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Pharmaceutical Nanotechnolgy

A Taylor dispersion analysis method for the sizing of therapeutic proteins and their aggregates using nanolitre sample quantities

Wendy Hulse*, Rob Forbes

Biopharmaceutical Formulation group, School of Pharmacy, University of Bradford, Bradford BD7 1DP, United Kingdom

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ABSTRACT

The growing number of Biosimilars now being approved for development lends the need to develop new analytical techniques for rapid, cost effective analysis of these high value biotherapeutics. The presence of aggregates in biopharmaceutical products is undesirable for many reasons. A major concern is the potential immunogeneic response that aggregates can induce on administration. The detection of low levels of aggregated proteins in solution may only be determined by a limited number of techniques, many of which require in-depth method development, multi-stage sample preparation and lengthy time of analysis. We explore the use of a novel analytical instrument using UV area imaging and Taylor dispersion analysis (TDA) to determine the hydrodynamic radius of BSA in an aggregated state and monitor it with time. Protein aggregation and its reversibility over time has been measured for a number of BSA samples (stressed and unstressed) by TDA with the results obtained being compared to those obtained from dynamic light scattering (DLS) and microcalorimetry. Correlations between the techniques for investigating protein aggregation behaviour were explored. The reproducibility of TDA measurements enabled the stability and reversibility of BSA aggregates to be more readily monitored than by using the other techniques.

1. Introduction

The presence of aggregates in biopharmaceutical products is undesirable for many reasons. A major concern is the potential immunogeneic response that aggregates can induce on administration. The detection of low levels of aggregated proteins in solution may only be determined by a limited number of techniques, many of which require in-depth method development, multi-stage sample preparation and lengthy time of analysis.

Dynamic light scattering (DLS) has been widely utilised in the area of protein characterisation, standard examples include Lysozyme (Poznanski et al., 2005) human immunoglobulin G (Ahrer et al., 2006; Bermudez and Forciniti, 2004) β-lactoglobulin (Elofsson et al., 1996) and bovine serum albumin (Adel et al., 2008). Limited sample preparation is required and it provides a relatively quick analysis time compared to other methods. However, the use of DLS, however, often has to be combined with other analytical techniques as the higher sensitivity of the instrument to larger particles can make analysis of some solutions problematic. This is particularly so if larger particles or dust are present in the solution.

Size exclusion chromatography (SEC) can also be used to determine the hydrodynamic volume of molecules and is widely used in industrial applications. Here, however the position of the eluted peak does not only depend on protein size but also on its shape. A second effect that can change the peak elution position is if the protein interacts with the column matrix. The presence of certain excipients, i.e. carbohydrates in formulations has been reported lead to inaccurate results in SEC (Ye, 2006). A calibration curve is therefore required and a set of relative standards is used resulting in greater analytical time and effort. The coupling of SEC with light scattering techniques is being utilised as a method of overcoming many of the problems associated with SEC analysis (Beaullieu et al., 2005; Li et al., 2009; Wang and Lucey, 2003).

Analytical ultracentrifugation (AUC) represents the gold standard for determining the hydrodynamic properties of proteins (Lebowitz et al., 2008). The utility of the AUC for analysing monoclonal antibodies has been demonstrated by Lu et al. (2007) and Pekar and Sukumar (2007). However, its high cost and large size along with a time consuming procedure make this method of analysis impractical for routine analysis.

Taylor dispersion analysis (TDA) is a fast, absolute method based on the dispersion of a solute plug through a uniform cylindrical tube under laminar Poiseuille flow. The method, sometimes termed Taylor–Aris dispersion, was first described by Taylor (1953) and developed further by Aris (1956). A plug of solute is injected into a moving solvent stream in an open tubular column which disperses by a combination of radial diffusion and cross sectional velocity. Bello et al. (1994) reported the use of small bore glass capillaries,

* Corresponding author. Tel.: +44 0 7854 196842, fax: +44 0 1274 236155.
E-mail address: w.l.hulse1@bradford.ac.uk (W. Hulse).

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radii ≤50 μm, which enabled the reduction of analysis time and sample consumption.

The ActiPix TDA200 combines UV area imaging and Taylor dispersion analysis (TDA) for determining diffusion coefficients and hydrodynamic radii of proteins in solution. The detector monitors broadening of a band of a therapeutic protein or small molecule solution injected into a stream of buffer solution and driven through a fused-silica capillary. The band is imaged at two windows, the first on entry to and the second on exit from a loop in the capillary. The hydrodynamic radius follows from the measured differences between peak times (first moments) and variances (second moments) at the two windows. This technique was used to analyse various native, heat stressed and mixed BSA samples over periods of time. The results were compared to those obtained from DLS and microcalorimetry.

2. Materials and methods

2.1. Materials

Bovine serum albumin (A2153, batch 18K0663) and Phosphate buffered saline tablets were purchased from Sigma–Aldrich, Dorset, UK.

Fused silica capillary was purchased from Composite metal services, Shipley, UK.

2.2. Methods

2.2.1. Taylor dispersion analysis

TDA was performed on a TDA200 HT nano-sizing system (Paraytec Ltd., York, UK). Samples (56 nl) were injected into fused silica capillary under a continuous flow of buffer (2 mm/s) using a CE injection system (PrinCE, Prince Technologies B.V., Netherlands). The detector head is placed inside the CE which allows for temperature control. The total capillary length, with ID:OD dimensions of 75:360 μm, was 143.3 cm with length to first window being 46 cm and length between windows 48.5 cm. The capillary was cleaned between samples using a sodium hydroxide wash (1 M). The UV Detection wavelength used was 214 nm.

2.2.2. Dynamic light scattering

Dynamic light scattering results were obtained using a Malvern zetasizer nano-S system (Malvern, UK). Samples were placed in a semi-micro disposable cuvette and held at 25 °C during analysis. Each Sample was recorded three times with 7 sub-runs of ten seconds using the multimodal mode. The *Z* average diameter and polydispersity index were calculated from the correlation function using the dispersion technology software.

2.2.3. Protein preparation

BSA was dissolved to the required concentration in Phosphate buffered saline (PBS). PBS (10 mM, pH 7.4) was prepared by dissolving the required number of tablets in distilled, de-ionised water.

Aggregation was induced by heating 2 ml of a 10 mg/ml BSA solution to 66 °C in a thermovac (Microcal, UK) for 20 min before being cooled rapidly to 4 °C.

3. Results and discussion

In order to evaluate any new instrument or analytical technique for industrial applications it is of utmost importance to select a model protein and characterise it using relevant processing conditions. Many of the current techniques employed involve large sample volumes, sample dilutions and multiple preparation steps which introduces a sample for analysis that does not reflect the properties of the sample in the process flow. Here we present the results obtained for a series of BSA samples analysed using the TDA200 instrument. Sample dilution is not required, only 60 nl of sample is used for each analysis and the time taken for analysis is as little as 3 min. Samples are analysed without any potentially disruptive preparation and/or dilutions and can be run in industrially relevant media and formulation buffers.

Fig. 1 shows a typical TDA trace from the TDA200 of BSA (10 mg/ml). Peak 1 represents the passing of the protein sample past the sensor which is narrower in width than in peak 2 where the sample has had time to diffuse due to Taylor dispersion.

The hydrodynamic radius of BSA measured using the TDA200 was 4.18 nm with a standard deviation of 0.24% ($n = 5$). The results obtained are within the range of reported the literature values of 3.3–4.3 nm (Jachimska et al., 2008) and were in very good agreement to those obtained using DLS (4.17 nm with a standard deviation of 2.23%).

Protein aggregation has been linked to several diseases including Alzheimers disease, Parkinsons disease, Prion disease, Huntingdons disease, Downs syndrome, Cataracts and Sickle cell disease (Sharma et al., 2010). Aggregation is also a major problem in the bioprocessing industry due to the potential immunogeneic response that aggregates can potentially induce when administered to a patient. With many new biosimilars being developed for market due to expiring patents the need for the development of novel technologies to determine low levels of aggregates is required. The high value of these biotherapeutics lends the need to use techniques that use minimal sample amount. Here we heat stressed BSA to induce aggregates to determine if the TDA200 was able to detect changes in hydrodynamic radius due to aggregation. BSA was chosen as the model for the investigation as its aggregation behaviour has been well characterised (Adel et al., 2008; Brahma and Mandal, 2005; Maruyama et al., 2001; Militello et al., 2004; Shanmugam and Polavarapu, 2004; Squire et al., 1968) and it is a pharmaceutically relevant molecule (Geisow, 1992; He and Carter, 1992).

Two samples of BSA were heated and then cooled rapidly to induce aggregation. The results are shown in Table 1.

The results show that after heat stressing both samples had a degree of aggregation.

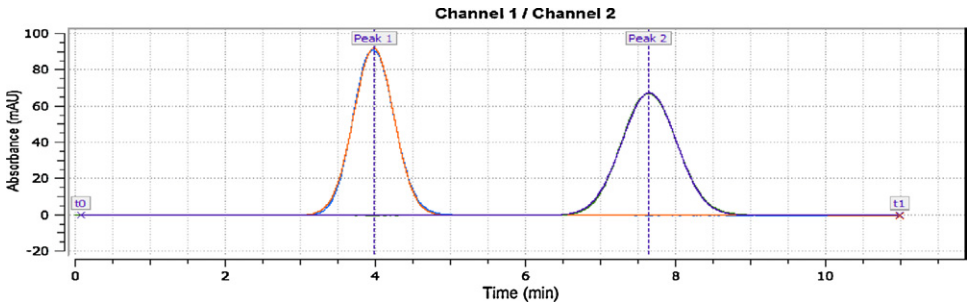

Fig. 1. TDA analysis of BSA (10 mg/ml).