Raman-based analysis of dispersed versus aggregated drug particles in MDI formulations for chemically-specific sizing and polymorphic purity assessment

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Summary

In this study, Raman Chemical Imaging (RCI) was used to investigate the Active Pharmaceutical Ingredients (API) particle size distribution (PSD) and degree of drug particle aggregation in a commercial MDI, Flixotide, which contains an anti-inflammatory corticosteroid (fluticasone propionate). Assessment of free-standing API particles and aggregated API particles was done by visual inspection of Raman-identified API particles as it relates to the spatial location of all detected fluticasone particles on a the brightfield and Raman Chemical fusion Images. By using RCI it is possible also to identify presence of foreign particulates in the formulations and obtain spectrally and spatially resolved images of each particle for accurate particle size determination and recognition of aggregated versus stand-alone particles. RCI can easily separate different chemical species or polymorphic/hydrate forms of the same chemical within single particle or aggregate for identification of polymorphic impurity/unwanted phase transition of the drug.

Introduction

Inhalation drug testing as it relates to dose delivery profile and drug particle size distribution (PSD) is essential for establishing the desired therapeutic effect of inhalable products. Physical properties of the drug molecules, particularly aerodynamic particle size distribution of MDIs and dry powder inhalers (MDIs) may be assessed by cascade impaction tests such as Next Generation Pharmaceutical Impactor (NGI), Andersen Cascade Impactor (ACI), and Multistage Liquid Impinger (MSLI). Recent studies however suggested that manual actuations (operator errors), flow rates and induction ports variations affect drug PSD and dose delivery profile [1]. It was shown that it is challenging to achieve good repeatability and reproducibility of inhalation drug testing using these techniques. Presence of large particles, aggregated particles and foreign contaminants may greatly affect in vivo deposition profiles and thus FDA recommends microscopic evaluation of the samples to access morphology changes of the drug particles and crystal growth especially for release and stability testing [2]. Accurate assessment of active pharmaceutical ingredient PSD in the MDI and DPI formulations is a challenging task to be addressed by microscopy or cascade impaction analysis alone due to the subjectivity and lack of chemical specificity of these methods. For inhalation route of administration, efficacy of the drug also relates to its polymorph stability and purity since it directly effect adhesion-cohesion interaction of the drug molecules between themselves and to excipient and or carrier. Due to increasing interest to develop and manufacture combination drug products, evaluation of polymorphic forms of the engineered particles and degree of agglomeration of the drug to drug or drug to carrier/excipient becomes especially important.

Raman Chemical Imaging (RCI) is a novel Raman-based technology for determination the PSD of drug-only particles based on their chemical make-up. RCI has been successfully applied for PSD analysis for Orally Inhaled and Nasal drug products (OINDPs) containing corticosteroids [3-4]. Raman imaging was shown to be especially beneficial to study the particle morphology, solvates and hydrates, clathrates, crystalline forms, polymorphs, amorphous forms, co-crystals [5] in suspensions as well as complex microstructure/agglomeration nature of combination dry powder inhaler formulations containing fluticasone and salmeterol xinafoate, as it relates to surface interfacial interactions between components of the formulation [6]. It was shown by means of Raman imaging that particle size and chemical composition of agglomerated systems of both fluticasone propionate and salmeterol xinafoate were different. Thus the mechanisms or engineering approaches to create chemically-specific particles of desired size and desired crystalline structure may be tested to understand dispersion of the drug particles and aerosolization performance [7]. Chemically-specific techniques such as RCI have potential to be used to assess bioavailability(BA)/bioequivalence(BE) of OINDPs and investigate less stable forms of polymorphs (i.e. hydrate forms, such as mometasone) [8] for which other methods are not suitable.

Experimental

Flixotide (fluticasone propionate + HFA 134a) - 125mcg dose strength was utilized in this study. Sample for analysis was provided by collaborator. A Raman spectral library of all pure component materials as well and RCI data were collected on a ChemImage Falcon II™ RCI System.

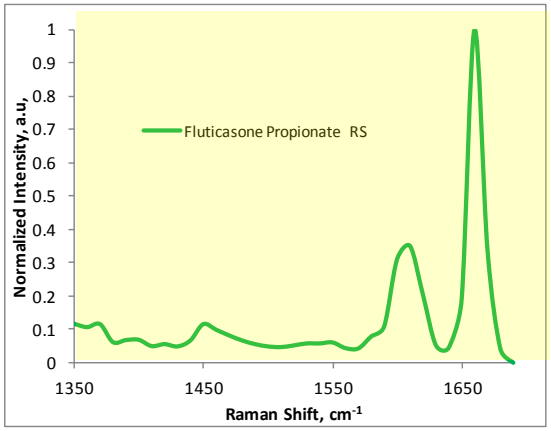

Figure 1. Raman dispersive spectrum of fluticasone propionate, API of Flixotide MDI, where the Raman Chemical Imaging spectral range has been highlighted in yellow.

Fluticasone propionate may be spectroscopically identified by the Raman peak at 1660 cm^{-1}. Raman Chemical Imaging data was collected over a portion of the fingerprint spectroscopic region from 1350-1690 cm^{-1} which encapsulates the distinct fluticasone propionate band observed from the Raman dispersive spectroscopy library development. 200 Fields of View (FOV) were measured resulting in 0.1mm^2 area measured for MDI sample. A final API-specific particle image was analyzed for particle number and size* based on Equivalent Circular Diameter (ECD), the diameter of a circle that would have the equivalent area as the particle. Table 1 summarizes the ECD size number-wise (not volume weighted) distribution for the measured sample, and the corresponding histogram is shown in Figure 2.

Table 1. Fluticasone propionate API number-wise (not volume weighted) PSD statistics in MDI sample based on Equivalent Circular Diameter*.

Particles	D10 (µm)	D50 (µm)	D90 (µm)	Std. Dev. (µm)	Min. (µm)	Max. (µm)
34	1.7	4.5	5.6	1.6	0.3	6.7

*Post ISPS analysis, spectral revision of FOVs with detected API particles was performed by analyst. Identified API particles that exhibited API-specific peaks were retained (Figure 3).

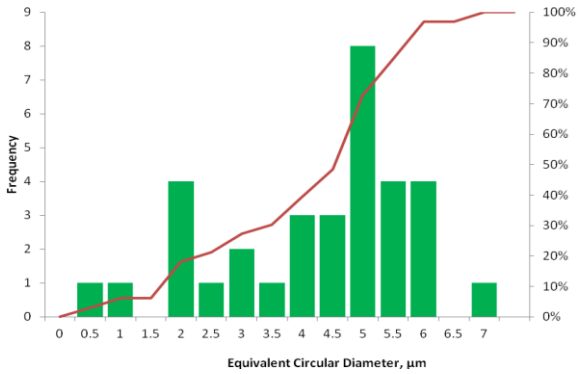

Figure 2. Fluticasone propionate Equivalent Circular Diameter histogram and cumulative percentage (red line) (Flixotide).

As it related to the confirmation of the polymorphic purity of all stand-alone and associated drug particles, following examination was performed on each identified by Raman Imaging drug particle/drug aggregate. The Raman spectrum may be easily derived from RCI for every single identified drug particle or drug aggregate to evaluate the Raman band shape and Raman peak position for polymorphic purity screening. This is done by applying image processing algorithms that allow obtaining spectral information along with sizing information for each detected particle. Figure 3 presents plot of the Raman spectra over fluticasone-specific Raman band at 1660cm^{-1} for all

identified fluticasone particles reported in sizing statistics. It is evident that Raman shape and peak position for all particles are identical, thus polymorphic purity of all sized drug particles may be confirmed.

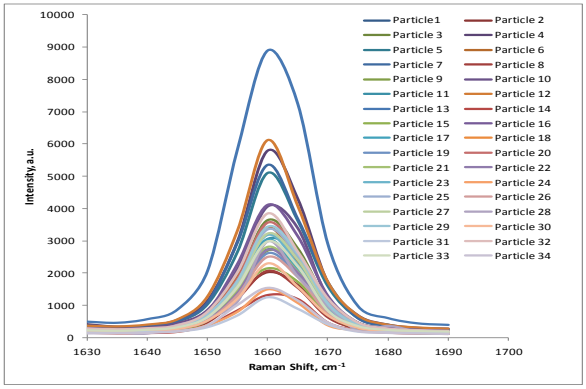

Figure 3. Raman spectra of all 34 identified fluticasone propionate particles in Flixotide.

RCI approach not only allows for obtaining accurate and chemically specific particle sizing (Table 1), but also provides means to collect information about any drug to drug or drug to excipient agglomerates that may form in the formulation and cause an unwanted drug polymorph transition. The API-API agglomerates were manually counted and sized for the sample. This process resulted in a qualitative understanding of the API-API agglomeration, which also was confirmed by spectral information for detected agglomerate. The following procedure was performed. Each FOV containing an API particle as confirmed by Raman Chemical Imaging was visually compared with an optical microscopy/Raman Chemical Fusion image. Example image is illustrated in Figure 4.

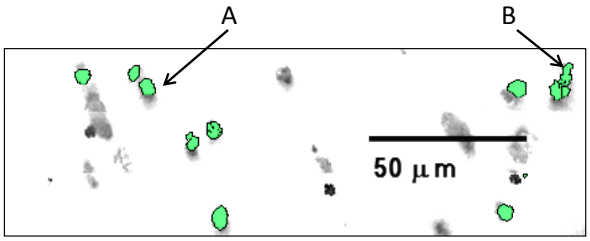

Figure 4. Representative Bright Field Reflectance (BFR)/RCI Fusion image of API particles (A-free standing fluticasone; B-agglomerated fluticasone) in Flixotide MDI where fluticasone is false-colored in green.

Assessment of free-standing fluticasone API particles and bound to each other API particles (API-API agglomerates) was done on the Fusion BFR/RCI images. If the identified API particle was determined to be an agglomerate based on visual inspection, the equivalent circle diameter was retained from reporting. Tables 2-4 summarize the API-API agglomerate observations and sizing statistics.

Table 2. Fluticasone propionate API-API agglomerates in sample based on ECD.

API Agglomerates (% of total API particles)	D10 (µm)	D50 (µm)	D90 (µm)	Std. Dev. (µm)	Min. (µm)	Max. (µm)
9 (26%)	4.8	6.2	8.3	1.5	4.3	9.2

Table 3. Fluticasone propionate API-API agglomerates size distribution in sample based on ECD.

<1(µm)	1-2 (µm)	2-3 (µm)	3-4 (µm)	4-5 (µm)	≥5(µm)
0	0	0	0	2	7

The benefit of applying RCI for aggregate analysis is that RCI may be applied to the sample prepared via dispersion and analyzed as is, or to samples from the cascade impactor stages to evaluate degree of aggregated particles and investigate stability parameters or hygroscopic growth of aggregates with temperature and relative humidity condition changes and relate morphological parameters obtained from RCI analysis to aerodynamic PSD obtained from impaction analysis. Also, effect of the actuation device may be evaluated to obtain dispersion characteristic of the drug particles as opposed to sampling from the bulk. All PSD analysis and agglomeration degree assessment may be performed without additional sample preparations. In addition, since RCI method is non-destructive, the HPLC or other analytical methods may be performed on the same sample to evaluate the drug dose, etc.

Table 4. Statistics of free standing versus agglomerated API particles.

API	Free Standing (% of total detected)	Agglomerated API (% of total detected)
Fluticasone propionate (based on 34 particles)	74%	26%

Conclusions

Raman Chemical Imaging and agglomerate-identification image processing analysis was used to differentiate stand-alone fluticasone particles from co-associated fluticasone particles in Flixotide MDI. Obtained results show that the mechanisms or engineering approaches to create drug particles of desired size and desired crystalline structure as well as device affect on drug delivery profile can be tested by this non-distractive method. Such analysis may aid in understanding dispersion of the drug particles and inhalation product aerosolization performance without time-consuming impaction analysis testing. Therefore, it may be concluded that Raman imaging is powerful analytical method to differentiate particles by their chemical make-up to provide means to evaluate degree of drug-specific aggregates, which is especially effective in analysis of combination inhalation products.

References

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