Chemical Imaging by Raman Spectroscopy: A Powerful Analytical Technique for Surface Morphology Investigation of Inhaled Products

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Introduction

Pulmonary disorders are a growing healthcare problem that are expected to worsen as the population ages. Regular use of inhaled bronchodilators to prevent and relieve symptoms is the mainstay of many pulmonary disorders. Inhalers may contain drugs either alone or combined with other Active Pharmaceutical Ingredients (API), for example, the long-acting beta 2-agonist and corticosteroid inhaler, which is mainly used for exacerbated chronic obstructive pulmonary disease (COPD) [1].

Particle interactions are of great importance where the dispersion of API particles from carrier particles is critical for lung deposition. Poor API homogeneity is undesirable since homogenous drug content is essential to achieve consistent emitted dose of the drug during inhalation[2]. Moreover, not only must the force of adhesion be strong enough to maintain the blend homogeneity during manufacturing process, but it must also allow the detachment on inhalation to effectively deposit API into the deep lung. Thus, blending API with carrier is a critical stage that determines the blend homogeneity and is the first step towards obtaining the final quality of the powder blend[3]. Raman spectroscopy and its powerful chemical imaging capability enables understanding of many of these key attributes for inhaled formulations[4].

Chemical imaging is a method that consists of acquiring a collection of responses obtained from a laser monochromatic light at multiple wavelengths of positions spatially distributed across the sample surface[1]. As a result, each mapped sample point (pixel) contains a wealth of information, and the recorded signal reflects chemical and physical properties such as ingredient identity, concentration, crystallinity, orientation, domain size and particle size. The components of inhaled formulations (API, carrier & excipients) are usually micronised and/or processed, to allow for the successful transportation down into the deep lung. Therefore, this needs to be monitored during sample processing and formulation.

Aim

The aim of this study was to demonstrate whether chemical imaging by Raman spectroscopy could detect any variation in product content in two different marketed products (Fobumix 320µg Budesonide/9µg Formoterol Fumarate Dihydrate® and WockAIR 320µg Budesonide/9µg Formoterol Fumarate Dihydrate®). In addition, the percentage of each component present in each product and particle size distribution of respective components remained key interests in this research.

Experimental

The chemical imaging of the dry powder inhaler (DPI) products was carried out using RA802 Pharmaceutical Analyser (Renishaw, UK). A thin layer of product powder contained in 5 capsules (lactose monohydrate, budesonide, and formoterol fumarate dihydrate) was placed onto a mirror slide and flattened using another slide, subsequently put it onto the Raman stage. LiveTrack focus-tracking ensured optimum focus was maintained over the rough split surface of the powder. Renishaw's fast mapping technique Streamline Image Acquisition was used with LiveTrack to acquire Raman images of the surface of the powder. Three maps from each product were chemically imaged in random areas of prepared sample.

Table 1: Streamline Image Acquisition Configuration

Laser Wavelength	785 nm
Laser Power	100%
Grating	1500 l/mm
Objective	HiMag (x50)
Focus-tracking	LiveTrack
Spectral range	149.3 cm^{-1} to 1885.85 cm^{-1}
Mapped area	350 µm (x) by 350µm (y)
Step size	1 µm (x) by 1µm (y)
Total spectra	122,500 each map

Reference spectra were previously acquired of each component: lactose monohydrate, budesonide, and formoterol fumarate dihydrate.

Multivariate data analysis technique, using reference spectra for the powder components, were used to acquire the spectrum dataset. Non-negative least squares (NNLS) component analysis was used to generate the chemical images and quantify the content of the materials within the powder layer. Images are based on NNLS scores, which indicate the correlation between each map spectrum and each reference spectrum. NNLS component analysis was performed using reference spectra for lactose monohydrate, budesonide, and formoterol fumarate dihydrate.

Results

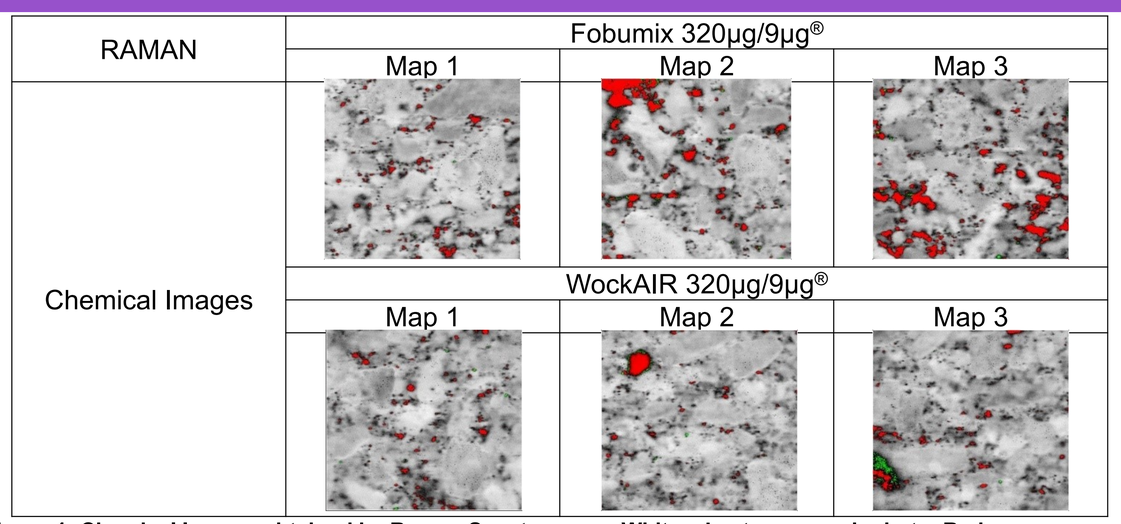

Figure 1: Chemical Images obtained by Raman Spectroscopy. White = Lactose monohydrate; Red = Budesonide; Green = Formoterol Fumarate Dihydrate.

Table 2: Particle Size Distribution Data for WockAIR 320µg/9µg® and Fobumix 320µg/9µg®.

WockAIR 320µg/9µg®	Budesonide				Formoterol Fumarate Dihydrate			
Particle Size Distribution (µm)	Map 1	Map 2	Map 3	Mean	Map 1	Map 2	Map 3	Mean
D90	4.84	7.48	4.56	5.63	3.28	3.17	2.95	3.13
D50	3.43	4.91	3.12	3.82	2.46	2.39	2.01	2.29
D10	2.24	2.59	2.01	2.28	1.64	1.42	1.29	1.45
Fobumix 320µg/9µg®	Budesonide				Formoterol Fumarate Dihydrate			
Particle Size Distribution (µm)	Map 1	Map 2	Map 3	Mean	Map 1	Map 2	Map 3	Mean
D90	4.17	6.97	5.93	5.69	2.95	3.75	3.60	3.43
D50	2.89	3.06	4.09	3.35	2.39	2.95	2.78	2.71
D10	1.92	1.92	2.59	2.14	1.53	1.53	1.74	1.60

Table 3: % Mass quantitation of components for WockAIR 320µg/9µg® and Fobumix 320µg/9µg®

	WockAIR 320µg/9µg®				Fobumix 320µg/9µg®			
% of the components	Map 1	Map 2	Map 3	Mean	Map 1	Map 2	Map 3	Mean
Lactose	81.03%	80.86%	83.06%	81.65%	80.45%	78.96%	78.70%	79.37%
Budesonide	18.89%	19.05%	16.85%	18.26%	19.50%	20.97%	21.26%	20.58%
Formoterol Fumarate Dihydrate	0.08%	0.09%	0.09%	0.09%	0.05%	0.07%	0.04%	0.05%

Discussion

In this study, an established chemical imaging method was used to compare and quantify the uniformity and homogeneity of two different marketed inhaler products which claim to use the same DPI formulations. Both formulations showed similar distribution based on the mapping data obtained here in Figure 1, Tables 2 and 3. However, the particle size of budesonide was higher than the formoterol fumarate dihydrate in both products.

Raman was able to measure small differences between Fobumix 320µg/9µg® and WockAIR 320µg/9µg® although they were not statistically significant (Two-way ANOVA, $\alpha = 0.05$).

The percentage comparison was carried out to reflect how these two products differ from each other. The percentage of components is not related to the relative concentration of these in the product. In theory, approximately 96% of lactose and 4% of budesonide was expected however, less % of lactose and consequently higher % of budesonide was detected. The exact reason is not understood at this stage which demands extensive data analysis using various statistical tools where data are normalised, truncated, and fine-tuned. That would require larger map over a longer period which this author plans to do that as a future study.

Conclusion

The objectives were to see if Raman was able to identify each component, % of each component and particle statistics of same in a multiphasic system which it did with some limitations of % of each component not accurately reflecting theoretical expectations. However, the results obtained in this study show that chemical imaging by Raman spectroscopy might be a suitable technique to analyse and monitor inhalation formulations. This technique would allow scientists to enrich their knowledge in understanding surface morphology of inhaled formulations, specially developing generic products and comparing them with innovators. The data analyses to reflect the relative percentage of these two marketed products were outside the scope of this study, however, this can be considered as a future study in conjunction with high performance liquid chromatography (HPLC) analysis.

References

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