Protein Aggregation

Nicomp DLS System

OVERVIEW

Aggregation in protein solutions has been demonstrated to have deleterious effects. Measurement of these aggregates has been possible for larger aggregates, but smaller aggregates in the range of 0.150 nm to 2 microns have been difficult to quantify. The Nicomp® dynamic light scattering (DLS) techniques can demonstrate the existence of aggregation, but does not provide any information on the absolute concentration of the aggregates.

DISCUSSION

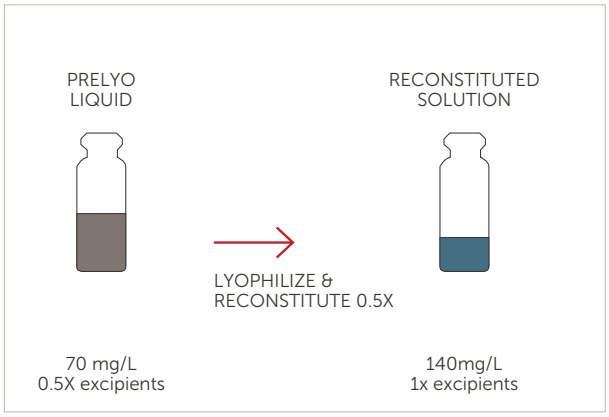
Biotherapeutics have been shown to be susceptible to inducing the elicitation of antidrug antibodies (ADA). Evidence suggests that protein aggregations have the ability to enhance immunogenicity and therefore enhanced immune responses to the monomeric form of the protein.

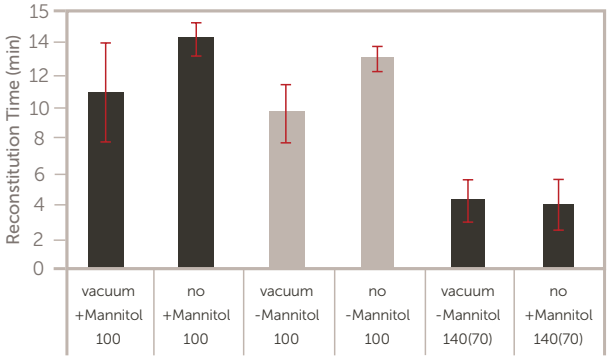
Manufacturers of the biotherapeutic proteins often prepare the drug for injection though a series of steps such as:

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 to the therapeutic course of treatment.

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





In Figure 1 the analysis of a vaccine with a population at 200 nm displays. Aggregation is clearly visible at about 300 nm. The three runs clearly demonstrate the loss of material at 200 nm with concomitant increases in the peak at 300 nm.

Figure 2 shows that there is also aggregation visible at approximately 600 nm. Note that the concentration of material at 200 nm is on the order of a hundred thousand times more concentrated than the aggregation at 600 nm.

Figure 3 displays a more accurate concentration measurement made available by changing the scaling of the display. From this figure it is possible to see that Vaccine 2 presents 5 times the concentration of aggregates at 600 nm than Vaccine 1.

With this information in hand, it is possible to determine the best formulation conditions for this vaccine preparation to avoid aggregation and reduce the risk of unwanted immune responses to the treatment.

The final figure shows another set of analyses demonstrating that some proteins will show low levels of aggregation even in the range of 5 microns, while the actual concentration might be so low relative to the native protein as to avoid detection by other methods.

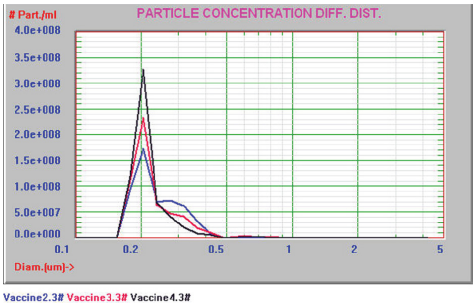


Figure 1. Vaccine with aggregates at 300nm

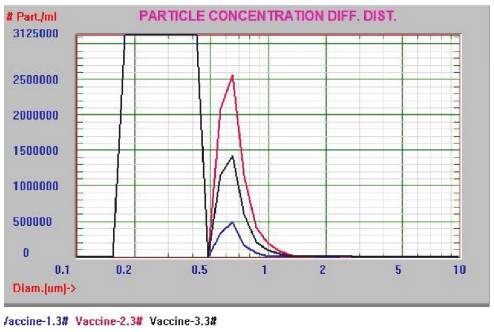


Figure 2. Vaccine with aggregates at 600nm

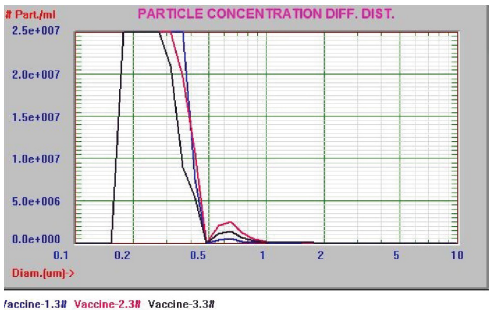


Figure 3. Comparison of Vaccine 1 to Vaccine 2

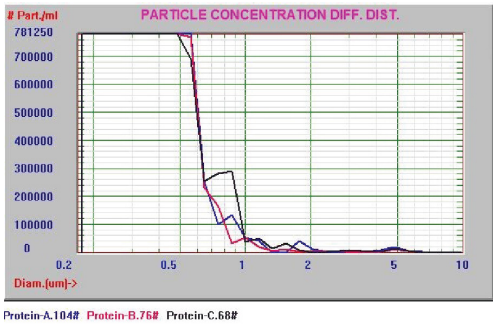


Figure 4. Low levels of aggregates in 5 micron range

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