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Research Paper

Investigations into the Formulation of Metered Dose Inhalers of Salmeterol Xinafoate and Fluticasone Propionate Microcrystals

Darragh Murnane,[1] Gary P. Martin,[1,2] and Christopher Marriott[1]

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Purpose. To investigate the aerosolization and behaviour of microparticles of salmeterol xinafoate (SX) and fluticasone propionate (FP) suspended in hydrofluoroalkane (HFA) propellant.
Methods. Microcrystals of SX and FP were produced from poly(ethylene glycol) by antisolvent crystallization. The suspension behaviour and aerosolization of the microcrystals when formulated as metered dose inhalers (MDIs) in HFA 134a propellant was compared with that of microparticles produced by micronization (mSX and mFP) using a glass twin stage impinger and by laser light diffraction using a pressurized cell.
Results. FP microparticles underwent non-reversible aggregation in suspension as seen by a doubling in the volume median diameter compared to the raw material. The degree of aggregation of SX particles in suspension was found to decrease as the particle size of the original particles increased. However, because the SX aggregate size was lowest for the particles with the smallest initial size (mSX), the highest fine particle fraction (FPF) of SX was obtained from a suspension of mSX. The FPFs following aerosolization of FP suspensions were similar although the FPF was lowest for particles with the largest original size.
Conclusions. The size of the aggregates in the HFA suspensions was found to correlate directly with the FPFs determined by impaction.

KEY WORDS: aggregation; fluticasone propionate; metered dose inhaler; microcrystals; salmeterol xinafoate; suspension.

INTRODUCTION

The localized delivery of aerosolized medicaments to the respiratory tract using either pressurized metered dose inhalers (MDIs) or dry powder inhalers is the mainstay management of asthma and chronic obstructive pulmonary disease. Few patients, however, realize the full benefit of localized pulmonary therapy because they cannot successfully inhale the medicaments such that the intended target is reached (1). The principal determinant of an inhalable aerosol being deposited in the respiratory tract is its aerodynamic size and polydispersity. It is generally accepted that for an aerosol cloud to be deposited in the respiratory tract it must possess an aerodynamic diameter <6 μm (2).

MDI formulations may be either solutions or particulate suspensions of the API. The droplet size upon atomization from the inhaler device can be controlled by altering the actuator orifice (3), (4) or by controlling the viscosity and evaporation rate of the propellant by formulation modification (4–6). In the case of suspension pMDIs, whilst factors governing atomization are important, the volume concentration of the particles in the propellant, the original size distribution and shape of those particles, the occurrence of aggregation and the homogeneity of particle dispersion are also crucial (7,8).

A stable suspension is a principal determinant of the size distribution of the inhaled aerosol as well as the dose reproducibility (9). The stability of a suspension is determined by the compatibility of the particles with the propellant, canister lining (10) and valve components (11,12). pMDI suspension instability can manifest as creaming, flocculation, sedimentation and Ostwald ripening. An MDI suspension with poor stability can rapidly lead to poor product performance (13) by directly affecting the respirable dose upon actuation. MDI suspensions may therefore require stabilization with excipients (*e.g.* surfactants). The changeover from chlorofluorocarbon (CFC) to hydrofluoroalkane (HFA) propellants following the Montreal Protocol has made the stabilization of suspension formulations problematic. Principally, traditional stabilizers display poor compatibility with the more polar (aprotic) HFAs (5).

APIs such as salbutamol (14) have been reformulated as a suspension in HFA 134a. Salmeterol xinafoate (SX) (12) and fluticasone propionate (FP) (14) have also been reformulated as excipient-free drug particle suspensions in HFA 134a. The above three commercially available products represent the approach of aiming to match the aerodynamic and clinical performance achieved by the earlier CFC product. However such an approach does not take advantage of the enforced

[1] King's College London, Drug Delivery Research Group, Pharmaceutical Science Division, 150 Stamford Street, London, SE1 9NH, UK.
[2] To whom correspondence should be addressed. (e-mail: gary.martin@kcl.ac.uk)

CFC–HFA changeover, whereby the stability, aerodynamic performance and reproducibility of MDI inhalers may be improved. For example rapid flocculation has been reported for SX and FP in both CFC and HFA 134a suspensions (15).

Particles in the respirable size-range are typically produced by crystallizing drug particles in an uncontrolled fashion followed by a high energy comminution process. The inefficient milling process exercises little control over particle shape or size distribution. Additionally, the energization and creation of amorphous surface regions serves as a source of physical instability (16,17). Aggregation has been reported to result in order to protect such high energy sites from Ostwald ripening (18). It is envisaged that the direct crystallization of drug particles in the respirable size range (microcrystals) would avoid such potential MDI suspension instability. The aim of the current work was to investigate the stability and aerosolization of microparticles of SX and FP formulated as suspension MDIs in HFA 134a produced by micronization and microcrystallization. This was achieved by applying a laser light diffraction technique recently developed by Jones *et al.* (21) to investigate the suspension behaviour of SX and FP microparticles suspended in HFA 134a and correlating the observations to *in vitro* impinger studies.

MATERIALS AND METHODS

Materials and Reagents

PEG 400, Analar® grade cyclohexane and HiPerSolv® grade ammonium acetate were purchased from BDH (VWR International Ltd., Poole, UK). High performance liquid chromatography grade methanol was purchased from Fisher Scientific Ltd (Loughborough, UK) or from VWR International Ltd. (Poole, UK). A Luna® ODS 2 column (3 μm, 150×4.6 mm i.d) was obtained from Phenomenex Ltd. (Macclesfield, UK). Span 80 was purchased from Sigma-Aldrich Company Ltd. (Gillingham, UK). Nylon filters (0.45 μm pore size, 47 mm diameter) and cellulose acetate syringe filters (0.45 μm pore size, Schleicher and Schuell brand) were obtained from Whatman Intl. Ltd. (Maidstone, UK). Water was produced by reverse osmosis using an ElgaStat unit (Elga LabWater, Marlow, UK). Silica gel was supplied by Prolabo (VWR International Ltd., Poole, UK) and compressed helium by BOC gases (Guildford, UK). HFA 134a was purchased as Solkane 134a (from Solvay Fluor und Derivate, Hanover, Germany) or Dymel 134aP (from DuPont de Nemours, Dordrecht, The Netherlands) or Zephex 134a (from Ineos Fluor, Cheshire, UK). Salmeterol xinafoate and fluticasone propionate were generous gifts from GlaxoSmithKline Pharmaceutical Development (Ware, UK). A Flixotide® Evohaler® (Lot D039083), and a Serevent® CFC MDI (Lot D038933) (Allen and Hanbury Brand) were both obtained from AAH Pharmaceuticals (Coventry, UK).

Production of SX and FP Microcrystals

Microcrystals of SX and FP were produced according to the process of amphiphilic crystallization (19) from solutions of API in PEG 400 and PEG 6000. Briefly, solutions of SX in PEG 400 (4% and 4.5% *w*/*w*) and PEG 6000 (4% *w*/*w*); and of FP in PEG 400 (0.65 *w*/*w* or 0.7% *w*/*w*) and PEG 6000 (0.65% *w*/*w*) were prepared as reported previously (19). Crystallization was performed according to the operating conditions detailed in Table I. Crystals were harvested by filtration on 0.45 μm Nylon filters and dried overnight at 50°C (Vacutherm, Heraeus GmbH, Hanau, Germany).

The dried powders were washed with 200 mL of filtered SX- or FP-saturated water at a temperature of 4°C by stirring continuously in a 250 mL glass beaker for 5 min. The washed drug crystals were recovered by filtration (cakes were washed on the filter with 100 mL of unsaturated filtered cold water. These washed crystals were dried *in vacuo* overnight at 50°C. The dried cakes were transferred to sealable glass vials and 15 mL of cyclohexane (BDH Ltd-VWR International, UK) was added to each. The vials containing the suspensions of SX or FP in cyclohexane were sonicated for 6 min in a Decon FS300B bath (Decon Laboratories Ltd, UK) to break up the filter cake. The cyclohexane was removed by heating the vials in the vacuum oven at 50°C overnight. The de-caked crystals were stored over silica gel in sealed glass vials at room temperature.

Characterization of SX and FP Microparticles

The purity of the microparticles and the reference micronized material was determined by a validated HPLC

Table I. Experimental Conditions for the Production of Salmeterol Xinafoate and Fluticasone Propionate Microcrystals and pMDI Suspension Formulation Compositions

	Crystallization conditions					Suspension formulations	
Formulation name	Batch size (g)	Solution concentration (% *w*/*w*)	Solvent: antisolvent ratio	Addition (g min^{-1})	Stirrer (rpm)	Concentration SX/FP (% *w*/*w*)	Final weight (g)
Micronized FP (mFP)	–	–	–	–	–	0.07	20
FP PEG 400	800	0.65	1:7	2,000	1,000	0.07	20
FP PEG 6000	800	0.65	1:7	2,000	1,000	0.07	20
FP PEG 400B	790	0.7	1:5.5	200	600	0.07	12
Micronized SX 25 μg (mSX)	–	–	–	–	–	0.05	12
Micronized SX 50 μg (mSX)	–	–	–	–	–	0.10	20
SX PEG 400	168	4.0	1:11	350	1,100	0.10	20
SX PEG 6000	183	4.0	1:11	350	650	0.10	20
SX PEG 400B	505	4.5	1:16	200	550	0.10	20

assay following the methods developed for SX and FP (20). A Luna ODS-2 150 mm column was used for high-throughput of the samples in place of the previously utilized reported Inertsil ODS-2 200 mm column, while the other analytical conditions were identical. The particle size distributions (PSD) of FP and SX microparticles was determined by laser diffraction (LD) using a Malvern Mastersizer X (Malvern Instruments Ltd, Malvern, UK) equipped with a 100 mm lens validated according to ISO 13320 (1990) standards. The dispersant liquid was an SX-saturated or FP-saturated solution of 0.5% *w/v* Span 80 in cyclohexane.

Powder densitometry was performed using a helium AccuPyc 1330 pycnometer (Micromeritics, Dunstable, UK). The instrument was calibrated according to the manufacturer's instructions using an AccuPyc 1330 1 mm^3 calibration sphere (certified volume of 0.7818537 cm^3). Each sample was analyzed five times, at a purge pressure of 14.5 psi helium, with ten purges and an equilibration rate of 0.005 psig min^{-1}.

Microscopy was performed using an FEI Quanta 200F field emission scanning electron microscope (FEI Company, Eindhoven, The Netherlands). Samples of SX and FP microcrystals, mSX and mFP were dispersed in cyclohexane in 5 mL stoppered glass vials, and sonicated briefly. Approximately 0.5 mL of the SX or FP suspensions was pipetted onto glass coverslips, which were adhered to microscope stubs with double-sided adhesive tape. The cover-slips were coated with the microparticles by removing the cycohexane suspending solvent under vacuum at 50°C (Vacutherm, Heraeus GmbH, Hanau, Germany). The coverslips were then sputter coated with a layer of gold (Polaron E51000, Polaron Equipment Ltd., UK).

Formulation of MDI Suspensions of FP and SX

Aliquots of micronized drug (mSX and mFP) or the microcrystals were weighed directly into either 19 mL aluminium MDI canisters (donated by Bespak Europe Ltd, UK) for aerodynamic assessment or into 19 mL poly(ethylene terephthalate) (PET) vials (donated by AstraZeneca Ltd, UK) for laser diffraction particle size analysis. The aluminium canisters were sealed with 63 μL, 20 mm diameter valves (DF 31/63 RCU CS, donated by Valois Division Pharmacie, France). The PET vials were sealed with a 20 mm continuous valve (donated by Bespak Europe Ltd, UK). HFA 134a was filled into the sealed containers until the desired weight was attained using a high pressure aerosol filler (Pamasol Willi Mäder, Pfäffikon, Switzerland). The suspensions were sonicated for 5 min to ensure dispersion of the powder in the HFA, and were stored valve-down at room temperature for between 10 and 25 days prior to aerodynamic testing. The canisters containing the SX and FP formulations were fitted with a Seretide® Evohaler® or a Flixotide® Evohaler® mouthpiece, respectively. Table I details the formulations which were prepared.

Pressure Cell Particle Size Analysis of MDI Suspensions

A 'flow-through' high pressure cell system was combined with a closed continuously circulating pump system (both Malvern Instruments Ltd, Malvern, UK), which had been specially modified in-house (21). The circulatory system was filled with HFA 134a through a specially designed valve. Samples of the suspensions for inhalation were injected directly into the circulatory system via a high pressure sample input valve. Prior to testing, each test formulation was sonicated in its PET vial for three minutes to ensure thorough dispersion of the microparticles. Samples of the HFA formulation in the PET vials were introduced into the circulatory pressure cell system with the pump set at 10 U (arbitrary units assigned by the pump manufacturer). Once the desired optical concentration of particles in the circulatory cell had been achieved (approx. 20% obscuration), the resulting suspension was equilibrated for 30 s before determining the particle size *in situ*. Each measurement sequence of particle size using the pressure cell, therefore, represented the preparation of a unique suspension. A minimum of three separate determinations were performed for each batch of microparticles. Particle sizing was carried out by laser diffraction using a Malvern Mastersizer X equipped with a 100 mm focal length lens. The 2OHD presentation was employed for data analysis and 2,500 measurement sweeps were performed for each individual measurement. The particle size was determined at 10 U of shear.

Dose Content Analysis

The emitted dose per actuation of the MDI suspensions was assessed using the dose uniformity sampling apparatus (DUSA) of the British Pharmacopoeia (BP) (22). The MDI actuator and valve assemblies were primed by firing five shots to waste before testing. A vacuum pump (Copley Scientific, Nottingham, UK) was used to draw air through the apparatus at 28.3 L min^{-1}. The inverted MDIs were shaken by hand for 5 s between each dose actuation. For both SX and FP suspensions, three actuations were collected in the device per determination. In the case of FP the washing solvent was HPLC grade methanol (Fisher Scientific Ltd, UK), and in the case of SX, the HPLC mobile phase was utilized. The filter and its support grid from the DUSA device were sonicated for 3 min in 10 mL of washing solvent. This was added to the washings from the inhaler mouthpiece and DUSA device and the volume constituted to 50 mL. Drug content was assayed in the washing solutions by HPLC. Qualification of the testing method was achieved by determining the dose content from a Flixotide® Evohaler® by firing two shots and a Serevent® CFC MDI by firing four shots, per determination.

Aerodynamic Assessment of the MDI Suspensions

The glass twin stage impinger (TSI) of the British Pharmacopoeia (22) was used to assess the aerodynamic performance of the pressurized metered dose inhalers. The TSI was set up and operated according to the BP with a flow rate of 60 L min^{-1}. The MDIs were primed by shaking for 5 s and discharging a dose to waste. Following a 5 s pause a further four doses were discharged in this manner. MDI suspensions were shaken for 5 s, and a dose was discharged immediately into the TSI "mouthpiece" and the pump was left to run for 7 s and then switched off. In the case of FP inhalers, ten doses were actuated in total. In the case of SX, ten doses were actuated for the suspensions formulated at a dose of 50 μg per actuation containing the microcrystal

batches SX PEG 400, SX PEG 400 B and micronized SX. Fifteen and 30 doses were actuated for suspensions containing SX PEG 6000 microcrystals (50 μg per actuation) and micronized SX (25 μg per actuation), respectively. Ten doses from a Flixotide® Evohaler® and 30 doses from a Serevent® CFC inhaler were used to check the recovery of FP from the TSI.

In the case of SX and FP inhalers, 7 and 30 mL of HPLC mobile phase were introduced onto stage 1 and stage 2 of the TSI respectively. Each stage was carefully washed and the washings were combined with the receptor liquid from the appropriate stage and diluted to volume. For SX inhalers, HPLC mobile phase was used to wash the device (to 20 mL), throat (to 20 mL), stages 1 and 2 (both to 50 mL) of the TSI. For FP inhalers HPLC grade methanol was used to wash the device (to 20 mL), throat (to 20 mL) and stages 1 and 2 (each constituted with the receptor liquids to 50 mL). The drug content of the solutions derived from the various stages was quantified by HPLC assay for SX and FP.

The recovered dose (RD) was the sum of the weights of drug (microgram) recovered from the inhaler device and the upper and lower stages of the TSI, whilst the emitted dose (ED) was the dose emitted from the inhaler device and depositing in the upper and lower stages of the TSI. Fine particle dose (FPD) was the amount of drug recovered from the lower stage of the impinger, which has a diameter less than the cut-off diameter of the upper stage of a TSI (drug particles <6.4 μm at an air flow rate of 60 L min^{-1}). The fine particle fraction (FPF) was calculated as the ratio of the FPD to RD and or the FPD to ED (both expressed as a percentage). The percent emission was calculated as the ratio of the ED to RD.

RESULTS

Microparticle Characterization

The purity, density and a summary of the PSD of the manufactured microparticles of SX and FP are presented in Table II. The data for the starting micronized SX and FP are also listed. Figs. 1 and 2 show the morphology of the API microparticles investigated in the study. No significant differences were demonstrated between the purity of the material recrystallized from PEG solvents and the raw materials (ANOVA, $p=0.249$ for SX and $p=0.510$ for FP). The crystal batch FP PEG 400B was shown to be of significantly higher density than those of the other FP microparticles (Tukey's test, $p<0.05$). The densities of SX microcrystal batches SX PEG 400 and SX PEG 6000 were demonstrated to be higher than those of the micronized raw material and SX PEG 400B (Tukey's test, $p<0.05$). The values of mSX and SX PEG 400 B density agree with the calculated crystal density for the form I polymorph of SX (23). The differences identified between the density values and the reproducibility of density determinations by pycnometry for all SX microparticle batches as well as FP PEG 400, FP PEG 6000 and mFP batches, were within ranges identified in previous studies of micronized particles (24). The value for FP PEG 400B were significantly different to the other FP microparticles, however, it was confirmed that the material was of an identical polymorph to all other batches (25).

The presence of sub-micron microparticles and small number of larger particles was evident from scanning electron micrographs (Figs. 1 and 2). SEM also demonstrated the assumption of sphericity inherent to laser diffraction-based sizing methods was not strictly appropriate in the case of SX or FP microparticles. The morphology of FP microcrystals was acicular and substantially different to mFP. The morphology of all SX crystals was similar demonstrating two-dimensional plate-like crystals. Microparticles crystallized from PEG solvents presented as plates which were thinner than those of mSX.

High Pressure Particle Sizing of MDI Suspensions

The measured PSD of microparticles when suspended in HFA 134a was compared to that of the microparticles when measured in an ideal liquid dispersant, cyclohexane (Table II). A significant difference in the particle median diameter for HFA suspensions was only found between mSX and SX PEG 400 (Tukey's test, $p<0.05$). No differences were observed between the other pair-wise comparisons. There was no significant difference between the 90% cumulative undersize diameter ($D_{(v,\ 0.9)}$) of mSX and SX PEG 400B in

Table II. Characteristics of Salmeterol Xinafoate and Fluticasone Propionate Microparticles Employed in the Inhalation Formulations (mean ± SD, $n\geq4$) and Particle Size Distribution of the Microparticles Suspended in HFA 134a (mean ± SD, $n\geq3$)

	Purity (% recovery)	Density (g mL^{-1})	Dispersant	$D_{(v,\ 0.1)}$ (μm)	$D_{(v,\ 0.5)}$ (μm)	$D_{(v,\ 0.9)}$ (μm)
SX PEG 400	100.8±1.33	1.301±0.039	Cyclohexane	0.66±0.01	3.66±0.22	10.78±0.51
			HFA 134a	3.40±0.59	12.82±2.75	34.00±5.73
SX PEG 6000	100.56±0.97	1.308±0.018	Cyclohexane	0.59±0.01	1.23±0.06	8.72±0.22
			HFA 134a	2.00±0.45	9.50±1.49	30.35±6.42
SX PEG 400B	102.13±0.88	1.257±0.017	Cyclohexane	1.06±0.06	6.59±0.31	14.55±0.63
			HFA 134a	3.53±0.05	9.97±0.30	20.90±1.04
mSX	99.96±2.71	1.239±0.005	Cyclohexane	0.59±0.01	1.13±0.12	3.69±0.23
			HFA 134a	1.57±0.58	7.03±0.95	18.36±2.67
FP PEG 400	99.22±1.07	1.399±0.004	Cyclohexane	0.85±0.02	2.50±0.10	6.73±0.19
			HFA 134a	0.98±0.02	5.32±0.11	18.04±2.50
FP PEG 6000	100.08±2.91	1.416±0.012	Cyclohexane	0.92±0.05	3.21±0.19	9.13±0.38
			HFA 134a	0.96±0.04	5.78±0.95	22.84±5.25
FP PEG 400B	101.93±0.72	1.682±0.289	Cyclohexane	1.94±0.03	6.14±0.17	17.98±0.96
			HFA 134a	2.25±0.21	12.13±1.11	31.09±3.27
mFP	100.38±0.48	1.391±0.067	Cyclohexane	1.01±0.02	3.06±0.05	7.20±0.15
			HFA 134a	1.44±0.11	5.26±0.16	12.78±0.84

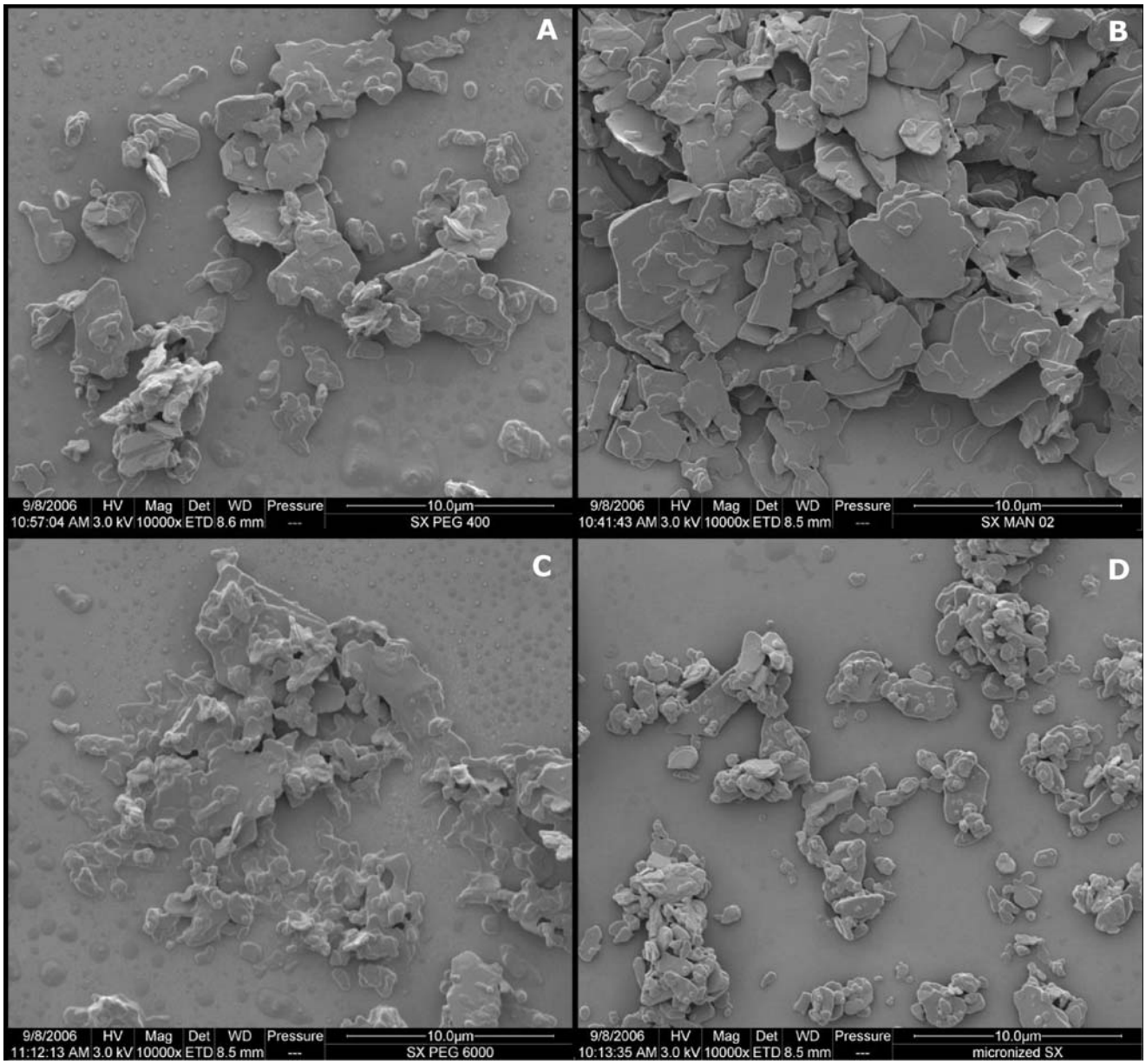

Fig. 1. Scanning electron micrographs of SX microparticles formulated as suspensions in HFA 134a: SX crystallized rapidly from PEG 400 (**A**); SX crystallized slowly from PEG 400 (**B**); SX crystallized from PEG 6000 (**C**); and commercial micronized SX (**D**).

HFA, while both SX PEG 400 and SX PEG 6000 crystal suspensions were larger.

When FP microparticles were dispersed in HFA 134a, the median diameter of FP PEG 400B particles was found to be significantly larger than the micronized material and microcrystals produced from PEG 400 and PEG 6000 solvents (Tukey's test, $p<0.05$). No significant differences were observed between the median diameters of mFP, FP PEG 400 or FP PEG 6000 batches. The $D_{(v,\ 0.9)}$ of FP PEG 400B microparticles when suspended in HFA 134a was significantly larger than that of suspensions of the three other microparticle batches.

All SX and FP microparticles displayed significantly larger particle sizes when suspended in HFA than the original microcrystals (pair-wise Student's t test, $p<0.046$). There was no significant difference between the growth in the median diameters of mSX and SX PEG 6000 microparticles (Student's t test, $p>0.05$), whilst both underwent significantly greater growth than SX PEG 400 and SX PEG 400B ($p<0.05$). The lowest increase in median diameter when the dispersing solvent was changed from cyclohexane to HFA 134a was for SX PEG 400B. The growth of the median diameters of FP particles was similar for all batches of microparticles upon formulation as suspension in HFA.

Qualification of TSI Analysis of SX and FP Suspensions

The recovered dose of salmeterol per actuation of the commercial Serevent® CFC inhaler from the TSI was 22.80± 0.80 µg ($n=3$), lower than the stated amount (25 µg, $p=0.042$). This corresponds to a recovery of 91.19±3.21% which

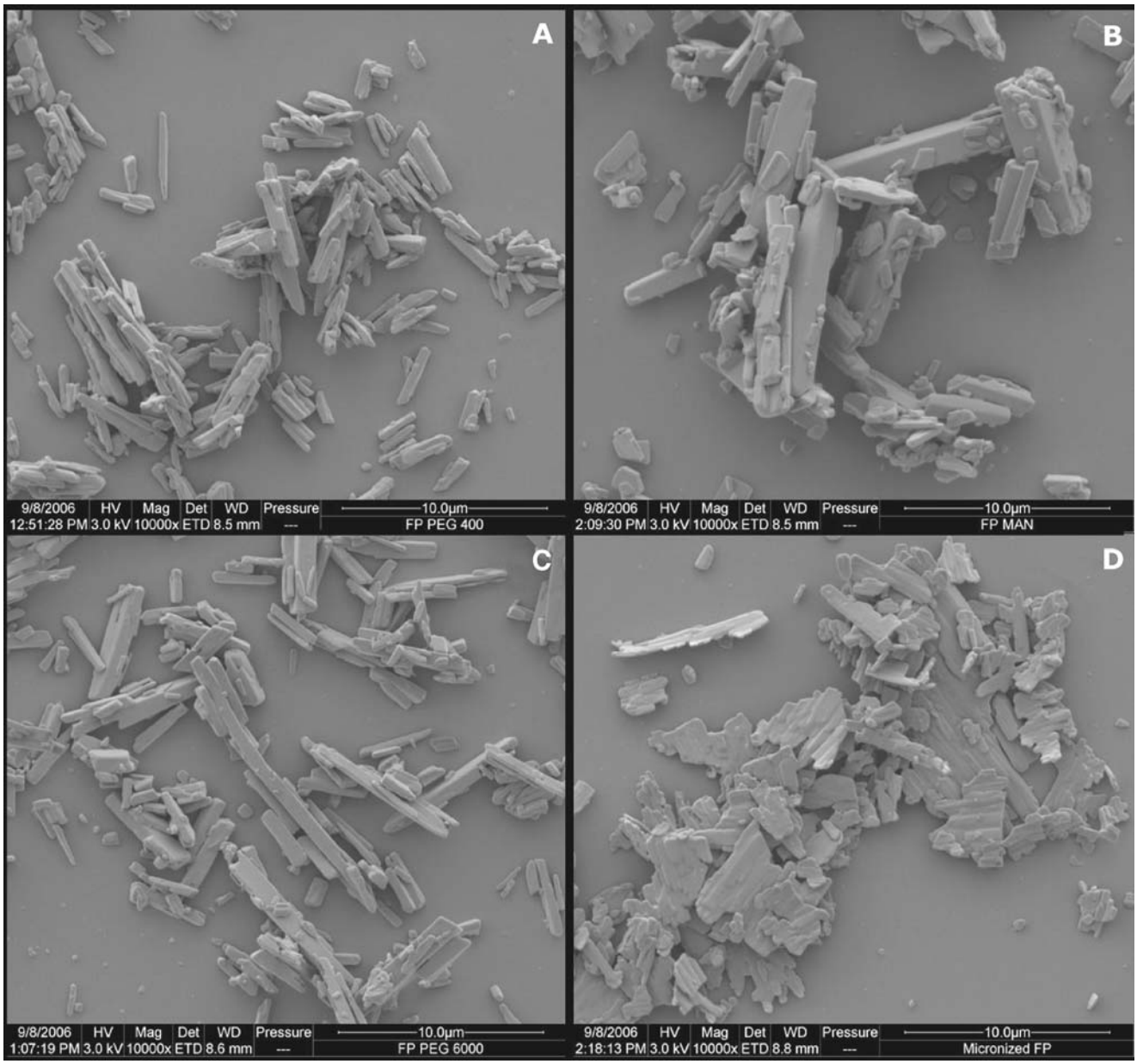

Fig. 2. Scanning electron micrographs of FP microparticles formulated as suspensions in HFA 134a: FP crystallized rapidly from PEG 400 (**A**); FP crystallized slowly from PEG 400 (**B**); FP crystallized from PEG 6000 (**C**); and commercial micronized FP (**D**).

was significantly lower than the dose recovered from the DUSA (28.48±2.48 μg, $n=4$, $p<0.05$). The recovery was, however, within the 75–125% limits of the British Pharmacopoeia (22). There was no significant difference between the recovered dose of FP from the TSI from a 50 μg Flixotide® Evohaler® and the labelled dose content ($n=3$, $p=0.07$). The drug recovery was 92.05±3.86%. There was no significant difference between the recovered dose of FP from the TSI and the dose content determined using the DUSA (47.84± 4.32 μg, $n=7$, $p=0.389$).

Aerodynamic Assessment of SX MDI Formulations

The *in vitro* deposition of SX from MDI suspension formulations in HFA 134a was studied using the TSI (Table III) and differences were observed in the deposition profiles of the different formulations of SX. The fine particle fractions (FPF) of two suspensions manufactured using commercial micronized SX were determined: one containing a dose of 25 μg and the second 50 μg per actuation, respectively. No significant differences were observed between the FPFs (Student's *t* test, $p=0.269$) or the emission ($p=0.403$) of these two formulations. Due to the limit of detection of the assay method for analyzing SX and FP (20) and in order to avoid the need to perform an excessive number of actuations, suspensions were prepared containing an actuated dose of 50 μg salmeterol in comparison to 25 μg (the commercial formulation (12)). The latter increase did not alter the FPF or the emitted fraction of the actuated dose (Table III). The FPF from the 25 μg suspension was similar to the value obtained with a commercial Serevent® Evohaler® formulation. The use of suspensions in this study

Table III. Metrics of MDI Formulations of SX and FP Microparticles Suspended in HFA 134a Propellant

Microparticle component	Recovered dose (RD) (μg)	DUSA dose (μg)	Fine particle fraction		Emission (%)
			% RD	% ED	
mSX (25 μg)[a]	21.01±0.54	Not determined	43.44±3.60	52.48±3.76	82.73±0.90
mSX (50 μg)	48.29±1.27	48.29±2.50	45.74±2.28	54.27±2.28	84.29±2.20
SX PEG 6000 (50 μg) batch 1	53.13±2.66	52.76±2.42	26.26±2.36	30.73±2.68	85.44±0.47
SX PEG 6000 (50 μg) batch 2	51.30±3.20	51.34±2.08	28.47±5.09	32.18±5.59	88.44±1.48
SX PEG 400 (50 μg) batch 1	54.65±2.07	52.59±1.64	22.80±1.04	25.70±1.21	88.71±0.79
SX PEG 400 (50 μg) batch 2	54.26±4.88	54.27±0.33	23.21±3.60	25.83±3.65	89.77±3.21
SX PEG 400B (50 μg)	55.48±3.75	55.85±1.14	22.61±3.35	26.22±4.47	86.56±2.54
mFP (50 μg)	48.17±1.75	50.06±3.76	43.16±2.79	48.87±2.96	88.32±1.10
FP PEG 6000 (50 μg) batch 1	46.88±1.49	50.09±0.49	38.09±3.39	45.25±4.31	84.22±0.83
FP PEG 6000 (50 μg) batch 2	32.83±2.51	43.98±2.54	40.21±6.38	40.21±6.38	91.96±2.26
FP PEG 400 (50 μg)	44.73±2.45	46.08±2.66	45.86±2.72	53.12±3.43	86.36±1.69
FP PEG 400B (50 μg)	52.09±1.81	Not determined	21.30±1.63	23.69±1.97	89.98±0.67
Flixotide® (50 μg)[a,b]	46.02±1.93	47.84±4.32	36.53±6.31	42.15±7.48	86.72±0.53

Dose content measured by the dose uniformity sampling apparatus (DUSA), recovered dose and deposition of SX and FP measured by the twin stage impinger (TSI: mean±SD, n=4; DUSA: mean±SD, n=3).
[a] TSI, n=3
[b] DUSA, n=7

with the higher concentration of microparticles was therefore judged to be appropriate.

The FPF of salmeterol was significantly higher when the micronized material was formulated as a suspension in HFA 134a than when microparticles crystallized from PEG solvents were formulated in a similar way (ANOVA and Tukey's tests, $p \leq 0.05$). No significant difference was demonstrated in the FPFs determined for suspensions of any of the three batches of microcrystals ($p \geq 0.05$). There was also no significant difference between the emissions of the microcrystals from HFA 134a aerosol formulations.

Aerodynamic Assessment of FP MDI Formulations

Significant differences were observed in the FPFs of FP aerosolized from the different suspension formulations (Table III). All emission values were found to be in the range 84–90%. The TSI deposition profiles were similar for all formulations with the exception of FP PEG 400B which produced a significantly lower FPF but a higher deposition in the throat than all other formulations with the exception of mFP (Tukey's test, 95% confidence intervals). The following ranking of FPFs was shown to be statistically significant using ANOVA and Tukey's test at the 95% confidence interval: FP PEG 400B < Flixotide® = FP PEG 6000 = mFP < FP PEG 400.

Reproducibility of MDI Formulation Performance

Three representative formulations were manufactured in duplicate to compare the batch-to-batch reproducibility of aerosolization including two SX formulations (SX PEG 6000 and SX PEG 400) and one FP formulations (FP PEG 6000). The formulation assessment is detailed in Table III. In the case of SX formulations, the only significant difference (Student's t test, p<0.05) was the lower emission for Batch 1 compared to Batch 2 of the SX PEG 6000 formulations. In the case of FP PEG 6000 formulations, there was a significantly higher percent emission for batch 2 while a higher recovered dose was determined for batch 1. In all cases, no differences were observed in the determined fine particle fractions.

LD Size Analysis and *In Vitro* Deposition Performance

The particle diameter equivalent to the aerodynamic diameter of 6.4 μm (the cut-off for the lower stage of the TSI), D_{calc}^{aer} was calculated using (1):

$$MMAD = MMD\sqrt{\frac{\rho}{\rho_0}} \quad (1)$$

where: MMAD is mass median aerodynamic diameter, MMD is the geometric mass median diameter, ρ is particle density and ρ_0 is unit density (*i.e.* 1 g cm^{-3}).

The percent <6.4 μm calculated from the size distributions of the original microparticles suspended in cyclohexane, from the size distributions of the microparticles suspended in HFA and from the impinger data (FPF as a percent of the recovered dose) are presented in Table IV. In the case of all SX microparticle formulations, there was a significant difference between the fractions of particles below D_{calc}^{aer} when suspended in cyclohexane and the determined FPFs (Tukey's test, $p \leq 0.05$). No significant difference was observed between the FPF and the D_{calc}^{aer} of the SX microparticles, when determined from *in situ* particle size determination of the microparticles suspended in HFA.

However, for FP, with the exception of FP PEG 400B, the FPF expressed as a % RD was significantly lower than the fraction below D_{calc}^{aer} as determined from the *in situ* sizing of the FP microparticles suspended in HFA ($p \leq 0.05$) (Table IV). A significant difference was observed between the fraction below D_{calc}^{aer} of the original microparticles dispersed in cyclohexane and the determined FPF for all suspension formulations of FP ($p \leq 0.05$). No significant difference was shown, however, between the FPF (as a % ED) for any of the FP microparticle formulations, and the

Table IV. The Particle Size of SX and FP Microparticles Measured by Laser Diffraction (Percent <6.4 μm (1)) in an Ideal Liquid Dispersant (Cyclohexane, Original) and Suspended in Hydrofluoroalkane 134a (HFA) and by Aerodynamic Assessment in the Twin Stage Impinger (TSI) (Percent < Aerodynamic Diameter of 6.4 μm) (mean ± SD, $n \geq 3$)

	Particles <6.4 μm original (%)	Particles <6.4 μm HFA (%)	Particles <6.4 μm TSI (% RD)
mSX	97.69±0.48	39.75±6.66	45.74±2.28
SX PEG 6000	77.88±1.13	27.57±2.30	26.26±2.36
SX PEG 400	64.58±1.95	20.15±5.12	22.80±1.04
SX MAN 02	43.12±2.19	22.45±0.83	22.61±3.35
mFP	80.24±0.71	52.69±1.89	43.16±2.79
FP PEG 6000	71.25±2.16	48.25±5.16	38.09±3.39
FP PEG 400	83.43±0.99	50.46±0.43	45.86±2.72
FP MAN 001	43.27±2.29	21.15±1.85	21.30±1.63

fraction below D_{calc}^{aer} from the *in situ* size distribution of the microparticles suspended in HFA ($p \geq 0.05$) (cf. Table III).

DISCUSSION

Microcrystallization of SX and FP resulted in microparticles possessing a geometric particle diameter measured by laser diffraction which might be considered suitable for aerosol delivery to the respiratory tract. LD analysis of raw materials in ideal dispersion media (such as were employed in this study) and validated in accordance with ISO 13320 is frequently performed to indicate the suitability for inhalational formulation and pulmonary deposition. A rank order relationship was observed between primary FP particle size measured by LD and the FPF: the lowest FPF resulted from a suspension of FP PEG 400B which demonstrated the largest particle size of the primary particles. No such relationship with primary particle size was observed in the case of SX deposition.

The current work identified that the validated LD analysis of raw materials provided a poor indication of the respirable fraction of microparticles. The effects of formulation (*e.g.* pMDI suspension) on the final aerosolized PSD are, by definition, excluded. Accordingly, the FPFs of SX and FP microparticles following suspension in HFA 134a were lower than predicted from LD analysis of the unformulated micron-sized particles (Tables III and IV). This indicated suspension instability of the primary particles, which can result from particle–particle or particle–device interactions (26).

In situ LD particle sizing employing a pressure cell and a pump capable of circulating the suspension under conditions of high shear revealed the origin of the decreased FPFs of SX and FP microparticles suspended in HFA 134a. The aggregation of primary FP and SX particles in HFA 134a was revealed to occur in the current study (Tables III and IV). Aggregation limited the efficiency of aerosolization of FP and, in particular, SX such that the FPF (% RD) agreed with the percent <6.4 μm calculated from the PSD of SX particles suspended in HFA in the high pressure cell (Table IV). Suspensions must be judged to be unstable if the primary particles are not maintained in suspension under moderate shear forces (such as shaking of the canister). The agreement between the FPF (% ED) rather than the FPF (% RD) and the PSD data (Table IV) suggested that in the case of FP pMDI suspensions, post-actuation events have a significant impact on the fine particle fraction.

SX and FP particles have been reported to flocculate in HFA and CFC suspension (15). The 'flocculation' of SX and FP, however, has been shown in the current work to consist of an irreversible component, and as such is more correctly termed aggregation. Aggregation was found to be detrimental to the efficiency of particle dispersion. The reported flocculation was most probably of the stable aggregates identified in the current study. The PSD of these aggregates was demonstrated to predict the respirable dose of SX and FP suspension pMDI formulations in HFA 134a (Table IV).

Particulate aggregation is common in pMDI suspension formulations (*e.g.* beclometasone dipropionate (27)). Suspension stability is essentially a function of the physicochemical properties of the material interface with the propellant (26). Stability depends on van der Waals' dispersive forces and electrostatic interactions (27) between particles which can lead to strong short-range cohesive forces (13). Cohesive forces due to incompatibility with the dispersion medium leads to particulate aggregation. Strong, cohesive inter-particulate forces account for the strength of aggregates of SX and FP, which were observed to behave as functional units within the suspension.

The degree of aggregation in HFA 134a of FP was significantly lower than that of SX. The latter was shown to decrease as the particle size of the primary particles increased (and hence specific surface area decreased). Such observations indicate the existence of an interfacial tension between SX particles and HFA 134a. mSX possesses a high surface energy (28) composed of high polar and H-bonding forces which are incompatible (Hansen solubility parameter, δ_t= 30 $MPa^{0.5}$) with those of HFA (δ_t=6.6 $MPa^{0.5}$, (6)). Aggregation of FP was essentially identical regardless of the microparticle type which was formulated. This contrasted to a previous study, where elongated FP particles (identified to be of a different solid state form) demonstrated greater aggregation than mFP in HFA 227 (29).

CONCLUSIONS

The manufacture of microparticles by antisolvent crystallization of SX and FP from PEG solutions resulted in microcrystals that could be employed in MDI formulations. Low FPFs of SX and FP aerosolized from HFA 134a were observed, regardless of whether micronized material or microcrystals were employed. The low FPFs were attributable to the irreversible aggregation of microparticles identified

during LD analysis of the high pressure suspensions. SX and FP aggregates appeared to behave as the functional drug particle units in suspension and the improved suspension stability anticipated for the microcrystal formulations at the outset of the study was not observed.

The results from this study underlined the importance of particulate and formulation factors in determining the availability of a fine particle dose from MDIs. For example, the deposition of FP was more influenced by particulate aggregation than the fibre-like shape of the primary particles. The current HFA-changeover approach of matching the previous (poor) characteristics of CFC formulations eschews the opportunity for improving the performance of inhalation formulations. A greater understanding of the fundamental interactions operating in MDI suspensions is required, to identify the physicochemical origins of suspension instability and enable successful suspension formulation approaches to be employed.

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