Influence of pressure on the properties of chromatographic columns II. The column hold-up volume

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Abstract

The effect of the local pressure and of the average column pressure on the hold-up column volume was investigated between 1 and 400 bar, from a theoretical and an experimental point of view. Calculations based upon the elasticity of the solids involved (column wall and packing material) and the compressibility of the liquid phase show that the increase of the column hold-up volume with increasing pressure that is observed is correlated with (in order of decreasing importance): (1) the compressibility of the mobile phase (+1 to 5%); (2) in RPLC, the compressibility of the C_{18}-bonded layer on the surface of the silica (+0.5 to 1%); and (3) the expansion of the column tube (<0.001%). These predictions agree well with the results of experimental measurements that were performed on columns packed with the pure Resolve silica (0% carbon), the derivatized Resolve-C_{18} (10% carbon) and the Symmetry-C_{18} (20% carbon) adsorbents, using water, methanol, or *n*-pentane as the mobile phase. These solvents have different compressibilities. However, 1% of the relative increase of the column hold-up volume that was observed when the pressure was raised is not accounted for by the compressibilities of either the solvent or the C_{18}-bonded phase. It is due to the influence of the pressure on the retention behavior of thiourea, the compound used as tracer to measure the hold-up volume.
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1. Introduction

In order to increase the number of analyses performed per unit time, chromatographers need to operate columns at flow-rates as high as compatible with the achievement of the desired separation. This requires the combined use of small-size particles (in order to obtain a sufficiently high column efficiency, particularly at high flow rates) and of large inlet pressures (in order to force at a sufficiently high velocity the percolation of the mobile phase through a bed packed with these small particles). Typical particle sizes now advocated are between 1 and 2 µm, leading to pressures in the low kilobar range [1]. The successful operation of a chromatographic column under these unconventional conditions

requires new, advanced pump technology, new injection devices that can reproducibly deliver very small samples and do it very fast, new fittings and connecting tubes of very small inner volumes, a detector having a cell of small volume, a very fast, yet high response, and a low noise level, and a thorough optimization of the whole chromatographic system, in order to achieve the expected performance under these high pressures.

So far, the effects of the high pressures that are now advocated [1] on the experimental results have been considered with benign contempt by the establishment. Long ago, Martin and Guiochon [2,3] reviewed and discussed the effects of the pressure on the properties of liquids (viscosity, specific volume, diffusion coefficient, . . .) and on the physical characteristics of the tubes of chromatographic columns (tube diameter and length) and of the packing material itself (volume, internal and external porosities, and permeability).

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This work forecasted a significant, linear dependence of most of the conventional parameters of a separation (hold-up volume, retention factor, possibly separation factor, and certainly van Deemter efficiency parameters) on the pressure drop applied to the column. However, this work was undertaken at a time when inlet pressures were not expected to exceed 0.4–0.5 kbar. Although the physico-chemical basis of the problem has not changed, this work needs to be updated and adapted to the new requirements of HPLC [4].

Under steady-state conditions, the mass flow of the mobile phase is constant along the column, at every cross-section of the column, equal to the mass flow rate delivered by the pump. Accordingly, because liquids are compressible, the volume flow-rate varies along the column [2,4]. The pumping systems of good instruments are designed to deliver a constant flow rate at the outlet of this system, whatever the inlet pressure. This determines the volume flow rate at the column exit. Elsewhere, the volume flow rate, hence the linear velocity of the mobile phase depends on the local pressure and on the compressibility of the mobile phase.

To a degree, pressure affects all the physico-chemical properties of matter [2–11]. As a result of all these effects, the properties of a chromatographic system are not uniform but vary along the column. This includes the diameter and the length of the column, the volumes occupied by the silica packing material and by the bonded layer of alkyl chains in RPLC columns, hence the column hold-up volume, the column external and total porosities, its permeability, and the retention data, i.e., the retention and separation factors. Because these parameters are all local functions of the pressure, their average values, i.e., the values that are derived from the chromatographic data, are all functions of the pressure gradient along the column, that is of the flow rate. In principle, the relationships between the local pressure, the pressure gradient, and the magnitude of the pressure dependence of the chromatographic parameters are known. However, these relationships depend on physico-chemical properties that are not always accurately known. For example, the compressibilities of only a few solvents are known and that of almost none of their mixtures is available in the literature. It is important to measure these parameters in order to be able to assess the exact influence of pressure in practical cases.

A systematic increase of the column hold-up volume with increasing pressure (by a few percent) has already been reported [5]. Important effects of pressure have also been observed and reported regarding the adsorption behavior of analytes having relatively large molecules, such as proteins [6,7], peptides [8], and fullerenes [9]. It was shown that the pressure dependence of the retention factor arises from the difference between the partial molar volume of the solute in the two phases and that the apparent value of $\ln k'$, the logarithm of the retention factor, is proportional to the pressure drop of the column [7,8,10]. It is often assumed in the mass balance equation of chromatography [11] that the properties of the column bed are axially (and radially) uniform. Actually, they

are not. However, as the results shown below demonstrate, the effects of this pressure dependence are negligible in the pressure range used in preparative HPLC.

The column hold-up volume is an important chromatographic property that is derived from the elution volume of a non-retained compound under normal temperature and pressure conditions. This is an average property that depends on the pressure gradient, on the compressibilities of the mobile and the stationary phases, and on the elasticity of the column wall. Because there is no phase equilibrium with an unretained compound, and because the elastic properties of the column tube and of the packing material are independent of the nature of the solute and of the mobile phase composition (although this may not be true in for most mobile phases used in RPLC), the systematic measurement of the relationship between column hold-up volume and inlet pressure provides a relatively easy method of investigation of the validity of the current theoretical relationships between pressure and column characteristics and a practical method to assess the compressibility of solutions.

Thus, it has been established that the value measured for the column hold-up volume, derived from the elution time of a non-retained compound (thiourea) at constant outlet flow rate depends on the average column pressures, even in the range from 1 to 200 bar. The first goal of this work was to assess the relative importance of (1) the compressibility of the mobile phase, (2) the column tube elasticity, (3) the compressibilities of the silica, and (4) that of the C_{18}-bonded layer of the packing material on this dependence. Basic equations of solid elasticity and fluid compressibility are easily applied. They allow the calculation of the relative importance of each of the four contributions that affect the hold-up volume. Our second goal was the acquisition of experimental data with different mobile phases of different compressibilities (water, methanol, and n-pentane), and different columns having different carbon loadings (Resolve silica, 0% C; C_{18}-bonded Resolve silica, 10% C; and C_{18}-bonded Symmetry silica, 20% C). All possible combinations could not be used (e.g., water and C_{18}-bonded silica or n-pentane and pure silica) because they are incompatible or poorly compatible due to unfavorable values of the interfacial tension between a strongly polar liquid and a very apolar adsorbent (or conversely). The comparison between predicted and experimental results will be discussed in detail.

2. Theory

When a bed is percolated by a stream of a liquid, a resistance to this flow arises, due to the viscous forces. As a result, to keep the liquid flow rate constant at the column outlet (usually under atmospheric pressure), it is necessary to apply a steady pressure at the column inlet. The stationary regime reached at constant flow rate is characterized by a pressure profile $P(z)$ along the column length, z. If we consider a volume element of the column bed, between posi-
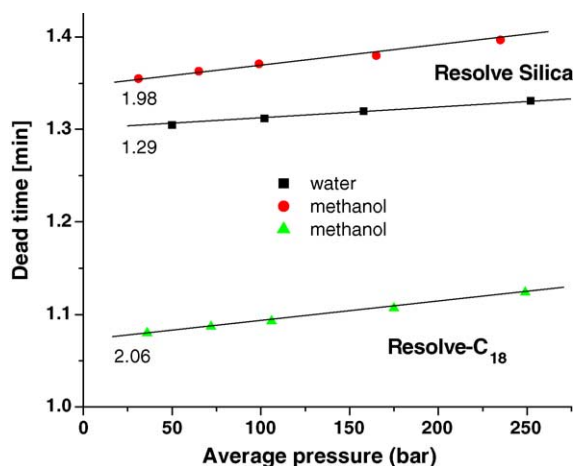
Fig. 1. Variation of the hold-up time of the Resolve silica and Resolve-C_{18} columns with either pure water or pure methanol as the mobile phase. The number on the left hand part of the plot is proportional to the slope of the linear fit of the experimental data. Note the higher value of the hold-up volume measured on the bare silica (+20%) and the larger slope with methanol. The hold-up times are derived from the elution time of thiourea (2 μL injection of a solution at less than 1 g/L, UV detection 265 nm) at a flow rate of 1 mL/min. $T = 295$ K.

tions z and $z + dz$ along the column (Fig. 1), the local Darcy law gives

$$\bar{u} = -\frac{k}{\eta}\frac{dP}{dz} \tag{1}$$

where $\bar{u}$ is the cross-section average fluid velocity (dimension $M\,S^{-1}$), k the permeability of the bed (dimension M^2) and η the fluid viscosity (1 cP $= 10^{-1}$ kg m^{-1} s^{-1}) under the local pressure, P (1 Pa $= 10^{-5}$ bar) at the abscissa z. Farkas et al. [12] have experimentally demonstrated the validity of the integrated form of Darcy law at the very low flow velocities usually achieved in HPLC (range of Reynolds number between 1×10^{-6} and 1×10^{-2}).

The variation of the internal pressure along the column has several impacts on the properties of the chromatographic system. It affects the column dimensions, the dimensions of the packing particles, hence column porosity and permeability, and the density of the mobile phase.

2.1. Definition of the measured column hold-up volume

There are many different definitions of the column hold-up volume. However, for the purpose of assessing the effect of pressure on the hold-up volume, they are quite similar. Two definitions are relevant to our problem and have physical significance. The first one is the free geometrical volume complementary to the volume of packing material (here the C_{18}-bonded silica) in contact with the mobile phase inside the column tubing under an average column pressure P. This geometrical volume depends only on the volume of the column tubing (V_C) and on that of the C_{18}-bonded silica (V_S):

$$V_0(P) = V_C - V_S \tag{2}$$

V_0 can be measured by a static method (e.g., pycnometry, by weighing the mass of liquid phase inside the column or difference between the masses of the column filled with the liquid and that of the dry column, knowing the volumetric mass of the liquid and that of the gas used to dry the column).

Actually, a large majority of chromatographers consider instead that the column hold-up volume is the elution volume of an ideal unretained compound. Under this assumption, the hold-up volume of a chromatographic column is measured dynamically and corresponds to the volume V_M of liquid phase that is required to elute the unretained compound. This volume V_M is measured at the column outlet (where the detection takes place), under NTP conditions. Because there is a pressure gradient along the chromatographic column (with an average column pressure P) when a flow is applied, the volume measured depends not only on the dimension of the column tubing and on the volume of adsorbent, but also on the compressibility of the liquid phase. The higher the compressibility, the larger the amount of solvent which can fill the free geometrical volume V_0 in the column and the larger the elution volume measured under NTP conditions at the column outlet. V_M is, by definition, necessary larger than V_0:

$$V_M(P) = \int_0^L \frac{\epsilon(z)S_c(z)}{\alpha(z)}dz \tag{3}$$

where $\epsilon(z)$ is the local total porosity of the column, $S_c(z)$ the cross-section area of the column tubing, and $\alpha(z)$ the contraction coefficient (<1) of the liquid at the position z along the column.

In addition, the so-called "unretained" compound may actually have a weak affinity for the adsorbent, so that the measured elution volume V is also larger than V_M. Later, in the experimental results, all the volumes measured are referred to this volume V, which take into account the simultaneous effects of the pressure on the column dimensions, of the compressiblity of the adsorbent, of the compressibility of the mobile phase, and the retention of the tracer.

$$V(P) = \left(1 + \frac{V_S}{V_0}H_{\text{tracer}}\right)V_M \tag{4}$$

where H_{tracer} is the Henry constant of the tracer between the liquid and the stationary phases at the average pressure.

The next sections discuss separately the different effects that affect the measurement of $V(P)$.

2.2. Column dimensions

The internal pressure applies a radial stress to the column tubing all along the z axis. A radial constraint is applied to the circular column wall ($\Delta P = P - P^0$, P and P^0 being the inner and the external atmospheric pressure) and the internal radius r_{in} becomes larger at the column inlet (where the inner pressure is the highest) than at the column outlet (where the inner pressure is close, if not equal, to the atmospheric pressure). We assume that the experiment is made under safe

conditions, hence that the deformations remain in the elastic range and are linear. Under such conditions, the effect is small and r_{in} can be written [2,4]:

$$\frac{r_{in}}{r_{in,0}} = 1 + a(P - P^0) \tag{5}$$

where $r_{in,0}$ and r_{in} are the internal radii at the pressure P^0 and P, respectively. The coefficient a will be obtained from calculation of the deformation of the internal radius as shown in the next equations.

Also, because of the back-pressure difference, $\Delta P = P_i - P^0$, between the inlet and the outlet of the column, the length L_0 of the column at atmospheric pressure is stressed in the longitudinal direction. In this treatment [4], the effects of the longitudinal and radial stresses are additive. The calculation of the actual effects on the dimensions of a tank resulting from the increase of the internal pressure is made complex by the fact that the radial expansion of the tube results in different increases of the internal and external radii, so that there is a slight decrease of the tube thickness, hence a slight extension of its length. The detailed calculation has been published [4]. The results for the three dimensions of the tube are given by these three equations

$$\frac{L}{L_0} = 1 + \frac{1}{3}\frac{r_{in,0}^2}{r_{ext,0}^2 - r_{in,0}^2}\frac{\Delta P}{2E} \tag{6a}$$

$$\frac{r_{in}}{r_{in,0}} = 1 + \left(\frac{1}{3} + \frac{4}{3}\frac{r_{ext,0}^2}{r_{in,0}^2}\right)\frac{r_{in,0}^2}{r_{ext,0}^2 - r_{in,0}^2}\frac{P - P^0}{E} \tag{6b}$$

$$\frac{r_{ext}}{r_{ext,0}} = 1 + \frac{5}{3}\frac{r_{in,0}^2}{r_{ext,0}^2 - r_{in,0}^2}\frac{\Delta P}{2E} \tag{6c}$$

where E is the Young's modulus of the column-wall and r_{ext} the external column diameter (in all numerical applications, the column will be assumed to be made of stainless steel and the Poisson coefficient was assumed equal to 1/3).

By identification of Eqs. (5) and (6b), we derive the coefficient a:

$$a = \left(\frac{1}{3} + \frac{4}{3}\frac{r_{ext,0}^2}{r_{in,0}^2}\right)\frac{r_{in,0}^2}{r_{ext,0}^2 - r_{in,0}^2}\frac{1}{E} \tag{7}$$

Because the two ends of the column are held by fitting bolts that are wider, thicker, and more rigid than the column tube, the shape of this tube becomes complex, with a diameter that is barely changed at both ends and a slightly conical shape of the main body of the tube. This shape, however, has little effect on the actual change in the column volume that remains small as shown later.

2.3. Compressibility of the mobile phase

The variation of the local pressure along the column axis induces a correlative variation of the local values of the specific volume of the liquid phase percolating through the pack-

ing bed and of its viscosity. Since the hold-up column volume is measured at the outlet of the column under normal conditions of pressure and temperature (e.g., at atmospheric pressure and ambient temperature), this is the average of the local specific volumes occupied by an element of the mobile phase during its migration along the column. It does not correspond to the actual geometrical void volume at the column outlet since the volume occupied by a given mass of the liquid phase depends on the local pressure and this pressure varies along the column. The equation that best describes the compressibility of liquids or change in their specific volume, V_{sp}, with the local pressure is the Tait equation [13]:

$$V_{sp} = V_{sp}^0\left[1 + c\ln\left(\frac{P^0 + b}{P + b}\right)\right] \tag{8}$$

where V_{sp}^0 is the specific volume of the liquid under NTP conditions (P^0, T), b and c are parameters of the Tait equation and are constant for a given liquid.

If we consider a slice of column of length δz with a total porosity ϵ_t, and if the pressure P remains constant in this slice, the volume dV^0 of liquid phase in the slice measured under the NTP conditions is:

$$dV^0 = \frac{\epsilon_t S_c\,dz}{1 + c\ln[(P^0 + b)/(P + b)]} \tag{9}$$

where the column porosity and its cross-section, ϵ_t and S_c, are assumed to be constant and equal to the value measured under NTP conditions (an approximation, see later). The hold-up volume or retention volume of a non-retained compound is the integral of this expression along the column, assuming a constant pressure gradient (which is approximate because the mobile phase viscosity is pressure dependent, causing a slight curvature of this pressure gradient).

$$\frac{V}{V^0} = \frac{1}{P_i - P^0}\int_{P^0}^{P_i}\frac{dP}{1 + c\ln[(P^0 + b)/(P + b)]} \tag{10}$$

where $S_c L_0$ is the geometrical volume of the column and $V^0 = \epsilon_t S_c L_0$ is the volume available to the mobile phase under NTP.

In fact, the very compressibility of the mobile phase leads to a non-linear profile of the pressure along the column, as it is obvious in gas chromatography, and even if the viscosity of the gases is independent of the pressure as it is for ideal gases. Eq. (9) cannot be integrated straightforwardly to give Eq. (10). Instead, the precedent equation should be written as [4]:

$$\frac{V}{V^0} = \frac{\int_{P^0}^{P_i} dP/[1 + c\ln[(P^0 + b)/(P + b)]]^2}{\int_{P^0}^{P_i} dP/[1 + c\ln[(P^0 + b)/(P + b)]]} \tag{11}$$

Note that the extra-column volume contribution is made of two parts, the extra-column volumes that take place upstream the column, the value of which is a linear function of the inlet pressure, and the extra-column volumes located downstream the column, that are practically constant, since the pressure there remains nearly constant.

2.4. Compressibility of the stationary phase

The volume V_S of the packing material inside the column is also a function of the pressure because all solids are compressible. In a column slice of length δz, the compressibility factor β of the homogeneous packing material is defined as:

$$\beta = -\frac{1}{\delta V_S} \frac{d(\delta V_S)}{dP} \tag{12}$$

Let us assume that the packing material is a heterogeneous adsorbent (e.g., RPLC phases), made of two distinct materials, characterized by the compressibility factors β_1 and β_2, respectively. The compressibility β_m of the mixture can be writen:

$$\beta_m = -\frac{1}{\delta V_{S,1} + \delta V_{S,2}} \frac{d(\delta V_{S,1} + \delta V_{S,2})}{dP} = \phi_1 \beta_1 + \phi_2 \beta_2 \tag{13}$$

where ϕ_1 and ϕ_2 are the volume fractions of the homogeneous materials 1 and 2, respectively ($\phi_i = \delta V_{s,i}/(\delta V_{s,1} + \delta V_{s,2})$). This model is useful to describe the compressibility of C_{18}-bonded silicas which are made of pure silica ($\beta_{silica} = 10^{-6}\,bar^{-1}$) and bonded octadecyl chains that can be considered as equivalent to octadecane ($\beta_{C_{18}} = 10^{-4}\,bar^{-1}$), although the bonded layer being less dense might be more compressible than a pure alkane.

From Eq. (12), the volume of silica, dV_S, contained in a slice of column of length dz under pressure P is

$$dV_S = (1 - \epsilon_t^0)S_c \exp(-\beta(P - P^0))\,dz \tag{14}$$

where $dV_{S,0} = (1 - \epsilon_t^0)S_c dz$, $dV_{S,0}$ being the volume of packing material contained in the same slice under pressure P^0 and ϵ_t^0, the total column porosity under atmospheric pressure. Note that we neglect the possible reorganization of a shrunk packing material under the viscous stress of the stream of the mobile phase because we assume that its deformation remains within the limits of its elasticity. Integration of Eq. (14) over the length L of the column, assuming again a constant gradient of pressure gives the relative decrease of the volume of packing material by reference to the volume measured under atmospheric pressure P^0:

$$\frac{V_S}{S_c L} = \frac{1}{P_i - P^0} \int_{P^0}^{P_i} \exp(-\beta(P - P^0))\,dP$$

$$= \frac{\exp(\beta P^0)}{\beta(P_i - P^0)}(\exp(-\beta P^0) - \exp(-\beta P^i)) \tag{15}$$

The same expression can be used to assess the shrinkage of the volume of the bonded layer on a C_{18}-bonded silica material, by substituting β by β_m, the compressibility of the mixture (pure silica + pure octadecylsilane) (see Eq. (13)).

2.5. Pressure dependence of the mobile phase viscosity

The change in hold-up volume arising from the compressibility of the mobile phase is not the same whether the mobile phase is isoviscous or its viscosity is pressure-dependent. For instance, the relative rates of variation of viscosity α with pressure for pure water (at 303 K), methanol (at 293 K), and n-pentane (at 303 K) are $0.053 \times 10^{-3}\,bar^{-1}$, $0.47 \times 10^{-3}\,bar^{-1}$, and $1.06 \times 10^{-3}\,bar^{-1}$. Accordingly, Eq. (11) becomes [4]:

$$\frac{V}{V^0} = \frac{\int_{P0}^{P_i} dP/[1 + \alpha(P - P^0)]}{\int_{P0}^{P_i} dP/[1 + \alpha(P - P^0)]} \times [1 + c\ln[(P^0 + b)/(P + b)]]^2 \times [1 + c\ln[(P^0 + b)/(P + b)]] \tag{16}$$

2.6. Retention of the tracer molecule

Finally, the value of V may be affected by a small retention of the tracer molecule, the retention factor of which depends on the average column pressure, according to [14]:

$$\left(\frac{\partial \ln k}{\partial P}\right)_T = -\frac{\Delta V}{RT} \tag{17}$$

where ΔV is the variation of the partial molar volume and T the temperature. In this equation, the column phase ratio is supposed to remain constant. Assuming $\Delta V = -10\,mL/mol$ (a typical value for low-molecular-weight compounds [7]) and a retention factor $k' = 0.1$ for the tracer, the elution volume of the tracer will increase by slightly less than 1% when the average column pressure increases from atmospheric pressure to 200 bar (0.8%). Although small this effect is easy to observe.

3. Experimental

3.1. Chemicals

The mobile phases used in this work were pure pentane, methanol, and water, all HPLC grade, all purchased from Fisher Scientific (Fair Lawn, NJ, USA). These solvents were filtered before use on an SFCA filter membrane, $0.2\,\mu m$ pore size (Suwannee, GA, USA). Thiourea and 1,3,5-tri-*tert*-butylbenzene were chosen to measure the column hold-up volume in water or methanol and in pentane, respectively. Both were obtained from Aldrich (Milwaukee, WI, USA).

3.2. Materials

Three different columns were used in this study. They were, respectively, packed with pure Resolve silica, non-endcapped Resolve-C_{18}, and endcapped Symmetry MS C_{18}. All three columns had been given by the manufacturer (Waters Corporation, Milford, MA, USA). These columns have the same dimensions, $150\,mm \times 3.9\,mm$. The main characteristics of these packing materials are summarized in Table 1. The hold-up times of these three columns were measured

Table 1
Physico-chemical properties of the Resolve, Resolve-C_{18}, and Symmetry-C_{18} packing materials (150 mm × 3.9 mm)

	Column		
	Resolve	Resolve-C_{18}	Symmetry-C_{18}
Diameter ratio (o.d./i.d.)	1.87	1.87	1.87
Particle shape	Spherical	Spherical	Spherical
Particle size (μm)	5	5	5
Pore size[a] (Å)	90	90	86
Pore volume[a] (mL/g)	0.50	0.50	0.90
Surface area[a] (m^2/g)	200	200	346
Total carbon (%)	0	10.2	19.6
Surface coverage (μmol/m^2)	0	2.45	3.18
Endcapping	No	No	Yes

The outer diameter (o.d,) of the stainless steel tube is 7.3 mm.

[a] Data for the packings before derivatization.

at different pressures. They were derived from the retention times of three consecutive injections of thiourea or 1,3,5-tri-*tert*-butylbenzene, provided that they agreed within less than 0.2%. The corresponding volume of mobile phase required to elute the tracer was derived by multiplying the hold-up time and the constant flow rate F_v. Various PEEK tubing were purchased from Upchurch Scientific (Oak Harbor, WA, USA) and connected between the column outlet and the detector cell, to achieve different average pressures along the column, according to the combination of their length (10–50 cm) and their inner diameter (0.0025 or 0.001 in.).

3.3. Apparatus

The hold-up times data were acquired using a Hewlett-Packard (Palo Alto, CA, USA) HP 1090 liquid chromatograph. This instrument includes a multi-solvent delivery system (tank volumes, 1 L each), an auto-sampler with a 250 μL sample loop, a diode-array UV-detector, a column thermostat, and a data station. Compressed nitrogen and helium bottles (National Welders, Charlotte, NC, USA) are connected to the instrument to allow the continuous operations of the pump, the auto-sampler, and the solvent sparging. Each extra-column volume contribution was measured according to the PEEK capillary placed before 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 23 °C and 1 mL/min during 50 min, from each pump head successively, into a volumetric glass of 50 mL. Whatever the back-pressure imposed between the pumps and the detector (from 20 to 350 bar), the flow rate measured after the detector, under NTP conditions, remained unchanged (at 0.9922 mL/min for a 20 bar back-pressure and 0.9928 mL/min for a 350 bar back-pressure, for a set value of 1.00 mL/min). All the measurements were carried out at a constant temperature of 23 °C, fixed by the laboratory air-conditioner. The daily variation of the ambient temperature never exceeded ±1 °C.

3.4. Measurements of the hold-up volume under NTP conditions

Two microliters of a 0.5 g/L solution of tracer were systematically injected, with or without a column. The detection of the tracer was made by UV detection at a wavelength of 265 nm. The column hold-up time is calculated by the difference between the elution time of the tracer in presence of the column and the elution time of thiourea measured with the connecting union and the PEEK capillary used to fix the average column pressure.

4. Results and discussion

4.1. Theoretical predictions

The theory section describes the three main sources of influence of the pressure gradient along the column on the hold-up volume of the column. These sources are (1) the variation of the geometrical volume of the stainless steel column tube (expansion of both its local diameter and its length); (2) the variation of the compressibility of the mobile phase inside the column; (3) the variation of the volume occupied by the packing material; and (4) the slight retention of the "unretained" tracer. The relative importance of these four contributions is now assessed independently. Our goal is to determine, from a theoretical point of view, which phenomenon plays the major role on the hold-up volume of the packed bed.

4.1.1. Expansion of a stainless steel column tube

The pressure affects the diameter and the length of the column tube, according to Eqs. (6b) and (6c), and (6a), respectively. Combining the radial and longitudinal deformations of the stainless steel column tube, it is possible through Eqs. (6a), (6b), and (6c) to assess the average relative variation of the volume of the whole column. The Young's modulus E of stainless steel material is about 2×10^6 bar. The parameter a given by Eq. (7) is 3.5×10^{-6} bar^{-1} for this value of E and for the ratio $r_{in,0}/r_{ext,0} = 0.53$ of the inner to the outer diameter of the column tube. Results are given in Table 2. Within the pressure range studied, 1–400 bar (the highest inlet column pressure at which the instrument can be used), the relative variation of the length of the column, proportional to the pressure difference (Eq. (6a)), is of 4.7×10^{-5} for 400 bar. The relative variation of the column tube internal cross-section radius (Eq. (6b)), also linear, is of 1.4×10^{-3}. These calculations show that the influence of the pressure on the length of the column tube is almost negligible by comparison to its effect on the column cross-section dimension (by almost a factor of 100). In other words, more than 98% of the volume change of the column is caused by the expansion of its diameter. For this reason, it is legitimate to neglect the coupling between these two effects, as we did earlier. As a matter of fact, the

Table 2
Theoretical relative variation of the length L_0 and the internal cross-section radius r_{in} of the column for a pressure difference $\Delta P = P - P^0$ (reference P^0 the atmospheric pressure)

	$\Delta P = P - P^0$			
	1–101 bar	1–201 bar	1–301 bar	1–401
$(L - L_0)/L_0$ ($\times 10^6$)	11.7	23.3	35.0	46.6
$r_{in}/r_{in,0}$ ($\times 10^6$)	350	700	1050	1400

changes in length and even in diameter of the column can be considered as negligible as long as the limit of elasticity of the metal tube is not reached and it is reasonable to assume that the dimensions of the column remain constant in conventional chromatography even at very high pressures.

Extrapolation of the two figures given above suggest that the changes in column length and cross-section area would be of only 0.011 and 1% for an inlet pressure of 2 kbar. Only the second figure is significant and should be taken into account in numerical calculations.

4.1.2. Mobile phase compressibility

The volume of mobile phase compressed in the column during elution is given by Eqs. (10), (11), or (16), depending whether we assume that the pressure gradient inside the column is linear, whether the non-linear pressure profile is taken into account, or whether the viscosity of the mobile phase is considered to be pressure-dependent, respectively. Because we measure the retention time of thiourea and the corresponding volume of mobile phase eluted at the column outlet, under NTP conditions, the free volume inside the column is necessarily overestimated. The volume of liquid phase that should percolate through the column when a constant back pressure, $P_i - P^0$, is applied is given by Eq. (10). This equation assumes that there is a linear gradient of pressure along the column, $(P_i - P^0)/L$, which is not exact because, in its derivation, we neglected both the real non-linear pressure profile and the change in viscosity of the mobile phase with increasing pressure. It also assumes that the column has a constant cross section S (a reasonable assumption, see above) and a constant total porosity, ϵ_t (which is only approximate, see below). Then, the NTP volume of mobile phase dV^0 contained in the column slice of width dz is given by Eq. (9).

Results of numerical calculations are given in Table 3 for four different values of the average column pressure, e.g., 50, 100, 150 and 200 bar, and using the three different Eqs. (10), (11), and (16). Three different liquids were considered (water, methanol, and n-pentane), chosen depending on their compressibility, β, which increases from water ($\beta = 0.46 \times 10^{-4}$ bar^{-1}), to methanol ($\beta = 1.23 \times 10^{-4}$ bar^{-1}) and n-pentane ($\beta = 3.14 \times 10^{-4}$ bar^{-1}). The effect of the compressibility on the hold-up volume of the fluid eluted at constant flow rate is obvious. The higher the liquid compressibility, the higher this volume because, under a given pres-

Table 3
Theoretical relative variation V^0/V_c between the volume of the column measured under NTP conditions (V^0) by dynamic chromatography and the true geometric column volume (V_c) for three different liquids (water, methanol and n-pentane) and different average column pressure ($P_{av} = (P_i - 1)/2$)

	Average column pressure (bar)			
	50	100	150	200
From Eq. (10), mobile phase compressibility with a linear pressure profile and isoviscous solvent				
Water[a]	1.002	1.004	1.007	1.009
Methanol[b]	1.006	1.0012	1.017	1.023
n-Pentane[c]	1.014	1.027	1.038	1.048
From Eq. (11), mobile phase compressibility with a non-linear pressure profile and isoviscous solvent				
Water[a]	1.0011	1.0022	1.0034	1.0045
Methanol[b]	1.0030	1.0059	1.0089	1.0118
n-Pentane[c]	1.0074	1.0144	1.0209	1.0271
From Eq. (16), mobile phase compressibility with a non-linear pressure profile and pressure-dependent viscosity				
Water[a]	1.0011	1.0022	1.0034	1.0044
Methanol[b]	1.0030	1.0059	1.0089	1.0116
n-Pentane[c]	1.0073	1.0142	1.0204	1.0263

[a] $c = 0.1368$ and $b = 2996$. Compressibility $\beta = 0.46 \times 10^{-4}$ bar^{-1}.
[b] $c = 0.148$ and $b = 1210$. Compressibility $\beta = 1.23 \times 10^{-4}$ bar^{-1}.
[c] $c = 0.0.0943$ and $b = 299.6$. Compressibility $\beta = 3.14 \times 10^{-4}$ bar^{-1}.

sure, the volume occupied by the more compressible liquid is lower.

For instance, for an average column pressure of 200 bar (which is about the maximum normally used in HPLC), the true geometrical void-volume is overestimated by 0.9, 2.3, and 4.8% when using water, methanol, or n-pentane as the eluent, respectively. The effect of the non-linear pressure profile results in a lower estimation of the true geometrical void-volume, e.g., 0.45, 1.2, and 2.5%. The effect of the pressure-dependence of the viscosity is almost zero but tends still to decrease the ratio V/V_0. Comparing with the results in the previous section, the effect of the mobile phase compressibility offsets completely that of the column tube elasticity, being more than 1000 times larger. Any variation in the elution volume of a tracer with the mobile phase flow rate must be first and foremost explained by the compressibility of the solvent, not by the deformation of the column wall.

4.1.3. Compression of the packing material

A shrinking of the packing material under pressure will affect the column total porosity in the same way as an expansion of the column tube, it will increase it. Eq. (13) gives the apparent volume occupied by the packing material in the column. We consider here pure silica and two different but typical silica-C_{18} materials for RPLC, containing, respectively, 10 and 20% of carbon (w/w). We will consider that the density of pure silica is 2.0 g/cm^3 and taking for the density of the octadecyl chains that of pure octadecane 0.777 g/cm^3, and assuming that the volumetric fractions, ϕ_1, of the C_{18} chains in the packing material considered are 0.23 and 0.41,

Table 4

Theoretical relative variation ($V_S/V_{S,0}$) between the volume of three packing materials (pure silica and two C_{18}-bonded silica containing 10 and 20% of carbon in mass) measured under pressure stress (V_S) and the same volume measured at atmospheric pressure P^0 (V_c) for different average column pressures ($P_{av} = (P_i - 1)/2$) calculated from Eq. (15)

	Average column pressure (bar)			
	50	100	150	200
Pure silica[a]	0.99995	0.99990	0.99985	0.9998
Silica-C_{18} (10%)[b]	0.9988	0.9977	0.9965	0.9953
Silica-C_{18} (20%)[c]	0.9980	0.9959	0.9938	0.9918

[a] Compressibility $\beta_{silica} = 10^{-6}\,bar^{-1}$.
[b] Compressibility $\beta_m = 0.238 \times 10^{-4}\,bar^{-1}$.
[c] Compressibility $\beta_m = 0.416 \times 10^{-4}\,bar^{-1}$.

respectively. Then, using Eq. (13), we have

$$\beta_{silica} = 10^{-6}\,bar^{-1}$$

$$\beta_m(10\%) = 0.23 \times 10^{-4} + 0.77 \times 10^{-6}$$
$$= 0.238 \times 10^{-4}\,bar^{-1}$$

$$\beta_m(20\%) = 0.41 \times 10^{-4} + 0.59 \times 10^{-6}$$
$$= 0.416 \times 10^{-4}\,bar^{-1}$$

The numerical results derived from Eq. (15) are summarized in Table 4 for these three packing materials. For average column pressures between 1 and 200 bar, the relative shrinkage of the volume of the pure silica does not exceed 0.02%. By contrast, the presence of the much more compressible layer of C_{18} chains bonded onto the silica leads to a more important volume decrease, up to about 0.5 and 0.9% between 1 and 200 bar for the two materials considered. This is an important variation of the internal packing porosity, one that is comparable in magnitude, albeit smaller than that due to the mobile phase compressibility.

4.1.4. Retention of the tracer molecule

Table 5 shows the contribution of the retention of the tracer independently of the variation of the phase ratio, V_S/V_0. As explained earlier, if we assume a variation of the partial molar volume $\Delta V = -10\,mL/mol$, a value that is typical for low-molecular weight compounds, and a retention factor $k' = 0.1$, the increase of the retention factor is less than 1%. This is an overestimate because the retention factor of an unretained compound in RPLC should be less than 0.1.

Table 5

Theoretical variations of the retention factor of a solute when the average column pressure is increased from atmospheric to 200 bar, according to Eq. (17) and assuming that the variation of the partial molar volume of the compound is $-10\,mL/mol$ and a retention factor $k' = 0.1$

	ΔP (bar)			
	50	100	150	200
$\Delta \ln k'$	0.0204	0.0408	0.0612	0.0815
$\Delta k'$	0.002	0.004	0.006	0.008

4.2. Conclusion of the theoretical predictions

From the theoretical and numerical calculations discussed earlier in this section, it is possible to classify as follows the contributions of the three different sources of influence of the pressure on the hold-up volume. These are, by order of increasing importance, the expansion of the column tube, the mobile phase compressibility, and that of the packing material. More specifically:

(1) The pressure stress acting on the column tube results in an expansion that is very small, almost non-existent, and certainly always negligible in practice. The column diameter increases by 0.0005% and its length by 0.00005% when the pressure increases by 1 bar, by 0.5 and 0.005% when it increases by 1000 bar, respectively.
(2) The decrease in the volume of the packing material under pressure has a measurable effect when RPLC packing materials, e.g., C_{18}-bonded silica, are considered. The column hold-up volume may increase by as much as 1% for the densely bonded packing materials.
(3) When it is not negligible, the adsorption of the tracer on the stationary phase has a significant impact on the increase of its elution volume with increasing the average column pressure. The effect should be less than 0.8% for $\Delta V = -10\,mL/mol$ and $k' = 0.1$.
(4) The compressibility of the liquid mobile phase has the major impact on the elution volume of tracer compounds under NTP conditions. Depending on the actual value of the compressibility of the mobile phase, the increase in the apparent hold-up volume can be between 1% (water) and 5% (n-pentane) for a variation of the inlet pressure of 200 bar.

4.3. Experimental measurements

Three different columns and three different mobile phases were used. The three columns are the pure Resolve silica, the non-endcapped derivatized Resolve-C_{18} silica and the end-capped derivatized Symmetry column. These three columns are suitable to account for packing materials having increasing carbon contents (0, 10.2, and 19.6%, respectively). The three liquids, water, methanol, and n-pentane were chosen for their different compressibility, 0.46, 1.23, and 3.14 bar^{-1}, respectively. However, n-pentane cannot be used with the pure Resolve silica column because the apolar n-pentane solvent does not wet the polar surface silica surface. For similar reason, water was not used with the bonded silica columns. Another expected limitation is the solvation of the C_{18}-bonded layer by the n-pentane solvent which vanishes the effect of pressure on the volume of the C_{18} chains.

The experimental results are given in Figs. 1 and 2. They show the evolution of the hold-up time measured with either thiourea (with water and methanol solvents) or tributylbenzene (with n-pentane) as a function of the average column pressure calculated from the mean of the inlet and
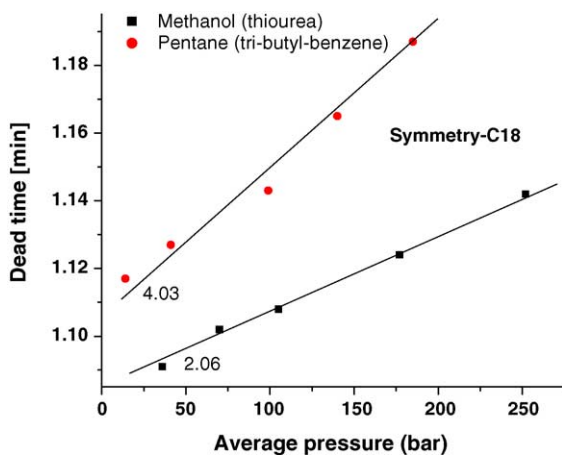
Fig. 2. Same as Fig. 1, except Symmetry-C$_{18}$ column with *n*-pentane or methanol as the mobile phase. Note how fast the hold-up volume increases with increasing pressure with the highly compressible *n*-pentane.
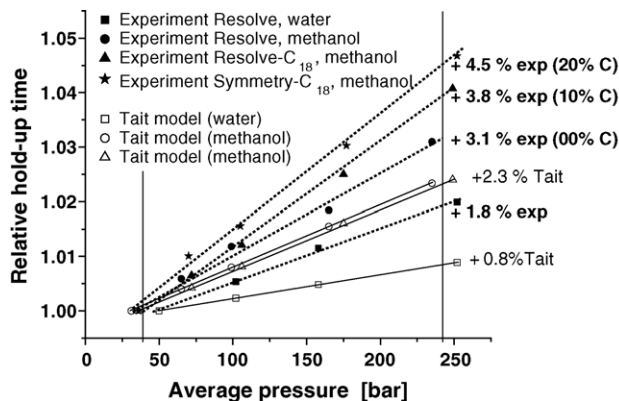

Fig. 3. Relative hold-up time (the reference time is the hold-up time measured for the lowest pressure drop) measured on the three columns (Resolve silica, Resolve-C$_{18}$, and Symmetry-C$_{18}$) vs. the column average pressure. The full symbols connected with dotted lines are experimental data. The empty symbols connected with solid lines represent calculated values (derived from the Tait equation in Eqs. (10) and (12)) of the solvent compressibility to the column hold-up volume measured under NTP conditions. The percentage on the right part of the graph gives the relative increase of the hold-up volume for a pressure increase from 35 to 240 bar. Note that the increase of the hold-up volume is larger for the columns having the higher carbon loading (0, 10, and 20%) and that the compressibility of the solvent does not account for the whole effect observed (about 1% is missing in the calculation).

the outlet column pressures. Fig. 1 shows the plots of the hold-up volume versus the average column pressure for the pure Resolve silica and for the Resolve-C$_{18}$ silica. The two columns have the same dimensions. Obviously, the hold-up volume of the Resolve column is larger than that of the bonded material because the C$_{18}$-bonded layer occupies a significant volume. The volume difference is nearly constant over the whole pressure range, at 0.275 mL. This represents a volume fraction of 40% of the total volume occupied by the solid material ($0.275/(1.792 - 1.093)$), or twice the value assumed in Section 4.1.3. This large value could be explained if the bonding of the alkyl chains in the neck regions of the mesopores obstructs the access of molecules of solvent and unretained tracer to narrow pores, which remains filled with air. It is also important to observe that the slope of the plots is higher with methanol than with water (around 1.3 and 2.0 for water and methanol, respectively). This is consistent with the higher compressibility of methanol. It is also consistent with the theoretical considerations reported earlier that the slopes of the two plots obtained with methanol on the two columns are very close (1.98 and 2.06).

Fig. 2 shows the similar results obtained with Symmetry-C$_{18}$ and with methanol and *n*-pentane as solvents. Note that, as was expected, the NTP volume measured with *n*-pentane is much larger than that derived from methanol. This is so because pentane is significantly more compressible than methanol. The slopes of the two plots are about 2.0 and 4.0 for methanol and pentane, respectively, consistent with the compressibility of pentane being 2.5 times that of methanol. The retention time of tributylbenzene in pentane increases much faster than when it is measured in methanol, a phenomenon that is essentially a consequence of the compressibilities of these solvents.

Fig. 3 compares the experimental hold-up times (normalized to the lowest hold-up time measured, when no restrictor is on-line, downstream the detector) and the hold-up times that are calculated from Eq. (10). The pressure limits used in

the integration are those that were measured experimentally. Tait equation (Eq. (8)) was used to account for the solvent compressibility. It appears from this figure that the compressibility of the solvent alone cannot explain fully the variation of the column hold-up volume with the average column pressure. Tait equation predicts that the NTP volume of water in the Resolve column should increase by about 0.8% when the average column pressure increases from 35 to 240 bar. Measurements show that it increases by 1.8%. The same difference is observed with methanol on that same Resolve column, Tait equation predicts 2.3%, measurements give 3.1%. With the C$_{18}$-bonded Resolve column, the difference is even larger, 2.3% calculated versus 3.8% measured. With either water or methanol, an increase of about 1% of the column hold-up volume must be explained by another phenomenon than the solvent compressibility. This difference is not small since it accounts for 50% of the change in hold-up volume of the column. The expansion of the internal column volume due to the elasticity of the tube cannot exceed 0.30% (Table 2) when the pressure differential along the column is 400 bar. The compressibility of silica can account for at most a 0.02% increase of the hold-up volume for an average column pressure of 200 bar (Table 4). Together, the volume expansion of the column tube and the volume compression of silica cannot account for the excess observed. By contrast, the variations of the hold-up volumes measured on Symmetry-C$_{18}$, Resolve-C$_{18}$, and Resolve silica can be explained by the compressibility of the C$_{18}$-bonded layer (Table 4).

The most probable explanation is the slight increase of the retention of thiourea or 1,3,5-tri-*tert*-butylbenzene

with increasing pressure [9]. This is qualitatively consistent with the theoretical prediction made in Section 4.1.4 of an increase of 0.8% when the average column pressure increases by 200 bar for a tracer molecule whose associated ΔV is -10 ml/moL and the retention factor $k' = 0.1$. Other possible explanations are the effect of the pressure on the organization, structure, and density of the monolayer of solvent against the surface of the adsorbent or a progressive increase of the availability of small mesopores or of possible micropores with increasing pressure. An error in the values found in the tables seems improbable given the consistency of the results obtained with the different solvents.

5. Conclusion

This work demonstrates that the influence of the pressures on the properties of a chromatographic system and, more particularly on the hold-up volume of the column is significant and that it depends quite significantly on the solvent and on the column used. The effect of the pressure on the dimensions of the column tube, usually made of stainless steel, is at most 0.3% for a pressure change of 400 bar, hence, practically negligible. The influence of the compressibility of the solvent used and that of a bonded layer, hence the carbon content of RPLC adsorbents, e.g., the C_{18}-bonded silicas, are physical parameters that have a strong influence on the value of the hold-up volume under NTP conditions. The higher the compressibility of the solvent the higher the hold-up volume and its variations with increasing pressure. Compared to that in water, the NTP hold-up volume in methanol and *n*-pentane increase 1.5 and 3 times faster, respectively, for a given increase of the local pressure. To a lesser degree, increasing the amount of C_{18}-bonded chains or the carbon content of RPLC materials leads to a further increase of the column hold-up volume because of the compressibility of the layer of alkyl chains. A direct consequence of this influence of the pressure on the column hold-up volume is that all column parameters depend on the actual flow rate of the mobile phase stream. This effect is particularly important with compressible mobile phases and with stationary phases having a high carbon load.

The measurement of all thermodynamic parameters, e.g., the retention factors, the separation factors, and the equilibrium isotherm need the accurate and precise determination of the hold-up volume under NTP conditions. It is important to assess the effect of pressure on these data, depending on the accuracy required. New issues become relevant, such as the assessment of the possible effect of pressure on adsorption isotherms. The degree of heterogeneity of the surface of C_{18}-bonded layers may depend on the density of the layer. Finally,

calculations based on the known values of the compressibility of liquids do not account completely for the observed variations of the NTP volumes with increasing pressure. Further investigations are needed to understand the origin of this effect. It might suggest that the high pressure gradient that can take place in a chromatographic column may cause the total porosity of the bed to vary significantly along the column.

This study dealt only with the experimental measurements of the effects of the pressure in the usual range of back pressure applied in HPLC, up to a maximum of 400 bar. However, because the effects of pressure are linear up to a few kilobars [4], it provides fair estimates of the properties of chromatographic systems in the so-called *ultra-high pressure* range, e.g., up to 1–1.5 kbar. It can be expected that effects about 3–3.5 times larger than those reported here will be observed under these conditions. This certainly deserves careful attention and can even cause unexpected difficulties if ignored.

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