International Journal of Pharmaceutics 380 (2009) 80–88

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

journal homepage: www.elsevier.com/locate/ijpharm

Understanding the effect of lactose particle size on the properties of DPI formulations using experimental design

Estelle Guenette[b], Andrew Barrett[a], Debbie Kraus[a], Rachel Brody[a], Ljiljana Harding[a,*], Gavin Magee[a]

[a] Pfizer Global R&D, Sandwich, Kent, CT13 9NJ, UK
[b] Ecole Nationale Supérieure de Chimie de Mulhouse, 3 rue Alfred Werner, 68200 Mulhouse, France

ARTICLE INFO

Article history:
Received 19 February 2009
Received in revised form 13 June 2009
Accepted 1 July 2009
Available online 9 July 2009

Keywords:
Dry powder inhaler
Salbutamol sulphate
Experimental design
Multivariate data analysis

ABSTRACT

Medicines for delivering therapeutic agents to the lung as dry powders primarily consist of a carrier and a micronised active pharmaceutical ingredient (API). The performance of an inhaled formulation will depend on a number of factors amongst which the particle size distribution (PSD) plays a key role. It is suggested that increasing the number of fine particles in the carrier can improve the aerosolisation of the API. In addition the effect of PSD upon a bulk powder is also broadly understood in terms of powder flow. Other aspects of functionality that different size fractions of the carrier affect are not clearly understood; for example, it is not yet clearly known how different size fractions contribute to the different functionalities of the carrier. It is the purpose of this investigation to examine the effects of different lactose size fractions on fine particle dose, formulation stability and the ability to process and fill the material in the preferred device. In order to understand the true impact of the size fractions of lactose on the performance of dry powder inhaled (DPI) products, a statistically designed study has been conducted. The study comprised various DPI blend formulations prepared using lactose monohydrate carrier systems consisting of mixtures of four size fractions. Interactive mixtures were prepared containing 1% (w/w) salbutamol sulphate. The experimental design enabled the evaluation of the effect of lactose size fractions on processing and performance attributes of the formulation. Furthermore, the results of the study demonstrate that an experimental design approach can be used successfully to support dry powder formulation development.

1. Introduction

The development of dry powder inhaler (DPI) formulations continues to be a scientific challenge. Drug particles used in DPI formulations require an aerodynamic diameter below 5 μm for deposition into the lungs and are often micronised in order to achieve the desired particle size range. In order to achieve accurate dose metering and enable processing using industrial scale equipment, good flow properties are also required. Micronised drug particles are usually highly cohesive and exhibit poor flow properties. They are often formulated with larger carrier particles, thus forming a homogeneous interactive mixture that is required to redisperse upon device actuation in order to achieve lung deposition of drug particles (Ganderton, 1992). Achieving adequate aerosolisation efficiency is another major challenge for DPI formulations: the majority of DPI formulations currently available on the market exhibit a relatively low efficiency with fine particle fraction (FPF) rarely exceeding 20% (Ganderton, 1992; Steckel and Müller, 1997). Formulation scientists have used a number of different approaches to increase understanding and improve aerosolisation performance of DPI drug products, and have shown that the product efficiency is dependent not only on the formulation properties, but also on the device design and patient's inspiratory force.

α-Lactose monohydrate is the most commonly used carrier (Adi et al., 2007) due to its favourable toxicological profile, physico-chemical stability, availability and compatibility with the majority of low molecular weight drugs. A variety of lactose particle size grades are available from suppliers. The physico-chemical characteristics of the lactose carrier such as surface roughness, moisture sorption, shape factors, surface area and particle size play a key role in the aerosolisation performance of the active pharmaceutical ingredient (API) (Larhrib et al., 1999; Bosquillon et al., 2001; Louey et al., 2003; Chew et al., 2005).

The particle size distribution (PSD) is one of the crucial parameters for DPI formulation. In order to achieve the desired particle size distribution, manufacturing steps such as milling, sieving and/or air classification are employed. Conflicting reports can be found in the literature over the effect of carrier particle size distribution and

* Corresponding author. Tel.: +44 (0)1304 644957; fax: +44 (0)1304 656 091.
E-mail address: ljiljana.harding@pfizer.com (L. Harding).

0378-5173/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.ijpharm.2009.07.002

drug dispersion—whilst a correlation has previously been reported by some authors (Steckel and Müller, 1997), others report no difference in mass median aerodynamic diameter (MMAD) of drug when varying carrier particle size (Podczeck, 1998). It is, however, widely recognised that formulations with higher contents of fine lactose particles (<15 μm in diameter) show improved aerosolisation performance (Zeng et al., 1999; Tee et al., 2000; Louey and Stewart, 2002; Louey et al., 2003; Islam et al., 2005; Steckel et al., 2006; Young et al., 2007a). Fine carrier particles responsible for improved performance are usually added into the formulation (thus forming 'ternary' interactive mixtures), or could be present as 'intrinsic fines', generated as a result of the comminution process.

The mechanisms by which fine excipient particles increase the performance of DPI formulations are unclear at present (Shur et al., 2008). Several theories have been offered in support of experimental evidence, the most prevalent two being the "active site theory" (Tee et al., 2000) and "agglomeration theory" (Louey and Stewart, 2002; Young et al., 2007b). The first theory suggests that fine lactose particles occupy high-energy sites, thus enabling drug adhesion to lower-energy locations of the coarse carrier. The agglomeration theory suggests redistribution of drug particles between the coarse carrier and fine excipient thereby forming mixed agglomerates that can be dispersed more easily due to facilitated detachment of agglomerates from the carrier surface.

Young et al. (2007b) observed a linear relationship between fine particle fraction (FPF) and the fine lactose content when fine lactose content was below 15%, with both freshly milled and recrystallised samples showing similar trends. Larhrib et al. (1999) highlighted the importance of establishing appropriate control parameters for lactose carriers. They investigated the effects of lactose carrier grades on the deposition profiles of salbutamol sulphate. Using lactose grades with differing particle size and intrinsic fines content was shown to affect the drug product performance.

Despite the unequivocal evidence that the fine lactose particles improve performance of DPI formulation, the challenge remains in finding the optimal size distribution of fine material and fine/coarse ratio that will not inadvertently affect the flow properties of the powder formulation (Zeng et al., 1998). It is not yet clearly known how different size fractions contribute to the different functionalities of the carrier. In order to be able to specify optimal lactose for a given product, the contribution of different size fractions of the distribution to the different functionalities of the carrier needs to be understood. More specifically, identifying size fractions that are correlated with fine particle dose, formulation stability, ability to process and fill the material in the preferred device are crucial for the success of DPI formulation strategy. The likelihood of obtaining an "off the shelf" lactose carrier that would exert optimal effects when used with different drug molecules is very small. Therefore, a systematic approach to establish the optimal formulation composition is needed, with a view to assess the effect of lactose particle size cuts on flow properties, aerosolisation performance and stability upon storage for various formulations.

The aim of the present study was to examine the *in vitro* drug deposition, the flow and the stability of interactive lactose mixtures and to investigate the functionality within the particle size distribution using an experimental design. The experimental design tool has been previously used in micronisation development (Magee et al., 2008) and formulation development (Lewis et al., 1999; Gonzalez-Rodriguez et al., 2007) for formulation screening and optimisation. Its application facilitates logical evaluation of critical parameters, helps resolve issues and provide direction during product development. The design used in this work presents a new approach to assessing the particle size distribution effects of lactose carrier in order to support dry powder formulation development.

Table 1
Experimental design outlining formulation composition (runs 1 and 10 are center points run at mid-setting for each of the variables).

Run	<10 μm (%)	10–40 μm (%)	40–80 μm (%)	>80 μm (%)
1	6	13	30	51
2	12	6	20	62
3	12	20	40	28
4	12	20	20	48
5	0	20	40	40
6	0	6	20	74
7	12	6	40	42
8	0	20	20	60
9	0	6	40	54
10	6	13	30	51

2. Materials and methods

Lactose carrier blends were prepared from samples provided by Friesland Foods Domo. Lactose sieve fractions were chosen to cover the following three nominal particle size ranges of interest: median size <10 μm, 10–40 μm and 40–80 μm. A fourth fraction of >80 μm was added as a coarse carrier fraction. In order to evaluate the effect of different lactose portions on formulation properties, a full factorial randomised experimental design with two centre points (at the mid-setting) was applied to the three particle size components (<10 μm, 10–40 μm and 40–80 μm) with the last component (>80 μm) being set at a level which makes the sum to 100%. The formulation details are shown in Table 1 .

Interactive mixtures of lactose size cuts were prepared using a Turbula T2F mixer. The material was blended in a 0.5 L stainless steel tub for 10 min at 46 rpm. Micronised salbutamol sulphate (Namsi Labs Ltd.; D[v, 0.1] = 0.47 μm, D[v, 0.5] = 1.28 μm, D[v, 0.9] = 2.86 μm) was used in this study as API. Blends with salbutamol sulphate (1%, w/w) were produced in a Diosna P1/6 blender/granulator. The material was mixed for 5 min at 1000 rpm (no chopper action) using a 0.5 L stainless steel bowl. All blends were produced in 100 g quantities.

Homogeneity of the powder blends was assessed by high performance liquid chromatography (HPLC). Ten randomly selected samples (20 ± 2 mg) were dissolved in methanol/water 60/40 (v/v) using 100 mL volumetric flasks for the analysis. The relative standard deviation (RSD) of the average content was used as a measure for the homogeneity of the powder mixture. All salbutamol sulphate samples were analysed by a validated HPLC method using an analytical column (Capital HPLC Ltd., ODS chromatographic column, 50 mm × 4.6 mm, 3 μm mean particle size) which was maintained at 30 °C. A methanol/0.0025 M SDS (aq)/1 M orthophosphoric acid mixture in a 60/40/0.5 ratio (v/v) was used as mobile phase. Using a flow rate of 1.0 mL/min and an injection volume of 20 μL, salbutamol sulphate typically elutes at 1.7–2.5 min. UV-detection was carried out at a wavelength of 225 nm. The system was calibrated using a 2 μg/mL external standard.

The *in vitro* deposition was determined using a Next Generation Impactor (Copley Scientific Limited, UK) at an airflow rate of 60 L/min. Aerosolisation performance of salbutamol sulphate was determined using a Monohaler device with a size 3 capsule manually filled with 20 mg (±10%) of blend. The fine particle fraction (FPF), fine particle dose (FPD), emitted dose (ED) and the total recovery were assessed. FPD was defined as the mass of particles <4.46 μm aerodynamic diameter (sum of deposition from impactor stages 3–8). The powder collected in each stage was dissolved in methanol/water 60/40 (v/v) and the solutions analysed by HPLC. Formulations were characterised initially and after 28 days of storage. Two conditions of storage were chosen: approximately 5 g of each blend was stored in amber glass vials (covered with muslin) in the stability chamber at 40 °C/75% RH and in open amber glass

vials placed in a desiccator filled with silica gel at room temperature (RT).

Particle size analysis was performed using a HELOS laser diffraction instrument (Sympatec GmbH, Germany) using a R4 lens (1.8–350 μm). Approximately 5 g of blend was used for each measurement and the measurement performed in triplicate.

Powder particles were imaged using a conventional scanning electron microscope (SEM; Carl Zeiss SUPRA 40VP, Cambridge, UK). Samples were mounted onto 12.5 mm diameter aluminium stubs using adhesive tabs. The uncoated specimens were examined at a magnification of 200× using an electron beam accelerating voltage of 10 kV with specimen chamber pressures of 1 Pa and 10 Pa.

Tapped density of powder blends was measured using a Caleva TDT2 Tapped Density Tester (Dorset, UK). The sample was introduced without compacting into 100 mL glass cylinder and the unsettled apparent volume recorded. The sample was tapped until the volume reading was the same for three consecutive measurements. Powder density at each step can be calculated by dividing the mass of powder by the measured density. Carr's index (CI) is calculated using the following formula:

$$\mathrm{CI} = \frac{\text{Tapped Density} - \text{Initial Density}}{\text{Tapped Density}} \times 100$$

Flow testing was conducted using a ring shear tester (Dietmar Schulze Schuttgutmesstechnik, Germany). Powder was carefully poured from a spatula in a clockwise motion until the sample cell was overflowing with powder. The flow function coefficient (FFC) was then measured at 4000 Pa.

Statistical analysis of the results obtained was performed using the Statgraphics® software package. In this work, the data analysis has been summarised by main effects plots and interaction plots. The main effects plot consists of lines showing the effect of each factor. Each line joins two points whose values equal the mean response at the low and high setting of the factor. The larger the difference between these two means, the steeper the line on the plot, implying a larger effect due to the factor.

Multivariate data analysis was performed using SIMCA-P+ (Umetrics AB). Principal component analysis is used to visualise all results from the experimental design. In this paper, a plot showing both scores and loadings for the first two principal components is used to summarise the design of experiments. The scores show the correlation between the observations and the loadings show the relative influence of each variable on the scores.

Table 2
Particle size distribution of lactose size cuts used in the experimental design (mean, $N = 3$).

Particle size cuts	D[v, 0.1] (μm)	D[v, 0.5] (μm)	D[v, 0.9] (μm)
<10 μm	1.19	3.94	9.02
10–40 μm	2.31	16.16	40.45
40–80 μm	24.64	47.19	74.19
>80 μm	40.31	128.57	221.18

3. Results and discussion

3.1. Particle size distribution

Particle size distribution for individual particle size cuts, described by the 10th, 50th and 90th percentiles (D[v, 0.1], D[v, 0.5] and D[v, 0.9], respectively) is shown in Table 2. Fig. 1 shows particle size distribution for blends produced using size cuts in proportions outlined in Table 1. Results indicate that different lactose blends were generated with respect to particle size and that sufficient difference in the size areas of interest was created. It can be seen from Fig. 1 that the two design centre point blends (blends 1 and 10) showed good reproducibility in terms of the particle size profile.

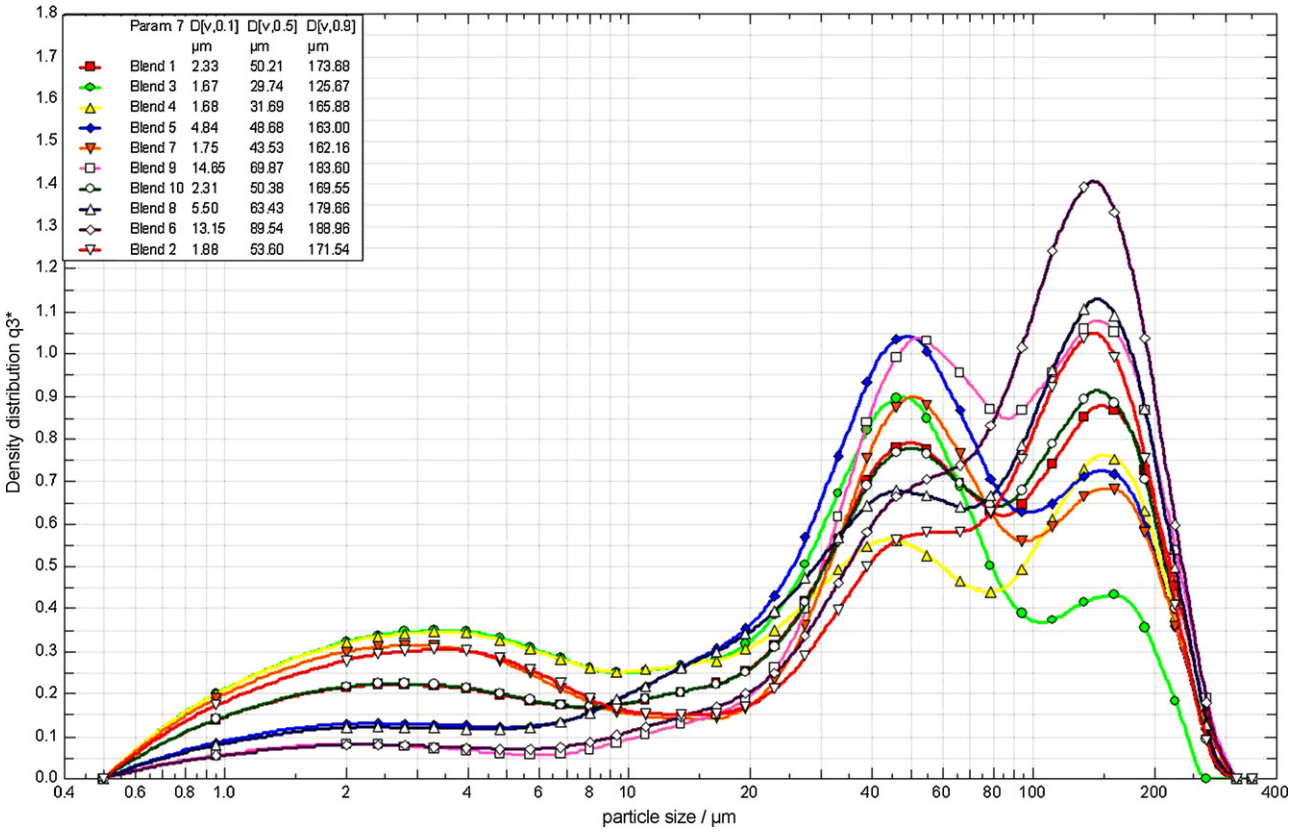

Fig. 1. Particle size distribution of salbutamol sulphate 1% (w/w) blends (mean, $N = 3$).

Table 3
Comparison of theoretical formulation composition based on lactose size fractions and Sympatec particle size distribution.

Blend number	Theoretical amount in each lactose fraction (%)			Sympatec cumulative distribution (%)		
	<10 μm	10–40 μm	40–80 μm	<10 μm	10–42 μm	42–86 μm
1	6	13	30	22	22	22
2	12	6	20	29	15	18
3	12	20	40	34	27	23
4	12	20	20	34	22	15
5	0	20	40	15	29	28
6	0	6	20	8	17	23
7	12	6	40	30	19	24
8	0	20	20	14	24	21
9	0	6	40	8	20	30
10	6	13	30	22	22	22

It is important to note that the final blends contained more undersized particles than set out by the design (Table 3). The presence of 'intrinsic' fines in each lactose portion, as well as the addition of 1% (w/w) of salbutamol sulphate to the blend amounted to that effect. Consequently, the portion of coarser particles was significantly lower than predicted, especially for blends 3, 5, 7 and 9 where the theoretical fraction of 40–80 μm particles was 40%.

3.2. Flow properties

Two methods of measuring the flow properties of the blends were applied, CI and FFC, with a view to compare the two methods and correlate the results. The mean results are presented in Table 4. The results seem to follow expected trends as the blends with less fine lactose exhibit better flow properties than those with higher percentage of finer size cuts. Blends 6 and 9 had the best flow due to the absence of ultrafine (<10 μm) or fine (10–40 μm) lactose fractions. Furthermore, for the blends 5 and 8 with the highest amount of 10–40 μm lactose fractions, a decrease in flow was observed. The two methods showed comparable results. A detailed statistical analysis is shown only for the FFC method, as this method showed better reproducibility for the centre points.

The main effects plot (Fig. 2) shows that the ultrafine lactose fraction (<10 μm) has the largest effect on flow properties. Increasing this fraction within the blend led to a significant reduction in the flow properties; an average decrease in FFC of 3 units. An increase in the second smallest lactose fraction (10–40 μm) also had a negative influence on flow although to a lesser extent; an average decrease in FFC of 1.7 units. Varying the amount in the fractions 40–80 μm or >80 μm (in the range studied) had no effect on the powder flow properties.

3.3. Homogeneity and potency

Homogeneity and potency of the powder blends were assessed by taking ten random samples through the blend. All blends demonstrated good homogeneity (RSD < 5%). Potency results were in the range of 95.3–101.5% of the nominal dose. The particles of lactose smaller than 10 μm in size seemed to have the largest effect on mean potency as shown in the analysis summary plot in Fig. 3 where an average potency of all the blends that used no ultrafine lactose was 96% compared with an average close to 100% of nominal for the blends using 12% ultrafines. An increase in specific surface area for blends containing a larger proportion of ultrafine lactose particles could be attributed to this effect, especially given the adhesive nature of salbutamol sulphate (Begat et al., 2004). Adhesive API particles are more likely to be 'swept up' by ultrafine lactose particles in a blend, thus minimising product losses and increasing product potency. It should be noted here that one of the batches with % <10 μm = 0 had a high potency result, which may indicate the natural variability in blend potency and would hence reduce the significance of this effect. Conversely, if that outlier was removed, the effect of particle size <10 μm on potency would increase further.

Table 4
Flow properties; mean (±SD).

Blend number	CI (%)	CI classification	FFC	FFC classification
1	22.60 (0.94)	Poor	3.35 (2.11)	Poor
2	22.20 (4.46)	Poor	2.51 (1.78)	Poor
3	27.50 (4.63)	Poor	2.30 (0.00)	Poor
4	25.40 (0.56)	Poor	2.30 (0.00)	Poor
5	19.45 (4.00)	Fair	4.10 (0.00)	Marginal
6	14.02 (3.56)	Good	6.95 (2.65)	Good
7	21.70 (5.87)	Fair	2.65 (2.67)	Poor
8	17.90 (2.37)	Fair	4.35 (1.63)	Marginal
9	14.35 (3.45)	Good	7.35 (4.81)	Good
10	22.15 (4.15)	Poor	3.00 (0.00)	Poor

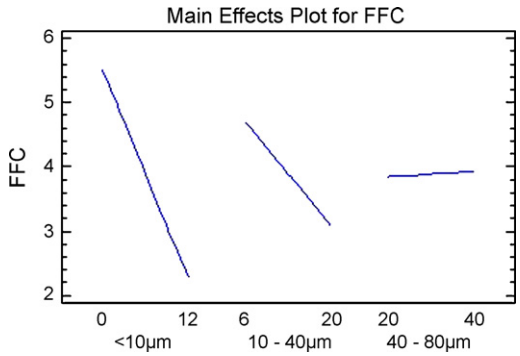

Fig. 2. Main effects plot showing the effect of each of the particle size cuts on flow properties (FFC).

3.4. Aerosolisation performance

The *in vitro* aerosolisation performance testing was performed using NGI equipment and results are shown in Table 5. A clear difference between the ten blends was noticed with the two centre point blends (blends 1 and 10) showing reproducible FPD results.

Blends 2, 3, 4 and 7 gave the best overall performance with mean FPD values ranging from 75.55 to 91.80 μg. These blends contained the largest amount of ultrafine lactose (<10 μm). Blends 3 and 4 also contained the highest amount of 10–40 μm fraction, thus rendering them the two highest performing blends (FPD 89.75 and 91.80 μg, respectively) This result is consistent with expectations as it is well understood that an increase in fine particles leads to increased performance.

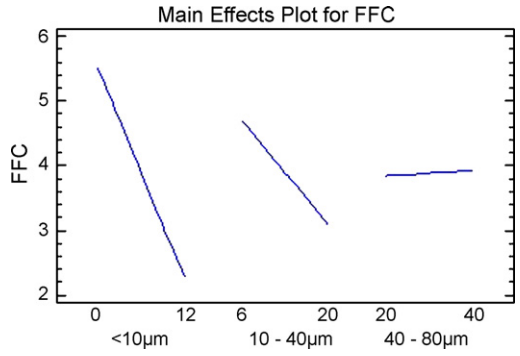

Fig. 3. Main effects plot for the potency (% nominal) of lactose blends showing the effect of each of the particle size cuts.

Table 5
NGI testing data for salbutamol sulphate blends (mean, $N = 2$).

Blend number	Emitted dose (μg)	Fine particle dose (μg)	Fine particle fraction (% emitted)
1	166.55	68.00	40.85
2	167.90	75.55	45.20
3	177.40	89.75	50.60
4	169.55	91.80	54.15
5	151.10	46.55	30.85
6	177.80	36.90	20.75
7	165.25	84.55	51.65
8	169.35	51.50	30.40
9	169.45	39.45	23.40
10	152.15	63.45	41.60

Blends 5 and 8 were intermediate performers with FPD values around 50 μg. These blends contained the same fine lactose composition; the only difference being the content of 40–80 μm fraction. Blends 6 and 9 were the lowest performers with FPD values below 40 μg and FPF less than 25%. Again the content of the ultrafine and fine lactose fractions could be used to explain this effect.

Statistical analysis of the NGI results was performed. There was no evidence of any of the lactose fractions having an effect on the emitted dose and the total recovery. The main effects plot for the FPD results is shown in Fig. 4. The graph shows that all lactose fractions examined in this study positively affected the FPD results, the effects being more pronounced for finer lactose fractions. A strong linear relationship is noted when plotting performance against the cumulative amount of particles up to 12 μm in size, after which point the linear correlation reduces. A plot of FPD against the cumulative particles size distribution at 10 μm is shown in Fig. 5, as an example, which has a correlation coefficient (R^2) of 0.9826. The correlation coefficients for similar plots for different particle size fractions up to 36 μm are plotted in Fig. 6. The correlation coefficient is above 0.98 for all fractions up to and including 12 μm; the highest being $R^2 = 0.9827$ for the cumulative fraction at 8.6 μm. There is still a reasonable correlation for larger size fractions, however, the correlation coefficients are clearly decreasing. This further confirms that the FPD is directly related to the amount of fine lactose within the formulation.

3.5. Influence of stability

Potency of blends after 28 days of storage at two conditions was assessed by taking 5 samples of 20 ± 2 mg from each blend for HPLC analysis. The results are shown in Table 6. There was a clear reduction in the mean potency upon storage (significant at the 95% confidence interval using paired *t*-tests), with blends exposed to elevated temperatures and humidity resulting in a more pronounced potency drop, probably due to chemical degradation of the drug (Aboul-Enein and Surmeian, 2000; Felix et al., 2008). A

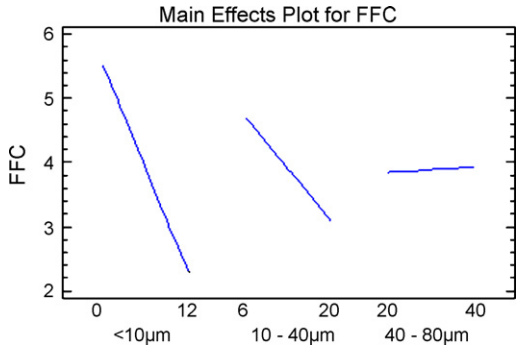

Fig. 4. Main effects plot for the FPD results showing the effect of each of the particle size cuts.

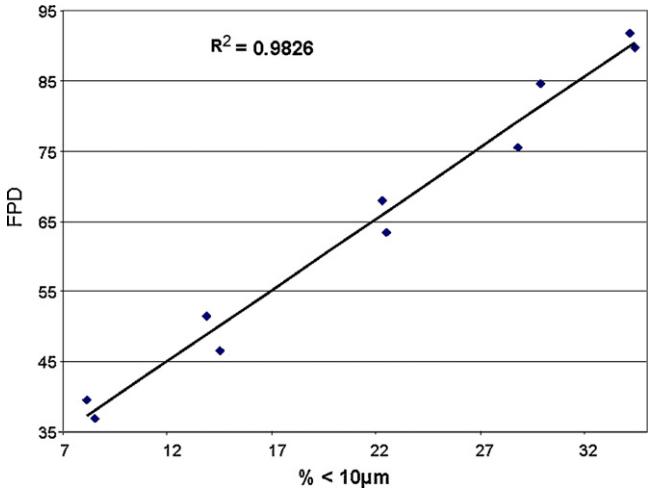

Fig. 5. Relationship between the cumulative amount (%) of lactose particles <10 μm and fine particle dose (μg).

mean decrease from initial (as % nominal) of 3.8% points (95% confidence interval 1.9 to 0.6% points) was seen for blends stored at room temperature and a mean decrease of 5.3% points (95% confidence interval 2.7 to 7.8% points) was observed. The decrease was not affected by any of the lactose portions.

NGI results presented in Fig. 7 show the effect of storage on aerosolisation performance. The only formulations that showed a clear reduction in FPD when stored at room temperature were

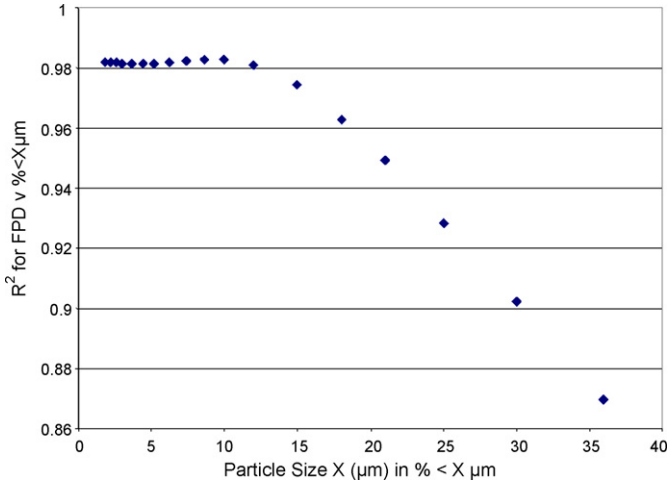

Fig. 6. Correlation coefficients (R^2) for plots of cumulative amount (%) of lactose particles <*X* μm and fine particle dose (μg).

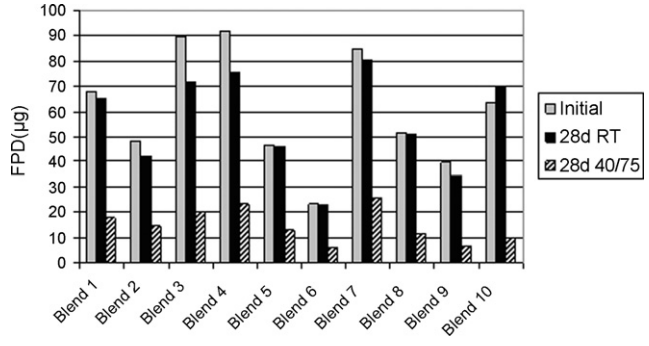

Fig. 7. Mean fine particle dose results for initial and 28 day timepoints.

Table 6
Blend potency results (mg/g) at initial timepoint and after 28 days of storage.

Conditions	Blend 1	Blend 2	Blend 3	Blend 4	Blend 5	Blend 6	Blend 7	Blend 8	Blend 9	Blend 10
Before stability	9.66	10.05	10.15	9.85	9.74	10.07	9.98	9.55	9.53	9.62
28 days RT	9.46	9.52	9.63	9.52	9.24	9.50	9.70	9.31	9.08	9.42
28 days 40/75	9.26	9.35	9.59	9.56	9.06	9.42	9.74	9.08	8.72	9.15

those with the highest levels of lactose in the <10 μm and the 10–40 μm fraction; both blends 3 and 4 showed a decrease in FPD from around 90 μg to around 75 μg. All other formulations gave consistent results after 28 days at RT and no drop off in performance. When stored at 40 °C/75% RH for 28 days, all formulations gave mean FPD results of less than 25 μg; the reduction in performance was not found to be related to any particular lactose size fractions.

The significant reduction in performance for blends stored at 40 °C/75% RH could be explained by particle agglomeration that resulted in reduction of the fine lactose content within the blend, as shown in SEM microphotographs (Fig. 8). This was confirmed by the laser diffraction measurements—a shift in particle size distribution towards larger particle sizes was noted for all samples (Fig. 9).

Table 7 outlines the effect of storage at elevated temperature and humidity conditions (28 days at 40 °C/75% RH) on powder flow properties. According to the FFC classification, after 28 days of storage at 40 °C/75% RH all blends were in the poor to marginal flow area with the FFC values in the range of 2.3–4.2. The most pronounced

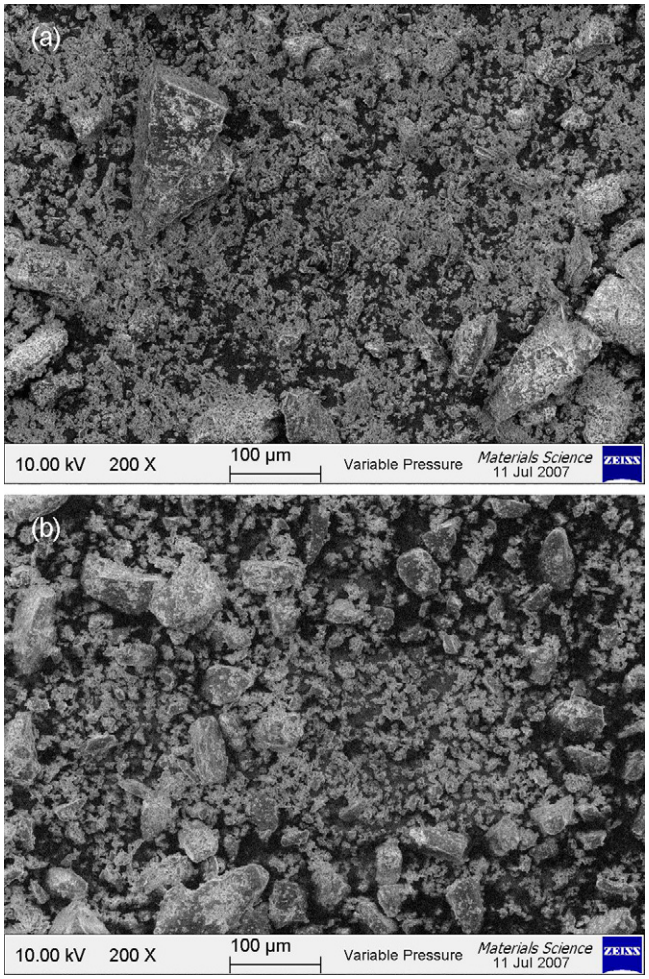

Fig. 8. SEM microphotographs of blend 3: (a) before and (b) after 28 days at 40 °C/75% RH.

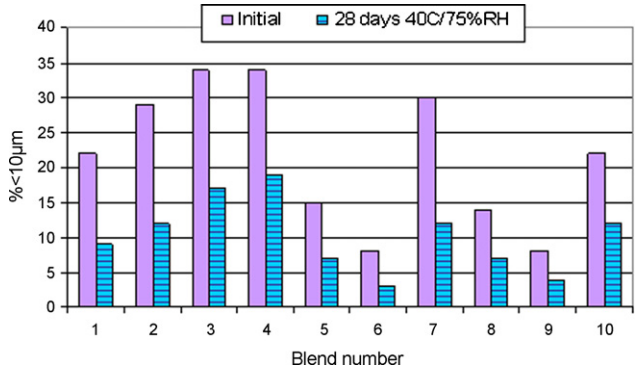

Fig. 9. Effect of storage (28 days at 40 °C/75% RH) on particle size distribution (% particles <10 μm).

Table 7
Effect of storage (28 days at 40 °C/75% RH) on function flow coefficient.

Blend number	FFC initial	FFC 28 days 40/75
1	3.35	2.90
2	2.51	2.80
3	2.30	2.45
4	2.30	1.95
5	4.10	2.85
6	6.95	3.65
7	2.65	2.30
8	4.35	2.85
9	7.35	4.20
10	3.00	2.30

reduction in flow was noted for the blends with largest particle size; as illustrated in Fig. 10, a direct correlation was noted between the reduction in flow properties on storage and the initial FFC results. It is thought that instrument sensitivity is rendering a change in flow properties for poorly flowing powders more difficult to detect.

3.6. Multivariate data analysis

As a means of visualising the results generated from the DoE, principal component analysis (PCA) was performed. The complete

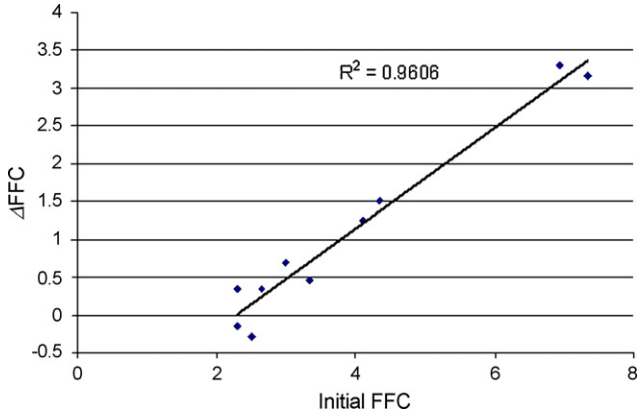

Fig. 10. Relationship between a change in function flow coefficient upon 28 days of storage at 40 °C/75% and initial FFC results.

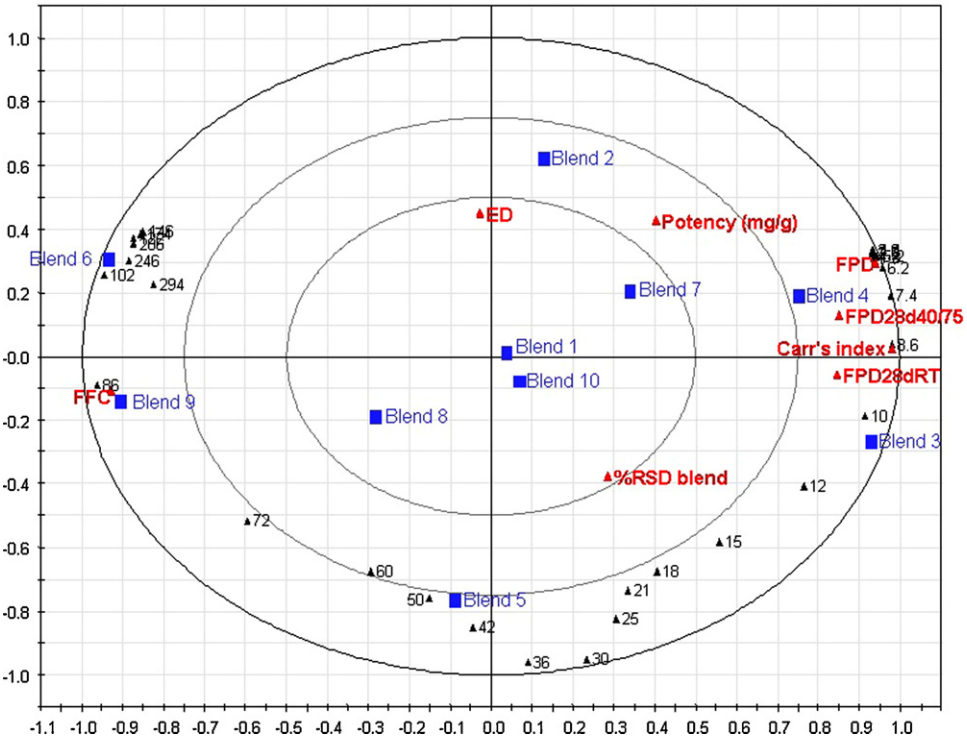

Fig. 11. Principal component plot for scores (shown in blue and labelled blends 1–10) and loadings (shown in black for the particle size classes and red for the other response variables) for the first two principal components for the DoE results (58% and 22% of the total variance in the data set is captured by PCs 1 and 2 respectively). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

particle size density distributions were included in this analysis, rather than the theoretical cumulative size fractions. This allows the variability in the batches over the full particle size range to be assessed. A plot for the first two principal components showing both the scores and loadings is shown in Fig. 11. To allow both the scores and loadings to be displayed on one plot the values have been scaled to between −1 and +1. The first component (PC1) captures 58% of the total variance in the data set whilst the second (PC2) captures 22%. Potency, homogeneity (%RSD) and ED are poorly modelled suggesting there is less variability in these results and that the variability of the other factors included dominate the PCA. This is expected from the standard statistical analysis of the results. As has been discussed, there were no significant trends in the homogeneity results and although an effect of the lactose portion % <10 μm on potency was detected, the significance of the effect was small in practical terms. ED was found to be highly variable but the variation is random and attributed to operator error and inherent method variability.

PC1 captures the particle size variation in terms of fine and coarse particles—the fine particle size classes are on the right of the plot and the coarse to the left. Therefore, blends 6 and 9 on the right of the plot will have a larger coarse component and blends 3 and 4 on the left of the plot a larger fine component. Blends 1 and 10 are found, as expected, at the centre of the plot. The fine size classes (<10 μm) are seen to positively correlate with the FPD and Carr's Index and negatively with the FFC. The plot indicates that blends 3 and 4 have high FPDs and Carr's Indices and low FFC values, whilst blends 6 and 9 will have low FPDs and Carr's Indices and high FFC values. The FPD values obtained on stability are correlated with the initial FPD, indicating that blends with a high FPD value will also have a higher FPD, relative to other batches, on stability at both conditions (a conclusion supported by the plot of the FPD results on stability, Fig. 7).

PC2 highlights the lesser effects of the mid-range of the particle size distributions and to some extent the variation in potency, homogeneity (%RSD) and ED. As already stated, PCA does not model the latter three variables well; PC2 being dominated by the variations in the mid-range particle size classes. Blends 2 and 5 are shown to vary the most significantly in their PC2 scores. Blend 5 is shown from the PCA plot to have large amounts in the size classes 20–60 μm whilst Blend 2 is shown to have small amounts in these classes. The particle size distributions for blends 2 and 5 are shown in Fig. 12 and the difference in the mid-range particle size classes is clear. There is less variation in these mid-range particle size classes for the remaining batches so their PC2 scores are found to be closer to zero. The mid-range particle size classes are not strongly correlated with any of the other response variables. This is in agreement

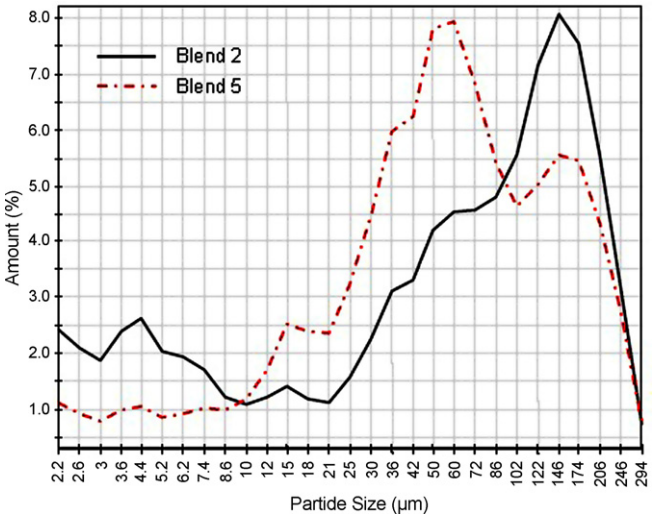

Fig. 12. Particle size distributions for blends 2 and 5.

with the findings from the standard analysis of the DoE, as discussed earlier.

4. Conclusions

This study offered a comprehensive approach to studying the effect of carrier particle size by using a statistical design to address the effects of relevant size cuts. Ten blends were prepared in this study using different carrier particle size fractions. The resulting formulations showed clear difference in terms of both physico-chemical properties and aerosolisation performance. Powder flow measurement showed that the smallest lactose size fraction had the greatest negative effect on powder flowability. In order to achieve at least 'fair' flow properties, the amount of ultrafine lactose needs to be kept to a minimum although it may be possible to have up to 20% in the 10–40 μm fraction.

The assessment of blend potency suggested that the content of ultrafine (<10 μm) carrier particles had a large positive effect. This was indeed an interesting finding, despite the fact that all blends had potency within the acceptable range (9.53–10.15 mg/g). It is postulated here that the ultrafine lactose particles increase powder aggregation as they are highly cohesive in nature. However, it was also noted that some natural variability in potency result exists that needs to be taken into account when interpreting these results.

The *in vitro* deposition study demonstrated that the presence of fine carrier particles influenced the dispersion of salbutamol sulphate in dry powder inhaler formulations. The study indicated that the aerosolisation performance improved when the amount of fine lactose particles was increased. For example, an improvement in the FPD values of approximately 40 μg was seen when the amount of ultrafine lactose was increased from 0 to 12%. The observed improvement is consistent with a hypothesis that ternary formulations achieve greater particle dispersion and surface detachment as outlined in Section 1. A good linear relationship was observed between the FPD results and the cumulative amount of particles under 10 μm, which is in agreement with previous studies (Larhrib et al., 1999; Louey et al., 2003; Young et al., 2007a). Larger lactose particles do not appear to have an effect on drug product performance.

Pharmaceutical formulations should be stable upon storage in a range of environmental conditions. Stability testing of blends after 28 days of storage at ambient conditions revealed a notable decrease in aerosolisation performance for the blends with high levels of fine lactose carrier. The reduction in FPD values was particularly pronounced for blends with high content of particles in the <10 μm and the 10–40 μm particle size range. This effect can be explained by an increase in the surface area for carriers with higher fines content; over the four-week period the powder absorbed moisture from the ambient air which led to an increase in capillary forces between the drug and the carrier. The capillary forces may have reduced the ability of the carrier–drug system to be dispersed by the drag forces and hence led to a reduction in the FPD values. Storing the blends at the conditions of elevated temperature and humidity (40 °C/75% RH) resulted in a greater decrease in aerosolisation performance that could not be correlated to any particular lactose size fractions.

In addition to the capillary effects described above, it is postulated that the finer particle size fractions of α-lactose monohydrate used here may contain a fraction of amorphous material as a result of mechanical processing. The processing method for the production of ultrafine lactose involved jet-milling, hence this carrier fraction is particularly prone to surface damage and creation of disordered regions. Data obtained from the manufacturer suggest that the amorphous content for ultrafine lactose is approx. 8% (when measured using dynamic vapour sorption). Absorption of water vapour by the amorphous regions can influence product stability. Furthermore, surface recrystallisation and the formation of solid bridges could also account for the reduction in drug product performance.

Lactose is known to promote chemical degradation of certain active pharmaceutical ingredients (Wirth et al., 1998) when used in formulations as an excipient. Furthermore, evidence exists that certain drug/lactose DPI mixtures are susceptible to increasing inter-particle adhesion forces upon exposure to elevated humidity (Podczeck et al., 1997). It can be anticipated that increasing the ratio of fine lactose particles within the formulation and the resulting increase in the surface area may exacerbate water absorption and product degradation effects for certain compounds.

This study was aimed at investigating the functionality within the particle size distribution using an experimental design. The need to understand and control the physico-chemical properties of the lactose carrier has been highlighted, as they strongly affect the efficiency of DPI formulations. The results obtained show that the beneficial effect that finer carrier size cuts bring to aerosolisation performance must be balanced with their negative effects on flow properties and stability upon storage. Robust and reliable measurement techniques for both surface and bulk powder measurement should be in place during the DPI formulation development. This is particularly important when large industrial scale equipment is used for powder processing as it can be anticipated that intra- and inter-batch variations in carrier properties may lead to variation in drug product performance and storage stability.

Acknowledgements

The authors would like to thank John Swarbrick (Inhalation Products, Friesland Foods Domo) for his help and guidance in selecting and providing the lactose sieve cuts used in this study. Gary Nichols is acknowledged for generating SEM images and Caroline Berry and Rachel Yorath for their help in generating the particle size data.

References

Aboul-Enein, H.A., Surmeian, M., 2000. Analysis of salbutamol and related impurities by derivative spectrometry. Arch. Pharm. 333, 75–78.
Adi, H., Larson, I., et al., 2007. Adhesion and redistribution of salmeterol xinafoate particles in sugar-based mixtures for inhalation. Int. J. Pharm. 337, 229–238.
Begat, P., Morton, D.A.V., et al., 2004. The cohesive-adhesive balances in dry powder inhaler formulations. I. Direct quantification by atomic force microscopy. Pharm. Res. 21, 1591–1597.
Bosquillon, C., Lombry, C., et al., 2001. Influence of formulation excipients and physical characteristics of inhalation dry powders on their aerosolization performance. J. Control Release 70, 329–339.
Chew, N.Y.K., Tang, P., et al., 2005. How much particle surface corrugation is sufficient to improve aerosol performance of powders? Pharm. Res. 22, 148–152.
Felix, F., Cides da Silva, L., et al., 2008. Thermal behavior study and decomposition kinetics of salbutamol under isothermal and non-isothermal conditions. J. Therm. Anal. Calorim., published online.
Ganderton, D., 1992. The generation of respirable clouds from coarse powder aggregates. J. Biopharm. Sci. 3, 101–105.
Gonzalez-Rodriguez, M.L., Barros, L.B., et al., 2007. Application of statistical experimental design to study the formulation variables influencing the coating process of lidocaine liposomes. Int. J. Pharm. 337, 336–345.
Islam, N., Stewart, P., et al., 2005. Surface roughness contribution to the adhesion force distribution of salmeterol xinafoate on lactose carriers by atomic force microscopy. J. Pharm. Sci. 94, 1500–1511.
Larhrib, H., Zeng, X.M., et al., 1999. The use of different grades of lactose as a carrier for aerosolised salbutamol sulphate. Int. J. Pharm. 191, 1–14.
Lewis, G.A., Mathieu, D., Phan-Than-Luu, R., 1999. Pharmaceutical Experimental Design. Marcel Decker Inc., New York.
Louey, M.D., Razia, S., et al., 2003. Influence of physico-chemical carrier properties on the in vitro aerosol deposition from interactive mixtures. Int. J. Pharm. 252, 87–98.
Louey, M.D., Stewart, P.J., 2002. Particle interactions involved in aerosol dispersion of ternary interactive mixtures. Pharm. Res. 19, 1524–1531.

Magee, G., Vaughan, C., et al., 2008. Proposing a design space–a case study using design of experiments and jet milling. RDD 1, 259–270.
Podczeck, F., 1998. The relationship between physical properties of lactose monohydrate and the aerodynamic behaviour of adhered drug particles. Int. J. Pharm. 160, 119–130.
Podczeck, F., Newton, J.M., et al., 1997. Influence of relative humidity of storage air on the adhesion and autoadhesion of micronized particles to particulate and compacted powder surfaces. J. Colloid Interf. Sci. 187, 484–491.
Shur, J., Harris, H., et al., 2008. The role of fines in the modification of the fluidization and dispersion mechanism within dry powder inhaler formulations. Pharm. Res. 25, 1631–1640.
Steckel, H., Markefka, P., et al., 2006. Effect of milling and sieving on functionality of dry powder inhalation products. Int. J. Pharm. 309, 51–59.
Steckel, H., Müller, B.W., 1997. In vitro evaluation of dry powder inhalers. II. Influence of carrier particle size and concentration on in vitro deposition. Int. J. Pharm. 154, 31–37.
Tee, S.K., Marriott, C., et al., 2000. The use of different sugars as fine and coarse carriers for aerosolised salbutamol sulphate. Int. J. Pharm. 208, 111–123.
Wirth, W.D., Baertschi, S.W., et al., 1998. Maillard reaction of lactose and fluoxetine hydrochloride, a secondary amine. J. Pharm. Sci. 87, 31–39.
Young, P.M., Chan, H.-K., et al., 2007a. The influence of mechanical processing of dry powder inhaler carriers on drug aerosolization performance. J. Pharm. Sci. 96, 1331–1341.
Young, P.M., Chan, H.K., Chiou, H., Edge, S., Tee, T.H.S., Traini, D., 2007b. The influence of mechanical processing of dry powder inhaler carriers on drug aerosolization performance. J. Pharm. Sci. 96, 1331–1341.
Zeng, X.M., Martin, G.P., et al., 1999. Effects of particle size and adding sequence of fine lactose on the deposition of salbutamol sulphate from a dry powder formulation. Int. J. Pharm. 182, 133–144.
Zeng, X.M., Martin, G.P., et al., 1998. The role of fine particle lactose on the dispersion and deaggregation of salbutamol sulphate in an air stream in vitro. Int. J. Pharm. 176, 99–110.