SPATIALLY-RESOLVED **RAMAN IMAGING** FOR **INGREDIENT-SPECIFIC CHARACTERIZATION** OF COMBINATION **DRY POWDER INHALERS**

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INTRODUCTION

Since the approval of Dry Powder Inhalers (DPI) containing Fluticasone Propionate (FP) and Salmeterol Xinafoate (SX) in 1999, combination inhaled therapies have dominated the COPD market. However, due to the lack of specific guidance of the Office of Generic Drugs (OGD) as to the Abbreviated New Drug Application (ANDA) submission, there was very little attempts of generic manufactures to come up with bioequivalent DPI formulations until September 2013 [1].

Success of an ANDA submission to OGD as it is stated in recently published draft Guidance on FP and SX [2] depends on ability of the manufacturer to prove that their test product is qualitatively and quantitatively the same as the reference product, as it relates to both the formulation and the device. DPI and MDI (Metered Dose Inhaler) products typically combine a bronchodilator, often a long-acting beta agonist, with a maintenance drug, usually an inhaled corticosteroid or added long-acting muscarinic antagonist. Thus completing *in vitro* and *in vivo* studies to establish bioequivalence of the inhaled combination therapies can be difficult without accurate and precise data on the Particle Size Distribution (PSD) of the individual ingredient. Later may be impossible to determine accurately using standard methods such as cascade impaction and optical microscopy since those techniques are not chemically-specific.

Any differences in the PSD of each of the active ingredients will almost certainly result in differences in deposition, preventing co-deposition of each active in the correct ratio in each region of the lungs. Even if the drugs PSDs were identical, formulations may be affected by numerous mechanical and environmental factors. External factors that may cause imbalances/variation on deposition/inefficacy due to the contribution of the excipients used (i.e. the grade/size of milled lactose which often is acted as a carrier). One active component of a dry powder formulation may have more surface energy or may be more hygroscopic than the other, leading to differences in how each detaches from the carrier or aggregates with other particles.

Because the interactions of the various components depend largely on the surface energies of the particles, some researchers have attempted to gain a better understanding of DPIs aerosolization behavior by using the colloid probe method of Atomic Force Microscopy (AFM) [3]. Such analysis to determine the Cohesive-Adhesive Balance (CAB) between each of the components of a dry powder formulation however is very time consuming and expensive.

METHODS

Raman Chemical Imaging (RCI) was shown to have a potential to address the need to accurately identify and size not only primary drug particles but also aggregates based on their unique spectroscopic properties. RCI method [4, 5] allows extraction of a Raman spectrum from every pixel in the image (spatial resolution approaching 350 nm can be achieved). Thus every image provides spectral and spatial information about any drug to drug or drug to excipient agglomerates that may form [6].

The RCI system combines spectral information gathered by Raman spectroscopy with morphological information obtained by optical imaging. The RCI microscope collects data from hundreds of randomly selected Fields-of-view (FOVs) from across each sample. The system then overlays the spectral data with an optical image of the sample, producing a montage of data correlated on a pixel-by-pixel basis that clearly shows which particles consist of excipients or any of the active ingredients.

With the ability to detect spectral shifts as small as 2 cm^{-1}, the method is even sensitive enough to identify individual polymorphs of drugs, which is important because different polymorphs have different dissolution rates, and the presence of particular polymorphs in the formulation can therefore affect product bioavailability.

RESULTS

RCI was shown as a powerful characterization methodology for complex combinational therapeutics as it relates to product aerosolization performance [6]. In the current study, RCI method was applied to investigate the microstructure of commercial Seretide® Accuhaler® with conjunction to CAB approach to colloid probe AFM. Assessment of free-standing API particles (including same drug agglomerates) and bound to lactose API particles (including different drugs agglomerates) can be done on the fusion BFR/RCI images by visual inspection of all API particles: all identified API particles are selected for visual evaluation as free standing versus bound to carrier/other API particles (Figure 1).

Related studies [3] showed that it is possible to establish the link between the microstructure and the dispersive mechanism of the formulation. Comparison of the CAB ratio of FP with respect to lactose and SX with respect to lactose suggested that the adhesion of FP to lactose was significantly ($p<0.05$) greater than SX to lactose. Furthermore, the cohesive SX interactions were 1.85 times greater than the adhesive SX-FP interactions, whilst the FP-SX interactions were 1.2 times greater than the cohesive FP-FP interactions.

RCI analysis of particles deposited on stage 3 of the next generation impactor suggested that the presence of finer free standing SX than FP and the presence of FP-lactose agglomerates vary (Table 1, Figure 2). These differences may be related to the surface interfacial properties of the components of the formulation as suggested by CAB-AFM analysis, which may have resulted in a specific formulation microstructure that aids dispersion of the different drugs using different mechanisms.

RCI was also successfully used to investigate the API PSD, carrier (Lactose) PSD and access qualitatively degree of API-carrier aggregation in a commercial DPI, ADVAIR DISCUS® 500/50 post multistage cascade impaction analysis (Figure 3) [7].

The percentage of free-standing API particles on Stage 3 was determined to be 90% whereas much more API-Lactose agglomerates were detected on Stage 4, and percentage of free-standing API was determined to be 60% out of all detected API particles. The size of agglomerates may be estimated by the D90 values and maximum size of the particles detected from each stage of the cascade impactor and was shown to be correlated for D90 values with cut-off ranges of the stages 3 and 4 from where DPI was sampled for RCI analysis. As shown on (Tables 2 and 3), the largest drug particles detected from Stage 3 and 4 were of sizes 5.6 and 4.5µm respectively, and the largest carrier particles detected from Stage 3 and 4, respectively were of sizes 4.6 and 3.4 µm (Figure 4).

RCI and agglomerate-identification image processing analysis was successfully used to differentiate stand-alone Fluticasone Propionate particles from co-associated FP particles in Flixotide MDI (Figure 5).

FIGURE 1

Representative fusion image of free standing Fluticasone propionate particles versus bound to carrier particles in Seretide® where Fluticasone Propionate is false-colored in Green; Lactose is false-colored in Red; Salmeterol Xinafoate is false-colored in Blue.

API	Free Standing (% of total detected)	Bound to Lactose (% of total detected)	Bound to other API (% of total detected)
Fluticasone Propionate	46%	20%	2%
Salmeterol Xinafoate	50%	10%	5%

TABLE 1

Statistics of free standing versus bound to carrier API particles.

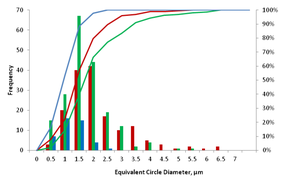

FIGURE 2

Equivalent circular diameter histogram and cumulative percentage for: Fluticasone Propionate (Green); Lactose (Red); Salmeterol Xinafoate (Blue).

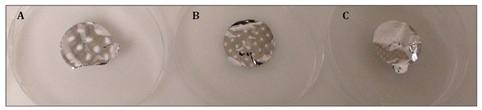

FIGURE 3

ADVAIR® samples analyzed by RCI: from stage S3 (A), from stage S4 (B), from stage S5(C).

SAMPLE	Stage	Particles	D10 (µm)	D50 (µm)	D90 (µm)	Std. Dev. (µm)	Min. (µm)	Max. (µm)
ADVAIR Discus® 500/50	S3	123	1.2	2.1	3.3	0.8	0.3	5.6
ADVAIR Discus® 500/50	S4	148	1.0	1.7	2.9	0.8	0.3	4.5
ADVAIR Discus® 500/50	S5	172	1.1	1.9	2.8	0.6	0.3	3.5

TABLE 2

Fluticasone Propionate API number-wise (not volume weighted) PSD statistics in nasal spray sample based on equivalent circular diameter.

SAMPLE	Stage	Particles	D10 (µm)	D50 (µm)	D90 (µm)	Std. Dev. (µm)	Min. (µm)	Max. (µm)
ADVAIR Discus® 500/50	S3	49	1.0	1.6	3.4	0.9	0.5	4.6
ADVAIR Discus® 500/50	S4	55	0.9	1.6	2.7	0.7	0.3	3.4
ADVAIR Discus® 500/50	S5	46	1.1	1.8	3.1	0.8	0.5	4.1

TABLE 3

Lactose API number-wise (not volume weighted) PSD statistics in nasal spray sample based on equivalent circular diameter.

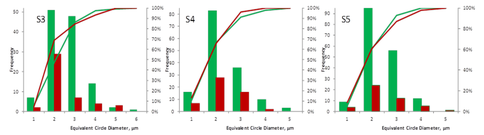

FIGURE 4

Fluticasone Propionate equivalent circular diameter histogram and cumulative percentage histograms for ADVAIR® samples collected from stage 3 and 4, and 5 where Fluticasone Propionate is false-colored in Green and lactose is false-colored in Red.

SAMPLE	API Agglomerates (% of total API particles)	D10 (µm)	D50 (µm)	D90 (µm)	Std. Dev. (µm)	Min. (µm)	Max. (µm)
Flixotide 125	9 (26%)	4.8	6.2	8.3	1.5	4.3	9.2

TABLE 4

Fluticasone Propionate API-API agglomerates in sample based on equivalent circular diameter.

SAMPLE	<1(µm)	1-2 (µm)	2-3 (µm)	3-4 (µm)	4-5 (µm)	>5 (µm)
Flixotide 125	0	0	0	0	2	7

TABLE 5

Fluticasone Propionate API-API agglomerates size distribution in sample based on equivalent circular diameter.

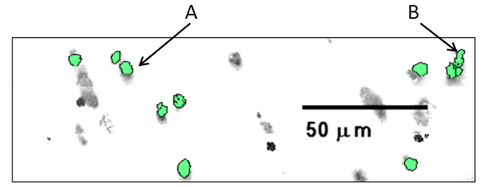

FIGURE 5

Representative BFR/RCI Fusion image of API particles (A-free standing Fluticasone Propionate; B-agglomerated Fluticasone Propionate) in Flixotide MDI where Fluticasone Propionate is false-colored in Green.

API	Free Standing (% of total detected)	Agglomerated API (% of total detected)
Fluticasone Propionate	74%	26%

TABLE 6

Statistics of free standing versus agglomerated API particles (Figure 5).

Results showed that desired size and desired crystalline structure of all components can be tested by non-destructive RCI method to evaluate product delivery profile (Tables 5, 6). Mechanisms or engineering approaches to create drug particles of desired physico-chemical properties (i.e. engineered particles) as well as the device effect on drug particles de-agglomeration were successfully investigated by this high fidelity imaging method without time-consuming cascade impaction analysis [8]. 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.

CONCLUSIONS

Therefore, it may be concluded that RCI allows obtaining in-depth solid-state characteristics of dry powder inhalers, differentiate particles by their chemical make-up, acquire sizing information for each component and evaluate the degree of drug-specific aggregates, which is especially effective in the analysis of complex combination inhalation products. The impact of agglomerates in DPI formulation on dissolution profile and permeability of the drug within the sites of action (i.e. patient's lungs) is critical. 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 identified and sized particle for accurate particle size determination and recognition of aggregated versus stand-alone particles. Such analysis may aid in understanding dispersion of the drug particles and inhalation product aerosolization performance without time-consuming impaction analysis testing.

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