Measuring silicone oil interaction with protein using Hound

Introduction

Many biologic drugs are lipophilic, meaning they are prone to strong negative interactions with the silicone oil used to lubricate prefilled syringes and vials. Protein – silicone oil interactions can cause protein aggregation and the formation of visible particles during storage which can lead to a decrease in product potency. Information about these interactions on a microscopic level is highly valuable for biologic drug stability and safety. With a complete understanding of what conditions cause these protein – silicone oil interactions, it is possible to minimize the chance of aggregation due to silicone oil in a formulation.

Hound counts, sizes and identifies particles by automated, imaged directed Raman or Laser-Induced Breakdown spectroscopy (Figure 1). Hound can distinguish between pure protein aggregates and particles made of silicone and protein, providing insight into the root cause of particle formation. In this application note, Hound is used to determine the chemical composition of active pharmaceutical ingredient (API) particles in a protein formulation where silicone oil interactions were a suspected cause of aggregation.

Figure 1: Hound images, sizes, counts and identifies particles with Raman or Laser-Induced Breakdown Spectroscopy.

Methods

A biologic drug formulation was filtered through a filter round with a 5 µm membrane cutoff. The filter round was then washed 3 times with 10 mL of particle free WFI (0.45 µm syringe filtered). The filter round was analyzed by Hound with automated microscopy and image directed Raman spectroscopy. Integrated dark field microscopy and subsequent image analysis found sub-micrometer thick layers on the surface of the filter round and counted these layers as particles. Hound analyzed an effective filtration area (EFA) of 5.6 x 5.6 mm to acquire the particle size distribution of the sample in 3 minutes.

Automated image directed Raman Spectroscopy was carried out on a subset of particles in the sample. Raman spectra were collected with a 532 nm Raman laser with an exposure time of 5 seconds per particle. In 3 hours Hound determined particle count, size distribution, and chemical composition.

Results

Particles of various compositions, some of which were very large visible particles, were identified in the protein sample (Figure 2). Of particular interest in this study were particles composed of pure protein API and those composed of silicone oil mixed with protein API. Raman spectra of protein particles were successfully matched to the pure protein API reference spectrum by Hound (Figure 3). Mixed particles of silicone oil and protein API were also identified (Figure 4).

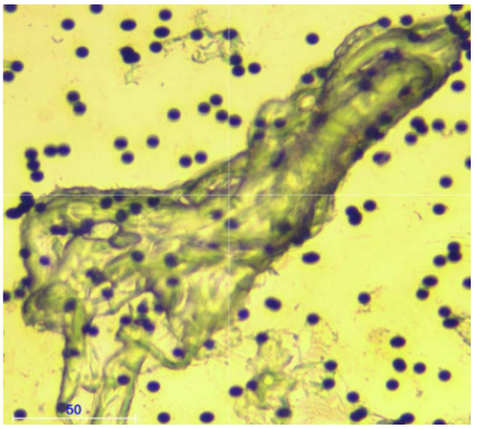

Figure 2: A dark field image of a 335 µm particle identified from a protein API sample.

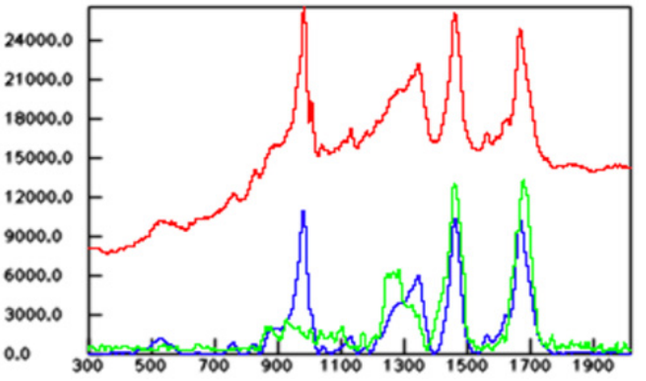

Figure 3: Raman spectra of a protein API particle matched by Hound to the reference Raman spectrum of the protein API with the original Raman spectra (red), processed spectrum (blue) and API reference (green).

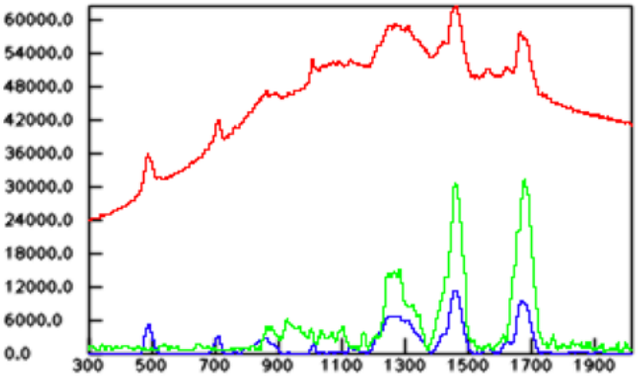

Figure 4: Raman spectra of silicone oil mixed with a protein API particle matched to the reference API spectrum with the original Raman spectra (red), processed spectrum (blue) and API reference (green).

The size distribution and identity of the particles are shown in Table 1. The majority of particles in the sample were identified as silicone mixed with protein API, indicating that silicone oil contamination in the sample was a contributor to particle formation. While other contaminants were identified (such as glass, rubber stopper and Tween 80), most particles in the sample were silicone oil, protein API, or a mixture of the two. Knowing that silicone oil is the cause of most protein aggregates in this formulation, the thickness and distribution of silicone oil on the syringes and vials used with this protein formulation can be investigated and optimized.

Particle size	2-5 µm	5-10 µm	10-25 µm	25-50 µm	50-100 µm	≥ 100 µm
Silicone	380	54	18	5	0	0
Glass	143	24	3	1	0	0
Stopper	53	12	8	3	0	0
Silicone + API	1,278	678	287	153	73	15
Protein API	167	76	32	6	0	0
Tween 80	45	13	4	1	0	0
Cellulose	2	19	13	5	1	0
Total	2,068	876	365	174	74	15

Table 1: Particle size distribution by chemical composition of each particle.

Summary

Hound quickly determines the chemical or elemental composition of particles in protein formulations to get to the root cause of protein aggregation. Automated particle analysis and image directed Raman spectroscopy with Hound allows scientists to investigate particle formation due to interactions with the silicone oil used to lubricate syringes and vials. Hound allows for root cause analysis of protein aggregation, making it possible to take the necessary steps to prevent aggregate formation.

Unchained Labs
6870 Koll Center Parkway
Pleasanton, CA 94566
Phone: 1.925.587.9800
Toll-free: 1.800.815.6384
Email: info@unchainedlabs.com

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