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Richard E. Cavicchi, Jason King, Dean C. Ripple

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Measurement of Average Aggregate Density by Sedimentation and Brownian Motion Analysis

Richard E. Cavicchi[1], Jason King[1,2], and Dean C. Ripple[1]
[1]Bioprocess Measurements Group
National Institute of Standards and Technology, Gaithersburg, MD 20899
[2]now with XSOLIS, 301 Plus Park Blvd. Nashville, TN 37217 Suite 411

ABSTRACT
The spatially averaged density of protein aggregates is an important parameter that can be used to relate size distributions measured by orthogonal methods, to characterize protein particles, and perhaps to estimate the amount of protein in aggregate form in a sample. We obtained a series of images of protein aggregates exhibiting Brownian diffusion while settling under the influence of gravity in a sealed capillary. The aggregates were formed by stir-stressing a monoclonal antibody (NISTmAb). Image processing yielded particle tracks, which were then examined to determine settling velocity and hydrodynamic diameter down to 1 μm based on mean square displacement (MSD) analysis. Measurements on polystyrene calibration microspheres ranging in size from 1 μm to 5 μm showed that the MSD diameter had improved accuracy over the diameter derived from imaged particle area, suggesting a future method for correcting size distributions based on imaging. Stokes' law was used to estimate the density of each particle. It was found that the aggregates were highly porous with density decreasing from 1.080 g/cm^3 to 1.028 g/cm^3 as the size increased from 1.37 μm to 4.9 μm.

Keywords: Flow imaging, Image analysis, Microscopy, Particle Sizing, Physical Characterization, Protein aggregates,

INTRODUCTION

The use of orthogonal methods to characterize the size distribution of protein aggregates in biopharmaceuticals is useful to cover broader size ranges with increased confidence. However, it can be difficult to reconcile results obtained through different measurement methods, which—based on their respective calibration and operational protocols—may provide seemingly conflicting results. The spatially averaged density is a key parameter for comparing size distributions obtained by the resonance mass method, particle tracking, flow image analysis, or light obscuration. Extracting particle diameter from a resonance mass measurement requires knowledge of the particle density. Optical methods also depend on particle density in an indirect manner: a higher particle density corresponds to a higher optical scattering or image contrast, which in turn can affect the particle diameter measurement. The density of protein aggregates is also important for estimating the amount of aggregated protein in a sample.

The density of a protein (as a molecule or pure substance) has long been considered a parameter that is—to a good approximation—independent of the protein type, and is commonly used in the analysis of X-ray structure data from protein crystals. A protein density of 1.35 g/cm^3 is a commonly accepted value based on early sedimentation[1] and compressiblity[2] measurements over a variety of different proteins)[3]. Quillin and Matthews[3]