In situ Raman study on Polysorbate induced protein agglomeration with Hound

Introduction

Polysorbate is a surfactant commonly used to stabilize protein formulations. Degradation of polysorbates via hydrolysis causes the formation of fatty acids. This degradation is a potential trigger for protein aggregation since insoluble fatty acid particles can induce agglomeration. This application note describes how you can use Hound to count, size and identify protein particles resulting from surfactant degradation (Figure 1).

Figure 1: Hound counts, sizes and identifies particles by automated image directed Raman and Laser-Induced Breakdown Spectroscopy.

Methods

Polysorbate 20 (PS20) was degraded by heating a 0.05% (w/v) solution with 300 ppm hydrogen peroxide at 80 °C for 7 days. Bovine serum albumin was mixed with the degraded PS20 and loaded onto a Hound wet round. The sample was analyzed by automated Raman spectroscopy at 532 nm. Fully automated binarization algorithms performed particle sizing, localization and morphological analysis for sub-visible and visible particles in the suspension. Hound analyzed an area of 20.3 x 20.3 mm within 15 minutes to generate a particle size distribution (Figure 2).

Results

Hound automatically picked a selection of particles, aligned and exposed them to the Raman 532 nm laser at a power of 5 mW, processed the spectra and compared them with the spectra library to complete particle identification. A total of 3,004 particles were identified by Hound (Table 1). Of the selected particles scanned by Raman, the majority of particles, from 2 µm to >50 µm, were identified as protein aggregates (Figures 3 & 4). A small population of contaminants with strong fluorescence profiles and several air bubbles were also identified.

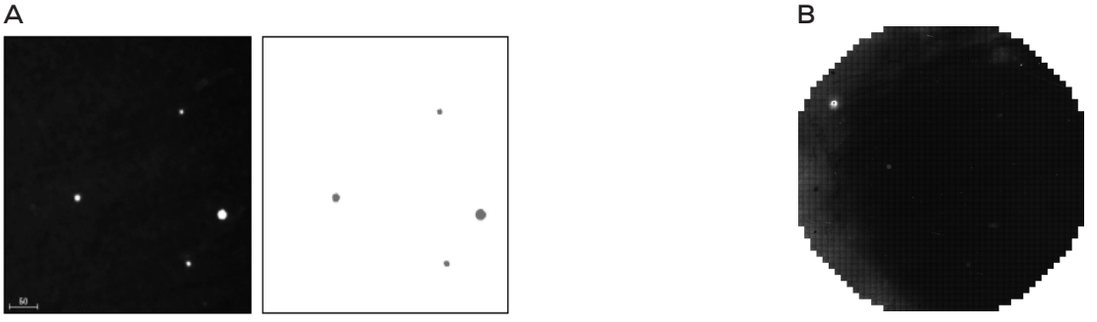
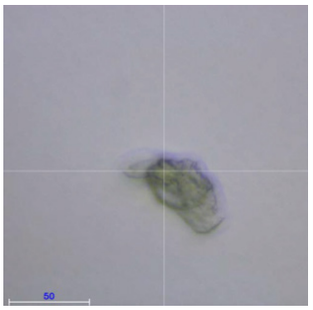


Figure 2: **(A)** Original dark-field grayscale image. **(B)** Processed binarized image.

A



B

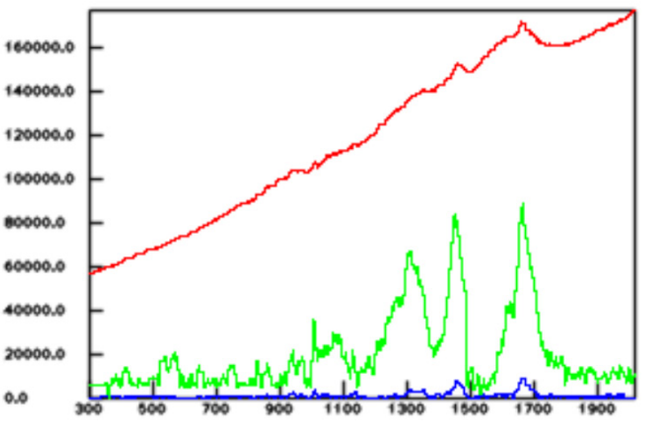
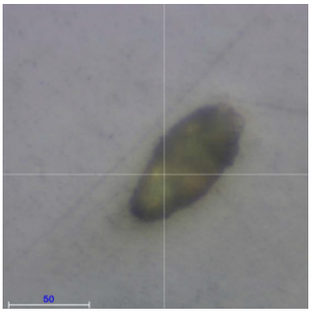


Figure 3: **(A)** 50x microscopic image of a 60 µm protein aggregate. **(B)** Raman (532 nm), 30s spectrum of the protein aggregate (red original, blue optimized, green database match).

	2-5 µm	5-10 µm	10-50 µm	>50 µm
Protein	45	12	2	1
Air bubbles	0	0	2	12
Fluorescence	4	1	0	1
Total	2,107	523	335	39

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

A



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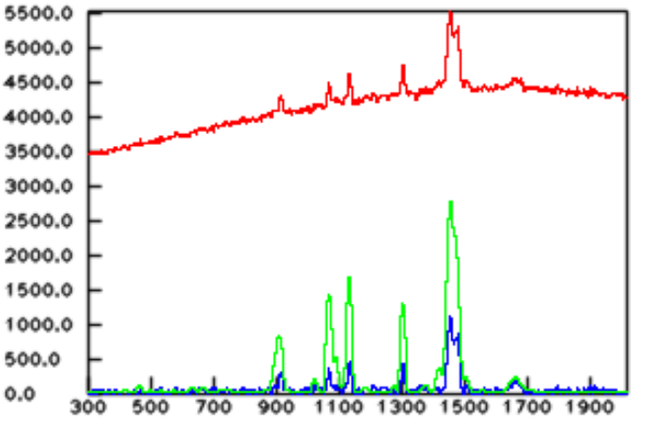

Figure 4: **(A)** 50x microscopic image of a 100 µm lauric acid particle. **(B)** Raman (532 nm), 10s spectrum of the 100 µm lauric acid particle (red original, blue optimized, green database match).

Summary

Sample preparation onto filters of amorphous particles, like protein agglomerates, can be challenging. These particles can be drawn through the filter pores, lose their shape and appear as thin films. Shear forces during filtration may cause aggregation of proteins prior to or during collection. Sample integrity can be maximized by keeping the particles in suspension.

By using a wet round with Hound equipped with a 532 nm Raman laser, amorphous particles can be counted, sized and chemically identified. This generates valuable information on the root cause of particle formation.

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