Journal of Pharmaceutical Sciences xxx (2018) 1-8

Contents lists available at ScienceDirect

Journal of Pharmaceutical Sciences

journal homepage: www.jpharmsci.org

Pharmaceutical Biotechnology

High-Throughput In-Use and Stress Size Stability Screening of Protein Therapeutics Using Algorithm-Driven Dynamic Light Scattering

Ashwinkumar A. Bhirde*, Meng-Jung Chiang, Ramesh Venna, Serge Beaucage, Kurt Brorson

Office of Biotechnology Products, Office of Pharmaceutical Quality, Center for Drug Evaluation and Research, U.S. Food and Drug Administration, 10903 New Hampshire Avenue, Silver Spring, Maryland 20993

ARTICLE INFO

Article history:
Received 10 January 2018
Revised 12 April 2018
Accepted 17 April 2018

Keywords:
high-throughput dynamic light scattering
therapeutic proteins
in-use stability
stress stability
algorithm
quality control
aggregates
particulates
particles

ABSTRACT

Stability of therapeutic proteins (TPs) is a critical quality attribute that impacts both safety and efficacy of the drug. Size stability is routinely performed during and after biomanufacturing. Dynamic light scattering (DLS) is a commonly used technique to characterize hydrodynamic size of the TPs. Herein, we have developed a novel method to evaluate in-use and thermal stress stability of TPs using algorithm-driven high-throughput DLS. Five marketed TPs were tested under the guidance of customized algorithms. The TPs were evaluated at relevant temperature conditions as well as under dilution and thermal stress for size stability. We found that the TPs were stable under the in-use conditions tested; however, sample loss due to evaporation can lead to large protein aggregates. A combined assessment of autocorrelation function and photos of sample well could be useful in formulation screening. Dilution of TPs also has an impact on the hydrodynamic size. Thermal stress experiments showed the importance of using different data processing methods to access size distribution. Polydispersity index was useful in evaluating sample heterogeneity. Herein, we show that algorithm-driven high-throughput DLS can provide additional supportive information during and after biomanufacturing and the potential to be used in a quality control environment.

Introduction

Biopharmaceuticals, especially monoclonal antibody (mAb) drugs, have shown tremendous use in the treatment of a variety of indications (e.g., cancer, inflammatory diseases).[1,2] However, one of the major challenges with biopharmaceuticals is immunogenicity.[3] In the context of biopharmaceuticals, immunogenicity is rarely desirable and can lead to loss of efficacy or adverse reactions. Unwanted immunogenicity can arise because of both proteinaceous and nonproteinaceous aggregates that might have formed during biomanufacturing, shipping, and handling. Unlike small-molecule drugs that are usually manufactured through the synthetic route, therapeutic proteins (TPs) manufacturing requires secretion by live cells in a bioreactor and subsequent large-scale protein purification.[4] Hence, bioprocessing is complex and has the potential to introduce particulates and/or aggregates during manufacturing.[5] Protein stability is a very important parameter that affects quality and physiochemical properties of biopharmaceuticals.[6,7] Hence, size and quantity of particulates has become a critical quality attribute for biopharmaceutical drugs.[8] Published research has associated TP aggregates with immunogenicity.[9] In current commercial practice, particles of size 10 μm and above are evaluated on a lot-by-lot basis for drug product (DP) and are controlled with clear upper limits following United States Pharmacopeia standards.[10,11]

Aggregates or particles/particulates found in protein therapeutic drugs have been categorized into 4 distinct categories namely nanometer (<0.1 μm), submicron (0.1-1 μm), subvisible (1-100 μm), and visible (>100 μm) aggregates.[12,13] A number of techniques are available to measure particulate or aggregates that cover parts of these size ranges; however, there is no single technique that can

Conflicts of interest: The authors declare no competing financial interest.
This article reflects the views of the authors and should not be construed to represent FDA's views or policies.
This article contains supplementary material available from the authors by request or via the Internet at https://doi.org/10.1016/j.xphs.2018.04.017.

* *Correspondence to:* Ashwinkumar A. Bhirde (Telephone: 240-4026234).
E-mail address: ashwinkumar.bhirde@fda.hhs.gov (A.A. Bhirde).

https://doi.org/10.1016/j.xphs.2018.04.017
0022-3549/

measure all particles in all size ranges.[14] Evaluating particles/particulates in the submicron to nanometer (0.01-1 μm) size range is particularly difficult as robust methods are few.[15,16] Size exclusion chromatography (SEC) is the industry standard for measuring protein therapeutic monomers;[17] however, it can only detect aggregates below 0.1 μm as larger particles get caught in the column frit.[18] More recently, analytical ultracentrifugation (AUC)[19] is also been used in the industry to measure size and aggregates of protein therapeutics. However, AUC can measure aggregates of size below 0.1 μm only; it is rather challenging and requires highly specialized expertise.[20] Measuring protein therapeutics in their native state (solution form) without dilution or reformulation is important to understand their true stability in the actual dosage form. Rigorous sample preparation methods and interaction with columns can alter the original protein conformations and result in misleading conclusions about actual DP stability. Fortunately, nanoparticle-tracking analysis and field-flow fractionation[21] can measure protein therapeutics in their native state.[21,22] However, they are not high throughput and have robustness issues. Dynamic light scattering (DLS) has emerged as a popularly adapted technique for size measurements, and more than 50% of today's biopharmaceutical industry uses DLS in some way or another.[23] DLS can be non-perturbing and can be run column-free. In this way, it can measure the protein therapeutic in its native solution state, without diluting the sample.[24] This mode prevents potential dissociation of reversible aggregates, is sensitive to small amounts of aggregates, and does not require prior knowledge of concentration and molecular composition.[25] Finally, it is a rapid measurement compared with other techniques, such as AUC, field-flow fractionation, nanoparticle tracking analysis, and SEC, and the instrumentation is more user-friendly, not requiring intense training and expertise. Thus, DLS is generally regarded as high throughput.

DLS is generally used as a characterization technique (vs. lot release), and size is reported in terms of Z-average. With newer operating capabilities and programmatic graphical user interface, DLS has potential in the field of protein therapeutics to expand beyond limited characterization. Recently, we have shown that DLS can be used to evaluate colloidal stability of nanoparticles under physiologically relevant preclinical conditions if time, temperature, repetitions, and overall experimental duration are optimized.[26,27] Another potential application is forced-degradation studies of biopharma DPs, including thermal stress stability of the DPs.[28,29] High-throughput DLS (HTS-DLS) methods using plate readers can address important issues concerning efficient DLS measurements of TPs: (1) Industry is developing protein therapeutic (e.g., mAb DPs) formulations at high active pharmaceutical ingredient (API) concentrations and can have high viscosity which may impact hydrodynamic (HD) size measurements obtained using DLS.[30] Comprehensive study can bolster confidence in the wide-spread use of DLS for protein therapeutics with high concentrations. Also, (2) often DLS data reported publicly are from Z-average known to skew (project smaller size) the HD size. Data obtained thus can instead be analyzed using different data-processing methods (e.g., regularization) to achieve reliable conclusions.

Here, we present a novel algorithm-driven approach to screen 5 typical biopharmaceuticals for in-use and thermal stress stability conditions using a HTS-DLS plate reader method. This study shows the possibility of using DLS in a quality control (QC) environment beyond simple and straightforward size measurements.

Materials and Methods

Materials

TP DPs (cetuximab, golimumab, panitumumab, bevacizumab, and etanercept) were obtained from commercial pharmacies. Formulation buffers, as specified by labeling, were made in-house using dry chemicals supplied by Sigma and Fischer. Ultrapure water (prepared in-house), infrared spectroscopy–grade paraffin oil (Sigma-Aldrich), 384-well plates with lids (Corning; shipped and sold by Thermo Fisher Scientific, Pittsburgh, PA), 16- to 25-mL troughs, multichannel pipettors, and 50-μL pipette tips were all supplied by Finnpipette Systems (Thermo Fisher Scientific). Concentrations of the 5 TPs were measured using NanoDrop ONE from Thermo Scientific.

HD Size Measurements

A DynaPro II Plate Reader DLS instrument from Wyatt Technologies (Santa Barbara, CA) was used to measure the HD size of the TPs under simulated in-use and thermal stress conditions. Dynamics software from the same vendor was used to record and analyze the light scattering data for HD size and polydispersity index (PDI) of the TPs in solution.

Size-Exclusion Chromatography

SEC experiments were performed on Agilent 1290 UPLC system that was equipped with a quaternary solvent delivery pump, an auto sampler, and a UV detector (excitation at 280 nm and emission at 360 nm, 20 Hz) with an injection volume of 1 μL. Data acquisition and instrument control were performed by LC OpenLAB software (Agilent).

Algorithm-Driven HTS-DLS Method

Task-specific algorithms were developed in our laboratory using the Dynamics graphical user interface to evaluate the size stability of the TPs. The 4 different algorithms to evaluate the HD size of TPs are under in-use condition at (1) 23°C and (2) 40°C, (3) under thermal stress condition, and (4) dilutions (3 different concentrations of the stock sample) conditions.

Change in HD size with time under controlled temperature is a marker for protein aggregation in solution. A diluted and reconstituted TP in transit or in custody at an infusion center could be exposed to conditions from 23°C to 40°C; this reflects different conceptions and realities of ambient temperature that can depend on location and patients' living conditions (e.g., an air-conditioned room at a western hospital vs. the clinics in tropics in developing countries). Algorithms for the high-throughput plate reader–based DLS developed were implemented to screen several TPs simultaneously under same conditions.

The TPs (30 μL/well) were added to a black clear-bottom 384-well plate (3540; Corning) followed by paraffin oil (15 μL/well). Checking vendor literature ensured that the plates were compatible with the paraffin oil. This step was performed before performing any longer duration and high-temperature experiments to rule out the risk of loss of sample. The use of oil is an optional measure to prevent sample evaporation. We did perform control experiments with and without oil. Most studies with test samples overlayed with the paraffin oil did not trend toward changes in the HD size of mAbs, arguing that the 2 components are not interactive. For high elevated temperature and longer duration studies, the vendors recommend the use of an oil overlay. Also, according to the instrument vendor, the laser reads from the bottom of the plate and should not reach the oil layer which is on top of the sample. Hence, we do not expect to see the interference from oil in our experimental readout.

The plate was centrifuged at 3000 rpm for 2 min at 23°C. The centrifugation step can be performed after adding the protein DPs and again after adding the oil or as a single step after adding both