58	3.38
310.0	94.0	0.65	2.51	6.07
320.0	124.0	0.65	2.13	4.95
340.0	186.0	0.65	1.36	2.94
360.0	249.0	0.65	0.90	1.81
310.0	121.0	0.75	1.54	3.64
320.0	163.0	0.75	1.26	2.80
340.0	248.0	0.75	0.79	1.60

Table 2
Retention factors of anthracene and pyrene on the neat silica column.

Temperature (K)	Pressure (bar)	Density (g/mL)	k_1	k_2
310	81.0	0.35	1.90	3.04
320	93.0	0.35	3.30	5.41
340	117.0	0.35	5.51	9.35
360	141.0	0.35	4.94	8.95
310	84.0	0.45	1.62	2.60
320	100.0	0.45	2.21	3.56
340	134.0	0.45	2.63	4.28
360	167.0	0.45	2.32	3.73
310	86.0	0.55	1.46	2.33
320	108.0	0.55	1.59	2.53
340	154.0	0.55	1.47	2.32
360	200.0	0.55	1.18	1.83
310	94.0	0.65	1.14	1.79
320	124.0	0.65	1.05	1.63
340	186.0	0.65	0.82	1.25
360	249.0	0.65	0.63	0.95
310	121.0	0.75	0.70	1.07
320	163.0	0.75	0.61	0.92
340	248.0	0.75	0.45	0.66

reversal of the plots of the retention factor versus the temperature along isopycnic lines.

4.2. Possible role of the solute molecules

Both compounds considered in our earlier study [3] were PNAs. To check out whether the trend reversal or early elution was specific of PNA compounds, the retention factors of two aliphatic compounds, octylbenzene and octadecene, were measured. They were selected because both have a chromophore and can be detected by the photo-diode array detector. The retention factors of both compounds are plotted versus the temperature in Fig. 8. The measurements were made under isopycnic conditions, at 0.35, 0.45, 0.55, and 0.65 g/mL, at temperatures of 320, 340 and 360 K. These data show for both compounds the same type of trend reversal or early elution as previously observed for the two PNAs. This result confirms the general character of the phenomenon, independently of the molecular structure of the component considered.

4.3. Possible role of the mobile phase

Since the cause of the phenomenon investigated cannot be related to either the stationary phase or the solute molecules, the mobile phase needs to be considered. Several factors related to the particular properties of the mobile phase could lead to the elution of the compounds studied earlier than expected. This could be due to: (1) the high compressibility of CO₂ in the zone considered which could affect the thermal homogeneity of the column; (2) the adsorption of small amounts of water contained in the stream of CO₂, which could affect retention; (3) the presence of a significant concentration of helium; or (4) the adsorption of CO₂ itself on the stationary phase, which could modify the solid/fluid interface.

The expansion along the column of the high compressibility mobile phase may absorb significant amount of heat, depending upon the pressure and density gradients along the column. The center of the column cools down. It absorbs heat from the peripheral region, which does absorb heat from the column wall and from the atmosphere around the column [32]. When this heat effect becomes sufficient, a radial thermal gradient forms across the column, resulting in radial gradients of the bed temperature, the mobile phase viscosity hence its velocity, and the analyte retention factors. Bands become warped during their migration along the column, the peripheral part of these bands moving faster than their central part; this broadens their elution profiles and drastically reduces their efficiency [33]. Even when the heat effect is so moderate that band profiles are barely affected, the apparent retention factor could be decreased because the average temperature of the column would be lower than the set temperature, leading to a retention factor lower than it should be, which is the effect observed. Another possible factor could be that some of the water contained in the mobile phase could adsorb on the stationary phase surface, forming an aqueous layer and leading to an early elution of the solutes. Since water adsorption depends much on the temperature, this might explain the phenomenon studied. The possible role of helium on the retention of solutes seems to be related to its dilution of the mobile phase (see Section 4.3.3). Finally, the effect investigated might be related to the adsorption of the mobile phase itself on the stationary phase. In this section we analyze each of these plausible factors to understand what could be their effects on the apparent retention of solutes.

4.3.1. Role of the mobile phase expansion in the column

The data reported in our previous study [8] and shown in Fig. 5 were acquired with a 0.46 × 15 cm Atlantis C18 column (Waters, Milford, MA, USA) packed with 3 μm particles, eluted at a flow rate of 3 mL/min. Due to its low permeability, this column has a

significant pressure drop and these data had been acquired in the most compressible zone of the CO₂ pressure–temperature plane investigated [8]. Under these conditions, the pressure drop along the column might have led to a significant density drop, hence to thermal heterogeneity throughout the column, with the formation of radial and axial temperature gradients. The amplitude of these gradients could be substantial in the region of interest since the coefficient of thermal expansion increases exponentially in the critical region of CO₂ [34].

We made new measurements with a column of the same size, packed with 10 μm Prototype XBridge C₁₈ particles, at a flow rate of 2 mL/min. Although this flow rate is higher than the one corresponding to the maximum efficiency, it is the lowest at which the back pressure regulator of the instrument operates satisfactorily. The permeability of the new columns are 11 times higher and the pressure drop about 17 times lower, which markedly reduces the drop of mobile phase density along the column, hence the amount of heat absorbed due to the mobile phase expansion. The difference between the inlet and the outlet pressures of the column was moderate, less than 4 bar. This still might result in a loss of heat due to expansion of the mobile phase, an endothermal process [32,33,35]. However, under the experimental conditions selected for these new measurements, the heat effect is much less than that observed by Kaczmarek et al. [33] when they recorded band profiles at 322 K with an average density equal to the critical density and a pressure difference between the column inlet and outlet of 30 bar ($P_i = 120.3$ bar, $P_o = 91.0$ bar). The outlet density in our column was 0.35 instead of 0.46 g/cm³, the eluent compressibility similar, and the pressure drop eight times less. Under similar conditions, Kaczmarek et al. had observed no effect on the band profiles [33]. This suggests that the retention factors measured with our high-permeability column are not significantly affected by the mobile phase expansion.

To verify that a heat effect does not affect the pattern of retention factor variation, we measured the retention factors of anthracene in different points close to those for which the earlier data had been reported on the 0.35 g/mL isopycnic line (the points at 320 K, 93 bar (Point 1) and at 340 K, 117 bar (Point 2), see Fig. 9). These points were selected because a strong trend reversal was observed between Points 1 and 2 and both points are in an area on the pressure–temperature plane where the compressibility is moderate.

The pressure drop across the new column when measuring the retention factors in Points 1 and 2 was 3 bar. The estimated temperature drops, obtained from numerical calculations [36] for the current experimental conditions, were less than 1 K. Due to the design of the SFC instrument, when the back pressure is set to a certain value (e.g., 93 bar), the average pressure in the column is actually higher. By setting the back pressure to slightly different values (by 1–2 bar), we measured the retention factors under a range of operating conditions close to the intended 93 bar. Similarly, due to the mobile phase expansion, the average operating temperature may be slightly lower than the set temperature. So by setting the column temperature to 1 K higher than the previously set temperature, we assess the possible influence of a temperature drop. All the experimental points around Points 1 and 2 are shown in Fig. 9a. The data provided in Fig. 9b, which are the retention factors of anthracene in the selected experimental points, show that the retention factor of anthracene varies around 8 but is always less than 9.21 in the neighborhood of Point 1. In other words, even if the actual experimental pressure in the column is 2 bar higher than the set pressure and the actual temperature 1 K lesser, the true retention factor of anthracene at 320 K and 93 bar does not exceed 9.21. Actually, our records show that the pressure varies by less than 2 bar and the column temperature by less than 1 K. So, the retention factor of anthracene at 320 K, under 93 bar is between 6.67

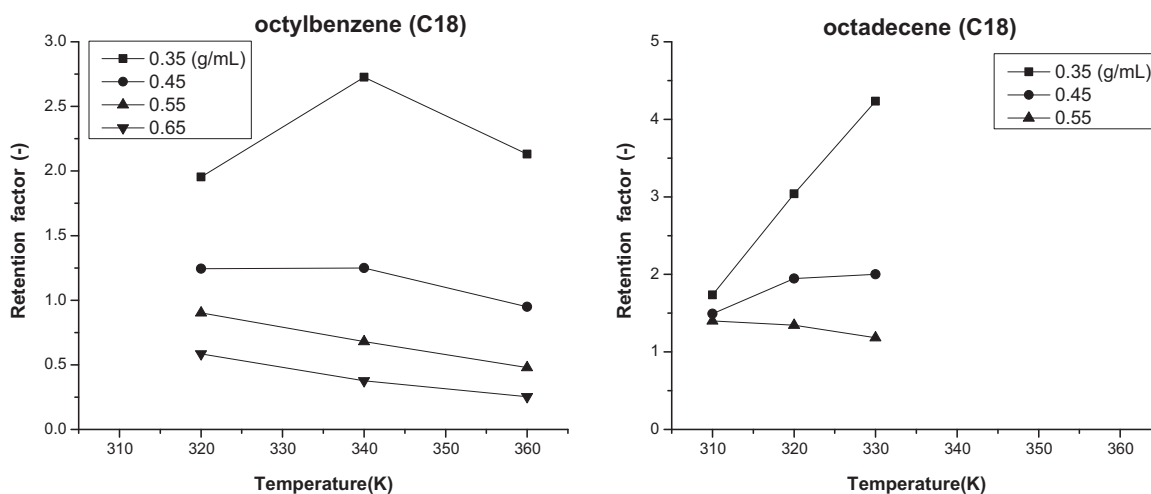


Fig. 8. Plots of the retention factors of octylbenzene and octadecene versus the temperature along isopycnic lines in a C18 column.

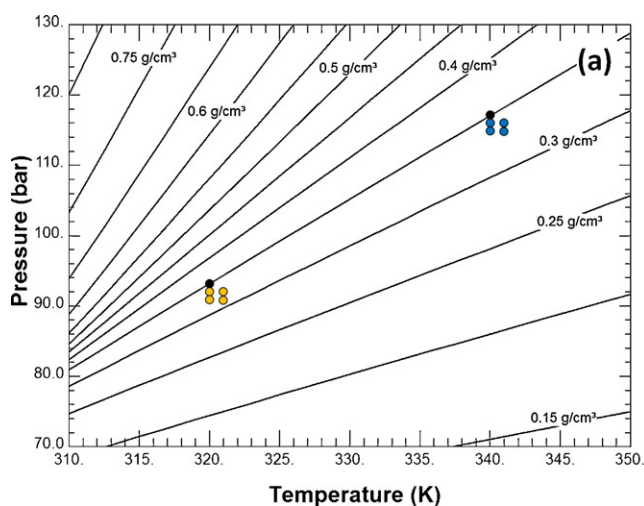
and 9.21. Following a similar approach, the true retention factor at 340 K, under 117 bar is between 9.4 and 11.6. Thus, the highest possible k value at 320 K, under 93 bar is lower than the lowest possible k value at 340 K, under 117 bar (Fig. 9b). This demonstrates that the compressibility of the mobile phase cannot cause the trend reversal of retention factors.

4.3.2. Possible role of water adsorption

Water strongly adsorbs on silica surfaces, interfering with analyte adsorption, decreasing its energy. The extent of water adsorption on silica depends on the temperature and the pressure [37]. Water is weakly soluble in carbon dioxide and there is always some water in the commercial product used as the mobile phase in SFC. From the mobile phase, water may adsorb onto the stationary phase in the column. To understand the influence of water adsorption on the retention factors we carried out two sets of experiments.

First, we saturated the silica surface with water, by pumping HPLC-grade water (Fisher Scientific) through the neat silica column at 1 mL/min, for 2 h, at 22 °C. The column was then fitted to the SFC instrument set at 320 K and the retention times of benzene, anthracene and pyrene were measured, with an inlet pressure of 93 bar. Due to the presence of liquid water blocking the flow of CO₂, which is compressible, the elution profiles were very noisy

preventing the identification of the peak of benzene. Although the base line correction method implemented by the Empower software allowed the measurement of the retention times of anthracene and pyrene, we were unable to calculate their retention factors. However their elution times were significantly shorter than those measured before introducing water inside the neat silica column. The column was continuously purged with neat CO₂ for 5 h while measuring the retention times of anthracene and pyrene every 1 h. The data are shown in Fig. 10. This plot shows that the initial elution times of both anthracene and pyrene were significantly shorter than those measured at 320 K, 93 bar, before introducing water inside the neat silica column (represented by the straight lines in the figure). This was expected because, initially, the pore accessibility of the solute molecules is severely restricted by the water molecules occupying all the pores and inter-particle spaces, causing significantly faster elution. Continuous purging with CO₂ resulted into increasing availability of the silica surface for adsorption, as shown by the increasing retention times of both compounds, which finally become equal to the pre-treatment retention times. This result shows that, irrespective of the initial water content of the column, an equilibrium between the water contents of the mobile and the stationary phases is achieved rather rapidly, given the limited solubility of water in carbon



(b)

320 K, 93bar		340 K, 117bar	
6.67		9.40	
320 K, 92bar	321 K, 92bar	340 K, 116bar	341 K, 116bar
7.53	8.81	10.18	10.89
320 K, 91bar	321 K, 91bar	340 K, 115bar	341 K, 115bar
7.87	9.21	11.13	11.60

Fig. 9. Retention factors of anthracene around some neighboring points of 320 K, 93 bar and 340 K, 117 bar. (a) The location of the points on the pressure–temperature plane of CO₂ to provide an estimate of the relative compressibility of the mobile phase around the two experimental points. (b) The retention factors corresponding to the points shown in (a). The data displayed in the table are arranged in the order of the respective positions of the neighboring points around the main points (shown in dark color).

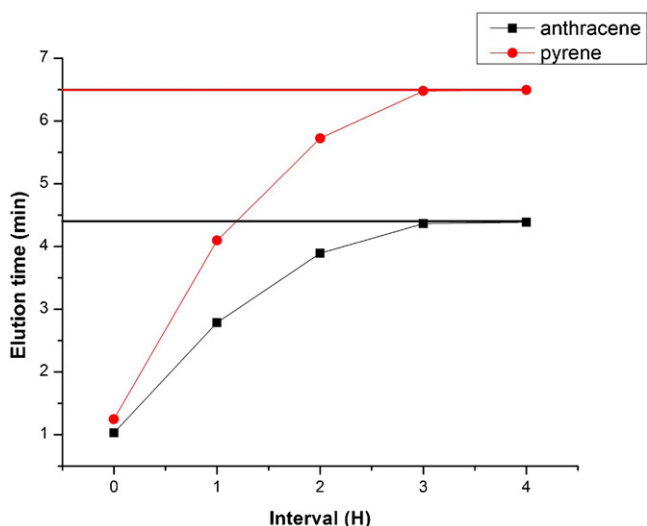


Fig. 10. Retention times of anthracene and pyrene on the neat silica column eluted with neat CO₂. The column was first saturated with water and then purged with neat CO₂ at 320 K, under 93 bar, over a period of 5 h. The X-axis shows the increment in retention time due to the continuous purging of the adsorbed water. The straight lines show the retention times of these two compounds measured before saturating the column with water, under the same set of experimental conditions.

dioxide. This also shows that the presence of water as an impurity in the mobile phase does not cause any permanent change of the stationary phase–mobile phase interface and cannot result in an earlier than expected elution of the compounds.

Equilibration processes were not studied under other experimental conditions but we measured the equilibration time when the column was initially dry. For this purpose, the neat silica column was placed in a oven at 200°C, continuously purged with dry nitrogen, then immediately fitted to the SFC instrument and measurements were made at 320 K, under 93 bar. From the elution times of anthracene and pyrene it was clear that the column equilibrated quickly with the mobile phase and the retention times returned rapidly to their initial values. The experimental results are not shown. These two experiments confirm that the water content of the silica surface is controlled by the water content of the mobile phase and that both phases reach equilibrium rather rapidly.

To further verify the influence of the water content of the mobile phase on the retention factor, we replaced the industrial grade

CO₂ used in other experiments (water concentration <200 ppm) by a research grade CO₂ from Airgas (water concentration <2 ppm). The retention factors were measured at only two temperatures 320 and 340 K since it is between these two temperatures that the phenomenon of early elution takes place most strongly, at low densities. The results of these experiments are shown in Fig. 11. The data show that the retention factors measured with research grade CO₂ (solid lines) were always slightly larger than those measured with industrial grade CO₂. This result is explained by the smaller amount of water being adsorbed on the silica surface at equilibrium with its lower concentration in the purer grade carbon dioxide. Even at 0.35 g/mL, the density for which the difference between the retention factors measured with the research grade and the industrial grade experiments is the most significant, the ratio of the retention factors at 320 and 340 K is nearly constant. This demonstrates that, although the amount of water present in the CO₂ mobile phase controls the retention times of the solutes, the changes are small and cannot explain the phenomenon observed, of an elution earlier than expected under specific experimental conditions, leading to a trend reversal.

The influence of water on the retention of the anthracene and pyrene on the C₁₈ bonded silica was not investigated, since its influence on their retention on silica was found to be unimportant and certainly not able to affect the trend of the retention pattern reported earlier.

4.3.3. Influence of helium

Helium has often been added to cylinders of carbon dioxide to pressurize the head space. It has been reported that its presence may influence retention but these reports are limited and contradictory. Porter et al. [38] reported serious reproducibility problems of retention times and peak area associated with the use of a mixture of helium and carbon dioxide as the mobile phase. In contrast, neither Schwartz et al. [39] nor Rosselli et al. [40] experienced any other effect than a slight decrease in retention times.

Perrut et al. [41] reported that the influence of the helium concentration in mixtures with carbon dioxide depends much on the helium concentration. At a concentration of ca. 5%, they found that the retention factors of methyl oleate, methyl linolenate, dibenzyl and methyl benzoate were about twice as much than they are in pure carbon dioxide. However, when the concentration is only 3%, as was the mobile phase used by Rosselli et al. and Porter et al., the effect is markedly less. This effect is explained by the density of the mixtures, which is lower than that of pure carbon dioxide at the same temperature, pressure and flow rate.

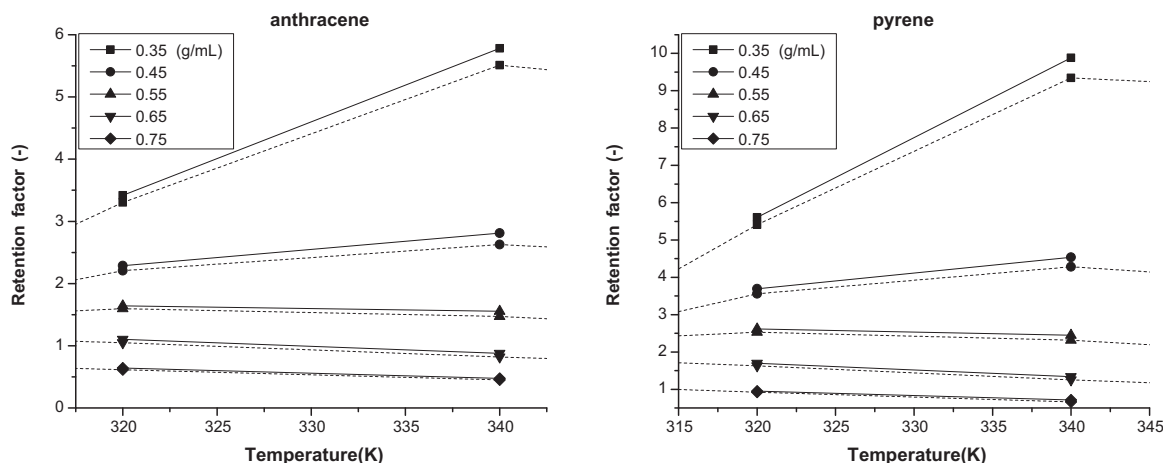


Fig. 11. Comparison between the retention factors of anthracene and pyrene measured with research grade (solid lines) and industrial grade (dashed lines) CO₂ in a neat silica column. The lesser water content of research grade CO₂ leads to a marginally higher retention factor, but the variation trend remains the same.

The carbon dioxide that we used as the mobile phase did not contain any helium nor any significant concentration of any other light gases.

4.3.4. Influence of the adsorption of carbon dioxide on the stationary phase

As explained earlier in the Theory section, the adsorption of CO₂ on the stationary phase surface becomes very important near the critical temperature [24,28–30]. The presence of CO₂ adsorbed on the stationary phase influences the adsorption behavior of all analytes. The most striking feature of the results of Strubinger et al. [30] and of Giovanni et al. [29] is that the location of the maxima of the excess adsorption isotherms of CO₂ on both stationary phases studied take place between 0.2 and 0.4 g/mL on each column, slightly below the critical density (0.47 g/mL), in precisely the density region where we observed the maximum deviation in the retention times from the expected linear correlation. It is also important that the excess amount adsorbed decreases rapidly with increasing pressure, density, and temperature, consistent with our observation that the maximum deviation of the retention factors from the expected trend also takes place at a density of about 0.35 g/mL and a temperature of 310 K. This last deviation decreases progressively with increasing density and/or temperature. The similarities, between the experimental conditions under which the excess isotherms measured by Strubinger et al. [28] on an ODS and a silica column and by Giovanni et al. [29] on neat silica, and those under which the unexpected deviations observed in the plots of the retention factors vs. the temperature take place, is striking. Based on this observation, we may now assume that the retention factor variation that we noted near the critical point of CO₂ might be related to the phenomena that control the excess adsorption of CO₂ on the stationary phase in that region. More accurate investigations, however, need to be performed to clarify this link.

5. Conclusion

The retention factors of all solutes decrease constantly with increasing temperature along the isopycnic lines in supercritical fluid chromatography. This trend, however, is reversed near the critical region of CO₂. In that region, the retention factors increase with increasing temperature along the isopycnic lines, they reach maxima and then begin to decrease again. Our investigations of the plausible reasons behind this unexpected behavior demonstrate that this effect is independent of the stationary phase used and of the analytes considered. This leaves the mobile phase as the possible origin of the effect. We could identify three plausible features of the mobile phase that can contribute to such phenomenon: (1) the high compressibility of the mobile phase in the region where the phenomenon is observed; (2) the water content of the mobile phase which could form an aqueous layer on the stationary phase; and (3) the formation of multiple layers of adsorbed CO₂ on the stationary phase. These last two factors could cause an earlier elution of analytes than expected, depending on the thickness of the multilayer formed. However, experimental results prove that the first two factors cannot play any role in the unexpected phenomenon. Based on published data on the adsorption of CO₂ molecules on a

neat silica and an ODS column, the last factor seems to be closely related to the observed change in the retention factor variation in the near critical region. A further study is required to provide a more precise physical explanation of the effect observed.

Acknowledgments

This work was supported in part by grant CHE-1108681 of the National Science Foundation, by financial and technical support by Waters Technologies Corporation, and by the cooperative agreement between the University of Tennessee and the Oak Ridge National Laboratory. We thank Donald Poe (University of Minnesota, Duluth, MN) whose comments on the column behavior was critical in developing a clearer understanding of the role of density on retention and Martin Gilar (Waters Technology Corporation) for fruitful discussions and for his support.

References

- [1] D.H. Desty, A. Goldup, G.R. Luckhurst, W.T. Swanton, in: M.V. Swaay (Ed.), *Gas Chromatography*, Butterworths, London, UK, 1962, p. 67.
- [2] T.L. Chester, D.P. Lnnis, *J. High Resolut. Chromatogr. Chromatogr. Commun.* 8 (1985) 561.
- [3] A. Tarafder, G. Guiochon, *J. Chromatogr. A* 1218 (2011) 4576.
- [4] D. Leyendecker, F.P. Schmitz, E. Klesper, *J. Chromatogr.* 315 (1984) 19.
- [5] K.D. Bartle, A.A. Clifford, J.P. Kithinji, G.F. Shilstone, *J. Chem. Soc. Faraday Trans. 1* 84 (12) (1988) 4487.
- [6] T. Berger, *J. Chromatogr.* 478 (1989) 311.
- [7] M. Roth, *J. Chromatogr.* 641 (1993) 329.
- [8] A. Tarafder, G. Guiochon, *J. Chromatogr. A* 1218 (2011) 4569.
- [9] M. Roth, *J. Phys. Chem.* 96 (1992) 8552.
- [10] P.A. Peaden, M.L. Lee, *J. Chromatogr.* 259 (1983) 1.
- [11] D. Bartmann, G.M. Schneider, *J. Chromatogr.* 83 (1973) 135.
- [12] U. van Wasen, G.M. Schneider, *Chromatographia* 8 (1975) 274.
- [13] U. van Wasen, I. Swaid, G.M. Schneider, *Angew. Chem. Int.* 19 (1980) 585.
- [14] C.B. Kautz, U.H. Dahlmann, G.M. Schneider, *J. Chromatogr. A* 776 (1997) 305.
- [15] A. Rajendran, T.S. Gilkison, M. Mazzotti, *J. Sep. Sci.* 31 (2008) 1279.
- [16] P.J. Schoenmakers, *J. Chromatogr.* 315 (1984) 1.
- [17] M. Perrut, J. Dellacherie, in: M. Perrut (Ed.), *Proceedings of International Symposium on Supercritical Fluids (Nice)*, ENSIC, Nancy, France, 1988, p. 447.
- [18] E.W. Lemmon, M. Huber, M.O. McLinden, D.G. Friend, *National Institute of Standards and Technology, Standard Reference Data Program*, Gaithersburg 8.0.
- [19] U. van Wasen, G.M. Schneider, *J. Phys. Chem.* 84 (1980) 229.
- [20] A. Tarafder, G. Guiochon, *J. Chromatogr. A* 1218 (2011) 1037.
- [21] H. Liu, E.A. Macedo, *Ind. Eng. Chem. Res.* 34 (1995) 2029.
- [22] J.C. Wheeler, *Ber. Bunsenges. Phys. Chem.* 76 (1972) 308.
- [23] C.A. Eckert, D.H. Ziger, K.P. Johnston, S. Kim, *J. Phys. Chem.* 90 (1986) 2738.
- [24] M. Roth, *J. Chromatogr.* 641 (1993) 329.
- [25] B. Subramanian, M.A. McHugh, *Ind. Eng. Chem. Process. Des. Dev.* 25 (1986) 1.
- [26] R. Fernández-Prini, M. Japas, *J. Phys. Chem.* 96 (1992), 5150–5121.
- [27] I.M. Abdulagatov, A.R. Bazaev, E.A. Bazaev, S.P. Khokhlichev, M.B. Saidakhmedova, A.E. Romazanova, *J. Solut. Chem.* 27 (1998) 731.
- [28] J.R. Strubinger, H.S. Song, J.F. Parcher, *Anal. Chem.* 63 (1991) 98.
- [29] O.D. Giovanni, W. Dörfler, M. Mazzotti, M. Morbidelli, *Langmuir* 17 (2001) 4316.
- [30] J.R. Strubinger, J.F. Parcher, *Anal. Chem.* 61 (1989) 951.
- [31] H.H. Lauer, D. McManigill, R.D. Board, *Anal. Chem.* 55 (1983) 1370.
- [32] K. Kaczmarek, D.P. Poe, G. Guiochon, *J. Chromatogr. A* 1217 (2010) 6578.
- [33] K. Kaczmarek, D.P. Poe, G. Guiochon, *J. Chromatogr. A* 1218 (2011) 6531.
- [34] A. Tarafder, G. Guiochon, *J. Chromatogr. A* 1218 (2011) 7189.
- [35] D. Poe, J. Schroden, *J. Chromatogr. A* 1216 (2009) 7915.
- [36] K. Kaczmarek, D.P. Poe, G. Guiochon, *J. Chromatogr. A* 1217 (2010) 6578.
- [37] R.K. Taylor, *Ind. Eng. Chem.* 37 (7) (1945) 649.
- [38] N.L. Porter, B.E. Richter, D.J. Bornhop, D.W. Later, F.H. Bayerlein, *J. High Resolut. Chromatogr. Chromatogr. Commun.* 10 (1987) 477.
- [39] H.E. Schwartz, P.J. Barthel, S.E. Moring, *J. High Resolut. Chromatogr. Chromatogr. Commun.* 10 (1987) 668.
- [40] A.C. Rosselli, D.S. Boyer, R.K. Houck, *J. Chromatogr.* 465 (1989) 11.
- [41] T. Görner, J. Dellacherie, M. Perrut, *J. Chromatogr.* 514 (1990) 309.