FlowCam® Nano provides counts, sizes and images of nano-and microparticles: Application to a therapeutic protein pumping study.

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Sub-visible particle characterization is becoming a more important method in assessing drug substance and drug product stability. However, the current USP protocol does not require any characterization of particles below 10 μm. This greatly hampers our ability to properly assess protein stability as a function of particulate formation and obscures most sub-visible particles and various morphological properties.

In this study, we assess the capabilities of the new FlowCam Nano from Fluid Imaging Technologies, in conjunction with a pumping study that focuses on particulate formation generated during fill operations. We emulated filler processes of those seen in pharmaceutical filling lines using 1mg/mL of IVIG in PBS pH 7.4 and 0.25M glycine pH 4.2. For these experiments evaluating oil immersion flow microscopy, a 500 mL fill volume was used, along with Pharmed BPT (Saint-Gobain), due to this particular tubing generating larger amounts of nanoparticles than other tubing tested previously. Post-pumping agitation was done using an ATR Rotamix at 15 RPM. Particle analysis was done using oil-immersion flow imaging microscopy (FlowCam Nano).

As seen in Figures 1 and 2, the FlowCam Nano showed that as IVIG is pumped, the size distribution shifts toward larger microparticles in both buffers. This is substantiated by an increase in the mean spherical diameter for the entire population. In Figures 3A and 3B, IVIG is shown to be much more colloidally stable in glycine than PBS. The size distribution of particles for IVIG in glycine is more concentrated in the submicron population and the size distribution of particles for IVIG in PBS is more evenly distributed up to 25 μm.

Initial experiments show that the new FlowCam Nano can generate highly resolved images of nanoparticulates, allowing the simultaneous assessment of particulate morphology for nano- and microparticulates. This is extremely useful, as currently unpublished data (for the pumping study) show that as a function of post-pumping agitation, a significant increase in microparticle concentration coincides with a significant depletion of the nanoparticle concentration. This suggests that agglomeration (and probably not nucleation) is the more likely mechanism for larger microparticulate formation.

The ability to characterize the nano- and microparticulate morphology simultaneously, will allow us to better assess the cause of particulate formation (such as observing the transition of nanoparticles to microparticles and seeing if specific morphological properties of larger nanoparticulates are conserved in microparticulates). In addition, the FlowCam Nano is also sensitive enough to assess differences in the colloidal stability of a drug product in different formulations. During filling operations, and accelerated degradation studies, FlowCam Nano showed the differences in size distributions of IVIG in PBS and 0.25 M glycine buffer. Since IVIG is less colloidally stable in PBS, the size distribution varies significantly more per size bin and the overall mean effective spherical diameter is also ~ 600 percent greater after 24 hours of agitation in comparison to the size distribution data of IVIG in glycine. Thus,

Flow Cam Nano has capabilities that are very useful in assessing drugs in the substance (e.g. optimizing drug formulations) and product (e.g. assessing particulates post fill & finish operations) phases.

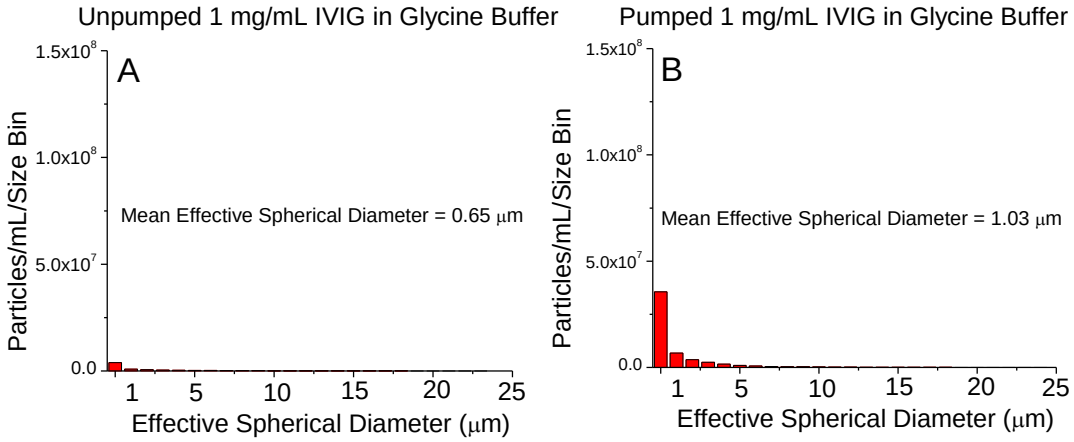

Figure 1. Particle size distribution data of unpumped (A) and pumped (B) 1 mg/mL IVIG in 0.25M glycine buffer pH 4.2 as measured by the FlowCam Nano

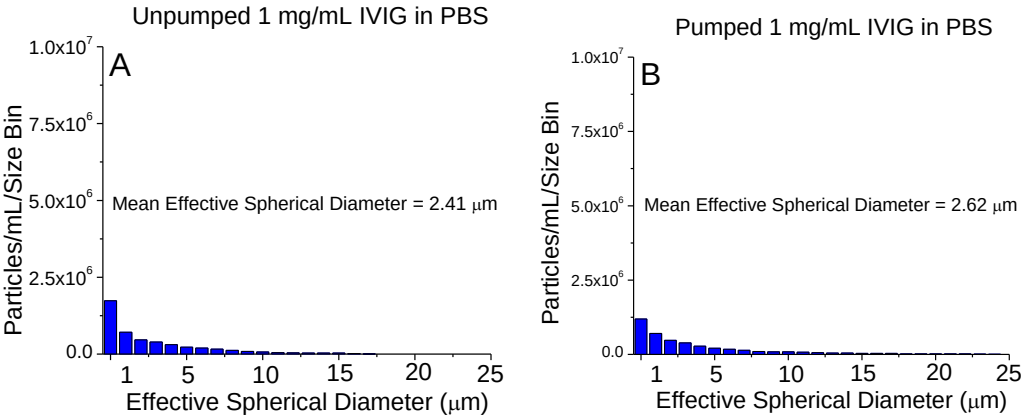

Figure 2. Particle size distribution data of unpumped (A) and pumped (B) 1 mg/mL IVIG in PBS pH 7.4 as measured by the FlowCam Nano

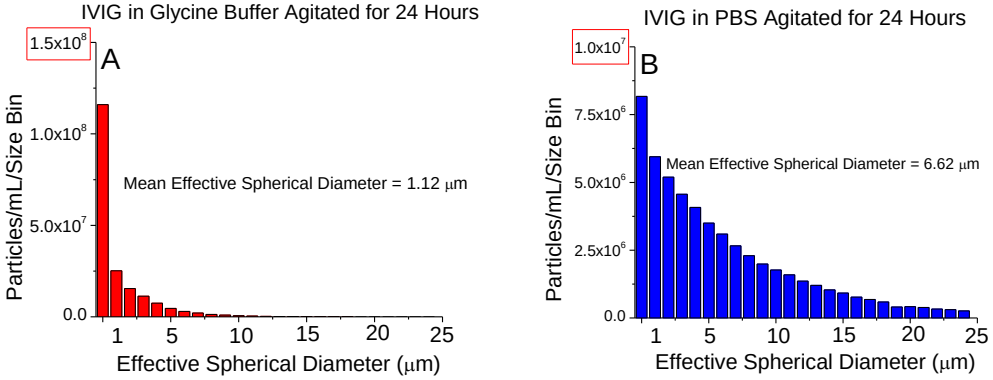

Figure 3. Comparison of pumped IVIG agitated for 24 hours in glycine (A) and PBS (B). Note that Y-axes are not normalized for these two graphs.