CHEMICALLY SPECIFIC ANALYSIS OF OINDPs: DIFFERENTIATION OF DRUG PARTICLE AGGLOMERATION BY RAMAN CHEMICAL IMAGING

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INTRODUCTION

Proper characterization of pharmaceutical products with respect to particle size and distribution requires that the extent and size of drug particle agglomerates is determined, as well as the size and number of primary Active Pharmaceutical Ingredients (API) particles. Such characterization may also be necessary for assessing product quality, establishing Bioequivalence (BE), evaluating out-of-specification issues, batch-to-batch comparison studies and addressing stability of the product during scale-up, and long-term shipping/storage in varying environmental conditions. By providing chemically specific identification for each particle in addition to Particle Size Distribution (PSD), Raman Chemical Imaging (RCI) [1] is an effective tool for identifying whether observed aggregates/agglomerates are combinations of multiple primary API particles, API-excipient particles or multiple excipient particles. In concert with other particle sizing techniques, RCI analysis of agglomerates can benefit developers of Orally Inhaled and Nasal Drug Products (OINDPs), containing multiple APIs and/or excipients such as nasal suspensions, dry powder inhalers and metered dose inhalers.

METHODS FOR IDENTIFICATION AND PSD ANALYSIS OF AGGLOMERATES

Aqueous Nasal Spray (NS) suspension samples containing micronized Beclomethasone were subjected to temperature treatments in order to replicate mailing conditions that might occur in the US Midwest (Room Temperature (RT) 21-23°C, 60°C [2], and -20°C [3]) for 12-14 hours. They were then analyzed by optical microscopy and RCI [1]. A commercial aqueous NS suspension, Beconase ® (Beclomethasone), (held at RT) was also analyzed. All tested aqueous NS suspensions contained microcrystalline cellulose as a solid excipient. NS samples were prepared for analysis by direct transfer of approximately 2 µL of the well-mixed suspension onto aluminum coated microscope slides. A quartz cover slip was then applied to obtain a thin layer of suspension for microscopy analysis. Multiple fields-of-view (FOVs) containing particles were randomly selected on the microscope slide for each prepared suspension sample. Brightfield Reflectance (BFR) and RCI images were obtained for each FOV using the Falcon II™ RCI System (ChemImage Corp., Pittsburgh, PA) at 50x magnification. Auto focusing was employed at each FOV. More than 500 FOVs (each measured 41x41µm) were analyzed per sample.

RESULTS AND DISCUSSION

The qualitative and semi-quantitative estimation of the degree of agglomeration in suspensions after various temperature treatments was performed by analysis of BFR and RCI of each identified drug particle and compared to room temperature formulations. RCI method [1,4] allows extraction of a Raman spectrum from every pixel in the image (spatial resolution approaching 350 nm can be achieved) and thus provides spectral and spatial information about any drug to drug or drug to excipient agglomerates that may form. Automated spectral and image processing algorithms [4] were used to obtain accurate and chemically specific particle sizing of suspended drug particles. Final API-specific particle images (Figure 1) were analyzed for PSD [4]. Raman spectrum in the spectral range from 1630 cm^{-1} to 1700 cm^{-1} was obtained from RCI for each detected API particle [4] and evaluated by an analyst. All identified particles which exhibited an API-specific peak at 1665 cm^{-1} (Beclomethasone) [1] were retained for statistical analysis (Figure 2).

The procedure for agglomerates assessment was performed as follows: the FOV containing an API particle as confirmed by RCI [4] was visually compared to an optical microscopy/RCI fusion image. Example image sets are illustrated in Figure 3. If the identified API particle was determined to be part of an agglomerate, based on visual inspection of the BFR image, the RCI fusion image associated with each particle, the Raman spectrum, and the size of the particle was retained.

These data suggest that the NS heated to 60°C possesses the highest degree of API-excipient agglomeration and larger values for API-API agglomerates, whereas primary API particles have very similar PSD for all NS samples. The highest percentage of API-API and API-excipient agglomeration (as compared to number of total detected API particles) was observed for Commercial NS (Table 1).

We also observed that most API-API agglomerates contain small particles. This may be because larger particles have a smaller surface area/volume ratio and weaker adhesion forces. This may explain why the size of observed agglomerates is larger than mean size for primary particles. It was noted that cooling to minus 20°C did not significantly affect particle size and degree of agglomeration as compared to RT NS.

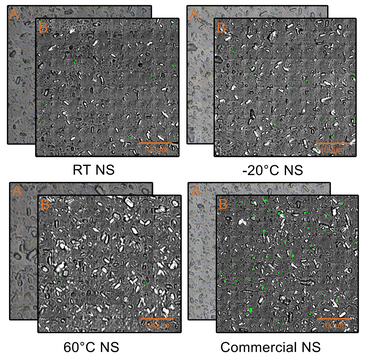

FIGURE 1
Representative BFR (A) and RCI Fusion (B) images of NS samples analyzed. Drug particles have been colored in green.

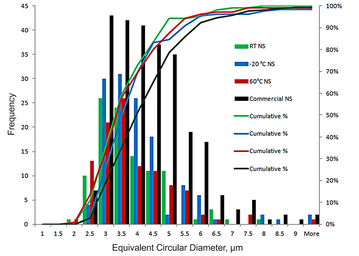

FIGURE 2
API equivalent circular diameter histograms for all NS analyzed: Green-RT NS; Red-heated to 60°C NS; Blue-cooled to -20°C NS; Black-Commercial NS.

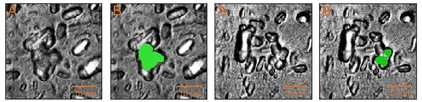

FIGURE 3
Representative RCI fusion image of API-API agglomerate (A), and API-excipient agglomerate (B) in Commercial NS.

Sample ID	Total #	% Agglomerates API-API / API-Excipient	Primary Particles			API-API Agglomerates			API-Excipient Agglomerates		
			D50	STD	Mean	D50	STD	Mean	D50	STD	Mean
RT NS	103	14/15	3.3	1.0	3.5	3.6	1.4	4.2	3.7	1.0	3.9
-20 °C NS	131	10/11	3.4	1.2	3.7	4.0	1.7	4.5	3.6	0.8	3.8
60°C NS	105	9/19	3.4	1.2	3.6	5.3	1.6	4.7	3.9	1.2	4.1
Commercial NS	261	23/25	3.8	1.3	4.0	4.8	1.6	5.3	4.2	1.2	4.4

TABLE 1
PSD statistics in samples based on equivalent circular diameter.

CONCLUSIONS

It was shown that monitoring the degree of agglomeration in aqueous suspensions subjected to various environmental conditions is possible by RCI method. The drug particles in the aerosolized formulation adhere to the larger particles (i.e. excipient) and consequently may not reach intended site of action possibly losing its effectiveness. Knowledge of the conditions promoting drug particles adhesion to each other or to excipients may aid understanding of OINDPs bioavailability, stability and efficacy.

REFERENCES

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