Analysis of Particle Agglomeration and Content Uniformity by Raman Imaging

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ABSTRACT SUMMARY

Content uniformity and drug particle agglomeration affect dissolution and product performance of most controlled-release pharmaceutical products. In this work, methods and results of an assessment of drug particle size and the extent of drug particle agglomeration will be shown using wide-field and confocal Raman imaging methods.

INTRODUCTION

Examination of drug particle size distributions (PSD), the extent of drug particle aggregates and content/blending uniformity is an important part in characterization of pharmaceutical products as it directly correlates with the dissolution rate of the Active Pharmaceutical Ingredient (API). Such characterization is also necessary for measuring product quality as well as establishing bioequivalence (BE), evaluating out of specification issues and addressing pharmaceutical anti-counterfeiting. This task is particularly critical for controlled release products with multiple actives and excipients since traditional analytical methods are not suitable for in-depth characterization of these complex formulations. Drug-specific PSD reporting is a challenging task for multiple ingredient formulations, including controlled-release capsules, beads, bi-layered tablets, stents and other advanced delivery systems.

We analyzed a pharmaceutical product with one active ingredient and numerous excipients to demonstrate that Raman imaging provides more objective measurement for ingredient-specific PSD. Morphology-driven confocal Raman spectroscopy and wide-field Raman Chemical Imaging (RCI) have shown promise for drug-only sizing by distinguishing particles by chemical makeup [1]; however, for drug particles agglomerate and aggregate assessment diffraction-limited imaging resolution is needed for accurate assessment of agglomerates, especially in suspension-like formulations.

In this work, confocal Raman spectroscopy and hyperspectral Raman imaging were employed to measure and compare populations of API-specific particles as well as size and number the drug aggregates in the dosage form of a nasal spray formulation.

EXPERIMENTAL METHODS

Rhinocort Aqua® nasal spray suspension (32 mcg Budesonide, AstraZeneca, Wilmington, DE) containing an insoluble corticosteroid API (Budesonide) and multiple excipients was analyzed to identify drug particles and aggregated drug particles in the sample using Wide-Field Raman Chemical Imaging System (FALCON II™ ChemImage Corporation, Pittsburgh, PA) with 532 nm laser excitation and a Bruker Senterra Confocal Raman microscope with 532 nm laser excitation, 400 g/mm grating (40-4450 cm^{-1} spectral range) and Opus 6.5 software.

An actuated sample (Figure 1) was analyzed as opposed to bulk samples in order to characterize the potential influence of the actuation device on deagglomeration.

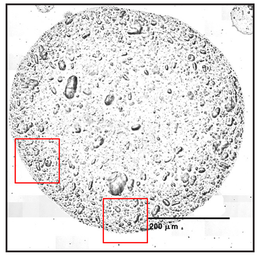

Figure 1. Brightfield image of the Rhinocort Aqua® droplet and Fields of View (FOV) in red used to illustrate ISPS analysis and Agglomerate Identification and Sizing analysis.

Confocal Raman spectra of several particles possessing morphological properties of the API were obtained (Figure 1) and evaluated against a spectral library of Budesonide (Figure 2) for chemical identification of the particles.

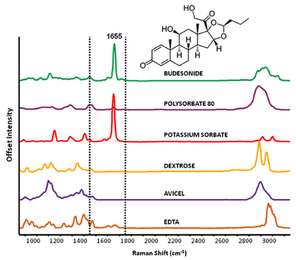

Figure 2. Raman dispersive spectra of Rhinocort Aqua® formulation components. The vertical dashed lines define the spectral boundaries used for the Raman Chemical Imaging measurements

Optical microscopy and RCI were used to measure the Budesonide PSD in the suspension. The spectral range for the RCI measurements was chosen to include a characteristic C=C feature at 1655cm^{-1} that can be used to discriminate Budesonide from all other excipients in this particular formulation. Imaging data was analyzed using the ChemImage Xpert™ software package (Version 2.3.1, ChemImage Corporation, Pittsburgh, PA) yielding both the Raman / brightfield fusion images. Resulting data was assembled to yield a representative PSD for Budesonide API/API agglomerates.

RESULTS AND DISCUSSION

Figure 3 shows a representative field of view of the suspension sample evaluated for drug particle size and aggregated drug particle size using confocal Raman imaging.

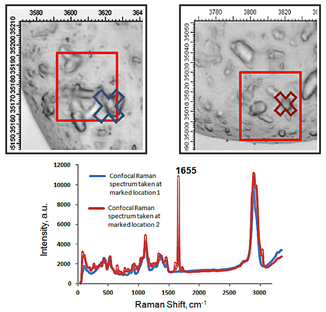

Figure 3. Representative fields of view of a Rhinocort Aqua® droplet showing a Budesonide particle identified by morphology-guided confocal Raman spectroscopy (red) and corresponding confocal Raman spectrum (red spectral trace) of examined particle.

Raman spectra of market location 1 (shown in blue) and location 2 (marked in red) confirm the chemistry of the particles possessing morphological parameters of the API; however, it is challenging to address drug-to-drug or drug-to excipient agglomeration, especially if drug particle edges are not well defined in the optical image due to placebo/other excipient co-location. In addition, not all particles possessing morphological properties of API can be successfully identified as drug particles by their Raman spectrum (marked location 1).

This challenge is easily addressed by wide-field Raman imaging. Brightfield/ Raman Chemical fusion images of the same field of view measured by confocal Raman imaging was selected for ISPS analysis by RCI (Figure 4). Automated particle detection and sizing [2] was performed using the RCI hypercube collected at these FOVs from 1640 to 1680cm^{-1}. The intensity within the Budesonide spectral peak was integrated at each pixel to create a working image with a higher signal-to-noise ratio than the peak intensity plane alone. The resultant working image showed potential API particles as bright regions on a dark background (Figure 3). Sizing the objects of interest was performed by individually processing the edges and brightness of each detected object based on Raman scattering detected at each pixel.

The presence of placebo or co-localized excipients in neighboring pixels does not interfere with detection of the API-specific Raman signal from the drug particle alone, thus making API particles identification more accurate and objective than morphology guided Raman confocal microscopy.

Neighboring objects are separated individually by applying a unique local threshold based on the intensity at object edges, which is iteratively determined for each detected object. Standard image analysis routines then used to compute the sizes and shapes of detected objects: the spectral shape of each object is verified after detection and sizing; a "shape" constraint is imposed on the average spectrum of an object so that it must have a continuously rising leading edge, and a continuously falling trailing edge (Figure 4). In comparison to morphological-driven confocal Raman imaging, many more API particles per single field of view can be detected and sized (Figure 3- 4), resulting in much more effective PSD evaluation.

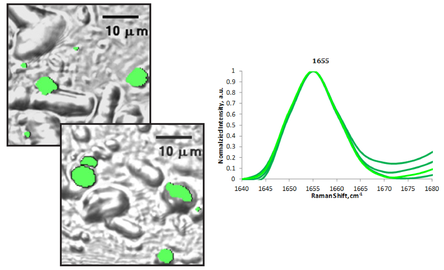

Figure 4. Representative fields of view of a Rhinocort Aqua® droplet showing Brightfield reflectance/ processed RCI at 1655 cm^{-1} and normalized Raman spectra of the identified Budesonide particles-spectra taken from the neighboring grouped pixels that poses high Raman signal at 1655cm^{-1} for spectroscopic verification of each detected particle (false-colored in green).

After the RCI data is collected for more than 100 FOVs, image processing techniques are employed to convert the RCI information into discrete API particles and API particles agglomerates for counting and sizing based on Equivalent Circular Diameter (ECD), the diameter of a circle that would have the equivalent area as the particle. Each FOV containing an API particle as confirmed by RCI can be visually compared with an optical microscopy/Raman Chemical Fusion image to determine number of API agglomerates/co-localized particles (Table 1).

Particles	#	D10 (µm)	D50 (µm)	D90 (µm)	Std. Dev (µm)
API	76	2.1	2.6	4.1	0.9
Agglomerates	9	4.1	5.6	5.8	0.8

Table 1. Budesonide API and API agglomerates PSD statistics in nasal spray sample based on ECD.

RCI-based approach to identify and accurately size agglomerated particles can be easily challenged and verified for the sizing precision and accuracy on NIST-traceable sizing standards such as Polystyrene Microspheres (PSMS) of same sizes (Figure 5) or of mixed sizes agglomerates (Figure 6).

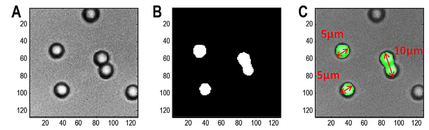

Figure 5. Representative Raman White Light (RWL) Image (A); Binary Mask-Processed RCI image (B) and RWL/RCI fusion Image of 5µm sizes PSMS (false colored in green).

Each FOV containing an PSMS as confirmed by Raman Chemical Imaging is visually compared with an optical microscopy, Raman White Light and Raman Chemical Fusion image, if the identified PSMS is determined to be an agglomerate based on visual inspection, the agglomerate is being sized. Figure 5 features 2 stand-alone 5 µm PSMS and 1 agglomerated 5 and 5 µm PSMS. Max chord of agglomerate should be a 10 µm. PSMS particles touching the image boundaries are removed.

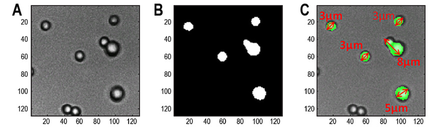

Figure 6. Representative Raman White Light (RWL) Image (A); Binary Mask-Processed RCI image (B) and RWL/RCI fusion Image of 3 and 5µm sizes PSMS (false colored in green).

Figure 6 features 3 stand-alone 3µm PSMS, 1 stand-alone 5 µm PSMS and 1 agglomerated 3and 5 µm PSMS. Max chord of agglomerate should be an 8 µm.

CONCLUSION

Evaluation of the same fields of view for drug-specific particle sizing in a suspension by confocal and wide-field RCI shows that accurate evaluation of API PSD and degree of drug particles agglomeration by confocal imaging is much more challenging as compared to wide-field Raman imaging. High-fidelity RCI with superior spectral and spatial resolution shows advantages in identification of agglomerates and particle association, which correlates with dissolution rate of the API and thus can provide valuable information about drug content uniformity and product quality.

REFERENCES

1. Doub, W.H. et al. Pharm. Research, 2007, 24 (5), 934-945.
2. U.S. Patent Pending: "Automation of Ingredient-specific Particle Sizing Employing Raman Chemical Imaging," Inventors: Ryan Priore, Oksana Olkhovyk, Oksana Klueva, and Michael Fuhrman.