Hound identifies visible protein agglomerates in solution

Introduction

The development of protein based formulations presents a number of challenges as proteins can undergo a variety of degradation pathways. One of the most common challenges that protein formulators face is aggregation that results in the formation of sub-visible and visible particles, which can negatively impact drug efficacy. Protein agglomeration can be induced by various factors. Some common factors include temperature, light, shake stress, degradation of polysorbates, silicone induced aggregation and tungsten induced aggregation from syringe contamination.

Methods

Protein aggregation was induced by adding 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 A small portion of the mixture containing a visible particle was then withdrawn with a pipette and transferred into a vial with particle free water for *in situ* imaging. The particle was transferred to a wet round and was loaded into Hound. The particle was located and Raman spectroscopy was performed with 532 nm laser excitation, laser power of 100% and exposure time of 30 s.

Results

A particle in the vial was photographed (Figure 1). An inverted microscope was then used to capture a picture of the particle *in situ*. A portion of the solution (100 µL) containing the visible particle was withdrawn with a pipette and deposited onto the wet round. The sample was covered with a glass window and placed into Hound. The particle was manually located and photographed with a 50x objective (Figure 2). The particle was identified as a mix of protein aggregate and silicone by matching to a reference spectrum and observing silicone peaks at 500 and 700 cm^{-1} (Figure 3).

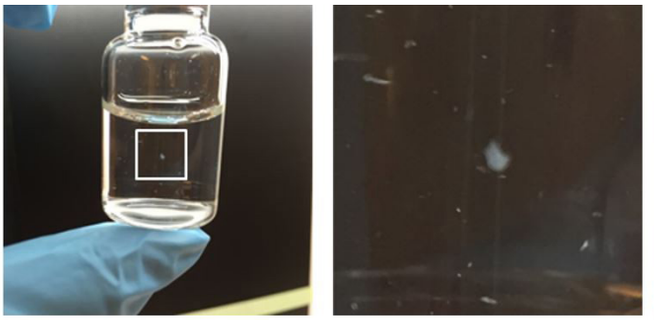

Figure 1: Photograph of a visible protein particle in a vial. The image on the right is a magnification of the white frame area on the left.

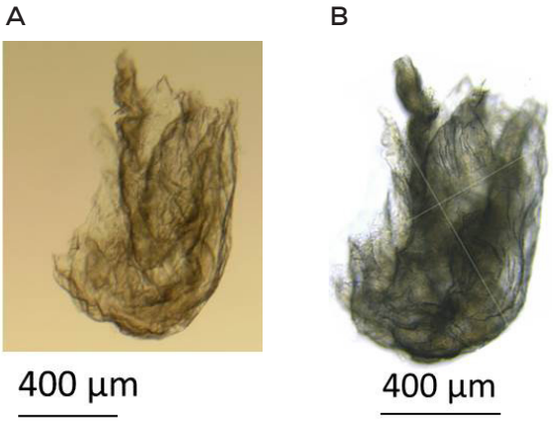

Figure 2: **(A)** *In situ* photograph of the closed vial; **(B)** Hound 50x photograph of the visible protein aggregate in the wet round.

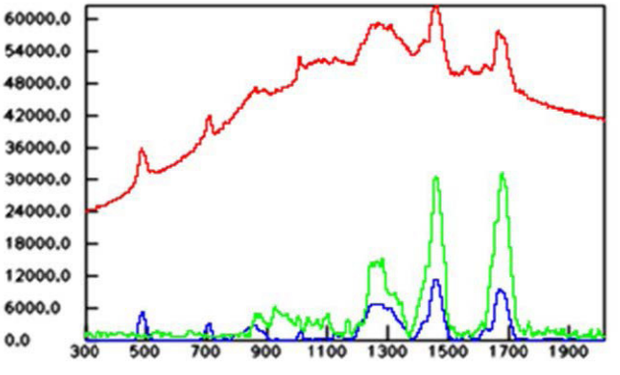

Figure 3: Raman spectrum of the visible protein agglomerate. Laser intensity: 100%, integration time: 30s. Match: Protein Rank 882 with silicone signals at 500 and 700 cm^{-1}.

Summary

A 500 µm visible particle was easily transferred onto a wet round for identification by image analysis and Raman on Hound. Use of a wet round in Hound allowed for the direct correlation between particles observed and particles analyzed in a container by visual inspection processes according to USP ‹1790›. Within 30 seconds Raman spectroscopy revealed the chemical nature of the particle to be a mixture of silicone and protein resulting in a root cause investigation that took 10 minutes.

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