International Journal of Pharmaceutics 453 (2013) 351–357

Contents lists available at SciVerse ScienceDirect

International Journal of Pharmaceutics

journal homepage: www.elsevier.com/locate/ijpharm

# Evaluating the inter and intra batch variability of protein aggregation behaviour using Taylor dispersion analysis and dynamic light scattering

Wendy L. Hulse[a,*], Jason Gray[a], Robert T. Forbes[b]

[a] Allembis Ltd, 300 St Mary's Road, Liverpool L19 0NQ, UK
[b] School of Pharmacy, University of Bradford, Bradford BD7 1DP, UK

ARTICLE INFO

*Article history:*
Received 2 April 2013
Received in revised form 27 May 2013
Accepted 28 May 2013
Available online 7 June 2013

*Keywords:*
Taylor dispersion analysis
Hydrodynamic radius
Dynamic light scattering
Aggregation
Biosimilar

ABSTRACT

Biosimilar pharmaceuticals are complex biological molecules that have similar physicochemical properties to the originator therapeutic protein. They are produced by complex multi-stage processes and are not truly equivalent. Therefore, for a biosimilar to be approved for market it is important to demonstrate that the biological product is highly similar to a reference product. This includes its primary and higher order structures and its aggregation behaviour. Representative lots of both the proposed biosimilar and the reference product are analysed to understand the lot-to-lot variability of both drug substances in the manufacturing processes. Whilst it is not easy to characterise every variation of a protein structure at present additional analytical technologies need to be utilised to ensure the safety and efficacy of any potential biosimilar product. We have explored the use of Taylor dispersion analysis (TDA) to analyse such batch to batch variations in the model protein, bovine serum albumin (BSA) and compared the results to that obtained by conventional dynamic light scattering analysis (DLS). Inter and intra batch differences were evident in all grades of BSA analysed. However, the reproducibility of the TDA measurements, enabled the stability and reversibility of BSA aggregates to be more readily monitored. This demonstrates that Taylor dispersion analysis is a very sensitive technique to study higher order protein states and aggregation. The results, here, also indicate a correlation between protein purity and the physical behaviour of the samples after heat shocking. Here, the protein with the highest quoted purity resulted in a reduced increase in the measured hydrodynamic radius after heat stressing, indicating that less unfolding/aggregation had occurred. Whilst DLS was also able to observe the presence of aggregates, its bias towards larger aggregates indicated a much larger increase in hydrodynamic radii and is less sensitive to small changes in hydrodynamic radii. TDA was also able to identify low levels of larger aggregates that were not observed by DLS. Therefore, given the potential for immunogenicity effects that may result from such aggregates it is suggested that TDA may be suitable in the evaluating detailed batch to batch variability and process induced physical changes of biopharmaceuticals and biosimilars.



## 1. Introduction

Within the pharmaceutical industry the generic variants of small molecule drugs have long been approved and commercially available. For a generic drug product to be approved an abbreviated new drug application (ANDA) or similar has to be submitted to the relevant regulatory authorities. Each application has to contain data showing that the generic drug product is pharmaceutically equivalent, bioequivalent and hence therapeutically equivalent to the brand leader product. In the case off small molecules bioequivalence has to be established in a cross over volunteer study (Schellekens et al., 2011) and is defined by the Food and Drug Administration (FDA) as the absence of a statistically significant difference in the rate extent to which the active pharmaceutical ingredient in pharmaceutically equivalent products becomes available at the site of action when administered to subjects at the same molar dose under similar conditions (21CFR320 – 2004). Therefore, the major difference between a new drug application (NDA) submitted by the innovator and the abbreviated form (ANDA) required for generic products is that pre-clinical and clinical data does not need to be repeated as the safety and efficacy of the drug product has already been established in the NDA.

In the case of small molecules an ANDA can be accepted for a generic product consisting of an active that is a different solid state form to the innovator as long as bioequivalence can be

* Corresponding author. Tel.: +44 07854 196842.
*E-mail addresses:* wendy.hulse@allembis.com, wendy-louise@hotmail.co.uk (W.L. Hulse).

0378-5173/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.ijpharm.2013.05.062

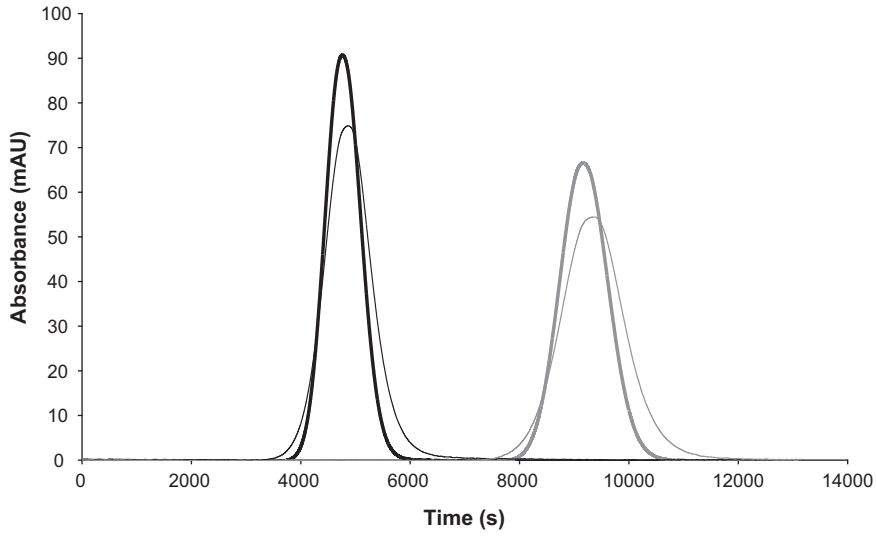


**Fig. 1.** Taylor dispersion analysis profile for BSA (A2153, 10 mg/ml) in PBS showing first (black) and second (grey) passes through the Actipix detector. Initial analysis (bold line) and heat shocked sample (fine line) ($n = 10$).

demonstrated, the product is adequately stable, suitably labelled and is manufactured according to current good manufacturing practice (cGMP) guidelines (Raw et al., 2004). However, given that different solid state properties of polymorphs can affect bioavailability, bioequivalence, stability and control of the manufacturing process, careful consideration is therefore given to these solid state differences. Guidelines have now been adopted (Raw et al., 2004; Yu et al., 2003) that map out the steps that should be taken to assess these issues with regards to product efficacy, bioequivalence, stability and the instigation of appropriate analytical testing and monitoring of the drug products during manufacture.

Similar to that of small molecules many biological molecules are now coming off patent and a new opportunity for the generic (biosimilars) market has opened. However, given the structural complexity coupled with the inherent variability of manufacturing methods and lengthy purification processes of biological therapeutics means that the general rules applied to the classification of generic small molecules cannot be applied to biological drugs. Therefore, biosimilars may not be truly equivalent and cannot obtain market approval through the procedures that are applied to small molecule generic products (Combe et al., 2005). Within the European union a generic biological drug that has been granted a marketing authorisation is defined as a 'similar biological medicinal product' or 'biosimilar' (Minghetti et al., 2012). In 2012 the FDA issued its Guidance for Industry-Scientific Considerations in Demonstrating Biosimilarity to a Reference Product (FDA, 2012). Here a *Biosimilar* or *biosimilarity* is defined as "the biological product is highly similar to the reference product notwithstanding minor differences in clinically inactive components," and that "there are no clinically meaningful differences between the biological product and the reference product in terms of the safety, purity, and potency of the product."

Therefore, any application must include information demonstrating biosimilarity based on, state of the art analytical studies that demonstrate that the biological product is highly similar to the reference product. Any applicant is also expected to use an appropriate analytical methodology with adequate sensitivity and specificity for structural characterisation and comparison to the reference product. This includes comparison of primary structures, such as amino acid sequence and higher order structures, including secondary, tertiary, and quaternary structure (including aggregation). It is also expected that any such study will be performed on multiple representative lots of the proposed product and the reference product to understand the lot-to-lot variability of both drug substances in the manufacturing processes.

**Table 1**
Summary of the SDS PAGE starting materials.

| Sample | Monomer | | Dimer | |
|---|---|---|---|---|
| | MW (kDa) | Area (%) | MW (kDa) | Area (%) |
| BSA (A8806) | 58.948 | 83.20 | 113.387 | 16.80 |
| BSA (A2058) | 53.580 | 67.58 | 112.479 | 32.42 |
| BSA (A1253) | 57.718 | 85.19 | 113.387 | 14.81 |

Comparability studies between biosimilars are complex and difficult due to the further issue of immunogenicity. The immunogenicity of a protein drug can cause hypersensitivity, anaphylaxis, infusion reactions, anaphylactoid reactions, cross-reactivity with and neutralisation of natural endogenous counterparts and a decreased efficacy of the drug (Barbosa, 2011; Buttel et al., 2011). The challenges of assessing and comparing biosimilars has been reported by Schellekens (2009) who showed through a case study of epoetins manufactured by different companies that bioequivalence cannot be applied to protein drugs. Jeske et al. (2012) also report on the lack of bioequivalence of low molecular weight heparins Longstaff et al. (2009) highlight the analytical problems associated with the classification of the biotherapeutics streptokinase, heparin and TGN1412 as biosimilars and it is therefore recommended that clinical trials are required rather than just the bioequivalence studies required to support the registration of a generic small molecule drug product (Zuniga and Calvo, 2010).

It is not possible to detect and/or quantify all variations of a protein, even with a combination of highly sensitive techniques, therefore some differences in protein product characteristics may not be detectable by analytical characterisation (Kresse, 2009). The development of new analytical technologies aims to bring developments into protein characterisation and aid in the full physical determination both inline and offline of these highly valuable biopharmaceutical products.

We have previously reported the development of Taylor dispersion analysis as a highly sensitive technique requiring only limited sample preparation for determining the hydrodynamic radius of proteins and monitoring aggregate behaviour both in solutions and unadulterated formulated drug products (Hulse and Forbes, 2011a,b). Therefore, here we evaluate the use of TDA and its use in determining the batch to batch variability and process induced