Analysis and Immunogenic Potential of Aggregates and Particles

A Practical Approach, Part 1

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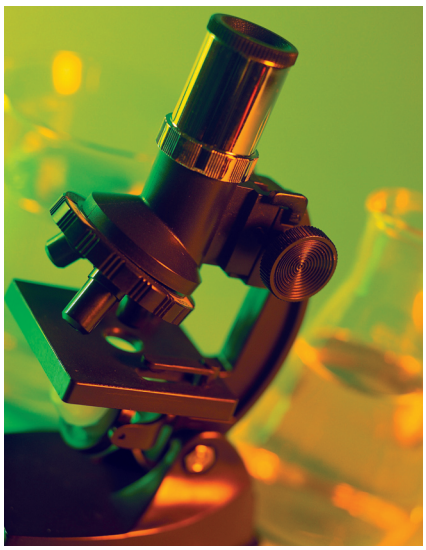
The number of biotherapeutics on the market has rapidly increased during the past several years. Such proteins commonly exhibit a concentration-dependent propensity for self-association, which often leads to the formation of aggregates that range in size from nanometers (oligomers) to microns (subvisible and visible particles). Publications two years ago focused attention on the potential immunogenicity of active-ingredient aggregates (**1–4**). The authors discussed lack of specificity of compendial measurements and inability of other current methods to address potential effects of large protein aggregates on the safety and efficacy of therapeutics. Discussions between regulators and industry have led to development of novel techniques to detect and characterize aggregates and increased research into the role of protein aggregates of all sizes in immunogenicity. In addition, the pharmacopoeias have been revising monographs to improve subvisible-particle testing of biotherapeutics and clarify terms such as *practically or essentially free of particles*.



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This CMC Strategy Forum focused on the latest developments in detection and characterization of protein aggregates. The meeting included real-life examples of using a historical database to gain product-specific knowledge necessary to conduct thorough risk assessments of aggregates' effects on product quality, safety, and efficacy. Participants also discussed implementation of appropriate control strategies for lot release, stability, and comparability of protein aggregates. The meeting ended with a session covering the most recent experiments probing the role of protein aggregates in immunogenicity, with discussions of the best models to use and initial results.

Panel discussions focused on issues such as the approaches for particle detection and testing (development, validation, and execution), methods and their application, and appropriate data analysis. Immunogenicity topics included potential thresholds for immunogenicity, linking laboratory and clinical data, and predicting and testing potential immunogenicity of products throughout a development lifecycle.

Morning Session Presentations

During morning sessions, speakers presented methods to detect and better understand the nature of protein aggregates and particles in biotechnology products. Discussions focused on how such methods should be validated and how they can characterize products during their lifecycles. Speakers also reported on the current thinking on setting limits on particles.

Testing Validation: Linda Narhi and Yijia Jiang (Amgen Inc.) reported on the validation and implementation of subvisible-particle testing throughout product development. They described some mechanisms that can lead to protein aggregation and the types of aggregates that can form from stresses during manufacturing and delivery. For example, protein aggregates are differentiated based on size, conformation, modification, morphology, and ability to dissociate. Particles in final products also can be

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Process Focus: Formulation, QA/QC

Who Should Read: Analytical development, formulation, and manufacturing

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Level: Intermediate

THE CMC STRATEGY FORUM SERIES

The CMC Strategy Forum series provides a venue for biotechnology and biological product discussion. These meetings focus on relevant chemistry, manufacturing, and controls (CMC) issues throughout the lifecycle of such products and thereby foster collaborative technical and regulatory interaction. The forum committee strives to share information with regulatory gencies to assist them in merging good scientific and regulatory practices. Outcomes of the forum meetings are published in this pee-reviewed journal with the hope that they will help assure that biopharmaceutical products manufactured in a regulated environment will continue to be safe and efficacious. The CMC Strategy Forum is organized by CASSS, an International Separation Science Society (formerly the California Separation Science Society), and is cosponsored by the US Food and Drug Administration (FDA).

classified based on their origin: *intrinsic* (originating from the manufacturing process, components of the final formulation, or from the product itself through inherent self association) or *extrinsic* (originating from outside normal equipment used to produce a drug, such as insect parts). Particles can range in size from submicron to visible, and various analytical tools are used to detect and characterize particles of all sizes.

USP chapter <788> describes the current test for subvisible particles. Such testing is intended mainly for small-molecule drugs and is based on light-obscuration technology as the primary analytical method and light microscopy as an alternative. The chapter focuses on detection of extrinsic particles and does not account for the inherent thermodynamic property of proteins to self-associate, which forms aggregate in biotechnology products — especially at high concentrations.

Consequently, the method is not optimal for biotherapeutics analysis. Narhi and Jiang described a small-volume light obscuration (HIAC) method for analyzing smaller volumes of biotechnology products. It includes sample-handling processes (such as vacuum degassing and diluting when appropriate) to reduce false-positive and false-negative results. In addition, particles sized ≥2, 5, 10, and 25 µm are detected and measured, beyond just the ≥10- and 25-µm particles in the current USP/EP method. That also relies on statistical analysis to develop sampling plans.

Method qualification and validation parameters were similar to those used for most methods (e.g. specificity, accuracy, precision) but had to be applied specifically for the small-volume HIAC method. The presentation focused specifically on a need to qualify the method for each product because not all protein products are the same. Degassing is essential to remove bubbles, and dilution may be required depending on the number of subvisible particles present. For some samples, high concentration results in undercounting the micron-range aggregate, but that is not the case for all products. Caution must be taken when selecting water to reconstitute lyophilized product or dilute material into the assay because various water sources can themselves contain subvisible particles. Milli-Q water (EMD Millipore) was recommended. A new USP monograph for a small-volume HIAC method is under development and will include many of these considerations.

Narhi and Jiang recommended that subvisible particle testing be carried out early in development to ensure appropriate history and consistency during development. They also suggest selecting processes that minimize aggregate formation and the importance of understanding clinical experience. Users should begin acquiring a data set in case specifications are required beyond the current USP limits. Creating a data set of subvisible particle numbers is essential for future comparability studies regardless of whether specifications are set.

Regulatory Perspective: Barry Cherney (Division of Therapeutic Proteins, CDER) presented on current regulatory considerations for the assessment of subvisible particles. He described the high-level relationship between immunogenicity and protein aggregation, which was further considered in the second half of the forum. Induction of immunogenicity is associated with highly repetitive arrays of native protein — particularly for 1–10 µm particles. However, although some convincing data have shown that protein particulates can enhance immunogenicity of a protein, no definitive data have been provided showing that subvisible protein particulates typically present in pharmaceutical products enhance immunogenicity.

Arguments that the amount of protein in particles is too small to induce an immune response were countered by the fact that the level required for any particular product to induce an immune response is unknown, as is the impact of multiple dosing. Cherney pointed out that the lack of data does not eliminate risk but simply creates an unknown and uncontrolled risk. He concluded that in the absence of convincing data showing that subvisible particulates are not critical, the types and amounts of subvisible particles should be considered critical quality attributes (CQAs) and monitored and controlled as appropriate.

Cherney said that most companies were not using methods that could detect and monitor all ranges of protein aggregates, but he acknowledged that it is difficult to distinguish between protein and other particles and that there were analytical gaps for quantifying 0.1–2 µm particles. Cherney emphasized the need to understand the protein aggregate profile during development and particularly on stability and after process changes. That provides information for a potential link to clinical outcomes and establishes a target profile when implementing manufacturing changes.

Measuring those particles during development or postapproval is necessary to establish a direct link to clinical outcomes. Not doing so makes manufacturers rely on process robustness alone to control particle levels and ensure consistency. In addition, every product has a risk–benefit profile, and understanding and controlling the protein particle level could shift the profile in a beneficial manner, or at least ensure that it remains stable.

Cherney recommended that manufacturers use at least two orthogonal methods to quantitate the amount sand sizes of subvisible particles >2.0 µm, because different methods

detect different particle properties. Variability of results from a method can be quite high and should be controlled as much as possible. Silicon oil from containers can interfere in some techniques, but other methods can be used to differentiate silicon oil droplets from protein. In addition, submicron particles should be assessed during product characterization, especially after process changes. Manufacturers should use multiple stress conditions to understand the propensity to form particles and the ability of analytical methods to detect various types of aggregates that may form. Cherney mentioned that correlating results derived from routine tests (such as HIAC) with those of more sensitive, orthogonal methods can help establish a suitable control strategy that may not necessarily involve adding tests to the specifications, but rather action limits. Other techniques should be used to characterize the nature of particles and their protein content.

In essence, a company should conduct a robust risk assessment during and after product development on the potential impact of subvisible particles on quality, safety, and efficacy. Because every product has a unique risk–benefit profile, a product-specific strategy should be proposed to provide adequate control of perceived risks that subvisible particles may pose.

Particles in MAb Solutions: Alla Polozova of MedImmune presented case studies on detection and characterization of subvisible particles. The first was a monoclonal antibody product in which visible particles formed less than a month after manufacturing. Continued increase in counts of the submicron and micron aggregates correlated with rapid formation of visible (>100 µm) aggregates. Colloidal instability and hydrophobicity of the surface of the protein aggregates were potential causes of visible-particle formation. This observation led to the decision to reformulate the product. After reformulation, both flow cytometry and flow microscopy showed a significant reduction in the formation of subvisible particles over time.

The second study illustrated effects dilution can have on quantitation of the submicron and subvisible aggregates present in different MAbs (similar to what was described in the first talk). Two different antibodies exhibited a rapid increase in particle numbers after dilution that gradually decreased over time in the first buffer tested. However, in a different formulation one antibody continued to show the slow decrease in submicron aggregates with time, whereas the other exhibited a steady increase in particle number over the same time period. That study illustrated that if dilutions are used for particle counting assays, then the effect of the dilution on particle count must be assessed for each product and each set of conditions.

The final case study demonstrated that the native charge of a MAb can affect the formation of particles, depending on the pH of the buffer in which the product was diluted. A low native charge can result in increased particle formation.

Evolving USP Chapter: Russell Madsen (chair of the USP Visual Inspection of Parenterals Expert Panel) provided the last presentation of the morning session. He described the current pharmacopoeia chapters' definitions of *essentially free* (USP),

Speakers and Their Presentations

The morning session consisted of presentations followed by interactive discussion among industry experts on the panel and the audience.

"Detection and Characterization of Aggregates and Particles," chaired by Ruth Cordoba-Rodriguez (CDER, FDA) and Michelle Frazier-Jessen (MacroGenics, Inc.)

"Validating and Implementing Subvisible Particle (SbVP) Testing for Biotechnology Products Throughout Development," by Linda Narhi and Yijia Jiang (Amgen, Inc.)

"Current Regulatory Expectations for the Assessment of Subvisible Particles," by Barry Cherney (DTP, CDER, FDA)

"Simple Tales of Difficult Particles: Case Studies of Particle Analysis in Monoclonal Antibody Solutions," by Alla Polozova (MedImmune)

"Visual Inspection of Parenterals and USP Chapter <1>: An Update," by Russell Madsen (The Williamsburg Group, LLC)

Morning presenters were joined on a panel by Mike Amos (NIST) and Mary Cromwell (Genentech, a Member of the Roche Group).

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practically free (PhEur), and *readily detectable* (JP) in terms of lighting levels and limits. Having zero particles in a product is inconsistent with those terms, so it is recognized that manufacturing processes will not ensure zero defects but instead need to reduce visible particles to an acceptable level. Existing chapters do not account for protein products with visible particles that do not have an impact on safety and efficacy.

A proposed revision designed to address those concerns was published in *Pharmacopoeial Forum* (September–October 2009). The tests described are intended for products that have been 100% inspected. Sampling and inspection should be carried out using ANSI/ASQ Z1.4 General Inspection Level II, a single sampling plan for normal inspection with an AQL of 0.65. That is consistent with results of the most recent PDA survey on visual inspection practices for parenteral products. Following input received as a result of the *Pharmacopoeial Forum* publication, the proposal was modified to include a two-stage inspection for products that have been shipped to customers:

• Sample and inspect 20 units; if no unit contains particles, then those 20 units are determined to be essentially free.

• Sample and inspect 20 units; if one unit has particles, inspect another 80 units. The product is deemed essentially free of particles if none of the 80 units contain visible particles.

Some protein products may contain inherent protein particles, and requirements should be derived from regulatory approval and individual monographs. Special care should be taken with protein-solution inspections to prevent creation of proteinaceous particles (e.g., gentle swirling).

Morning Panel Discussion

A panel consisting of Mike Amos (NIST), Barry Cherney, Mary Cromwell (Genentech, a member of the Roche Group), Russell Masden, Linda Narhi, and Alla Polozova discussed as series of questions that were also posed to the audience.

Which of the current techniques can be validated and used at lot release, stability, and/or nonvalidated but qualified for characterization? The HIAC method has been validated for routine particle-size distribution analysis in quality control (QC) laboratories to test for subvisible particles. It has been used for years, so a large body of historical data is available. A Micro-Flow Imaging system can also be validated for particle-size distribution. Morphology analysis is used for determining what type of particles (e.g., proteins or silicon oil droplets) are being analyzed, so it remains in the characterization arena. Coulter counting can be validated for particle-size distribution as well under appropriate solution conductivity. For visible particles, automation can be "validated" (for example, with an Eisai system), and manual inspectors can be "qualified" using challenge sets.

Optical assessments for nonproteinaceous particles larger than 10 µm using microscopes can be validated according to USP, but isolation of protein particles can be problematic with this technique. Field flow fractionation, light scattering, scanning electron microscope–energy dispersive spectroscopy (SEM–EDS), Fourier transform infrared (FTIR) microscopy, Raman microscopy, and atomic force microscopy (AFM) are generally considered useful for characterization. Particle analysis using flow cytometry with or without fluorescent labeling is another useful technique that could potentially be used in QC environments.

Technology from NanoSight uses laser-light scattering and particle movement tracking (due to Brownian motion) to analyze submicron aggregates and particles in solution and is useful for characterization, although its results can be confounded by high concentration or

opalescence of background solution. The method appears promising but has not yet been widely used in pharmaceutical applications. Izon, an instrument based on the Coulter principle, and Archimedes, a technology that determines the mass of the submicron particles, are also promising new techniques that are in development.

One of the biggest challenges in selecting techniques for quantifying versus characterizing particles is in understanding the relationships among the numbers that each method produces. Each method is likely to detect different aspects (e.g., density, light reflection, and shape), so numbers and sizes of particles may not necessarily need to be in agreement. The ability to show that two methods correlate with each other and are thus predictive of the relative aggregate content may indicate that only one method is necessary as a routine test. Of course, users should understand why the numbers are different and justify the methods they select.

What parameters can and should be validated in an assay for subvisible particles, and what are the challenges?

• Specificity — Particles should be measured in different matrices.

• Accuracy — Measurement of known numbers of beads at specific concentrations should be used because you can't actually know the true number of protein particles in a solution.

• Precision — Repeatability and intermediate precision should be estimated using both artificial beads and real protein samples.

• Linearity and range — Linearity can be determined with beads, and dilution effects should be assessed with the protein sample.

• Limit of detection (LOD) and limit of quantification (LOQ) — Simulation and experimental confirmation should be carried out.

• Robustness — Effects of altering sample handling must be assessed (degassing time, vacuum, swirling, settling time, pooling effects, temperature, flushing processes, sensor-to-sensor variability, and so forth).

Challenges to validation can originate from method design itself and the nature of the protein particles. Handling the sample is critical: Degassing, temperature, swirling to prevent settling before analysis, and dilution can all affect assay results. Initial tare and volume and number of cleaning/flushes between samples are important to control. Use both standards and actual material for a particular aspect of validation (e.g., limit of detection versus specificity). Protocols for good manufacturing practice (GMP) qualification and validation must be tailored for an individual product because each method seems to detect different types of particles differently, complicating nalytical development (e.g., which stress condition causes protein aggregates most similar to those seen in the product during development and stability?). Intensity and circularity seems to be an issue. Companies should consider having laboratory analysts handle samples that mimic what will be done by physicians and patients.

Challenges to validation can also originate from method design and the nature of protein particles. Dilution in particular seems to be molecule and formulation dependent. Some products are linear, and particle counts increase upon dilution; other products appear to lose particles (perhaps dilution of loosely associated particles "dissolves" them, or the aggregates themselves are the results of reversible association driven by protein concentration). Materials that patients may be exposed to are also factors, especially for products that go through transport, reconstitution (if lyophilized), and delivery through syringes (involving passing through needles, skin, stored in bags, and so forth). Even the water and buffer used for dilution or reconstitution are important because silicon oil coating syringes or vials (e.g., water for injection) can introduce particles. Software used to analyze data also must be considered and evaluated for compliance to 21 *CFR* Part 11.

How and when would a universal standard rather than internal product-specific test samples be used to validate? What is an appropriate universal standard (beads? actual product? a new standard)? It will not be easy to develop aggregate standards per se. They will need to be highly stable, of recognized size and number, and made available to everyone regardless of position in industry, academia, or regulatory agencies. The morphology and optical properties of such standards need to mimic those of amorphous protein particles. A lot more discussion is needed. NIST is taking this on, and any material will be tested through a collaborative study. Standards using materials that mimic protein particles are also being examined.

Table 1: Presented classifications of particles and aggregates

Category	Classification
Size	Submicron 1–100 µm >100 µm
Reversibility	Reversible Irreversible Dissociable Nondissociable Dissociable under physiological conditions
Secondary and Tertiary Structure	Folded Partially unfolded Unfolded Amyloid
Covalent Modification	Cross-linked Intramolecular chemical modification
Morphology	Aspect ratio Surface roughness Optical properties Internal morphology

In validation, the product itself can be used as a "standard" — although the challenge is determining the real number of particles, so this material is best for validation parameters such as specificity and reproducibility. For accuracy and precision, it is best to use spherical beads because their actual number and size are known.

Actual protein material can be used for determining specificity (no interference of different matrices or dilution, and measurement over monomer), linearity, and range (showing numbers of particles can be diluted appropriately and determining LOD). For robustness assessments, analysts must understand how sample handling and subtle differences in running methods — including different sampling volumes and time between sample preparation, measurement, and washes — affect results obtained with actual products.

Combining beads and protein particles can produce some unexpected results. Sometimes beads interact with protein in a sample and either aggregate or trigger new particle formation. Studies have shown that larger-sized beads and/or protein particles can

"block" detection of smaller particles. One assumption — at least for Micro-Flow Imaging (MFI) and HIAC, in which light is used to detect the particles — is that smaller particles can hide behind larger particles passing through a detector at the same time, thereby preventing detection. Without an actual standard, a product-specific particle containing material can be created by stressing it. However, different types of stress create different types of aggregates with different characteristics, including reversibility. So choosing a stress that causes protein aggregates representative of particles found in actual drug products is a very difficult task.

Can we decide on a common nomenclature for aggregates, particles, etc.? Linda Narhi of Amgen Inc. presented a table (Table 1) created by a subgroup of the American Association of Pharmaceutical Scientics (AAPS) focus groups on protein aggregation and biological consequences to further the standardization of nomenclature around the description of particles. The manuscript and table have been accepted as a commentary (5).

To what extent do we need to identify what particles are when we find them, and when during the lifecycle do we do this? One ultimate goal of this type of analysis is to ensure product consistency while minimizing the safety risk to patients. So, we need to understand what is in our products and whether those aggregates present a safety issue. It is necessary to develop a particle characterization program throughout development to help address concerns and inform choices during process and formulation development that will minimize aggregate formation. The risks for immunogenicity seem to be more associated with protein particles, but data have shown that nonprotein particles coated in protein can be highly immunogenic as well. Significant differences in particle levels among lots or processes must be investigated. Identification is important for method development and data analysis to identify whether air microbubbles and/or silicon oil droplets, for example, are being measured. Such methods can be used to help identify sources of contamination or issues for root-cause investigations as well.

Multiple characterization and identification methods are available such as SEM–EDS, micro-x-ray fluorescence, Raman microscopy (with different detection systems), microspectroscopy, microchemistry, micro-FTIR, and "rapid ID." Such technologies can detect extremely small quantities of elements and components. For definitive identification, particles must first be isolated. Analysts must be extremely careful not to affect particles themselves or contaminate samples during this process. Substrates used for analysis are important (e.g., filters, aluminum slides, potassium bromide disks). For organic materials, analysts usually need 10- to 20-µm particles for FTIR. Raman can be used for 1-µm particle identification. Transmission electron microscopy (EM) can be used to go below 1 µm. For all methods, a good comparative database is essential.

Extrinsic and intrinsic particles are nonproteinaceous. Identifying them is an important part of root cause analysis to determine their source (cellulose, rubber, steel, glass). An understanding of their origin and developing processes to remove them should occur at all stages of product development. For inherent particles (those from a drug product itself), the nature of a product dictates the type and extent of investigation necessary. Because toxicology studies have some relevance to humans (although often are questionable for immunogenicity), you should at least understand the number of protein aggregates/particles in a product even at early stages. As a drug moves through its development cycle, process and product changes can occur, including scale up, formulation (from first-in-human to commercial), frozen to liquid storage, increases in concentration, and delivery device (e.g., vials to syringes). Including the size distribution and characterization of protein particles in comparability studies is important.

All those changes can affect the number and type of particles that can form, so particles should be counted to determine whether any changes have occurred. Characterizing particle type is also important, in case changes in the properties of the aggregate (size, conformation, stability, reversibility) have occurred from manufacturing to delivery. During product and process development, data from the levels and nature of subvisible/visible particles can be used to understand how they form (where in the process) and whether they can be controlled. Those data can help with selection of conditions to minimize particle formation. The data may also be useful to determine whether subvisible particle levels can correlate to the appearance of visible particles.

Using more than one orthogonal test is valuable because tests often provide different results, and each test may measure a different attribute. Stressing products can provide an idea of the propensity to form particles over time. Different tests should be used for the protein aggregates of different sizes, from nanometer to the micron sizes. For nanometer aggregates, techniques such as SE-HPLC with a light scattering detector, multiangle laser light scattering (MALLS), dynamic light scattering (DLS), field flow fractionation (FFF), and inductively coupled plasma (ICP) can be used, but more quantitative versions still need to be developed. In addition, understanding submicron particle morphology is difficult (AFM could be used). Newer technologies such as nanoparticle tracking analysis (by NanoSight) could be the technology of the future for looking at submicron particles, and flow cytometry is very promising. Moreover, many vendor–user collaborations are being explored to address this gap.

What control mechanisms should be developed around subvisible particles: specifications, IPCs, or alternatives? How do we develop those controls? What happens if the particle profile changes? The need for lot-release or stability tests for particles beyond compendia requirements would depend on process capability, lot-to-lot variability, method capability, and a risk assessment as to the potential impact of specific particles on safety and efficacy. It appears that biopharmaceutical industry is not currently comfortable setting specifications for subvisible particles until more is known about those factors. In-process controls (IPCs) may be required, depending on where in a process subvisible protein particles appear and/or are controlled or removed — the "validated out" concept. Action limits could be applied to ensure that at least a process is under control.

If the presence of subvisible particles

is identified as a critical quality attribute (CQA) for products in development, then the same approach for setting specifications or IPCs can be used as for any other CQA. Data from toxicology material lot release through various manufacturing phases and stability testing (consistent with clinical use) are accumulated and statistically analyzed. Regardless of specification and IPC development, analysts should obtain data for comparability studies. For legacy products, subvisible data should be accumulated for investigational purposes until a significant amount is acquired and a meaningful risk assessment can be performed. However, when and where to take that data are important considerations (e.g., before or after transport, if transport has an effect on protein particle numbers).

Whether to have a control at the drug substance step is again depends on the product, the relationship among drug substance (DS) particles and the drug product (DP). For example, if there is a clear correlation between DS particulates and visible particles in DP, then it may well be valuable to set some control at the DS stage.

If visible particles are present in DP, should you develop a semiquantitative method to at least provide some control over them? Two main methods can be used to assess particles: manual and automated inspection (e.g., Eisai). Each has its advantages and disadvantages in its ability to be controlled and qualified or validated.

Manual Inspection: People have different abilities for differentiating numbers and sizes of particles. Particle-size determination depends on several factors, including the analyst and the length of time and lighting conditions used to conduct an assay. The definition of a *visible particle* is usually one sized between 50 and 150 µm, but that remains nonstandardized across the industry, with each company deciding what level of certainty it wants. The visual manual inspection test is nondestructive but cannot count more than 10–20 particles accurately. The results for each device inspected are usually reported as accept or reject, and interassay variability is medium to low. The test is hard to "validate," usually through certification and challenge of inspectors with training sets.

Automated Inspection: The automated inspection method for visible particles is good at number and color assessment but not sizing. The technology is reproducible but is affected by product viscosity, particle mobility, and rheology of solution. Current automated inspection methods are not useful for distinguishing particle types, although they can be "trained" on the basis of buoyancy and movement in solution. Automated methods are easier to validate than manual inspections, and in the future they may be able to count more visible particles (in the range of 0–100 particles) than manual inspection. Automated instrumentation could theoretically count subvisible particles, although it would be difficult (too many particles and not easy to track) and could require improved hardware and software. In addition, container effects (e.g., fill volume, meniscus effect) must be considered, and background (e.g., scratches, glare) must be substracted. Automated technology can distinguish most particle types using various types of light sources at various positions. Automated inspection methods also are a probabilistic assay and based on acceptance quality level (AQL).

Controlling the appearance of visible particles depends on their nature. As described, extrinsic visible particles are unwanted and should be controlled once their sources are identified. Removal of all small-particle sources from manufacturing processes is practically impossible, however, and the inspection processes to remove units that contain visible particles are not 100% effective. So the concept of *practically free of particles* is essential.

The presence of protein particles is not always consistent from unit to unit. They may or may not appear on stability, and the potential risk to patients is product specific. So control (e.g., a filter before dosing) is product dependent. Some products do include terminology such as "Product may contain small, translucent protein particles." Companies often need to define what constitutes "normal" for QC lot release and stability so that deviations from normal lot-to-lot variability can be detected. A document describes the approximate number of inherent particles typically observed per unit, approximate percentage of units per lot (if not in all units), particle shape, color, and so forth.

Part 2 of this article will describe presentations given during the afternoon sessions of the forum. Speakers and panel experts discussed the immunogenicity of particles among other topics.

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