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Taylor Dispersion Analysis as a promising tool for assessment of peptide-peptide interactions

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ABSTRACT

Protein-protein and peptide-peptide (self-)interactions are of key importance in understanding the physiochemical behavior of proteins and peptides in solution. However, due to the small size of peptide molecules, characterization of these interactions is more challenging than for proteins. In this work, we show that protein-protein and peptide-peptide interactions can advantageously be investigated by measurement of the diffusion coefficient using Taylor Dispersion Analysis. Through comparison to Dynamic Light Scattering it was shown that Taylor Dispersion Analysis is well suited for the characterization of protein-protein interactions of solutions of α-lactalbumin and human serum albumin. The peptide-peptide interactions of three selected peptides were then investigated in a concentration range spanning from 0.5 mg/ml up to 80 mg/ml using Taylor Dispersion Analysis. The peptide-peptide interactions determination indicated that multibody interactions significantly affect the PPIs at concentration levels above 25 mg/ml for the two charged peptides. Relative viscosity measurements, performed using the capillary based setup applied for Taylor Dispersion Analysis, showed that the viscosity of the peptide solutions increased with concentration. Our results indicate that a viscosity difference between run buffer and sample in Taylor Dispersion Analysis may result in overestimation of the measured diffusion coefficient. Thus, Taylor Dispersion Analysis provides a practical, but as yet primarily qualitative, approach to assessment of the colloidal stability of both peptide and protein formulations.

1. Introduction

Protein-protein (self-)interactions (PPIs) are of key importance in understanding the physicochemical behavior of proteins in solution. For example, PPI measurements can be used to determine the likelihood of success of protein crystallization experiments, where solution conditions leading to moderately attractive PPIs are most likely to yield crystals (Wilson and Delucas, 2014). Protein solubility is also related to PPIs, with the highest solubility generally observed under conditions resulting in the most repulsive PPIs (Wilson and Delucas, 2014). Finally, PPIs are indicative of the colloidal stability of a protein. Under different solution conditions with highly similar conformational stability, aggregation kinetics are generally faster under conditions that result in attractive PPIs as compared to repulsive PPIs (Chi et al., 2003a). In pharmaceutical development there is a strong focus on preventing aggregation since aggregates may cause severe adverse effects to the patients (Kahn and Rosenthal, 1979; Moore and Leppert, 1980).

The magnitude and nature of PPIs are often evaluated by either the second virial coefficient, B_{22}, or the interaction parameter, k_D. These coefficients describe a solution's deviation from ideality, and are related as follows (Harding and Johnson, 1985; Saluja et al., 2007; Zhang and Liu, 2003):

$$k_D = 2B_{22}M_W - \zeta_1 - 2\nu_{sp} \quad (1)$$

where M_w is the molecular weight, ζ_1 is the first order concentration coefficient in the virial expansion of the frictional coefficient, and ν_{sp} is the protein partial specific volume. The value of B_{22} denotes the deviation from ideality of the solution with a negative value as an indicator of attractive interactions, and a positive value indicating repulsive interactions (Saluja et al., 2007). Likewise k_D can be used to assess the nature of PPIs, but due to the hydrodynamic contributions (ζ_1 and ν_{sp}) in Eq. (1) there may be instances where a slightly negative k_D is not indicative of attractive PPIs (Yadav et al., 2010b). However, k_D is still a useful indicator to rank PPIs in the sense that a more negative k_D value indicates more attractive PPIs, and a more positive k_D value indicates more repulsive PPIs (Saluja et al., 2007; Yadav et al., 2010a, 2010b).

Currently, a number of analytical techniques are commonly used to characterize B_{22} and k_D of protein solutions. B_{22} can be determined

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using e.g., self-interaction chromatography (SIC) using approximately 50 mg protein material (Binabaji et al., 2014; Le Brun et al., 2010; Tessier et al., 2002), size-exclusion chromatography (SEC) using between 20 and 100 μl sample (Bajaj et al., 2004, 2007; Bloustine et al., 2003), static light scattering (SLS) using approximately 50 μl sample (Alford et al., 2008a, 2008b; Yadav et al., 2010a, 2011a), analytical ultracentrifugation (AUC) using between 60 and 420 μl sample depending on detector (Cole et al., 2008; Saluja et al., 2010), and membrane osmometry using approximately 500 μl sample (Alford et al., 2008b). Dynamic light scattering (DLS) requires approximately 50 μl sample and is the most common method to determine k_D (Garidel et al., 2015; Raut and Kalonia, 2015; Yadav et al., 2011a). However, for peptides, investigations of PPIs are complicated due to their small size, since methods like membrane osmometry, light scattering techniques, and analytical ultracentrifugation are better suited for molecules in the protein size range (Mahler et al., 2009). SIC has been linked to analytical concerns around the preparation of the column and the interaction between the protein immobilized on the column and the protein in solution (Saito et al., 2012). SEC results may be inaccurate due to column adsorption and dissociation of aggregates during analysis (Carpenter et al., 2010). Thus, at present the characterization of peptide-peptide interactions represents a significant analytical challenge.

The interaction parameter, k_D is related to the diffusion coefficient as follows (Harding and Johnson, 1985; Saluja et al., 2007):

$$D_m = D_s(1 + k_D C) \tag{2}$$

where D_m is the mutual diffusion coefficient measured at a given concentration, C, and D_s is the solute diffusion coefficient of the protein or peptide at infinite dilution. Consequently, it is in principle possible to obtain k_D by any method which allows the measurement of D_m. Taylor Dispersion Analysis (TDA) (Aris, 1956; Taylor, 1953) allows measurement of diffusion coefficients and hydrodynamic radii of low-molecular weight compounds (Bello et al., 1994; Cottet et al., 2007a; Sharma et al., 2005; Ye et al., 2012), peptides (Hawe et al., 2011), proteins (Bello et al., 1994; Østergaard and Jensen, 2009), and nanoparticles (Cottet et al., 2007b; d'Orlye et al., 2008) in narrow capillaries using a Poiseuille flow. Also, complexation (Bielejewska et al., 2010; Jensen and Østergaard, 2010), self-association (Jensen et al., 2014), and aggregation (Hawe et al., 2011; Hulse and Forbes, 2011; Hulse et al., 2013) have been detected using TDA. Recently, the use of TDA for investigation of protein-protein interactions was introduced by Latunde-Dada et al. (2015a).

In this work we demonstrate that TDA can be applied for assessment of PPIs by measuring the diffusivity of both peptides and proteins at different concentrations in solution. To verify the suitability of TDA for PPI investigations, DLS was used as a comparator method for the analysis of the protein containing samples. To illustrate potential benefits of using TDA during pharmaceutical development, we investigated the PPIs of three selected peptides and used the same instrumentation for relative viscosity analysis of these peptide solutions. Moreover, we address the effect of dilution and viscosity differences in TDA and the impact on the D_m determination.

1.1. Taylor Dispersion Analysis

Extensive descriptions of TDA can be found elsewhere (Bello et al., 1994; Cottet et al., 2007a, 2007b, 2014; Østergaard and Jensen, 2009; Sharma et al., 2005). In brief, TDA is based on the works of Taylor (Taylor, 1953) and Aris (Aris, 1956) who studied the dispersion of analytes in a tube when subjected to Poiseuille laminar flow conditions. Due to different flow velocities across the tube cross section, the analyte molecule will show a special dispersion, Taylor dispersion, which is related to the diffusivity of the analyte. Assuming that the diffusion along the capillary axis is negligible compared to convection, the relation between the dispersion coefficient, k, and diffusivity, D, is given by the relation (Aris, 1956):

$$k = D + \frac{R_c^2 u^2}{48D} \tag{3}$$

where R_c is the capillary radius and u is the mean fluid velocity. The dispersion coefficient, k can also be related to t_R, the mean residence time required to reach the detector, and σ^2, the temporal variance of the concentration profile, observed by the detector as the peak width:

$$\sigma^2 = \frac{2kt_R}{u^2} \tag{4}$$

If $D \ll R_c^2 u^2/48D$, σ^2 and D are related through:

$$D = \frac{R_c^2}{24\sigma^2} t_R \tag{5}$$

A TDA setup with two detection windows is used in the current study (Østergaard and Jensen, 2009; Paraytec Limited, 2010). Dispersion is measured at two points along the capillary, and due to the diffusivity of the sample, the UV signal will show two peaks with identical areas, but different heights and temporal variances (Fig. 1, see also: Paraytec Limited, 2010).

Using data from the two detection windows, D can then be calculated from (Chapman and Goodall, 2008; Hawe et al., 2011; Hulse and Forbes, 2011; Hulse et al., 2013; Paraytec Limited, 2010):

$$D = \frac{R_c^2(t_2 - t_1)}{24(\sigma_2^2 - \sigma_1^2)} \tag{6}$$

where t_1 and t_2 are the time required for the analyte to reach detection window 1 and 2, respectively, and σ_1^2 and σ_2^2 are the peak variance of the peak at window 1 and 2, respectively.

TDA is an absolute method, and hence calibration is not needed (Cottet et al., 2014). To ensure that Eq. (5) is valid, the dimensionless residence time, τ, and Peclet number, Pe, are calculated. Taylor's conditions are considered satisfied if $\tau > 1.4$ and $Pe > 69$ (d'Orlye et al., 2008).

1.2. Viscosity analysis

The instrumental setup for TDA with two detection windows also allows measurements of the relative viscosity. The transit time between the two detection windows of a flow marker is measured first in a reference solution with a known viscosity, and then in a sample solution with unknown viscosity. The relative viscosity of the sample solution can then be calculated (Ye et al., 2012; Paraytec Limited, 2011):

$$\eta = \frac{\eta_{ref} \times (t_{2,s} - t_{1,s})}{t_{2,ref} - t_{1,ref}} \tag{7}$$

where η_{ref} and η are the viscosity of the reference and sample solution, respectively, $t_{1,s}$ and $t_{2,s}$ are the time required for the analyte in the sample solution to reach detection window one and two, respectively, and $t_{1,ref}$ and $t_{2,ref}$ are the time required for the analyte in reference solution to reach detection window one and two, respectively.

2. Materials and methods

2.1. Materials

Lyophilized type I α-lactalbumin from bovine milk ≥85% (Sigma-Aldrich product number: I5385, M_w 14 kDa) and lyophilized Human Serum Albumin (HSA) ≥97% (agarose gel electrophoresis, Sigma-Aldrich product number: A9511, M_w 66 kDa) were purchased from Sigma-Aldrich (St. Louis, MO). The following peptides were kindly