analytical chemistry

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Taylor Dispersion Analysis in Coiled Capillaries at High Flow Rates

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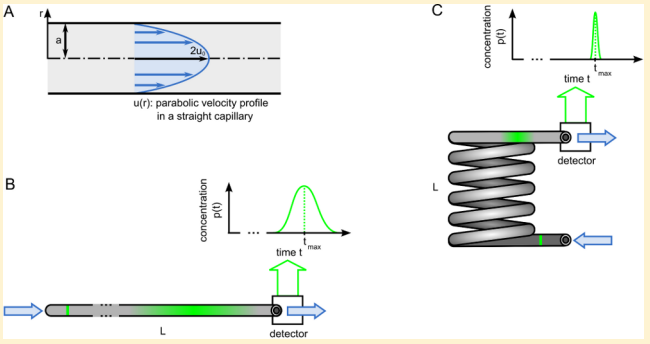
ABSTRACT: Taylor Dispersion Analysis (TDA) has been performed for analytes moving at high flow rates in long, coiled capillaries. A thin injection zone of the analyte is stretched by the flow and final distribution of concentration of the analyte at the end of the capillary has the Gaussian shape. The high flow rates in coiled capillary generate vortices. They convectively mix the analyte across the capillary. This mixing reduces the width of the Gaussian distribution several times in comparison to the width obtained in a straight capillary in standard TDA. We have determined an empirical, scaling equation for the width as a function of the flow rate, molecular diffusion coefficient of the analyte, viscosity of the carrier phase, internal radius of the cylindrical capillary, and external radius of the coiled capillary. This equation can be used for different sizes of capillaries in a wide range of parameters without an additional calibration procedure. Our experimental results of flow in the coiled capillary could not be explained by current models based on approximate solutions of the Navier−Stokes equation. We applied the technique to determine the diffusion coefficients of the following analytes: salts, drugs, single amino acids, peptides (from dipeptides to hexapeptides), and proteins.



Taylor Dispersion Analysis (TDA) is used in analytical chemistry for measurements of diffusion coefficients and sizes of analytes.[1−12] This method is also applied for determination of equilibrium constants for the ligand−selector complex formation[13] without separation of a free ligand from the complex. Leclercq and Cottett[14] have used capillary electrophoresis (CE) with TDA for characterization of polyelectrolytes complexes (i.e., their charge stoichiometry). Generally, CE coupled with TDA allows one to separate unknown compounds in the mixture to determine simultaneously the diffusion coefficient, hydrodynamic radius, and charge of each component.[15]

TDA describes a flow, driven by applied pressure, of injected analyte in a carrier phase. The narrow zone of the analyte is stretched by the Poiseuille flow (Figure 1A), and the final distribution monitored at the end of long, straight capillary is the Gaussian function (Figure 1B). Its width is proportional to the average velocity squared and inversely proportional to the diffusion coefficient (D). D is determined with high accuracy in a straight capillary providing that two conditions are fulfilled:[16−21]

$$\frac{u^2R^2}{48D} \gg D \tag{1}$$

$$\frac{L}{u} \gg \frac{R^2}{D} \tag{2}$$

where u is the average velocity (m/s) (averaged over the cross section of the capillary), R is the internal capillary radius (m), D is the diffusion coefficient of the analyte (m^2/s), and L is the capillary length (m). Equation 1 states that diffusion narrows the distribution of the analyte stretched by the flow. The second condition ensures that the analyte is very well mixed across the capillary of radius R during the time of the flow which equals L/u. From eq 2, it follows that TDA requires a small velocity of the carrier phase. For accurate measurements, u should decrease with decreasing D. We have verified that for a capillary of radius R = 0.13 mm and length L = 30 m, the flow rate should be smaller than u = 1.26 cm/s (0.04 mL/min) for small ligands (i.e., salts) and smaller than u = 0.31 cm/s (0.01 mL/min) for proteins. Small flow rates are difficult to obtain in typical chromatographic equipment. Long capillaries or a special nanosizing system with a very thin capillary[1] solve this problem. In our paper, we show that small velocities of carrier phase are not necessary for TDA to work well in analytical chemistry. We demonstrate how to determine diffusion coefficients of proteins for flow velocity as high as u = 31 cm/s (1 mL/min) (or higher) in a coiled capillary. As discussed by Johnson and Kamm,[22] curvature of a capillary induces a centrifugal force during the flow. The force generates vortices across the capillary which mix the analyte (Figure 1C). This mixing relieves the condition described by eq 2, allowing larger velocities in TDA. The influence of capillary coiling on the deformation of the analyte zone was also described in the literature[23,24] but has not been tested thoroughly as it has in our experiments.

The paper is organized as follows. First of all, we describe the existing models for flows in straight and coiled capillaries. In the

Received: January 7, 2013
Accepted: March 17, 2013
Published: March 17, 2013

   dx.doi.org/10.1021/ac4007792 | *Anal. Chem.* 2013, 85, 4051−4056

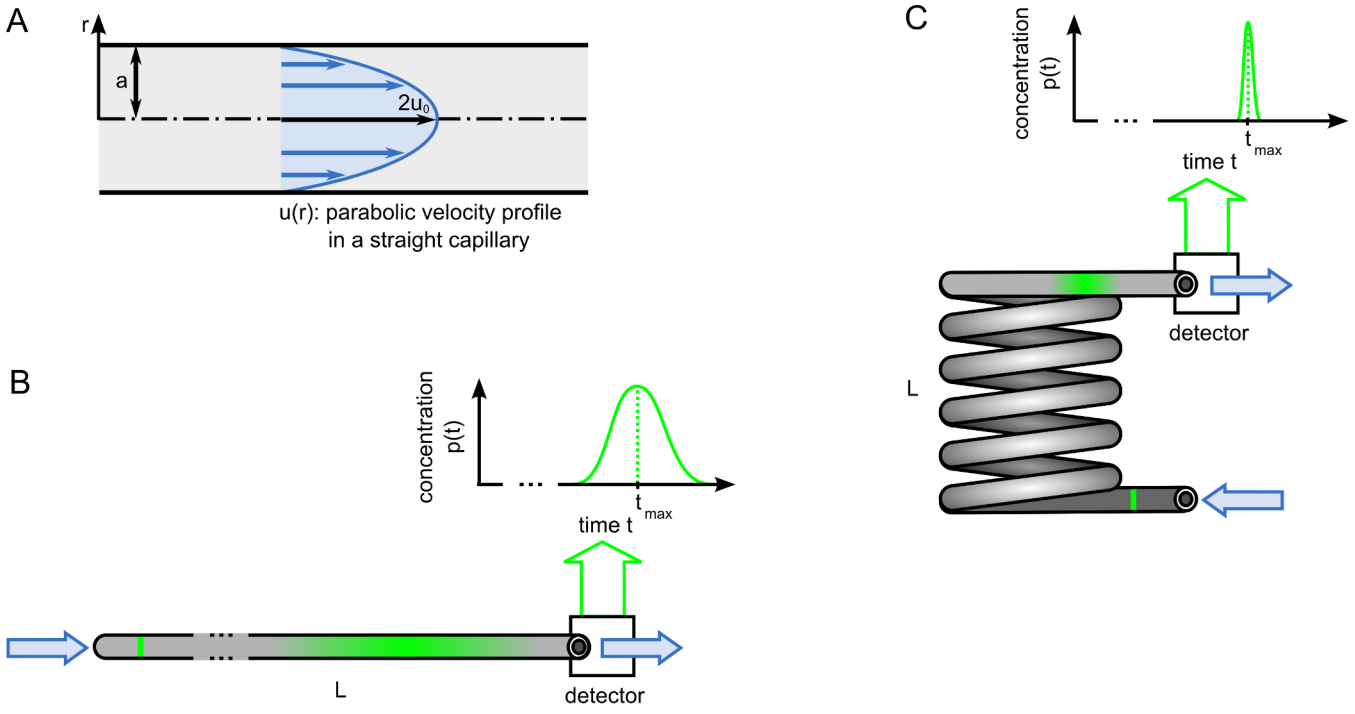


Figure 1. Taylor dispersion analysis in straight and coiled capillaries.

next part, we show our experimental results and analyze the sources of the errors in classical TDA. Finally, we present our conclusions.

Theory for Flow of Analytes in Straight and Curved Capillaries. We analyze the dispersion of the analyte injected into a carrier phase, which flows through a cylindrical, straight, long, and thin tube under a laminar Poiseuille flow. During the experiment, the dispersion of the studied compound is measured by the UV−vis detector at wavelength λ. The concentration distribution is given by the Gaussian distribution:[1,17,25]

$$P(t) = \frac{1}{2\sqrt{\pi\sigma t}} \exp\left[-\frac{(L - ut)^2}{4\sigma t}\right] \tag{3}$$

where σ is the dispersion coefficient and t is time. In accordance with the Taylor theory, we calculate the molecular diffusion coefficient D from the measured dispersion coefficient σ. For the straight capillary, $\sigma = \sigma_s$ and

$$\sigma_S = \frac{u^2R^2}{48D} \tag{4}$$

In typical experiments, long tubes (L > 15 m) are usually coiled. The centrifugal force is generated due to the curved path of flow in a coiled capillary. But for very small flow rates, this force is negligible and the classical Taylor analysis, given by eqs 3 and 4, is applicable. Experiments and computer simulations show that curvature of the coiled capillary is negligible for flows satisfying the following condition:[22]

$$(\mathrm{Dn})^2\mathrm{Sc} < 100 \tag{5}$$

where the Dean (Dn) and Schmidt (Sc) numbers are

$$\mathrm{Dn} = \frac{2\rho uR}{\mu}\left(\frac{R}{r}\right)^{1/2} \tag{6}$$

$$\mathrm{Sc} = \frac{\mu}{\rho D} \tag{7}$$

here ρ is the density of the carrier phase, μ is the viscosity of the carrier phase, R is a capillary radius, and r is the external radius of curvature of the coiled capillary. Condition 5, similarly to condition 2, requires a very small velocity (u) of the flow.

A theoretical analysis of high flow rates in a coiled capillary was done by Johanson and Kamm.[22] Their calculations were based on approximate solutions of the Navier−Stokes equation in coiled capillaries. From the theory, it follows that apart from the Poisseulle flow along the capillary, the centrifugal force arising from the coiling generates vortices across the capillary. The vortices mix the analyte perpendicular to the Poisseulle flow better than diffusion alone and in this way reduce the width of the analyte distribution along the capillary. The reduction of width in a coiled capillary in comparison to a straight capillary is considerable. The theory[22] predicted that,

$$\frac{\sigma_s}{\sigma_c} = 5 \tag{8}$$

for $\mathrm{Dn}^2\mathrm{Sc} > 10^4$, where σ_s is the dispersion coefficient in a straight capillary given by eq 4 and σ_c is the dispersion coefficient in a coiled capillary. Equation 8 shows that at large velocities the width of the Gaussian distribution given by σ_c is reduced 5 times in comparison to σ_s characteristic for the same flow velocity but in a straight capillary. In this paper, we show that the prediction given by eq 8 does not comply with experiments. We have found that σ_s/σ_c is a linear function of ln $\mathrm{Dn}^2\mathrm{Sc}$ for $\mathrm{Dn}^2\mathrm{Sc}$ ranging from 10^3 to 10^6. In the next section, we present a new solution for dispersion in a coiled capillary.

EXPERIMENTAL SECTION

Apparatus. Experiments were conducted using a Shimadzu (Kyoto, Japan) HPLC pump model LC-20AD and autosampler model SIL-20 AHT. The capillaries were made of PEEK

dx.doi.org/10.1021/ac4007792 | Anal. Chem. 2013, 85, 4051−4056

(polyether ether ketone) with a 0.25 mm inner diameter (length 30 m), 0.18 mm inner diameter (length 20 m), or 0.5 mm inner diameter (length 50 m). Capillaries were coiled, and the radii of curvature were 8 or 15 cm. All capillaries were thermostatted at 25 ± 0.1 °C by column oven CTO-20AC. The absorbance was measured by a Shimadzu UV−vis detector SPD-20A connected to a PC computer using LC solution, version 1.25. The carrier phase was degassed using a degassing unit DGU-20A3R and was transported through a capillary with a different flow rate in the range from 0.01 to 1 mL/min with the precision of 0.002 mL/min. We also tested a wide range of injection volumes from 1 to 100 μL. The injection volume for all experiments was 12 μL.

Reagents. Ribonuclease A, apotransferrin, lysozyme, warfarin, L-phenylalanine, alpha-tris-(hydroxymethyl)-methylamine (Tris), the peptides: Phe-Leu-Glu-Val, Val-Glu-Pro-Ile-Pro-Tyr, Tyr-Tyr-Tyr-Tyr-Tyr-Tyr, Val-Tyr-Val, Z-Phe-Leu, Z-Gly-Phe, Phe-Gly-Gly, Ala-Phe, Tyr-Ile-Gly-Ser-Arg, Gly-Leu-Tyr, and Gly-Gly-Tyr-Arg were purchased from Sigma-Aldrich Chemie (Steinheim, Germany). Potassium nitrate (KNO_3) and ortho-phosphoric acid (H_3PO_4) were from POCH (Gliwice, Poland).

Determination of Capillary Radius. Commercial capillaries are not uniform and therefore the effective radius of the capillary was measured experimentally. To determine the capillary radius, the time of filling a dry capillary by the carrier phase with a known density was measured. The obtained values of the effective radius were 0.13 mm for a 30 m capillary, 0.095 mm for a 20 m capillary, and 0.26 mm for a 50 m long capillary. We used an 18 mM TRIS solution as the carrier phase adjusted to pH = 7.4 with H_3PO_4.

Study of the Impact of Capillary Coiling on Concentration Distribution. The following substances were examined: ribonuclease A, apotransferrin, lysozyme, warfarin, potassium nitrate, and L-phenylalanine. The concentration and the wavelength for absorbance measurements for those compounds are shown in Table 1. In all experiments, the carrier phase was the 18 mM buffer Tris at pH = 7.4 and the volume of injection was 12 μL. The density and viscosity of the carrier phase were the same as for water: ρ = 997.044 kg/m^3, μ = 0.89 × 10^{-3} Pa s at 25 °C.

Table 1. Concentration of the Examined Substances

solute	concentration (mol/L)	wavelength λ (nm)
ribonuclease A	7.38 × 10^{-4}	280
apotransferrin	1.26 × 10^{-5}	280
lysozyme	4.55 × 10^{-8}	280
warfarin	2.73 × 10^{-4}	308
potassium nitrate	2.97 × 10^{-7}	220
L-phenylalanine	4.96 × 10^{-4}	214

Study of the Impact of Capillary Diameter on Obtained Results. We tested three different capillaries and two radii of curvature for coiling. The inner radius of the capillary was 0.26 mm (length 50 m), 0.13 mm (length 30 m), 0.095 mm (length 20 m), all with the radii of curvature for coiling equal to 8 cm; the capillary had an inner radius of 0.095 mm, length of 20 m, and a radius of coiling of 15 cm. The substances used for testing were L-phenylalanine, warfarin, apotransferrin, and ribonuclease.

Calculation Procedure. The concentration distribution given by eq 3 has been corrected to take into account a finite volume of injected sample:[13]

$$P(t) = \frac{1}{\sqrt{2\pi}} \frac{Ah}{\sqrt{2\sigma t + m_2}} \exp\left[-\frac{(L - ut + m_1)^2}{2(2\sigma t + m_2)}\right] \quad (9)$$

where A is the amplitude and h is the injection length in the capillary given by $h = LV_{inj}/V_{cap}$, where V_{inj} is the volume of injection and V_{cap} is the volume of the capillary. The parameters m_1 and m_2 denote corrections for the rectangular injection. They are defined in the following way: $m_1 = 1/(2h)$ and $m_2 = 1/(12h^2)$. Finally, we get

$$P(t) = \frac{2\sqrt{3}}{\sqrt{2\pi}} \frac{Ah}{\sqrt{24\sigma t + h^2}} \exp\left[-\frac{6\left(L - ut + \frac{h}{2}\right)^2}{(24\sigma t + h^2)}\right] \quad (10)$$

Only three parameters are fitted: the amplitude (A), the dispersion coefficient (σ), and the velocity (u). The fit, using the least-squares method, to experimental data is presented in Figure 2. The fit of the velocity was only performed to test the precision of the pump. In all presented experiments, the same results were obtained for the fixed velocity, with only two fitting parameters, A and σ.

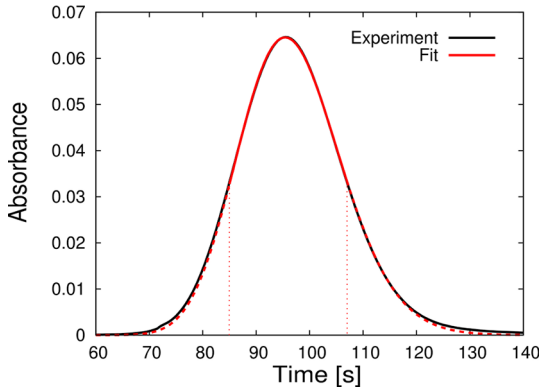


Figure 2. Absorbance as a function of time at a high flow rate, u = 30 cm/s, in a L equals ∼30 m long capillary. The distribution is described by eqs 3 and 10. The fitting range was from 85 to 107 s (i.e., it covered the upper half of the peak).

RESULTS AND DISCUSSION

Influence of Capillary Coiling at High Flow Rates on Determination of the Diffusion Coefficients. We analyzed the impact of capillary coiling on analyte dispersion. We conducted experiments for three reference substances (L-phenylalanine, warfarin, and apotransferrin), using a 30 m long capillary. We recorded the absorbance as a function of time for several different flow velocities. Fitting the function $P(t)$ (eq 10) to the experimental data, we obtained the values of the experimental dispersion coefficients (σ_c) and the experimental flow velocities (u). Next, we calculated the theoretical dispersion coefficient (σ_s) that would appear if the capillary were straight (eq 4). The diffusion coefficients D substituted in eq 4 were taken from the literature (Table 2). Similarly, we used the literature values of D to calculate the Dean (Dn) (eq 6) and Schmidt (Sc) (eq 7) numbers. In Figure 3, we

dx.doi.org/10.1021/ac4007792 | Anal. Chem. 2013, 85, 4051−4056

Table 2. The Diffusion Coefficients for Three Reference Substances from Literature Data (D_{ref})

solute	$D_{ref} \times 10^{10}$ (m^2/s)
L-phenylalanine	7.10 (ref 10)
warfarin	4.90 (ref 26)
apotransferrin	0.57 (ref 27)[a]

[a]The value of the diffusion coefficient was calculated as described in ref 27 from the Stokes–Sutherland–Einstein equation.

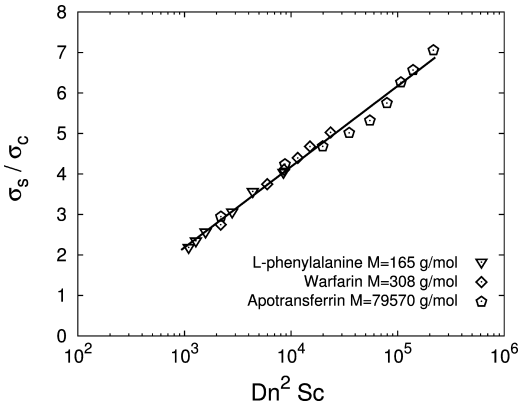


Figure 3. The dependence of the ratio of the coefficients in a straight capillary (σ_s) and a curved capillary (σ_c) on the product of the Dean number squared and the Schmidt number, for three substances of different molar masses and different molecular diffusion coefficients.

plotted the ratio of the experimental dispersion coefficient (σ_c) in a coiled capillary to the theoretical dispersion coefficient (σ_s) in a straight capillary, as a function of Dn^2Sc.

All data in Figure 3 fall onto a single curve as predicted by the simulations of Johnson and Kamm,[22] but σ_s/σ_c does not approach a constant value for $Dn^2Sc > 10^4$, as predicted in ref 22 (see eq 8). In our measurements, the data are very well described by the linear relation:

$$\frac{\sigma_s}{\sigma_c} = A \ln(Dn^2Sc) + B \tag{11}$$

where $A = 0.87 \pm 0.02$ and $B = -3.8 \pm 0.2$ are fitted parameters. We tested eq 11 for different capillaries (inner diameter, length, and radius of coiling) and different substances in a wide range of parameters (volume, flow rate, and concentration). Below, we show all these tests as a proof that eq 11 is universal and can be used without any further calibration to predict the unknown diffusion coefficient from a single measurement. When the diffusion coefficient D for a given substance is unknown, it can be determined from eq 11. Solving eq 11 for an unknown D, we get

$$D = -\frac{1}{48}\frac{u^2R^2}{\sigma_c A \,\mathrm{Lambert}W\left(-1, -\frac{1}{192}\frac{r\mu e^{-B/A}}{R\rho\sigma_c A}\right)} \tag{12}$$

Equation 12 can be simplified by replacement of the function Lambert W $(-1,x)$ with its asymptotic expansion:[28]

$$\text{Lambert } W(-1, x) \approx W(x) = L_1 - L_2 + \frac{L_2}{L_1} + \frac{L_2(-2 + L_2)}{2L_1^2} \tag{13}$$

where $L_1 = \ln(-x)$ and $L_2 = \ln[-\ln(-x)]$. When the expansion contains 4 terms, as in eq 13, and the flow velocities are as high as in the present study, then the approximation introduces only a very small additional error of 0.3%. Further on, we use eq 12 with the substitution of eq 13 to calculate the diffusion coefficients of other substances. We tested the sensitivity of eq 12 to errors in various parameters and we found that, within the accuracy of our measurements, a significant error was introduced only by the parameters A and B. The goodness of fit of eq 11 is therefore essential for an accurate determination of the diffusion coefficient. In order to test whether the presented method of determination of the diffusion coefficient can be used for a given substance, one has to check if the relationship between u and σ_c for that substance fulfills the dependence in eq 11. Below we present positive results of such a test for three other substances: ribonuclease, lysozyme, and potassium nitrate.

Equation 11 that was previously fitted to the reference data in Figure 3 was used as follows: we measured the values of the experimental dispersion coefficient (σ_c) and the experimental flow velocity (u) for new substances at several different flow velocities. Then, for each substance, we took the value $\sigma_{c,max}$ for the highest measured velocity (u_{max}) and substituted them into the eq 12 together with the values of $A = 0.87$ and $B = -3.8$, that were previously fitted to the reference data. In this way, we obtained the diffusion coefficients (D_{exp}) (Table 3). The

Table 3. The Diffusion Coefficients Calculated from the Formula12 (D_{exp}) and Literature Data (D_{ref})[a]

solute	$D_{exp} \times 10^{10}$ (m^2/s)	$D_{ref} \times 10^{10}$ (m^2/s)	R_H (nm)
lysozyme	1.26 ± 0.08	1.17 (ref 29)	1.95
ribonuclease A	1.21 ± 0.08	1.21 (ref 30)	2.03
potassium nitrate	17 ± 2.00	11.7–19.3 (ref 31)	0.15

[a]The reference diffusivities (D_{ref}) were collected from the literature.

calibrated data points corresponding to D_{exp} are marked with black solid symbols in Figure 4. Note that, from the definition, they lie on the calibration curve. For the other data points, we calculated σ_s, Dn, and Sc from the formulas 4, 6, and 7, always substituting the previously determined value of the diffusion coefficient (D_{exp}). Since these data points also fall onto the straight line in Figure 4, the outcome of the test is positive, which means that the linear eq 12 indeed correctly describes the relationship between u and σ_c for ribonuclease, lysozyme, and potassium nitrate.

Finally, we determined the diffusion coefficients of peptides (Table 4) for which the diffusion coefficients have not been measured to date. As expected, they also passed the test of linearity (data not shown), since their molar masses are between the masses of the previously examined substances. Using the measured diffusion coefficients, we determined the values of hydrodynamic radii for these substances, using the Stokes–Sutherland–Einstein equation:[27]

$$R_H = \frac{k_B T}{6\pi\mu D} \tag{14}$$

where k_B is the Boltzmann constant, T is the temperature, and R_H is the hydrodynamic radius of the compound.

Influence of Capillary Diameter on Value of Diffusion Coefficient. In order to prove that eq 11 can be used for different capillaries without any additional calibration, we checked the impact of the capillary diameter, length, and radius

dx.doi.org/10.1021/ac4007792 | Anal. Chem. 2013, 85, 4051–4056

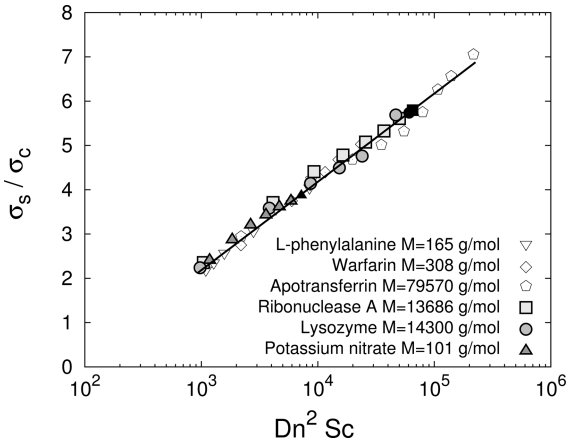


Figure 4. The ratio of the dispersion coefficient for a straight capillary (σ_s) (see eq 4) to that for a curved capillary (σ_c) as a function of the Dean (Dn) (eq 6) and Schmidt (Sc) (eq 7) numbers for chemical compounds of molar mass ranging from 10^2 to 10^5 g/mol. The data points for ribonuclease, lysozyme, and potassium nitrate have been calculated based on the calibration line (eq 11): the calibrated data points are marked with black solid symbols and lie on the calibration line (see the explanation in the text); the other data points for these substances also fall onto the same curve, which indicates that the method of determination of diffusion coefficients is correct. The data points for L-phenylalanine, warfarin, and apotransferrin are the same as in Figure 3 and have been shown for reference.

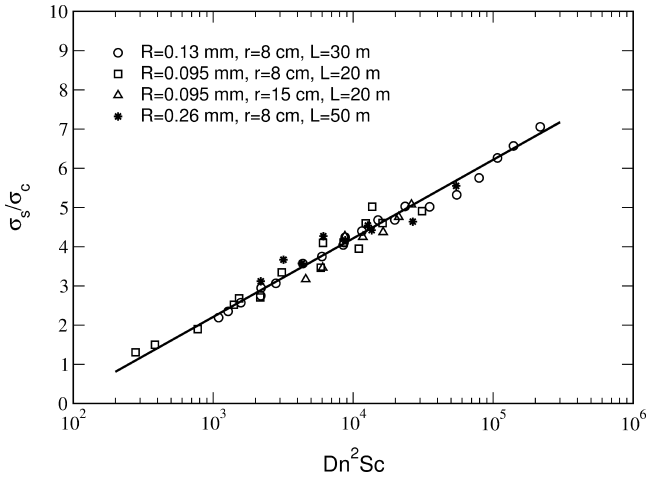


Figure 5. Comparison of the dependence of the ratio of the coefficients in a straight capillary (σ_s) and a curved capillary (σ_c) on the product of the Dean number squared and the Schmidt number for different capillaries (inner radius 0.095, 0.13, and 0.26 mm; length 20 m, 30 m, and 50 m, respectively) and two radius of coiling (for 0.095 mm), r = 8 and 15 cm, and different substances (warfarin, L-phenylalanine, apotransferrin, and ribonuclease). This figure shows without any doubt that eq 11 and following eqs 12 and 13 are universal for the range of parameters studied in this paper and as such can be used in other experiments without any additional calibration.

Table 4. The Values of the Diffusion Coefficients and Hydrodynamic Radii of Peptides

substance	λ (nm)	M (g/mol)	$R_H \times 10^{10}$ (m)	D_{exp} (10^{10} m^2/s)
Ala-Phe	214	236.27	4.50	5.5 ± 0.4
Phe-Gly-Gly	214	279.29	4.84	5.1 ± 0.3
Gly-Leu-Tyr	275	351.4	5.45	4.5 ± 0.3
Z-Gly-Phe*	214	356.37	5.34	4.6 ± 0.3
Val-Tyr-Val	275	379.45	5.90	4.2 ± 0.3
Z-Phe-Leu*	214	412.48	6.81	3.6 ± 0.2
Gly-Gly-Tyr-Arg	275	451.48	6.18	4.0 ± 0.3
Tyr-Ile-Gly-Ser-Arg	275	594.66	7.90	3.5 ± 0.2
Phe-Leu-Glu-Glu-Val	214	635.71	7.59	3.2 ± 0.2
Val-Gly-Pro-Ile-Tyr	275	716.82	8.04	3.0 ± 0.2
Tyr-Tyr-Tyr-Tyr-Tyr-Tyr	275	997.05	14.6	1.7 ± 0.1

*Z = carboxybenzyl group.

of coiling on our results. We measured the distribution of concentration of L-phenylalanine, warfarin, apotransferrin, and ribonucease for different diameters of tubes, in a wide range of flow velocities. For one of the capillaries, we also changed the curvature radius of coiling to estimate the impact of this parameter on the results. We determined the dependence of the ratio of the dispersion coefficient for a straight capillary (σ_s) to that for a curved capillary (σ_c) as a function of the Dean (Dn) and Schmidt (Sc) numbers, according to the procedure described earlier. The results are shown in Figure 5. We obtained the same dependence (marked with a straight line) for all tested capillaries. This dependence is reproducible with a high fidelity for all capillaries.

CONCLUSIONS

We have studied the Taylor Dispersion Analysis in coiled capillaries at high flow rates and showed its potential applications in analytical chemistry. We presented an empirical equation which allows one to considerably shorten the analysis typically from one hour (standard TDA in long capillary and low flow rates) to minutes (at high flow rates). In this way, we have opened a new direction of research, especially because theoretical predictions are not in accordance with experimental results described in our paper (eqs 11 and 12 and Figure 4). Equation 11 works well for small analytes and proteins for a wide range of parameters [i.e., velocities ranging from 1 to 31 cm/s, volume of injection ranging from 1 to 100 μL, concentrations on the order of 10^{-8} to 10^{-4} M, different inner radii of the capillary from 0.095 to 0.26 mm, and different radii of coiling (8 and 15 cm)] and under these conditions does not require additional calibration. However, from our experience we know that some problems in TDA arise when very high flow rates and large volumes of injection are used in shorter capillaries for substances with small diffusion coefficients. In our Experimental Section, we have shown the values of diffusion coefficients for different types of substances, such as salts, drugs, peptides, and amino acids. The results for lysozyme, ribonuclease A, potassium nitrate, apotransferrin, warfarin, and L-phenylalanine are in good agreement with the literature data. We also determined the diffusion coefficients of peptides for which the diffusion coefficients have never been measured.

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Notes

The authors declare no competing financial interest.

dx.doi.org/10.1021/ac4007792 | Anal. Chem. 2013, 85, 4051−4056

■ ACKNOWLEDGMENTS

This research was supported by the National Science Centre grant, Maestro no. 2011/02/A/ST3/00143. A.O.M. acknowledges the support of the National Science Center grant, Sonata no. 2011/01/D/ST3/00751.

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