rap.ID Single Particle Explorer® Application Note

Chemical ID of visible protein agglomerates in wet-dispersion.AID

Formation of protein agglomerates

Development of protein based formulations exhibit a number of challenges as protein can undergo a variety degradation pathways. One of the most common changes that protein formulators experience is aggregation, resulting in formation sub-visible and visible particles that can negatively impact the drug's performance. Protein agglomeration can be induced by various factors. Some common factors factors include temperature, light and shake stress; degradation of polysorbates followed by formation of fatty acids and a decrease in polysorbate concentration; silicone induced protein aggregation; and presence of tungsten particles in prefilled syringes.

Identification of protein agglomerates with the Single Particle Explorer

Protein aggregation was induced by introducing an acidic tungstate solution to a protein solution. A small portion of the mixture containing a visible particle was then withdrawn with a pipette and transferred into a vial with particle free water. A picture of a particle in the vial was then taken with a photo camera (Figure 1). Inverted microscope was then used to capture a picture of the particle *in situ* (Figure 2). A portion of the solution (100 µL) containing the visible particle was withdrawn with a pipette and deposited into the wet-dispersion.AID. Sample was covered with a glass window and introduced to the Single Particle Explorer. Particle was manually located and photographed with 50x objective (Figure 2). Raman Spectroscopy was performed on the particle with 532 nm laser excitation, laser power of 100% and integration time of 30s. Particle spectrum matches reference spectrum of a protein with a rank value of 882.

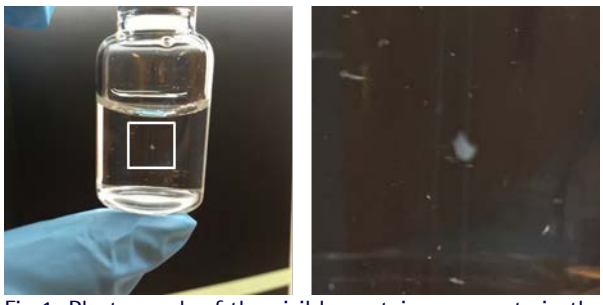

Fig.1: Photograph of the visible protein aggregate in the container. The image on the right is a zoom-in of the section indicated by white frame on the left.

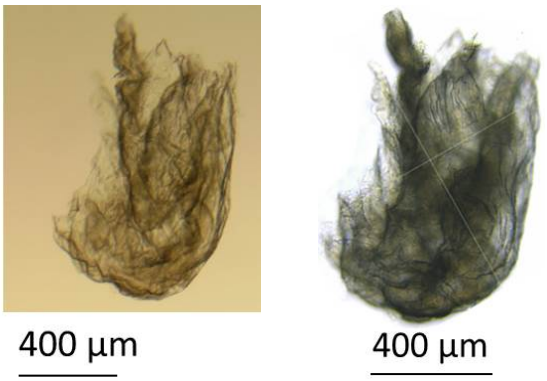

Fig.2: Left: *In situ* photograph in the closed vial; Right: SPE 50x photograph of the visible protein aggregate in the wet-dispersion.AID.

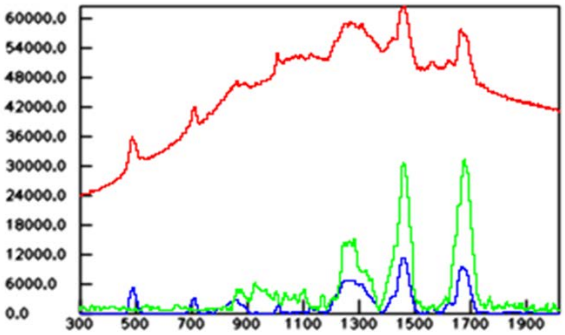

Fig.3: Raman spectrum of the visible protein agglomerate. Laser intensity: 100%, integration time: 30s. Match: Protein Rank 847. Silicone signals at 500 and 700 cm^{-1}

Easy sample prep and particle preservation

- Wet-dispersion.AID allows direct correlation between particles observed in a container by visual inspection processes according to the updated USP <1790> and particles being analysed.
- In our example a visible particle was photographed *in situ* and transferred to a wet cell. Image of the particle taken with SPE is identical to *in situ* image as can be seen in Figure 2.
- The 500 µm large clearly visible particle could be transferred easily into the wet-dispersion.AID and was found by the image analysis of the SPE within minutes.
- Raman spectroscopy revealed within 30 seconds the chemical nature of the particle. It is a mixture of Silicone and Protein, therefore the root cause investigation just took 10 minutes.
- The use of wet-dispersion.AID ensures the sample integrity and identification of the particles of the interest.

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