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Research Paper

Engineering of Crystalline Combination Inhalation Particles of a Long-Acting β_2-agonist and a Corticosteroid

Chonladda Pitchayajittipong,[1] Jagdeep Shur,[1] and Robert Price[1,2]

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Purpose. Engineering of inhalation particles incorporating, in each individual particle, a combination of a long-acting β-agonist and a glucocorticosteroid in a pre-determined and constant ratio for delivery via a dry powder inhaler (DPI).
Methods. Individual crystalline particles containing both the glucocorticosteroid fluticasone propionate (FP) and long-acting β-agonist salmeterol (SX) were prepared, in a ratio of 10:1, using the solution atomization and crystallization by sonication (SAX) process. Combination drug particles were characterized by particle size, morphology, crystallinity and aerosolisation efficiency using inertial impaction.
Results. Combination drug particles were spherical and crystalline, with a median diameter of 4.68± 0.01 μm. Aerosolisation of formulations containing combination drug particles resulted in greater uniformity in delivery ratios of both actives across all stages of the impactor before and after storage.
Conclusions. Actives in a pre-determined dose ratio can be crystallised in a single particle using the SAX process.

KEY WORDS: combination products; dry powder inhaler; glucocorticosteroid; long-acting β-agonist; SAX.

INTRODUCTION

Asthma and COPD are complex disease conditions of the airways that share some similarities: both are characterized by air flow limitation and airway inflammation (1–3). In order to manage these respiratory disease conditions, therapies are required to control symptoms, reduce exacerbations and improve health status in patients. The first-line treatment for both conditions is long-acting β_2-agonists (LABA) and inhaled corticosteroids (ICS), which are employed to aid bronchodilation and reduce inflammation, respectively (4,5).

Inhalation dosage forms combining a LABA and ICS are available in both pressurised metered dose inhaler (pMDI) and dry powder inhaler (DPI) platforms (6). Of the inhalation products available, the combination of salmeterol xinafoate (SX, LABA) and fluticasone propionate (FP, ICS) (Seretide®/Advair®, GlaxoSmithKline, UK) has gained widespread acceptance with physicians and patients and is currently listed amongst the top ten best-selling pharmaceutical products with annual sales of approximately £5 billon forecasted for 2009 (7). This combination-based therapy shows greater efficacy compared with monotherapy treatments with the individual components (8), with reduced mortality rates in COPD above and beyond that achieved by individual therapies (9).

The enhanced clinical benefit of combining both classes of compounds in a single formulation may be related to additive effects of administering both agents simultaneously to the lung, because they have complementary modes of action and target different aspects of the underlying disease pathophysiology (8,10). However, the increased clinical efficacy of inhaled therapies combining FP and SX have been reported to be more than just additive effects of co-administering both agents, but may be due to synergistic interactions of the two classes of compounds at the receptor, molecular and cellular level (11).

The synergistic action between FP and SX is thought only to occur when both drugs reach the same target cell in the required concentrations (11). The administration of both drugs from a combination inhalation dosage form is, therefore, likely to enhance the probability of co-deposition in comparison to monotherapy treatment with the individual active agents. Furthermore, recent studies have shown that the opportunity for co-deposition of SX and FP particles in the airways is further enhanced in the Advair® HFA formulation as a result of particle co-association within the delivery device (11–13).

Current combination drug DPI formulations are prepared as a physical mixture of the micronised actives and coarse carrier particles of lactose monohydrate. During inhalation, the patient's inspiratory force is employed to aerosolise the formulation and elutriate the drug particles from the carrier particles for lung deposition (14). However,

[1] Pharmaceutical Surface Science Research Group, Department of Pharmacy and Pharmacology, University of Bath, Claverton Down, Bath, BA2 7AY, UK.
[2] To whom correspondence should be addressed. (e-mail: r.price@bath.ac.uk)

the probability of co-depositing both actives at the same site of action from combined DPI dosage forms is limited owing to the complex relationship between formulation aerosolisation behaviour, device de-aggregation properties and patient inspiratory action. This may ultimately affect the possibility of synergistic action between the actives at the cellular level, which may in turn affect the efficacy of the treatment. Thus, there is a requirement of processes that may enable the preparation of combination DPI products that will allow both drugs to be delivered efficiently and independent of dose variations via the patient's inspiratory force at the same site of action.

One possible approach to circumvent formulation problems associated with the development of combination drug DPI products is to load the formulation of the individual actives in different compartments in a single DPI inhaler device (15). Upon inhalation, both formulations are aerosolized into an inhaled air-stream, thereby enabling delivery of both actives in the same aerosol cloud. Another possible approach would be to co-precipitate the actives from a solution, as suggested by Westmeier and Steckel (16). They were able to co-precipitate particles of SX and particles of FP in the presence of one another in the respirable size range using a mixture of polysorbate and HPMC as crystal growth inhibitors. However, the resultant product was found to be partly crystalline following isolation of particles from the anti-solvent (16). Whilst the use of dual particles such as those suggested by Westmeier and Steckel may enhance the probability of co-association of the actives, it does not guarantee co-delivery of precipitated agglomerates containing both actives in the respirable size range upon aerosolisation and deaggregation. In order to achieve such a goal, particles need to be engineered such that each individual particle contains both actives in the required concentration and in the respirable size range. Such an approach may be possible via a solution-to-droplet particle engineering approach (17,18).

One of these technologies is the solution atomization and crystallization by sonication (SAX) process, which produces high-purity, micron-sized and sphere-like crystalline particles in a single-step operation (18). The atomisation of a solution containing both actives in a common solvent in a defined ratio will result in the formation of droplets that will contain very limited amount of solvent and in which the ratio of the two actives in each droplet will be the same as in the solution. With limited influence of any residual solvent, crystallisation within the highly supersaturated droplets will lead to the formation of respirable-sized particles that contain both actives in the desired ratio.

The aim of the present study was to prepare crystalline respirable combination drug particles in which each particle contained both SX and FP in the desired ratio. The aerosolisation efficiency of combination drug particles containing SX and FP was investigated, with particular interest in the uniformity of delivery of both actives following aerosolisation from combination DPI formulations.

MATERIALS AND METHODS

Materials

Micronised fluticasone propionate (FP) and salmeterol xinafoate (SX) were obtained as a gift from Merck Generics (Potters Bar, UK). Acetone (Fisher Chemicals, Loughborough, UK) and perfluorodecalin (F2 Systems, Preston, UK) were utilised in the fabrication of SAX particles and were 99.9% pure. Other solvents such as hexane, cyclohexane, methanol, ethanol and acetonitrile were all supplied by Fisher Chemicals (Loughborough, UK) and were 99.9% pure. Water was prepared by MilliQ from reverse osmosis (Molsheim, France). Surface dissolved lactose monohydrate was produced using Lactohale® (Friesland Foods, Domo, Borculo, Netherlands).

Methods

Engineering of Crystalline Combination Drug Particles Containing FP and SX

The operational methodology of the SAX process has been previously described elsewhere (18). Combined drug particles of FP/SX were prepared upon atomisation of a 2% *w*/*v* solution containing a ratio of FP to salmeterol base of 10:1 in acetone, which represented an equivalent to a Seretide®/Advair® 500/50 Discus device that contains 500 μg FP and 50 μg salmeterol. Atomisation was conducted using a SU11 co-axial two-fluid atomiser with internal mixing (Spraying Systems Co., Illinois, USA) with a solution flow rate of 4 $ml.min^{-1}$ and air pressure at 2.5 bars over a distance of 60 cm. An additional volumetric air-flow, set at 30 $L.min^{-1}$, was focused into the drying chamber of the system to aid evaporation of solvent from the generated droplets. The resultant droplets were collected in a non-solvent of perfluorodecalin at 5°C, and exposed to sonic energy using a sonic horn (P100, Sonic Systems, Somerset, UK) operating at a fixed wavelength of 20 kHz and capable of inducing a maximum power output of 750 W. The ultrasonic horn (horn diameter ~13 mm), which was immersed 5 cm into the solution, was continually operated during droplet collection to induce nucleation and crystal growth of the drugs within the supersaturated droplets. Insonation has been previously demonstrated to aid nucleation and crystal growth of highly viscous solutions and reduce metastable zone-width for nucleation (19).

Extraction of Crystalline Particles from Non-solvent

The extraction of perfluorodecalin and isolation of combination drug particles containing FP and SX were carried out using a supercritical CO_2 fluid extraction vessel. The supercritical fluid delivery system consisted of a liquid CO_2 pump (P-500, Thar Technologies, Inc., Pittsburgh, PA, USA) and a heat exchanger, which provided supercritical CO_2 to the bottom of extraction column via a 0.2 μm porous membrane. This porous membrane maximized the mass-transfer efficiency during extraction. The suspension of SAX particles in non-solvent was charged into a 150 mL extraction vessel to which supercritical CO_2 was delivered via the liquid CO_2 pump into the bottom of extraction vessel at a constant flow rate of 10 $g.min^{-1}$. The extraction temperature was maintained constant at 40°C within an air-heated oven (Thar Inc., USA), whilst an automated backpressure regulator BP-1580-81 (Thar Inc., USA) maintained a constant working pressure of 100 bar during the run. The effluent supercritical

CO_2 was vented from the top of the column. The extraction process ran for a total of 3 h, following which the vessel was depressurized slowly at a rate of 2 bar.min^{-1}. Following completion of the extraction process, particulates were removed from the vessel and stored over silica gel.

Physicochemical Characterisation

Particle Size. The particle size distribution of micronised FP, micronised SX, and combination drug particles of FP/SX and coarse carrier lactose monohydrate particles were measured by laser diffraction (HELOS, Sympatec, Clausthal-Zellerfeld, Germany) using a wet dispersing system (CUVETTE, Sympatec, Clausthal-Zellerfeld, Germany). Approximately 5 mg of powder samples were suspended in 5 ml of dispersion media, which was composed of cyclohexane containing 0.1% *w/w* lethicin. Previous studies have shown that this dispersion media does not result in dissolution of the drug materials (18). The suspension was sonicated for 180 s prior to sizing. The suspension was added into 50 ml of dispersion media in a glass cuvette and stirred with a magnetic bar at 1,000 rpm. Sizing was triggered when the optical concentration was greater than or equal to 20%, and measurements were performed for duration of 20 s. The particle size analysis was performed and analysed using the Windox 5.0 software (Sympatec, Clausthal-Zellerfeld, Germany), by which means the cumulative undersize particle diameters, d_{10}, d_{50} and d_{90}, were calculated.

Scanning Electron Microscopy. The morphology of all formulations was investigated using scanning electron microscopy (SEM, Jeol 6310, Tokyo, Japan) at 10 kV. Samples were mounted on carbon sticky tabs and gold-coated for 5 min to provide a thickness of 20 nm using an Edwards Sputter Coater (Crawley, UK) prior to analysis.

Differential Scanning Calorimetry. The thermal properties of micronised FP, micronised SX and combination drug particles of FP/SX were investigated using a differential scanning calorimeter (DSC 2910, TA Instruments, Surrey, UK) calibrated with indium and tin standards. Approximately 3–6 mg of sample were accurately weighed into an aluminium pan (TA Instruments, Surrey, UK) and then hermetically sealed. The sample and reference (identical, but empty, hermetically sealed aluminium pan) were heated at a rate of 10°C.min^{-1} from 30°C to 350°C. The calorimeter head was purged with a steady stream of nitrogen (BOC gases, Guilford UK, 99.998% pure) at 25 mL.min^{-1} during all measurements. Measurements and subsequent analysis of each sample were performed in triplicate, and the data was collected following the first heating scan.

X-ray Powder Diffraction. X-ray powder diffraction (XRPD) was employed to compare the crystal structure of micronised FP and SX and combination drug particles of FP and SX. XRPD diffractograms were obtained using a Phillips PW1710 Reflection X-ray powder diffractometer (Cambridge, UK). X-ray powder diffractograms were recorded by mounting the samples in the window of an aluminium specimen holder and then exposing it to the X-ray beam with a CuKα source (λ=1.5418 Å) operated at 40 kV and 25 mA. A single sweep between diffraction angles (2θ) 5° and 30° was employed for each measurement using a slit-detector at 25°C.

Production of Surface-Dissolved Lactose Monohydrate

The carrier lactose monohydrate used in this study was subjected to a temperature-controlled dissolution process to reduce the proportion of intrinsic fines. This technique is described in detail elsewhere (20).

Briefly, the coarse carrier lactose monohydrate (Lactohale®) was sieved to obtain a 63–90 μm sieve fraction using stainless steel sieves (Endecotts Limited, London, UK) and an Analysette 3 PRO vibratory sieve shaker (Fritsch GmbH, Idar-Oberstein, Germany) set to an amplitude of 1 mm. A 400 ml saturated aqueous lactose monohydrate solution was prepared and continuously stirred at the constant temperature of 20°C in a water-jacketed vessel. 100 g of the 63–90 μm lactose monohydrate was added to the saturated lactose monohydrate solution and the lactose monohydrate surface dissolved by increasing the temperature to 25°C. From knowledge of the solubility *versus* temperature profile of lactose monohydrate, this combination of variables was calculated to dissolve 10% of the initial mass of lactose monohydrate (20). After 24 h of stirring at 25°C, the surface-dissolved lactose monohydrate was removed by filtration and washed several times with lactose monohydrate saturated ethanol. Finally, it was allowed to air dry under ambient laboratory conditions (20–25°C and 30–40% RH) for seven days before use.

In-Vitro Aerosol Dispersion Performance

The aerosolization performance of combination drug DPI formulations containing micronised FP and SX with lactose monohydrate was compared to formulations containing combination drug particles containing FP and SX.

Formulation Preparation. A 25 mg unit dose delivering 500 μg FP and 50 μg SX required the addition of 1.6% *w/w* combination drug particles of FP/SX particles. The formulation was prepared by sandwiching the drug material between two layers of equal quantity of surface-dissolved lactose monohydrate. The formulation was then mixed in a Turbula for 40 min (Type T2F, Bachofen AG, Basel) at 46 rpm. To compare the *in-vitro* inhalation performance of formulations containing combination drug particles of FP/SX and micronized FP and SX, a combination drug formulation containing 1.45% *w/w* micronized FP and 0.15% *w/w* micronized SX was also prepared by sandwiching micronised FP and SX between two layers of the surface-dissolved lactose monohydrate, which was subsequently blended in a Turbula mixer at 46 rpm for 40 min.

High Performance Liquid Chromatography (HPLC) and Content Uniformity Analysis. Determination of drug content and analysis of next generation impactor (NGI) measurements were carried out using HPLC methodology adapted from Westmeier and Steckle (16). The HPLC consisted of a pump (Jasco PU-980, Jasco Corp., Japan) coupled to a UV detector (Jasco UV-975) set at 228 nm for parallel detection

of both APIs (t_r for salmeterol 1.30 min; t_r for fluticasone propionate 3.75 min). The mobile phase was a mixture of 45% *v/v* methanol, 35% *v/v* acetonitrile and 20% *v/v* water adjusted to pH 3.1. The pump flow rate was set to 1.5 ml. min^{-1} through 4.6 mm×250 mm C-18, 5 μm Hypersil column (Thermo Electron Corporation, Waltham, MA, USA), which was conditioned to 40°C in a column oven. Quantification was carried out by an external standard method. Linearity was checked between 0.1 and 50 μg/mL for each individual API. The relationship between drug concentrations and peak area for each drug was linear with linear regression analysis yielding a coefficient of determination (R^2) of 0.9999 for both drugs.

Following blending, the drug content uniformity of all the formulations was assessed. From each formulation, ten random samples of 25±1 mg were taken from random positions of the powder bed and dissolved in 100 ml of mobile phase. The proportion of drug in each sample was calculated and the content uniformity expressed by percentage relative standard deviation.

Inertial Impaction Testing of Prepared Blends. Following content uniformity testing, 25±1 mg of each blend was loaded into size three hydroxypropylmethyl cellulose capsules (HPMC, Shionogi Qualicaps SA, Basingstoke, UK). The capsules were stored at 44% RH for 24 h prior to *in vitro* performance testing. Testing was performed using a Next Generation Impactor (NGI) with pre-separator, which was connected to a vacuum pump (GE Motors, Michigan, USA). Prior to testing, the pre-separator was filled with 15 ml of mobile phase, and the cups of the NGI cups were coated with 1% *v/v* silicone oil in hexane to eliminate particle bounce. For each experiment, ten individual capsules of the same formulation were discharged into the NGI at 90 $L.min^{-1}$ for 2.8 s via a Monohaler® (Miat® SpA, Milan, Italy) DPI device. At this flow rate, a 4 kPa pressure drop is created across the device. Following aerosolization, the NGI apparatus was dismantled, and the inhaler, capsules and each stage of the NGI were washed down into known volumes of HPLC mobile phase. The mass of drug deposited on each stage of the NGI was determined by HPLC. This protocol was repeated three times for each blend, following which the emitted dose (ED), mass median aerodynamic diameter (MMAD), geometric standard deviation (GSD), fine particle dose (FPD) and fine particle fraction of the emitted dose ($\%FPF_{ED}$) were determined. The MMAD was determined by the % cumulative undersize on probability scale *versus* logarithmic aerodynamic diameter data as described previously (21). The d_{50} cut-off diameter of each stage of the NGI at 90 $L.min^{-1}$ is shown in Table I. The FPD and %FPF were calculated by the mass of drug collected on stages 2–8 of the NGI.

In Vitro Aerosol Dispersion Analysis of Combination DPI Drug Formulations Following Storage at 25°C/75% RH. The *in vitro* aerosol dispersion characteristics of combination DPI drug formulations containing micronised FP and SX and combination drug particles of FP and SX was evaluated following storage of the respective formulations at 25°C/75% RH for two and four weeks.

Capsules containing the respective formulations were stored in sealed containers, which contained saturated solutions of sodium chloride to produce an environment with 75% RH. The sealed containers were then placed into an oven controlled at 25°C, which provided the conditions of 25°C/75% RH. The *in vitro* aerosol dispersion analysis of the formulations was determined following storage for 2 and 4 weeks. In each case, ten capsules of each formulation was discharged into a NGI at 90 $L.min^{-1}$ for 2.8 s via a Monohaler® (Miat® SpA, Milan, Italy) DPI device, following which the %FPF was determined. In addition, the ratio of FP:SX on stages 2–5 of the NGI deposited upon aerosolization of each formulation was determined.

Table I. Aerodynamic Cut-Off Diameters of Stages 1–7 of the NGI at 90 $L.min^{-1}$

Stages of the NGI	d_{50} (μm)
1	6.48
2	3.61
3	2.30
4	1.37
5	0.76
6	0.43
7	0.26

Statistical Analysis. Linear regression analysis was used for the assessment of HPLC calibration. Statistical analysis between different populations was carried out using one-way analysis of variance. Comparison of the mean values was performed by Tukey's multiple comparison. All statistical analyses were performed using GraphPad Prism software (GraphPad Software Inc, California, USA). Error bars in graphical representations of data show ± 1 standard deviation in all cases.

RESULTS AND DISCUSSION

Combination drug DPI products are notoriously difficult to manufacture and have been reported to exhibit significant variability in the delivery of multiple active therapeutic agents present in a formulation (22). This can limit both the successful development of a combination drug DPI product and the synergistic action of the actives at the cellular level. The objective of the present study was to employ the SAX technology to engineer an ICS (FP) and a LABA (SX) into a single inhalable crystalline particle. To investigate the possible benefits of engineering two actives into a single particle, *in vitro* deposition characteristics of a conventional combination drug DPI formulation, prepared by blending the two micronised active therapeutic agents, was compared to a carrier-based formulation prepared using SAX-engineered combination FP/SX drug particles.

Physicochemical Characterisation

Scanning electron micrographs of micronized FP, micronized SX and SAX-engineered combination drug particles of FP/SX are shown in Fig. 1. In addition, the particle size distribution of the materials are shown in Fig. 2 and summarised in Table II.

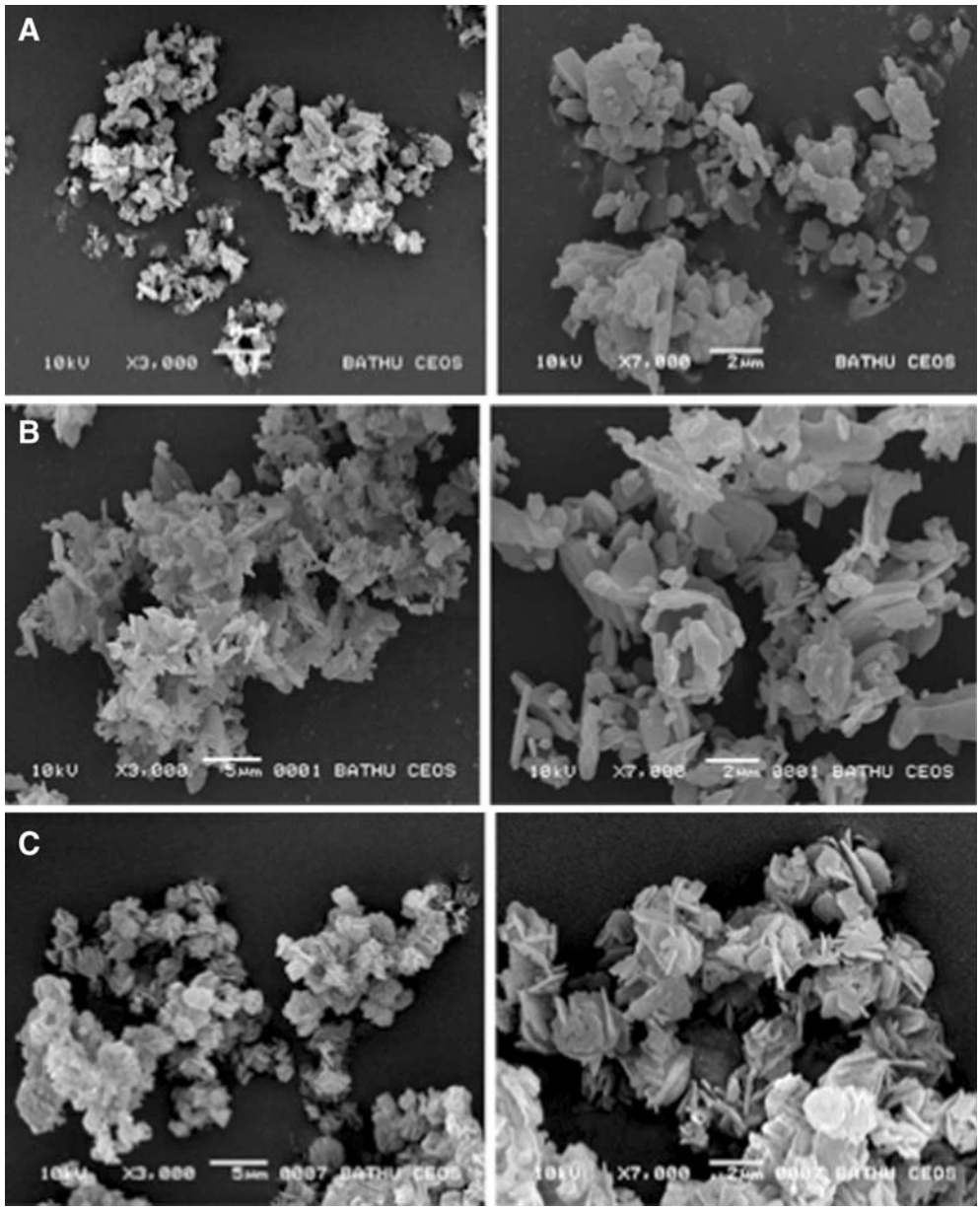

Fig. 1. Scanning electron micrographs of micronized FP (**A**) and SX (**B**) and SAX FP/SX (**C**).

The morphology of combination FP/SX drug particles appeared different from micronised FP and micronised SX as shown in Fig. 1. Whilst micronised FP and SX particles exhibit a similar plate-like morphology, qualitative assessment of SEMs of combination drug particles of FP/SX suggest that the particles are spherical and possess a corrugated morphology.

Particle size analysis of combination drug particles suggested a mono-modal particle size distribution with a median equivalent volume diameter (d_{50}) of 4.68±0.01 μm and d_{90} of 8.53±0.01 μm. In comparison, particle size analysis of micronized FP and SX determined a median volume diameter of 3.28±0.01 μm and 2.13±0.02 μm, respectively. Furthermore, the d_{90} of micronized FP and SX were 5.98± 0.01 μm and 4.09±0.02 μm, respectively. These data suggest that despite the larger particle size of combination drug particles of FP/SX in comparison to the micronized products, these particles remain within a suitable size range for pulmonary drug delivery.

Representative DSC thermograms of micronised FP and SX and combination drug particles of FP/SX are shown in Fig. 3A and B, respectively. The thermogram of micronized FP presented an endothermic peak at ~292°C related to the melting/degradation temperature of form I of FP (Fig. 3A). The thermogram of micronized SX (Fig. 3A) displays two endothermic events at ~124°C and ~139°C. Previous studies have shown that SX exists in two enantiotropic polymorphic forms (Form I and II), which are present in the micronized SX samples as evidenced by the endotherm at ~124°C, which represents the melting of SX form I that subsequently re-crystallises to SX form II at ~130°C before the melt at ~139°C (23–25). Micronisation of SX form I has been reported to induce the formation of trace seeds of SX form II, which enabled this re-crystallisation process to occur (24). In contrast, the DSC thermogram of SAX-engineered combination drug particles of FP/SX shows three endothermic peaks (Fig. 3B). The first endothermic peak exhibited at ~122°C

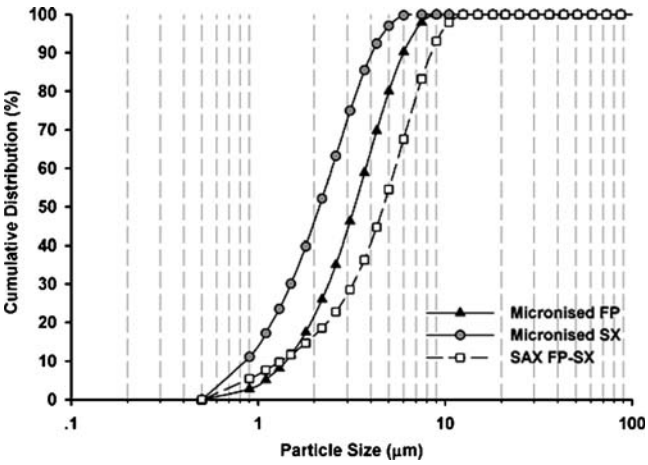

Fig. 2. Particle size distribution of micronised FP and SX and combination drug particles of FP and SX produced using SAX.

suggests the melt of form I of SX, the second endothermic peak exhibited at ~139°C represented the melt of form II of SX while the third endotherm at ~294°C suggests the onset of the melting/degradation of form I of FP. Hence, the thermogram of the combination FP/SX drug particles suggests that both FP and SX have been successfully co-processed using the SAX process. Furthermore, thermograms of the SAX particles did not indicate to a glass transition or re-crystallisation event over the heating scan range investigated, which suggests that the material maybe predominately crystalline.

The crystalline nature (*i.e.* the degree of molecular long-range and short-range order which indicate degree of crystallinity and non-crystallinity, respectively) of micronized FP, micronized SX and combination drug particles of FP/SX were further investigated using XRPD. The XRPD patterns in Fig. 4 show sharp diffraction peaks associated with micronised FP, micronised SX and combination drug particles of FP/SX, which suggest that the materials were predominately crystalline. The XRPD diffractograms of micronised FP and SX are similar to those reported previously (23,26). The XRPD diffractogram of combination drug particles of FP/SX possessed similar peaks to micronized FP, which was to be expected as the combination drug particles of FP/SX contain a significantly greater amount of FP than SX. These data in combination with DSC studies indicate that combination FP/SX drug particles are predominately crystalline.

In Vitro Aerosol Dispersion Performance

The aerosol dispersion performance of combination drug particles containing FP and SX was evaluated. Of particular interest was the uniformity of delivery of FP and SX across the stages of impactor. These data would provide a measure of the efficiency of co-delivery of actives on the stages of the impactor following aerosolization of combination drug particles, providing an indication to the potential benefit of co-

Table II. Particle Size Distribution of Micronized FP and SX and SAX-Engineered Combination Drug Particles of FP/SX

	$d_{10\%}$ (μm)±SD	$d_{50\%}$ (μm)±SD	$d_{90\%}$ (μm)±SD
Micronised FP	1.41±0.01	3.28±0.01	5.98±0.01
Micronised SX	0.86±0.01	2.13±0.01	4.09±0.01
SAX FP/SX	1.33±0.01	4.68±0.01	8.53±0.01

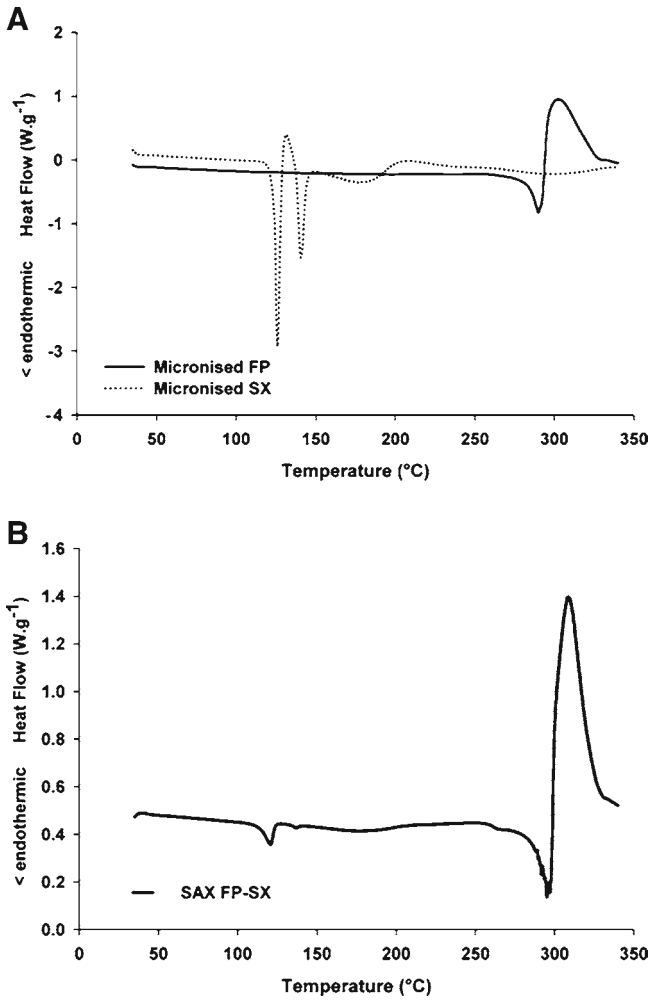

Fig. 3. DSC thermograms of **A** micronized FP and SX and **B** combination drug particles of FP/SX.

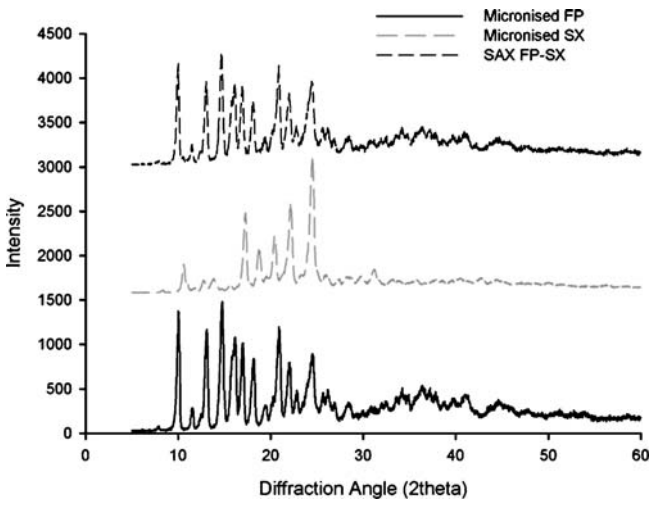

Fig. 4. XRPD diffractograms of micronized FP, micronized SX and combination drug particles of FP/SX.

processing the actives in a single particle over micronised mixtures in terms of dose delivery.

Drug Content Uniformity

The mean mass of drug and percentage relative standard deviation (%RSD) of the drug content of two combination DPI formulations containing either micronized FP and SX or SAX FP/SX are shown in Table III. It was confirmed that both formulations contained FP and SX in a ratio of 9.8 to 1, respectively. Furthermore, the %RSD of both actives in either formulation was less than 5%, which suggests homogeneous distribution of the actives in the formulation blend. These data provide further evidence to suggest that the SAX process was successful in co-processing FP and SX into a single particle.

In Vitro Aerosol Dispersion Performance of Combination DPI Formulations of Micronized FP and SX and SAX FP/SX Particles

The aerodynamic assessment of combination DPI formulations of micronized FP and SX and combination drug particles of FP/SX as determined by inertial impaction are shown in Fig. 5A and B, respectively. Furthermore, the mean emitted dose (ED), fine particle dose (FPD) and percentage fine particle fraction of the emitted dose (%FPF_{ED}) along with the mass median aerodynamic diameter (MMAD) and geometric standard deviation (GSD) of FP and SX following aerosolisation of the formulations are shown in Table IV.

The emitted dose of the micronised FP/SX formulation was determined as 315.00±5.13 μg for FP and 36.99±0.25 μg for SX (Table IV). In comparison, the emitted dose of formulations of combined SAX engineered FP/SX particles were significantly ($p<0.05$) lower for both FP (226.78±3.09 μg) and SX (25.66±2.44 μg). The FPD of FP and SX upon aerosolization of the micronised FP/SX formulation were 24.83±1.76 and 2.48±0.21 μg, respectively (Table IV). The FPD of FP and SX upon aerosolization of the formulation containing combination drug particles of FP/SX were 24.21±0.53 and 3.05±0.14 μg, respectively. These data showed that there was no significant difference between the FPD of FP and SX upon aerosolization of either the micronised FP/SX preparation or the formulation containing combination drug particles of FP/SX ($p>0.05$), and the ratios were maintained upon emission from the device.

The %FPF_{ED} of the micronized FP/SX formulation was 7.88±0.46% for FP and 6.70±0.53% for SX. In comparison, the formulation containing combination drug particles of FP/SX showed a significant increase in the %FPF_{ED} of FP (10.7±0.3%) and SX (11.6±1.1%) ($p<0.05$). There was no statistical difference between the %FPF_{ED} of both FP and SX following aerosolization of combination drug particles of FP/SX, which further suggests that both FP and SX materials were successfully co-processed into individual particles. The significant increase in the performance of the SAX engineered combination drug particles of FP/SX in comparison to the micronized material may be attributed to the corrugated morphology of the SAX particles as suggested by Fig. 1 and/or reduction in surface and interfacial free energy, which may have resulted in reduced drug-carrier adhesion and thereby increased drug liberation (27).

However, whilst these data suggest that the total dose delivered from the formulations was consistent with the formulated dose, they do not indicate uniform dose delivery of both actives throughout the impactor stages of the NGI. In order to investigate this further, Fig. 5A and B show the stage-by-stage deposition of both actives following aerosolisation of combination DPI formulations containing micronized FP and SX and combination drug particles of FP and SX on all stages of the impactor, respectively. Aerosolization of combination drug DPI formulations containing micronized forms of each drug resulted in significant ($p<0.05$) differences between the amount of FP and SX delivered on the preseparator and stages 2–8 of the NGI (Fig. 5A). In contrast, Fig. 5B shows that following aerosolization of combination drug particles of FP and SX, both actives were delivered consistently together across the stages of the impactor, with no significant differences observed between the amount of FP and SX delivered on all stages. These data suggest that delivery of the actives following aerosolization of the formulation containing micronised FP and SX may give rise to non-uniform delivery of the actives at a stage-by-stage level, which may limit possible synergistic action at a local level within the respiratory tract. In contrast, combination drug particles are likely to result in consistent delivery of both actives, which may enhance the opportunity for synergistic action between the two class molecules and, therefore, result in improved therapeutic outcomes.

Effect of Storage on the In Vitro Aerosol Dispersion Performance of Combination DPI Formulations of Micronized and Combination Drug Particles of FP/SX

The *in vitro* aerosol dispersion performance of formulations comprising micronized FP/SX and combination drug particles of FP/SX particles were also re-evaluated following storage of the respective formulations at 25°C/75% RH for two and four weeks. The fine particle dose delivery of combination drug DPI formulations containing micronized FP/SX and combination drug particles of FP/SX following storage at 25°C/75% RH are shown in Fig. 6.

Storage of the micronized FP/SX combination drug formulation for two weeks at 25°C/75% RH led to a significant ($p<0.05$) decrease in the %FPF_{ED} of FP, as shown by Fig. 6. There was, however, no significant effect of storage on the %FPF_{ED} of SX. In contrast, there was a small yet significant ($p<0.05$) increase in the %FPF_{ED} of FP following aerosolization of the formulation containing combination

Table III. Content Uniformity of Combination Drug Formulation Blends Containing Micronized FP and SX and SAX FP/SX Particles ($n=10$)

	Mean Mass of Drug (μg ± SD)		% RSD	
Formulation	FP	SX	FP	SX
Micronised FP/SX	360.21 (5.23)	36.59 (2.33)	4.27	3.89
SAX FP/SX	358.11 (4.11)	36.50 (2.18)	3.77	4.40

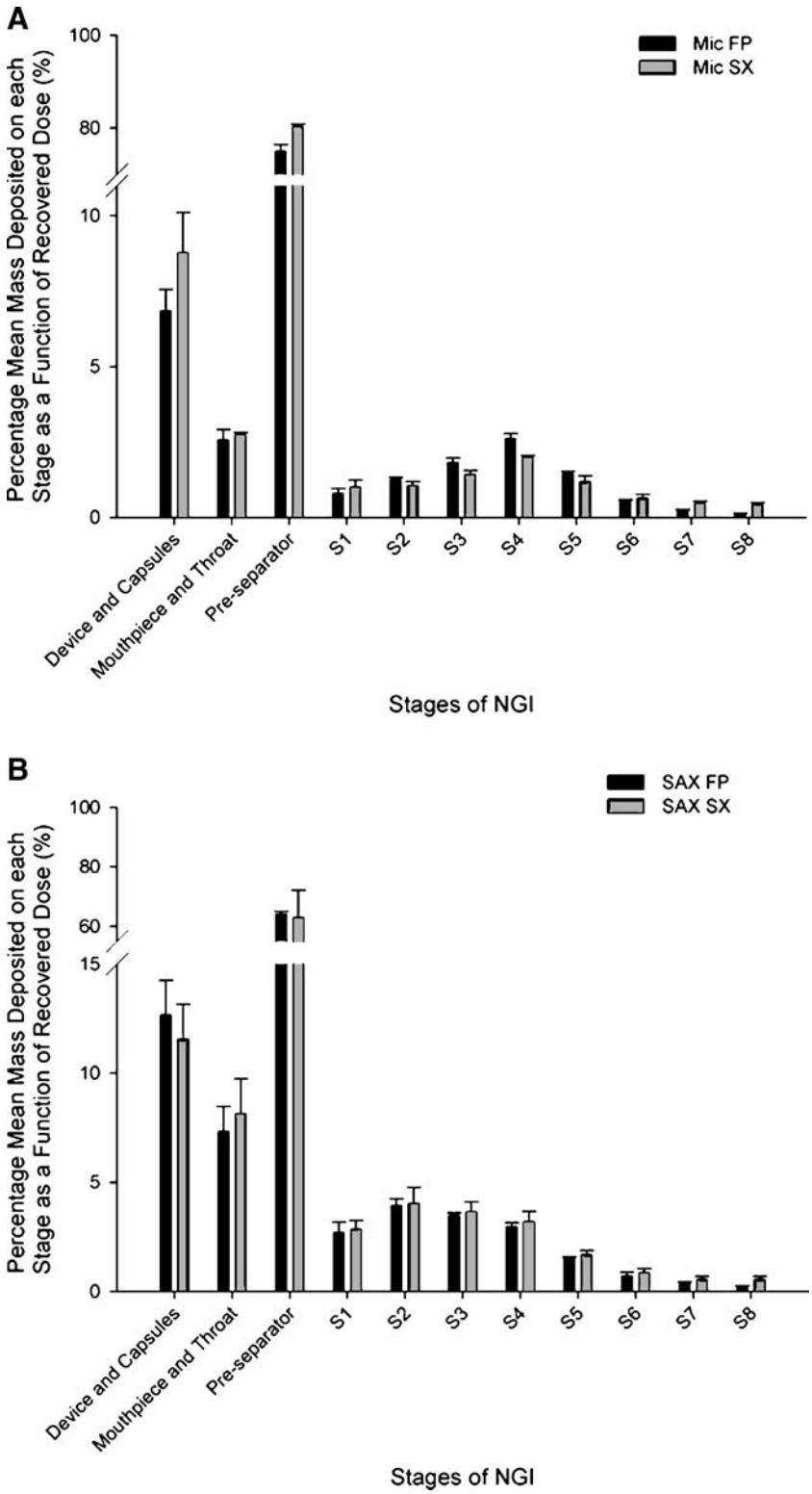

Fig. 5. Mean mass deposition of FP and SX on each stage of the NGI expressed as a percentage of recovered dose following aerosolization of combination DPI formulations containing **A** micronized FP and SX and **B** combination drug particles of FP/SX particles (n=3).

drug particles of FP/SX, whilst the $\%FPF_{ED}$ of SX was unaffected. Moreover, following storage of the formulations at 25°C/75% RH for two weeks, the $\%FPF_{ED}$ of FP and SX were significantly ($p<0.05$) greater upon aerosolization of the formulation containing combination drug particles of FP/SX than the micronised FP/SX formulation.

Upon aerosolization of the micronized FP/SX formulation after storage at 25°C /75% RH for four weeks, the %

Table IV. *In-Vitro* Aerosol Dispersion Performance of Combination DPI Formulations of Micronised FP and Micronized SX and SAX FP/SX (n=3). *FP* Fluticasone Propionate, *SX* Salmeterol Xinafoate

Formulations	Mean ED (μg ± SD)	Mean FPD (μg ± SD)	Mean FPF_{ED} (% ± SD)	MMAD (μm ± GSD)
Micronised (FP)	315.00±5.13	24.83±1.76	7.88±0.46	2.50±2.40
Micronised (SX)	36.99±0.25	2.48±0.21	6.70±0.53	2.30±3.00
SAX (FP)	226.78±3.09	24.21±0.53	10.68±0.32	3.60±2.80
SAX (SX)	25.66±2.44	3.05±0.14	11.96±1.09	3.30±3.10

FPF_{ED} of FP and SX had significantly ($p<0.05$) decreased (Fig. 6). In contrast, the $\%FPF_{ED}$ of either active from the SAX FP/SX formulations showed no significant change following storage. The decrease in the aerosolization performance of the micronized material may be attributed to relaxation of process-induced surface disorder present on the micronized particles, which may have affected their surface interfacial interactions and therefore product performance. Previous studies have suggested that metastable (non-crystalline, higher free energy sites) present on the surface of micronised materials molecularly relax to a lower free energy state, which affects their surface interfacial properties (28,29). As the SAX particles may be devoid of such process-induced surface disorder, the performance of the material is not significantly affected upon storage.

The sensitivity to variation in fine particle delivery of both FP and SX from the formulation containing micronized forms of both actives as a function of storage conditions may also effect the uniformity in delivery of both actives across the stages of the impactor. This is demonstrated in Fig. 7A and B, which shows the mean normalised mass ratio of FP:SX as a function of emitted dose deposited on stages 2–5 (representing cut-off diameters of 3.61, 2.30, 1.37 and 0.76 μm at stages 2–5 of the NGI at 90 $L.min^{-1}$, respectively) following storage of the formulations for two and four weeks at 25°C /75% RH, respectively. The mean normalised mass ratio of FP:SX following aerosolisation of the formulation containing combination drug particles of FP/SX after two weeks storage resulted in consistent delivery of both actives across stages 2 to 5, with values ranging from 0.85 to 1.2 (Fig. 7A), and remained similar following storage for four weeks (Fig. 7B). In contrast, it is evident that the mean normalised mass ratio of FP:SX deposited on stages 2–5 varied between approximately 0.85 and 1.6 on aerosolization of the micronized FP/SX formulation following storage of the formulation at 25°C /75% RH at two and four weeks.

These data suggest that the dose delivery of both micronized actives across the impactor stages can be greatly affected and thereby result in non-uniform dosing situations. Furthermore, the inability to maintain consistent delivery of

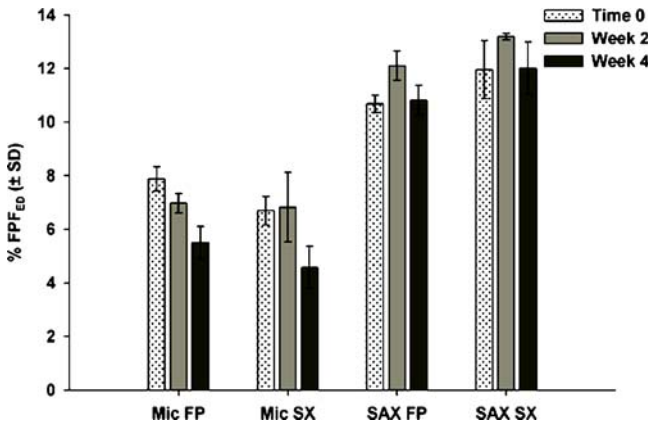

Fig. 6. Fine particle fraction of the emitted dose ($\%FPF_{ED}$) of FP and SX following aerosolization of combination formulations comprised of micronized FP/SX or SAX FP/SX following storage at 25°C/75% RH for 2 and 4 weeks.

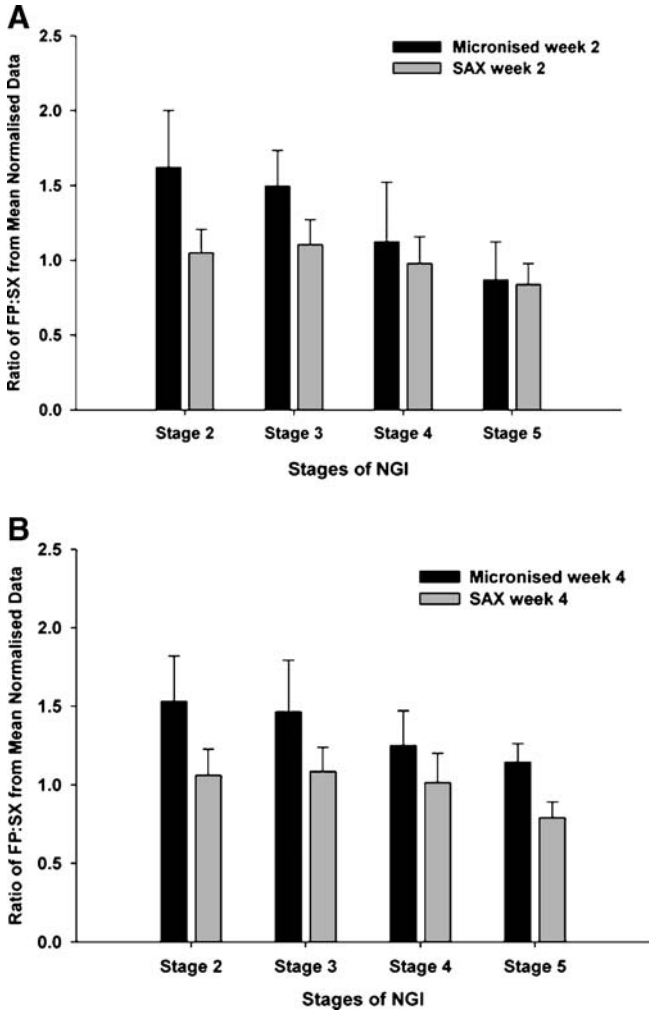

Fig. 7. Mean mass ratio normalized as a function of recovered dose of FP:SX on stages 2–5 of the NGI following aerosolization of combination DPI formulations containing **A** micronized FP and SX and **B** combination drug particles of FP/SX particles following storage of formulations at 25°C/ 75% RH for **A** 2 and **B** 4 weeks (n=3). The value at one indicates uniform deposition of FP and SX.

both actives in the correct ratio may reduce possible synergistic action of both actives at a local level. In contrast, delivery of the combination drug particles maintained uniformity in delivery of both actives to the lower stages of the impactor and was not directly influenced by storage conditions. These data suggest that the delivery of both actives in the form of combination drug particles is likely to enhance the probability of co-deposition of both actives in the ratios required to elicit a synergistic anti-inflammatory response. This approach, therefore, may afford itself as a novel development for the engineering of combination DPI formulations.

CONCLUSIONS

The SAX process has been utilized to produce individual crystalline particles containing two chemical entities in the respirable size range. Formulation performance analysis suggested that combination formulations comprised of SAX FP/SX particles have greater and more consistent fine particle delivery in the correct ratio than formulations produced using micronised drug actives. Furthermore, upon formulation stability testing, SAX particles may have improved stability profile following storage with little variation in performance or fine particle dose than formulations comprised of micronised material. Hence, the SAX process may be utilized to produce individual particulates of two active ingredients, which will allow the delivery of combination medicaments effectively and independently of dose variation. This approach presents itself as novel means to produce inhaled combination dosage forms.

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