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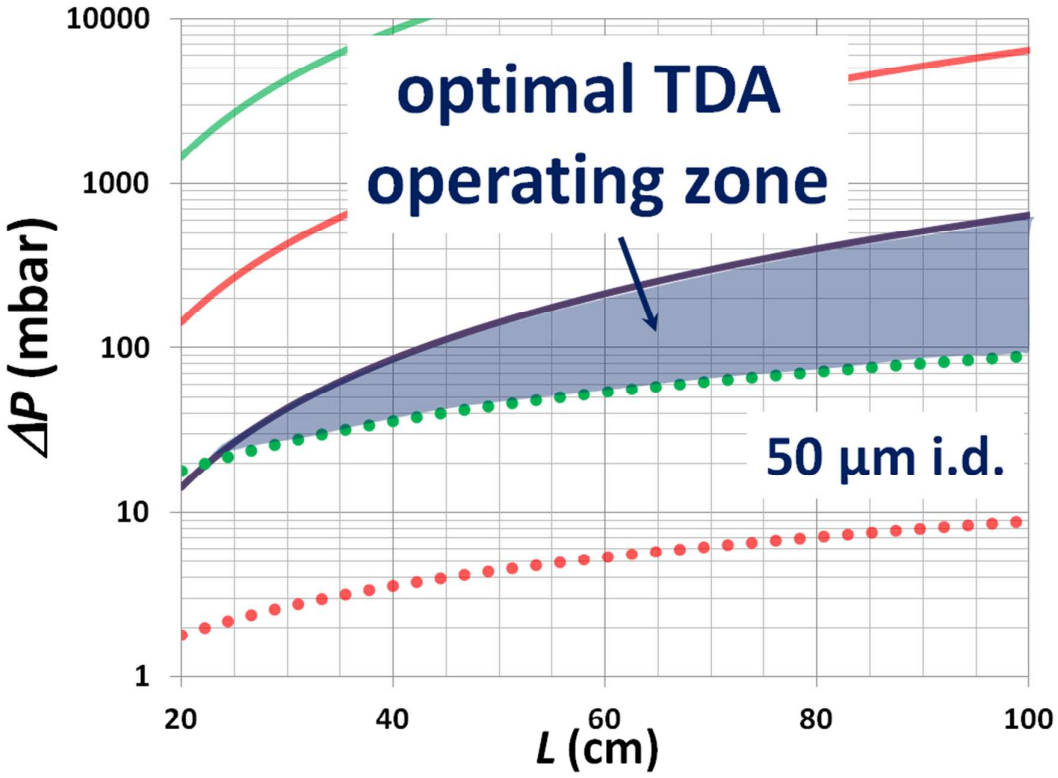

On the Optimization of Operating Conditions for Taylor Dispersion Analysis of Mixtures

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ABSTRACT

In this work, we investigate the possibility to optimize the operating conditions, namely mobilizing pressure, capillary length and capillary radius for doing Taylor Dispersion Analysis on solutes having hydrodynamic diameters between 1 and 100 nm. Optimizing Taylor Dispersion Analysis means finding the set of operating conditions that verify the conditions of validity of this method, and finding the most appropriate conditions that may enhance or maximize the separation performances. Our conclusion is that the performances of Taylor Dispersion Analysis are independent of the operating conditions, as far as the conditions of validity of the method are verified. The inequalities defining the set of acceptable operating conditions are given in this work as a function of the maximal relative error on the diffusion coefficient fixed by the user. These inequalities define operating zones that were represented for three typical capillary diameters (25, 50 and 100 μm). Within these zones, all experiments should lead to similar results on D (or R_h) and similar separation performances. It was concluded that assuming a 3% relative error on the determination of D, a 60 cm × 50 μm i.d. capillary can be used by default for doing TDA of analytes in the 1-100 nm diameter range with mobilizing pressure in the 40-100 mbar range.

INTRODUCTION

Taylor Dispersion Analysis (TDA) is an absolute and straightforward method for determining diffusion coefficient and thus, hydrodynamic radius. This method is based on the seminal work of Taylor[1] and Aris[2], describing the dispersion of a solute plug in an open tube under Poiseuille laminar flow. The dispersion is due to the combination of the dispersive parabolic velocity profile with the molecular diffusion that redistributes the molecules in cross section of the capillary. When axial diffusion is negligible compared to Taylor dispersion, the molecular diffusion coefficient, D, is related to the temporal variance of an analyte, σ_t^2, the average elution time t_o and the capillary radius, R_c, of the tube by the following equation[3]:

$$D = \frac{R_c^2 t_o}{24 \sigma_t^2} \quad (1)$$

Knowing the capillary radius, this simple equation allows the absolute determination of the solute diffusion coefficient from the experimental values of the temporal variance and the average elution time t_o.

TDA was first applied to the determination of gaseous diffusion coefficients[4], then to liquid diffusion coefficients[5-7], in relatively long open tubular columns (14 to 170 m long, with about 0.4 - 0.5 mm inner diameter). More recently, owing to the development of capillary electrophoresis instrumentations, TDA was performed in small internal diameter capillaries requiring the injection of only a few nL of sample[8-13]. TDA is applicable on small molecules, polymers, proteins and nanoparticles of virtually any size from Angstrom to sub-micron. It can be applied either to monodisperse or polydisperse samples[14-15], and basically leads to a weight-average hydrodynamic radius of the mixture when using a mass-sensitive detector[16]. In the case of binary mixtures, the deconvolution of taylorgrams allowed a fast monitoring of polymerization reaction[14]. Since TDA is an absolute method, no calibration is required and the knowledge of the sample concentration is neither needed.

The elution time and temporal variance of unretained analytes in capillary tubes are functions of three main operating parameters[17]: (*i*) the applied pressure, (*ii*) the capillary effective (and total) lengths, and (*iii*) the capillary radius. The question then arises: is there a particular set of operating parameters that will optimize the analysis by TDA? Let's first discuss what does optimization of TDA mean. TDA was shown to provide the individual diffusion coefficients and the relative amounts of a mixture of two analytes[14]. It can thus be considered as a separation method for which the selectivity is based on dispersion rather than on retention. TDA for the analysis of a binary mixture can be envisioned as finding the optimal experimental conditions in terms of pressure drop (or linear velocity) and capillary dimensions (lengths and diameter) that would lead to the best separation/discrimination between the two populations of the mixture (as in the case of the monitoring of a polymer reaction).

On another point of view, when TDA is used for the determination of the diffusivity (and of the related molecular or particulate size) of a single compound, its optimization might be also thought of finding the range of operating parameters in which the conditions required for the validity for eq. (1) are satisfied. In his seminal work, Taylor pointed out two conditions to be fulfilled for performing TDA. A first condition is that the elution time t_o (or time of displacement from capillary inlet to detector position) must be much longer than the characteristic time of diffusion of the analyte across the capillary radius[1]. A second condition is that the contribution due to the parabolic velocity profile to the peak variance must be much larger than the dispersion due to the longitudinal molecular diffusion[18].

Two additional conditions that are linked to the practical measurement procedure (determination of the temporal, rather than spatial, variance of the peak and use of the time of the peak apex for the elution time) are also considered in the following. The constraints on the

operating parameters that should be fulfilled for a given maximal relative error on the determination of D are established.

THEORETICAL BACKGROUND

Normalization of the taylorgram of a single component

Let assume that we are analyzing a single component, labelled 1, in solution in the carrier liquid. Its taylorgram, i.e. the detector signal S_1 vs. time t, depends on various operating parameters (flow rate, pressure drop, capillary radius, temperature, effective capillary length, sample volume, analyte concentration in the sample...). Let assume that the taylorgram can reasonably well be approximated by a Gaussian curve with mean elution time t_o and temporal standard deviation $\sigma_{t,1}$. The signal $S_1(t)$ is assumed to be that of a concentration-sensitive detector, i.e.:

$$S_1(t) = k_1 c_1 \quad (2)$$

where c_1 is the analyte concentration in the detector cell, k_1 the response factor which depends on the detector characteristics and on the nature of the analyte. Thus, the elution curve is:

$$S_1(t) = h_1 \exp\left[-\frac{(t-t_o)^2}{2\sigma_{t,1}^2}\right] \quad (3)$$

where h_1 is the peak height equal to :

$$h_1 = \frac{k_1 c_1^o V_{inj}}{Q \sigma_{t,1} \sqrt{2\pi}} \quad (4)$$

where c_1^o is the analyte concentration in the sample, V_{inj} the injected sample volume and Q the carrier flow rate. Changing any operating parameter will change the peak height and standard deviation. One can normalize the peak shape by plotting y_1 equal to :

$$y_1 = \frac{S_1}{h_1} \quad (5)$$

versus a reduced time x given by:

$$x = \frac{t - t_o}{\sigma_{t,1}} \quad (6)$$

Then, whatever the values of the operating parameters, the expression of the normalized peak is:

$$y_1(x) = e^{-\frac{x^2}{2}} \quad (7)$$

In the reduced coordinates y_1 and x, the peak height is equal to 1, the mean x value is 0 and the standard deviation equal to 1.

Normalization of the taylorgram of a mixture of two components

Let assume that we now add a second component, labelled 2, to the component 1 described above. The taylorgram, $S(t)$, of the binary mixture is made of the sum of the two signals of the individual components 1 and 2, S_1 and S_2:

$$S(t) = S_1(t) + S_2(t) \quad (8)$$

since the detector is assumed to be linear. The signal S_2 is equal to:

$$S_2(t) = h_2 \exp\left[-\frac{(t - t_o)^2}{2\sigma_{t,2}^2}\right] \quad (9)$$

with, by comparison with eq 4:

$$h_2 = \frac{k_2 c_2^o V_{inj}}{Q \sigma_{t,2} \sqrt{2\pi}} \quad (10)$$

h_2 and $\sigma_{t,2}$ being respectively the height and standard deviation of the individual peak of component 2, c_2^o the concentration of component 2 in the sample.

We now attempt to normalize the taylorgram as it was done above using the characteristic values h_1 and $\sigma_{t,1}$ of the peak of the first component:

$$y(x) = \frac{S(x)}{h_1} = \frac{S_1(x)}{h_1} + \frac{S_2(x)}{h_1} = y_1(x) + y_2(x) \quad (11)$$

It was shown above that y_1 does not depend on the values of the operating conditions. For y_2, from eqs 6, 9 and 11, we have:

$$y_2(x) = \frac{h_2}{h_1} \exp\left[-\frac{x^2}{2\left(\sigma_{t,2}/\sigma_{t,1}\right)^2} \right] \quad (12)$$

i.e., with eqs 4 and 10:

$$y_2(x) = \frac{k_2}{k_1} \frac{c_2^o}{c_1^o} \frac{\sigma_{t,1}}{\sigma_{t,2}} \exp\left[-\frac{x^2}{2\left(\sigma_{t,2}/\sigma_{t,1}\right)^2} \right] \quad (13)$$

The ratio k_2/k_1 of the response factors depends on the nature of the two analytes. It is then independent of the operating conditions. The ratio c_2^o/c_1^o of the component concentrations in the sample depends solely on the sample at hand and is independent of the TDA operating conditions. The standard deviation ratio, $\sigma_{t,2}/\sigma_{t,1}$, might depend on the operating conditions. However, if one is operating in required conditions for TDA, $\sigma_{t,2}/\sigma_{t,1}$ is equal to $\sqrt{D_1/D_2}$ according to eq. (1), so that it depends only on the nature of the components 1 and 2, but not on the operating conditions. Therefore, if, upon changing the operating parameters, one normalizes the signal of component 1 so that it is invariant, then the signal of component 2 is also invariant. So is obviously their sum. One is led to conclude that the TDA separation is independent of the operating conditions and cannot be optimized.

Such a conclusion can be substantiated by looking at the chromatographic resolution. If the normalization of the chromatogram of two components is performed along the same transformation rules as those used above, it is easily shown (see Appendix 1) that the resolution is unchanged and that it depends on the relative position of the centers of mass of the two peaks and on the ratio of their standard deviations. TDA can be viewed as the limiting case of a chromatographic separation in which the two centers of mass converge to the same position. Even if the numerical value of the resolution becomes then zero, the important point is that, in this case, it solely depends on the ratio of the standard deviations of the two peaks, hence, according to eq. (1), on the ratio of the diffusion coefficients of the two analytes, independently of the operating conditions.

Clearly, such a conclusion derived above from the analysis of the behaviour of a mixture of two components can be extended for a mixture of any number of components. Experimental confirmation of this demonstration is given below for a bimodal mixture.

It should be noted, however, that the above conclusion relies on the independency of the $\sigma_{t,2}/\sigma_{t,1}$ ratio on operating parameters, as a result of Taylor expression of the peak variances for straight tubes. It cannot be generally extended to the case of coiled or helicoidal tubes as the $\sigma_{t,2}/\sigma_{t,1}$ ratio then depends, in a relatively complex way, not only on the sample components and mobile phase properties but also on the flow velocity, tube internal radius and tube radius of curvature, through dimensionless numbers (Dean and Schmidt numbers, ratio of the capillary internal radius to its radius of curvature)[19]. However, the smaller are the flow velocity and the ratio of the capillary internal radius to its radius of curvature, the smaller is the effect of the tube curvature. For small enough values of these numbers, it can be concluded that the TDA separation is independent of the operating conditions. In the experimental conditions of the present study, the values of the dimensionless numbers are far below the maximum threshold values allowing to neglect the influence of the tube curvature[3].

EXPERIMENTAL SECTION

Reagents

Borax (disodium tetraborate decahydrate) was purchased from Prolabo (Paris, France). Phthalic acid was obtained from Aldrich (Milwaukee, WI, USA). Standard of poly(styrene sulfonate) (PSS, weight average molecular masses M_w 29 × 10^3 g/mol) was purchased from Polymer Standards Service (Mainz, Germany). The polydispersity index of the PSS is below 1.2. The degree of sulfonation of the PSS is higher than 90%. The water used to prepare all buffers was further purified with a Milli-Q-system from Millipore (Molsheim, France). The borate buffers were directly prepared by dissolving the appropriate amount of borax in water.

Taylor dispersion analysis

Taylor dispersion analysis (TDA) experiments were performed on a PACE MDQ Beckman Coulter (Fullerton, CA) apparatus. Capillaries were prepared from bare silica tubing purchased from Composite Metal Services (Worcester, United Kingdom). Various capillary dimensions were used and are given in the figure captions. New capillaries were conditioned with the following flushes: 1 M NaOH for 30 min, 0.1 M NaOH for 30 min and water for 10 min. Before sample injection, the capillary was filled with the buffer (80 mM borate buffer, pH 9.2). Phthalate / PSS sample mixtures were dissolved in the buffer. Sample injection was performed hydrodynamically on the inlet side of the capillary (see figure captions for the injection time and pressure). Between two TDA analysis, the capillary was successively flushed with: (i) water (50 psi, 1 min); (ii) 1M HCl (50 psi, 2 min) and (iii) buffer (50 psi, 3 min). Solutes were monitored by UV absorbance at 200 nm. The temperature of the capillary cartridge was set at 25 °C.

The elution time was corrected from the delay in the application of the pressure using the following equation (in min)[16]:

$$t_R = t_{R,obs} - 0.125 \quad (14)$$

The corrections due to the finite injection plug on the observed elution time and on the peak variance remain very small since the injected volume was lower than 1% of the capillary volume to the detector (see Table 1).

Deconvolution of bimodal taylorgrams were performed by non-linear curve fitting (Microcal Origin 6.0) with two Gaussian functions having the same average elution time.

RESULTS AND DISCUSSION

Optimizing Taylor dispersion analysis of mixtures

To investigate the influence of the operating parameters (mobilizing pressure, capillary length, capillary diameter) on the TDA of mixtures, a binary mixture containing a small molecule (phthalate) and a standard of polymer (PSS, 29×10^3 g/mol) was analyzed. Taylorgrams obtained from this mixture are the sum of two Gaussian peaks (one solute being monodisperse and the second having a low polydispersity). Nevertheless, the variances of the two Gaussian peaks constituting the whole signal depend on t_o and R_c, and thus vary according to the experimental conditions as stated by eq. (1). We thus experimentally investigated the influence of the main operating parameters (mobilizing pressure, capillary length and diameter) on the taylorgrams to see if the separation or discrimination between the two components of the sample can be optimized / maximized. According to the theoretical section (see Eq. 11 and 12), all the taylorgrams obtained for a bimodal mixture should lead, after normalization, to a general curve that depends only on the ratio of the diffusion coefficients of the two components and on the relative composition (mass proportion) of the two components in the mixture. We propose here to experimentally verify this feature. All the experimental operating conditions studied in this work are presented in Table 1. The injected volume was kept as low as possible and was always lower than 1% of the capillary volume to

the detector (see last column in Table 1) to limit the contribution of the finite injected volume to the global variance of the elution peak.

The mobilization pressure was first studied since it controls the linear velocity of the mobile phase in the capillary. Figure 1A displays four different taylorgrams obtained for the analysis of binary Phthalate / PSS mixture, at different mobilizing pressures (from 0.8 to 2.0 psi, i..e. from ~ 50 to 150 mbar) on a 60 cm (50 cm to the detector) × 50 µm i.d. fused silica capillary. As expected, elution times and peak variances decrease with increasing mobilizing pressures. For each taylorgram, the signal $S(t)$ was converted into $y(x)$ using equation (11) by dividing the signal $S(t)$ by h_1, the peak height corresponding to the contribution of phthalate, and by changing the variable from t to $x = \frac{t - t_0}{\sigma_{t,1}}$. $\sigma_{t,1}$ and h_1 were obtained by deconvolution of the taylorgram into the sum of two Gaussian peaks. The peak variances and the ratio of the two peak areas (A_1/A_2) obtained by curve fitting are gathered in Table 1. While $\sigma_{t,1}$ and $\sigma_{t,2}$ values decrease with increasing mobilization pressure, similar $\sigma_{t,2}/\sigma_{t,1}$ ratios were obtained ($\sigma_{t,2}/\sigma_{t,1} \sim 3.0$) for the different experiments. The invariance of A_1/A_2 ratio is just representative of the conservation of the relative proportion of components 1 and 2 in the mixture for each experiment. The four $y(x)$ signals obtained from the taylorgrams at different pressures were superposed in Figure 1B showing a good overlay of the normalized taylorgrams and demonstrating that the intrinsic separation between the two populations does not depend on the mobilization pressure used to perform the TDA.

The influence of the capillary length at constant mobilization velocity was next investigated on the same bimodal mixture. Two effective lengths from the injection point to the detector were tested (30 cm and 50 cm) and the corresponding taylorgrams are displayed in Figure 2A. Similar variance ratios and peak area ratios were obtained for the two experiments, but also in comparison to the previous set of experiments at different

mobilization pressures (see Table 1). The overlay of the y(x) signals displays comparable normalized taylorgrams, as expected by the theory.

Finally, we investigated the influence of the capillary diameter (on 50, 75 and 100 μm i.d.) at constant capillary lengths and for similar linear velocities (see Figure 3A for the taylorgrams). Elution times are not exactly the same because the applied mobilization pressures could not be exactly matched to the desired value (commercial CE instrumentation only allows increment of 0.1 psi for the setting pressure). As expected, the peak variances were found to increase with the capillary diameter (see Table 1 for numerical values) but the ratio was still similar to what was previously observed $\sigma_{t,2}/\sigma_{t,1} \sim 3$. After normalization, Figure 3B displays a common trace for the three taylorgrams demonstrating that the capillary diameter cannot change the intrinsic separation between the two populations of the mixture.

Choosing the optimal operating conditions for TDA

If the operating conditions are not intrinsically modifying the separation obtained by TDA between the different components of the sample mixture, they should be chosen in such a way that the different conditions of validity of TDA are fulfilled. As mentioned in introduction, the first Taylor condition for performing TDA is that the elution time t_o must be much larger than the characteristic diffusion time of the solute in the capillary cross section. This leads to a minimum value for t_o in order to perform the determination of D within an acceptable error. The expression of this minimum t_o value is given in Appendix 2.1. In fact, two other conditions, linked to the practical procedure of determination of D, also lead to a minimum value of t_o. One is due to the fact that the time of apex is often used for t_o. As discussed in Appendix 2.2, the error associated with this approximation decreases with increasing t_o. The other practical condition is linked to the limited validity of the relationship between the spatial variance, computed by Taylor[1], and the temporal variance which makes eq 1 rather simple. It

is discussed in Appendix 2.3. These three conditions are associated to systematic errors in the value of D which partially compensate each other. Their resulting effect is discussed in Appendix 2.4 and is expressed as follows for the minimum value of t_o:

$$t_o \geq \frac{3R_c^2}{80D\varepsilon} \quad (15a)$$

where ε is the relative error on the determination of D that can be tolerated. If one takes $\varepsilon = 3\%$, which corresponds to the typical relative standard deviation obtained by studying the repeatability on D determination by TDA, ineq 15a becomes:

$$t_o \geq \frac{1.25R_c^2}{D} \quad (15b)$$

Noting that $t_o = l/u$ and that the mean velocity, u, of the mobile phase is related to the pressure drop ΔP applied for mobilizing the mobile phase by means of the Poiseuille law, $u = R_c^2 \Delta P / (8\eta L)$, the condition 15b is fulfilled if ΔP satisfies the following condition

$$\Delta P \leq \frac{6.4\ D\eta l L}{R_c^4} \quad (16a)$$

where L is the total capillary length, l is the effective capillary length (to the detector), η is the mobile phase viscosity. Ineq 16a can also be expressed as a function of the hydrodynamic radius, R_h, of the solute, independently of the solvent viscosity:

$$\Delta P \leq \frac{0.34kT\, l\, L}{R_h R_c^4} \quad (16b)$$

The second Taylor condition that should be fulfilled for eq 1 to be valid is that the mobilizing linear velocity u should be fast enough so that the longitudinal molecular diffusion could be

neglected compared to the Taylor dispersion term as expressed in ineq 17a (see Appendix 3 for more details):

$$u \geq \frac{D}{R_c}\sqrt{\frac{48}{\varepsilon}} \quad (17a)$$

Taking ε=3% in ineq 17a leads to:

$$u \geq \frac{40D}{R_c} \quad (17b)$$

Combining ineq 17b with the Poiseuille law gives:

$$\Delta P \geq \frac{320\,D\eta L}{R_c^3} \quad (18a)$$

$$\Delta P \geq \frac{17\ kTL}{R_h R_c^3} \quad (18b)$$

Ineqs 16b and 18b define a zone of operating applied pressure drop that depends on the capillary length, capillary diameter and solute hydrodynamic radius. To select the appropriate pressure drop for a given capillary length, Figure 4 displays the operating zones between the plain and dashed lines for different solute sizes (0.5, 5 and 50 nm hydrodynamic radius) on a 25 µm (Figure 4A), 50 µm (Figure 4B) or 100 µm (Figure 4C) i.d. capillary. A logarithmic scale was selected for ΔP since the ranges of validity are very different depending on the solutes. These graphs are of high practical interest since they set the range of accessible operating conditions (ΔP *vs* L) for sizing (macro)molecules with diameters between 1 and 100 nm by TDA. It is interesting to notice that on a 60 cm long × 50 µm i.d. capillary (see Figure 4B), the 40-100 mbar range, which is the typical range accessible experimentally using CE apparatus, is optimal for sizing solutes in the 1-100 nm diameter range by TDA using eq

1. For that reason, a 60 cm × 50 µm i.d. capillary can be considered as an optimal choice for doing TDA in the 1-100 nm diameter range. Using a 60 cm × 25 µm i.d. capillary would require operating in a significantly higher pressure range (500-3000 mbar) which is not always accessible on commercial CE apparatus. On the other hand, Figure 4C demonstrates that longer capillaries ($L \sim 2.5$ m) would be required for doing TDA on 100 µm i.d. in the same diameter range and using $\Delta P \sim$ 30-200 mbar range. For a given pressure drop ΔP, the analysis time scales as $t_o \sim \frac{Ll}{R_c^2}$ according to Poiseuille law. Therefore, for a given pressure drop, a 2.5 m × 100 µm i.d. capillary would lead to an analysis time five times longer than on a 60 cm × 50 µm i.d. capillary, assuming that the detector is positioned 10 cm before the capillary outlet. For that reason, a 50 µm i.d. appears to be a good compromise for the typical pressure drop range accessible on CE apparatus.

Ineqs 16b and 18b show that the extent of the pressure range within which TDA can be implemented depends on the capillary length and on the solute hydrodynamic radius. There is a minimum value of the effective capillary length below which the conditions 16b and 18b for ΔP cannot be fulfilled simultaneously. It corresponds to the point where the upper and lower curves in the graphs of Figure 4 cross. It is given as a function of the tolerated error on D by (see Appendix 4)

$$\frac{l}{R_c} \geq \frac{3\sqrt{3}}{20\,\varepsilon^{3/2}} \qquad (19)$$

i.e. l/R_c larger than 50 for a 3% error. The required length increases strongly with decreasing tolerated error. Although this condition is very largely satisfied with present-day CE instruments, it should be kept in mind if TDA is to be performed using microfluidic or nanofluidic technologies.

CONCLUSION

In conclusion, TDA, which can be considered as a separative method based on dispersion (and not on retention as for chromatography), leads to separations that are not affected by the operating conditions (capillary length and diameter, mobilizing pressure), as far as the conditions of validity of eq. (1) are satisfied. This is an important feature of TDA, since it means that, in practice, there is no interest to spend time in trying to optimize TDA separation by changing the operating conditions. All acceptable operating conditions will give similar TDA separations. However, operating conditions should be chosen so that conditions of validity of eq. (1) are fulfilled. Assuming a 3% relative error on the determination of D, this work demonstrates that, by default, it seems preferable to use 60 cm × 50 µm i.d. capillary for doing TDA of 1-100 nm analyte diameters using mobilizing pressure in the 40-100 mbar range. It is also important to keep in mind that the injected volume should be lower than 1% of the capillary volume to the detector.

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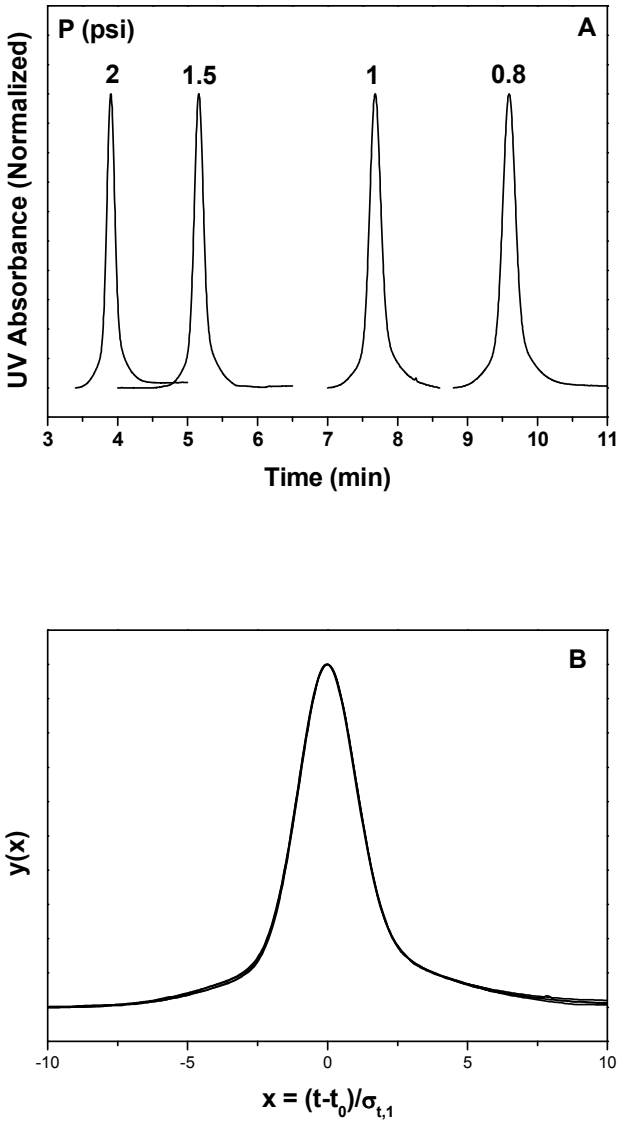


Figure 1. Influence of the mobilization pressure on taylorgrams in time-scale (A) and in normalized coordinates (B). Experimental conditions: Fused silica capillary 60 cm (50 cm to the detector) × 50 µm i.d. Mobile phase: 80 mM sodium borate buffer, pH 9.2. Hydrodynamic injection: 0.3 psi, 3 s. Mobilization pressure: 0.8; 1.0; 1.5 and 2.0 psi as stated on the graph. Temperature: 25 °C. Sample: 2g/L phthalate + 2g/L PSS29000 in the mobile phase. Normalized taylorgrams (Figure 1B) were obtained using eq 11, σ_1 and h_1 being calculated by deconvolution of the experimental taylorgrams of Figure 1A.

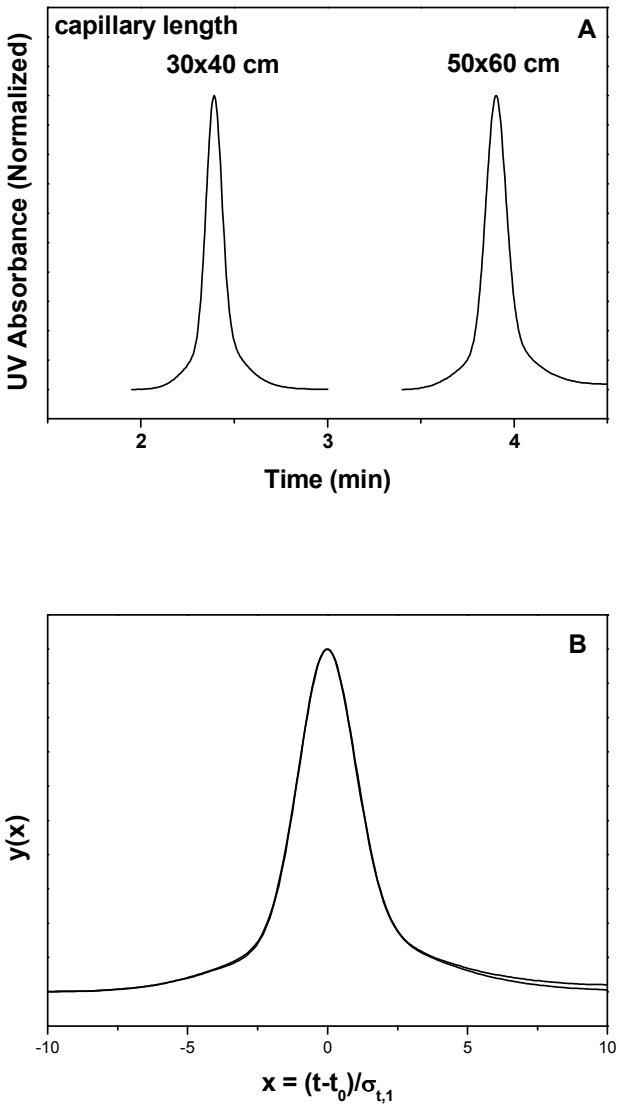


Figure 2. Influence of the capillary length on taylorgrams in time-scale (A) and in normalized coordinates (B). Experimental conditions: Fused silica capillary 50 µm i.d. × 40 cm (30 cm to the detector) or 60 cm (50 cm to the detector) as stated on the graph. Mobile phase: 80 mM sodium borate buffer, pH 9.2. Hydrodynamic injection: 0.3 psi, 3s. Mobilization pressure: 1.3 psi for 40 cm capillary and 2.0 psi for the 60 cm capillary. Temperature: 25 °C. Sample: 2g/L phthalate + 2g/L PSS29000 in the mobile phase.

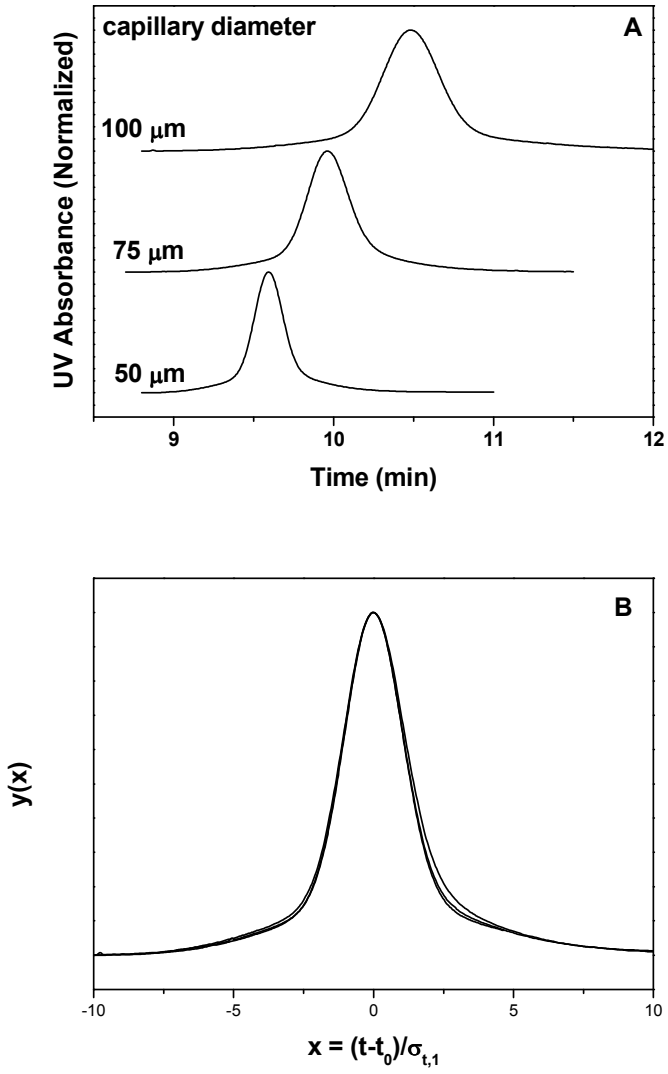
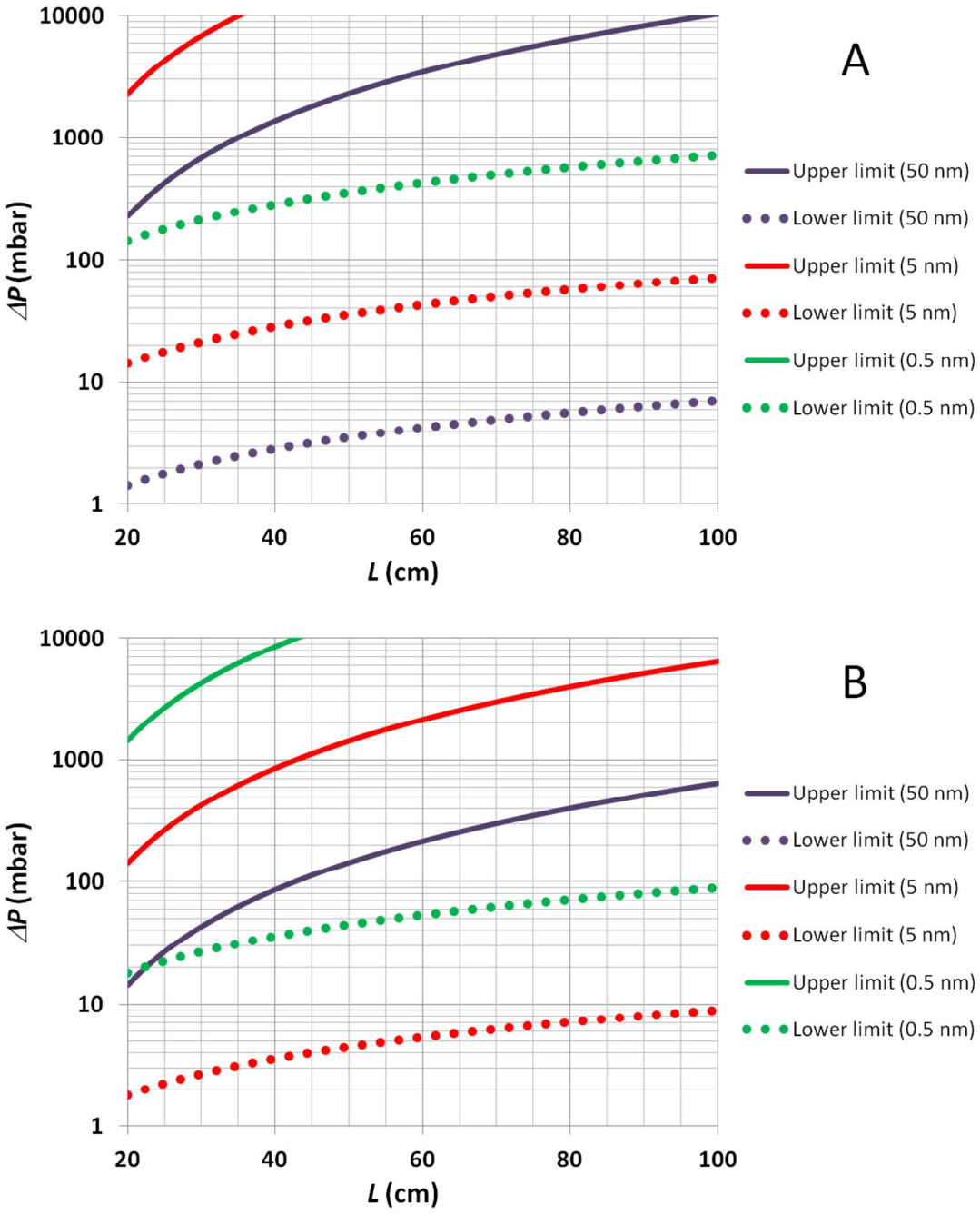


Figure 3. Influence of the capillary diameter in time-scale (A) and in normalized coordinates (B). Experimental conditions: Fused silica capillary, 60 cm (50 cm to the detector) × 50 μm; 75 μm or 100 μm i.d. as stated on the graph. Mobile phase: 80 mM sodium borate buffer, pH 9.2. Hydrodynamic injection: 0.3 psi, 3s on 50 μm; 0.2 psi, 3s on 75 μm; 0.1 psi, 2s on 100 μm. Mobilization pressure: 0.8 psi on 50 μm; 0.4 psi on 75 μm and 0.2 psi on 100 μm. Temperature: 25 °C. Sample: 2g/L phthalate + 2g/L PSS29000 in the mobile phase.


A
10000
1000
100
10
1
ΔP (mbar)
20
40
60
80
100
L (cm)
Upper limit (50 nm)
Lower limit (50 nm)
Upper limit (5 nm)
Lower limit (5 nm)
Upper limit (0.5 nm)
Lower limit (0.5 nm)
B
10000
1000
100
10
1
ΔP (mbar)
20
40
60
80
100
L (cm)
Upper limit (50 nm)
Lower limit (50 nm)
Upper limit (5 nm)
Lower limit (5 nm)
Upper limit (0.5 nm)
Lower limit (0.5 nm)

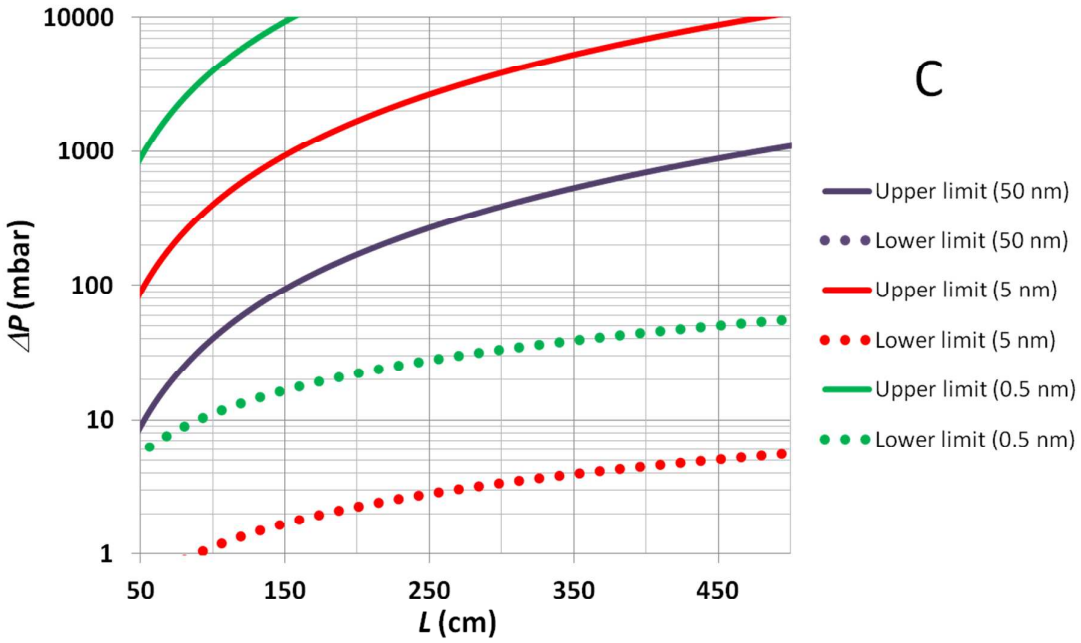
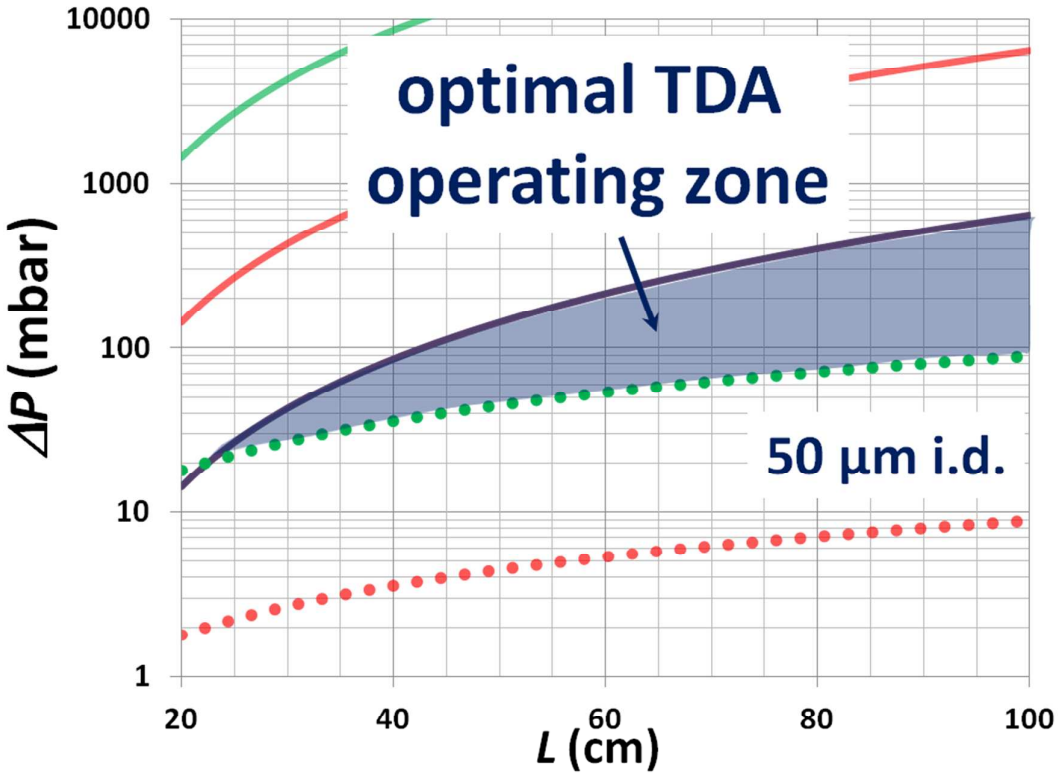


Figure 4: Selection of the operating pressure drop as a function of the capillary length on a 25 µm (A), 50 µm (B) and 100 µm (C) i.d. capillary and for different solute sizes (R_h=0.5 nm; 5 nm and 50 nm). Taylor validity expressed by eq 16b is displayed by plain lines (upper limit). The condition of validity of eq. (1) relative to low axial diffusion and expressed by eq 18b is displayed by dotted lines (lower limit). These calculations are based on a maximal relative error on D of 3%. Note that the solvent viscosity η is not required for these calculations. The effective capillary length l from the injection point to the detector is supposed to be equal to the total capillary length L minus 10 cm.

Table 1 : Experimental conditions, peak variances of the two populations, ratio of the two peak area, and ratio of injected volume to the capillary volume obtained for different taylorgrams. σ_1 and A_1 are respectively the peak variance and the peak area corresponding to the contribution of the phthalate. σ_2 and A_2 are respectively the peak variance and the peak area corresponding to the contribution of the PSS. σ_1, σ_2, A_1, A_2 were determined by deconvolution of the experimental taylorgrams by curve fitting with the sum of two Gaussian peaks. V_i/V_c is the ratio of injected volume to the capillary volume to the detector.

	Capillary diameter (μm)	**Capillary lengths** (cm)	**Mobilization pressure** (psi)	$\sigma_{t,1}$ (min)	$\sigma_{t,2}$ (min)	$\sigma_{t,2}/\sigma_{t,1}$	A_1/A_2	V_i/V_c (%)
Influence of the mobilization pressure	50	50 × 60	0.8	0.083	0.250	3.01	1.83	0.18
	50	50 × 60	1	0.074	0.224	3.03	2.09	0.18
	50	50 × 60	1.5	0.060	0.183	3.05	2.01	0.18
	50	50 × 60	2	0.052	0.158	3.04	1.89	0.18
Influence of the capillary length	50	30 × 40	1.3	0.041	0.127	3.10	1,89	0.45
	50	50 × 60	2	0.052	0.158	3.04	1.89	0.18
Influence of the capillary diameter	100	50 × 60	0.2	0.165	0.530	3.21	1.80	0.16
	75	50 × 60	0.4	0.118	0.375	3.18	1.56	0.27
	50	50 × 60	0.8	0.083	0.250	3.01	1.83	0.18

For TOC only:


10000
1000
100
10
1
ΔP (mbar)
optimal TDA
operating zone
50 µm i.d.
20
40
60
80
100
L (cm)

Appendix 1 – Influence of the change of the variables on the resolution in chromatography.

Let consider the chromatographic separation of two Gaussian peaks in chromatography. The overall signal, $S(t)$, is given by eq 8 as the sum of the two individual signals, $S_1(t)$ and $S_2(t)$, which are themselves given, respectively, by eqs 3 and 9, except that the mean elution times are now t_1 and t_2, respectively, instead of t_o. We normalize the ordinate by the height of peak 1, as in eq 11, and the abscissa time axis by x, now based on the first moment and second central moment of peak 1:

$$x = \frac{t - t_1}{\sigma_{t,1}} \tag{A1}$$

The resolution, R_s, is defined as

$$R_s = \frac{t_2 - t_1}{2\left(\sigma_{t,1} + \sigma_{t,2}\right)} \tag{A2}$$

In the reduced coordinate system, the mean elution reduced time are x_1, equal to 0 according to eq A1 and x_2 equal to

$$x_2 = \frac{t_2 - t_1}{\sigma_{t,1}} \tag{A3}$$

Let τ_1 and τ_2 be the standard deviations of peaks 1 and 2 in the reduced coordinate system. In this system, the resolution, R_s^*, is defined as

$$R_s^* = \frac{x_2 - x_1}{2\left(\tau_1 + \tau_2\right)} = \frac{x_2}{2\left(\tau_1 + \tau_2\right)} \tag{A4}$$

Their variances are defined as

$$\tau_1^2 = \frac{\int y_1 x^2 \,\mathrm{d}x}{\int y_1 \,\mathrm{d}x} = \frac{\int \frac{S_1}{h_1}\left(\frac{t - t_1}{\sigma_{t,1}}\right)^2 \frac{\mathrm{d}t}{\sigma_{t,1}}}{\int \frac{S_1}{h_1}\frac{\mathrm{d}t}{\sigma_{t,1}}} = \frac{1}{\sigma_{t,1}^2}\frac{\int S_1 \left(t - t_1\right)^2 \mathrm{d}t}{\int S_1 \,\mathrm{d}t} \tag{A5}$$

The last fraction in the right-hand side (RHS) term of this equation is recognized as the variance $\sigma_{t,1}^2$ of peak 1. Hence τ_1 is equal to 1. Similar, the variance of the second peak in the dimensionless system is given as

$$\tau_2^2 = \frac{\int y_2 \left(x - x_2\right)^2 \mathrm{d}x}{\int y_2 \,\mathrm{d}x} = \frac{\int \frac{S_2}{h_1}\left(\frac{t - t_2}{\sigma_{t,1}}\right)^2 \frac{\mathrm{d}t}{\sigma_{t,1}}}{\int \frac{S_2}{h_1}\frac{\mathrm{d}t}{\sigma_{t,1}}} = \frac{1}{\sigma_{t,1}^2}\frac{\int S_2 \left(t - t_2\right)^2 \mathrm{d}t}{\int S_2 \,\mathrm{d}t} \qquad \text{(A6)}$$

The last fraction in the RHS of eq A5 is the variance $\sigma_{t,2}^2$ of peak 2. Hence,

$$\tau_2 = \frac{\sigma_{t,2}}{\sigma_{t,1}} \qquad \text{(A7)}$$

Combining eqs A3, A4 and A7, it comes

$$R_s^* = \frac{x_2}{2\left(\tau_1 + \tau_2\right)} = \frac{\frac{t_2 - t_1}{\sigma_{t,1}}}{2\left(1 + \frac{\sigma_{t,2}}{\sigma_{t,1}}\right)} = \frac{t_2 - t_1}{2\left(\sigma_{t,1} + \sigma_{t,2}\right)} \qquad \text{(A8)}$$

Comparing eqs A2 and A8 shows that R_s^* is equal to R_s. Hence the resolution in chromatography is not changed by the transformation of the actual coordinate system into the dimensionless coordinate system by means of eqs 5 and A1. Hence the resolution in chromatography depends on the reduced time and on the ratio of the standard deviations of the two peaks.

Appendix 2 – Conditions leading to a lower value of the migration time

2.1. Condition associated with the long-term approximation of the dispersion coefficient (first Taylor condition)

In his seminal work[1], Taylor computed the spatial variance, $\sigma_{z,lim}^2$, at time *t* after injection of an infinitesimally narrow analyte band in the capillary tube under laminar flow as

$$\sigma^2_{z,lim} = \frac{R_c^2 u^2 t}{24 D} \quad \text{(A9)}$$

In this expression, $\sigma^2_{z,lim}$ is the spatial variance of the zone at time t_o, the time required for the centre of mass of the analyte zone to reach the detector at position l along the tube. However, eq A9 is a long-time asymptotic expression, hence the subscript "lim" given to this spatial variance. Physically, it assumes that the analyte molecules spend enough time in the capillary tube to sample uniformly the various flow streamlines, hence that their characteristic diffusion time across the capillary radius is much shorter than the migration time. In a detailed investigation of the time dependence of the spatial variance, Alizadeh et al.[3] found out that the actual spatial variance, $\sigma^2_{z,act}$, is expressed as

$$\sigma^2_{z,act} = \frac{R_c^2 u^2 t}{24 D} - \frac{128 K R_c^4 u^2}{D^2} + R(t) \quad \text{(A10)}$$

with $K = 2.1701\ 10^{-5}$. $R(t)$ is a residual term which is equal, in absolute value, to the second term of the righ-hand side of eq A10-1 for $t = 0$, so that the initial spatial variance is zero. This residual term decreases faster than exponentially with increasing time. It can be calculated that the relative contribution of this residual term to the spatial variance is less than 1%, 0.1% or 0.1% when $Dt/R_c^2 \geq 0.23$, 0.36 or 0.50, respectively As shown in the following, we are, in practice, concerned with elution times that are larger than these time values. Therefore, this residual term can be neglected and, in combination with eq A9, the actual spatial variance at time $t = t_o$ can be written

$$\sigma^2_{z,act} = \sigma^2_{z,lim}\left(1 - \frac{R_c^2}{15 D t_o}\right) \quad \text{(A11)}$$

since $24 \times 198\ K$ is very close to 1/15.

It is interesting to compare this result with the empirical expression obtained by Atwood and Golay[20] from the fitting of numerical simulation data of dispersion of peaks in short tubes, i.e. (eq 5 of [19])

$$\frac{\sigma_{z,act}}{l} = N^{-1/2}\left(1+\frac{3}{N}\right)^{-1/4} \quad \text{(A12)}$$

where N is the theoretical plate number, equal to $l^2/\sigma_{z,lim}^2$ Combing eqs A9 and A12, noting that $t_o = l/u$, and developing the result in a Maclaurin series limited to the first order in $R_c^2/(Dt_o)$, this gives

$$\sigma_{z,act}^2 = \sigma_{z,lim}^2\left(1-\frac{R_c^2}{16Dt_o}\right) \quad \text{(A13)}$$

Taking into account the limited accuracy of the empirical expression A12, the agreement between eqs A11 and A13 is remarkably good.

If a relative error lower than ε on the measurement of D from eq 1, which derives from A9, can be tolerated, the relative error on $\sigma_{z,act}^2/\sigma_{z,lim}^2$ must be lower than ε, which, according to eq A11 implies that t_o must obey the following condition

$$\frac{Dt_o}{R_c^2} \geq \frac{1}{15\varepsilon} \quad \text{(A14)}$$

Accepting a 3% error, this gives $Dt_o/R_c^2 \geq 2.2$. The interest of eqs A11 and A14, which derive from the expression of Alizadeh et al.[3] is that a tolerated error can be associated with the condition for t_o. For example, it shows that the somewhat arbitrary condition given by Taylor[1] ($Dt_o/R_c^2 \geq 1.4$) corresponds to a 4.8% error on D. However, the condition given by inequality A14 can be combined with other conditions which lead to a lower value of t_o (see Appendix 2.4).

2.2. Condition linked to the use of elution time of peak apex for t_o

In TDA, the taylorgram of a single analyte looks usually symmetrical. In fact, it is not exactly so and the time of elution of the peak apex, t_{apex}, which is usually taken as t_o is not exactly equal to t_o.The cross-sectional average concentration distribution of the analyte in the TDA process can be modelled by a one-dimensional convection-diffusion equation for an analyte injected as a Dirac pulse at position $z = 0$ along an infinite tube in the flow, at velocity u, of the carrier fluid and having a dispersion coefficient $\mathcal{D}$, defined as[21]

$$\mathcal{D} = \frac{\sigma_{z,lim}^2}{2t} \quad \text{(A15)}$$

If m is the amount of analyte injected, the concentration profile, $c(z,t)$, is expressed as[3,22-26]

$$c(z,t) = \frac{m/\left(\pi R_c^2\right)}{\sqrt{4\pi\mathcal{D}t}} \exp\left[-\frac{(z-ut)^2}{4\mathcal{D}t}\right] \quad \text{(A16)}$$

where m is the amount of analyte injected in the tube. Letting $z = u\,t_o$ in this equation provides the temporal concentration distribution given by a concentration-sensitive detector located at $z = l$. Differentiating the resulting equation with respect to t, then solving the derivative for its positive root provides the expression of t_{apex} in combination with eqs A9 and A15 as[22]

$$t_{apex} = t_o\left[\sqrt{\left(\frac{R_c^2}{48\,D\,t_o}\right)^2 + 1} - \left(\frac{R_c^2}{48\,D\,t_o}\right)\right] \quad \text{(A17)}$$

and a development in Maclaurin series limited to the first order in $R_c^2/(Dt_o)$ gives

$$t_{apex} = t_o\left(1 - \frac{R_c^2}{48\,D\,t_o}\right) \quad \text{(A18)}$$

It should be noted that the use of the first moment, t_1, of the taylorgram would not be an exact estimate of t_o. Indeed, it can be shown that t_1 is related to t_o as[3,22-24,26,27]

$$t_1 = t_o\left(1+\frac{R_c^2}{24\,D\,t_o}\right) \quad \text{(A19)}$$

While t_{apex} undersestimates t_o by a given amount, t_1 overestimates it by a double amount. Accordingly, t_o can be estimated from the determination of t_{apex} and t_1 as

$$t_o = t_{apex} + \frac{1}{3}\left(t_1 - t_{apex}\right) \quad \text{(A20)}$$

2.3. Condition linked to the use of the temporal variance instead of the spatial variance

The temporal standard deviation, σ_t, of the concentration distribution recorded by a concentration detector located at $z = l$, i.e. $c(l,t)$, is generally related to the spatial standard deviation of the zone profile taken at the time t_o at which the centre of mass of the analyte zone reaches the detector position, by the following relationship

$$\sigma_t = \frac{\sigma_z}{u} \quad \text{(A21)}$$

which considers that σ_t is the time required to displace the zone by a distance σ_z in the tube. This relationship, however, is correct as a limiting expression for very narrow zones. Indeed, the second central moment of the concentration distribution expressed by eq A16, for $z = u\,t_o$, is given as[3,22-24,26,27]

$$\sigma_t^2 = \frac{2\mathcal{D}\,t_o}{u^2} + \frac{8\mathcal{D}^2}{u^4} \quad \text{(A22)}$$

Combined with eq A15 for $t = t_o$, this gives

$$\sigma_t^2 = \frac{\sigma_z^2}{u^2}\left(1+\frac{R_c^2}{12\,D\,t_o}\right) \quad \text{(A23)}$$

Hence, the temporal variance classically obtained from eq A21 is undersestimated.

2.4. Combination of the three above conditions

In practice, the experimentally determined diffusion coefficient is obtained as

$$D_{exp} = \frac{R_c^2 t_{apex}}{24\sigma_{t,meas}^2} \quad \text{(A24)}$$

where $\sigma_{t,meas}^2$ is the temporal variance directly obtained from the distribution of the concentration recorded by a concentration detector located at position $z = l$ along the tube. The true diffusion coefficient that results from the genuine Taylor method is equal as

$$D_{true} = \frac{R_c^2 t_o}{24\left(\frac{\sigma_{z,lim}^2}{u^2}\right)} \quad \text{(A25)}$$

The relationship between t_{apex} and t_o is given by eq A18. The relationship between the measured temporal variance and the actual spatial variance in the tube at time t_o, $\sigma_{z,act}^2$, is, according to eq A23,

$$\sigma_{t,meas}^2 = \frac{\sigma_{z,act}^2}{u^2}\left(1+\frac{R_c^2}{12 D t_o}\right) \quad \text{(A26)}$$

and the relationship between $\sigma_{z,act}^2$ and $\sigma_{z,lim}^2$ is given by eq A13.

From eqs A13, A18, and A24-A26, it appears that the effects of the use of the time of apex, of the temporal variance instead of the spatial one and of the finite rate of radial diffusion, but neglecting the contribution of axial molecular diffusion combine together to the following expression for D_{exp}

$$D_{exp} = D_{true}\frac{\left(1-\frac{R_c^2}{48 D t_o}\right)}{\left(1+\frac{R_c^2}{12 D t_o}\right)\left(1-\frac{R_c^2}{15 D t_o}\right)} \quad \text{(A27)}$$

or, making a series development limited to the first order in $R_c^2/(D t_o)$,

$$D_{exp} = D_{true}\left(1-\frac{3R_c^2}{80\,D\,t_o}\right) \quad \text{(A28)}$$

which leads to the following condition for t_o

$$\frac{D\,t_o}{R_c^2} \geq \frac{3}{80\,\varepsilon} \quad \text{(A29)}$$

Appendix 3 – Condition leading to an upper value of the migration time

The expression of the spatial variance obtained by Taylor (eq A9) accounts solely for the contribution to the analyte dispersion due to the non-uniformity of the velocity profile of the laminar flow of carrier in the tube cross-section. It does not include the contribution of the axial molecular diffusion. When the latter is accounted for, the expression of the spatial variance becomes, as intuitively suggested by Taylor[18] and demonstrated by Aris[2]

$$\sigma_{z,lim}^2 = 2\left(D+\frac{R_c^2\,u^2}{48\,D}\right)t \quad \text{(A30)}$$

instead of eq A9. For $t = t_o$, this can be written as

$$\frac{\sigma_{z,lim}^2}{u^2} = \frac{R_c^2\,t_o}{24\,D}\left(1+\frac{48\,D^2}{R_c^2\,u^2}\right) \quad \text{(A31)}$$

When comparing eqs A9 and A31, the second term in the brackets of eq A31 appears as the relative error made when neglecting the contribution due to the axial molecular diffusion. Letting this error be lower than ε, the condition to be satisfied for neglecting this contribution (second Taylor condition) is then

$$\frac{R_c\,u}{D} \geq \sqrt{\frac{48}{\varepsilon}} \quad \text{(A32)}$$

The condition $R_c\,u\,/\,D \geq 69$ suggested by Taylor[18] corresponds to a relative error of 1% on D. Noting that $u = l\,/\,t_o$, the inequality 14 to an upper limit to t_o expressed as

$$\frac{D t_o}{R_c^2}\left(\frac{R_c}{l}\right) \leq \sqrt{\frac{\varepsilon}{48}} \quad \text{(A33)}$$

This upper limit for t_o increases with increasing error, but only as the square root of the error.

Appendix 4 – Condition on capillary tube geometry

In order to perform the determination of D by means of TDA with an error lower than or equal to ε, the elution time, t_o, must be found between the two limits given by ineqs A29 and A33, i.e.

$$\sqrt{\frac{\varepsilon}{48}}\left(\frac{l}{R_c}\right) \geq \frac{D t_o}{R_c^2} \geq \frac{3}{80\varepsilon} \quad \text{(A34)}$$

This double condition shows that a time window for t_o can be found in order to perform TDA using eq 20 only if

$$\sqrt{\frac{\varepsilon}{48}}\left(\frac{l}{R_c}\right) \geq \frac{3}{80\varepsilon} \quad \text{(A35)}$$

$$\frac{l}{R_c} \geq \frac{3\sqrt{3}}{20\varepsilon^{3/2}} \quad \text{(A36)}$$

The larger l / R_c, the larger the t_o window for performing TDA at a constant error level.