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# Subvisible (2–100 μm) particle analysis during biotherapeutic drug product development: Part 2, experience with the application of subvisible particle analysis

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ABSTRACT

Measurement and characterization of subvisible particles (including proteinaceous and non-proteinaceous particulate matter) is an important aspect of the pharmaceutical development process for biotherapeutics. Health authorities have increased expectations for subvisible particle data beyond criteria specified in the pharmacopeia and covering a wider size range. In addition, subvisible particle data is being requested for samples exposed to various stress conditions and to support process/product changes. Consequently, subvisible particle analysis has expanded beyond routine testing of finished dosage forms using traditional compendial methods. Over the past decade, advances have been made in the detection and understanding of subvisible particle formation. This article presents industry case studies to illustrate the implementation of strategies for subvisible particle analysis as a characterization tool to assess the nature of the particulate matter and applications in drug product development, stability studies and post-marketing changes.



## 1. Introduction

The compendial method for subvisible particle testing, based on USP <788> and <787>, uses light obscuration for monitoring

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particles having an equivalent circular diameter (ECD) of ≥10 μm and ≥25 μm [1,2]. As discussed in a previous manuscript [3], health authorities often request testing beyond the limitations of the compendial method and investigation of particles smaller than 10 μm for more complex biotherapeutic parenteral drug products, which may have the propensity to form proteinaceous particulate matter. Consequently, the application of particle analysis across the entire subvisible particle range has become a key consideration during the development of a biotherapeutic drug product [3–17]. In this article, we provide examples demonstrating how a variety of subvisible particle analysis techniques can be applied in practice during clinical and commercial development. The term "subvisible" applies to particulate matter of the size range defined as 2–100 μm, which may be proteinaceous or non-proteinaceous.

Subvisible particle analysis requires significant long-term data under actual conditions of drug product storage, with material made at different times and from different facilities, to understand the drug product and method variability. The key to understanding such variability in the particles and amounts seen is to have sufficient data to understand product trends in subvisible particulate matter and the ability to determine if the particulate matter is protein-based or originates from other intrinsic or extrinsic species that are also counted in quantitative subvisible particle assessments [18–20]. Collection of information on particle morphology and composition may be additional components of particle analysis and trending during development.

The case studies presented are a compilation of the selected experiences of the companies that participated in the preparation of this manuscript, and illustrate ways in which this strategy can be applied. The case studies are organized into two sections: (1) Particle Characterization and (2) Applications of Particle Characterization in Drug Product Development, Stability Studies and Post-Marketing Changes.

While the information presented is illustrative of the types of approaches being taken to evaluate subvisible particles also below 10 μm, the examples are not intended to prescribe strategies or the application of any specific techniques considered necessary to meet requirements in relation to regulatory applications for product development or licensure.

## 2. Section 1: particle characterization

### 2.1. Introduction

Light obscuration particulate matter analysis has been a key analytical tool for development of a suitable injectable drug product consistent with current pharmacopeia requirements. The development of new particle analysis techniques has led to a better understanding of particle characteristics. In particular, morphology information can facilitate deeper understanding of the origin and differentiation of the types of subvisible particles for characterization and identification of particles in samples [11,21–29].

The ability to characterize particle morphology with flow imaging technologies has led to efforts to differentiate specific particle types using a variety of image processing filters [24,27–29]. Silicone oil microdroplets and air bubbles are examples of particles that have morphological features (e.g. shape, contrast pattern) that make them relatively straightforward to identify, with reasonable accuracy. Other types of particles, including both proteinaceous and non-proteinaceous ones, can be much more challenging to identify because a unique set of morphological features is difficult to define. Flow cytometry is another technique which is being explored for its ability to differentiate between particle populations. One major difficulty that remains to be addressed is the assessment of the accuracy of the different classification procedures. The latter is typically performed using artificially generated pure samples which makes it challenging to evaluate the relevance to the mixtures present in "real life" and which so far have precluded their wide application.

In some cases further particle characterization is needed, and it can be performed with microspectroscopy techniques such as Fourier Transform Infrared (FTIR) microspectroscopy and Scanning Electron Microscopy with Energy Dispersive Spectroscopy detectors (SEM-EDS). These investigational tools can provide information on chemical composition of particles and, in some cases, give insights into their structure [2,4,5]. Identification and analysis of particles by such techniques can help to classify particles more definitively (e.g. inherent, intrinsic, and extrinsic) [5]. Particle composition and structure information can be useful for understanding root causes of particle formation [7,8] in process optimization studies.

The case studies in this section show the application of particle characterization in the following areas: (1) minimization of artifacts in particular matter analysis via optimization of a sample preparation technique [1,2,21,22], (2) classification and differentiation of particles and (3) identification with further characterization [14,16,23,30,31].

### 2.2. Case study 1.1: optimization of sample preparation for a lyophilized drug product

#### 2.2.1. Experimental

Three recombinant proteins rP 1, rP 2, and rP 3 (lyophilized drug product presentations) and formulation buffers 1, 2, and 3 (liquids) were used in the study. Lyophilized products were slowly reconstituted with sterile water for injection (SWFI) to avoid foam and bubble formation. Ten vials of each were pooled for analysis and analyzed immediately after pooling (initial), 2 h later, and after 5 min or 10 min exposure to a 20 kPa vacuum. Samples were analyzed in one replicate using a Liquid Particle Counting Systems Hiac/Royco 9703 with HRLD-400 liquid sensor, following the harmonized light obscuration particle count procedures [2,20]. Samples were also analyzed in two replicates using a Microflow Imaging™ DPA4100 instrument (Protein Simple). The MFI View Analysis Suite (MVAS) software filter screen was used to obtain separate count of subvisible particles and air bubbles.

#### 2.2.2. Results and discussion

The selective results from an optimization study of sample preparation techniques for light obscuration and flow imaging analysis using a 2 h sample hold, and 5 min and 10 min of vacuum application for rP 3 are shown in Fig. 1A, B. As has been observed by others, the number of particles counted by flow imaging was significantly higher than that obtained by light obscuration [9,16]. When the frequency counts were allocated into 1 μm bins, the highest particle count was observed in the particle range of 2–3 μm for rP 3. The number of particles was significantly reduced after application of vacuum or 2 h sample hold in rP 3, while it remained unchanged in rP 2 and rP 1 (not shown). There were also no significant differences between particle counts for all samples of rP 1, rP 2, and rP 3 exposed to vacuum for 5 min or 10 min. In some cases, vacuum application was more efficient in removing the air bubbles than the 2 h sample hold. The high particle count of the initial samples of rP 3 was due to the subvisible air bubbles, which was confirmed by light microscopy analysis of liquid samples immediately after reconstitution and after application of different sample preparation techniques (not shown).

Based on the sample optimization data discussed above, two techniques, the 10 min vacuum application and the 2 h sample