

Influence of the pressure on the properties of chromatographic columns I. Measurement of the compressibility of methanol–water mixtures on a mesoporous silica adsorbent

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Abstract

The compressibilities of aqueous solutions of methanol or acetonitrile containing 0, 20, 40, 60, 80 and 100% (v/v) organic solvent were measured with a dynamic chromatographic method. The elution volumes of thiourea samples (2 μ L) in these solutions were measured at different average column pressures, adjusted by placing suitable capillary restrictors on-line, after the detector. The reproducibility of the measurements was better than 0.2%. In the range of average pressures studied (10–350 bar), the maximum change in elution volume of thiourea is 1.3% (in pure water) and 4.0% (in pure methanol). This difference is due to the different compressibilities of these pure solvents. For mixtures, the plots of the elution volume of thiourea versus the pressure are convex downward, which is inconsistent with the opposite curvature predicted by the classical Tait model of liquid compressibility. This difference is explained by the variation of the amount of thiourea adsorbed with the pressure. The deconvolution of the two effects, adsorption of thiourea and solvent compressibility, allows a fair and consistent determination of the compressibilities of the methanol–water mixtures. A column packed with non-porous silica particles was also used to determine the compressibility of methanol–water and acetonitrile–water mixtures. A negative deviation by respect to ideal behavior was observed.

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1. Introduction

Today, industrial analysts are under the unremitting pressure of ceaselessly increasing needs for always faster analyses carried out with more efficient columns. Thus, conventional RPLC tends to use fast monolithic columns or to order shorter conventional columns than in the recent past, packed with finer particles, using higher and higher pressures, now typically up to 400 bar. This evolution is required to face the demands of an industry searching intensely for higher productivity levels. This is particularly true in the pharmaceutical industry under pressure to decrease its costs and faced with the requirements of combinatorial chemistry, a field in which a new chemical synthesis delivers now

scores of 96-sample trays when it used to present the analyst with only a few samples.

Unlike the effects of an increase in the column temperature or a change in the mobile phase composition, which are well known, the effect of an increase in the average column pressure has not yet been the topic of strong and systematic interest [1,2]. The reason for this lack of interest is that analysts have been using temperature and mobile phase composition for nearly half a century to adjust retention times and resolution. On the other hand, pressure is adjusted reflexively to set the mobile phase flow rate in the desired range. Analysts tend to ignore or discount the effect of pressure on retention. Admittedly, pressure has been shown to affect merely the retention of high molecular-weight compounds [3–6]. A change in the average column pressure does affect the retention of analytes having relatively large molecules (peptides, fullerenes, proteins, etc.), whose partial molar volumes are

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different in the liquid and in the adsorbed phase. For instance, the difference in partial molar volumes ($\bar{V}_s - \bar{V}_l$) of insulin [3], bradykinin [4] and C₆₀ buckminsterfullerenes [5] on C₁₈-bonded silica are -100 , -35 and $+12$ mL/mol, respectively.

However, for how important the influence of pressure on the retention times and the resolution of analytes is, there is a variety of other effects in HPLC that are more subtle and often completely neglected. Pressure affects the dimensions of the column (the cross-section area and the length of the tube increase with increasing pressure), the volume occupied by the stationary phase in the column (the densities of the C₁₈-bonded layer and of the silica particles increase with increasing pressure), and the density and viscosity of the mobile phase, which is compressible. It may even raise significantly the melting point of the mobile phase or force the liquid phase to penetrate (intrusion) into hydrophobic micropores of the RPLC adsorbent [6]. All these effects will cause noticeable variations of the apparent column hold-up volume, an important parameter which determines the values of many other chromatographic parameters. For all these reasons, it is important to assess the importance of these effects which turn out to be small but are reproducible and easily observable using the current modern HPLC instruments. In a previous work [7], we measured and reported the effect of the column average pressure on the hold-up column volume under NTP conditions, using columns packed with particles of neat silica and of C₁₈-bonded silica adsorbents. We used three pure solvents of different compressibilities (water, methanol and *n*-pentane) as the mobile phase. This earlier work had two main conclusions: (1) the higher the mobile phase compressibility, the higher the increase of the column hold-up volume observed when the average column pressure is increased by a fixed value; and (2) the presence of alkyl-bonded chains on the silica surface leads to a steeper increase of the void volume with increasing pressure because the C₁₈-bonded layer is far more compressible than the solid silica particles, by a factor of nearly 100. For example, the apparent hold-up volumes of columns packed with Resolve silica (0% carbon), Resolve-C₁₈ silica ($\approx 10\%$ carbon) and Symmetry-C₁₈ ($\approx 20\%$ carbon) and percolated with methanol increase by 3.1, 3.8 and 4.5%, respectively, when the average column pressure increased from 40 to 240 bar. The higher the carbon content, the higher the relative increase of the hold-up volume.

In this work, we used an inverse method in order to determine the compressibilities of mixtures of methanol and water by measuring the evolution of the elution volume of thiourea on packed chromatographic columns. We had two goals. First, the role of the mobile phase compressibility in LC is often neglected but, given the precision and accuracy of the measurements which can easily be achieved with modern instruments, it is detectable, it can be measured with a reasonable accuracy, and it should often be taken into account. Thus, we need to assess the influence of this compressibility on the hold-up column volume and on the chromatographic parameters derived from it, especially the retention factors k' and the other isotherm coefficients. Second, while the compress-

ibilities of pure solvents can be found in handbooks, there are almost no data available in the literature on the compressibilities of mixtures, nor combination rules allowing an extension of the Tait equation. Only a few data measured at different temperatures and for few methanol contents are available [8,9]. They were acquired using a piezoelectric manometric gauge. These data will be helpful to validate the results that we obtained with a new dynamic chromatographic method and that we report in this work. A chromatographic column packed with an underivatized mesoporous silica (Resolve, Waters, Milford, MA, USA) was used to measure the elution volume of thiourea under different flow rates, average column pressures, and temperatures, with six different mixtures of methanol and water. The results will be compared to those given by a classical model of compressibility (Tait model), with a few experimental values of the mixture compressibility found in the literature [8,9], and with values obtained with the same method as described here, using a column packed with fine solid particles. These results are then discussed as well as the limits of the present method.

2. Theory

Under constant pressure, the elution volume of the marker thiourea on a packed silica column depends on three independent factors. First, it depends on the geometrical volume of the bed, V_0 , that is accessible to the tracer (the difference between the geometrical volume of the tube and the volume of the solid packing material). Second, because LC operates at constant flow rate and the hydraulic resistance to the flow caused by the finite permeability of the packed bed, the compressibility of the mobile phase will affect the result of measurements of the elution volume of thiourea, which is the volume of eluent needed to elute a pulse through the column, measured at the column outlet. Third, because thiourea might not be completely unretained on silica, its elution volume may depend on the composition of the mobile phase. This last effect will probably be small but it is not necessarily negligible.

For a mobile phase of constant composition, the influence of an increasing pressure on the elution volume of thiourea may have different origins. Their contributions must be discussed and compared.

- (1) The pressure stress acting on the column tube results in its expansion. Calculations show [6] that this effect is very small, almost non-existent, and certainly negligible in practice. Within the pressure range studied, 1–400 bar, the relative increase of the column length is proportional to the pressure and is between 5.6×10^{-6} and 2.3×10^{-5} (depending on the tube material and the assumptions made in the calculation). The average column tube cross-section increases also linearly with increasing pressure. This relative increase is between 4.6×10^{-4} and 1.8×10^{-3} when the pressure increases from 1 to

400 bar. Thus, the deformation of the column tube is very small, its volume increases by less than 0.1% when the pressure increases from 1 to 400 bar. This effect will be neglected.

- (2) The pressure stress acting on the silica adsorbent results in its shrinkage. When underivatized silica is used, the relative variation of the silica volume V_S is less than -2.0×10^{-4} (0.02%) when the pressure increases from 1 to 400 bar [6]. Thus, the shrinkage of the silica is also completely negligible.
- (3) The pressure stress acting on the mobile phase may force its intrusion inside the smallest pores of the adsorbent (see Washburn equation). This effect depends on the contact angle between the solid surface and the mobile phase used. If this angle is less than 90° , the liquid wets the surface and no pressure is required for the liquid to penetrate into the smallest pores. The process is spontaneous (capillary forces). In this study, we used water and methanol as the liquids. The contact angles reported between a silica surface and water or alcohol (ethanol) suggest that the wetting of the surface may be partial or complete depending on the pretreatment of the silica surface [10]. Whether dehydroxylated silica (a surface mainly covered with hydrophobic siloxane bridges) or silica saturated with water (a surface mainly covered with hydrophilic silanols with some physisorbed water), the contact angle is always less than 90° [11] (Wilhelmy measured 45° and 0° on quartz, in these two cases, respectively, using the dynamic technique [12]). The intrusion of water or methanol inside the silica adsorbent is then spontaneous and would not require the application of any pressure. Liquid intrusion cannot be responsible for a significant increase of the elution volume of thiourea with increasing average column pressure.
- (4) Pressure may influence the adsorption of thiourea onto the silica surface as was observed with proteins. This effect is large and easily detected for large molecular weight compounds which have quite different partial molar volumes in the liquid and in the adsorbed phases [3–5]. In this work, we use thiourea which has a relatively low molecular size ($M_w = 76$) and is generally considered to be not retained. Accordingly, the effect of pressure should be very small compared to that measured with large molecules. However, the precision of the measurements is approximately 0.2% and a very small effect could be significant in this work.
- (5) Pressure acting on the mobile phase compresses it, decreases its specific volume inside the column. The specific volume of the mobile phase in the column decreases linearly from the column inlet to its outlet. As a result, the volume of eluent measured at the outlet during the elution of a peak, under NTP conditions, is larger than the volume occupied by the liquid inside the column under atmospheric pressure (no flow rate) and the elution volume increases with increasing pressure. This is certainly the main source of variations of the elution volume of

thiourea on a silica adsorbent when the average column pressure changes.

The relative increase of the hold-up volume by reference to the geometrical free volume was derived earlier [7]. It can be quantified fairly well using the Tait model [13] for the liquid compressibility. The Tait equation is:

$$V(P) = V^0 \left[1 + c \ln \left(\frac{P^0 + b}{P + b} \right) \right] \quad (1)$$

where b and c are the two numerical parameters of the Tait model. V^0 is the volume of liquid phase contained in the column under NTP conditions. The Tait model applied for each elementary volume inside the column, under the local pressure P . We assume in this work that the pressure gradient along the column is linear. A detailed investigation of the (small) effects of a nonlinear gradient and of the dependence of the mobile phase viscosity on the pressure will be addressed elsewhere. The effect of a linear pressure gradient along the column can be easily calculated. The elution volume of solvent that percolates through the free geometrical volume V_0 . Note that V_0 is different from V^0 . It is the geometrical volume accessible to the mobile phase inside the column, under the pressure stress (average column pressure P) necessary to achieved the required flow rate:

$$\frac{V(P)}{V^0} = \frac{1}{P^{\text{il}} - P^{\text{ot}}} \int_{P^{\text{ot}}}^{P^{\text{il}}} \frac{dP}{1 + c \ln((P_0 + b)/(P + b))} \quad (2)$$

where $V^0 = \epsilon_t S_C L$ is the volume of the column bed that is accessible to the liquid phase under NTP conditions, P^{il} and P^{ot} are the inlet and the outlet column pressures.

Using the experimental results, we will plot the relative increase of the column hold-up volume by reference to the NTP hold-up volume measured for the lowest possible outlet pressure (ca. 10 bar), because V^0 is not known a priori. If P_1^{il} and P_1^{ot} are the lowest inlet and outlet pressures at which $V(P)$ was measured and P_i^{il} and P_i^{ot} are the same pressures for the i th measurement, then

$$\frac{V(P_i)}{V(P_1)} = \frac{P_1^{\text{il}} - P_1^{\text{ot}} \int_{P_1^{\text{ot}}}^{P_i^{\text{il}}} dP / (1 + c \ln((P_0 + b)/(P + b)))}{P_i^{\text{il}} - P_i^{\text{ot}} \int_{P_i^{\text{ot}}}^{P_1^{\text{il}}} dP / (1 + c \ln((P_0 + b)/(P + b)))} \quad (3)$$

The plots of the elution volume of thiourea $V(P)$ versus the pressure P measured on the mesoporous silica adsorbent followed an empirical second-order polynomial:

$$V(P) = \alpha_0 + \alpha_1 P + \alpha_2 P^2 \quad (4)$$

The term $\alpha_1 P + \alpha_2 P^2$ is the apparent increase in elution volume due to the pressure increase and to the compressibility of the mobile phase when the pressure increases from P^0 to P . α_0 is the elution volume of thiourea extrapolated at a pressure of zero (see Fig. 2). If n_0 is the number of moles of liquid

per unit volume at pressure P^0 , the number of moles N_0 contained in the porous material under pressure P^0 is n_0V^0 . The number of moles $N(P)$ contained in the same porous volume under pressure P is:

$$N(P) = n_0(V^0 + \alpha_1 P + \alpha_2 P^2) \quad (5)$$

The specific volume at pressure P^0 and P are:

$$V_{P^0,T} = \frac{1}{n_0} \quad (6)$$

$$V_{P,T} = \frac{V_0}{N(P)} = V_{P^0,T} \frac{V_0}{V^0 + \alpha_1 P + \alpha_2 P^2} \quad (7)$$

The compressibility at pressure P and temperature T is defined as:

$$\beta_T(P) = -\frac{1}{V_{P,T}} \frac{dV_{P,T}}{dP} = \frac{\alpha_1 + 2\alpha_2 P}{V^0 + \alpha_1 P + \alpha_2 P^2} \quad (8)$$

The compressibility of methanol–water mixtures can be calculated from the P – ρ – T relationship [8] derived using standard thermodynamic analysis programs which lead to:

$$P = A\rho^2 + B\rho^8 + C\rho^{12} \quad (9)$$

where A , B and C are three numerical coefficients, all functions of the temperature T and the methanol mole fraction x , with:

$$\begin{aligned} A &= \sum_{i=1}^5 T^i \sum_{j=0}^4 a_{ij} x^j \\ B &= \sum_{i=0}^4 T^i \sum_{j=0}^4 b_{ij} x^j \\ C &= \sum_{i=0}^4 T^i \sum_{j=0}^4 c_{ij} x^j \end{aligned} \quad (10)$$

Here, a_{ij} , b_{ij} and c_{ij} are the coefficients of the polynomial expressing the P – ρ – T relationship for the mobile phase considered to which were fitted to experimental data with deviations less than 0.1% [9]. The compressibility calculated from this equation is:

$$\beta_T(P) = \frac{1}{\rho} \left(\frac{\partial P}{\partial \rho} \right)_T^{-1} \quad (11)$$

3. Experimental

3.1. Chemicals

The mobile phases used in this work were aqueous solutions of methanol or acetonitrile (0, 20, 40, 60, 80 and 100% (v/v) organic solvent), all 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). All the mobile phases (except for the pure solvents, i.e., water, methanol and acetonitrile)

Table 1

Physico-chemical properties of the Resolve silica packing material (150 mm \times 3.9 mm)

Resolve column	
O.D./I.D. ratio diameter	1.87
Particle shape	Spherical
Particle size (μm)	5
Pore size (\AA)	90
Pore volume (mL/g)	0.50
Surface area (m^2/g)	200
Total carbon (%)	0
Surface coverage ($\mu\text{mol}/\text{m}^2$)	0
Endcapping	No

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

were prepared by mixing the two solvents in the required proportions, prior to pressurizing their solution by using a single pump head. This experimental procedure avoids the need of a correction for non-ideal mixing. Thiourea was obtained from Aldrich (Milwaukee, WI, USA).

3.2. Materials

Two different columns were used in this work. The first chromatographic column was the Resolve (Waters, Milford, MA, USA) mesoporous silica previously used [5,7]. Its dimensions are 150 mm \times 3.9 mm. The main characteristics of the packing material are summarized in Table 1. The volume of mobile phase required to elute the marker thiourea was derived by multiplying the elution time and the constant flow rate F_v (1 mL/min). The second column was made of non-porous silica particles given by Phenomenex, Torrance, CA, USA. Its dimensions are 100 mm \times 4.6 mm. The average size of these particles is 4.8 μm . The specific surface area of these particles is less than 1 m^2/g . The solid silica particles were packed in our laboratory, using the slurry method. They were suspended in methanol, also used as the pushing solvent and consolidated under a constant pressure of 2000 psi. Various mobile phase velocities were applied in order to reach similar back-pressure for all the mobile phases. Previous calibrations showed the flow rate delivered by the pumps of the instrument to be accurate within 0.04%. Several PEEK tubings (inner diameter, 0.0025 in.) were purchased from Upchurch Scientific (Oak Harbor, WA, USA) and connected next to the detector cell to achieve different average column pressures, according to the combination of their length (1.66, 3.33 and 5.0 ft).

Using the Resolve column, the following method for data acquisition was applied. Because the viscosity of a methanol–water mixture depends much on its composition (it is at its maximum at about 50/50, v/v), the number of data points acquired and the range of average column pressures used could not be the same for all the mobile phase compositions. To avoid damaging the inner surface of the capillary tubes, they were not cut exactly to achieve target average column pressures. For these reasons, 5, 4, 3, 3, 4 and 8 data points were acquired with mobile phases containing 0, 20, 40, 60, 80 and 100% of methanol, respectively.

Using the non-porous silica column, the mobile phase velocity could be adjusted in order to generate the same back-pressures for all the mobile phases used. Then, flow rates of 0.75, 0.50, 0.40, 0.45, 0.60 and 1.20 mL/min were applied for methanol contents of 0, 20, 40, 60, 80 and 100% (v/v), respectively.

3.3. Apparatus

The elution times of thiourea were acquired using a Hewlett-Packard (Palo Alto, CA, USA) HP 1100 liquid chromatograph. This instrument includes a binary-solvent delivery system, an auto-sampler with a 100 μ L sample loop, a UV detector with a detector cell withstanding back-pressures up to 400 bar, a column thermostat and a data station. The extra-column volume contributions were measured for each PEEK capillary placed after the detector. All the retention data were corrected for this contribution. The flow rate accuracy was controlled with and without back-pressure by pumping the pure mobile phase at 22 °C and 1 mL/min during 50 min, from each pump head successively, into a volumetric flask of 50 mL. Whatever the back-pressure imposed between the pumps and the detector (from 30 to 400 bar), the flow rate measured after the detector, under NTP conditions, remained unchanged, because the solvent is pumped under atmospheric pressure. All the measurements were carried out at a constant temperature of 22 °C, fixed by the column thermostat.

3.4. Measurements of the elution volume under NTP conditions

The NTP conditions refer to the normal conditions of pressure and temperature of the laboratory, e.g., the atmospheric pressure P^0 and $T = 295$ K. Samples of 2 μ L of a less than 0.5 g/L solution of thiourea were systematically injected into the equipment set with or without a column to measure the hold-up and the extra-column volumes, respectively. The solutions of thiourea used were prepared in the same mobile phase as that used to run the experiments. The composition of the injected sample was the same as that of the mobile phase, except for the dilute thiourea. The detection of thiourea was made by UV detection at a wavelength of 270 nm. The elution time of thiourea was calculated as the difference between the elution time of thiourea on the equipment set with the column and the elution time of thiourea measured when the column was replaced with a connecting union.

3.5. Solubility measurements of thiourea under NTP conditions

The solubility of thiourea was measured for three different solvents (pure water, pure methanol and the methanol–water mixture (50/50, v/v)). A 0.400 g of thiourea were inserted in a 15 mL conical vial. A 1.5 mL of solvent were added and the vial was mechanically shaken at room temperature for 1 h using a vortex. The vial was then centrifuged for an additional

hour and the supernatant was collected for UV analysis, 2 μ L of the supernatant being injected three times successively and the peak area measured. The wavelength was set at 305 nm in order to keep the UV absorbance in the concentration–absorbance plot within the linear range (less than 100 mAU). No calibration was made beforehand because we wanted only to compare the relative solubilities in the different solvents tested, not their absolute values.

4. Results and discussion

4.1. Elution volume of thiourea measured under NTP conditions

The experimental results are in Table 2. In this table are reported the inlet, outlet, average column pressures, and the extra-column volumes. Note that there is practically no effect of the mobile phase composition on the value of the extra-column volume, except possibly with pure methanol. The volumes of the connection tubes are too small (less than 0.1 mL) to permit the detection of any significant dependence of the extra-column volumes on the mobile phase composition. This indicates that methanol is markedly more compressible than the other liquid mixtures.

Fig. 1 shows plots of the elution volume of thiourea versus the average column pressure for the six different mobile phases. It is noteworthy that these curves do not converge toward the same value of the elution volume when the average column pressure tends toward zero (or rather toward the atmospheric pressure). This volume should be the geometrical free volume V^0 (Eq. (2)) because all the solvent mixtures used fill spontaneously all the pores of the adsorbent, hence should occupy the same volume. Fig. 1 indicates that this is not so. Accordingly, the compressibility of the bulk mobile phase is not the only factor affecting the elution volume of thiourea under NTP conditions. Because the difference between the elution volumes measured with pure methanol and with the methanol–water mixtures with 60/40 and 40/60 (v/v) is almost 10% while the experimental accuracy is better than 0.2%, these differences cannot be accounted for by experimental errors. Fig. 2 shows the plot of the elution volume of thiourea extrapolated to a zero pressure versus the mobile phase composition. There is a minimum value for a methanol concentration of approximately 50/50 and maxima in pure water and pure methanol. There is one major explanation for this non-monotonous behavior, adsorption of thiourea.

The retention factor of thiourea is related to its distribution coefficient between the liquid and the solid phases. In principle, there should be competitive adsorption between water, methanol, and thiourea. The initial slope of the competitive isotherm of thiourea in the presence of a water–methanol solution depends on the composition of this solution. The solubility of thiourea also changes with this composition. We measured the amount of thiourea dissolved in pure water, in pure methanol and in their 50/50 (v/v) mixture (see proce-

Table 2

Corrected elution time of thiourea measured under NTP conditions on the Resolve 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)
0	0	11	89	50	0.087	1.297
	5/3	64	144	104	0.087	1.299
	10/3	118	196	157	0.087	1.301
	5	215	294	255	0.087	1.309
	5 + 5/3	275	347	311	0.087	1.315
20	0	16	134	75	0.086	1.278
	5/3	95	218	157	0.086	1.283
	10/3	176	296	236	0.086	1.291
	5/3 + 10/3	260	375	318	0.086	1.302
40	0	20	162	91	0.085	1.268
	5/3	115	258	187	0.085	1.278
	10/3	213	354	284	0.085	1.289
60	0	18	149	84	0.086	1.269
	5/3	109	241	175	0.086	1.278
	10/3	202	336	269	0.086	1.290
80	0	13	109	61	0.086	1.286
	5/3	80	178	129	0.086	1.294
	10/3	149	248	199	0.086	1.305
	5	272	372	322	0.086	1.326
100	0	7	54	30.5	0.088	1.362
	5/3	41	90	65.5	0.089	1.364
	10/3	76	125	101	0.089	1.368
	5	139	188	164	0.090	1.374
	5 + 1/3	176	225	201	0.091	1.380
	5 + 10/3	211	261	236	0.091	1.387
	5 + 5	246	296	271	0.092	1.394
5 + 5 + 5/3	317	367	342	0.092	1.412	

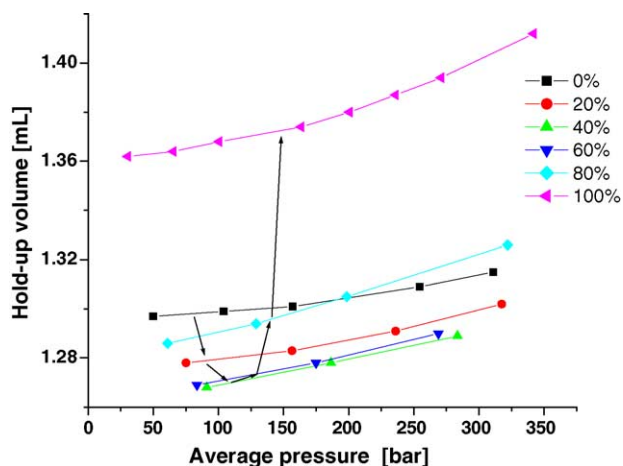


Fig. 1. Variation of the elution volume of thiourea on the Resolve silica adsorbent using different mixtures of methanol and water as the mobile phases. The elution volumes are measured from the elution time of thiourea ($2 \mu\text{L}$ injection of a solution at less than 1 g/L , UV detection 270 nm) at a flow rate of 1 mL/min . $T = 296 \text{ K}$. Note that decreasing and increasing elution time of thiourea when the methanol content increases in the mobile phase. The arrows show the subsequent data curve corresponding to a step increase of 20% in the volumetric fraction of methanol.

ture in Section 3). The results show a higher solubility in the mixture than in the two pure solvents and a higher solubility of thiourea in water than in methanol. This confirms the results of measurements made seventy years ago by Shnidman [14] showing a higher solubility of thiourea in pure water (177.7 g/L) than in pure methanol (108.3 g/L). The adsorp-

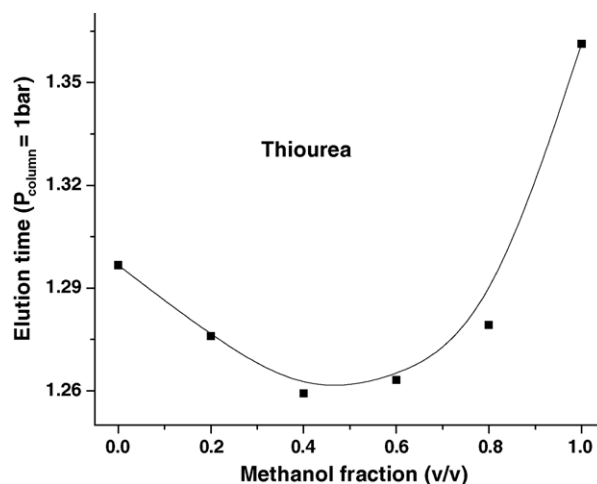


Fig. 2. Evolution of the elution volume of thiourea extrapolated at the hypothetical pressure of 0 bar vs. the mobile phase composition.

tion equilibrium of thiourea is governed by the mobile phase solubility, which explains why the elution volume of thiourea differs significantly with the mobile phase composition and why it is minimum in the solution in which thiourea is the most soluble (Figs. 1 and 2). The slight retention of thiourea was confirmed by its retention time in pure water, measured at three different temperatures, 25, 45 and 65 °C, and by the values of the column hold-up volume measured by a static method.

The elution volume of thiourea in pure water decreases from 1.296 to 1.272 and to 1.249 mL with increasing temperature from 25 to 45 and to 65 °C, respectively. This decrease is consistent with a small retention of thiourea on silica, a retention which is expected to decrease with increasing temperature.

The various definitions and methods of measurement of the column hold-up volume and the reasons for the choice of the most appropriate method have been reviewed by Scott and co-workers [15] and more recently by Dorsey and co-workers [16]. In this study, the column hold-up volume was determined by pycnometry, i.e., by filling successively the column with two solvents of different densities and weighing it. Assuming that the total volume V^0 taken up by the two liquids is the same under NTP conditions, which is highly probable since both solvents wet at least partially the silica surface (with a wetting angle well below 90°), and that the partial volume of the solvent is the same in the adsorbed and mobile phases, it can be calculated that [17]:

$$V^0 = \frac{m_1 - m_2}{d_1 - d_2} \quad (12)$$

where m_1 and m_2 are the weights of the column filled with solvents 1 and 2, respectively, and d_1 and d_2 are the densities of the two solvents under NTP conditions. Water and methanol constitute a good solvent pair for this measurement because they both wet well the solid surface of the adsorbent and they have significantly different densities under NTP conditions (0.9998 and 0.7914 g/cm³, respectively). So, they provide well distinct masses for the filled columns. Combined with the balance accuracy (± 0.00005 g), this combination provides an accurate determination of V^0 . The weights of the column filled with water and methanol were 62.93045 and 62.67765 g, respectively. The geometrical free column volume V^0 was then 1.213 mL. This value is slightly but clearly lower than the elution volumes of thiourea shown in Fig. 2, where the minimum volume measured was about 1.26 mL. In conclusion, thiourea is definitely retained onto the Resolve silica adsorbent from all aqueous solutions of methanol. In pure methanol, the retention factor is slightly less than 0.12. In the 50/50 mixture, k' is about 0.04. In pure water, it is close to 0.15.

4.2. Measurement of the mobile phase compressibility

In the previous section, we demonstrated that the relative positions and shapes of the curves in Fig. 1 depend not only on

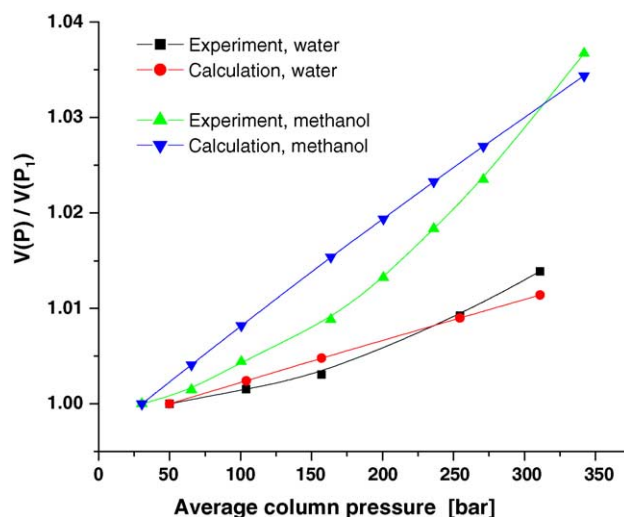


Fig. 3. Comparison between the measured and the calculated relative increase of the elution volume of thiourea when the pressure increases from 40 to 325 bar. The calculations were performed by using Eq. (2) and the Tait model for the compressibility model of pure water and pure methanol. The Tait parameters of these solvents are given in Table 5. Note the opposite curvature of the experimental and calculated profiles for both solvents.

the compressibility of the solvent but also on the solubility of thiourea in the mobile phase. The elution volume of thiourea is a function of both retention and compressibility. As seen in Fig. 1, for all the mixtures used, the elution volume of thiourea does not increase linearly with increasing pressure. The plots are rather parabolic functions, with a downward convexity. The empirical Tait model (see Eq. (1)) is generally accepted as accounting well for the compressibility of liquids. Its validity was confirmed by earlier results obtained with pure water and pure methanol [6]. The values of the two numerical coefficients of this model are accurately known for these solvents. The variation of the elution volume of an inert, truly unretained tracer with the average column pressure can be calculated and compared with the experimental values obtained [7]. The measurements reported earlier in this work were made in a range of average column pressures between 40 and 330 bar. At this stage, they were not corrected for the retention of thiourea. The calculations were made using Eq. (3) in which the pressure limits for the integration were the experimental ones given in Table 2. Fig. 3 compares the results for water and methanol [6].

The agreement between the results of the calculations and the experiments is only qualitative. The total relative increases in the retention volumes observed and calculated are comparable (+1.2 and +3.5% for water and methanol, respectively), if they appear to be slightly underestimated by the model. The main disagreement concerns the curvature of the plots. The directions of the curvatures of the experimental and the calculated plots are opposite. The experimental plot is convex downward and it is more strongly curved than the calculated plot which is convex upward, in agreement with Tait equation that states that the compressibility of liquids decreases slowly with increasing pressure. The compressibility

β derived from this equation is:

$$\beta(P) = \frac{c}{P + b} \quad (13)$$

As explained in Section 2, there is no contribution to the variation of the elution volume of thiourea with pressure that could be found in the expansion of the column tube (this would be less than 0.1% of the effect observed) nor in the shrinkage of the silica particles (an effect less than 0.02%), nor in the forced intrusion of the liquid into small mesopores or micropores (water and methanol both wet the silica surface). Besides, these three contributions are purely mechanical and are of a magnitude that is independent of the chemical composition of the mobile phase. Since the accelerated increase of the elution of thiourea that is observed at high pressures differs whether water, methanol, or one of their solutions is used, the origin of the discrepancy between experimental and calculated plots in Fig. 3 must be related to the composition of the mobile phase. The unavoidable conclusion is that the unusual pressure dependence of the plots of the retention volume of thiourea versus the average column pressure shown in Fig. 1 arises through the adsorption of thiourea onto the silica and the possible effect of pressure on this adsorption.

The slopes of the experimental curves are always lower between 0 and 150 bar and higher beyond for pure water and pure methanol (Fig. 3). These slopes were calculated by fitting the curves to the second-order polynomial in Eq. (4). Table 3 shows the best parameters α_0 , α_1 , and α_2 of the polynomial that fits the best the plot of the elution volume of thiourea $V(P)$ versus the average column pressure in Fig. 1. Table 4 gives the compressibility of the six different mobile phase mixtures derived from Eq. (8) which assumes that the pressure does not affect the retention of thiourea. These values appear to be unrealistic. They are inconsistent with the empirical Tait model for the two pure solvents, water and methanol, because the liquid compressibility is supposed to decrease with the pressure while they show the opposite trend, an increase of the solvent compressibility with increasing pressure. The equation of state of methanol–water mixtures have been derived using standard thermodynamic analysis programs that lead to Eqs. (9) and (10). The compressibilities calculated from the relationship between the pressure and the density of the liquid (Eq. (11)) measured at a constant temperature leads to a monotonous decrease of the compressibility of methanol–water mixtures with increasing pressure [9], a result in contradiction with an interpretation

of our experimental data that assumes that thiourea is not adsorbed.

4.3. Separation of the contributions of thiourea adsorption and mobile phase compressibility

Thermodynamics teaches that the dependence of the retention factor k' on the local pressure is given by [6]:

$$\left(\frac{\partial \ln k'}{\partial P}\right)_T = -\frac{\Delta V}{RT} + \left(\frac{\partial \ln \phi}{\partial P}\right)_T \quad (14)$$

where ΔV is the difference between the partial molar volume of the solute in the two phases, ϕ is the column phase ratio, and T is the temperature. The phase ratio $\phi = V_S/V_0$ of the packing material is nearly independent of the pressure according to the points (1) and (2) of our earlier discussion, in Section 2. Then,

$$\left(\frac{\partial \ln k'}{\partial P}\right)_T \simeq -\frac{\Delta V}{RT} \quad (15)$$

The single effect of the solvent compressibility on the elution volume of thiourea, as predicted by Tait equation, are plotted in Fig. 3. They do not match the curvature of the experimental profiles.

We need to separate the effects of the solvent compressibility from those of thiourea adsorption. We know the former, at least in pure water and methanol, from the empirical Tait model. To estimate the second, we will assume that the true hold-up volume of the column is the value given by the pycnometric measurements, $V^0 = 1.213$ mL at atmospheric pressure and derive the retention factor from the classical equation:

$$k' = \frac{V_R - V_M}{V_M} \quad (16)$$

According to Tait equation, we can now derive V_M for all pairs of inlet and outlet column pressures:

$$V_M = \frac{V^0}{P^{il} - P^{ot}} \int_{P^{ot}}^{P^{il}} \frac{dP}{1 + c \ln((P_0 + b)/(P + b))} \quad (17)$$

Fig. 4 shows plots of the retention factor of thiourea versus the average column pressure for pure methanol (full circles), pure water (full squares) and four solutions. Clearly, the trend is not linear, as it was often observed with proteins or peptides which have much larger retention factors. Surprisingly, the retention factor of thiourea decreases first for

Table 3

Empirical parameters α_0 , α_1 and α_2 of Eq. (7) which fit the best the experimental elution volumes of thiourea vs. the pressure for different mobile phase composition

	Methanol content					
	0%	20%	40%	60%	80%	100%
α_0	1.2967	1.2760	1.2592	1.2631	1.2792	1.3613
α_1	-0.00087	0.00939	0.0922	0.05753	0.10036	0.01852
α_2	0.19243	0.22863	0.04514	0.15795	0.14054	0.37813

Table 4
Experimental compressibilities ($\times 10^5$) of mixtures of methanol–water for different pressures

Pressure (bar)	Methanol content							
	0%	0% Tait	20%	40%	60%	80%	100%	100% Tait
100	3.1	4.4	4.5	8.3	7.5	10.5	7.7	11.3
200	6.2	4.3	8.2	8.9	9.8	12.6	13.8	10.5
300	9.3	4.2	11.8	9.6	12.2	14.7	19.6	9.8
400	12.3	4.0	15.3	10.2	14.6	16.7	25.1	9.2

The comparison with data predicted with Tait model of solvent compressibility is also given for pure water and pure methanol. Note the reversed trend.

average column pressure less than 175 bar, then increases at higher pressures. In other words, the difference between the partial molar volumes of thiourea in the adsorbed and the liquid phases changes sign within the pressure range usually applied in chromatography. This explains why the curvatures of the plots of the elution volume of thiourea in Fig. 1 are all convex downward. At low pressures, the variation of the elution volume is the result of two opposite effects, the retention of thiourea decreases with increasing pressure, which tends to decrease V_R , while the compressibility of the mobile phase tends to increase V_R . Thus, the slope $\frac{dV_R}{dP}$ is lower than the one predicted by the compressibility model. On the other hand, under high pressures, the two effects become additive, the increasing adsorption of thiourea and the compressibility of the mobile phase contribute both to the increase of V_R . As a result, under high pressures, the experimental slope, $\frac{dV_R}{dP}$, exceeds the one predicted by the Tait equation.

For all the solutions used, it is possible to derive the parameter b from the experimental data by writing that the relative increase of the elution volume of thiourea (between the lowest and the highest pressure applied) measured is given by the Tait model (Eq. (2)). This assumption is in agreement with the experimental data and the Tait curves giving almost the same

results in Fig. 3. Since the parameter c is almost the same for pure water (0.1368) and pure methanol (0.1480), we may assume a simple linear interpolation to assess c at every mobile phase composition. The best parameters calculated for the Tait model are given in Table 5. The validity of these parameters was tested by plotting the logarithm of the retention factor, k' , versus the pressure (Fig. 3), after correcting it using Eq. (15). These plots are given in Fig. 4. As expected, they exhibit a minimum retention of thiourea for an intermediate pressure around 175 bar. It is noteworthy that the parameter b decreases continuously from pure water to pure methanol. The values of the compressibility calculated with Eq. (4) are shown in Fig. 5. Unlike the viscosity of methanol–water mixtures, their compressibility is a monotonous function of the methanol concentration. It is minimum for water and maximum for methanol.

These results are qualitatively in good agreement with those of the P – ρ – T measurements performed by another group [8,9] who used a different experimental approach, incorporating a constant volume cell and a pressure gauge. They provided compressibility data at different temperatures (from 298.15 to 523.15 K) for three methanol volume fractions (0.25, 0.50 and 0.75). We compared in Fig. 6 these data measured at 298.15 K with our experimental values of the

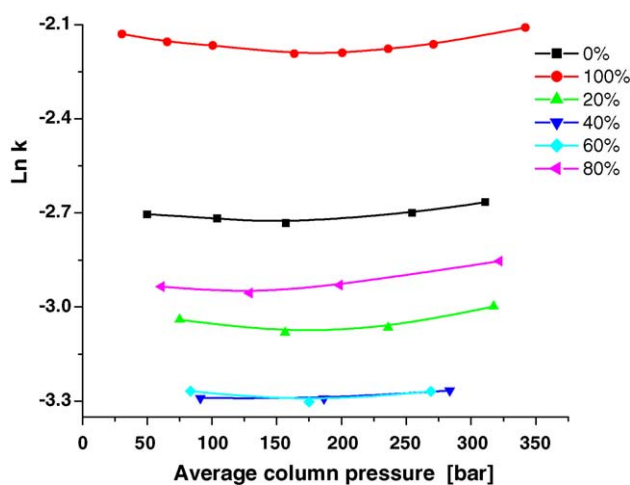


Fig. 4. Plot of the retention factor of thiourea ($V_R - V_M$)/ V_M on the Resolve silica adsorbent for different mobile phase compositions after recalculating the true thermodynamical column void volume V_M under NTP conditions using the Tait model of solvent compressibility and the parameters given in Table 5. Note that the retention decreases at low pressure (positive ΔV) and increases at high pressures (negative ΔV).

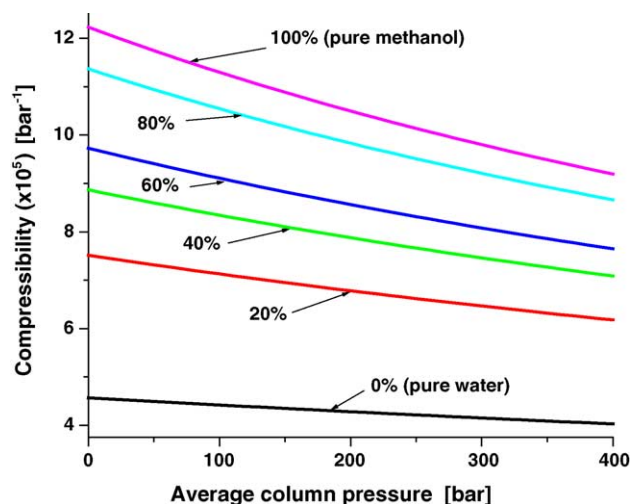


Fig. 5. Compressibility curves measured by chromatography (Tait model with parameters given in Table 5) for the different mixtures of water and methanol at 295 K. Note the continuous increase of the solvent compressibility when the methanol content increases.

Table 5

Best Tait model parameters obtained by fitting Eq. (2) to the largest relative increase of the elution volume of thiourea measured (Fig. 1)

	0%	20%	40%	60%	80%	100%
<i>b</i>	2996	1850	1590	1470	1280	1210
<i>c</i>	0.137	0.139 ^a	0.141 ^a	0.143 ^a	0.1455 ^a	0.148

Note the continuous decrease of the parameter *b* when the methanol content increases.^a Calculated by linear interpolation of the literature coefficient given for pure water and pure methanol.

compressibility found in this work at 295 K and for methanol fractions of 0.20, 0.40, 0.60 and 0.80. There is an excellent agreement between these sets of data. The compressibility of methanol solutions increases with increasing methanol concentration in the mobile phase. The chromatographic measurements are accurate enough to measure this trend. The rate of increase of the compressibility is very similar between the two sets of data (20% compared with 25%, 40% compared with 50%, 80% compared with 75%). The absolute values of the compressibility differ somewhat, however. They seem to have been overestimated by the chromatographic method coupled with the Tait compressibility model. Nevertheless, the present data gave a good estimate of the compressibility of methanol–water mixtures despite the spurious adsorption of the elution volume marker, thiourea.

Similar results could have been obtained by chromatographic methods based on the measurement of the true column void volume using either the minor disturbance methods [18] or deuterated eluent components [19], with a refractive index detector. These methods are much more time-consuming.

4.4. Measurements of the compressibilities of mixtures with a non-porous material

Similar experiments were made with a column packed with non-porous silica particles. The specific surface area and

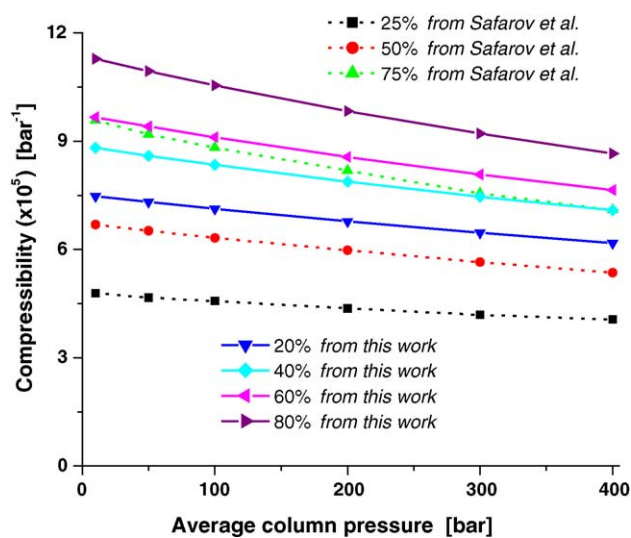


Fig. 6. Comparison of the compressibility values measured in this work with the Resolve mesoporous silica ($T = 295$ K) with those of the literature [9] ($T = 298.15$ K).

the average particle diameter of this material were $0.6 \text{ m}^2/\text{g}$ and $3.8 \mu\text{m}$, respectively. The advantage of these particles resides in their very low specific surface area, making legitimate to neglect the adsorption of thiourea on this adsorbent and simplify the interpretation of the measurements. The total void volume of the column was determined by pycnometry using methanol and water as the two solvents, like for the direct determination of the hold-up volume of the Resolve column (see Section 4.1). The weights of the column filled with methanol and water were 69.47960 and 69.62175 g, respectively. Thus, the void volume was $V_0 = 0.6801 \text{ mL}$, corresponding to a total porosity of this column of 0.409, a value consistent with the external porosity (≈ 0.37) of packed mesoporous silica columns, commonly determined by inverse size-exclusion chromatography (ISEC). Fig. 7 shows the elution profile of thiourea on this column. The peak is not perfectly Gaussian but exhibits a small hump on its rear front, suggesting that the bed is not radially homogeneous and that the different streamlines along the bed are not all equivalent. This void volume is larger than the elution volume of thiourea measured at the apex of the peak (Fig. 7) but corresponds approximately to the elution volume of the hump. In all cases, however, the hold-up volume measured was the retention volume of the peak maximum, which is narrow, so accurate and reproducible results were obtained. All the

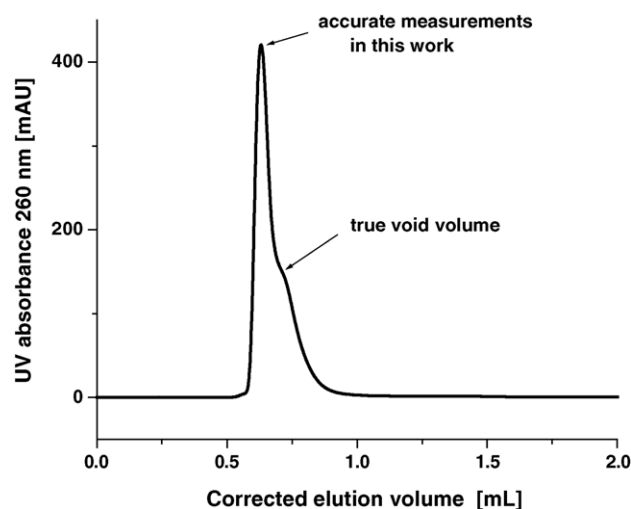


Fig. 7. Elution profile of thiourea obtained after the injection of $2 \mu\text{L}$ of a less than 1 g/L solution of thiourea in pure methanol using the column packed with the non-porous material. Flow rate 1.2 mL/min , $T = 295$ K. The elution volume is obtained after subtraction of the extra-column volume 0.0955 mL . Note the presence of a hump on the rear part of the profile suggesting a packing heterogeneity.

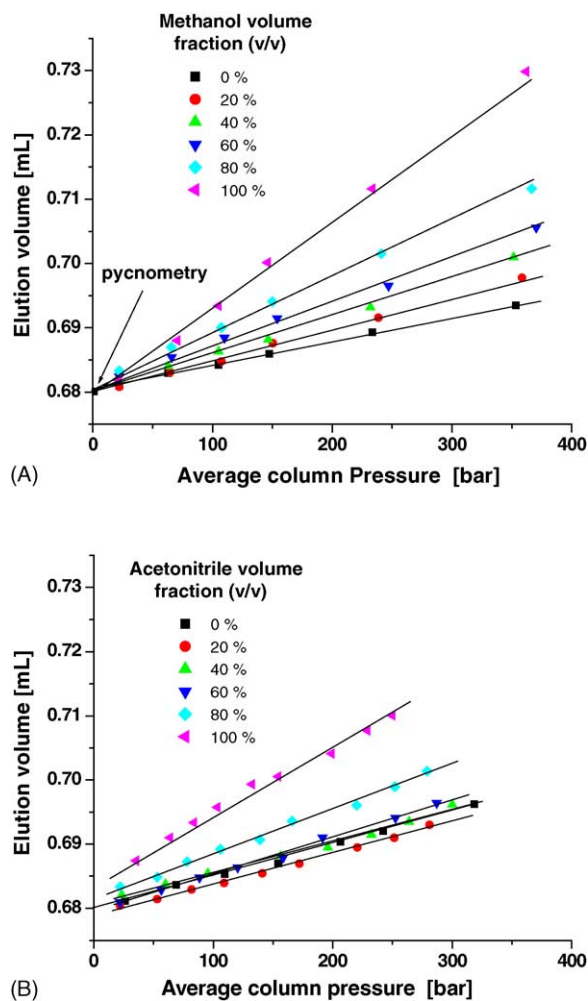


Fig. 8. Evolution of the elution volume of thiourea measured on the non-porous silica packed column for methanol–water (A) and acetonitrile–water (B) mixtures, respectively. Note the quasi-linearity of the plots by comparison to Fig. 1 because of the negligible impact of the adsorption of thiourea.

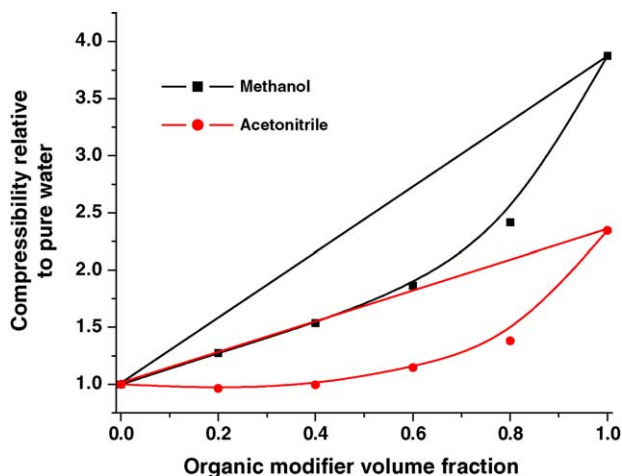


Fig. 9. Plots of the compressibilities (relative to pure water) measured on the non-porous particle adsorbent at constant mobile phase composition and constant pressure. Note the negative deviation of the mixtures' compressibility.

elution volumes were corrected for the contribution of the extra-column volumes measured under the same experimental conditions as the retention volume of the thiourea peak but without the column, replacing it with a zero-volume connector. The results obtained are summarized in Fig. 8A and B for methanol–water and for acetonitrile–water mixtures, respectively.

The convergence at low pressures of all the curves in the two figures is striking compared to the similar curves shown in Fig. 1. The adsorption of thiourea is now completely negligible. Also for this reason, the convex downward curvature of the plots has disappeared. The curves can be fitted to a linear function. The void volume determined by pycnometry is in very good agreement with the elution volume of thiourea derived by extrapolation to zero pressure of all the curves corresponding to the different mobile phases (Fig. 8A and B). Each plot was fitted to Eq. (2), in order to derive the best parameters of the Tait model. The parameter c was fixed at 0.1368, the value given for pure water [6], because the curves are practically insensitive to small variations of this parameter. The compressibilities of the methanol–water and acetonitrile–water mixtures calculated from the best values of the b parameter found by regression are shown in Fig. 9. First, it is important to observe that all the experimental data are consistent with the compressibilities of the three pure solvents. The compressibilities of water [6], acetonitrile [20] and methanol [6] are 460, 820 and 1320 TPa⁻¹, respectively. Second, the data in Fig. 9 show that the compressibility of methanol–water and acetonitrile–water mixtures does not follow an ideal behavior pattern. A significant negative deviation is observed, as was observed for mixtures of acetonitrile with various ketones [20].

5. Conclusion

This work demonstrates that within the range of pressures conventionally applied in HPLC (1–400 bar), aqueous mobile phases of methanol or acetonitrile have a significant compressibility. The immediate consequence is that the column hold-up volume depends on the average column pressure selected, i.e., on the flow rate applied during chromatographic experiments. Although small, this effect is larger than the accuracy of the measurements of the retention factors and may even affect the separation factor. The actual importance of the effect depends on two parameters, the true geometric void volume (the difference between the volume of the column tube and the volume of the packed particles) and the compressibility of the mobile phase used, which is itself a function of the chemical nature and the composition of this liquid. The order of magnitude of the increase in the column hold-up volume is about 1.5, 5 and 7% with pure water, pure acetonitrile and pure methanol, respectively, when the pressure increases from atmospheric pressure to about 350 bar (the effect would exceed 10% with the organic mobile phases used in NPLC). Since most mobile

phases used in RP-HPLC are mixtures of methanol or acetonitrile with water, great care should be taken in the measurement and handling of chromatographic retention properties when an accuracy better than a few percent is desired. This applies to both linear and preparative chromatography (although, in this latter case, lower pressures are usually applied).

The effect of pressure on parameters of the retention mechanism should be of particular concern to two groups of chromatographers. Analysts considering ultra-fast analyses should be concerned by this effect, given the present trend in the evolution of HPLC instruments toward ultra-high pressure systems capable of operating with inlet pressures up to several kbar. They will have carefully to take into account the compressibility of the liquid phase, which will drastically affect the accuracy and reproducibility of retention data if it is neglected. Even when operating with inlet pressures of a few hundred bar, those interested in the determination of adsorption isotherms should pay careful attention to the compressibility of the mobile phase because the determination of the amount of a compound adsorbed on the stationary phase in the presence of a solution of known concentration depends directly on the value of the hold-up time t_0 [21].

The compressibilities of aqueous solutions of methanol or acetonitrile are not easily found in the literature as are, e.g., viscosities, densities, or even excess volumes of these mixtures. Yet, their knowledge is essential to derive correct estimates of chromatographic parameters. This report shows that the regular HPLC instrumentation can provide an accurate method of measurement of these compressibilities. It requires only a column packed with solid silica particles (in order to eliminate the slight but spurious influence of the marker's adsorption on the silica surface) and the systematic determination of its hold-up time as a function of the average column pressure, using a conventional hold-up time marker. This method gives results of sufficient accuracy if the HPLC instrument can be operated in the pressure range of 1 to 400 bar, for solutions that have a compressibility exceeding that of water.

6. Nomenclature

NTP	normal conditions of pressure (atmospheric pressure P^0) and temperature ($T = 295$ K)
P^{il}	experimental inlet column pressure (Pa)
P^{ol}	experimental outlet column pressure (Pa)
S_C	cross-section area of the column (m^2)
V_0	geometrical accessible free volume in the column under pressure P (L) (see [7])
V^0	geometrical accessible free volume in the column under atmospheric pressure P^0 (L) (see [7])
$V(P)$	elution volume of solvent which percolates through the free geometrical volume V_0 under pressure P (L)

Greek letters

α_1	first-order fitting parameter of the second-order polynomial to $V(P)$ (L Pa^{-1})
α_2	second-order fitting parameter of the second-order polynomial to $V(P)$ (L Pa^{-2})
$\beta_T(P)$	compressibility of the liquid at temperature T and pressure P (Pa^{-1})
ϵ_T	total porosity of the column (V_0/V_C) (V_C is the volume of the column tube)
ρ	liquid density

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