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Effect of the pressure on pre-column sample dispersion theory, experiments, and practical consequences

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ABSTRACT

The effect of the pressure on the dispersion of a low molecular weight compound along 0.508 and 1.016 mm i.d. × 50 cm long open circular tubes was investigated theoretically and experimentally. The theoretical predictions were based on the early models of dispersion derived by Aris and Taylor (1953) and on the approximate model of Alizadeh for the time moments (1980). Experimentally, the system pressure was increased at constant flow rate (0.15 mL/min) from less than 20 to nearly 1000 bar by using a series of capillary tubes (25 μm i.d. PEEKSIL tubes) of increasing flow resistances placed upstream the detection cell of a commercial very high pressure liquid chromatograph (vHPLC) but downstream the 50 cm long tube. Theoretical and experimental results agree that the peak variance increases linearly with increasing pressure in the tube volume. The relative increase of the peak variance is 7% above that measured at low pressure (<20 bar) for each 100 bar increment in the tube volume. This result confirms that accurate measurements of the column efficiency corrected for extra-column contribution cannot be made by replacing the column with a zero dead volume union connector, because the pressures applied in the pre-column volume are significantly different in these two cases. This work shows also that increasing the pressure in the pre-column volume by increasing the flow rate affects the apparent column efficiency that is measured, independently of the direct effect of the flow rate. For a 2.1 × 50 mm column packed with 1.3 μm core–shell particles run with a classic Acquity system, the associated relative decreases of the column efficiency are expected to be −30%, −20%, and −5% for retention factors of 1, 3, and 10, respectively. The column HETP is no longer independent of its length.

1. Introduction

A recent report on the kinetic performance of prototype narrow-bore columns packed with 1.6 μm CORTECS-C_{18} core–shell particles illustrated the impact of the high pressures used in modern very-high pressure liquid chromatograph (vHPLC) on the intrinsic reduced plate height of columns [1]. This work highlighted the importance of the contribution of the vHPLC system to analyte band broadening between the injection port and the detection cell exit. The classical method used to measure the extra-column band variance consists in replacing the chromatographic column with a zero dead volume (ZDV) union connector. This method ignores that the back pressure to the pre-column volumes (those of the injection port, the needle seat capillary, the injection valve, and the inlet connector) is much smaller in the presence of the ZDV union than in that of the column. Then, the measurement of the true extra-column peak variance is in error and this error increases with increasing system dispersion volume. As a result, while the corrected HETPs should be independent of the vHPLC instrument used, they differ for different commercial vHPLC systems used for the measurement, because these instruments cause different pre-column dispersion [1]. A non-invasive method was proposed to cope with this issue by keeping the column on the instrument [2,3].

The explanation given for this discrepancy is that the local pressure influences directly and significantly the sample dispersion along the pre-column volume. To be consistent with the expectation that the corrected HETP is independent of the instrument used, it was concluded that the use of a union connector underestimates the true pre-column volume variance, because the radial concentration gradients across the pre-column volumes are relaxed more rapidly at low pressures (in the absence of a column) than at high pressures (in the presence of the column), since diffusion coefficients decrease with increasing pressure, e.g., with increasing eluent viscosity. Additionally, the linear velocity along the pre-column connectors decreases with increasing the pressure, due to the eluent compressibility.

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The goal of this work is to validate this interpretation both theoretically and experimentally. Remarkably, there are few experimental investigations reported in the literature on this issue. Most often studied are the effects of pressure on the physical properties of the mobile phase in HPLC and vHPLC [4–9] and on retention factors [10–13]. Additionally, the local pressure gradient along chromatographic columns was taken into account for the calculation of apparent retention factors and column efficiency in HPLC [14–17] and in supercritical fluid chromatography (SFC) [18–24]. This work investigate the effect of the pressure on the band dispersion of a low molecular weight compound along 0.508 and 1.016 mm i.d. × 50 cm open circular tubes. The inlet pressure was increased from less than 20 bar to nearly 1000 bar by using a series of flow restricting capillary tubes (25 μm i.d.) placed upstream the detection cell and downstream the 50 cm long tube. The eluent was pure acetonitrile, the temperature ambient, and the flow rate was kept constant at 0.15 mL/min. The peak variance was measured for increasing pressures in the tube and analyzed theoretically, based on the general theory of dispersion in open tubes. The influence of pressure on the column efficiency and performance is analyzed and discussed in detail.

2. Theory

2.1. Density and viscosity of pure acetonitrile

The variations of the density, the viscosity, and the heat capacity of the eluent with temperature and pressure affect the average linear velocity along the tube, the diffusion coefficients of analytes in the bulk mobile phase, and the intensity of frictional heating in the column. These dependencies are quantitatively described below with pure acetonitrile used as the eluent.

2.1.1. Density and linear velocity

The semi-empirical expression of the density $\rho(T, P)$ of pure acetonitrile as a function of temperature and pressure is written [25]:

$$\rho(T,P)=\rho(T_{ref},P^0)\left(\frac{P+b+b_1T}{1+b+b_1T}\right)^c\exp(\alpha[T-T_{ref}]+\beta[T^2-T_{ref}^2]) \tag{1}$$

The numerical values of the parameters P^0, T_{ref}, b, b_1, c, α, and β are listed in Table 1. T and P are expressed in Kelvin and bar units, respectively.

Table 1
List of parameters used for the calculation of the density, viscosity, and heat capacity of pure acetonitrile as a function of temperature and pressure from Ref. [25].

System	Parameter value [unit]
Density Eq. (1)	
T_{ref}	298.25 [K]
P^0	1 [bar]
$\rho(P^0, T_{ref})$	776.6 [kg/m^3]
α	-3.304×10^{-4} [K^{-1}]
β	-1.756×10^{-6} [K^{-1}]
b	3.403×10^3 [bar]
b_1	-7.53 [bar K^{-1}]
c	0.125
Viscosity Eq. (3)	
A	-1.757
B	386 [K]
ζ	6.263×10^{-4} [bar^{-1}]
Heat capacity per volume unit Eq. (5)	
$c_{p,m}(P^0, T_{ref})$	1.762×10^6 [J m^{-3} K^{-1}]
a_1	-2.116×10^3 [J m^{-3} K^{-2}]
b_2	0.528 [J m^{-3} K^{-3}]

At constant temperature T, the average linear velocity $u(P)$ at a pressure P for a given flow rate F_v applied under atmospheric pressure P^0 is given by:

$$u(P)=\frac{F_v}{\pi r_c^2}\frac{\rho(P^0,T)}{\rho(P,T)} \tag{2}$$

where r_c is the radius of the open circular tube.

2.1.2. Viscosity and diffusion coefficients

The semi-empirical expression of the viscosity $\eta(T, P)$ of pure acetonitrile as a function of temperature and pressure is written [25]:

$$\rho(T,P)=10^{\left(A+\frac{B}{T}\right)}\left(1+\xi[P-1]\right) \tag{3}$$

The numerical values of the parameters A, B, and ξ are listed in Table 1.

At constant temperature T, the diffusion coefficient at pressure P can be obtained from the diffusion coefficient at normal pressure P^0 [26]:

$$D_m(P,T)=D_m(P^0,T)\frac{\eta(P^0,T)}{\eta(P,T)} \tag{4}$$

2.1.3. Heat capacity and frictional heating

The pressure dependence of the heat capacity per volume unit $c_{p,m}(T)$ of pure acetonitrile is negligible. Its semi-empirical expression as a function of temperature is [25]:

$$c_{p,m}(T)=c_{p,m}(T_{ref})+a_1(T-T_{ref})+b_2(T^2-T_{ref}^2) \tag{5}$$

The numerical values of the parameters $c_{p,m}(T_{ref})$, a_1, b_2, and T_{ref} are listed in Table 1.

The temperature increase, ΔT, along a column operated under adiabatic conditions is given by [25]:

$$\Delta T=(1+\alpha_pT_{av})\frac{\Delta P}{c_{p,m}(T_{av})} \tag{6}$$

where T_{av} is the average temperature along the column, $\alpha_p=\frac{1}{\rho}\left(\frac{\partial\rho}{\partial T}\right)_P$ is the isobaric expansion coefficient, and ΔP is the pressure drop.

2.2. Dispersion theory along tubes of finite length

The general theory of dispersion of a pulse of analyte along a laminar solvent stream flowing through the section of a cylindrical tube was derived initially by Taylor [27] and later by Aris [28]. Alizadeh used this theory of dispersion along a tube to measure liquid diffusivity. He distinguished different conditions, whether the asymptotic dispersion regime (for infinitely long tube, infinitely fast diffusive compounds, or infinitely long dispersion time) is reached [29].

2.2.1. General expression

The most general expression for the cross-section averaged spatial second moment, σ_z^2, at time t after the pulse has begun to move in the parabolic flow is given by [29]:

$$\sigma_z^2=2\left(D_m+\frac{u^2r_c^2}{48D_m}\right)t-128\left(\frac{u^4r_c^4}{D_m}\right)\times\sum_{n=1}^{\infty}\frac{1}{\alpha_{0n}^8}\left[1-\exp\left(-\frac{-\alpha_{0n}^2D_mt}{r_c^2}\right)\right] \tag{7}$$

where D_m is the diffusion coefficient of the molecule in the solvent, u is the average cross-section linear velocity, r_c is the inner radius of the circular tube, and α_{0n} is the nth root of the first derivative