AIM™ for Aggregation Analysis

Introduction:

A large percentage of modern industrial processes involve the use of colloidal systems, where a mixture of dispersed insoluble particles are suspended throughout another substance. Colloidal systems can be found in diverse applications, such as material sciences, food sciences, environmental, pharmaceutical, and toners/inks areas. In most of these systems, *colloidal stability* is a critical quality attribute (CQA) that must be monitored.

For a variety of reasons, particles in a colloid can have a propensity to aggregate, whereby multiple single particles are groped together to form an aggregate. In most colloidal systems, aggregation is an undesirable effect, and great pains are taken to create a stable system that is not prone to aggregation. This is not a simple undertaking, since many different factors within a process can lead to aggregation (Brownian motion, electrostatic forces, etc.).

This preponderance of causes for aggregation means that measurement of aggregation (colloidal stability) becomes a CQA for these processes. While there are many different techniques for measuring colloidal stability, the most common are zeta potential and light scattering/ transmission-based particle analysis. These are indirect methods: the ideal method is to directly measure the "particle size distribution" (PSD) of the particles in the colloid.

Using AIM to measure aggregation:

Sebago Scientific's AIM system is ideally suited for measuring aggregation. It produces a true PSD based upon individual, direct measurements of particle size and concentration. The aggregation percentage is merely the number of particles greater than a specific input size divided by the total number measured. Since AIM directly measures each particle's size individually, no bias is introduced such as that found in ensemble techniques such as light scattering (DLS and SLS in particular).

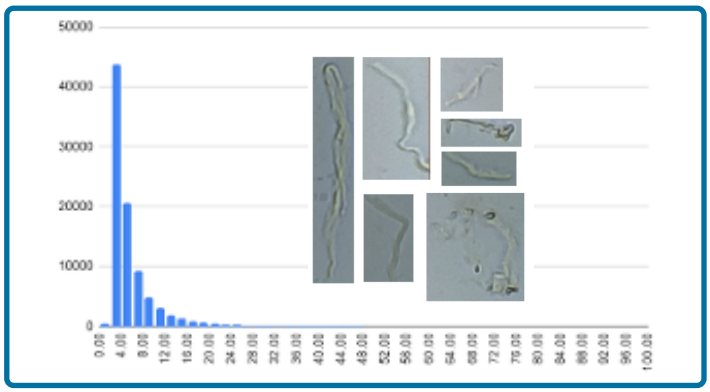
The following two examples discuss different applications of AIM for measuring aggregation under very different constraints:

Example 1: Measuring protein aggregation in biotherapeutics:

Protein therapies (referred to as "large molecule", to differentiate from classical "small molecule" therapies) are a relatively new but rapidly growing application in pharmaceutics. Unfortunately, proteins are very susceptible to aggregation; this aggregation is highly undesirable as it can lead to reduced efficacy and adverse

effects (immunogenetic reactions) in patients. Because of this, much effort is placed in formulation towards eliminating aggregation, and aggregation must be monitored throughout the process of creating and delivering the therapy.

Single proteins are in the 2-10nm size range, so well below AIM's limit of detection (LOD). However, protein aggregates can aggregate to very large sizes, with compendial regulations requiring testing for aggregates greater than 10µm. AIM is perfect for measuring any aggregation above 2µm.

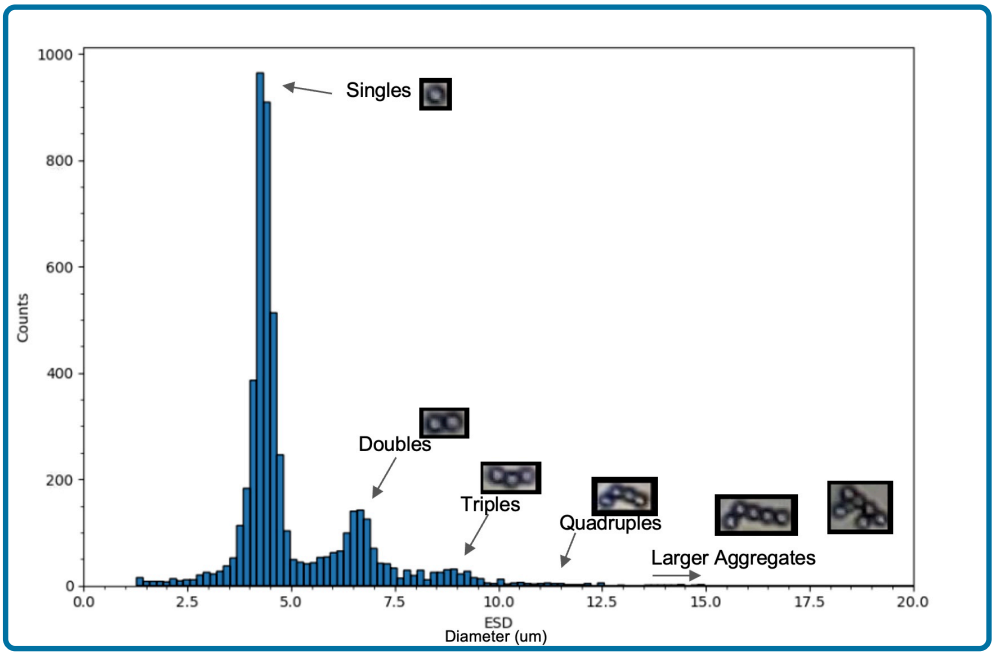


Like many other natural systems, the distribution of particle sizes in protein aggregation follows a power law, whereby the frequency of occurrence decreases exponentially as you move to higher order aggregates. Figure 1 (above) shows the results of an AIM measurement on a stressed protein sample, along with sample images of larger aggregates. The power law distribution is clearly visible, and actual concentration at any size can be quantified.

Example 2: Counting monomers in aggregates for accurate concentration:

Unlike the previous example, many colloidal systems use particles that are large enough to be seen individually by the AIM system. The AIM analysis software can actually count individual particles within an aggregate for yielding more accurate aggregation percentages. In other systems, the aggregate is counted as only a single (large) particle.

An excellent example of this is shown in Figure 2, where 4µm polystyrene spheres were run through AIM for QC purposes. In this particular application, it was found that the type of buffer used for storing the spheres affected if they aggregated over time. The manufacturer's object was to find the best storage buffer (least prone to aggregation).



As can be seen in the particle size distribution (PSD), the particles are predominantly 4µm in size (single beads). However, secondary peaks can be seen in the distribution, representing doublets, triplets and larger aggregates. The AIM software allows the user to easily interrogate the PSD, and to look at images anywhere in the distribution. Example images have been added to the PSD for demonstration.

Normally, the software would count each aggregate as a single particle, which would cause a lower than actual concentration to be calculated (remembering that concentration is simply (total number particles/volume imaged). However, if aggregate detection (available in optional test plan upgrade) is turned on on the AIM software, it will "break up" the aggregates into single particles for counting purposes when calculating total number of particles for the concentration. This yields a more precise concentration calculation.

How AIM™ Works

The diagram at right shows an overview of the AIM system. Sample is pipetted into a disposable sample cup; between 500µl and 3ml of sample is required, depending on sample characteristics. The internal pump pulls the sample through a narrow flow cell. At designated intervals the flow is stopped and imaged through the microscope optics.

The acquired images are then stored and processed by the embedded computer, producing reports such as particle size distributions and tabular results of the statistics, as determined by the particular test plan/SOP.