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Taylor dispersion analysis with two detection points on a commercial capillary electrophoresis apparatus

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ABSTRACT

This work describes a simple technical modification for doing Taylor dispersion analysis with two UV detection points on a commercial capillary electrophoresis apparatus. So far, double UV detection was only possible using specific detectors that are external to the capillary electrophoresis apparatus. In this work, the detection interface of the capillary electrophoresis apparatus was easily modified to allow the introduction and the superposition of two capillary windows in the same interface (at the same detection point). This modification made possible the double detection of the sample zone in Taylor dispersion analysis using a loop. The peak dispersion using the modified interface was similar to that obtained on a non-modified UV interface. Diffusion coefficients (and the corresponding hydrodynamic radii) of small molecule and proteins were determined in good agreement with values of the literature and with RSD lower than 5%.

1. Introduction

Taylor dispersion analysis (TDA) based on the seminal work of Taylor [1] and later of Aris [2] consists on the analysis of peak broadening of a solute plug in a laminar Poiseuille flow for the determination of the diffusion coefficients (D), and consequently of the hydrodynamic radius (R_h). This technique was first applied to the determination of gaseous diffusion coefficients [3] and then to liquid diffusion coefficients [4–6]. More recently Bello et al. [7] demonstrated the possibility to use capillary electrophoresis instrumentation to perform TDA in thin capillaries. TDA is a simple and absolute method that requires only minute amount of sample (few nL injected). It can be applied for the sizing of solutes of virtually any size from angstroms to sub-microns. TDA has been used for a wide variety of applications including peptides and proteins [8–11], small molecules [12,13], macromolecules [14–17], and nanoparticles [18,19]. In the case of polydisperse samples, TDA generally leads to weight-average R_h for UV detection sensitive to the mass concentration of the solute [15].

The band broadening resulting from Taylor dispersion is easily quantified via the temporal variance of the elution profile. Nevertheless, corrections due to the pressure ramp and to the finite injection volume should be considered. Extensive work was done to correct the elution time and the peak variance for the injected volume and pressure ramp errors [12,14,16,20]. Another approach to avoid any correction is based on a differential measurement of the peak dispersion using two detection windows as recently proposed by Chapman and Goodall [21]. The use of an external detector (Actipix D100, Paraytec, York, GB) having two detection points allows the direct determination of the diffusion coefficient and R_h using the following equations:

$$D = \frac{R_c^2(t_2 - t_1)}{24(\sigma_2^2 - \sigma_1^2)} \quad (1)$$

$$R_h = \frac{k_B T}{6\pi\eta D} \quad (2)$$

where D is the diffusion coefficient, R_c the capillary radius, t_1 and t_2 are the average elution times at the first and second detection windows respectively. σ_1 and σ_2 are the standard deviations of the peak at the first and second detection points. k_B is the Boltzmann constant, T is the temperature and n is the viscosity of the carrier liquid. It is noteworthy that Eq. (1) is valid when the peak appearance time t_R is higher than the characteristic diffusion time on a distance equal to the capillary radius as verified by Eq. (3). Axial diffusion should also be negligible compared to convection as verified by Eq. (4), where P_e is the Peclet number:

$$\tau = \frac{Dt_R}{R_c^2} > 1.4 \quad (3)$$

$$Pe = \frac{uR_c}{D} > 69 \quad (4)$$

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where u is the linear mobile phase velocity. It should be noted that in all TDA experiments presented in this work, the conditions of validity of Eq. (1) [1,12,22] were fulfilled i.e. $\tau > 1.4$ and $P_e > 69$ (Eqs. (3) and (4)). Using two-detection point external detector, Forbes et al. [8–10] studied the hydrodynamic radii of proteins and small molecules and found good correlations with DLS measurements on these monodisperse samples. Recently, Ye et al. [23] studied the diffusion of drug samples in pharmaceutical media using the double detection method. They concluded that the use of a double detection simplifies the TDA analysis compared to single point detection since no corrections are needed making the analysis more robust when the injection volume or the flow rate were modified.

In this paper, we demonstrate the possibility to perform TDA using two-detection points directly on a commercial CE apparatus by modifying the UV detection interface. External detector is no longer required using this simple approach. Hydrodynamic radii obtained on proteins and small molecules are compared with data from the literature.

2. Experimental

2.1. Reagents and materials

Bovine serum albumin (BSA) (batch A0299196) was purchased from Acros (France), human serum albumin (HSA) (batch 090M7001V), caffeine (batch 070M1642V), sodium hydrogenophosphate and sodium dihydrogenophosphate were purchased from Sigma Aldrich (France). Sodium chloride was from Acros (Belgium). Sodium hydroxide was from Merck (Germany). The water used to prepare all buffers was purified with a Milli-Q system from Millipore (France). Adhesive gum (RadeFix) was purchased from Kores (Austria).

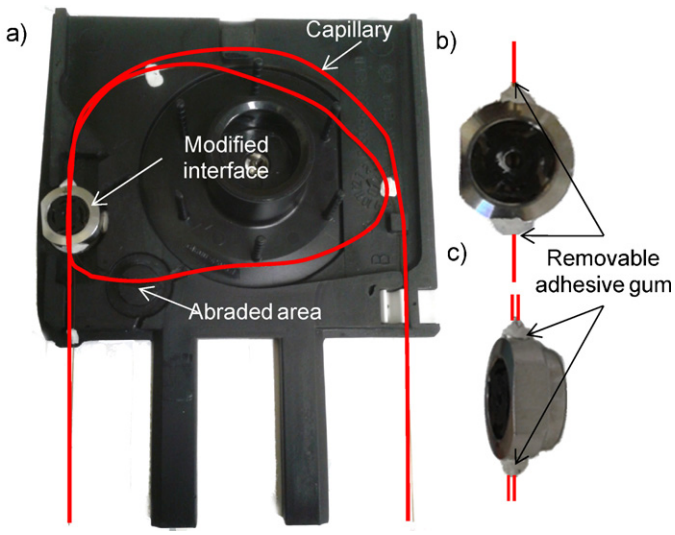

Fig. 1. (a) Representation of the cassette with a looped capillary and double detection points at the same interface. (b) Front view of the modified interface, (c) side view of the modified interface showing the position of the two capillary detection windows one on top of the other.

2.2. Buffers

Phosphate buffer (ionic strength 160 mM; pH 7.4; 1.09×10^{-3} Pa s viscosity at 25 °C) was prepared by dissolving the appropriate amounts 15.7 mM Na_2HPO_4, 3.2 mM NaH_2PO_4 and 110 mM of NaCl in water. BSA (5 g/L), HSA (5 g/L) and caffeine (2 g/L) were prepared directly in the phosphate buffer.

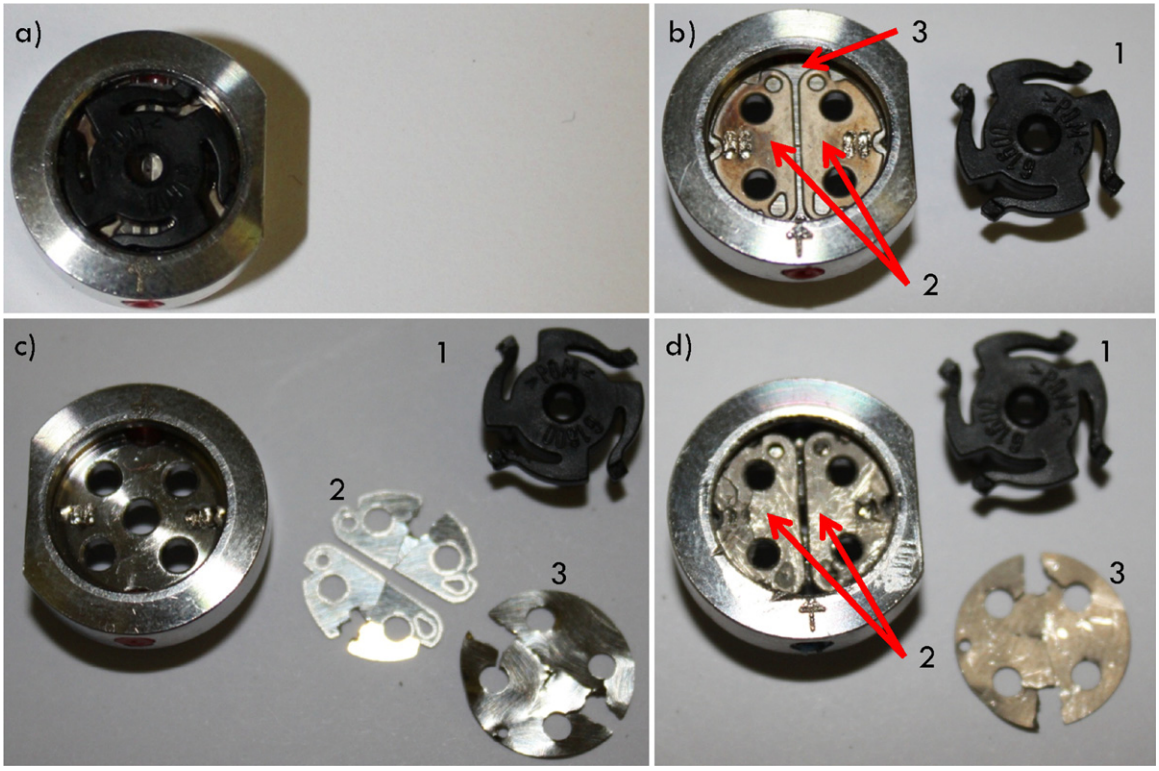

Fig. 2. Modification of the interface for double detection. Intact interface (a). Removal of the plastic part #1 (b). Removal of the aluminum parts #2 and #3 (c). Part #2 was returned to its place and glued on the interface while part #3 was discarded. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

2.3. Taylor dispersion analysis

TDA was performed on an Agilent CE instrument (Agilent technologies, Waldbronn, Germany) with a modified detection interface using bare fused silica capillaries (Polymicro technologies, USA) having 60 cm × 50 μm i.d. dimensions and two detection windows at 24.5 cm and 51.5 cm. New capillaries were conditioned with the following flushes: 1 M NaOH for 30 min; 0.1 M NaOH for 30 min; water for 10 min and buffer for 15 min. Between each analysis, capillaries were rinsed with water (3 min) and buffer (4.5 min). Samples were injected hydrodynamically on the inlet side of the capillary (30 mbar, 9 s, 6.33 nL injected volume). The injected sample volume was kept at a value lower than 1% of the capillary volume (to the second detection window; 0.63%). Experiments were performed using two different mobilization pressures (30 and 50 mbar). The temperature of the capillary cartridge was set at 25 °C. The solutes were monitored by UV absorbance at 200 nm. The taylorgrams were recorded with the Agilent Chemstation, exported to Microsoft Excel for subsequent data processing using Microcal Origin 6.0. The Gaussian fits were realized with Microcal Origin 6.0.

2.4. Modification of the detection interface

The Agilent detection interface was modified in order to get a double detection at the same interface. The capillary was placed in the cassette as shown in Fig. 1a with a capillary loop. The two detection windows were positioned one on the top of the other inside the interface as shown in Fig. 1b and c. They were immobilized with removable adhesive gum to impede them from moving when positioned inside the cassette. The cassette was also modified by abrading the plastic in order to create a channel for the capillary to reenter in the cassette and generate the loop (see Fig. 1a).

The modification of the detection interface was performed as shown in Fig. 2. After removing the black plastic part #1 protecting the window, parts #2 and #3 become visible (Fig. 2b). Part #2 which serves as a guide for the capillary, was removed with care (Fig. 1c). The aluminum foil containing the detection slit (part #3) was removed and discarded. Part #2 was then reassembled by gluing them onto the interface (Fig. 1d). The colored (green, red, blue, or black depending on the detection slit dimensions adapted to the capillary internal diameter) plastic entrance and exit of the capillary in the interface were enlarged using an appropriate syringe needle (1.2 mm diameter) to allow the entrance of two capillaries in the same interface. Plastic part # 1 was finally replaced.

3. Results and discussion

To perform TDA with two-detection points on a commercial CE apparatus, without the use of external detector, the modification of the detection interface was essential to allow the positioning of two detection windows in the same interface. The positioning of the capillary loop in the cassette and in the interface is presented in Fig. 1. Effective capillary lengths from the inlet end to the two detection points are 24.5 cm and 51.5 cm. The modifications of the detection interface are described in Section 2 (see also Fig. 2). They are based on the removal of the detection slit (part #3 in Fig. 2) that blocks the entrance of two capillaries at the same time. Since TDA is not a separation but a dispersive technique and peak broadening is relatively large. Therefore, the absence of detection slit in the modified interface was not crucial and it did not change significantly the elution profiles (and thus, the temporal variance of the peak) compared to a non-modified interface for a given capillary length, as shown in Fig. 3 for HSA sample. It should be noted that in Fig. 3b, the taylorgrams obtained with the modified interface were renormalized for a good superposition with the one derived

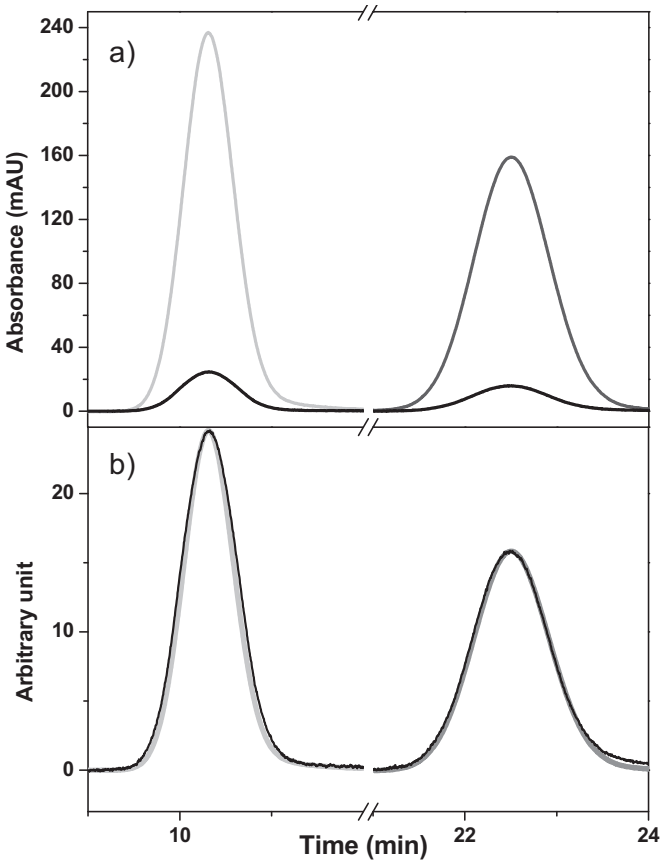

Fig. 3. Taylorgrams of HSA obtained with a capillary loop and double detection with the modified interface (a) raw data; (b) normalized data. Experimental conditions: 50 μm i.d. × 60 cm (24.5 cm to the first 1st detection window, 51.5 cm to the 2nd detection window) fused silica capillary. Mobile phase: 50 mM sodium phosphate buffer, pH 7.4 containing 110 mM NaCl. Mobilization pressure: 30 mbar. Results obtained for a double detection on the modified interface (black trace). Results obtained with a non-modified interface and single detection (light grey) for the same capillary lengths and for independent experiments.

from the non-modified interface. Indeed, the sensitivity of detection obtained with the modified interface was about 10 times lower than the one currently observed with the non-modified interface (Fig. 3a). This is due to the fact that, in the modified interface, the light beam was no longer limited to the internal section of the capillary since the detection slit was removed (part #2 in Fig. 2). Nevertheless, the peak variance did not change as seen in Fig. 3b by the almost perfect superposition of the traces. This moderate loss in detection sensitivity is not so crucial in TDA since it is not a separation technique and the injected concentration of the sample is generally sufficient to ensure good detection (sample concentration generally ranges between 0.5 and 10 g/L). Nevertheless, for poorly soluble organic compounds (e.g. hydrohpobic drugs), the loss in sensitivity of detection could be an issue [23].

The validation of the modified interface for TDA with two detection points was performed by choosing monodisperse solutes which were well studied in the literature: a small molecule (caffeine) and two proteins (BSA and HSA). Two mobilization pressures were used (30 and 50 mbar). Table 1 gathers the experimental results (average values based on 10 determinations) compared to the literature values. Experimental values are given with a confidence interval at 95% confidence level. R_h obtained at 30 mbar and 50 mbar are very close and are not significantly different. For all determinations, RSD on R_h are lower than 5%. The results obtained for caffeine were in good correlation with the literature values. The relatively high R_h value (0.46 nm) is consistent with the fact

Table 1
Diffusion coefficients and corresponding hydrodynamic radii obtained by TDA with two detection points obtained with the modified detection interface and compared to the literature values.

Sample	30 mbar		50 mbar	
	D (m^2 s^{-1}) (n = 10)	R_h (nm) (n = 10)	R_h (nm) (n = 10)	Literature values R_h (nm)
Caffeine (2 g/L)	$(4.3 \pm 0.1) \times 10^{-10}$	0.462 ± 0.013 (RSD 2.9%)	0.436 ± 0.017 (RSD 4.0%)	0.32–0.49 [10,24]
BSA (5 g/L)	$(4.9 \pm 0.1) \times 10^{-11}$	4.10 ± 0.12 (RSD 2.9%)	4.13 ± 0.13 (RSD 3.1%)	3.3–4.9 [9,10,25]
HSA (5 g/L)	$(4.8 \pm 0.1) \times 10^{-11}$	4.19 ± 0.09 (RSD 2.1%)	4.25 ± 0.14 (RSD 3.2%)	3.3–4.1 [25]

that caffeine tends to form dimers at high concentrations [10]. For albumin proteins, the values were also in good correlation with the literature values. These results demonstrate the validity of this approach based on the double detection with the modified interface.

4. Conclusion

This work demonstrates that it is possible to easily modify the detection interface to perform TDA analysis on two detection points on commercial CE apparatus, without the use of external detector. Advantages of this approach include low cost, rapid modification, good temperature control since the capillary is maintained in the cassette with the thermostated air cooling, TDA with multi-wavelengths detection using the UV diode array detector. The obtained R_h results were in good agreement with the literature with RSD lower than 5%. This simple modification of the interface opens also new perspectives to perform two detection points CE.

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