Analytical method development to characterize subvisible particles using morphologically directed Raman spectroscopy

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Abstract/Introduction

Particles are a critical quality attribute of injectable protein therapeutics having potential risks associated with drug safety and efficacy. Particles are categorized into visible (> 100 µm), subvisible (1-100 µm), submicron (100-1000 nm), and nanometer (<100 nm) particles. Particles could be proteinaceous and non-proteinaceous in composition with various shapes. To control subvisible particles (SVP) in protein therapeutics, it is important to evaluate particle size, shape, and composition as well as the size distribution and counts. The current compendial method Light Obscuration (LO) and its orthogonal methods such as Flow Imaging Microscopy (FIM) and Membrane Microscopy (MM) have been routinely utilized to analyze SVPs in protein therapeutics for lot release based on USP guidelines. Though the compendial methods can provide information on the particle size, shape, and count, none of them can distinguish particle composition or identification. Herein, we introduce a novel analytical tool, morphologically directed Raman spectroscopy (MDRS), for SVP characterization. The benefit of this method is that particles can be identified based on its Raman spectrum along with particle size, shape, and size distribution. Three particle standards – polystyrene beads, a traditional particle standard currently used in industry, and SU-8 and ETFE, new particle standards developed by NIST – were used for method development. The new standards have been developed by NIST as proteinaceous particle surrogates to support the development of SVP analysis methods by having appropriate standards. In our study, MDRS rendered high resolution images for ETFE standard (> 90%) ranging from 19 µm to 100 µm, covering most SVP range, and generated more accurate or comparable physical property evaluation data to FlowCam. Our data also reported that among all the standards, ETFE particle standard showed the closest morphology to proteinaceous particles. After establishing the feasibility of the MDRS method and appropriate standards, we analyzed proteinaceous SVPs. To our knowledge, this is the first report of characterizing proteinaceous SVPs in protein therapeutics through individual particle identification using MDRS. Our study shows that MDRS may contribute to improve the current SVP analysis system in protein therapeutics quality control.

Methods

Flow Imaging Microscopy (FIM) FlowCam 8400 (Fluid Imaging Technologies, Inc.) equipped with a multi-use flow cell (80 µm x 700 µm, d x w) and a 10X magnification lens was used for this study.

Morphologically-Directed Raman Spectroscopy (MDRS) ETFE, SU-8, polystyrene bead, and protein aggregates were analyzed using a Morphologi 4-ID MDRS instrument (Malvern Panalytical Ltd).

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Results and Discussion

Table 1. Comparison of ETFE optical images and their processed images generated by the MDRS system as shown on the top table. The percentage of high-resolution images were shown on the bottom table.

Particle ID	CE Dimeter (µm)	Optical Image	Processed Image
1689	56.6		
2523	25.1		
3031	20.1		
3399	17.4		
1176	16.1		
1958	15.3		

Particle Size	High-Resolution Image (%)
19 µm ≤ CE Diameter	> 90
18 µm ≤ CE Diameter < 19 µm	78.6
17 µm ≤ CE Diameter < 18 µm	76.9
16 µm ≤ CE Diameter < 17 µm	70.0

Table 2. Morphological data of SU-8 standard calculated by FIM and MDRS. SU-8 standard was developed to evaluate size and shape analysis algorithms on different instruments.

Method	FIM	MDRS
CE Diameter (µm)	75.92 ± 2.99	74.28 ± 0.97
Length (µm)	152.9 ± 2.52	148.67 ± 0.89
Aspect Ratio	0.27 ± 0.02	0.34 ± 0.01
Circularity	0.34 ± 0.02	0.25 ± 0.02
Convexity	0.78 ± 0.01	0.75 ± 0.02

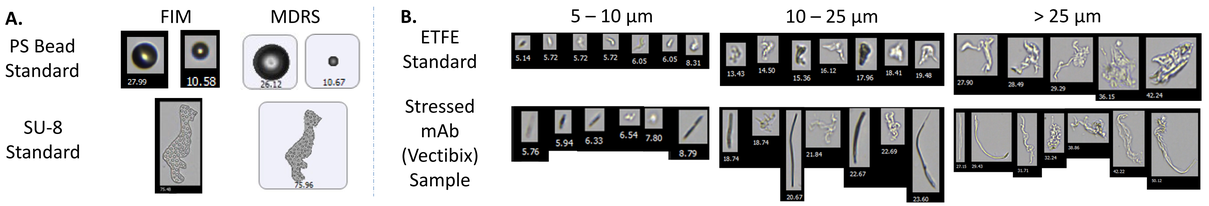

Figure 1. Representative images of polystyrene (PS) bead standard, SU-8 standard, ETFE standard, and particles found in a stressed mAb sample. **(A)** Images of monodisperse particles (PS beads on top and SU-8 on bottom) captured by FIM (left) and MDRS (right). **(B)** Images of polydisperse particles (ETFE on top and mAb on bottom) captured by FIM. Vectibix was subject to a combined stress (70 C and 300 rpm) for 30min.

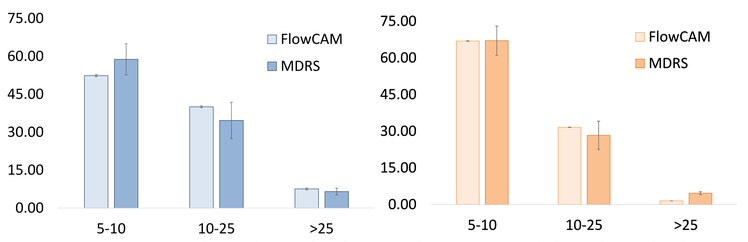

Figure 2. A comparison of particle size distributions of ETFE standard (left) and particles found in a stressed mAb (Vectibix) sample (right) calculated using FIM and MDRS.

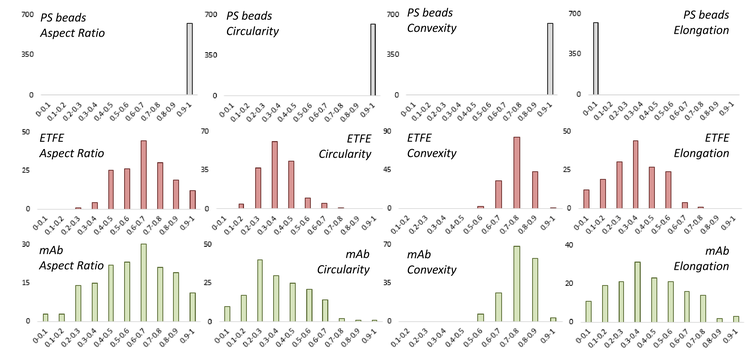

Figure 3. Morphological data histograms of polystyrene beads, ETFE standard, and a stressed mAb (Vectibix) stressed measured by MDRS.

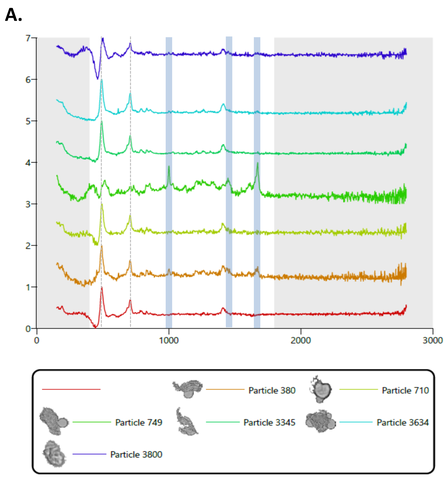

Figure 4. Identification of silicone oil-covered protein aggregates. Representative Raman spectra of silicone oil particles and protein aggregates formed in stressed insulin drug products. In Raman spectra, characteristic peaks of silicone oil were highlighted with gray dashed lines at 490 cm^{-1} and a doublet at 689 cm^{-1} / 710 cm^{-1} and those of protein aggregate were highlighted in blue regions at 1000 cm^{-1}, 1400 cm^{-1}, and 1670-1690 cm^{-1}.

Using polystyrene (PS) bead standard, two NIST particle standards, and a stressed mAb (Vectibix) sample, MDRS method to characterize proteinaceous subvisible particle has been developed. The performance of MDRS was compared with the FIM system and assessed in terms of image quality analysis (**Table 1-2, Figure 1**), size distribution analysis (**Figure 2**), and morphological data analysis (**Figure 3**). Chemical identification using Raman spectroscopy was applied to identify particles in the ETFE standard and the stressed protein drug product samples (**Figure 4**).

Conclusion

MDRS provided high-quality images for monodisperse particles and polydisperse particles having CE diameter > 17-19 µm, while FIM rendered high-resolution images for all the particles. Morphology and size distribution data processed by MDRS and FIM were compared, showing similar results. Our data supported ETFE standard as a suitable proteinaceous particle surrogate, compared to PS bead standard. Chemical identification using Raman spectroscopy was able to identify protein aggregates and excipient particles in the stressed protein drug product samples.