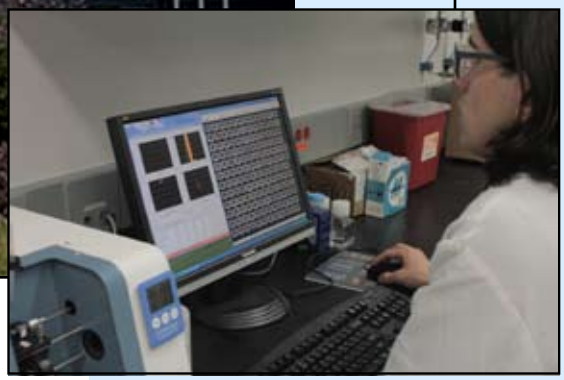
Vol. 14 No. 5

www.dddmag.com

DRUG DISCOVERY & DEVELOPMENT

STRATEGIES & TECHNOLOGIES DRIVING DRUG DISCOVERY TO MARKET



Instrumentation:

Particle Analysis

An imaging particle analyzer can give researchers a better picture of particles in parenteral formulations.

Safety Snapshot

An imaging particle analyzer can give researchers a better picture of particles in parenteral formulations.

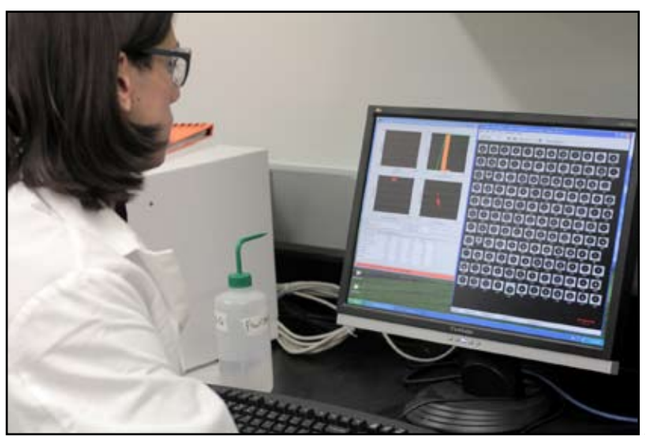
Gregory J. Morrone
Associate Scientist, Biophysics
Biopharmaceutical Analytical Sciences
Wasfi Al-Azzam
Investigator
Biopharmaceutical Analytical Sciences
GlaxoSmithKline
King of Prussia, Pa.

Counting visible particles in small samples of parenteral formulations has traditionally been performed primarily with visual inspection by holding a vial up to a light and subjectively grading the count as high, moderate, or low. A secondary method uses a light obscuration instrument, which provides a total count of all particles detected greater than 10 um and 25 um. Though both methods meet U.S. Food and Drug Administration (FDA) requirements for counting particles under United States Pharmacopeia (USP) 1 and USP 788, many pharmaceutical fomulators, analysts, and quality control professionals are increasingly concerned about the shortcomings of these methods. Many pharmaceutical companies now realize that knowing exactly what particles may be hiding in samples--and how many of each type--is vital to meeting strict quality demands, accelerating development timetables, minimizing risk and, ultimately, to the success of the product.

After using light-obscuration instruments to count particles in a new parenteral product formulation, a project team at GlaxoSmithKline (GSK) in King of Prussia, Pa. found that light obscuration method yields a read-out of particles counted in the sample but is unable to shed light on the nature of the particles. This technology has difficulty determining the actual number of particles present as transparent or translucent particles may be excluded from the analysis. Additionally, al light obscuration instrument is not capable of differentiating one type of particle from another particle, such as an air bubble or foreign fiber from an aggregated protein. If the particles are not identified, differentiating the actual number of product-related particles can be a difficult task.

A GSK team member with expertise in particle analysis reasoned an imaging particle analyzer that takes digital images of each individual particle detected in a sample would reveal the nature of the particles and assist in a better assessment of the product quality. The project team evaluated a range of available technologies from different vendors. The list of criteria relevant to particle analysis was extensive, including the volume of sample needed to obtain statistically significant data, how much set-up time is required, the adjustments needed to yield useful images, and whether additional steps were required for qualification and validation.

After testing, the team concluded that the FlowCAM® particle imaging and analysis system from Fluid Imaging Technologies, Yarmouth, Maine, set up quickly and with small samples, yielded high-resolution images in real-time along with the particle size, count, shape, and other measurement parameters. The system provided images of aggregated proteins, longitudinal fibers, round, silicone oil droplets, and air bubbles, as opposed to counts and graphs offered by other systems. It also could detect opaque, translucent, and transparent particles and automatically discern one from the other using pattern recognition software. The FlowCAM could also determine why the particles were present and whether the particles detected were related to the product formulation or to sampling technique.



Researchers review images of air bubbles detected by the FlowCAM® particle imaging and analysis system. Individual images may be selected for further analysis and emailed to colleagues for collaborative review. (Image: GlaxoSmithKline)

The system takes measurements based on actual images of each particle as opposed to measuring based on estimated particle counts and sizes. For particle analysis in the range of detection of 2.5 um to 3 mm, the FlowCAM yielded the highest quality images of any instrumentation tested, the team concluded. The accompanying VisualSpreadsheet©software enables users to filter and sort particle data based on different criteria while a sample was running and save them in libraries for future comparison. Individual images of interest could be selected with a mouse click; irrelevant images such as air bubbles could be deleted, while the raw data remained intact.

Based on the evaluations, GSK decided to install a FlowCAM VS-1, which was developed to analyze parenterals for proteinacous particles. The model's autoimage mode triggers the camera to capture an image each time a particle passes through the field of view in the flow cell.

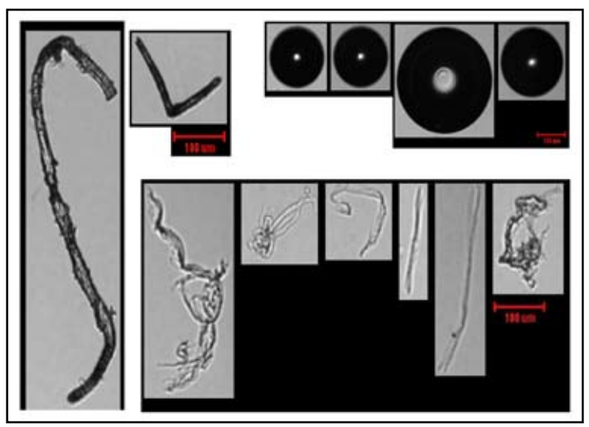
Since installation in February 2010, the FlowCAM has been in continuous operation to automatically uncover any trends in the number and/or type of visible particles present in the samples. The project team shares information with colleagues in other departments around the world by emailing the images and data. Quality control managers, who rely heavily on particle analysis data, view the particles on monitors. The FlowCAM data is also being used to verify the accuracy of the data generated by visual inspection.

For particle analysis in the range of detection of 2.5 um to 3 mm, the FlowCAM yielded the highest quality images of any instrumentation tested.

While light obscuration systems require at least a 25 mL sample to yield enough data to be considered statistically significant, the FlowCAM requires only 1 mL of sample to run an analysis. With samples often provided in 300 uL ampoules, the FlowCAM has reduced the number of required samples from more than 800 per batch to three, while also reducing sample handling and preparation requirements.

The ability to see the actual particles in parenteral formulations, to see their morphology, and to automatically differentiate one type from another marks an important advance in particle analysis. At GSK, imaging particle analysis using FlowCAM is being integrated into its testing program for development of protein-based parenteral drugs. ■

Gregory Morrone has performed higher order structure characterization work in Biopharmaceuticals R&D since 2010. Wasfi Al-Azzam is the leader of protein therapeutics higher order structure team at Biopharmaceutical Analytical Sciences in GSK-Biopharmaceuticals R&D.



Left: These actual images of proteinaceous particles, fibers and air bubbles were imaged by the FlowCAM® at GSK, King of Prussia, Pa. The FlowCAM's ability to detect opaque, translucent and transparent particles and automatically discern one from the other with its proprietary pattern recognition software provided dramatic advantages. It possessed not only the ability to see exactly what was present but also to determine why they were present and to understand whether the particles detected were related to the product formulation or to the sampling technique. Seeing actual images of aggregated proteins, longitudinal fibers, round, silicone oil droplets and air bubbles after years of being limited to simple counts and graphs astounded the entire project team. At right: Gregory J. Morrone and Wasfi Al-Azzam. (Image: GlaxoSmithKline)

In an investigation of available technologies, the Eyetech from Ankersmid, Netherlands; the Sysmex 3000 from Malvern Technologies, Worcestershire, UK; the MicroFlow Imaging instrument from Brightwell Technologies, Ottawa, Ont., Canada; and the FlowCAM® particle imaging and analysis system from Fluid Imaging Technologies, Yarmouth, Maine, USA were evaluated by the project team. Some of these demanded a host of adjustments, configurations, and set up time to yield images from the sample while requiring additional steps for qualification and validation. Additionally, some of these technologies may require substantially larger volumes of very precious samples to obtain statistically significant data. The FlowCAM® setup quickly, and with small samples yielded high resolution images in real-time along with the particle size, count, shape and other measurement parameters, according to Greg Morrone, shown with Wasfi Al-Azzam. (Image: GlaxoSmithKline)