rap.ID Single Particle Explorer® Application Note

in situ Raman study on Polysorbate Induced Protein Agglomeration

Polysorbate degradation in biopharmaceuticals can induce particle formation

Polysorbate is a surfactant commonly used in protein based pharmaceuticals to stabilize formulations. Degradation of polysorbates via hydrolysis causes the formation of fatty acids and results in a decrease in polysorbate concentration. This leads to a concern for protein solubility in the formulation. Further, due to the lipophility of the biopharmaceutical active ingredients the insoluble fatty acid particles might induce agglomeration.

In situ Raman identification

Polysorbate 20 (PS20) was degraded by heating 0.05% (w/v) solution of PS20 with 300 ppm hydrogen peroxide at 80°C for 7 days. A model protein solution based on bovine serum albumin was mixed with resulting degraded PS20 and placed into the wet cell.

The sample in the wet cell (wet-dispersion.AID, rap.ID) was evaluated by means of the automated 532 nm Raman system, the Single Particle Explorer (SPE), rap.ID. Dark field illumination delivers a strong contrast of particles and stainless steel background of the measurement chamber. Fully automated binarization algorithms (static image analysis) performed sizing, localization and further morphological analysis of even submicrometer particles in the suspension.

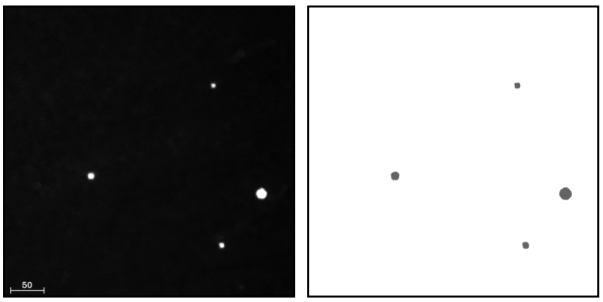

Fig. 1: Original dark-field grayscale image (left) and processed binarized image (right).

The SPE analyzed an area of 20.3 x 20.3 mm within 15 minutes and generated a particle size distribution table.

In a fully automated process the instrument picked a selection of particulates, aligned and exposed them to the Raman 532 nm laser at a power of 5 mW. It processed the spectra and compared them with the spectra library. Matches exceeding a quality level were in Tab. 1

Fig.2: Wet cell in a sample holder, layer thickness was set to 400 µm resulting in a sample volume of 700 µL

Results from the morphologically guided Raman identification

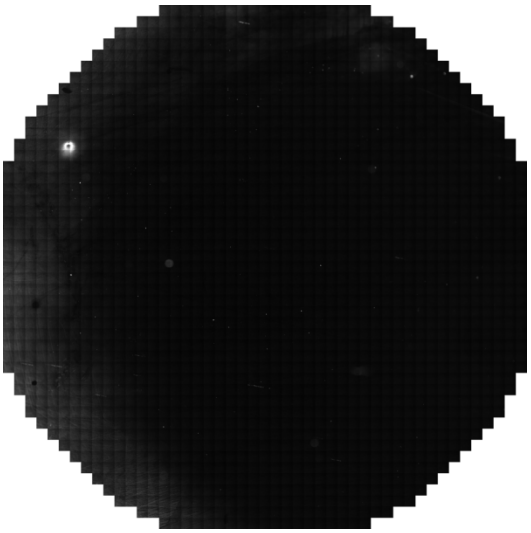

Fig.3: mosaic of dark field images of the wet cell. Total size: 20.3x20.3 mm.

The total number of particles detected and identified in 700 µL area of the wet cell is shown in Table 1.

Tab. 1: Particle size distribution and best match with the library

	2-5 µm	5-10 µm	10-50µm	> 50µm
Protein	45	12	2	1
Air bubbles	0	0	2	12
Fluorescence	4	1	0	1
All	2107	523	335	39

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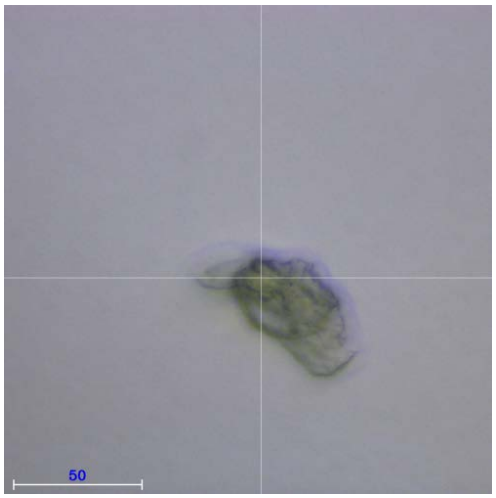

Fig. 4: 50x microscope image of a 60 µm protein agglomerate

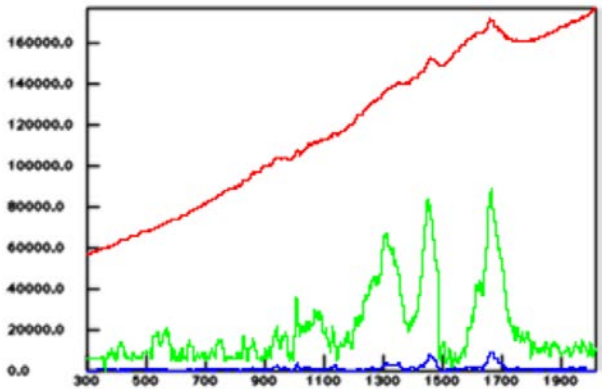

Fig. 5: Raman (532 nm), 30s spectrum of a 60 µm protein aggregate shown in Fig. 4 (red original, blue optimized, green database match)

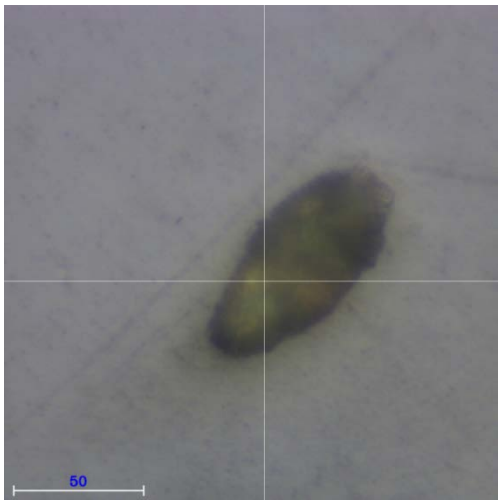

Fig. 6: 50x microscope image of a 100 µm lauric acid particle

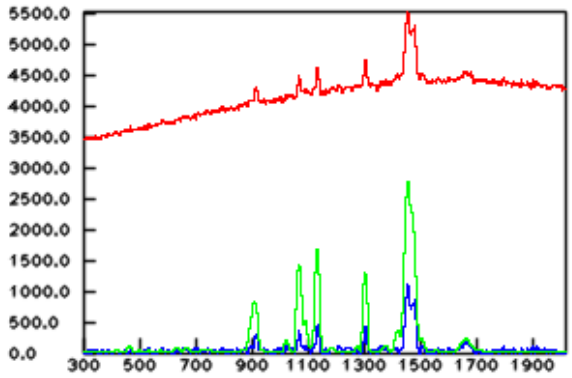

Fig. 7: Raman (532 nm), 10s spectrum of a 100 µm fatty (lauric) acid shown in Fig. 6 (red original, blue optimized, green database match)

Chemical ID with minimum sample preparation and maximum sample integrity

Sample preparation of semi solid or amorphous particles on a filter is a challenge. It is even more challenging in the control of protein based formulations. Particulates could be drawn through the filter pores or silicone droplets might spread on the surface. It is known that Protein particulates lose their shape and appear as thin films on filter membranes such as the filtr.AID, rap.ID.
There is furthermore discussion that sheer forces during filtration might cause coagulation and agglomeration of protein prior or during the deposition on a filter. Sample integrity is maximized when the particles are kept in the suspension as it is in the comparable Micro Flow Imaging, MFI technique.
It is shown that in the novel Raman optimized wet-dispersion.AID in combination with the green 532 nm SPE ls raman.ID opens the window to chemical information on particulate matter in protein based formulation. This generates valuable information on the pathways of particle formation as well as stability data with previously unseen speed and depth of information.