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Spray drying of budesonide, formoterol fumarate and their composites—II. Statistical factorial design and *in vitro* deposition properties

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ABSTRACT

The aim of this study was to investigate the effect of changing spray drying parameters on the production of a budesonide/formoterol fumarate 100:6 (w/w) composite. The systems were spray dried as solutions from 95% ethanol/5% water (v/v) using a Büchi 191-Mini Spray Dryer. A 2^{5-1} factorial design study was undertaken to assess the consequence of altering spray drying processing variables on particle characteristics. The processing parameters that were studied were inlet temperature, spray drier airflow rate, pump rate, aspirator setting and feed concentration. Each batch of the resulting powder was characterised in terms of thermal and micromeritic properties as well as an *in vitro* deposition by twin impinger analysis. Overall, the parameter that had the greatest influence on each response investigated was production yield – airflow (higher airflow giving greater yields), median particle size – airflow (higher airflow giving smaller particle sizes) and Carr's compressibility index – feed concentration (lower feed concentration giving smaller Carr's indices). A six- to seven-fold difference in respirable fraction can be observed by changing the spray drying process parameters. The co-spray dried composite system which displayed best *in vitro* deposition characteristics, showed a 2.6-fold increase in respirable fraction in the twin impinger experiments and better dose uniformity compared with the physical mix of micronised powders.

1. Introduction

Effective control of asthma symptoms and maintenance of optimal lung function are critical for the long-term management of patients with persistent asthma. Increasing the dose of inhaled glucocorticosteroid or, alternatively, the addition of a long-acting β_2-agonist to low-dose inhaled glucocorticosteroids are two therapeutic options. In 2001 evidence was found that the addition of a long-acting β_2-agonist such as formoterol to inhaled glucocorticosteroids may be more beneficial in terms of asthma control than increasing the dose of corticosteroids alone (Zetterström et al., 2001). Consequently, from a point of view of efficacy, a single device containing both, a steroid and β_2-agonist compound, would be the best solution.

The first commercial inhaler containing budesonide and formoterol was Symbicort® using the Turbuhaler® device produced by AstraZeneca. It contains an inhalation powder, which is a mixture of two active ingredients, budesonide and formoterol fumarate dihydrate and another inactive ingredient, lactose (Symbicort®, product leaflet). A few other combination inhalers are currently available on European markets for instance Seretide® (Advair®) containing dry powders of fluticasone propionate and salmeterol xinafoate and metered dose inhalers—Combivent® with salbutamol sulphate and ipratropium bromide and Berodual® (Duovent®) formulated with fenoterol hydrobromide and ipratropium bromide.

Ensuring the dose homogeneity can be problematic when a mixing process is employed to produce a blend of pharmaceuticals due to segregation of particles of different sizes or different adhesion to the inner walls of a mixer. The uniformity of the blend may not be achieved with sufficient precision especially for inhalable mixes containing very small doses of one of the drugs. Co-spray drying from solutions could be an alternative and more suitable method of producing composite powders ensuring constant composition of each of the particles (Corrigan et al., 2006). Preparation of combination formulations demonstrating suitability for pulmonary or nasal administration composed of a spray dried mixture of drugs or excipients have been reported (Woolfe et al., 2002; Tajber et al., 2008). It has also been shown that the *in vitro* deposition of such composite particles can be advantageous over the equivalent physical mixtures (Kawashima et al., 1998).

However, spray drying is a complex technological process and enables adjustment of many parameters. Understanding how changing processing parameters will affect the physicochemical properties of the product obtained is desirable in the production of solid dosage forms with controllable and predictable performance. With a Büchi 191-Mini Spray Dryer it is possible to alter a num-

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ber of parameters, such as inlet temperature (the temperature of the drying medium), airflow through the apparatus (amount of the drying medium needed to disperse the liquid), peristaltic pump speed (the rate at which the liquid is delivered to the atomiser), aspirator suction velocity (the rate at which the drying medium is drawn through the spray dryer) and concentration of the liquid being spray dried.

There is evidence that different conditions of spray drying impact on the final product. Chawla et al. (1994) investigated four factors: pump speed, aspirator level, heat level (equivalent to inlet temperature) and concentration of salbutamol sulphate and their effect on particle size and yield of the drug. Although an older model, a 190-Büchi spray dryer, was used, it was concluded that no individual parameter but rather a combination of factors was responsible for controlling the output. It was concluded that larger particles were produced when both the aspirator level and the feed concentration were at their highest levels, while an improvement in the percentage yield was obtained by increasing the aspirator level, feed concentration and pump speed (Chawla et al., 1994). Optimisation of production yield and moisture content by means of changing spray drying conditions in a NIRO Mobile Minor device were carried out by Billon et al. (2000). The most significant factors affecting the product characteristics found in this study were inlet temperature, pump feed rate and excipient concentration. Spray drying of human insulin was the subject of another study (Ståhl et al., 2002) and it was found that degradation of the protein was affected mainly by the process variables determining the outlet temperature, i.e. inlet air temperature, aspirator capacity and feed flow rate.

Micron-sized drug particles may be very adhesive and cohesive, which is reflected in poor flow properties (Hickey et al., 1994). In the design of dry powder inhalations it is essential to include studies on how to achieve good powder emissions from capsules and devices. Therefore the evaluation of the flow and packing properties of the inhalable powders is necessary. A few studies have been reported on the assessment of the aforementioned mechanical characteristics, focusing on the carrier-based powder mixtures (Kawashima et al., 1998; Iida et al., 2001). Kawashima et al. (1998) have shown a proportional relationship between the amount of drug emitted and the specific surface area. On the other hand, surface roughness of carrier was studied by Iida et al. (2001). It was shown that when this parameter decreased the flow properties of the mixture improved and this, in turn, correlated well with the improved fraction of drug emitted from the capsule and device.

The principles of evaluation of flowability as well as their mathematical correlations were first explained by Carr (1965). The estimation of flowability involves the use of properties that can be measured by the following methods: angle of repose, compressibility, angle of spatula and cohesion (Carr, 1965). Detailed accounts regarding these methods can be found in the literature and automated powder flowability analysers are available on the market.

However, to our knowledge, no studies on how the spray drying parameters may influence *in vitro* or *in vivo* pulmonary deposition have been conducted. Considering the above and that spray drying is an important unit process in pharmaceutical technology, it warrants such investigations.

In the above studies, statistical factorial experiments were employed as they can be very useful when investigating complex systems and the effects of more than one factor are investigated simultaneously in all their possible combinations. Therefore they are also of economic advantage saving time and reducing material costs. The choice of an experimental design depends on the objectives of the experiment and the number of factors to be investigated. In multi-factor experiments one can use fractional factorial designs, which involve only a fraction of full set of runs and a 2^{5-1} statistical design was chosen in our investigations.

Feasibility studies of spray drying and physicochemical characterisation of budesonide and formoterol fumarate dihydrate co-processed mixtures has been carried out previously (Tajber et al., 2009). The work of the present study has aimed at examining the effect of changing spray drying parameters on a budesonide/formoterol fumarate 100:6 composite. Each batch of the resulting powder was characterised in terms of micromeritic properties and *in vitro* deposition since, in the design of dry powder inhalations it is vital to achieve good pulmonary deposition.

2. Materials and methods

2.1. Materials

Micronised budesonide and micronised formoterol fumarate dihydrate were kindly donated by IVAX Pharmaceuticals (Waterford, Ireland). Ethanol was obtained from Cooley Distillery (Ireland). Methanol and acetonitrile were HPLC grade and purchased from Lab-Scan. Deionised and HPLC water were produced by a Purite Prestige Analyst HP water purification system.

2.2. Preparation of powder formulations

The composite systems were spray dried as solutions from 95% ethanol/5% water (v/v) using a Büchi 191-Mini Spray Dryer (Büchi Laboratoriums-Technik AG, Flawil, Switzerland) fitted with a standard 0.7 mm 2-fluid nozzle. The volume of solutions used in all experiments was 50 ml and the atomising air pressure at which the spray dryer was operated was set to 5 bar. The remaining process parameters were set according to the requirements of the individual runs as set in the factorial design studies outlined in Section 2.10.

For comparison purposes in the glass impinger studies the equivalent physical mixture of budesonide and formoterol fumarate dihydrate was prepared. Stoppered vial (10 ml), containing accurately weighted quantities of micronised substances, was placed in a Turbula mixer (Glen Greston Ltd., Middx, UK) and mixing was carried out for 15 min at 42 revolutions per minute. The median particle sizes for the powder raw materials were 1.4 and 2.0 μm for budesonide and formoterol fumarate dihydrate, respectively, as measured by laser diffraction particle sizer Malvern 2000 with a Scirocco dry powder attachment (Malvern Instruments, UK).

2.3. Thermal analysis

Differential scanning calorimetry (DSC) experiments were conducted using a Mettler Toledo DSC 821^e with a refrigerated cooling system (LabPlant RP-100). Nitrogen was used as the purge gas. Hermetically sealed 40 μl aluminium pans with three vent holes were used throughout the study and sample weights varied between 4 and 10 mg.

Thermogravimetric analysis (TGA) was performed using a Mettler TG 50 module linked to a Mettler MT5 balance in the furnace under nitrogen purge. Sample weights between 5 and 12 mg were used and placed into open aluminium pans. A heating rate of 10 °C/min was implemented in all DSC and TGA measurements. Analysis was carried out and monitored by Mettler Toledo STAR^e software (version 6.10) with a Windows NT operating system.

2.4. X-ray diffraction analysis (XRD)

Powder XRD analysis was conducted using nickel filtered Cu Kα ($\lambda = 1.54056$) monochromatic radiation on a Siemens D500 Diffractometer with a DACO MP wide-range goniometer. The anode X-ray tube was operated at 40 kV and 30 mA. Measurements were taken

from 5° to 35° on the 2θ scale at a step size of 0.05°/s. Low background silicon mounts (Bruker AXS, UK) were used to support the sample during measurements.

2.5. Visualisation of particles

Scanning electron microscopy (SEM) analysis was performed using a Hitachi S-3500N variable pressure scanning electron microscope. Samples were glued onto aluminium stubs and sputter-coated with gold prior to analysis.

2.6. Particle sizing

Particle size analysis of the co-spray dried composites was carried out using a Sympatec HELOS laser diffraction particle size analyser connected to a RODOS dry dispensing unit. The pressure applied to achieve a proper dispersion of particles was 3 bar and injector depression was 128 mbar. The feed rate used was 18%. Measurement was done using a lens with focal length equal to 100 mm and 51 ms measurement intervals.

2.7. Density measurements

A 5 cm^3 graduated cylinder was used in the bulk density determination of samples. The container was filled with accurately weighed sample and the top was levelled. The density was calculated as the ratio of the mass to the volume of the sample and called aerated density. The tap density was determined similarly to the aerated density, but the volume taken for calculations was that after 500 strokes.

The aerated and tap density values were then used to calculate the Carr's compressibility indices according to the following equation:

$$\text{Carr's compressibility index} = \left(\frac{\text{tap density} - \text{aerated density}}{\text{tap density}}\right) \times 100 \quad (1)$$

2.8. Glass impinger studies

The apparatus used was a twin stage glass impinger conforming to the specification in the British Pharmacopoeia (apparatus A, Appendix XII F, 2007) and European Pharmacopoeia (apparatus A, Section 2.9.18, 5th Edition, 2007). The lower stage of the twin impinger has a cut-off of 6.4 μm and the particles that reach this chamber are defined as "respirable" and are thought to penetrate into the deep lung (Hallworth and Westmoreland, 1987).

The separate analysis of each replicate sample from the factorial design was not possible due to an insufficient amount of material being available. Therefore sample blends were prepared by mixing for 15 min in a Turbula mixer equal weights of the two replicates and these blends were used for loading the capsules used in the twin stage impinger analysis. The analysis was carried out five times for each blended sample.

The powders were aerosolized using a dry powder inhalation device (Rotahaler®, Allen & Hanburys, UK). The aerodynamic particle deposition was investigated using the twin impinger (Model TI-2, Copley) containing 7 and 30 ml of 80% (v/v) ethanol for stages 1 and 2, respectively. A total of 50 ± 1 mg of powder was loaded into a size 3 hard gelatin capsule. After the Rotahaler® was connected to the mouthpiece of the twin impinger, a capsule was placed in the holder of the device. An air stream of 60 l/min was produced throughout the system by attaching the outlet of the twin impinger to a vacuum pump for 3 s. The powder deposited in stages 1 and 2, mouthpiece and device was collected by rinsing with fresh solvent. The rinsed solutions were diluted to appropriate volumes, filtered through 0.45 μm PVDF filters (Millipore) and the drug contents were determined by a HPLC method. The fraction considered as the respirable fraction (stage 2) was expressed as a percent of the dose emitted from the device.

2.9. HPLC analysis

The analysis of both of the drug contents in the aliquots collected from the twin impinger studies was carried out with a use of a Waters™ HPLC set up. It consisted of a Waters™ 600E pump, Waters™ 717 Autosampler and a Waters™ 2487 Dual λ Absorbance Detector. The analytical column used was a Waters™ Spherisorb Cartridge (150 mm length, diameter 4.6 mm, ODS1 packing, particle size 5 μm). The mobile phase consisted of methanol, acetonitrile and water (35:30:35, v/v). Separation was carried out isocratically at ambient temperature (about 22 °C) and a flow rate of 0.5 ml/min, with UV detection at 230 nm. The retention time of formoterol fumarate was 1.6 min and budesonide 8.8 min. The calibration curves were prepared from concentrations falling into the linear ranges of detection and were 0.5–50 μg/ml for formoterol fumarate and 2–100 μg/ml for budesonide.

2.10. Design study—structure

A 2^{5-1} factorial design study was undertaken to assess the effect of spray drying processing variables on particle characteristics. The processing variables that were studied using the Büchi 191-Mini Spray Dryer were (*A*) inlet temperature, (*B*) spray drier airflow rate, (*C*) pump rate/setting, (*D*) aspirator setting and (*E*) feed concentration. Each factor was studied at two levels as listed in Table 1.

The low and high setting for the airflow parameters were the greatest and lowest practically obtainable with the machine used (Büchi B-191 technical specification). The pump rate of 40% was selected as being the lowest practical level at which no condensation of the solution on the drying chamber occurred. As regards the aspirator suction, the rates were dictated by the balance between the degree of product separation in the cyclone and the moisture content in the product. The value of 1.75% (w/v) for the solution concentration was the realistic limit of budesonide solubility achievable without long stirring or using an ultrasonic bath to avoid possible chemical degradation of the actives in solution.

All runs of the experiment were performed in a randomised manner to eliminate any unknown possible sources of bias. The design matrix for the experiment is shown in Table 2.

Each system was prepared in duplicate. The response variables that were analysed by the factorial experiment were median particle size, outlet temperature, production yield, Carr's compressibility index and the *in vitro* respirable fraction obtained by twin stage impinger deposition studies. The statistical analysis was carried out with the assistance of Minitab™ software (version 13.32).

The type of factorial design used here is a resolution V design, which means that main effects are confounded with four factor and two factor interactions are confounded with three factor interac-

Table 1
Processing variables used in the factorial study.

Parameter	Low (−)	High (+)	Units
A (inlet temperature)	78	84	°C
B (airflow)	400	800	l/h
C (pump setting)	10 (~150)	40 (~600)	% (ml/h)
D (aspirator setting)	70 (−20)	100 (−40)	% (mbar)
E (feed concentration)	1	1.75	% (w/v)

Table 2
The design matrix consisting of one half fraction of the 2^5 design and the data collected from the analyses and used in the statistical factorial design.

Sample ID	Inlet temperature	Airflow rate	Pump setting	Aspirator setting	Feed concentration	Percentage yield (%)	Carr's index (%)	Outlet temperature (°C)	Median particle size (μm)	Respirable fraction (%)
1BF	−	−	+	+	+	32.2	53.0	48	3.73	4.2
2BF	−	−	−	+	−	52.7	44.8	61	2.86	5.5
3BF	+	−	+	+	−	24.8	36.7	54	3.59	6.6
4BF	−	+	−	−	−	38.4	28.2	56	1.94	4.1
5BF	+	−	+	−	+	24.0	38.0	48	4.86	4.4
6BF	−	+	+	+	−	58.4	42.3	51	1.97	1.8
7BF	−	−	−	−	+	41.6	48.4	58	5.04	4.2
8BF	+	+	−	−	+	57.7	49.4	60	2.09	2.8
9BF	+	−	−	+	+	51.5	60.0	68	4.09	4.3
10BF	−	+	+	−	+	58.4	47.7	44	2.48	1.7
11BF	+	+	+	+	+	62.3	47.5	55	2.20	2.6
12BF	−	+	−	+	+	60.9	46.1	62	1.76	4.8
13BF	+	−	−	−	−	41.3	43.6	62	4.62	12.1
14BF	+	+	−	+	−	57.9	43.6	66	1.91	4.3
15BF	+	+	+	−	−	61.4	45.6	49	2.16	3.3
16BF	−	−	+	−	−	21.6	44.8	42	4.42	5.9

Table 3
Coefficients of the quadratic equations linking the spray drying parameters (in terms of coded factors) with responses. R^2 values are given to indicate the goodness of fit of the theoretical models to the experimental values.

Term	Median particle size[a] (μm)	Term	Yield (%)	Term	Carr's index (%)
Intercept	+0.460	Intercept	+46.36	Intercept	+44.95
A	+0.013	*A*	+1.23	*A*	+0.58
B	−0.150	*B*	+10.15	*B*	−1.18
C	+0.016	*C*	−3.88	*C*	−0.52
D	−0.043	*D*	+3.72	*D*	+1.78
E	+0.022	*E*	+1.82	*E*	+3.78
A×*C*	−0.011	*A*×*B*	+2.05	*A*×*B*	+2.16
A×*D*	+0.015	*A*×*D*	−2.19	*A*×*C*	−3.07
B×*C*	+0.013	*B*×*C*	+6.69	*B*×*C*	+2.52
B×*D*	+0.021	*C*×*D*	−1.80	*C*×*D*	−1.35
B×*E*	−0.001			*C*×E	−1.68
				D×E	+1.12
R^2	0.9895	R^2	0.9417	R^2	0.9284

[a] The values contributing to this response were logarithmically transformed prior to the statistical analysis.

tions. However, only main effects and two-factor interactions were considered as the most likely to influence the response and only they constituted the statistical model.

The statistical analysis of variance, ANOVA, was carried out to determine the significance and impact of each main factor as well as their interactions on the product characteristics. The relationships linking the main factors and interactions with the response were determined and presented as quadratic equations of the general form in the following equation:

$$Y = \text{intercept} + \sum \text{main effects} + \sum \text{interactions} \quad (2)$$

with coefficients for each term of that equation i.e. for intercept, each main effect and interaction shown in Table 3.

The equation coefficients were calculated using the coded values, thus the various terms can be compared directly regardless of their magnitude. The coding used throughout the statistical analysis denotes that −1 was taken instead of the actual value for the factor on its lower level and +1 for the upper level. Therefore, a positive parameter coefficient indicates that the output increases with increasing variable level and a negative coefficient that the output increases with decreasing variable level. Numerical output of ANOVA includes *F*-value indicating the magnitude of impact of each factor and the statistical significance is shown as a *p*-value with smaller figures indicating greater importance.

3. Results and discussion

3.1. Particle size and morphology

Small spherical particles were observed by SEM for all co-spray dried budesonide/formoterol fumarate composites. The photomicrographs of various samples are presented in Fig. 1. Particle surfaces were generally smooth with monomodal particle size distributions (on a log-linear plot) in all cases. The median particle size determined by laser diffraction particle size analysis was in the range 1.76–5.04 μm (the details are given in Table 2) which lies within the span of 0.5–8 μm for the ideal particle size of the powders intended for inhalation purposes as set by Davies et al. (1976).

The largest particles were obtained for sample 7BF (spray dried with feed concentration on the highest levels) and the smallest for 12BF (airflow rate, aspirator and feed concentration on the highest levels). An interesting observation for some systems was the presence of apparently hollow particles. This was observed for systems

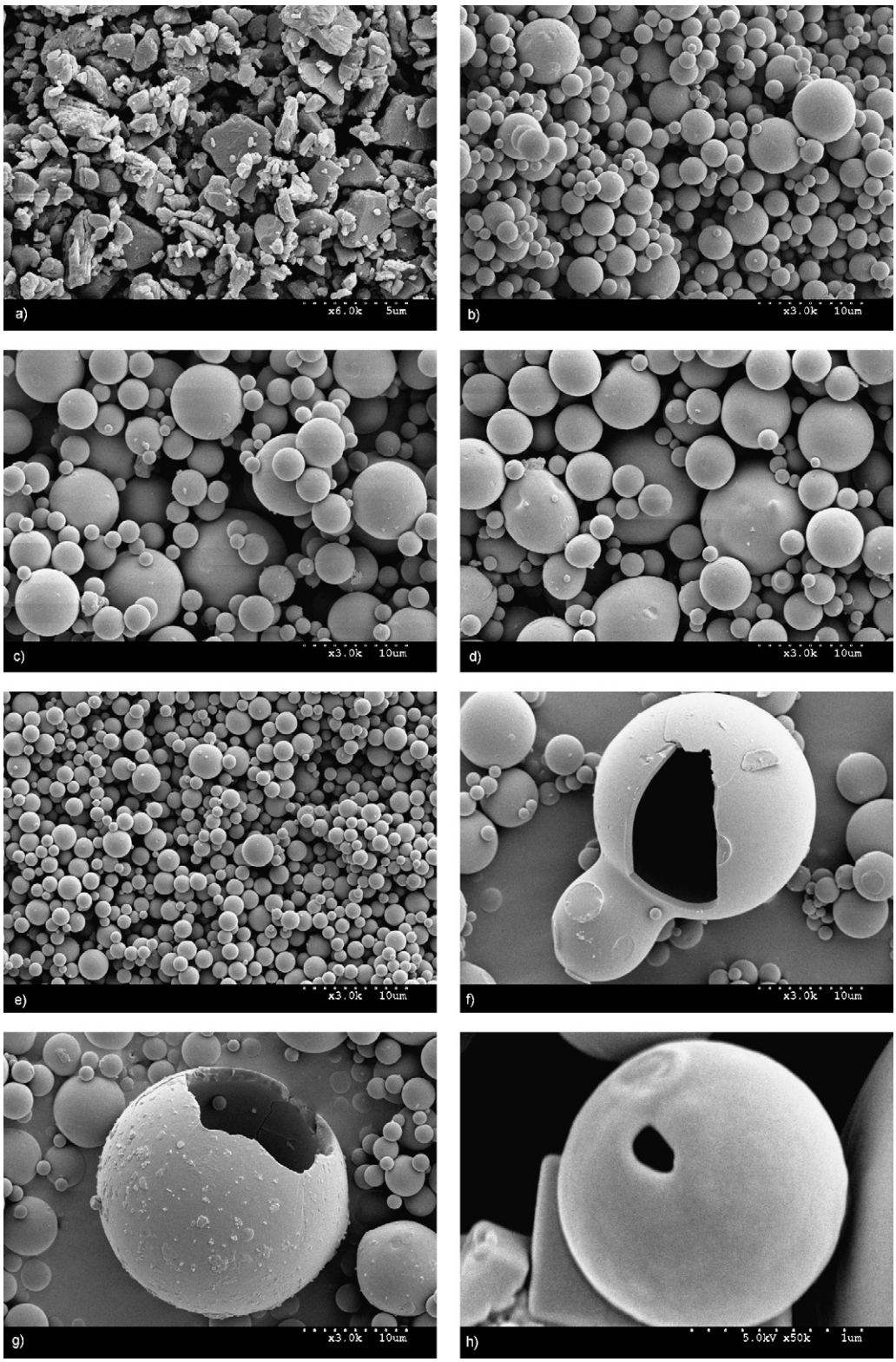

Fig. 1. SEM micrographs of (a) the physical mixture of budesonide-formoterol fumarate dihydrate 100:6, (b) 2BF, (c) 7BF, (d) 13BF, (e) 14BF, (f) 5BF, (g) 7BF and (h) 13BF. Note different magnifications of the presented micrographs.

5BF, 7BF, 9BF, 12BF and 13BF and SEM photomicrographs illustrating this phenomenon can be viewed in Fig. 1. What is striking is that not only were the largest spheres hollow, but as shown in the last SEM micrograph in Fig. 1, particles as small as 1 μm also had blowholes implying hollowness.

After examination of the median particle size values measured for the batches spray dried at a given set of parameters, the powder particles seem to be smaller in size when the drying air flow is high and this is in agreement with the technical specification of the apparatus (Büchi B-191 technical specification).

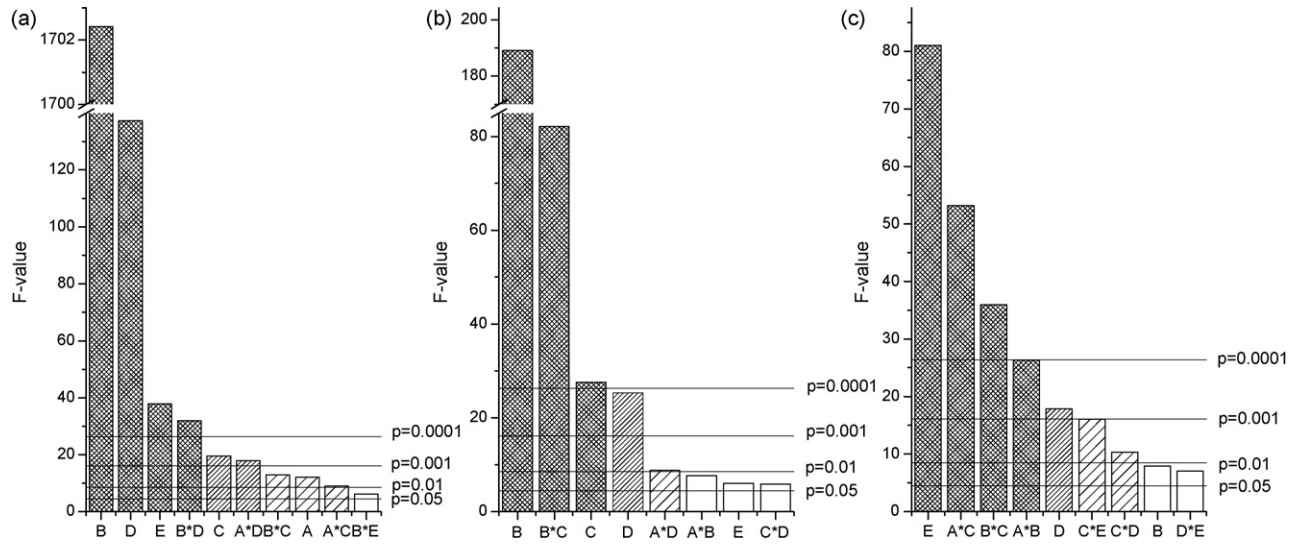

Fig. 2. Bar plots showing *F*-value and associated cut-offs for the *p*-value determining statistical significance of the main effects and interactions for the response: (a) median particle size, (b) yield and (c) Carr's compressibility index. Only terms with *p*-value equal or less than 0.05 were plotted.

Median particle size values were logarithmically transformed before statistical analysis as the experimental data for this response did not follow normal distribution. ANOVA indicated that all main effects and four two-level interactions can be regarded as impacting on the size of particles. The greatest influence on the particle size, from a practical point of view, is airflow through the apparatus and the aspirator value with *F*-value 1702.4 ($p < 0.0001$) and 137.3 ($p < 0.0001$), respectively, as seen in Fig. 2a. The higher atomisation flow needed for the production of smaller particles is associated with more energy supplied for breaking down the liquid droplets (Masters, 1985). Whereas this trend is commonly known and observed (Chawla et al., 1994; Ståhl et al., 2002), the importance of the aspirator is not that obvious and may also be connected with a higher degree of aerosolisation of the solution caused by the suction forces produced by aspiration. Importance of the feed concentration is often reported, for instance works of Corrigan et al. (2006), Chawla et al. (1994), Prinn et al. (2002) and Elversson et al. (2003) related larger particle sizes to more concentrated solutions used.

All the main effects are involved in interactions and analysis of variance revealed five relationships, four at the 99% level: inlet temperature and pump (*AC*), inlet temperature and aspirator (*AD*), airflow and pump (*BC*), airflow and aspirator (*BD*) and one at the 95% level—airflow and feed concentration (*BE*). The strongest interaction appears to be between airflow and aspirator (*BD*).

The coefficients of the equation (of the general form of Eq. (2)) that relates the particle size to the process variables are given in Table 3. Three of the main effects are positive i.e. inlet temperature (*A*), pump (*C*) and feed concentration (*E*) which means that when any of these factors increases, larger particles are produced and two are negative i.e. airflow (*B*) and aspirator (*D*) giving smaller particles when at higher levels.

Fig. 3a shows a response surface plot for the main interaction i.e. airflow (*B*) and aspirator (*D*). Clearly, smaller particles can be obtained if both of the parameters are on higher levels but the effect of aspirator is overshadowed by the profound impact of the airflow.

3.2. Outlet temperature

The outlet temperature of a spray drying process is a resultant of various factors including those related to the apparatus used and material being processed. In this study, the outlet temperatures typically varied between 42 and 68 °C.

It has been found that this response was primarily dictated by the pump settings ($p < 0.001$), as expected. The greater the speed of the pump, the more liquid is supplied to the drying chamber and more solvent vapour is generated, therefore decreasing the exhaust temperature. Out of the other parameters having an influence on the resulting outlet temperature, the aspiration capacity aiding to the airflow and inlet temperature have been found significant with both of them being positive i.e. higher setting resulted in higher outlet temperatures.

The outlet temperature data was examined in the context of associations with other characteristics of the spray dried samples. It has been found that a relationship may be present between the content of residual solvents left in the samples after spray drying, defined as the weight loss in TGA between 25 and 100 °C, and the average outlet temperature recorded during the drying sessions as

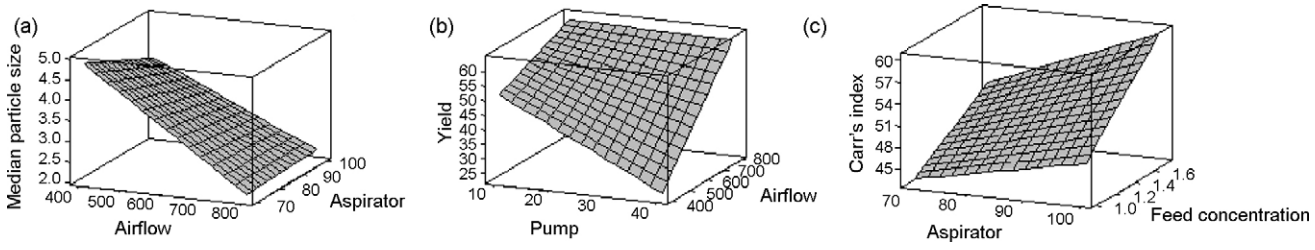

Fig. 3. Response surface plots showing the effects of the two main parameters having the greatest influence on the given response. The rest of parameters were kept at the levels ensuring the maximum response: (a) median particle size, (b) yield and (c) Carr's compressibility index.

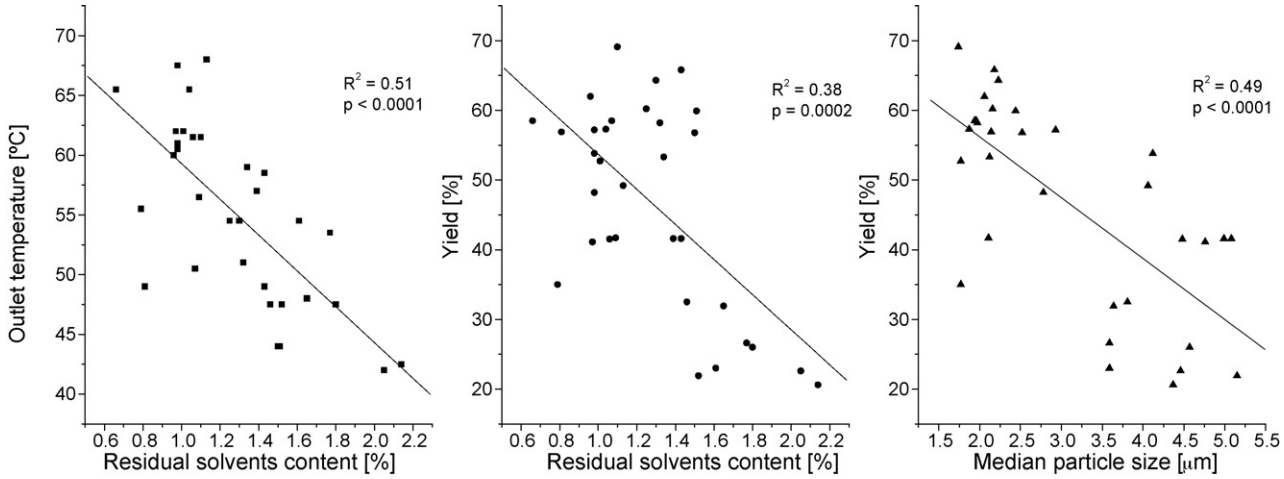

Fig. 4. Relationships between: (a) residual solvent content by TGA and outlet temperature, (b) residual solvent content by TGA and recovered yields and (c) recovered yields and their median particle sizes as measured by a laser diffraction particle sizer.

presented in Fig. 4a. The points on the graph are scattered but a trend can be observed implying that higher outlet values resulted in lesser solvent residues. This correlation has also been discerned by Billon et al. (2000) for the moisture content of spray dried acetaminophen-polymers formulations and Ståhl et al. (2002) for spray dried insulin.

3.3. Production yield

The weights of the powder collected after every spray drying run were expressed as the per cent of the initial amount of the solids taken for solution preparation. Table 2 shows the values obtained. The lowest yield achieved was for sample 16BF (around 22% of the starting amount) and the greatest was for 11BF (~62%).

A trend between the results of the thermogravimetric analysis and the yields recovered was seen (Fig. 4b). Generally, greater amounts of product were recovered when the powders contained less residual solvents. It may also be associated with the highly cohesive nature of the particles as the less water results in "stickier" products (Hickey et al., 1994). A similar phenomenon was described by Broadhead et al. (1994), who noticed that the greatest yields were acquired for the batches of β-galactosidase with the lowest moisture content. Static charge build-ups have also been noticed during powder collection. Adhesion to the cyclone walls was also apparent and triboelectrification phenomenon i.e. electrostatic charging upon contact and friction between particles and spray dryer glass walls could explain, at least to some extent, the powder deposits. Budesonide has been known for its static properties and charge production on the surface of budesonide particles has been reported for DPIs (Byron et al., 1997).

Statistical analysis of the product yield values demonstrated that among the main effects four were positive i.e. inlet temperature, airflow, aspirator and feed concentration and one negative—pump performance as showed in Table 3. The main effect of the inlet temperature was determined to be not significant. The greatest influence on the spray drying process was the airflow through the apparatus due to the greatest *F*-value equal to 189.0 (Fig. 2b). On the other hand, many literature sources point at the feed concentration as having the foremost impact on the yields in spray drying processes. The works of Chawla et al. (1994) and Prinn et al. (2002) stated that the feed solution concentration was the most significant factor affecting the efficiency of the spray drying process. However, the feed concentrations used by Chawla et al. (1994) were 10 and 20% (w/v) for the minimum and maximum values, respectively and the strengths of bovine serum albumin solutions employed by Prinn et al. (2002) differed by 50-fold (0.2 and 10%, w/v). The feed concentration parameter (*E*) achieved the *p*-value of 0.022 which indicates its importance between the 95 and 99% levels only, but in this study the difference between the lower and upper levels for the feed concentration is only 0.75%. This was limited by the solubility of budesonide and this may be the cause for the lesser significance of this variable.

All main effects, except feed concentration (*E*), influenced one another (i.e. there was an interaction between them) and this means that the effect of one factor was dependent upon a level of the second factor. The strongest interaction observed was between airflow (*B*) and pump rate (*C*) with the *p*-value lower than 0.0001. The *F*-value for this term was 82.1, being a 10-fold and over greater than the *F*-value of the rest of interactions. The other 2-way interactions were comparable with regard to the ability to influence the yield as indicated by the close values of *F*- and *p*-value (Fig. 2b).

Fig. 3b shows that better production yields can be obtained if both airflow (*B*) and pump (*C*) are on higher levels and the greatest effect on the percentage yield is pump rate especially when set on a higher level. This interaction overrides the meaning of the effect of pump alone, as Table 3 suggests that greater yield values can be obtained when the pump is set at a lower level.

Airflow is known to affect the particle size and higher airflows used result in smaller particles but higher pump rates have the opposite effect on the particle size as shown above. Ståhl et al. (2002) made approximate estimations of the amount of product passing the cyclone of a B-191-Mini Spray dryer with the outgoing air and observed that larger particles were retained in the cyclone and collecting device more readily. Maa et al. (1998) have estimated that the cyclone customarily fitted in this type of Büchi glassware was not effective in collecting fine particles below 2 μm, therefore it seems logical that smaller yields should be a consequence of smaller particles being flown to the filter. A correlation between the yield and mean particle size values was carried out here and is depicted in Fig. 4c. It contradicts this concept and surprisingly, it can be seen that greater yields were achieved with smaller particles and this may be due to their greater adhesiveness and cohesiveness.

3.4. Carr's compressibility index

Table 2 lists the Carr's compressibility indices calculated from the bulk and tap densities according to Eq. (2). The lowest value of

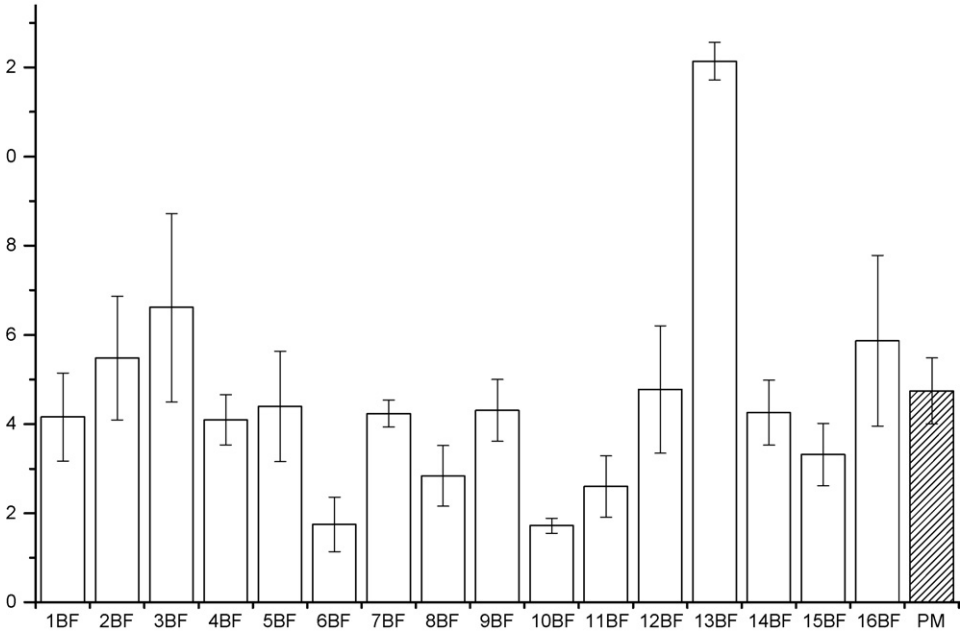

Fig. 5. The *in vitro* respirable fractions of the budesonide/formoterol fumarate composites spray dried at different conditions (empty bars). The physical mixture is also included (crossed bar).

28.2% was measured for one of the replicates of 4BF and the greatest equal to 60% for 9BF, but generally the values oscillated around 40% (Table 2) suggesting rather poor flow properties. In general, lower values of the index are desirable as they indicate better flow (Carr, 1965).

The factor having the greatest impact on this response is feed concentration (*E*) of the spraying solution with the *F*-value of 81.1 according to Fig. 2c. It is also connected to two interactions: pump-feed concentration (*CE*) and aspirator-feed concentration (*DE*). This term is positive as indicated by the sign "+" in Table 3 and it means that the greater the concentration of spray drying solution, the greater the Carr's compressibility index and bulk density. Generally, the effect of the drying conditions on bulk density is product dependant (Masters, 1985). Goula and Adamopoulos (2004) investigating the spray drying of tomato pulp observed that an increase in the feed concentration caused a decrease in bulk density of the resulting powder, but according to Nath and Satpathy (1998) an increase in the feed concentration should result in greater particle density.

This response has as many as five interactions with *p*-value above the 0.01 level indicating a great complexity of relations between the parameters. The most significant seem to be the following relationships: inlet temperature and airflow (*AB*), inlet temperature and pump (*AC*) and airflow and pump (*BC*) that may form a 3-way relationship.

To visualise how the Carr's compressibility index is affected, the values are presented in Fig. 3c as a surface plot drawn against the aspirator (*D*) and feed concentration (*E*) parameters.

3.5. *In vitro* deposition—twin impinger experiments

A Rotahaler® device was chosen for these experiments due to its simple construction allowing comparison of the dispersibility of loaded powders. The results of twin impinger analysis on the co-spray dried composites were compared to the results for the physical mix of budesonide and formoterol fumarate mixed in the ratio 100:6.

The emitted doses usually varied between 19.7% (sample 1BF) and 41.7% (sample 6BF) indicating that the great majority of the spray dried samples was retained in the device. The physical blend was not different in this respect. This is another indication of poor flowability as implied earlier by the high Carr's compressibility indices.

The respirable fraction (the lower stage) varied from about 1.8 to 12.1%. Thus a six- to seven-fold difference in respirable fraction could be achieved by changing the spray drying parameters. Sample 13BF showed the greatest deposition in the lower stage (Fig. 5). For the physical mixture there was $4.8 \pm 0.7\%$ deposition in the lower stage of the impinger. SEM and laser diffraction particle size analysis showed that the diameter of at least 60% particles is less than the cut-off diameter. Because the respirable fractions are much lower than 60% it is obvious that the influence of cohesive and adhesive forces is very strong. The possibility of a charge build-up arising was signalled earlier.

The sample with the greatest respirable fraction (13BF) was spray dried at the higher inlet temperature (84 °C) from 1% (w/v) solution and all other parameters of the spray drier were maintained at their lower settings (airflow 400 Nl/h, pump 10%, aspirator 70%). This resulted in relatively large particles (median size 4.6 μm according to the laser diffraction measurements) with a low content of solvents in the material (about 1% mass loss in the range 25–100 °C by TGA).

Statistical analysis showed that only the inlet temperature effect was positive and all others negative and they fully correspond with the settings of the spray drier used for processing of sample 13BF, which, as mentioned earlier, is the sample with the greatest respirable fraction. The factors having the largest coefficients, thereby most affecting the respirable fractions are the feed concentration (*E*) and airflow (*B*). Moreover, they are involved in an interaction emphasising their significance. The negative coefficients indicate that a low drying gas flow and concentration has to be used to obtain high respirable doses.

The significance of the feed concentration was shown for the response "Carr's compressibility index" and the same is observed

here, for the respirable fraction. The trend is identical, i.e. in both cases lower solution concentrations are needed to optimise the response. It would suggest that indeed, the bulk density of an inhalable powder can be a measure of its flowability (French et al., 1996; Steckel and Brandes, 2004). But the manner in which the respirable fraction is affected by the airflow is unexpected and one would anticipate that lower particle sizes are more desirable. It implies that cohesive attraction also has a great impact on the respirable fractions achieved and it is known that a powder inhaler containing a significant proportion of particles below 5 μm has poor dispersibility (Hickey et al., 1994). The cohesion between the particles by contact surfaces is proportional to the specific surface area, hence the particle size (Chew et al., 2000). Particle radius also appears in the expressions on the van der Waals and electrostatic cohesion forces (Hickey et al., 1994). Thus, by increasing the size of particles, the cohesiveness can be reduced and better dispersibility attained. Several authors report on low respirable *in vitro* doses obtained linked to the strong interparticulate forces (Bosquillon et al., 2001; Voss and Finlay, 2002).

SEM micrographs presented in Fig. 1 suggested that the processed powders may be mixtures of solid and hollow spheres. An empty interior can arise from expansion of gas in the droplets with a vapour-impervious film or from air entrained in the liquid feed (Elversson et al., 2003). In particles where the drying film is impermeable to vapour, an overpressure is created and blowholes or exploded particles may occur (Crosby and Marshall, 1958). Therefore, it is likely that some hollow spheres may be created on spray drying as budesonide is a hydrophobic material. Furthermore, the shell thickness increases with concentration of the feed solution (Elversson et al., 2003; Crosby and Marshall, 1958) and it is reasonable to assume that larger particles would have thinner shells. This may translate into the improved *in vitro* respirable fraction of 13BF (having large mean particle size and spray dried from low concentration solution) as hollow particles are known to have advantageous lung deposition (French et al., 1996) due to the small aerodynamic diameters.

The ratio between budesonide and formoterol fumarate in the lower stage for all spray dried samples was measured by the HPLC analysis and was found to be 100 to 6 (with deviation for both of the drug contents being 5% w/w). For the physical mix the mean ratio of budesonide versus formoterol fumarate for this fraction was 100 to 7.5 with 23 and 21% w/w deviation of content for budesonide and formoterol fumarate, respectively. *In vitro* aerosol deposition profile carried out with an Andersen Cascade Impactor on an MDI inhaler Ventide containing a suspension of micronised beclomethasone dipropionate and salbutamol sulphate revealed that the dose of beclomethasone delivered to the apparatus was lower than the labelled dose. The mass median aerodynamic diameter (MMAD) of beclomethasone was greater (3.7 μm) then that of salbutamol sulphate (2.4 μm) (Steele et al., 2004) implying different aerodynamic properties reflected in different fine particle doses. Also salmeterol xinafoate was found to posses a different deposition profile to fluticasone propionate from Seretide 100 and Seretide 500 inhalers in *in vitro* Multistage Liquid Impinger analysis (Taki et al., 2007). It was assumed that both of the drugs might also deposit differently *in vivo*.

Even though no comparisons with a commercial product were carried out in this study, it shows that with the development of novel combination inhalers based on binary or more complex mixtures of micronised actives, the task to preserve the accurate proportions between the drugs reaching the lungs can be quite difficult. Co-spray drying may offer a means to overcome the problem of the dose uniformity from both MDIs and DPIs.

3.6. Thermal properties of the budesonide/formoterol fumarate 100:6 composites

Thermal characteristics including examples of the DSC curves of co-spray dried budesonide/formoterol fumarate 100:6 and 400:6 composites have been presented earlier (Tajber et al., 2009). Very little has been published on how the spray drying variables affect solid-state characteristics of the product and for instance it has been revealed that different inlet temperatures may result in the difference in physical stability of an amorphous antibiotic substance (Ohta and Buckton, 2005).

The data collected during the DSC measurements was examined in terms of its potential associations with the responses. No evident trends were seen between either the onset or enthalpy of the melting endotherm and any of the factorial outputs. It is perhaps due to the fact that the amorphous co-spray dried powders had recrystallised before melting took place, thus making correlations unfeasible.

However, a good correlation ($R^2 = 0.80$) between the onset of the recrystallisation process and its enthalpy for the various samples has been identified as presented in Fig. 6a. This phenomenon may have two possible explanations. It concurs with similar observations for other pharmaceutical materials for which a shift to higher temperatures concurrently with an increase in the enthalpy of the exotherm has been assigned to an increased fraction of the amorphous material present (Mura et al., 2002). Differences in the onset temperatures of recrystallisation discernible for the composites can be explained on the basis that the samples with the higher amount of the amorphous material require more energy to transform into the crystalline state. It may also mean that DSC has been demonstrated to be a more sensitive tool for detecting small variations in the amorphous/crystalline nature of the composites, while XRD showed only a broad "halo" in all cases (Tajber et al., 2009). On the other hand, the different thermal properties of the powders might be a consequence of polyamorphism, i.e. the existence of two or more amorphous phases, each having a different energetic state (Hancock et al., 2002).

When the values of enthalpy of the recrystallisation process were subjected to the ANOVA test, it was revealed that the energy was predominantly influenced by the airflow through the spray dryer. Therefore, this would suggest a connection with a particle size of the composites, since it was demonstrated earlier that this parameter had the major effect on the size of particulates. A closer look at this association showed a weak relationship ($R^2 = 0.22$). Similarly, the enthalpy plotted against the production yields also gave a weak, but a somewhat better correlation ($R^2 = 0.29$).

The recrystallisation exotherm characterises the formation of the crystalline phase from the amorphous matrix. The enthalpy of this process is related to the content of the amorphous phase and it is believed that the amorphous form has a higher surface energy than the crystalline counterpart due to being in a higher energy state (Newell et al., 2001). The surface characteristic is particularly vital in dry powder inhalers as highly micronised powders exhibit a more cohesive nature. For that reason, it deemed interesting to investigate if the enthalpy of the recrystallisation process correlates with the results of the twin impinger analysis. Fig. 6b presents the outcome of this analysis and the fraction retained in stage 2 of the twin impinger apparatus was used as that reflecting the respirable fraction. Although the best line fitted through the points gave a weak correlation characterised by the R^2 value of 0.24, a much better fit was obtained when the outlying point for the greatest respirable fraction was omitted ($R^2 = 0.60$, Fig. 6b). For comparison, similar trends were investigated for the respirable fractions versus particle size, for which a value of R^2 equal to 0.27 when all data points were included and R^2 equal to 0.46 excluding the outlier

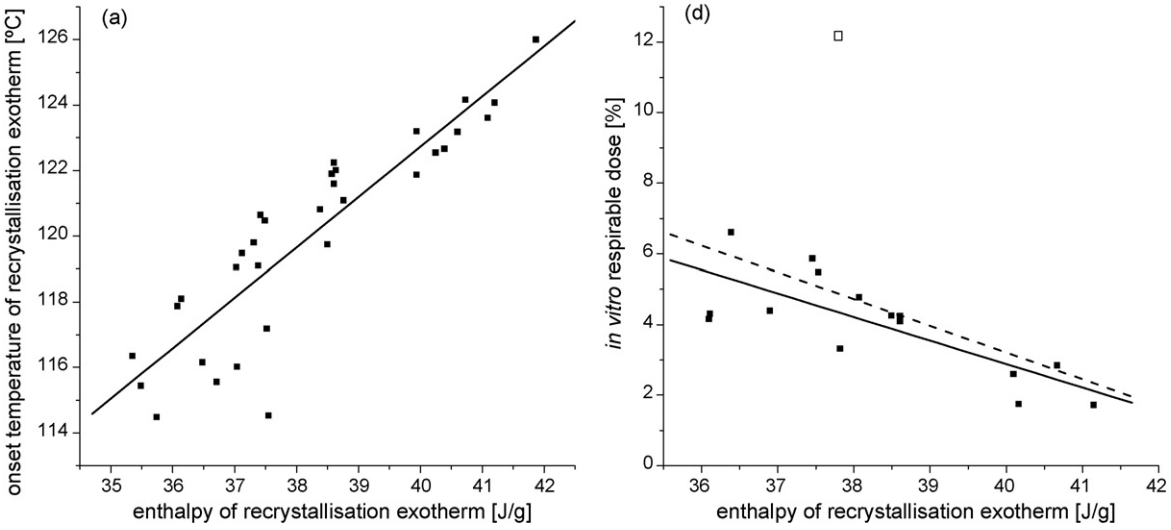

Fig. 6. Correlations between the enthalpy of the recrystallisation exotherm and: (a) onset of the recrystallisation peak, (b) *in vitro* respirable fraction. The solid lines correspond to the best linear fits. The dashed line in (b) is the best fit for the whole dataset, the solid line—when the open square representing the greatest respirable fraction was omitted.

were found. This may mean that in this case, a better link may exist between the energy of amorphous, spray dried powders and the respirable fraction than the median particle size. The size of particulates varied between around 1.8 and 5.0 μm (Table 2), a variation that could be too minor and overshadowed by the effects emerging form the physicochemical rather than micromeritic properties of the powders.

4. Conclusions

The effect of five variables (air inlet temperature, airflow, pump feed rate, aspirator capacity and feed concentration) on the physicochemical, micromeritic and aerodynamic properties of co-spray dried budesonide/formoterol fumarate dihydrate 100:6 were evaluated by a 2^{5-1} factorial experimental design. Various product characteristics were investigated and overall, the parameter that had the greatest influence on each of the property was production yield – airflow (higher airflow gives greater yields), median particle size – airflow (higher airflow gives smaller particle sizes), Carr's compressibility index – feed concentration (lower feed concentration gives smaller Carr's indices), *in vitro* respirable fraction – feed concentration (lower feed concentration gives greater respirable fractions).

One of the processed composites (13BF) gave a 2.6-fold increase in the powder deposition in the lower stage of the twin stage impinger compared with the micronised mix, probably due to the presence of hollow spheres, causing better flowability and dispersibility of this sample. Moreover, the twin impinger studies presented that the ratio in which the mechanical binary mixture of actives was prepared did not result in the same ratio being attained in the second stage of the apparatus (respirable dose). Spray dried powders may be a superior alternative in this regard as every particle contains the blend of drugs at a constant ratio minimising the dose uniformity issue.

The various batches of co-processed mix showed different DSC profiles and a correlation between the *in vitro* respirable fractions and the energy of recrystallisation (by DSC) of such amorphous spray dried powders was revealed.

This work has shown that when spray drying is involved in manufacture and development of an inhalable powder, the process conditions should be carefully studied as they can impact for example on the aerodynamic performance of such powder and could give rise to up to a 7-fold difference in respirable fraction of the spray dried product. Statistical factorial designs can be of significant benefit as they allow optimisation of process parameters and product characteristics.

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