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Analytical mitigation of solute–capillary interactions in double detection Taylor Dispersion Analysis

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ABSTRACT

Taylor Dispersion Analysis (TDA) in the presence of interactions between solutes and capillary walls yields inaccurate results for the diffusion coefficients of the solutes because the resulting concentration profiles are broadened and asymmetric. Whilst there are practical ways of mitigating these interactions, it is not always possible to eradicate them completely. In this paper, an analytical method of mitigating the effects of the adsorptions is presented. By observing the dispersion of the solute molecules at two detection points and using the expected relations between measured parameters, such as the standard deviations and peak amplitudes, the dispersive components of the profiles were isolated with a constrained fitting algorithm. The method was successfully applied to lysozyme and cytochrome C which adsorb onto fused silica capillary walls. Furthermore, this illustrates an advantage of using the fitting method for Taylor Dispersion Analysis.

1. Introduction

Taylor Dispersion Analysis (TDA) is a fast and absolute method for determining the diffusion coefficients, and hence the hydrodynamic radii of molecules. The method, sometimes referred to as Taylor-Aris dispersion, was first described by Taylor in his classic paper [1]. In 1956, Aris developed the method further by accounting for the longitudinal diffusion of the molecules [2].

This technique was first applied to the determination of gaseous [3] and then liquid diffusion coefficients [4–6]. With the use of fused silica capillaries, TDA regained interest and has been used to analyze amino acids, peptides, proteins, small molecules, macromolecules, nanoparticles and biosensors [7–23].

A requirement for the accurate determination of the diffusion coefficients of injected solutes is that the solute molecules do not adsorb onto the capillary walls. Such interactions can arise when there is a force of attraction between unshielded charges on the solute molecules and exposed charges on the capillary walls. For example, the surface of a fused silica capillary is covered in silanol groups (Si—OH) which can ionize at pH values of 2 and above to form negatively charged groups ($Si—O^-$) [24]. Methodologically, this poses a problem for TDA of molecules with net regions of positive charge, as the resulting electrostatic attraction between the molecular surface and the capillary wall alters the solute's dispersion as it travels through the capillary. As a result, the observed concentration distributions (taylorgrams) are asymmetric with pronounced tailing. Fig. 1 shows the taylorgrams obtained for a series of concentrations of lysozyme prepared in phosphate buffered saline solution which interact with a fused silica capillary. Note that the tailing observed becomes less pronounced with concentration.

A number of experimental approaches can be employed to minimize or prevent capillary interactions due to electrostatic attraction. These strategies focus on modification or attenuation of the charge on either the capillary, the solute medium or the molecule itself. Such approaches are well-documented in capillary electrophoresis literature and reviews on the subject are available [24–26]. These include the use of additives in the carrier medium to compete with the solute molecules for charged sites on the capillary and the use of coated capillaries. However, these approaches may not always be suitable or sufficiently successful.

The diffusion coefficient of injected solutes undergoing Taylor dispersion can be deduced by fitting Taylor's solution to the taylorgrams [27]. Alternatively, this can be achieved by calculating the moments of the profile [3–8,12,28,29] or by measuring its height and area [30]. The analysis can either be carried out at a single detection point or at two spatially separated detection points. These methods are referred to as single detection TDA and double detection TDA [31–33] respectively.

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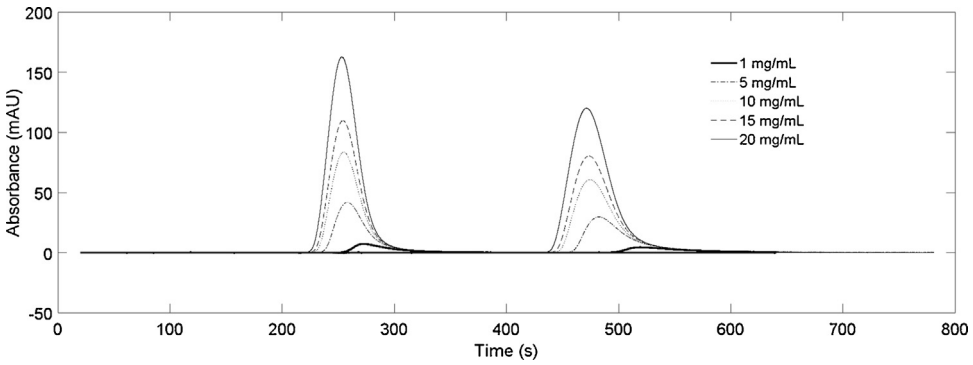

Fig. 1. Taylorgrams showing the effects of solute–capillary interactions.

The concentration distribution C which arises at a time t when a solute undergoes Taylor dispersion is given by [1]:

$$C \propto C_0 \sqrt{\frac{t_r}{t}} e^{-\frac{u^2(t-t_r)^2}{4kt}} \tag{1}$$

where C_0 is the initial solute concentration, t_r is known as the mean residence time, u is the mean flow speed of the carrier solution and k is the dispersion coefficient.

At large values of t, Eq. (1) is approximated by a Gaussian distribution [1]:

$$C \propto A e^{-\frac{(t-t_r)^2}{2\sigma^2}} \tag{2}$$

where A is the peak amplitude, the dispersion coefficient k is related to the standard deviation of the Gaussian σ by

$$k = \frac{u^2\sigma^2}{2t_r} \tag{3}$$

This is applicable to single detection TDA where only one standard deviation is measured. In double detection TDA, the dispersion coefficient is determined from the standard deviations of the taylorgrams, σ_1 and σ_2, at the points and the respective mean residence times, t_1 and t_2:

$$k = \frac{u^2(\sigma_2^2 - \sigma_1^2)}{2(t_2 - t_1)} \tag{4}$$

The diffusion coefficient D and hydrodynamic radius R_h are related to k by:

$$D = \frac{r_c^2 u^2}{48k} \tag{5}$$

$$R_h = \frac{k_B T}{6\pi\eta D} \tag{6}$$

where r_c is the capillary radius, k_B is Boltzmann's constant, T is the temperature and η is the viscosity of the carrier solution. By equating Eqs. (3) and (4), it can be seen that there are relationships between the measured parameters at the two detection points.

Fig. 2 shows a typical capillary geometry where l_1 and l_2 are the distances from the point of solute injection to the centers of the two detection points.

If the injection plug length l_i of the solute in a carrier solution moving at speed u is accounted for in Eq. (3), the following ratios are deduced to exist between the measured parameters at the two detection points in the absence of solute–capillary interactions:

$$\frac{t_1}{t_2} = \frac{l_1 - (l_i/2)}{l_2 - (l_i/2)} = r \tag{7}$$

$$\sqrt{\frac{\sigma_1^2 - \sigma_i^2}{\sigma_2^2 - \sigma_i^2}} = \sqrt{\frac{l_1 - (l_i/2)}{l_2 - (l_i/2)}} = \sqrt{r} \tag{8}$$

$$\frac{A_2}{A_1} = \sqrt{\frac{l_1 - (l_i/2)}{l_2 - (l_i/2)}} = \sqrt{r} \tag{9}$$

where $\sigma_i^2 = l_i^2/12u^2$ [13]. It should be noted that this estimate for σ_i is based on the assumption that the initial plug has a rectangular cross-section which is not strictly true for TDA. A more rigorous treatment would attempt to quantify the small amount of dispersion that occurs during the injection [34–36].

Agreement with these ratios (henceforth referred to as Taylor dispersion ratios) during measurements can be used to confirm that the solute has undergone ideal Taylor dispersion. Typically, deviations from these ratios are observed in the presence of solute–capillary interactions due to the asymmetric broadening of the Taylorgrams as shown in Fig. 1. This results in inaccuracies in the estimated diffusion coefficients. To date, a range of theories have been developed to account for the effects of a variety of interactions on the dispersion of a solute and include mass exchanges between phases of the solute [37,38], reactive gases [39], electrokinetic effects [40] and dead zone effects in streams [41]. These studies incorporate solute transport models which divide the solute into a dispersive component (or bulk flow region) which undergoes Taylor dispersion and an interactive component (or slow/dead zone) which contributes the asymmetry to the profile. If the two components are assumed to be independent (as can be the case when the solute–capillary interactions are moderate), it may be possible to isolate the dispersive components of the profile.

In this paper, the Taylor dispersion ratios will be used to constrain the fitting algorithm used in generic double detection TDA in order to isolate the dispersive components of the broadened profiles. This is the novelty of the method. In this way, only solutions which can arise feasibly as a result of Taylor dispersion will be considered by the algorithm when searching for the best fits to the taylorgrams. The ratios ensure that the parameters used to compute the diffusion coefficient are consistent across the two detection points and thus mitigate the effects of the interactions on the analysis. It may be described conceptually as the use of the solution

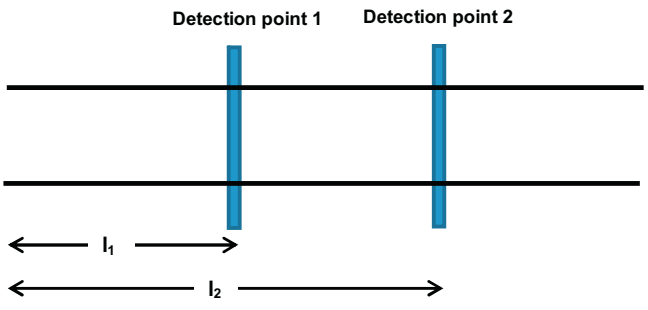

Fig. 2. Geometry of a capillary with two detection points.