International Journal of Pharmaceutics 552 (2018) 1–6

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

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

Mini review

Commentary: New perspectives on protein aggregation during Biopharmaceutical development

Maryam Shah

Division of Pharmacy and Optometry, School of Health Sciences, Faculty of Biology, Medicine and Health, University of Manchester, Manchester, UK

ARTICLE INFO

Keywords:
Protein aggregation
Monoclonal antibody
Particle
Technologies
Interface
Excipients
Characterisation

ABSTRACT

The occurrence of protein aggregation during bioprocessing steps such as purification, formulation and fill-finish, impacts yield and production costs, and must be controlled throughout the manufacturing process. Understanding aggregation mechanisms and developing mitigating strategies are imperative to ensure the clinical efficacy of the protein drug product and to reduce costs. This commentary reflects on recent progress made in the field of monoclonal antibody (mAb) aggregation with considerations on current and emerging measurement techniques, the use of novel excipients for preventing aggregation, interfacial phenomena and prediction of aggregation rates. The future direction of research is discussed based on academic and industrial perspectives.

1. Introduction to protein biopharmaceutical development

Biopharmaceutical proteins constitute a growing family of medicines for many therapeutic areas including oncology, inflammation and autoimmunity, infectious disease, and cardiovascular and metabolic disease. In the last decade more than 170 biologics have been approved for clinical use and around a third of these are monoclonal antibodies (mAbs) (Biopharma, 2017) – which are the focus of this commentary. There are, however, a number of associated challenges in their manufacturing and formulation including controlling and predicting the reversible and irreversible formation of protein aggregates. Aggregation can lead to loss of product recovery following production and purification, and places constraints on how the mAb is formulated for storage. mAb stability depends sensitively on the formulation components (i.e. salts, excipients (Kamerzell et al., 2011; Ugwu and Apte, 2004)) and storage conditions (i.e. temperature (Grant et al., 2012; Lazar et al., 2010)). There are strict regulations on the level of aggregation in protein biopharmaceuticals due to safety concerns (FDA, 1999; Pharmacopeia, 2012a,b) as aggregates have been linked to immunogenicity when injected in patients (Carpenter et al., 2009; Rosenberg, 2006; Wang et al., 2008). However, this is a highly debated area of science with some studies implying there is no direct link between aggregation development and adverse immune responses (Singh et al., 2010). Nevertheless, unwelcomed particles (aggregates, foreign particles or mixed complexes) need to be monitored. A position paper (Mathonet et al., 2016) reviewing visual inspection and quality control to meet the pharmacopeial description “practically free of particles” (Ph. Eur. monograph on ‘Monoclonal antibodies for human use’ (2031)) considers that the probability of a visible particle being present [in the parenteral product] cannot be completely eliminated.

Biopharmaceutical companies have in-house (proprietary) approaches to minimise aggregation, based on experience. For companies with both Research and Development arms, a wealth of bioprocess expertise has been accumulated, including the relationship between mAb stability and domain architecture to the amino acid sequence (Dobson et al., 2016). However, ushering in a non-empirical, rational approach *a priori* would be of great benefit when translating results for the aggregation behaviour of one protein family (e.g. mAbs) to another (e.g. bispecific mAbs or peptide fusions). Such an approach would require commensurate improvement of measuring and detecting aggregates, their kinetics, and accompanying predictive models. The ability to predict aggregation requires an improved understanding of the underlying inter- and intra-molecular mechanisms. To date, the strategy has been to bring together robust *in silico* models with quantitative analytical measurement to provide insight to the aggregation process; from nucleation to visible particulate, particularly across the so-called ‘gap region’ around 1 μm (Agrawal et al., 2016; Carpenter et al., 2009; Gross et al., 2016; Lauer et al., 2012). This strategy has been particularly productive under collaborative ventures between academic and industrial partners.

This commentary reflects on recent progress made in the field of (non-covalent) protein aggregation (i.e. physical, non-covalent changes), paying particular attention to mAbs. Additionally, from an academic perspective with industrial focus, speculations on the future

E-mail address: maryam.shah2102@gmail.com.

https://doi.org/10.1016/j.ijpharm.2018.09.049
Received 20 June 2018; Received in revised form 18 September 2018; Accepted 19 September 2018
Available online 22 September 2018

direction of research are reported, asking: what are scientists aiming to achieve when using a specific measurement technique or predictive model; and how important is our knowledge of the knowns and unknowns as related to protein aggregation?

2. Measuring protein aggregation: scope, limitations and future technologies

Much effort has been put into developing technologies able to detect and characterise protein aggregates. With the increased interest in the characterisation of smaller sub-micron sized aggregates (0.1–1 μm) (Pharmacopeia, 2011; Singh, 2013; Weinbuch et al., 2013), the need for technologies able to detect small aggregates in highly concentrated mAb formulations is a future requirement that will be paramount to predicting shelf life of liquid drug products.

A major advance was in the quantitative characterisation of aggregate size distributions. In particular, aggregates generated by the dynamic process of 'reversible self-assembly' (RSA); which, for mAbs, would typically be in the order of tens of nanometres in diameter. Analytical ultracentrifugation (AUC) is a useful technique for characterising such aggregates (e.g. monomer, dimer, trimer) and in quantifying protein association as a function of protein concentration; and thus calculating the respective equilibria that may exist (Sarangapani et al., 2016). Current AUC technology using optical detection systems (UV/vis absorption and Rayleigh interference) is limited to protein concentrations less than 50 g/L. Above this limit samples require dilution prior to measurement. One possible solution arises from protein detection technologies based on Schlieren optics, which do not require dilution and are compatible with AUC instruments. Ironically, such optical systems were formally available in earlier AUC instrumentation and therefore could be reintroduced in a relatively simple manner.

The requirement for sample dilution is not restricted to AUC: other technologies including conventional methods such as dynamic light scattering (DLS) and emerging techniques covering the sub-visible size-range such as resonant mass measurement (RMM) (Archimedes®, Malvern Ltd., UK) and nanoparticle tracking analysis (NTA), are also poorly suited to cope with characterising aggregates in highly concentrated mAb solutions (above 100 g/L) due to inherent ambiguity in the interpretation of data acquired (Amin et al., 2014; Funke et al., 2016; Panchal et al., 2014; Patel et al., 2012; Zolls et al., 2012). As a consequence, most analytical measurements require sample dilution, which can cause a change in the aggregate distribution depending on whether or not aggregates are reversibly or irreversibly formed. Reversible self-association (RSA) in concentrated protein solutions leads to formation of oligomers, which, will dissociate upon dilution (Kanai et al., 2008). The behaviour of RSA at low concentrations is clearly understood, however, at high concentrations it remains difficult to assess and predict (Weiss et al., 2007). Due to the limited material available during early formulation studies (and academia) and analytical limitations, models are used to extrapolate low concentration measurements to high protein concentrations, where aggregation is more likely to occur. With the complexity of aggregation mechanisms, which vary with protein concentration and solution properties, reliable methods/relationships have not yet been established (Philo and Arakawa, 2009; Saluja et al., 2010; Zangi, 2009). Dilution is a standard step in sample preparation for many analytics and there are studies where dilution would not be an issue. For example, in accelerated stability studies (Ammann, 2011) measurements as a function of stress conditions (including transport stress, formulation parameters i.e. not concentration as a parameter), dilution would not be an issue.

Measurement of aggregates in highly concentrated mAb solutions is relevant because high doses may be needed to meet a therapeutic response, and in the case of chronic administration via subcutaneous injection, a volume limit of around 1–2 ml is common (Shire et al., 2004). There are technologies which can be utilised at high protein concentrations to study aggregation mechanisms (Scherer et al., 2010; Tomar et al., 2016). Recent reports demonstrate the utility of 1H NMR spectroscopy in determining mAb behaviour in high concentration mAb solutions. For example, solution viscosity and mAb aggregation data acquired by NMR provide complementary information to accelerated stability studies (Kheddo et al., 2016a), and NMR has been used to assess protein self-association in both phases of a liquid/liquid phase-separated mAb formulation (Kheddo et al., 2017).

The majority of technologies focus on determining particle size or characteristics such as changes in secondary or tertiary structure giving a comprehensive picture of a product's aggregate profile (den Engelsman et al., 2011). However, more important than exact sizing of particulates, is determining protein aggregate counts. The United States Pharmacopeia (USP) chapter 'Particle Matter in Injections' ⟨7 8 8⟩ defines particle concentration limits in parental solutions that are larger than 10 and 25 μm (Pharmacopeia, 2012b). There is a recommendation to monitor particles smaller than 10 μm in USP chapter 'Subvisible Particulate Matter in Therapeutic Protein Injections' ⟨7 8 8⟩, and a supporting chapter with guidance on the expanded techniques (Pharmacopeia, 2012a,b). Particles larger than 100 μm can be assessed fairly accurately with visual inspection, with a move towards automated inspection instruments (Borchert et al., 1986; Knapp, 2003). For subvisible particles (1–100 μm), light obscuration is the most widely used method and known to produce accurate, reliable data. Microscopic particulate count tests are utilised when light obscuration has issues with particles which appear semi-transparent and/or highly viscous solutions (Das, 2012; Pharmacopeia, 2012b). The third commonly used (and emerging) method is coulter-counter. All three methods produced similar results when assessing the effect of particle concentration, with the exception of light obscuration at the higher concentration of 150 mg/ml mAb. Underestimation of particle counts is correlated with lower count limit of the techniques (Das, 2012; Demeule et al., 2010). Coulter-counter covers a wide size range (~0.4–1600 μm) and is unaffected by colour, shape or refractive index. However, one noted limitation is the formulation needs to be suspended in an electrolyte that may result in artefacts related to changes in composition and particle counts (Barnard et al., 2012; Kolewe et al., 2010).

A number of techniques have been developed to cover the subvisible size range, and although they are being used in the industry, their performance is still inadequate to be used in pharmaceutical development. The drive for smaller sample volumes is limited by less particle accuracy when using smaller sample sizes (Ríos Quiroz et al., 2015; Shah et al., 2017). Due to the difficulty in accurate particle counting for particles smaller than 10 μm, comparison between different techniques needs to be carried with caution, even more so with (diluted) high concentration samples and in the presence of contaminants (e.g. silicone-oil) (Demeule et al., 2010; Shah et al., 2017). Discrepancies between different particle counting methods have been reported multiple times. Nevertheless, good reproducibility has been illustrated for controlled instrument parameters. Thus, another aspect which requires consideration is developing agreement between different methods or between instrument settings; collaboration between manufacturers, industry and regulations would be paramount to achieve this (Ríos Quiroz et al., 2015; Ripple et al., 2015).

Another requirement in the advancement of technologies is the ability to differentiate between protein and foreign matter. For example, mAb solutions in pre-filled syringes often contain sloughed silicone-oil particles following agitation/transport and commercially available techniques detecting small aggregates do not have the ability to differentiate. The recently developed (RMM) Archimedes® system has the ability to differentiate between protein and foreign matter (based on particle buoyancy) although the approach has concentration limits. Archimedes has been used alongside micro-flow imaging (MFI), to cover a broad size range (Shah et al., 2017; Weinbuch et al., 2013). MFI has received much attention due to the volume and size-range matching regulations and providing useful information on the