Protein Aggregation

AccuSizer® FX Nano

OVERVIEW

Aggregation in protein solution based therapeutics may yield harmful immunogenicity. Measurement of these aggregates has been possible for the larger size aggregates, but smaller aggregates, in the range of 0.15 to 2 microns, have been difficult to quantify. Dynamic light scattering (DLS) techniques can demonstrate the existence of aggregation, but do not provide any information on the absolute concentration of the aggregates. Single particle optical sizing (SPOS) can now measure both the size and concentration of aggregated proteins, and is becoming a preferred technique for this application.

INTRODUCTION

Biotherapeutics can be susceptible to inducing the elicitation of anti-drug antibodies (ADA). Evidence suggests that protein aggregates and particles have the ability to enhance immunogenicity, and therefore trigger immune responses, to the monomeric form of the protein.

Manufacturers of the biotherapeutic proteins often prepare the drug for injection though a series of steps:

1. Protein synthesis and purification
2. Lyophylization for stabilization during shipment
3. Reconstitution prior to injection

While lyophilization has the benefit that it stabilizes the protein for transportation, it is not clear that the lyophilized protein will return to its monomeric state after reconstitution. If a small amount of the protein aggregates as a result of this process, it is possible to induce an immune response in the patient during course of treatment.

Developing a simple method to measure the degree of aggregation, enabling display of data in histogram of size versus concentration following reconstitution, would result in the ability to screen these drugs during the formulation process. This analysis could ensure that the reconstitution process results in the release of the monomer without a significant amount of aggregation.

PARTICLE COUNTING TECHNIQUES

There are few techniques capable of measuring both particle size and concentration in the size range of interest to quantify protein aggregation. The light obscuration method is familiar to many pharmaceutical scientists since it is used for the USP <788> particulate matter in injections and the USP <729> test to measure the tail of lipid emulsions. But classic light obscuration sensors typically have a lower particle size limit, near 1 µm, and have concentration limits too low for many protein aggregation samples.

The new PSS AccuSizer® FX Nano system (shown above) is designed to work at smaller particle sizes, and at higher concentration. This is accomplished by using two sensors; the FX Nano sensor to measure from 0.15 – 0.6 µm and the LE400 sensor to measure from 0.5 – 40 µm. The FX Nano sensor uses a focused beam to reduce the total volume inspected, thus increasing the concentration limit of the sensor. This sensor is coupled with the SIS sampler, modified to allow for sample volumes as low as 250 µL. This configuration passes all requirements in USP <787> subvisible particulate matter in therapeutic protein injections.

Aggregated proteins are sized and counted one at a time using the two sensors to cover a dynamic range of 0.15 – 40 µm. The LE sensor when used alone can operate from 0.5 – 400 µm, making this a very flexible system.

EXAMPLE DATA 1: IMMUNOGLOBULIN G BEFORE AND AFTER FILTRATION

Immunoglobulin G (IgG), ~150 kDa, 1% was prepared in PBS. The sample was measured undiluted using the AccuSizer FX Nano with both the FX and LE sensors and the SIS sampler. The result is shown in Figure 1. Total # particles counted = 109,343, concentration = 9.7x10^8 particles/mL.

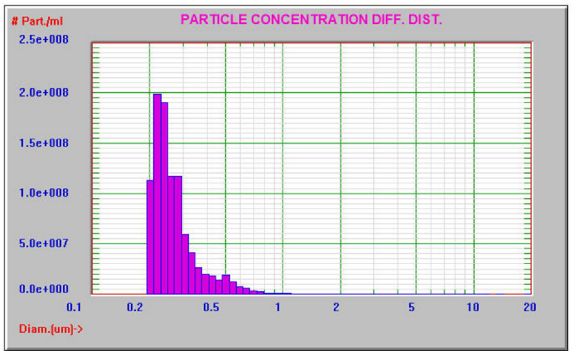

Figure 1. Unfiltered IgG

The sample was also analyzed on the Nicomp® 380 dynamic light scattering (DLS) system (see Figure 2), in order to asses the size of the monomer and to get a rough feel for the size of the aggregated protein. Peak #1 (native protein): 14.6 nm, 52% intensity (99.6% total mass). Peak #2 (aggregated tail): 395 nm, 48% intensity (0.4% total mass). It is the tail of aggregates at 200-500 nm and greater that the AccuSizer FX Nano is being used to study in detail.

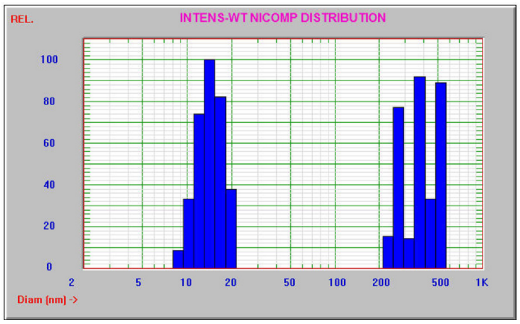

Figure 2. DLS Nicomp 380 data for unfiltered IgG

The protein sample was then passed through a 0.2 µm filter and analyzed again on the AccuSizer FX Nano system. The results comparing before and after filtration are shown in Figure 3. The results for the PBS is also shown in black. The concentration reduced from 9.7 to 3.1x10^8 particles/mL after the filtration step. The decrease in the tail of aggregated particles is clearly visible and easily identified by the AccuSizer FX Nano system.

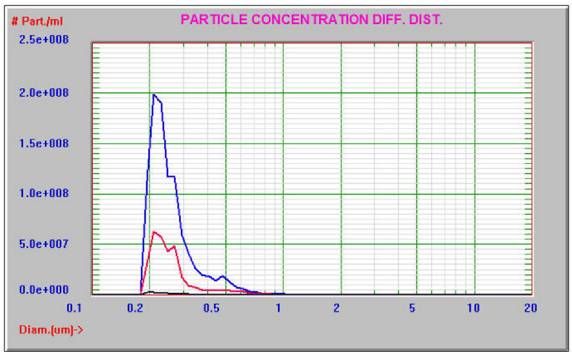

Figure 3. Before (blue) and after (red) filtration

EXAMPLE DATA 2: IGG BEFORE AND AFTER INCUBATION

The sample preparation was used to investigate the effect of incubation on the IgG protein sample. Figure 4 shows the particle size distribution of the IgG sample before incubation, and then after 1 and 6 hours of incubation at 37 C. The samples were measured using the AccuSizer FX system alone. Defining the aggregated protein as >0.7 µm, the concentration reduced as follows: 9.77, 7.75, to 5.08x10^5 particles/mL. Clearly, this extent of aggregation reduced with increased thermal incubation, suggesting improved dissolution. The tail of aggregates is expanded in Figure 5 for more detail.

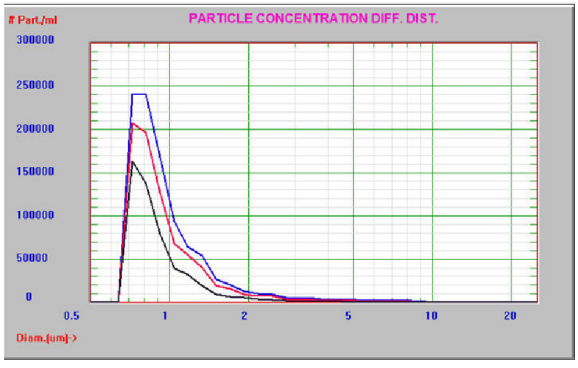

Figure 4. Time 0 (blue), 1 hour (red), and 6 hour (black)

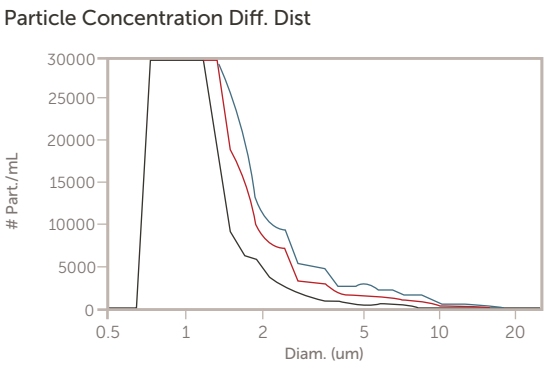

Figure 5. Expanded view of aggregated protein tail

EXAMPLE DATA 3: PROTEIN UNDER DIFFERENT STORAGE AND PROCESSING CONDITIONS

An unknown protein was supplied by a customer. The buffer, and three different preparation/storage conditions, were analyzed on the AccuSizer FX Nano to determine particle size and concentration of the aggregated protein. The concentration of particles >0.19 µm for the buffer and protein samples are shown in Table 1. The graphical results are presented in the differential count format in Figure 6 and cumulative format in Figure 7.

Sample	Particles/mL >0.19 µm
Protein A (red)	4.78×10^8
Protein B (green)	2.36×10^8
Protein C (blue)	1.10×10^8
Buffer (purple)	0.36×10^8

Table 1. Particle concentration of buffer and three protein samples

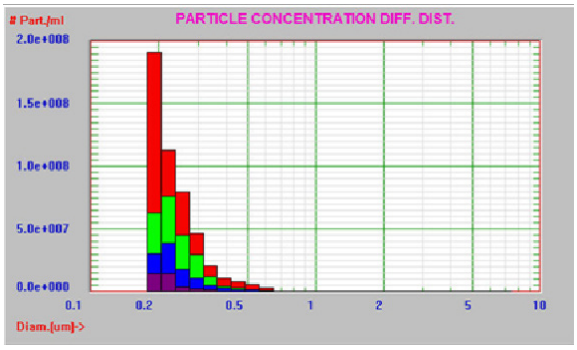

Figure 6. Differential distribution

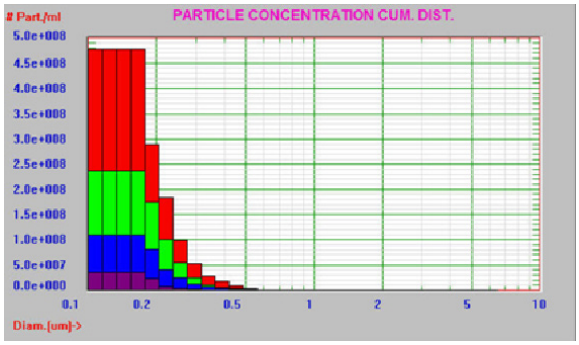

Figure 7. Cumulative distribution

Figures 8 and 9 show the same aggregated protein data but with the concentration plotted on a log scale for the y-axis to better show the large concentration range of results.

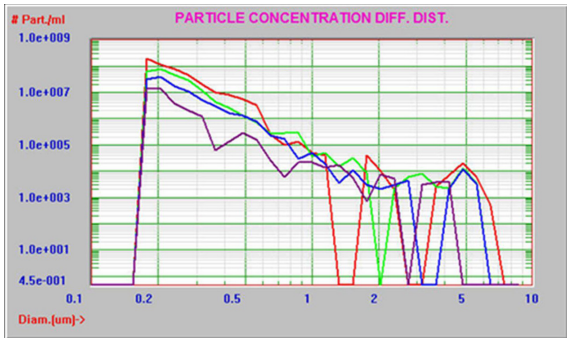

Figure 8. Differential distribution, log scale

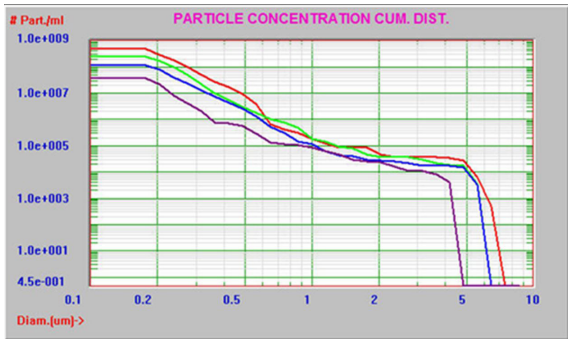

Figure 9. Cumulative distribution, log scale

CONCLUSIONS

The AccuSizer FX Nano provides an analytical tool capable of easily quantifying both the size and concentration of protein aggregates. The unique two sensor approach covers the broad dynamic range required to provide a detailed, high resolution view of the tail of aggregated proteins. Accurate count/mL data allows for comparison of results for samples at different concentrations. The SIS sampler can work with the small sample volumes required for this application. In addition, by removing the FX Nano sensor, the system could be used for standard USP <787> and <788>particulate matter in injections.

FOR MORE INFORMATION

Please call your Regional Customer Service Center today to learn what Entegris can do for you. Visit entegris.com and select the Contact Us link to find the customer service center nearest you.

TERMS AND CONDITIONS OF SALE

All purchases are subject to Entegris' Terms and Conditions of Sale. To view and print this information, visit entegris.com and select the Terms & Conditions link in the footer.

Entegris

Corporate Headquarters
129 Concord Road
Billerica, MA 01821
USA

Customer Service
Tel +1 952 556 4181
Fax +1 952 556 8022
Toll Free 800 394 4083