doi:10.1017/S1431927622005876
Microsc. Microanal. 28 (Suppl 1), 2022

Microscopic Analysis of Particulates in Pharmaceutical Products: A Case Study

Youlong Ma[1], Charudharshini Srinivasan[1]* and Muhammad Ashraf[1]

[1.] Division of Product Quality Research, Office of Testing and Research, Office of Pharmaceutical Quality, Center for Drug Evaluation Research, US Food and Drug Administration, Silver Spring, MD, USA
* Corresponding author: Charudharshini.Srinivasan@fda.hhs.gov

Complex parenteral products are prone to particulates issues that may pose safety and quality risks [1; 2]. There are compendial methods for characterization of particulates in a drug product described in USP<788> and USP <1788> [3; 4]. However, the USP <788> light obscuration (LO) and optical microscopy (OM) techniques for particulate measurements are limited by the morphological features of the particles if they are flaky in nature [5]. To address these challenges in this case study, three unique microscopy techniques were developed to evaluate the particulate matter in complex parenteral products including particle size, composition, and microstructure.

A stereomicroscope (Unitron Z6) was utilized for pre-screening the particles collected on a membrane filter. These particles are extremely thin, flaky and thus not visible under conventional illumination. This challenge was overcome by using the stereomicroscope equipped with a polarizer and a coaxial illuminator. To further analyze the composition and microstructure of the particles, the membrane filter was sputter coated and transferred to a field emission scanning electron microscope (SEM, TESCAN Mira3) with energy dispersive X-ray spectrometer (EDS, Oxford Max-80 SDD). The membrane filter with particles was embedded in epoxy and sectioned with ultramicrotome, the sectioned slices were placed on a grid and transmission electron microscopy (TEM, JEOL 1400) was also used to investigate the internal structure of the particles.

To demonstrate the advantage of using polarizer plus coaxial illumination over conventional oblique illumination in stereomicroscopy, images were taken with both instrumental set up and are represented in parallel (Figure 1). The optical image captured under oblique illumination barely show any contrast over the membrane filter (Figure 1A), while the one collected with the assistance of polarizer and coaxial illuminator was able to clearly visualize the thin, irregular flakes (Figure 1B). The particle flakes range in color from brown (for extremely thin flakes) to red and blue (for thicker flakes). With the stereomicroscopy confirming the existence of particles, the next step was to utilize SEM-EDS to obtain images of particles at high magnification as well as gather compositional information simultaneously. Figure 2A shows a typical region of the membrane filter covered with several particulate flakes. EDS spectrum revealed that these flaky particles are composed of silicon and other elements potentially from the glass container and iron (Fe) from the drug product formulation (Figure 2B). To investigate further, TEM-EDS was used that offers new opportunities for nanometer scale analysis of such particulate flakes. As shown in Figure 3, the cross-section of the particles comprises of two regions, the dark spots of iron and the flaky particle of iron and glass elements.

In summary, particulates could compromise the quality of the drug product and pose risks for safety concerns. Our investigation through this case study revealed that particulates can originate due to complex interaction between drug formulation and the container closure system. Light microscopy and chemical analysis using electron microscopy were valuable tools for identification of these unique flake-

like particle. TEM may be implemented to identify the nanostructures within the particle that further reveals the mechanism of particle generation. The combination of different high resolution microscopic techniques has proven its robustness on particulate identification that could be employed for the prospective investigation of particulates in drug products.

Disclaimer: This article reflects the views of the author and should not be construed to represent FDA's views or policies.

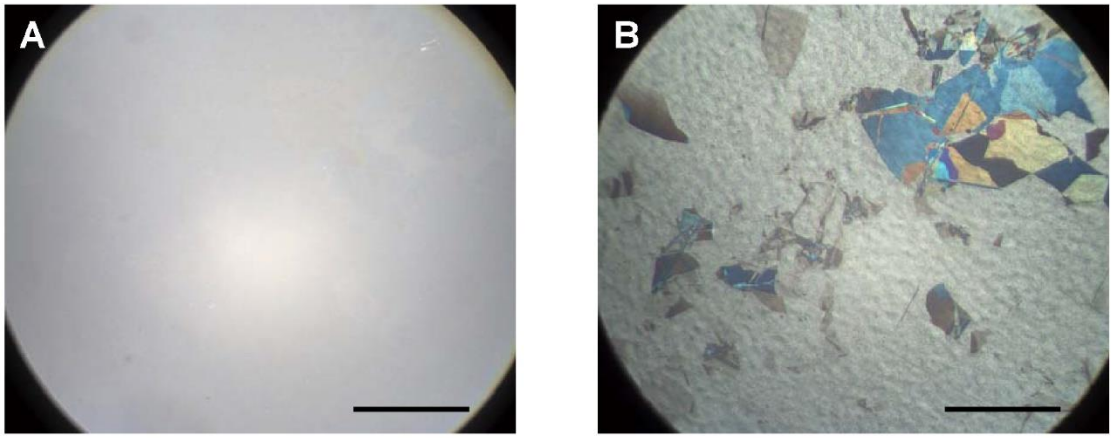

Figure 1. Stereomicroscopy images of particulates on membrane filter from a complex parenteral drug product. A) Micrograph of membrane filter captured under oblique illumination. B) Micrograph (same area as A) visualized with the aid of polarizer and coaxial illuminator. *Scale bar is 500µm.

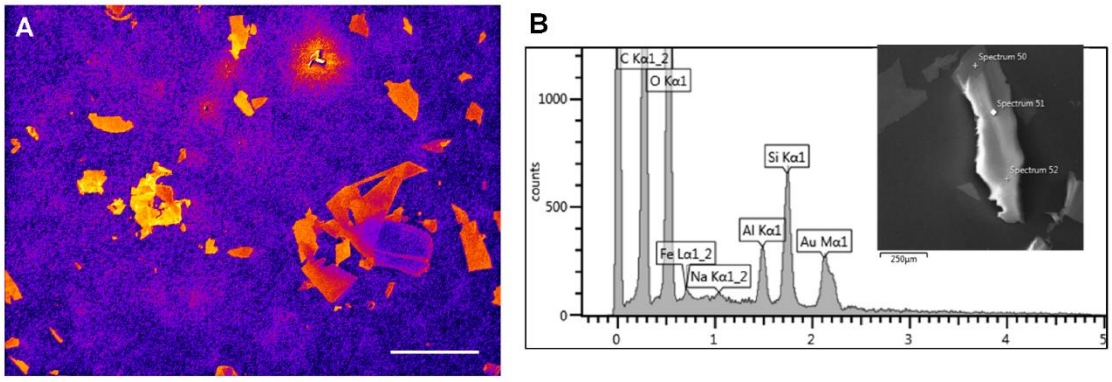

Figure 2. SEM-EDS Image and elemental analysis of particulates from a complex parenteral drug product. A) A SEM micrograph of flaky particles (pseudo colored) on membrane filter. B) Corresponding EDS spectrum shows that the particle is composed of Si, Fe, Al, and Na. *Scale bar is 500µm.

https://doi.org/10.1017/S1431927622005876 Published online by Cambridge University Press

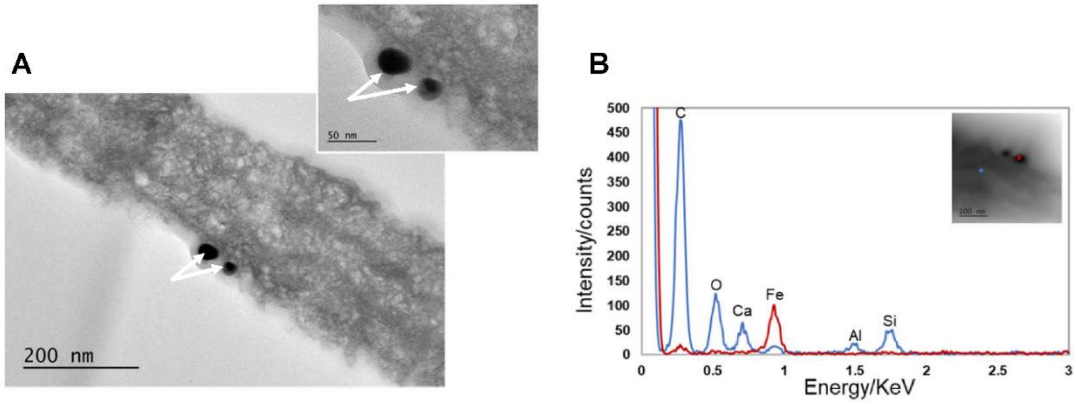

Figure 3. TEM-EDS analysis of particulates following ultramicrotomy. A) A TEM micrograph of colloid particle (highlighted by white arrows) embedded within a flaky particulate. B) Corresponding EDS spectrum confirms that the colloids are composed of iron (spectrum in red) within the flaky particulate, which is a complex of glass elements and iron (spectrum in blue).

References:

[1] Ef Schumacher, et al. Microscopy and Microanalysis 21 (2015), p. 395-396.
[2] C. Srinivasan, et al. International Journal of Pharmaceutics (2019), p. 118510.
[3] USP <788> Particulate Matter in Injections. Rockville, MD: US Pharmacopeia, (2013).
[4] USP <1788> Methods for the Determination of Particulate Matter in Injections and Ophthalmic Solutions. Rockville, MD: US Pharmacopeia, (2018)
[5] Y. Ma, et al. International Journal of Pharmaceutics 596 (2021), p. 120248.

https://doi.org/10.1017/S1431927622005876 Published online by Cambridge University Press