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Abstract

Taylor Dispersion Analysis (TDA) allows diffusion coefficient (D) or hydrodynamic radius (R_h) determination on a wide range of size between angstroms and about 300 nm. However, solute adsorption phenomena can affect the repeatability and reproducibility of TDA. Several numerical studies addressed the theoretical impact of solute adsorption in TDA, but very few experimental studies focus on this topic and no experimental methodologies were proposed so far to reduce the impact of adsorption in TDA. In this work, an experimental protocol, called plug-in-front TDA, consisting of adding the solute in the eluent at a lower concentration compared to the injected sample, was proposed to strongly limit the impact of adsorption on the R_h determination. This protocol was suggested based on the evidence that adsorption / desorption phenomena impacting narrow bore fused silica TDA in aqueous conditions are typically slow processes that can be counteracted by saturating the interaction sites during the experiments. Successful applications to proteins and mRNA lipid nanoparticles (LNP) in vaccine against Covid 19 and protein analysis were reported. TDA of proteins in conditions of strong interactions with the capillary surface was possible using the plug-in-front methodology. We anticipate that such experimental methodology will greatly help the experimentalist for implementing TDA in various applications.

Keywords: Taylor dispersion analysis, adsorption, lipid nanoparticles, hydrodynamic radius.

1. Introduction

Taylor Dispersion Analysis (TDA) is a powerful technique used to determine hydrodynamic radii (R_h) and diffusion coefficient (D) of numerous compounds [1]. Based on the pioneer work of Taylor [2,3] and Aris [4], R_h and D can be determined by analyzing the peak broadening of a solute plug in a laminar Poiseuille flow [3,4]. Firstly applied to gaseous solutes [5], determination of these characteristics was developed in aqueous media for small molecules such as ethanol, acetone or benzene and its derivatives [6–8]. At the end of the last century, a regain of interest in TDA was observed with the work of Bello et al. [9] who highlighted the use of capillary electrophoresis (CE) instrumentation to speed-up, miniaturize and automatize TDA in typically 50 µm i.d. capillaries.

Since TDA is an absolute method (no calibration is needed) with low sample consumption and a large range of size (from angstrom to about 300 nm in diameter) [10], it offers numerous advantages compared to other techniques such as dynamic light scattering (DLS) or diffusion ordered spectroscopy NMR (DOSY NMR) [11,12]. Indeed, TDA is not sensitive to the presence of dusts or large aggregates contained in the sample, contrary to what is observed in scattering techniques. From simple molecules [1,13,14] to (bio)(macro)molecules [15–18], as well as lipid nanoparticles [19–21], TDA is nowadays a recognized straightforward sizing method applicable to a huge variety of solutes.

Analytical conditions must be carefully chosen to correctly perform TDA. First, the solute must be prepared in the exact same buffer as the one used as the mobile phase to avoid any mismatch buffer signal perturbation [22]. The Peclet number Pe and the average elution time t_0 that depend on the capillary length, the mobilization pressure, and the solute size, must be controlled to remain in the conditions of validity of TDA (see theoretical background section for more de-

tails) [3]. It exists also a maximum sizing limit for TDA above which hydrodynamic chromatography occurs and can contributes to the peak dispersion [23]. It was also shown that for coiled capillaries with capillary i.d. higher than 100 µm, radial mixing of the solute may occur at high mobilization pressure. This phenomenon dramatically affects the TDA analysis because radial mixing generates thinner peaks leading to larger diffusion coefficient values [24,25].

TDA relies on the assumption of no physicochemical interaction between the solute and the capillary wall. In 1958, Golay theoretically studied the impact of retention on the peak broadening of a solute in an open tube the wall of which is coated with a thin retentive layer [26]. He derived a long-time asymptotic expression of the analyte dispersion coefficient, D_{disp}, (in $m^2.s^{-1}$)

$$D_{disp} = \frac{D}{1+k} + \frac{1+6k+11k^2}{(1+k)^3}\frac{R_c^2 u^2}{48D} \qquad (1)$$

where D is the analyte molecular diffusion coefficient in the carrier fluid (in $m^2.s^{-1}$), R_c is the capillary radius (in m), u the carrier fluid average velocity (in $m.s^{-1}$), and k the retention factor [26]. The Taylor-Aris result is recovered by setting $k = 0$ in this equation. The first term reflects the axial molecular diffusion, the second the Taylor contribution (mass transfer in the carrier fluid). An additional term accounting for the diffusional mass transfer within the stationary retentive layer can be added [26] but is often found negligible owing to the very small thickness of this layer and is not of concern in our case. Eq. (1) relies on instantaneous equilibrium at the interface between the fluid and stationary phases. Recognizing that equilibrium is not always reached at the interface, Aris [4], then Khan [27], extended the analysis by adding, to the right hand side of Eq. (1), a third term reflecting interfacial resistance and depending on the sorption rate constants. However, because the formulation of their kinetic model involved the thickness of the retentive layer, it is not directly applicable to the situation encountered in TDA capillaries which generally do not contain such a layer. The contribution of the mass transfer resistance due to adsorption on the wall was

obtained by Giddings using either the stochastic theory [28] or the nonequilibrium theory of chromatography [29], so that the dispersion coefficient can be expressed as

$$D_{disp} = \frac{D}{1+k} + \frac{1+6k+11k^2}{(1+k)^3}\frac{R_c^2 u^2}{48D} + \frac{k}{(1+k)^3}\frac{u^2}{k_d} \qquad (2)$$

with $k=k_a/k_d$, k_a and k_d being the adsorption and desorption rates (in s^{-1}), respectively. For adsorption on heterogeneous surfaces, $1/k_d$ in the last term of this equation should be replaced by $<\tau_a>$, the mean duration of a step spent by a molecule in the adsorbed state on the capillary wall [30].

Several theoretical models based on different approaches have been developed to account for the influence of solute interaction with the wall to the dispersion coefficient (or, equivalently, to the plate height). A summary of these works can be found in the introduction of the article by Hlushkou et al. [31]. Eq. (2) is retrieved when general models are applied to conditions pertaining in TDA (steady Poiseuille flow in a tube without irreversible adsorption or reaction on the tube wall) [32–37]. Using a random walk particle-tracking technique, Hlushkou et al. performed 3-D simulations of advective-diffusive solute transport in the carrier flow in a tube combined with a stochastic approach of the sorption process [31]. The finiteness of the adsorption rate is accounted for by using a fixed value of the probability that a solute molecule is adsorbed when it hits the wall. The value of the retention factor is obtained by proper adjustment of the adsorption probability and the mean adsorption time $<\tau_a>$. Whatever the flow velocity and the retention factor, they obtained perfect fit of the dispersion coefficient according to Eq. (1) (Golay equation) for an adsorption probability of 1, which corresponds to very fast sorption rates (equilibrium assumption). For a slow interfacial transfer (low value of the adsorption probability), their results fit well with Eq. (2) in the case of an exponential distribution of the duration of the adsorption step. However, if this distribution is uniform (all adsorption steps have then the same duration τ_a), the dispersion coefficient is lower and fits with a modification of Eq. (2) in which the last term is divided by 2. This clearly shows that the dispersion depends on the microscopic details of the adsorption pro-

cess, in agreement with the general stochastic theory of chromatography which predicts that the temporal variance of a solute peak depends on the variance of the number of adsorption steps and on that of the duration of an adsorption step [38,39]. The peak temporal variance is thus lower when the latter vanishes.

It should be noted that the derivation of Eqs (1) and (2) relies on a first order kinetic model, hence on a linear adsorption isotherm. Madras et al. investigated the influence of non-equilibrium dynamic adsorption between the solute and the capillary inner surface on the peak shape via numerical simulation using a Langmuir isotherm [40]. They observed deviations from Gaussian peak shapes depending on the kinetic rate constants and on the number of active sites on the wall, in agreement with the findings of TDA experiments on phenanthrene in a supercritical fluid performed by Smith et al. [41] and Hamilton [42].

Eqs. (1) and (2) give the asymptotic long-time expressions of the dispersion coefficient. If the capillary is not long enough, the peak elution profile may be strongly distorted and sometimes bimodal. This was observed by experiments and/or simulations on short tubes either without adsorption [43–45] or with adsorption [46–48]. Analytical solutions to the system of mass balance equations controlling the migration of a solute in a straight conduit with reversible and/or irreversible adsorption have been recently obtained [49,50]. They allow to get a highly detailed view of the complexity of the evolution of the solute concentration distribution at all positions within the capillary as a function of time. Especially, the instantaneous mean zone velocity and dispersion coefficient are obtained as the growth rates of the first moment and of half of the second central moment of the cross-sectional averaged distribution of the solute concentration along the tube, respectively. It is found that, depending on the values of the adsorption and desorption rates, the zone velocity and dispersion coefficient may, during a short period, be negative. The classical Taylor regime is asymptotically recovered at long times. The influence of the initial condition used to release the solute in the capillary has been investigated [49–51].

In this work, we study experimentally the adsorption of model small molecules and proteins. To limit the impact of slow desorption kinetics phenomena on TDA measurements, we propose a new methodological protocol. Applications to the sizing of lipid nanoparticles (LNP) used in mRNA vaccines are demonstrated.

2. Experimental section

2.1. Chemicals and Materials. 4-(2-hydroxyethyl)-1-piperazineethane sulfonic acid (HEPES, ≥ 99.5% purity), 2-(N-morpholino)ethane sulfonic acid (MES, ≥ 99.5% purity), tris(hydroxymethyl)aminomethane (Tris, ≥ 99.5% purity) and sodium tetraborate decahydrate (Borax ≥ 99.5% purity) were purchased from Sigma-Aldrich (Saint-Quentin-Fallavier, France). Sodium hydroxide (NaOH, 98 % purity) and hydrochloric acid (fuming, ≥ 37%) were purchased from Fluka (Saint-Quentin-Fallavier, France). Acetonitrile (ACN, HPLC grade) was purchased from Carlo Erba (Val de Reuil, France). Acetic acid (99.99% purity) was supplied by VWR Chemicals (Rosny-sous-Bois, France). Pfizer vaccine COMIRNATY™ Original/Omicron BA. 4-5 (lot number: GJ7181, with an expiration date in July 2023) against Covid 19 was generously given by Pharmacie des 4 Seigneurs (Montpellier, France) and kept at -80°C.

Toluene (analytical reagent grade) was purchased from Fisher Scientific (Loughborough, Leicestershire, UK). 1-Methyl-4-(1-methylethyl) benzene (*p*-cymene, purity < 98%) was purchased from Parfum Cosmetic World (Grasse, France). Cyclo(Ala-Ala) peptide was purchased from Bachem (Bubendorf, Switzerland). Triphenylphosphine (99% purity), Myoglobin (Myo, from equine skeletal muscle, 95% purity) were purchased from Sigma-Aldrich (Saint-Quentin-Fallavier, France). All aqueous solutions were prepared using deionized water (18 MΩ) delivered by a Synergy UV water purification system (Millipore, Fontenay sous Bois, France).

2.2. Eluent and sample preparation. 2 M acetic acid (pH 2.2, η = 1.12 cp) was prepared by dilution of acetic acid with deionized water (12.8 g for 100 mL). (50/10 mM) MES/NaOH buffer (pH 5.8, η = 0.99 cp) was prepared by mixing 1.95 g of MES with 1 mL of NaOH 1M in 100 mL volumetric flask. (20/10 mM) HEPES-NaOH buffer (pH 7.0, η = 0.96 cp) was prepared by mixing HEPES (0.477 g for 100 mL) with sodium hydroxide (10 mL of a 1M NaOH solution to a 100 mL volumetric flask). 10 mM sodium tetraborate decahydrate buffer (pH 9.1, η = 0.98 cp) was prepared by dissolving 0.191 g of Borax powder ($Na_2B_4O_7$, 10 H_2O) in a 10 mL volumetric flask. ACN/HEPES solution was prepared by mixing pure ACN with 40 mM HEPES / 10 mM NaOH pH 7.4 buffer with a ratio of 70/30 (w/w). The viscosity of this solution was measured at 0.39 cp. (20/17 mM) Tris-HCl buffer (pH 7.4, η = 0.92 cp) was prepared by mixing 0.24 g of Tris with 1.7 g of 1 M HCl in a 100 mL volumetric flask.

Cyclo(Ala-Ala), toluene, p-cymene and triphenylphosphine were diluted in ACN/HEPES solution at a concentration of 10 gL^{-1}, 6 gL^{-1}, 8 $gL^{-1}$and 0.7 gL^{-1}, respectively. Myoglobin (Myo) powder was diluted either in 2 M acetic acid, or in MES/NaOH buffer, or in HEPES/NaOH buffer, or in sodium tetraborate decahydrate buffer at a concentration of 2 gL^{-1}, and stored at 5°C. The sample was diluted twice to obtain the desired concentration (1 gL^{-1}) for TDA analysis. TDA of LNP was performed by injecting LNP solution without dilution. DLS analysis of LNP was performed by diluting 60 µL of LNP solution with 1 mL of filtered (0.02 µm filter) Tris-HCl buffer (20/17 mM).

For protein plug-in-front eluent preparation, myoglobin solution (named S_0, at 2 gL^{-1}) was diluted to the desired concentration (i.e., from 0.05 gL^{-1} up to 0.5 gL^{-1} for eluent preparation (S_2)) and to 1 gL^{-1} for the analyzed solution (S_1) using the pH 5.8 or 7.0 previously prepared background electrolytes.

For LNP plug-in-front preparation, 10 µL of LNP solution were mixed with 190 µL of (20/17 mM) Tris-HCl buffer (dilution factor 20) for the preparation of S_2 solution.

2.3. Taylor Dispersion Analysis Experiments. TDA experiment was performed on a 7100 Agilent system (Agilent Technology, Santa Clara, USA), piloted with ChemStation, using 50 µm i.d. capillaries. Fused silica capillaries (50 µm i.d., 375 µm o.d.) were purchased from Polymicro Technologies (Phoenix, AZ, USA). Coated DB-1 and DB-17 capillaries (50 µm i.d.) were from Agilent Technology (Santa Clara, USA). Fused silica capillaries were conditioned by flushing NaOH 1 M solution for 5 min at 1 bar, followed by a 5 min flush of pure water at 1 bar. Coated capillaries were used after a simple flush with the eluent. For TDA in the case of fast desorption kinetics, fused silica capillaries and DB-1 and DB-17 coated capillaries were 80 cm long (71 cm to the detector). For the rest of the study, fused silica capillaries were 40 or 60 cm long (31.5 and 51.5 cm to the detector, respectively).

For protein analysis, the presaturation protocol on 40 cm long fused silica capillaries consists in flushing the capillary with the sample containing the analyte (for 10 min at 100 mbar, 5 capillary volumes) before analysis. After presaturation, the capillary was flushed with the background electrolyte (BGE) without protein for 1 min at 1 bar (5 capillary volume) followed by a plug injection (30 mbar 4 s) and mobilization with the same BGE.

For protein plug-in-front experiments, the capillary was flushed with S_2 solution containing diluted proteins for 200 s at 100 mbar for the 40 cm long capillary (corresponding to 1 capillary volume). The analyzed protein solution (S_1) was injected at 30 mbar for 4 s and mobilized with S_2 solution. Between each run, the capillary was flushed with the BGE without protein for 100 s at 1 bar (5 capillary volumes).

For LNP plug-in-front experiment, the 60 cm long capillary was flushed with S_2 solution containing diluted LNP (LNP/Tris-HCl, 1/20 v/v) for 7 min at 100 mbar (1 capillary volume). The non-diluted LNP sample was next injected at 30 mbar for 12 s and mobilized with S_2 solution. Be-

193 tween each run, the eluent was flushed for 200 s at 1 bar (5 capillary volumes) with BGE without
194 LNP.

195

196 **2.4. Theoretical background.** In TDA, the diffusion coefficient *D* can be obtained using-the entire
197 Taylor-Aris expression (Eq. (1) for *k*=0), as follows [2–4,16]

198 $$D = \frac{1}{2}\left(D_{disp} - \sqrt{D_{disp}^2 - \frac{R_c^2 u^2}{12}} \right) \quad (3)$$

199 The dispersion coefficient can be experimentally determined as:

200 $$D_{disp} = \frac{l^2 \sigma_t^2}{2 t_0^3} \quad (4)$$

201 where σ_t is the temporal variance of the peak and l is the length of the capillary to the detector (in
202 m). Eq. 3 can be simplified when axial diffusion is negligible, leading to:

203 $$D = \frac{R_c^2 t_0}{24 \sigma_t^2} \quad (5)$$

204 The validity of Eq. 5 depends on two conditions. First, the axial diffusion must be negligible com-
205 pared to the dispersion induced by the parabolic velocity profile. Within 3% relative error (ε) on
206 *D*, solute velocity must fulfill the following condition [23]:

207 $$u \geq \frac{40 D}{R_c} \text{ (or Pe}\geq 40\text{)} \quad (6)$$

208 where *Pe* is the Péclet number. If *Pe* is under 40, the entire Taylor-Aris expression Eq. 3 should be
209 used to determine *D*. The second condition is that t_0 must be longer than the characteristic diffu-
210 sion time of the solute in the cross section of the capillary [23]. Keeping the same relative error of
211 3%, t_0 must fulfill the following condition:

212 $$t_0 \geq \frac{1.25 R_c^2}{D} \quad (7)$$

Finally, to determine the hydrodynamic radius R_h, the Stokes-Einstein is applied:

$$R_h = \frac{k_B T}{6\pi\eta_S D} \quad (8)$$

where k_B is the Boltzmann constant, T is the temperature (in K), and η_s is the fluid viscosity.

The peak asymmetry (A_s) was determined at 10% of the peak apex, using Eq. (9):

$$A_S = \frac{t_B - t_0}{t_0 - t_A} \quad (9)$$

where t_B and t_A represent the time at 10% of the maximum after and before peak apex, respectively.

2.5. Dynamic Light Scattering. DLS experiments were carried out on an Anton Paar Litesizer 500 (Anton Paar, Graz, Austria). The measurement angle of the DLS was set at 90° and temperature of the cuvettes was set at 25°C. LNP vaccine solution was diluted by adding 60 µL of vaccine solution into 1 mL of eluent (20/17 mM Tris/HCl eluent, pH 7.4). Cumulant fit was used to estimate the sample polydispersity. CONTIN-like fit provided by the Anton Paar software was used to obtain the size distributions.

3. Results and Discussion

3.1. Plug injection TDA of small molecules in the presence of adsorption. In this first part of this work, we focus on fast adsorption and desorption kinetics of small molecules on the capillary wall, leading to equilibrium adsorption. As predicted by the general theory of chromatography[26] fast adsorption/desorption kinetics should lead to an increase of the solute retention time and to higher peak broadening, keeping constant the symmetry of the taylorgram. Therefore, the diffusion coefficient obtained from the working Eq. (5) - in which the observed analyte retention time,

235 t_r, replaces the carrier residence time in the capillary, t_0 - is an apparent diffusion coefficient, D^*,
236 which differs from the true diffusion coefficient D_0. From the Golay expression of the peak vari-
237 ance [26] and Eq. (1), it can be shown that:

238 $$D^* = D_0 \times \frac{1+k}{1+6k+11k^2} \quad (10)$$

239 with $k=(t_r-t_0)/t_0$. In the case of low k values, applying a Taylor series expansion on Eq. (10) led to :

240 $$\frac{D^*}{D_0} \approx 1-5k \quad (11)$$

241 As stated by Eq. (11), fast adsorption/desorption kinetics tends to increase the peak broadening
242 and thus to decrease the apparent diffusion coefficient by a factor of -5k.

243 To our knowledge, there is no experimental demonstration of the validity of Eqs. (10) and
244 (11) in modern implementation of TDA (i.e. using narrow bore capillaries of about 50 µm I.D.).
245 Plug injection TDA of three small apolar molecules (toluene, *p*-cymene and triphenylphosphine)
246 was performed using two commercial 50 µm. i.d. capillaries coated with apolar phases
247 (dimethylpolysiloxane (DB-1) and (50% phenyl)-methyl polysiloxane (DB-17)) in ACN/HEPES
248 (70/30 w/w, 0.39 cp) eluent. In these conditions, the solutes were all separated from the non-
249 retained polar molecule cyclo(Ala-Ala) used as the void-time marker (see top inserts of Figure 1).
250 Average k values were determined at 28.6 ± 0.2 × 10^{-3}, 68.5 ± 0.3 × 10^{-3} and 95.4 ± 0.4 × 10^{-3} (on n
251 = 5 repetitions) for toluene (Figure 1A), *p*-cymene (Figure 1B) and triphenylphosphine (Figure
252 1C), respectively. The apparent diffusion coefficients D^* in presence of adsorption are gathered in
253 Table 1. Measuring diffusion coefficient D_0 in absence of adsorption was possible when using
254 ACN/HEPES (70/30 w/w) mobile phase on fused silica capillary (see bottom of Figure 1 for TDA
255 traces and Table 1 for D_0 values).

256 Table 1 demonstrates the validity of Eq. (11) for all small molecules, with experimental rati-
257 os of D^*/D_0 very close to 1-5k. A R_h difference up to 0.40 nm (corresponding to 88% relative error)

258 was obtained for triphenylphosphine. As a conclusion, fast adsorption/desorption kinetics in TDA
259 is expected to overestimate the R_h result without changing the Gaussian shape of the taylorgram. It
260 is however worth noting that fast adsorption kinetics with measurable retention is not very com-
261 mon in practical modern TDA using narrow bore fused silica capillary in aqueous solution.

262 For slow adsorption/desorption kinetics, non-Gaussian peak was generally observed, with
263 typical peak tailing due to non-equilibrium phenomena. This situation is much more common in
264 practice in TDA. However, Eq. (11) is not adequate to describe such a situation and practical solu-
265 tions are required to address this issue.

266

267 **3.2. Plug injection TDA of proteins in the presence and in the absence of adsorption.** When
268 performing simple plug TDA analysis of basic model proteins in acetic acid 2 M, pH 2.2 on a fused
269 silica capillary, no adsorption occurs due to the absence of electrostatic interaction (see Figure
270 2A). One way to generate solute adsorption is to increase the pH of the eluent from pH 2.2 (2M
271 acetic acid) to pH 5.8 using MES/NaOH (50/10 mM), to pH 7.0 using HEPES/NaOH (40/10 mM)
272 and finally to pH 9.1 using a 10 mM sodium tetraborate. The silica surface charge (pKa □ 6)
273 changes from neutral to negative when increasing the pH. Since the isoelectric point (pI) of Myo is
274 7 [52], adsorption is promoted at intermediate pH, as displayed in Figure 2A. At pH 5.8 and 7.0,
275 almost no signal was observed due to irreversible oppositely charged interactions. At pH 9.1, due
276 to electrostatic repulsion between negatively charged Myo and silica surface, no adsorption was
277 observed for Myo.

278 Since, in the case of slow adsorption/desorption kinetics, it is not possible to correct the im-
279 pact of adsorption onto the experimental determination of D (as Eq. (10) in the case of fast equi-
280 librium interactions), it is desirable to propose an experimental alternative to tackle adsorption
281 issue. Interestingly, strong interactions observed at pH 5.8 and 7.0 can be reduced using a

presaturation protocol of the capillary surface prior TDA analysis. Experimentally, this protocol consisted in flushing the capillary with the solute solution in order to saturate the active sites of adsorption. As shown in Figure 2B, Myo peak intensity increased owing to the presaturation of the capillary. However, strong peak tailing was observed as calculated from peak asymmetry (A_s = 4.0 ± 1.2 (n = 5) at pH 7.02, see Eq. (9)). The improvement of the TDA signal after capillary presaturation proves that desorption kinetics of the solute from the capillary wall is slow.

R_h of Myo with simple plug injection TDA at pH 2.2 was estimated around 3.43 ± 0.17 nm, with A_s equal to 1.48 ± 0.26 (n = 5). A similar R_h value was found using presaturation protocol, but with lower standard deviation and peak asymmetry values (R_h = 3.54 ± 0.09 nm and A_s = 1.17 ± 0.13, n = 5). In that case, presaturation helped to reduce protein adsorption onto the capillary wall by saturation of the adsorption sites, leading to more repeatable experiments. In contrast, no difference was observed between the two protocols at pH 9.1, i.e. R_h and A_s were similar (R_h = 1.83 ± 0.09 nm and A_s =1.13 ± 0.05 with plug injection protocol, and R_h = 1.89 ± 0.06 nm and A_s =1.33 ± 0.15 with presaturation protocol, n = 5), as shown in Table SI1. At pH 9.1, no adsorption occurs and presaturating the capillary was thus not useful.

R_h values were in perfect agreement with the literature: R_h of Myo was found 1.83 ± 0.09 nm (n = 5) at pH 9.1 (compared to 1.80-1.90 in literature [53,54]). In acidic conditions, R_h of Myo was almost twice higher than the native protein R_h value. This can be easily explained by the fact that Myo tends to aggregate in acidic conditions, leading to larger measured sizes [53].

3.3. Plug-in-front injection TDA of proteins. Based on the improved results obtained using presaturation of the capillary inner surface, a new methodology is proposed to perform TDA using what we name “plug-in-front” approach. In this new mode, TDA is performed by injecting a solute plug (at 1 gL^{-1}) in a capillary prefilled with an eluent containing the solute at a lower concentra-

306 tion (C_{front} from 0.05 gL^{-1} to 0.4 gL^{-1}, as indicated in Figure 3). Clearly, the peak symmetry im-
307 proved with increasing protein front concentration, as shown by the Gaussian fitting in Figure 3.
308 The influence of the solute concentration in the front on R_h and A_s was systematically investigated
309 and reported in Table 2. The A_s value decreased with increasing C_{front} and stabilized down to 1.0 –
310 1.2 above 0.4 gL^{-1}. It is also worth noting that the satellite peak observed after the main elution
311 peak disappeared above 0.4 gL^{-1}. Regarding the R_h value, an ANOVA test at 95% confidence level
312 proved that the results are not significantly different for C_{front} above or equal to 0.2 gL^{-1}. Average R_h
313 of Myo was 1.86 ± 0.04 nm at pH 5.8 and 1.84 ± 0.02 nm at pH 7.0 (n = 5), in good agreement with
314 the literature [53,54]. It is worth noting that the plug-in-front methodology allows analyzing pro-
315 teins on fused silica capillaries event at pH where electrostatic interactions occur. This results is
316 important in practice for TDA application and was not *a priori* expected. It can be explained by the
317 slow kinetics of desorption of the solute from the capillary surface.
318
319 **3.4. Optimizing the plug-in-front protocol to limit solute consumption.** One of the main ad-
320 vantages of using capillary electrophoresis apparatus to perform TDA experiments is its low sam-
321 ple consumption. With a 40 cm long fused silica (50 µm i.d.), a 30 mbar injection for 3 s only rep-
322 resents 3.5 nL of analyte solution, while capillary conditioning and TDA run typically consume
323 respectively 5 and 2 µL of eluent.
324 In the present study, the plug-in-front protocol was adapted to minimize the amount of sam-
325 ple used. In this optimized protocol, only 0.2 mg of Myo under solid form was required and dis-
326 solved in 100 µL of eluent to prepare the mother solution S_0 (see Figure 4A). S_0 was further diluted
327 to prepare 100 µL of S_1 and 200 µL of S_2 solutions at 1 and 0.5 gL^{-1}, respectively. These solutions
328 allowed up to 27 consecutive plug-in-front TDA analyses.

As shown in Figure 4B and 4C, the signal obtained using this optimized protocol was similar to that in Figure 3, with almost perfect Gaussian peaks and low A_s value (see Table 2). At pH 5.8, almost symmetrical peaks were obtained (A_s = 1.03 ± 0.08, n = 5), while at pH 7.0, no significant change was observed between the experiments at 0.4 gL^{-1} and 0.5 gL^{-1} C_{front} plug. R_h values were in very good agreement with the literature [53,54], with 1.89 ± 0.09 nm and 1.85 ± 0.05 nm at pH 5.8 and 7.0, respectively (n = 5). Low RSD values on both R_h and A_s proves the reliability of this protocol.

3.5. Application to the analysis of mRNA lipid nanoparticles. Finally, the previously optimized protocol was applied to plug-in-front TDA of LNP. Indeed, since COVID19 pandemy, mRNA LNP formulations have gained considerable interest for the development of mRNA vaccines and for new cancer gene therapy. In a previous study [55], TDA of LNP was performed on a μSIL-FC coated capillaries. The analysis of LNP on fused silica capillary was not an option due to irreversible adsorption onto capillary surface. This previous TDA method included a long rinsing protocol: 3-4 min rinsing with water and background electrolyte between each run, and a more intensive cleaning procedure every 10 runs composed of 20 min successive rinsing with Hellmanex / isopropanol / water followed by 10 min LNP presaturation. In this work, we propose to apply the plug-in-front protocol to the analysis of Pfizer Comirnaty™ vaccine against Covid 19. Compared to the "normal" plug injection on bare fused silica capillary (Figure 5A) and to the plug injection after presaturation of the capillary (see Figure 5B), the plug-in-front protocol leads to better signal asymmetry without the resort to a coated capillary (Figure 5C).

The vaccine solution was injected as is without any dilution. The exact composition of the vaccine solution being unknown, 20 mM /17 mM Tris-HCl buffer, pH 7.4 (η = 0.94 cp) was used as eluent. The plug-in-front optimized TDA protocol was implemented on a 60 cm long bare fused

353 silica capillary using as eluent a 20 times diluted LNP solution in Tris-HCl buffer. Figure 6A dis-
354 plays 5 plug-in-front TDA runs on Pfizer Comirnaty™ vaccine. The taylorgrams display a small thin
355 peak on the top of the elution profile, which can be attributed to small molecules and/or mismatch
356 buffer due to differences in composition between the sample and the front matrices. This small
357 molecule contribution corresponded to a hydrodynamic diameter D_h lower than 0.7 nm and was
358 not taken into account in the LNP size distribution. D_h distributions obtained by TDA using the
359 constrained regularized linear inversion algorithm (CRLI method as described in [56]) are dis-
360 played in Figures 6B and 6C, for five repetitions. Since hydrodynamic diameters are more general-
361 ly used than hydrodynamic radius in the pharmaceutical industry, LNP sizes are given in D_h. D_h of
362 56.60 ± 1.58 nm *PDI* of 0.0037 ± 0.0016 were determined (n = 5).

363 For a better comparison, the D_h distributions were represented both in linear and log scales
364 and were compared with those obtained by DLS. Figures 6B and 6C display the intensity weighted
365 D_h distributions centered at an average value of D_h=91.65 ± 2.64 nm (n = 5) obtained by DLS using
366 CONTIN-like analysis and the polydispersity index obtained from the cumulant fit (*PDI*=0.23) in
367 good agreement with the literature (80 to 100 nm and *PDI* going from 0.2 to 0.25) [57–59].

368 TDA led to lower D_h values compared to DLS as already observed for LNP analysis [21] and
369 for any polydisperse sample [11,60,61]. This is due to intrinsic differences in the principle of anal-
370 ysis of both techniques. TDA basically leads to a mass-weighted size distribution for a mass con-
371 centration sensitive detector [11], while DLS is an intensity-weighted distribution which weighs
372 more the larger nanoparticles. This also explains why the DLS size distribution is much broader in
373 DLS compared to TDA.

374 We anticipate that plug-in-front protocol is a convenient, straightforward and simple way to
375 improve TDA repeatability and to limit the impact of slow adsorption /desorption kinetics on size
376 determination. Compared to the previously published article on TDA of LNP, we significantly im-

proved the TDA protocol by replacing expensive coated capillaries by fused silica capillaries and by considerably reducing the rinsing times between runs down to 3.3 min rinse with the plug-in-front protocol and no additional rinsing protocol between series of experiments.

4. Conclusion

In this work, we experimentally confirmed that the relative error on D (or R_h) is $5k$ in the case of fast equilibrium adsorption/desorption, as predicted from the theory of chromatography. More complex and larger solutes, such as proteins, can interact on the capillary wall with slow desorption kinetics leading to peak tailing, a decrease of the signal intensity or even the absence of detection for strong interactions. Capillary presaturation significantly improved the shape/symmetry of the taylorgram. A new protocol, called plug-in-front TDA, consisting in adding the solute in the eluent at a lower concentration compared to the injected sample, was successfully tested and led to much better repeatability and shorter rinsing protocols. The interest of this new approach was demonstrated on the analysis of protein and lipid nanoparticles (LNP) in mRNA vaccines. Both peak asymmetry and standard deviation on R_h values were significantly reduced, making this protocol of great interest for many applications.

It is interesting to note that the plug-in-front protocol presented here bears some similarity with the step-and-pulse procedure used a long time ago in gas chromatography for the purpose of determination of sorption isotherms [62]. In the latter method, the eluent was made of a given concentration of solute in the carrier gas and the injected sample contained a slight concentration excess of solute over that of the eluent, giving access to the slope of the sorption isotherm at the solute concentration in the eluent. Repeating the process and various solute concentration in the eluent allowed the determination of the whole isotherm. By contrast, in the present plug-in-front

case, for the purpose of preventing adsorption of the analyte on the TDA capillary wall, the solute concentration in the carrier is significantly lower than that in the injected sample.

Supporting Information

Table SI1. Myoglobin R_h and A_s measurement of plug injection TDA at four different pH values.

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References

[1] H. Song, Y. Vanderheyden, E. Adams, G. Desmet, D. Cabooter, Extensive database of liquid phase diffusion coefficients of some frequently used test molecules in reversed-phase liquid chromatography and hydrophilic interaction liquid chromatography, J. Chromatogr. A. 1455 (2016) 102-112. http://dx.doi.org/10.1016/j.chroma.2016.05.05.

[2] G. Taylor, Dispersion of soluble matter in solvent flowing slowly through a tube, Proc. R. Soc. Lond. A 219 (1953) 186–203. https://doi.org/10.1098/rspa.1953.0139.

[3] G. Taylor, Conditions under which dispersion of a solute in a stream of solvent can be used to measure molecular diffusion, Proc. R. Soc. Lond. A 225 (1954) 473–477. https://doi.org/10.1098/rspa.1954.0216.

[4] R. Aris, On the dispersion of a solute in a fluid flowing through a tube, Proc. R. Soc. Lond. A 235 (1956) 67–77. https://doi.org/10.1098/rspa.1956.0065.

[5] J.C. Giddings, S.L. Seager, Rapid determination of gaseous diffusion coefficients by means of gas chromatography apparatus, J. Chem. Phys. 33 (1960) 1579-1580. https://doi.org/10.1063/1.1731448.

[6] E. Grushka, E.J. Kikta, Extension of the chromatographic broadening method of measuring diffusion coefficients to liquid systems. I. Diffusion coefficients of some alkylbenzenes in chloroform, J. Phys. Chem. 78 (1974) 2297–2301. https://doi.org/10.1021/j100615a024.

[7] A.C. Ouano, Diffusion in Liquid Systems. I. A simple and fast method of measuring diffusion constants, Ind. Eng. Chem. Fund. 11 (1972) 268–271. https://doi.org/10.1021/i160042a019.

[8] K. C. Pratt, W. A. Wakeham, The mutual diffusion coefficient of ethanol–water mixtures: determination by a rapid, new method, Proc. R. Soc. Lond. A 336 (1974) 393–406. https://doi.org/10.1098/rspa.1974.0026.

[9] M.S. Bello, R. Rezzonico, P.G. Righetti, Use of Taylor-Aris Dispersion for measurement of a solute diffusion coefficient in thin capillaries, Science 266 (1994) 773–776.

[10] B.M. Belongia, J.C. Baygents, Measurements on the diffusion coefficient of colloidal particles by Taylor–Aris Dispersion, Journal of Colloid and Interface Science 195 (1997) 19–31. https://doi.org/10.1006/jcis.1997.5131.

[11] H. Cottet, J.-P. Biron, M. Martin, Taylor Dispersion Analysis of mixtures, Anal. Chem. 79 (2007) 9066–9073. https://doi.org/10.1021/ac071018w.

[12] B.J. Berne, R. Pecora, Dynamic Light Scattering, with application to chemistry, biology and physics., Wiley Interscience, New York, NY, 1976.

[13] U. Sharma, N.J. Gleason, J.D. Carbeck, Diffusivity of solutes measured in glass capillaries using Taylor's Analysis of Dispersion and a commercial CE instrument, Anal. Chem. 77 (2005) 806–813. https://doi.org/10.1021/ac048846z.

[14] R. Niesner, A. Heintz, Diffusion coefficients of aromatics in aqueous solution, J. Chem. Eng. Data 45 (2000) 1121–1124. https://doi.org/10.1021/je0000569.

[15] E.P.C. Mes, W.Th. Kok, H. Poppe, R. Tijssen, Comparison of methods for the determination of diffusion coefficients of polymers in dilute solutions: The influence of polydispersity, J. Polym. Sci. B Polym. Phys. 37 (1999) 593–603. https://doi.org/10.1002/(SICI)1099-0488(19990315)37:6<593::AID-POLB11>3.0.CO;2-N.

[16] H. Cottet, M. Martin, A. Papillaud, E. Souaïd, H. Collet, A. Commeyras, Determination of dendrigraft poly- L -Lysine diffusion coefficients by Taylor Dispersion Analysis, Biomacromolecules 8 (2007) 3235–3243. https://doi.org/10.1021/bm070268j.

[17] H. Cottet, J.-P. Biron, L. Cipelletti, R. Matmour, M. Martin, Determination of individual diffusion coefficients in evolving binary mixtures by Taylor Dispersion Analysis: Application to the monitoring of polymer Reaction, Anal. Chem. 82 (2010) 1793–1802. https://doi.org/10.1021/ac902397x.

[18] T. Le Saux, H. Cottet, Size-based characterization by the coupling of capillary electrophoresis to Taylor Dispersion Analysis, Anal. Chem. 80 (2008) 1829–1832. https://doi.org/10.1021/ac702257k.

[19] F. d'Orlyé, A. Varenne, P. Gareil, Determination of nanoparticle diffusion coefficients by Taylor dispersion analysis using a capillary electrophoresis instrument, J. Chromatogr. A 1204 (2008) 226–232. https://doi.org/10.1016/j.chroma.2008.08.008.

[20] U. Franzen, C. Vermehren, H. Jensen, J. Østergaard, Physicochemical characterization of a PEGylated liposomal drug formulation using capillary electrophoresis, Electrophoresis 32 (2011) 738–748. https://doi.org/10.1002/elps.201000552.

[21] C. Malburet, L. Leclercq, J.-F. Cotte, J. Thiebaud, E. Bazin, M. Garinot, H. Cottet, Size and charge characterization of lipid nanoparticles for mRNA vaccines, Anal. Chem. 94 (2022) 4677–4685. https://doi.org/10.1021/acs.analchem.1c04778.

[22] S. Latunde-Dada, R. Bott, K. Hampton, J. Patel, O.I. Leszczyszyn, Methodologies for the Taylor dispersion analysis for mixtures, aggregates and the mitigation of buffer mismatch effects, Anal. Methods 7 (2015) 10312–10321. https://doi.org/10.1039/C5AY02094H.

[23] H. Cottet, J.-P. Biron, M. Martin, On the optimization of operating conditions for Taylor dispersion analysis of mixtures, Analyst 139 (2014) 3552–3562. https://doi.org/10.1039/C4AN00192C.

[24] D. Sadriaj, G. Desmet, D. Cabooter, Taylor-Aris methodology for the experimental determination of molecular diffusion coefficients: Tutorial with focus on large biomolecules, J. Chromatogr. A 1664 (2022) 462787. https://doi.org/10.1016/j.chroma.2021.462787.

[25] A. Lewandrowska, A. Majcher, A. Ochab-Marcinek, M. Tabaka, R. Hołyst, Taylor Dispersion Analysis in coiled capillaries at high flow rates, Anal. Chem. 85 (2013) 4051–4056. https://doi.org/10.1021/ac4007792.

[26] M.J.E. Golay, Theory of chromatography in open and coated tubular columns with round and rectangular cross-sections., Desty D.H., Butterworths, London, 1958.

[27] M.A. Khan, Non-equilibrium theory of capillary columns and the effect of interfacial resistance on column efficiency. In Gas Chromatography 1962; Van Swaay, M., Ed.; Butterworths, London, (1962) pp. 3-17.

[28] J.C. Giddings, Kinetic model for chromatographic dispersion and electrodiffusion, J. Chem. Phys. 26 (1957) 1755–1756. https://doi.org/10.1063/1.1743620.

[29] J. Calvin Giddings, Non-equilibrium and diffusion: a common basis for theories of chromatography, J. Chromatogr. A 2 (1959) 44–52. https://doi.org/10.1016/S0021-9673(01)86255-5.

[30] J.C. Giddings, Kinetic processes and zone diffusion in chromatography, J. Chromatogr. A 3 (1960) 443–453. https://doi.org/10.1016/S0021-9673(01)97028-1.

[31] D. Hlushkou, F. Gritti, G. Guiochon, A. Seidel-Morgenstern, U. Tallarek, Effect of adsorption on solute dispersion: A microscopic stochastic approach, Anal. Chem. 86 (2014) 4463–4470. https://doi.org/10.1021/ac500309p.

[32] T. Boddington, A.A. Clifford, The dispersion of a reactive species (atomic hydrogen) in a flowing gas, Proc. R. Soc. Lond. A 389 (1983) 179-186. https://doi.org/10.1098/rspa.1983.0102.

[33] V. Balakotaiah, H. Chang, Dispersion of chemical solutes in chromatographs and reactors, Phil. Trans. R. Soc. Lond. A 351 (1995) 39-75. https://doi.org/10.1098/rsta.1995.0025.

[34] Y. Chen, The statistical theory of linear capillary chromatography with uniform stationary phase, J. Chromatogr. A 1144 (2007) 221–244. https://doi.org/10.1016/j.chroma.2007.01.041.

[35] C.-O. Ng, N. Rudraiah, Convective diffusion in steady flow through a tube with a retentive and absorptive wall, Phys. Fluids. 20 (2008) 073604. https://doi.org/10.1063/1.2958322.

[36] M. Levesque, O. Bénichou, R. Voituriez, B. Rotenberg, Taylor dispersion with adsorption and desorption, Phys. Rev. E 86 (2012) 036316. https://doi.org/10.1103/PhysRevE.86.036316.

[37] A.M. Berezhkovskii, A.T. Skvortsov, Aris-Taylor dispersion with drift and diffusion of particles on the tube wall, J. Chem. Phys. 139 (2013) 084101. https://doi.org/10.1063/1.4818733.

[38] D.M. Scott, J.S. Fritz, Model for chromatographic separations based on renewal theory, Anal. Chem. 56 (1984) 1561–1566. https://doi.org/10.1021/ac00273a006.

[39] F. Dondi, M. Remelli, The characteristic function method in the stochastic theory of chromatography, J. Phys. Chem. 90 (1986) 1885-1891. 10.1021/j100400a029

[40] G. Madras, B.L. Hamilton, M.A. Matthews, Influence of adsorption on the measurement of diffusion coefficients by Taylor dispersion, Int. J. Thermophys. 17 (1996) 373–389. https://doi.org/10.1007/BF01443398.

[41] S.A. Smith, V. Shenai, M.A. Matthews, Diffusion in Supercritical Mixtures: CO2 + Cosolvent + Solute, J. Supercrit. Fluids 3 (1990) 175–179.

[42] B.L. Hamilton, University of Wyoming, 1992.

[43] J.G. Atwood, M.J.E. Golay, Dispersion of peaks by short straight open tubes in liquid chromatography systems, J. Chromatogr. A 218 (1981) 97–122. https://doi.org/10.1016/S0021-9673(00)82050-6.

[44] A. Shankar, A.M. Lenhoff, Dispersion in round tubes and its implications for extracolumn dispersion, J. Chromatogr. A 556 (1991) 235–248. https://doi.org/10.1016/S0021-9673(01)96224-7.

[45] M. Guan, G. Chen, Streamwise dispersion of soluble matter in solvent flowing through a tube, J. Fluid Mech. 980 (2024) A33. https://doi.org/10.1017/jfm.2024.34.

[46] A. Shankar, A.M. Lenhoff, Dispersion and partitioning in short coated tubes, Ind. Eng. Chem. Res. 30 (1991) 828–835. https://doi.org/10.1021/ie00053a004.

[47] M.W. Lau, C.O. Ng, On the early development of dispersion in flow through a tube with wall reactions, in: F.G. Zhuang, J.C. Li (Eds.), New Trends in Fluid Mechanics Research, Springer, Berlin, Heidelberg, 2009: pp. 670–673. https://doi.org/10.1007/978-3-540-75995-9_224.

[48] M.D. Beauchamp, M.R. Schure, Simulation and theory of open-tube dispersion in short and long capillaries with slip boundaries and retention, J. Chromatogr. A 1588 (2019) 85–98. https://doi.org/10.1016/j.chroma.2018.12.040.

[49] L. Zhang, M. Hesse, M. Wang, Transient solute transport with sorption in Poiseuille flow, J. Fluid Mech. 815 (2017) 295–332. https://doi.org/10.1017/jfm.2017.54.

[50] W. Jiang, L. Zeng, X. Fu, Z. Wu, Analytical solutions for reactive shear dispersion with boundary adsorption and desorption, J. Fluid Mech. 947 (2022) A37. https://doi.org/10.1017/jfm.2022.656.

[51] S. Debnath, W. Jiang, M. Guan, G. Chen, Effect of ring-source release on dispersion process in Poiseuille flow with wall absorption, Phys. Fluids. 34 (2022) 027106. https://doi.org/10.1063/5.0077957.

[52] D.G. Cornish, G.W. Froning, Isolation and purification of turkey heme proteins, Poult. Sci. 53 (1974) 365–377. https://doi.org/10.3382/ps.0530365.

[53] S. Ali, H. Farooqi, R. Prasad, M. Naime, I. Routray, S. Yadav, F. Ahmad, Boron stabilizes peroxide mediated changes in the structure of heme proteins, Int. J. Biol. Macromol. 47 (2010) 109–115. https://doi.org/10.1016/j.ijbiomac.2010.05.013.

[54] V. La Verde, P. Dominici, A. Astegno, Determination of hydrodynamic radius of proteins by Size Exclusion Chromatography, Bio Protocol 7 (2017). https://doi.org/10.21769/BioProtoc.2230.

[55] C. Malburet, L. Leclercq, J.-F. Cotte, J. Thiebaud, S. Marco, M.-C. Nicolaï, H. Cottet, Antigen-adjuvant interactions in vaccines by Taylor Dispersion Analysis: Size characterization and binding parameters, Anal. Chem. 93 (2021) 6508–6515. https://doi.org/10.1021/acs.analchem.1c00420.

[56] L. Cipelletti, J.-P. Biron, M. Martin, H. Cottet, Measuring arbitrary diffusion coefficient distributions of nano-objects by Taylor Dispersion Analysis, Anal. Chem. 87 (2015) 8489–8496. https://doi.org/10.1021/acs.analchem.5b02053.

[57] L. Kudsiova, A. Lansley, G. Scutt, M. Allen, L. Bowler, S. Williams, S. Lippett, S. Stafford, M. Tarzi, M. Cross, M. Okorie, Stability testing of the Pfizer-BioNTech BNT162b2 COVID-19 vaccine: a translational study in UK vaccination centres, BMJ Open Science 5 (2021). https://doi.org/10.1136/bmjos-2021-100203.

[58] F. Selmin, U.M. Musazzi, S. Franzè, E. Scarpa, L. Rizzello, P. Procacci, P. Minghetti, Pre-drawn syringes of Comirnaty for an efficient COVID-19 mass vaccination: Demonstration of stability, Pharmaceutics 13 (2021) 1029. https://doi.org/10.3390/pharmaceutics13071029.

[59] J. Szebeni, B. Kiss, T. Bozó, K. Turjeman, Y. Levi-Kalisman, Y. Barenholz, M. Kellermayer, New insights into the structure of Comirnaty Covid-19 vaccine: A theory on soft nanoparticles with mRNA-lipid supercoils stabilized by hydrogen bonds, Immunology, 2022. https://doi.org/10.1101/2022.12.02.518611.

[60] L. Leclercq, S. Reinhard, J. Chamieh, M. Döblinger, E. Wagner, H. Cottet, Fast Characterization of polyplexes by Taylor Dispersion Analysis, Macromolecules 48 (2015) 7216–7221. https://doi.org/10.1021/acs.macromol.5b01824.

[61] L. Leclercq, P. Saetear, A. Rolland-Sabaté, J.-P. Biron, J. Chamieh, L. Cipelletti, D.J. Bornhop, H. Cottet, Size-based characterization of polysaccharides by Taylor Dispersion Analysis with photochemical oxidation or backscattering interferometry detections, Macromolecules 52 (2019) 4421–4431. https://doi.org/10.1021/acs.macromol.9b00605.

[62] P. Valentin, G. Guiochon, Determination of gas-liquid and gas-solid equilibrium isotherms by chromatography: I. Theory of the step-and-pulse method, J. Chromatogr. Sci. 14 (1976) 56–63. https://doi.org/10.1093/chromsci/14.2.56.

LIST OF CAPTIONS

Figure 1: TDA experiment for toluene (A), p-cymene (B) and triphenylphosphine (C) in presence of adsorption on DB-1/DB-17 or in absence of adsorption on fused silica capillary (*n* =5). Experimental conditions: capillary of 80 cm total length (71.5 cm to detector) × 50 µm i.d. with or without DB-1/DB-17 coatings as indicated in the graph. Eluent: ACN/HEPES 70/30 (w/w). Hydrodynamic injection of solutes at 30 mbar for 4 s (0.16% capillary volume). 1: cyclo(Ala-Ala) used as the void-time marker, 2: toluene, 3: p-cymene, 4: triphenylphosphine. Mobilization pressures as indicated in the figure. Temperature: 25°C. UV detection: 214 nm. Dotted lines are Gaussian fits of the taylorgrams.

Figure 2: Plug injection TDA experiment on myoglobin, without (A) or with (B) capillary presaturation, at four different pH values (*n* =5). Black lines correspond to experimental traces and red dashed lines to Gaussian fits. Experimental conditions: fused silica capillary, 40 cm total length (30 cm to detector) × 50 µm i.d. Eluents: acetic acid (2 M, pH 2.2), MES/NaOH (50 mM/10 mM, pH 5.8), HEPES/NaOH (40 mM/10 mM, pH 7.0) and sodium tetraborate (10 mM, pH 9.1). Capillary rinsing: BGE 1 bar, 5 min (followed by solute solution injection for 10 min at 100 mbar in case of presaturation). Hydrodynamic injection of solutes: 30 mbar, 4 s (0.66% capillary volume). Mobilization pressure: 30 mbar. Myo concentration: 1 $g.L^{-1}$. Temperature: 25°C. UV detection: 214 nm.

Figure 3: Influence of Myo front concentration (C_{front}) in plug-in-front TDA at pH 5.8 (A) and 7.0 (B), *n* =5. Black lines correspond to experimental traces and red dashed lines to Gaussian fits. Experimental conditions: fused silica capillary, 40 cm total length (31.5 cm to detector) × 50 µm i.d. Eluents: MES/NaOH (50 mM/10 mM, pH 5.8) or HEPES/NaOH (40 mM/10 mM, pH 7.0), both containing Myo as indicated on the Figure. Capillary rinsing between runs: eluent at 1 bar, 5 min. Hydrodynamic injection: 30 mbar, 4 s (0.66% capillary volume). Mobilization pressure: 30 mbar. Myo concentration in the plug: 1 $g.L^{-1}$. Temperature: 25°C. UV detection: 214 nm.

Figure 4: Schematic representation of optimized plug-in-front TDA protocol (A) and five repetitions of the corresponding taylorgrams for myoglobin at pH 5.8 (B) and pH 7.0 (C). Experimental conditions: fused silica capillary, 40 cm total length (31.5 cm to detector) × 50 µm i.d. Eluents: MES/NaOH (50 mM/10 mM, pH 5.8) and HEPES/NaOH (40 mM/10 mM, pH 7.0) both containing 0.5 gL-1 Myo. Before the first injection, the capillary was rinsed with the spiked eluent for 200 s at 100 mbar (1 capillary volume) for saturation of the interacting sites. Between runs, the capillary was flushed with spiked eluent for 100 s at 1 bar (5 capillary volumes). Hydrodynamic injection: 30 mbar, 4 s (0.66% of capillary volume). Myoglobin plug concentration: 1 g.L-1. Mobilization pressure: 30 mbar. Temperature: 25°C. UV detection: 214 nm.

Figure 5: TDA experiments on Pfizer Comirnaty™ vaccine using plug injection without capillary presaturation (A), with capillary presaturation (B) and using plug-in-front protocol (*n* =5) (C). Experimental conditions: fused silica capillary, 60 cm total length (51.5 cm to detector) × 50 µm i.d. Eluent: 20 mM/17 mM Tris/HCl, pH 7.4 (A and B); 20 times diluted vaccine in Tris/HCl eluent (C). Hydrodynamic injection: 30 mbar, 12 s (0.81% of capillary volume). Vaccine solution was injected as is. Mobilization pressure: 45 mbar. Temperature: 25°C. UV detection: 214 nm. Rinsing between runs: 5 min eluent at 1 bar (A); 20 times diluted vaccine solution in Tris-HCl buffer for 200 s at 1 bar (5 capillary volumes) (B and C). Rinsing before the first run: 20 times diluted vaccine solution in Tris-HCl buffer for 420 s at 100 mbar (1 capillary volume) to saturate all the interacting sites (B and C). Gaussian fits of the taylorgrams are presented in red.

Figure 6: Plug-in-front TDA of Pfizer Comirnaty™ vaccine solution with CRLI fit of 5 runs (A), overlay of the corresponding size distributions obtained by DLS and TDA in linear size scale (B) and log scale (C). CRLI fit of the taylorgram is represented in red in Figure 6A. Repetitions of P(D_h) were normalized within each method to the same peak area. Repetitions of P(D_h) from TDA were then adjusted to the maximum of P(D_h) from DLS, to avoid too much spreading of the DLS distribution in the inter-method comparison. Experimental conditions as in Figure 5C.

623

624 **Table 1: Values of retention factor (k), diffusion coefficient in presence (D^*) and in absence (D_0) of**
625 **adsorption and hydrodynamic radius in presence (R_h^*) and in absence (R_{h0}) of adsorption, obtained**
626 **for toluene, p-cymene and triphenylphosphine to verify the validity of Eq. (11).** Experimental condi-
627 tions as in Figure 1 with DB-1/ DB-17 coatings.

628

629 **Table 2: Impact of presaturated capillary and plug-in-front protocol on R_h and A_s values for myoglo-**
630 **bin TDA.**

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632