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

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Abstract

Sub-visible particle characterization is a critical method for assessing drug substance and drug product quality and stability. In this study, we evaluated the capabilities of the new FlowCam Nano® from Fluid Imaging Technologies (which utilizes a patented oil immersion flow microscopy), in conjunction with a pumping study that focused on protein particle formation generated during filling pump operations. Initial experiments showed that the new FlowCam Nano® can generate highly resolved images of both nano and microparticles, allowing assessment of morphology for micron-sized and submicron particles. In addition, particle sizes and counts are obtained for particles as small as 0.3 microns. This capability is extremely useful. For example, the peristaltic pumping study showed that there were relatively high levels of both nano- and microparticles formed when intravenous immunoglobulin (IVIG) formulations were pumped. Also, it was observed that during post-pumping agitation there was a substantial increase in microparticle concentrations coinciding with a large depletion of nanoparticles. This result suggests that agglomeration was the likely mechanism for agitation-induced increases in microparticle concentrations. In addition, results obtained with the FlowCam Nano® were sensitive enough to observe manifestations in differences of colloidal stabilities in protein formulations in different buffers (Figures **3A** and **3B**). During pumping operations, and post-pumping agitation studies, FlowCam Nano® was able to show the differences in particle size distributions for samples in PBS versus 0.25 M glycine buffer. IVIG is much less colloidally stable in PBS than in glycine buffer, and presumably, particles of the protein are also. During agitation of pumped IVIG in PBS, the diameter of particles increased much more rapidly than was observed during the same treatment in glycine buffer. Overall, this study demonstrated that FlowCam Nano® has unique capabilities that are very useful for characterizing and quantifying nano- and microparticles in therapeutic protein products and for gaining insights into effects of processing steps and formulations. The ability to characterize and quantify nano- and microparticles simultaneously will allow us to assess better the causes of particle formation and develop more effective control strategies.

Methods and Materials

Using a Flexicon PF6 asceptic peristaltic filler pump, we emulated filler processes of those seen in pharmaceutical filling lines using 1 mg/mL of IVIG in PBS pH 7.4 and 0.25 M glycine pH 4.2. For these experiments evaluating oil immersion flow microscopy, a 500 mL fill volume was use, 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 micrscopy (FlowCAM Nano®).

FlowCam Nano® Sampling Procedure

For maximum accuracy in sizing small (less than 1 micron) particles, a segmentation threshold setting of 5.00 was used, segmenting on dark particles only. An Edge Gradient filter was applied to accept only particles with an edge gradient above 30, to insure that only accurately measurable particles were captured. A dilution ratio of 0.10 was applied to reflect the portion of imageable depth that the FlowCam Nano® was able to capture within the edge gradient filter's restrictions. A flow rate of 0.02 ml/min was used to maximize image clarity. Protein Samples were processed using the following method:

After previous run, clean flow cell with 0.2 ml of roughly 2% Contrad 70 solution. The cleaning solution ensured that no aggregates adhered to the flow cell between samples. Next, rinse flow cell with 0.2 ml DI water. Visually verify in Setup/Focus window that no cleaning solution or contaminants remain in flow stream. Vortex the sample using a Vornado Miniature Vortex Mixer. Pipette 0.12 ml of sample into sample introduction pipette tip (this varied by sample density, as high image counts made massive files). Using Setup/Focus, advance lower sample meniscus past the viewable area.

Size calibration standards were run using the same procedure, with the modification that only a rinse (not a cleaning) step was used. The size standard solutions were a 20:1 dilution of Duke Standards 0.350 um +/- 0.007 um Polystyrene Particle Counter Size Standards and a 20:1 dilution of Duke Standards 0.600 um +/- 0.009 um Polystyrene Particle Counter Size Standards.

Results

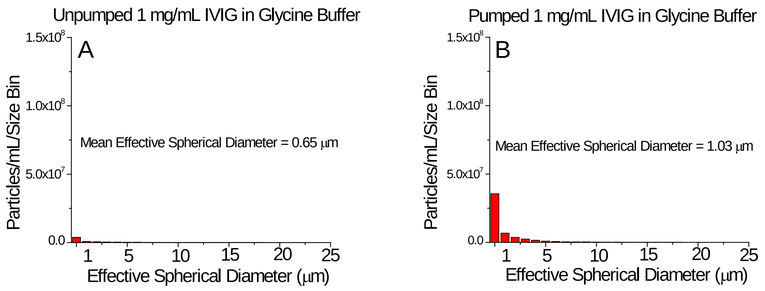

Figure 1 Particle Size Distribution Data of Unpumped (**A**) and Pumped (**B**) 1 mg/mL IVIG in 0.25 M Glycine Buffer pH 4.2 via FlowCam Nano®

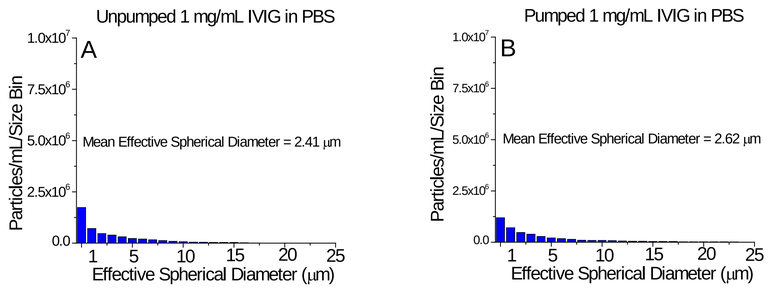

Figure 2 Particle Size Distribution Data of Unpumped (**A**) and Pumped (**B**) 1 mg/mL IVIG in PBS pH 7.4via FlowCam Nano®

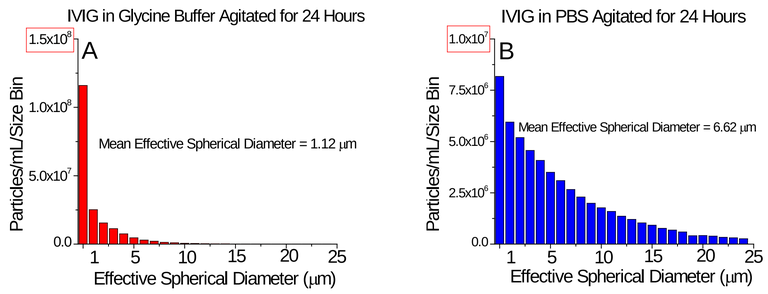

Figure 3 Comparison of Pumped IVIG Agitated for 24 Hours in Glycine (**A**) and PBS (**B**)
**Y-axes are not normalized for these two graphs

Submicron FlowCam Nano® Images

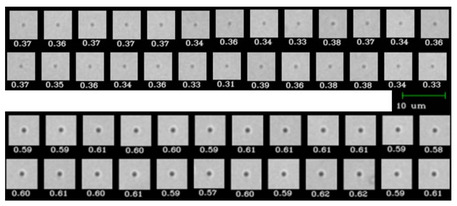

Figure 4 FlowCam Nano® images of polystyrene bead standards.
The certified ranges of those beads were 0.345 +/- 0.007 µm (**A**) and 0.600 +/- 0.009 µm (**B**)

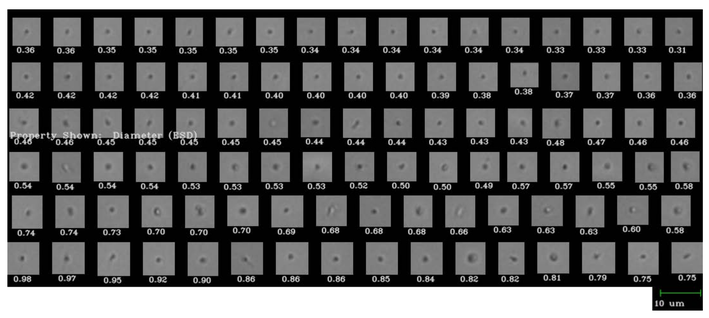

Figure 5 FlowCam Nano® images of a population of submicron particles from Pumped IVIG in PBS

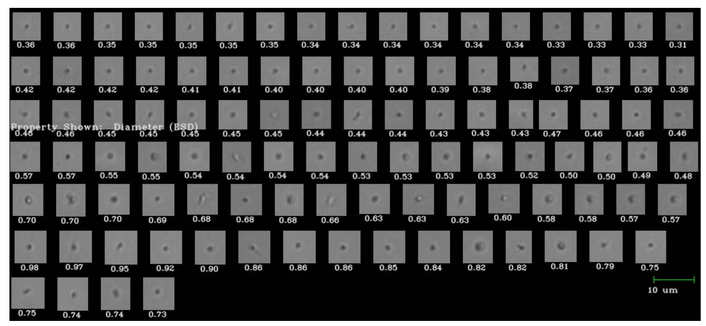

Figure 6 FlowCam Nano® images of a population of submicron particles of pumped IVIG in Glycine Buffer

Size Distribution by Particle Concentration

Sample (Fraction)	Particles ≥ 0.3 (particles/mL)	Particles ≥ 1 (particles/mL)	Particles ≥ 5 (particles/mL)	Particles ≥ 10 (particles/mL)
Control	4.0×10^6	9.1×10^5	2.8×10^5	1.2×10^5
1	3.6×10^7	6.7×10^6	9.2×10^5	2.3×10^5
16	1.1×10^8	1.4×10^7	1.2×10^6	2.4×10^5
32	4.8×10^5	2.3×10^5	4.7×10^4	2.0×10^4
48	1.2×10^8	2.5×10^7	4.6×10^6	6.4×10^5

Sample Agitated	Particles ≥ 0.3 (particles/mL)	Particles ≥ 1 (particles/mL)	Particles ≥ 5 (particles/mL)	Particles ≥ 10 (particles/mL)
Control	4.0×10^6	9.1×10^5	2.8×10^5	1.2×10^5
1 Hour	1.9×10^8	4.7×10^7	1.7×10^6	9.4×10^4
4 Hours	1.8×10^7	3.2×10^6	3.6×10^5	2.0×10^4
8 Hours	4.5×10^5	2.3×10^5	3.4×10^4	0
24 Hours	1.2×10^8	2.5×10^7	4.6×10^6	6.6×10^5

Figure 7 Size Distribution by particle concentration determined by FlowCam Nano® of 1 mg/mL IVIG in 0.25 M Glycine Buffer During the Pumping Run (**top**) and Post-Pumping Agitation (**bottom**)

Sample (Fraction)	Particles ≥ 0.3 (particles/mL)	Particles ≥ 1 (particles/mL)	Particles ≥ 5 (particles/mL)	Particles ≥ 10 (particles/mL)
Control	1.2×10^6	7.2×10^5	2.3×10^5	1.1×10^5
1	1.8×10^6	7.2×10^5	2.4×10^5	8.0×10^4
16	1.1×10^6	4.7×10^5	1.4×10^5	5.4×10^4
32	1.7×10^6	7.7×10^5	3.2×10^5	1.4×10^5
48	2.1×10^6	9.6×10^5	4.6×10^6	2.6×10^5

Sample Agitated	Particles ≥ 0.3 (particles/mL)	Particles ≥ 1 (particles/mL)	Particles ≥ 5 (particles/mL)	Particles ≥ 10 (particles/mL/size)
Control	1.2×10^6	7.2×10^5	2.3×10^5	1.1×10^5
1 Hour	1.9×10^6	9.5×10^5	3.4×10^5	1.3×10^5
4 Hours	1.4×10^6	9.0×10^5	4.8×10^5	3.2×10^5
8 Hours	3.2×10^6	2.2×10^6	1.3×10^6	8.4×10^5
24 Hours	8.2×10^6	5.9×10^6	3.5×10^6	1.8×10^6

Figure 8 Size Distribution by particle concentration determined by FlowCam Nano® of 1 mg/mL IVIG in PBS During the Pumping Run (**top**) and Post-Pumping Agitation (**bottom**)

Discussion

As seen in Figures **1** and **2,** 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 particle 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.

Conclusions

Overall, this study demonstrated that FlowCam Nano® has unique capabilities that are very useful for characterizing and quantifying nano- and microparticles in therapeutic protein products and for gaining insights into effects of processing steps and formulations. The ability to characterize and quantify nano- and microparticles simultaneously will allow us to assess better the causes of particle formation and develop more effective control strategies.

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