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Accurate measurement of dispersion data through short and narrow tubes used in very high-pressure liquid chromatography

Fabrice Gritti*, Thomas McDonald, Martin Gilar

Waters Corporation, Milford, MA 01157, USA

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ABSTRACT

An original method is proposed for the accurate and reproducible measurement of the time-based dispersion properties of short $L< 50$ cm and narrow $r_c< 50\,\mu$m tubes at mobile phase flow rates typically used in very high-pressure liquid chromatography (vHPLC). Such tubes are used to minimize sample dispersion in vHPLC; however, their dispersion characteristics cannot be accurately measured at such flow rates due to system dispersion contribution of vHPLC injector and detector. It is shown that using longer and wider tubes (>10 μL) enables a reliable measurement of the dispersion data. We confirmed that the dimensionless plot of the reduced dispersion coefficient *versus* the reduced linear velocity (Peclet number) depends on the aspect ratio, L/r_c, of the tube, and unexpectedly also on the diffusion coefficient of the analyte. This dimensionless plot could be easily obtained for a large volume tube, which has the same aspect ratio as that of the short and narrow tube, and for the same diffusion coefficient. The dispersion data for the small volume tube are then directly extrapolated from this plot. For instance, it is found that the maximum volume variances of 75 μm × 30.5 cm and 100 μm × 30.5 cm prototype finger-tightened connecting tubes are 0.10 and 0.30 μL^2, respectively, with an accuracy of a few percent and a precision smaller than seven percent.

1. Introduction

Within the last decade, the advances in chromatographic columns and sorbent reduced the peak volume variances of a weakly retained compound ($k'\simeq 1$) from about 500 μL^2 to only 5 μL^2 [1–4]. At the same time, manufacturers of modern vHPLC instruments reduced the system contribution of injector, connecting tubes, and detector typically to ranges from about 1 μL^2, at best, to values as large as 20 μL^2 [5–8]. For the sake of comparison, the system volume variance of traditional HPLC systems operating at 400 bar is historically on the order of 50–100 μL^2 [9,10,8,11]. Overall, the progress made in system technology is still lagging behind that achieved in column technology [2]. This has become a concern in vHPLC since the chromatographer is unable to utilize a full intrinsic efficiency of the chromatographic column [1].

Consequently, efforts have been devoted toward the reduction of the sample dispersion along vHPLC systems [12]. The system dispersion contribution for small detector cells, injector [13], impact of rectangular injection profile bias [14], tubes as well as connectors/fittings is relatively small, hence difficult to directly measure. The characterization of tubing dispersion becomes extremely challenging at largest flow rates (1.0–2.0 mL/min) when the tube volume (dispersion) becomes a minor contribution to total dispersion, system peak is very narrow and arrives to detector at very low time.

The theory of axial dispersion along circular tubes is well established in the spatial domain. It is not in the temporal domain for the lack of mathematical solution. It was first investigated by Taylor [15,16], then by Aris [17]. Later, Lighthill, Atwood, and Golay studied the same dispersion problem but for short tubes under pre-asymptotic conditions [18–20] where they studied the evolution of a peak shape during the zone initial movements through the tube using experimental data and computer simulation. Lenhoff added the effect of partitioning in short coated tubes [21]. Alizadeh discussed the conditions of validity of the Taylor's theory for liquid diffusivity measurements [22]. In particular, Alizadeh distinguished between the spatial and temporal variances of the concentration distribution since, in practice, mostly temporal data are recorded. The difference between spatial and temporal concentration distributions were simulated during the early development of dispersion [23], shedding light on the complex relationship between time and spatial variances for short tubes. Neue [24] proposed an original expression for the molecular dispersion through circular tubes based on the coupling theory of eddy

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* Corresponding author.
E-mail address: Fabrice_Gritti@waters.com (F. Gritti).

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diffusion as elaborated by Giddings [25]. It is clear that the proposed models overestimate the actual measurements at high flow rates for tubing with small aspect ratio. Such results were previously anticipated by Booras [26], who concluded that the Taylor-Aris dispersion theory may not be applicable for such cases. Unresolved issues are still remaining regarding the time-based dispersion along short tubes: beyond which reduced linear velocity the observed time-based variance starts deviating from the spatial-based variance predicted by Aris? What is the impact of the sample diffusivity on this threshold speed? How does the observed time-based variance change with increasing further the linear velocity? Finally, how can we measure accurately sample dispersion at such a high flow rate along short and narrow tubes? All these questions will be answered only if more detailed analyses are investigated.

The main goal of this work is to answer these unsolved riddles by accurately measuring the time-based dispersion of short and narrow tubes that are used as connectors with vHPLC instruments. The variance of such tubes (inner diameter < 100 μm and a length < 40 cm) is smaller than 1 μL^2, and cannot be measured accurately at high flow rates even with vHPLC instrument equipped with 250 nL detection cells and 10 nL injection loops. We propose first a method in which the variance of larger internal diameter/volume capillary (the same aspect ratio, L/r_c as the capillary of interest) is experimentally measured. The variance of smaller internal diameter/volume of the same L/r_c ratio is then directly extrapolated. We used this method to estimate the volume variance of prototype finger-tightened capillaries having internal diameters of 75 and 100 μm and length of 30.5 cm, but the approach can be applied to any other sizes of circular tubes. We examined flow rates typically used in vHPLC (0.05–1.50 mL/min). Secondly, this extrapolation method is tested based on the direct measurement of the band spreading on these capillaries of interest by coupling a nano-LC detector to the UHPLC system. Finally, it is shown how this work can quantitatively refine the dispersion models through open tubes in a range of flow rates where both flow and diffusion mechanisms govern the overall sample dispersion.

2. Theory

2.1. Exact dispersion theory along circular tubes

The general theory of sample dispersion through a cylindrical tube under laminar flow, e.g., for a Hagen-Poiseuille parabolic radial flow profile, was initially derived by Taylor [15] and later by Aris [17]. Alizadeh applied the results of this theory to measure liquid diffusivity [22]. In particular, he distinguished between conditions when the asymptotic Taylor dispersion regime applies (infinitely long tubes or infinitely long elution times) or when this dispersion model fails (short tubes and/or for large linear velocities).

The Taylor asymptotic model along circular tubes is acceptable when the number of theoretical plates, N, is larger than 30 as shown by Atwood and Golay from numerical calculations and experimental data [18,19]. When $N < 30$, peaks become clearly asymmetric and the observed variance is slower than that predicted by the linear Taylor behavior. The deviation from Taylor model is apparent at very large reduced linear velocity ν defined by Eq. (1)

$$\nu = \frac{2ur_c}{D_m} \tag{1}$$

where r_c is the inner radius of the tube and D_m is the diffusion coefficient of the sample in the eluent. Note that the optimum performance of the tube, $h_{min}=1/(2\sqrt{3}) \simeq 0.3$, is observed for a reduced linear velocity of $\nu_{opt} = \sqrt{192} \simeq 14$ [15]. u is the average linear velocity across the circular tube at a given flow rate F_v

$$u = \frac{F_v}{\pi r_c^2} \tag{2}$$

Alizadeh distinguished between the spatial variances (where the time is fixed, $\sigma_z^2(t)$) and the time or volume variances (where the tube length is fixed, $\sigma_t^2(L)$ or $\sigma_v^2(L)$ of the concentration distribution $c(z,t)$ [22]. In practice the experimenters most often observe the latter, since they record concentration distribution as a function of time at the tube outlet. The relationship between these two variances defined by Eqs. (3) and (4) is only straightforward when the band width is infinitely small with respect to the tube length [25].

$$\sigma_t^2 = \frac{1}{u^2}\sigma_z^2 \tag{3}$$

$$\sigma_v^2 = \left(\pi r_c^2\right)^2 \sigma_z^2 \tag{4}$$

When the bandwidth is not negligible compared to the tube length, the above-mentioned expressions are not rigorously valid. Alizadeh derived approximate and analytical solutions when the peaks become slightly asymmetric [22]. When the peak shape is severely distorted in the time domain [18,19,23], the spatial variance cannot be directly translated to time/volume domain variance by Eqs. (3) and (4).

In contrast, the general expression of the spatial variance along short tubes is known irrespective of the number of plates N. The average cross-section spatial variance, $\sigma_z^2(t)$, at the time $t = L/u$ after the pulse has begun to move through a parabolic stream flow is given by [17,22]:

$$\sigma_z^2 = 2\left(D_m + \frac{u^2 r_c^2}{48 D_m}\right) t - 128\left(\frac{u^2 r_c^4}{D_m^2}\right) \times \sum_{n=1}^{\infty} \frac{1}{\alpha_{0n}^8}\left[1 - \exp\left(-\frac{\alpha_{0n}^2 D_m t}{r_c^2}\right)\right] \tag{5}$$

where α_{0n} is the nth root of the first derivative of the zeroth-order Bessel function of the first kind. The first ten roots considered in this work were: $\alpha_1 = 3.83170597$, $\alpha_2 = 7.015586669$, $\alpha_3 = 10.173468135$, $\alpha_4 = 13.323691936$, $\alpha_5 = 16.47063005$, $\alpha_6 = 19.61585851$, $\alpha_7 = 22.76008438$, $\alpha_8 = 25.903672087$, $\alpha_9 = 29.046828534$, and $\alpha_{10} = 32.189679911$ [27].

The spatial domain variance can be conveniently expressed as the dimensionless reduced plate height, using Eq. (6)

$$h_z = \frac{\sigma_z^2}{2Lr_c}, \tag{6}$$

Rewriting Eq. (5) for the elution time expressed as $t = L/u$, after simplification, we obtain Eq. (7), which demonstrates that the dispersion properties (h_z) are fully determined once the reduced linear velocity, ν, and the aspect ratio of the tube, L/r_c, are known.

$$h_z = \frac{2}{\nu} + \frac{\nu}{96} - 16\nu^2 \frac{r_c}{L} \sum_{n=1}^{\infty} \frac{1}{\alpha_{0n}^8}\left[1 - \exp\left(-\frac{2\alpha_{0n}^2}{\nu}\frac{L}{r_c}\right)\right] \tag{7}$$

The limit of $h_z(\nu)$ when ν tends toward infinity can be determined mathematically. Using the second order Taylor development of the exponential function near 0 and the sums of the infinite series involving the zeros of Bessel functions [27], e.g.,

$$\exp(-x) = 1 - x + \frac{x^2}{2} + O(x^3) \tag{8}$$