Influence of the pressure on the properties of chromatographic columns III. Retention volume of thiourea, hold-up volume, and compressibility of the C_{18}-bonded layer

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Abstract

The influence of the average column pressure (ACP) on the elution volume of thiourea was measured on two RPLC columns, packed with Resolve-C_{18} (surface coverage 2.45 μmol/m^2) and Symmetry-C_{18} (surface coverage 3.18 μmol/m^2), and it was compared to that measured under the same conditions on an underivatized silica (Resolve). Five different methanol–water mixtures (20, 40, 60, 80 and 100% methanol, v/v) were used. Once corrected for the compressibility of the mobile phase, the data show that the elution volume of thiourea increases between 3 and 7% on the C_{18}-bonded columns when the ACP increases from 50 to 350 bar, depending on the methanol content of the eluent. No such increase is observed on the underivatized Resolve silica column. This increase is too large to be ascribed to the compressibility of the stationary phase (silica + C_{18} bonded chains) which accounts for less than 5% of the variation of the retention factor. It is shown that the reason for this effect is of thermodynamic origin, the difference between the partial molar volume of the solute in the stationary and the mobile phase, ΔV, controlling the retention volume of thiourea. While ΔV is nearly constant for all mobile phase compositions on Resolve silica (with $\Delta V \simeq -4$ mL/mol), on RPLC phases, it significantly increases with increasing methanol content, particularly above 60% methanol. It varies between -5 mL/mol and -17 mL/mol on Resolve-C_{18} and between -9 mL/mol and -25 mL/mol on Symmetry-C_{18}. The difference in surface coverage between these two RP-HPLC stationary phases increases the values of ΔV by about 5 mL/mol.
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1. Introduction

A progressive increase of the inlet pressure applied is a major trend recently observed in RPLC. This trend results from the rapidly increasing needs to perform faster and more efficient analyses to satisfy various demands, in organic synthesis, food safety, environmental pollution, biological chemistry, and molecular biology. On conventional instruments, chromatographic columns are conventionally subjected to pressure stresses that do not exceed 400 bar. A survey of recent publications suggests a rapid increase

of the number of applications run at pressures exceeding 300 bar and new instruments are now available that allow operation above 1 kbar. Yet chromatographers tend to neglect the influence of pressure on their data. This is dangerous because another important current trend in chromatography is the improvement in the accuracy of the measurements made. In most cases, the precision of chromatographic measurements (retention factors and peak areas) can now be better than 0.1%. We need to understand better the actual effects of the pressure in liquid chromatography.

To do this, it is important first to understand the effects of the pressure on the different physico-chemical properties of liquids (e.g., melting point, specific volumes, viscosity, diffusion coefficient) that are involved in the chromatographic

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process, as well as on the physical characteristics of the column (cross-section area, length, packing material compressibility). These theoretical aspects of the problem were reviewed by Martin et al. [1–3]. The effects of the pressure on the column tube, that deforms, on the packing materials, that shrink, and on the mobile phases, that are compressed, have been quantitatively assessed using the classical models of metal elasticity and fluid compressibility. This work demonstrated that the compressibility of the mobile phase and that of the bonded layer of RPLC packing materials should be the two main parameters affecting the column hold-up volume (hence the retention times and volumes). This conclusion was validated experimentally [4]. Since chromatography was found to be sensitive to the compressibility of the mobile phase, inversely, it must be possible to derive the compressibility of complex mixtures of liquids from the results of chromatographic measurements of the column hold-up volume [4]. This method was found to give very good estimates of the compressibility of aqueous solutions of methanol and acetonitrile, values in good agreement with the results of other, independent measurements, using a piezoelectric manometric gauge [5,6].

As aforementioned, the compressibility of the packing material, assumed to be a binary system made of noncompressible silica and compressible liquid octadecane, was significant. The volume of a C_{18}-bonded silica containing 10% carbon decreases by about 0.5% when the pressure increases from 1 to 200 bars. This value was derived from the rate of pressure dependence of the elution volume of thiourea on a Resolve-C_{18} column (containing 10% carbon, i.e., with a surface coverage of 2.45 μmol/m^2) and on a Symmetry-C_{18} column (containing 20% carbon, i.e., with a surface coverage 3.18 μmol/m^2) [4]. Using pure methanol as the mobile phase, relative differences of 0.7% were observed for the retention volumes of thiourea on Resolve and Resolve-C_{18}, and on Resolve-C_{18} and Symmetry-C_{18}. However, this increase of the elution volume of thiourea may have two different origins: first, as just suggested, it may be caused by a decrease of the volume of the packing material due to a finite compressibility. Second, if the marker is but slightly retained, it may have different partial molar volumes in the apolar C_{18}-bonded layer and in the polar mobile phase ($\overline{V_s} - \overline{V_l} \neq 0$). This issue has yet to be debated and checked from an experimental point of view if one expects to determine by chromatography the compressibility of the C_{18}-bonded layer.

The literature is still inconclusive regarding the degree of interaction between an organic solvent and the C_{18}-bonded layer on the surface of classical RPLC packing materials. In a recent paper, Rustamov et al. [7] characterized the geometry of chemically modified silica and found that the properties of the alkyl bonded chains were similar to those of the corresponding liquid alkanes. They measured pore volumes that were consistent with the assumption that the organic modifiers that they used (MeCN, MeOH, and THF) do not penetrate between the bonded chains at any water-organic

solvent composition. Henry et al. [8] studied the influence of the solvent on the degree of order of alkyl chains by sum frequency generation spectroscopy and found that the C_{18}-bonded chains were resistant to solvent induced disorder. McDonald showed that water and water-rich aqueous solutions of methanol (less than 10% methanol) do not wet hydrophobic surfaces [9]. Pore-dewetting prevents access of the mobile phase to the mesopore structure, depending to some extent on the average column pressure, when the contact angle between the mobile phase and the solid support exceeds 90°. On the other hand, nuclear magnetic resonance (NMR) and Fourier transform infrared spectroscopy (FT-IR) investigations demonstrated the existence of interactions between the organic modifier and the octadecyl chains [10–12]. Small angle neutron scattering was used to measure the volume fraction of the alkyl chains within the bonded phase layer [13]. Sander et al. found that approximately 35% of the bonded phase volume was occupied by methanol when pure methanol was used as the liquid phase. Indirectly, chromatographic measurements also imply that the organic modifier penetrates between the bonded chains [14], leading to a partition mechanism, at least for some appropriate analytes (small or planar). The void volume of RPLC columns, measured by recording the elution volume of a hold-up column marker, decreases significantly with increasing methanol concentration in the mobile phase [15], suggesting that the bonded chains expand in the presence of the organic modifier and quit their "collapsed state" in water-rich aqueous solvents. There is controversy regarding the nature of the "collapsed" state of bonded chains and this idea is not well supported by current thinking in column technology. It would be explained by pore de-wetting, as suggested by McDonald [9]. But this takes place only at very high water concentrations. Otherwise, chains tend to be in a more or less extended conformation. These contradictory conclusions found in the literature show how difficult it is clearly to interpret the data acquired and to give a clear physical description of the structure of the C_{18}-bonded layer interface in the presence of a liquid mobile phase.

To clarify this issue, we measured the influence of pressure on the retention volume of thiourea and the column hold-up volume using two different RPLC packing materials, Resolve-C_{18} and Symmetry-C_{18}, which have carbon loadings of 10 and 20% and chain densities of 2.45 and 3.18 μmol/m^2 C_{18}, respectively. The measurements were carried out using aqueous solutions of methanol containing 20, 40, 60, 80 and 100% methanol. The evolution of the elution volume of a compound that is "almost" unretained (thiourea) with increasing average column pressure will be discussed and the results compared to those already acquired on an underivatized silica adsorbent (Resolve) [16]. The data were corrected for the compressibility of the bulk liquid phase. The significant residual influence of the pressure could be either the compressibility of the bonded phase layer (mechanical origin) or the possible adsorption of thiourea on or in the alkyl bonded layer (thermodynamic origin).

2. Theory

2.1. Compressibility of the bulk liquid phase

2.1.1. The Tait equation

The compressibility of the mobile phase percolating through a chromatographic bed can be derived from Tait equation [17]. If P_0 and P are the reference and the actual pressure of the liquid, the volume occupied by a certain mass of liquid, $V(P)$, can be derived from its volume V_0 under the reference (e.g., normal) pressure

$$V(P) = V_0 \left[1 + c \ln \left(\frac{P_0 + b}{P + b} \right) \right] \tag{1}$$

where b and c are two model parameters. Some values of b and c are given for several pure solvents in reference [3].

2.2. Compressibility of the packing material

The compressibility of the stationary phase was calculated by assuming that the silica bonded phase consists in a simple, immiscible binary system made of pure silica (compressibility $\beta_{silica}=10^{-6}\,\text{bar}^{-1}$) and pure octadecane liquid (compressibility $\beta_{C_{18}} = 10^{-4}\,\text{bar}^{-1}$). The average compressibility of the material can be derived once the volume fraction occupied by the C_{18} chains in the packing material is known [4]:

$$\beta_m = \phi_{C_{18}}\beta_{C_{18}} + \phi_{silica}\beta_{silica} \tag{2}$$

According to this model, the compressibility of packing materials should range between 0.1×10^{-4} and $0.5 \times 10^{-4}\,\text{bar}^{-1}$ since the volume fraction occupied by the C_{18} chains in RPLC is almost always between 0.1 and 0.5. The compressibility of silica is negligible.

3. Experimental

3.1. Chemicals

The mobile phases used in this work were mixtures of methanol and water (20, 40, 60, 80 and 100% methanol, v/v). Both solvents were HPLC grade and purchased from Fisher Scientific (Fair Lawn, NJ, USA). These solvents were filtered before use on an SFCA filter membrane, 0.2 μm pore size(Suwannee, GA, USA). Thiourea was obtained from Aldrich (Milwaukee, WI, USA).

3.2. Materials

Three different columns were used in this work. The pure Resolve silica column serves as a reference because it contains no bonded alkyl chain. The properties and the results obtained with this column were given elsewhere [16]. The other two columns were packed with Resolve-C_{18} and

Table 1
Physico-chemical properties of the adsorbent materials packed in a stainless steel tube (150 × 3.9 mm)

	Resolve	Resolve-C_{18}	Symmetry-C_{18}
O.D./I.D. ratio diameter	1.87	1.87	1.87
Particle shape	Spherical	Spherical	Spherical
Particle size (μm)	5	5	5
Pore size (Å)	90	90	86
Pore volume (mL/g)	0.50	0.50	0.90
Surface area (m²/g)	200	200	346
Total carbon (%)	0	10.2	19.6
Surface coverage (μmol/m²)	0	2.45	3.18
Endcapping	No	No	Yes

The outer diameter (O.D.) of the tube is 7.3 mm.

Symmetry-C_{18} (Waters, Milford, MA, USA). Their physical properties are given in Table 1. Resolve-C_{18} was chose because it has exactly the same silica support as the reference column and it contains only 10% carbon. Symmetry-C_{18} was chosen because it has twice as much carbon (20%) as Resolve-C_{18} and is provided by the same company. Both columns have the same dimensions, 150 mm × 3.9 mm. Several PEEK tubings (inner diameter 0.0025 in., 1.66, 3.33 and 5.0 ft length) were purchased from Upchurch Scientific (Oak Harbor, WA, USA) in order to generate a high flow resistance and allow an easy change of the pressure profiles along the column. The experimental protocol was given earlier [16]. The HPLC apparatus was the same as this used in [16].

4. Results and discussion

4.1. Elution volume of thiourea measured under NTP conditions

The elution volumes of thiourea measured on Resolve-C_{18} and on Symmetry-C_{18} are listed in Tables 2 and 3, respectively. These tables give also the inlet, the oulet, and the average column pressures, and the extra-column volumes measured with a stainless steel union.

Figs. 1–3 show plots of the elution volumes of thiourea versus the average column pressure for the three adsorbents studied and for the five methanol–water mixtures successively used as the mobile phase. Note that pure water was not used because water does not wet properly the hydrophobic surface in the mesopore structure of the RPLC adsorbents [15]. It can enter the mesopores only if a suitably high pressure is applied and, then, it is instantaneous expelled upon any pressure release. This phenomenon, which is easily observed, leads to poorly reproducible results unless great care is applied to keep water in the mesopores. This is the same phenomenon as mercury intrusion used to measure the pore distribution of porous silica materials. The fundamental reason is that the contact angle of water on C_{18}-bonded surfaces like that of mercury on pure silica exceeds $90°$.

Table 2
Corrected elution time of thiourea measured under NTP conditions on the Resolve-C_{18} adsorbent as a function of the mobile phase composition and average column pressure

Methanol content (%, v/v)	Length of capillaries (ft)	Column outlet pressure (bar)	Column inlet pressure (bar)	Column average pressure (bar)	Extra column volume (mL)	Thiourea elution volume (mL)
20	0′	16	133	75	0.086	1.170
	5/3′	95	211	153	0.086	1.176
	10/3′	176	290	233	0.086	1.184
	5/3 + 10/3′	260	372	316	0.086	1.199
40	0′	20	164	92	0.085	1.108
	5/3′	115	261	188	0.085	1.119
	10/3′	213	360	287	0.085	1.134
60	0′	18	155	87	0.086	1.081
	5/3′	109	246	178	0.086	1.093
	10/3′	202	341	272	0.086	1.106
80	0′	13	112	63	0.086	1.073
	5/3′	80	181	131	0.086	1.083
	10/3′	149	254	202	0.086	1.095
	5′	272	377	325	0.086	1.117
100	0′	7	55	31	0.088	1.078
	5/3′	41	91	66	0.089	1.084
	10/3′	76	126	101	0.089	1.092
	5′	139	189	164	0.090	1.102
	5 + 1/3′	176	226	201	0.091	1.111
	5 + 10/3′	211	263	237	0.091	1.119
	5 + 5′	246	297	272	0.092	1.126
	5 + 5 + 5/3′	317	370	344	0.092	1.145

For each mobile phase and each adsorbent, the elution volume of thiourea was extrapolated to zero pressure. The values obtained for each column are plotted versus the methanol fraction in Fig. 4. Obviously, the elution volume increases from Symmetry-C_{18} to Resolve-C_{18} and to Resolve because the volume of the bonded layer is larger in Symmetry-C_{18} than in Resolve-C_{18} and is zero in Resolve, due essentially to their different carbon contents (Table 1). Also, the shape of the curves differs considerably if there is a C_{18}-bonded layer on the silica surface or not. With pure silica, the elution volume of thiourea is minimum for a mixture of water and methanol and maximum with pure methanol, due to the

Table 3
Corrected elution time of thiourea measured under NTP conditions on the Symmetry-C_{18} adsorbent as a function of the mobile phase composition and average column pressure

| Methanol content(%, v/v)	Length of capillaries (ft)	Column outlet pressure (bar)	Column inlet pressure (bar)	Column average pressure (bar)	Extra column volume (mL)	Thiourea elution volume (mL)
20	0′	16	155	86	0.086	1.099
	5/3′	95	232	164	0.086	1.108
	10/3′	176	312	244	0.086	1.119
	5/3 + 10/3′	260	393	327	0.086	1.141
40	0′	20	189	105	0.085	1.042
	5/3′	115	289	202	0.085	1.054
	10/3′	213	385	299	0.085	1.074
60	0′	18	178	98	0.086	1.014
	5/3′	109	270	190	0.086	1.025
	10/3′	202	365	284	0.086	1.039
80	0′	13	133	73	0.086	1.003
	5/3′	80	201	141	0.086	1.014
	10/3′	149	272	211	0.086	1.025
	5′	272	398	335	0.086	1.052
100	0′	7	64	36	0.088	0.995
	5/3′	41	100	71	0.089	1.003
	10/3′	76	136	106	0.089	1.009
	5′	139	200	170	0.090	1.022
	5 + 1/3′	176	236	206	0.091	1.03
	5 + 10/3′	211	273	242	0.091	1.037
	5 + 5′	246	309	278	0.092	1.046
	5 + 5 + 5/3′	317	379	348	0.092	1.068
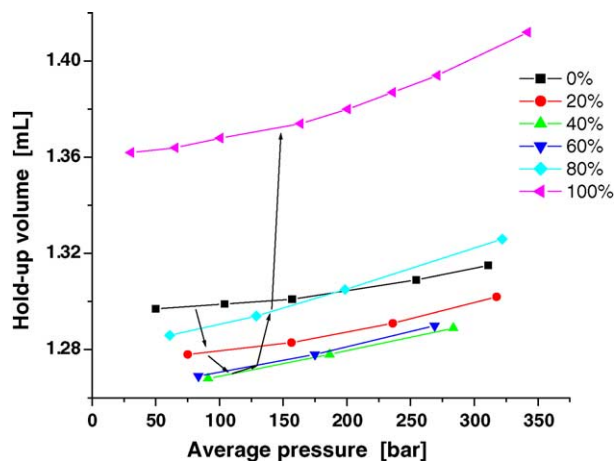
Fig. 1. Variation of the elution volume of thiourea on the Resolve silica adsorbent using different mixtures of methanol and water as the mobile phases. The elution volumes are measured from the elution time of thiourea (2 μL injection of a solution at less than 1 g/L, UV detection 270 nm) at a flow rate of 1 mL/min. $T = 295$ K. Note the decreasing and increasing elution time of thiourea when the methanol content increases in the mobile phase.
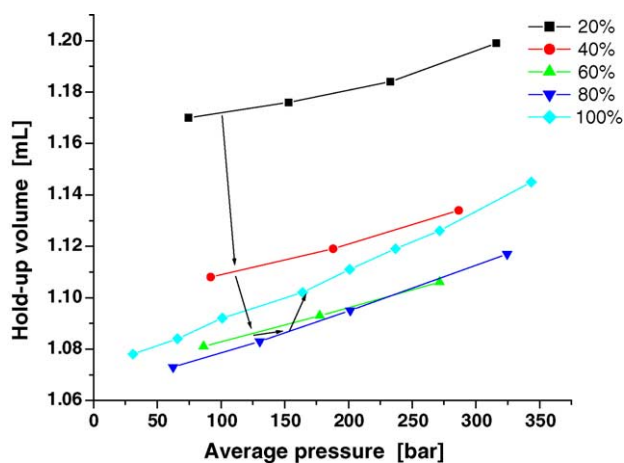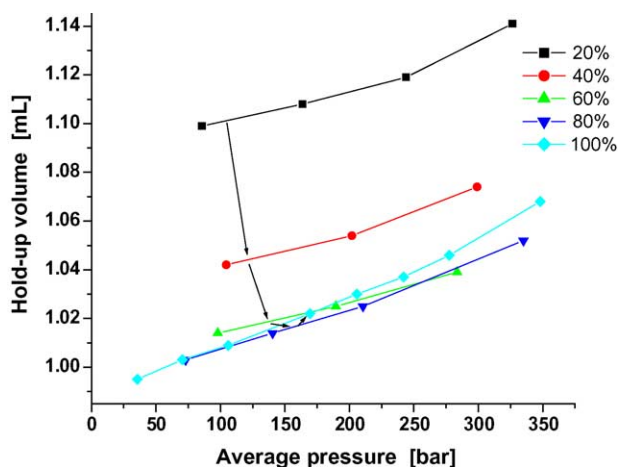

Fig. 2. Same as in Fig. 1 except for the Resolve-C_{18} adsorbent.

Fig. 3. Same as in Fig. 1 except for the Symmetry-C_{18} adsorbent.
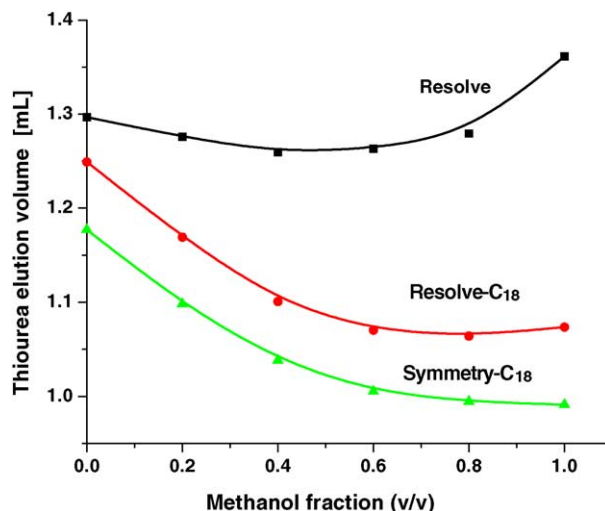
Fig. 4. Evolution of the elution volume of thiourea extrapolated at the hypothetical pressure of 0 bar vs. the mobile phase composition for the three different chromatographic adsorbents.

different solubilities of thiourea in these solutions [16]. With alkyl-bonded silicas, the elution volume of thiourea decreases with decreasing water content of the mobile phase. A possible explanation could be that the increasing solvation of the C_{18}-bonded chains with increasing organic modifier (methanol) content. Accordingly, the bonded layer would swell due to the penetration of adsorbed methanol molecules around the chains, it would occupy a larger volume which would lead to a decrease of the void volume (i.e., of the volume occupied by the free or bulk mobile phase).

However, as reported earlier, when Rustamov et al. [7] compared the void volumes of MeCN, MeOH, and THF measured by the minor disturbance method [18,19] on alkyl-bonded silicas having different alkyl chain lengths, they found no significant changes of void volume. They also measured the same pore volume independently of the method used, whether the minor disturbance method (in which the chains are in contact with a pure liquid) or low temperature nitrogen adsorption method (in which the chains are under vacuum and most probably collapsed). They concluded that the conformation of the alkyl chains on the silica is the densest, "collapsed" or "liquid like". They stated that the penetration of organic solvent molecules between the C_{18} chains is unlikely. Other authors [13] characterized various alkyl-modified silicas by small angle neutron scattering (using a CH_3OH/CD_3OD mixture to match the scattering length density of the silica). They showed that the thickness of the C_{18}-bonded layer is about 17 Å while in their most extended conformation, these chains are 23 Å long and that the volume fraction of the alkyl chains in the bonded layer was about 65%. The complementary volume described methanol associated with the alkyl chains and the degree of solvation of the octadecyl chains by methanol. The presence of methanol in water dramatically increases the solubility of hydrocarbons. The addition of 0.2% (w/w) of methanol to

water increases the solubility of hydrocarbons 10-fold [20]. Methanol is a classical cosolvent that enhances the solubility of heavy hydrocarbons in supercritical CO_2. Because the solubility, S_w (mol L^{-1}), of octadecane is almost zero in pure water (pS_w = 8.08 [21]), we may expect a progressive increase of the solvation of the octadecyl chains when methanol is added to the mobile phase.

The reason why Rustamov could show that increasing the concentration of methanol, acetonitrile, or THF in water does not change the column void volume measured by the minor disturbance method is that this method measures the thermodynamical void volume V_M [18], which assumes that the volume of the bonded phase is zero. Thus, it is independent of the nature of the eluent used to measure V_M. They used the concept of excess isotherm relative to the interface area between the liquid and the solid phase. V_M is calculated as the average of the elution volume of the organic modifier disturbance peak from methanol–water solutions in the entire composition range (from pure water to pure methanol) using the equation

$$V_M = \int_{x=0}^{x=1} V_R(x)\,dx \tag{3}$$

where $V_R(x)$ is the elution volume of a perturbation pulse of methanol injected on a concentration plateau of methanol at the volume fraction x.

Systematic measurements [19] show that the elution volume of the methanol perturbations decreases with increasing volume fraction of methanol from 0 to 70% methanol because the amount of methanol adsorbed between the bonded chains increases less rapidly than the methanol content (convex upward isotherm). A change of the curvature of the plot of the retention time of these perturbations around 20% methanol indicates the beginning of the intrusion of the liquid phase within the bonded layer and of the swelling of the collapsed chains. Beyond about 70% methanol, the retention volume of these perturbations increases because, the adsorption isotherm of methanol exhibiting an S-shaped behavior [18,19], the amount adsorbed in the solid phase increases faster than the methanol content. It seems that there is a C_{18} "solid–liquid" phase transition. The capacity of adsorption of methanol increases and the perturbations are more retained. The "U" shape of the plot of the retention volume of the minor disturbances versus the methanol content is inconsistent with the bonded phase having the same structure and a constant surface area of contact with the liquid phase. If there were monolayer adsorption of methanol, the elution time of the perturbation peaks of methanol would continuously decrease with increasing plateau concentration because the surface would get closer and closer to saturation. According to the literature on RPLC, however, when the content of methanol in an aqueous mobile phase increases, the C_{18}-bonded chains unfold leading to an increase of their surface area of contact with the liquid. This area is maximum with pure methanol and, according to reference [8], adsorbed methanol occupies

about 35% of the volume of the C_{18}-bonded layer. The shape of the plot of the retention times of minor disturbances of methanol showed in reference [19] seems most consistent with a swelling of the bonded layer due to the progressive intrusion of the liquid phase when the content of organic modifier increases.

When using thiourea as the hold-up volume marker, we must remember that we are now dealing with the ternary methanol–water–thiourea system. Since thiourea is injected in extremely small amount and its concentration remains infinitesimal, the concept of surface saturation does not apply and its isotherm has a linear behavior. However, thiourea is in strong competition with methanol for adsorption onto the bonded chains. So, the elution volumes of thiourea plotted in Fig. 4 are related to the initial slope (at zero thiourea concentration) of its competitive adsorption isotherm from water in the presence of methanol. The free geometrical volumes of the columns, V_0, measured by pycnometry, using methanol and dichloromethane as the two solvents to fill and weigh the column, were 0.970 and 0.905 mL for Resolve-C_{18} and Symmetry-C_{18}, respectively. Methanol and dichloromethane wet spontaneously the C_{18}-bonded surface, occupying all the accessible volume between the bonded chains, inside the mesopores, and between the particles. Yet, these volumes are smaller than the elution volumes of thiourea. We know that thiourea is adsorbed on the pure Resolve silica ($0.04 < k' < 0.12$) [16]. Similarly, thiourea is slightly retained on the derivatized adsorbents. Typically, k' varies between 0.10 and 0.20 when the methanol content decreases from 100 to 20%, as shown by measurements of the retention factor of thiourea made on Symmetry-C_{18} at these two mobile phase compositions and at different temperatures, and by taking into account the compressibility of the solvent contained in the column between the inlet, P^{ol}, and the outlet, P^{il}, pressures. The retention factor is given by

$$k' = \frac{V_R - V_M}{V_M} \tag{4}$$

The retention volume, V_R is measured. The hold-up volume is derived from the pycnometric volume, accounting for the compressibility of the mobile phase. Assuming that the pressure gradient is linear along the column, the integration of Eq. (1) from the outlet to the inlet pressure gives:

$$V_M = \frac{V_0}{P^{il} - P^{ol}} \int_{P^{ol}}^{P^{il}} \frac{dP}{1 + c\,\ln((P_0 + b)/(P + b))} \tag{5}$$

The Van't Hoff plots are given in Fig. 5. The Van't Hoff relationship can be written:

$$\ln k' = -\frac{\Delta H^0}{R}\frac{1}{T} + \frac{\Delta S^0}{R} + \ln \beta \tag{6}$$

If the adsorption enthalpy and entropy do not depend much on the temperature, a linear plot is expected.

The experimental results confirm that the retention of thiourea decreases with increasing temperature, demonstrating that the adsorption of thiourea on C_{18}-bonded phases is
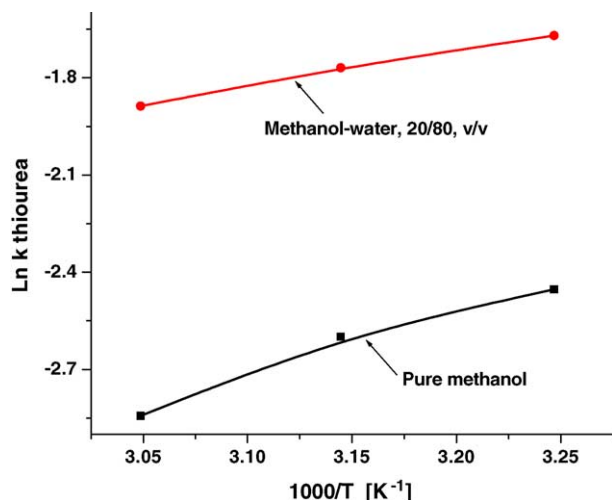
Fig. 5. Van't Hoff plots of thiourea in pure methanol and in a water rich mobile phase (80%, v/v) on the Symmetry-C_{18} adsorbent. Note that thiourea is retained and the slope of the plot is higher when the retention is lower suggesting an entropy governing mechanism.

finite. Surprisingly, although its retention is stronger in the 80/20 (v/v) methanol–water mixture than in pure methanol, the average slope of the plot of $\ln k'$ versus $1/T$ is lower (Fig. 5). The average variation of enthalpy between the stationary and the liquid phases are about -9 and -16 kJ/mol in the methanol–water mixture and in pure methanol, respectively. This means that the retention mechanism of thiourea is entropically driven. We can calculate from the data in Fig. 5 that the transfer of 1 mole of thiourea from the 80/20 methanol–water mixture to the C_{18}-bonded layer has an entropy of about -40 J K^{-1} mol^{-1}, lower than that of the same transfer from pure methanol, which is about -75 J K^{-1} mol^{-1}. This means that the degree of disorder of the adsorbed layer system is higher in an aqueous solution than in pure methanol. This suggests that the C_{18} bonded chains can adopt a larger variety of conformations (by combining trans and cis conformations) in an aqueous solvent than in a better solvent like methanol in which these chains are mainly in the trans conformation.

It is noteworthy also that the plots in Fig. 5 are not straight lines but are slightly curved. The slopes of these curves increase with increasing temperature. At higher temperatures the bonded chains are more mobile, especially in a good solvent like methanol, which allows a deeper penetration of the bonded layer and increases the number of adsorption sites. This phenomenon is less intense in methanol–water than in pure methanol because the bonded phase is more compact and more tightly folded onto itself.

In conclusion, the dependence on the mobile phase composition of the elution volume of thiourea on C_{18}-bonded phases (Fig. 4) is governed by its adsorption entropy while on pure silica, it is controlled by the solubility of thiourea in the mobile phase [16]. The number of possible chain conformations decreases with increasing methanol content. These

chains tend to adopt an ordered, comb-like structure. In the same time, the degree of solvation of the chains by methanol and the number of adsorption sites increase while the solubility of thiourea decreases. This explains why the elution volume of thiourea becomes constant around 70% methanol and increases at higher methanol contents. The physical properties and the structure of the bonded layer depend on the organic modifier concentration in the aqueous mobile phase as well as on the temperature.

The next section addresses the influence of the pressure on the properties of the bonded layer material.

4.2. Effect of pressure on the retention factor of thiourea

In the previous section, we assumed that the pressure was constant. We now consider the influence of the pressure on the retention of thiourea at constant mobile phase composition. First, the data must be corrected from the effect of the compressibility of the mobile phase inside the column. We derived the retention factors by considering the free geometrical volume, V_0, determined by pycnometry and by calculating the corresponding HPLC hold-up volume under the experimental conditions (i.e., under the applied pressure gradient) from Eq. (5), using the Tait model of liquid compressibility. All the parameters of this model for methanol/water mixtures were measured earlier and are given in reference [16]. The plots of the logarithm of the retention factor, k', versus the average column pressure, P, are given in Fig. 6. The trends observed are similar to those observed with pure silica, except for a slightly larger average slope. We know from basic thermodynamics [3] that:

$$\left(\frac{\partial \ln k'}{\partial P}\right)_T = -\frac{\Delta V}{RT} + \left(\frac{\partial \ln \phi}{\partial P}\right)_T \tag{7}$$

where ΔV is the difference between the partial molar volumes of the solute in the two phases, ϕ is the column phase ratio, and T is the temperature. By contrast to pure Resolve silica, the phase ratio, $\phi = V_S/V_0$, of Resolve-C_{18} and Symmetry-C_{18} depend on the pressure because the compressibility of silica is negligible, not that of the C_{18}-bonded layer. The compressibility of the bonded layer must be considered because that of liquid octadecane (10^{-4} bar^{-1}) is a hundred times that of solid silica (10^{-6} bar^{-1}). Calculations predict that the total volume of the stationary phase (silica + bonded chains) should decrease by about 1% for a change of the average column pressure of 200 bars and a carbon content of 20% [4].

The sum of the volumes of the stationary and the mobile phases, $V_S + V_0$, is supposed to be constant because the deformation of the stainless steel column tube is assumed to be negligible [4]. Accordingly, Eq. (7) can be rewritten as follows:

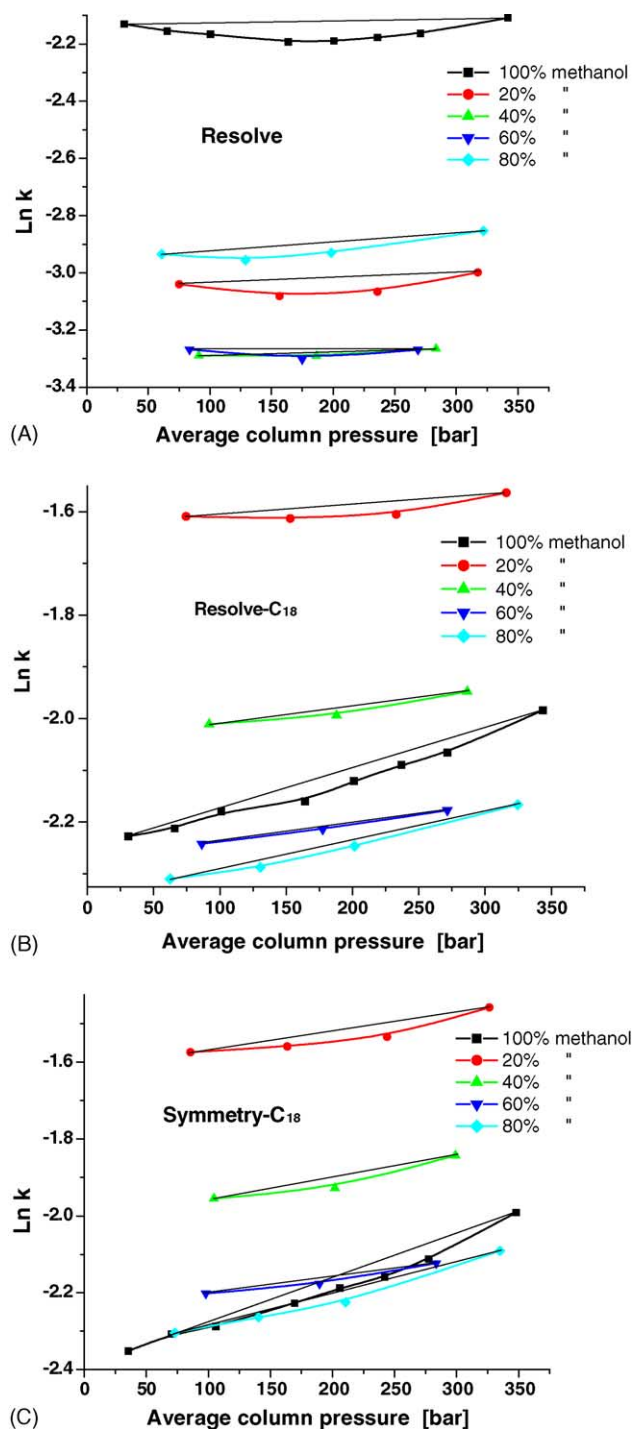$$\left(\frac{\partial \ln k'}{\partial P}\right)_T = -\frac{\Delta V}{RT} + (1 + \phi)\beta_m \tag{8}$$

where β_m is the average compressibility of the stationary phase derived from the volume fractions of silica (ϕ_{silica}) and octadecyl chains ($\phi_{C_{18}}$):

$$\beta_m = \phi_{silica}\beta_{silica} + \phi_{C_{18}}\beta_{C_{18}} \tag{9}$$

Thus, the slope of the variation of the logarithm of the retention factor of thiourea with pressure is the sum of two terms: a thermodynamic term that is due to the difference of the molar partial volumes of the marker in the stationary and the mobile phases and a compressibility term due to the change in the volume of the C_{18}-bonded chain. The curves obtained with pure silica correspond only to the thermodynamic term. According to our experimental results and to the similarity of the plots of $\ln k'$ versus P for pure silica and for C_{18}-derivatized silicas, we may separate thermodynamic and compressibility effects by assuming that the thermodynamic contribution of pressure is the same on Resolve and Resolve-C_{18} and that the compressibility effect is linear ($\ln k' = (1+\phi)\beta_m \times P + \ln k'_0$). The difference between the plots in Fig. 6A and B would then be related to the mere compression of the bonded phase, the Resolve column being considered as a reference system. The average value of the term $(1+\phi)\beta_m$ can then be assessed for each mobile phase composition by calculating the difference between the slopes of the straight lines joining the data measured for the lowest and the highest pressure (see Fig. 6). Assuming that $\phi = (V_C - V_0)/V_0 = 0.848$ and 0.981 for Resolve-C_{18} and Symmetry-C_{18}, respectively, the compressibility, β_m, of the stationary phase can be derived from our experimental data. Assuming that the densities of solid silica and of liquid octadecane are 2.0 and 0.777 g/cm^3, respectively, and that the density of the bonded layer is that of liquid octadecane, the volumetric fractions, ϕ_1, of the C_{18} chains in the packing materials considered are 0.27 and 0.47, respectively. From Eq. (9), we derive the average compressibility of the two materials

$$\beta_m(R) = 0.27 \times 10^{-4} + 0.73 \times 10^{-6} = 0.277 \times 10^{-4} \, bar^{-1}$$

$$\beta_m(S) = 0.47 \times 10^{-4} + 0.53 \times 10^{-6} = 0.475 \times 10^{-4} \, bar^{-1}$$

where R stands for Resolve-C_{18} (10% carbon) and S for Symmetry-C_{18} (20% carbon).

In order to assess the physical sense of the compressibilities found by this method, we described a simple model for the volume of the stationary phase. We consider here two different but typical silica-C_{18} materials for RPLC, containing respectively about 10 and 20% of carbon (w/w). It is obvious in Fig. 6A–C that, despite the correction of the data eliminating the influence of the solvent compressibility on the retention data, the slopes of the plots are not independent of the methanol content in the mobile phase, the higher the methanol content, the higher the average slope of the plots for the two bonded silicas. As a result, the values derived for the compressibility would increase from 2.5×10^{-4} to $7.8 \times 10^{-4} \, bar^{-1}$ for Resolve-C_{18} and from 4.5×10^{-4} to

Fig. 6. Plot of the retention factor of thiourea $(V_R - V_M)/V_M$ on the three adsorbents, Resolve (A), Resolvel-C_{18} (B) and Symmetry-C_{18} (C) for different mobile phase compositions. The true thermodynamical column void volume V_M was calculating under NTP conditions using the Tait model of solvent compressibility and the parameters given in reference [16] for each solvent. Note that the retention is almost constant on the pure silica but significantly increases on the derivatized silica.
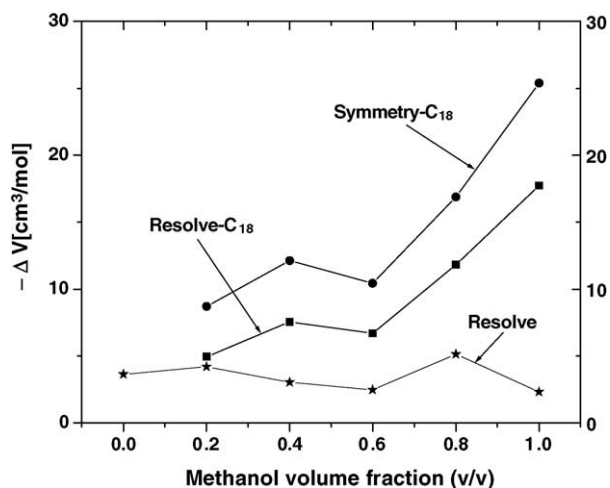
Fig. 7. Estimation of the average difference between the partial molar volumes of thiourea (ΔV) in the three stationary phases (Resolve, Resolve-C_{18} and Symmetry-C_{18}) and mixtures of methanol and water. The data were calculated from the average slope of the plots in Fig. 6, using the first right-hand term in Eq. (8) after they were corrected for the bonded layer compressibility (Eq. 9)).

$11.3 \ 10^{-4} \, bar^{-1}$ for Symmetry-C_{18}. These values are one order of magnitude larger than predicted by our model.

The large difference between the measured and predicted values of the compressibility falsifies the assumption made earlier, that the deconvolution between the contributions of the effects of pressure on the retention of thiourea and the solid phase compressibility could be made by assuming a constant difference between the partial molar volumes of thiourea in the two phases, whatever the pressure and mobile phase composition. This difference is unlikely to be the same when thiourea is adsorbed from the same solution onto a polar silica surface or a hydrophobic C_{18}-bonded surface.

Conversely, if we assume that the compressibility term in Eq. (8) remains constant, independently of the mobile phase composition, the average variation of the partial molar volume ΔV of thiourea can be assessed from the average slope of the curves shown in Fig. 6A–C. The results are plotted in Fig. 7. The values obtained are of the order of a few mL/mol (between -2 and -5 mL/mol) with pure Resolve, for which the plots $\ln k'$ versus P are almost horizontal (see Fig. 6A). Then, the mobile phase composition does not affect the difference between the partial molar volumes of thiourea in the two phases. The values obtained increase from -5 to -17 mL/mol and from -9 to -25 mL/mol, with Resolve-C_{18} and Symmetry-C_{18}, respectively, when the methanol content increases from 20 to 100%. These values are consistent with those reported for similarly small-molecule analytes, like nitrophenol isomers [22], typically around -10 mL/mol.

These results suggest that the variation of the retention factor of thiourea with the average column pressure is mostly controlled by the thermodynamics of its retention, not by the compressibility of the stationary phase. The latter effect accounts for less than 5% of the variation of the retention factor of thiourea. The presence of the layer of bonded alkyl chains affects ΔV, which depends on the mobile phase composition, especially at high methanol concentrations, when it is supposed that the alkyl chains begin to expand significantly. Finally it is reasonable to consider the compressibility of the bonded layer as independent of the mobile phase composition because this phenomenon is essentially mechanical and should not be related to the chemical nature of the fluid under pressure.

5. Conclusion

Our work demonstrates that both the density of the C_{18}-bonded layer of RPLC adsorbents and the methanol content of an aqueous mobile phase affect the difference between the partial molar volumes, ΔV, of small analytes in the stationary and the liquid phases. The compressibility of the layer of C_{18}-bonded chains does not contribute significantly to the increase of the analyte retention when the average column pressure increases. The essential of the variation observed is related to the thermodynamics of retention, i.e., to ΔV.

The variation of ΔV with the mobile phase composition comforts the idea that RPLC stationary phases and the associated mobile phases do not behave as two inert and independent phases. They interact with each other. The liquid phase penetrates to a significant extent into the bonded layer when the methanol content exceeds 70% methanol (v/v). The bonded phase structure is affected by the nature of the organic modifier which has a higher solubility for octadecane than for water. The literature is somehow undecided on this issue. Some authors suggest that the organic modifier does not penetrate inside the bonded layer but that methanol or acetonitrile are adsorbed at the top of the chains. Other, independent measurements, including those reported here, submit that the fraction volume of the C_{18}-bonded chain layer occupied by the organic modifier is about 35%. The presence of the organic components of the mobile phase inside the hydrophobic layer also explained why this layer was found to be heterogeneous regarding the adsorption of phenol or caffeine [23]. The wetting of the hydrophobic layer may also depend on the composition of the mobile phase and the ACP. This would affect the retention times of non retained compounds by increasing the accessible volume inside the column [9].

The average column pressure is an important parameter that affects the retention factors of all compounds, not only the high-molecular ones. It even affects to a measurable degree the retention times of small, poorly retained analytes in RPLC. Particular attention must be paid to this effect when the mobile phase has a large content of methanol, typically beyond 70%, since the C_{18} layer rearranges into a more ordered structure. Within the conventional range of pressures used in HPLC (0–400 bar), the retention times of most analytes in pure methanol may increase by nearly 10%.

Acknowledgements

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