

## Combination Particles Containing Fluticasone Propionate and Theophylline for Lung Delivery

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### Summary:

Theophylline has been identified as a compound that can help reverse steroid resistance in chronic obstructive pulmonary disease (COPD) as well as in asthmatic patients who appear to be resistant to inhaled corticosteroids (ICS). The solution atomisation and crystallisation by sonication (SAX) has been used to successfully produce a particle which contains a long acting  $\beta_2$ -agonist (LABA) as well as an ICS. This combination formulation can deliver the two compounds in a fixed ratio throughout a model lung. This study suggest that individual particles containing both theophylline and an ICS have been successfully produced using the SAX process.

### Introduction:

Over the last decade, there have been great advances in the knowledge and treatment for asthma. Yet there remain limited therapies available to treat chronic obstructive pulmonary disease (COPD). Both diseases are characterised by chronic inflammation, which if left untreated, can lead to long-term and permanent damage.

Corticosteroids recruit histone deacetylase (HDAC) in order to switch off inflammatory gene transcription in alveolar macrophages [1, 2]. Work by Ito *et al* has shown that COPD patients have a reduction in HDAC activity [3]. One theory for the mechanism by which HDAC activity is reduced is due to cigarette smoke causing an increase in oxidative stress resulting in increased nitration [1].

Traditionally, theophylline has been used an oral bronchodilator but its use has been reduced due to the introduction of safer bronchodilators, namely  $\beta_2$ -agonists and even more recently long acting  $\beta_2$ -agoists (LABAs) [4, 5]. Markham and Faulds reviewed three studies comparing the steroid sparing effect of theophylline. They found that using a low dose of theophylline with a low dose ICS provides the same protection in asthmatic patients as high dose ICS [6]. This suggests that theophylline works in conjunction with the ICS to have a greater anti-inflammatory effect. Ito *et al* have shown that theophylline induces HDAC activity and therefore, leads to a decrease in inflammatory gene expression [4].

We have previously shown that the solution atomisation and crystallisation by sonication (SAX) has the ability to produce particles, which contain both a LABA and an ICS. These particles, when delivered via a dry powder inhaler (DPI) formulation provide a fixed ratio of the two compounds to be delivered in an *in vitro* model of the lung and therefore, may potentiate the synergistic effect of the two compounds.

### Aims:

The aim of this study was to engineer particles contains both theophylline and an inhaled corticosteroid using the SAX process so that the two drugs are co-deposited in a fixed ratio throughout the lung.

### Materials:

All solvents were obtained from Fisher Chemicals (Loughborough, UK). Perfluorodecaline was obtained from F2 Chemicals (Lancashire, UK). The course carrier lactose monohydrate was obtained from Friesland Food Domo-Pharma (Zwolle, The Netherlands).

### Methods:

The SAX process was used to engineer particles of theophylline (THE) and fluticasone propionate (FP) described in detail elsewhere [7]. Briefly, a solution containing 2%w/w total drug, theophylline and fluticasone in a 1:1 ratio was prepared using dichloromethane and methanol 50:50. This was then sprayed at a rate of  $4\text{mLmin}^{-1}$  with an air pressure of 2.5bar and a drying air flow rate of  $30\text{Lmin}^{-1}$ . The particles were collected in perfluorodecaline which was maintained at  $5^\circ\text{C}$  with ultrasonic energy of  $5\mu\text{M}$  amplitude being provided by an ultrasonic probe.

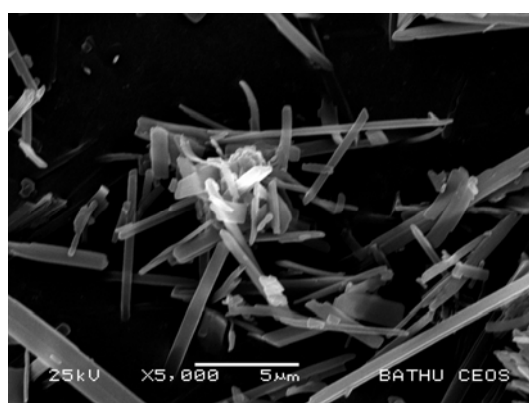
The particles were then isolated from the perfluorodecaline by using supercritical carbon dioxide ( $\text{SCO}_2$ ) before being analysed. The resultant SAX processed materials were characterised by X-ray powder diffraction (XRPD), nuclear magnetic resonance (NMR), differential scanning calorimetry (DSC, TA Instruments, Crawley, UK) and dynamic vapour sorption (DVS). Furthermore, the particle size distribution (PSD) of the materials was assessed by laser diffraction using a Sympatec (GmbH, Clausthal-Zellerfeld, Germany) and morphology by scanning electron microscopy (SEM, Joel, Japan).

A DPI formulation of SAX engineered particles containing both FP and THE was produced by blending 1.6 % w/w of active material with lactose. The content uniformity was assessed by high performance liquid chromatography (HPLC). The aerosolisation performance of the preparation was studied using a next generation impactor (NGI) with pre-separator via a Cyclohaler™ device at 90 L.min<sup>-1</sup>.

The HPLC method was adapted from Murnane *et al* [8]. Briefly, the mobile phase consisted of 0.6%w/v aqueous ammonium acetate with methanol in a 25:75 volume ratio. A flow rate of 1.5mL.min<sup>-1</sup> through a Phenomenex C18(2) 150x4.6 i.d mm 5µM Hypersil column maintained at room temperature was used. The detection wavelength was 235nm.

### Results and discussion:

As a solution containing both compounds is processed together using SAX, each droplet must contain both compounds, and therefore the final product must contain both compounds in the correct ratio. Figure 1 shows the SEM of the resultant particles. The particles all appear to be needle shaped. However, only one crystalline habit can be seen, which suggests the materials may have been co-processed into a single particle. Particle size analysis confirmed that the material had volume median diameter of 1.56 µm, with 90 % of particles less than 4.81 µm. Therefore, the material was suitable for pulmonary drug delivery.



**Figure 1:** Scanning electron microscope image of SAXS produced FPTHE particles.

Solution NMR confirmed that both THE and FP were present in the SAX particle in the correct ratio. Both DVS and XRPD showed that the material produced by SAX was crystalline. In addition, XRPD exhibited peaks for both FP and THE confirming the presence of both compounds. Interestingly, DSC analysis showed a single melting endotherm for the SAX material, which was distinct from either FP or THE (onset of melt 252.54°C, compared to 288.07°C and 271.75°C respectively).

Content uniformity of the blend, proved that the ratio of FP:THE was 1:0.98 w/w. The relative standard deviation of the mean drug content was sufficiently low to confirm that the blend was homogenous. The aerosolisation performance of the SAX FP:THE formulation is summarised in Table 1. The fine particle dose (FPD) of FP was 31 ± 2 µg, whereas the FPD of THE was 56 ± 15 µg. These data show that more THE was delivered upon aerosolisation than FP, which may be attributed to mechanical disruption of particles upon blending. However, the mass median aerodynamic diameter (MMAD) were similar for both FP and THE. However, upon formulating these particles in pressurised metered dose inhalers the fine particle delivery of both FP and THE were similar.

	Fluticasone Propionate		Theophylline	
	Mean	SD	Mean	SD
<b>Fine Particle Dose (mcg)</b>	31	2	56	15
<b>Fine Particle Fraction (%)</b>	15.5	1.1	33.4	7.7
<b>Mass Median Aerodynamic Diameter (µm)</b>	2.39	2.60	2.16	2.26

**Table 1:** Results calculated from NGI.

## Conclusion:

In conclusion, the SAX technology enables the processing of two actives into one individual crystalline particle, which enables the delivery of fixed ratios of two molecules. The potential of processing three drugs into one particle are under investigation, where preliminary data suggests this is possible. Current studies are also investigating the pharmacological action of these agents.

## Acknowledgments:

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