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The influence of dose on the performance of dry powder inhalation systems

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Abstract

The relationship between drug/lactose ratio and aerosolisation performance of conventional carrier based formulations was investigated using the twin stage impinger. A dose range of ~10–450 μg of drug in a 50 mg lactose carrier formulation was studied. Statistical differences in both the fine particle dose and fine particle fraction were observed across the dosage range (ANOVA, $p < 0.05$). In general, no statistically significant difference (Fishers Pairwise, $p < 0.05$) in fine particle dose was observed between drug levels of approximately 10 μg and 135 μg, whereas a linear decrease in fine particle fraction was observed across the same drug level range ($R^2 = 0.977$). Increasing the dose from ~135 μg to 450 μg resulted in a statistically significant increase in both fine particle dose and fraction (ANOVA $p < 0.05$). Such observations may be attributed to the occupation of 'active' carrier sites by drug particles at low drug concentration, since the quantity of drug particles liberated from the carrier during aerosolisation remains constant at the lower dosing regimes.

Keywords: Dry powder inhaler; Low dose; Active sites; Pulmonary delivery

1. Introduction

Dry powder inhalers (DPI) are routinely used in the treatment of respiratory diseases such as asthma. In general, the therapeutic agents employed in such systems are typically processed/milled to produce 'micron-sized' particulates that are of a size range suitable for respiratory deposition (0.5–8 μm, Ganderton and Kassem, 1992). Micron-sized particles have a relatively high surface area to mass ratio and consequently exhibit greater cohesiveness and adhesiveness compared to larger, unprocessed particles. For low dose drugs, this high adhesivity and cohesivity can result in product development hindrance in regard to issues such as content uniformity, stability and drug metering.

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A common approach used in order to combat these ever present formulation challenges, is to blend the micron-sized drug particles with a larger inert carrier material, such as lactose, to form an 'ordered mix' (Hersey, 1975). This approach results in improvement of handling and processing properties, accurate dosing by dilution of drug to mass ratio and an increase in device drug emptying.

In addition to the advantages that ordered mixes can offer, upon inhalation, the drug particles must be liberated from the formulation so as to penetrate the lower respiratory airways. This is achieved by the forces generated from the patient's inspiration and can be aided by device characteristics, for example, increased pressure drops or turbulent air flows.

The forces required to successfully remove drug particles from the surfaces of the carrier will be dependent on the physico-chemical properties of the drug and carrier, and the environmental conditions under which they are formulated, stored and used. Many studies have been conducted to investigate the influence of such factors on the performance of DPI based systems (Ganderton and Kassem, 1992). These include the study of carrier morphology/crystallinity (Kawashima et al., 1998; Zeng et al., 2000; Young et al., 2002; Larhib et al., 2003; Flament et al., 2004), the influence of carrier material, grade and size (Steckel and Muller, 1997; Larhrib et al., 1999; Louey et al., 2003; Steckel and Bolzen, 2004), the influence of carrier fines (Lucas et al., 1998; Zeng et al., 1998, 1999; Louey et al., 2003) and the addition of ternary force control agents (Young et al., 2002).

In general, the morphology and roughness of carrier particles are not uniform. This is in part due to the fact that carriers, such as lactose, are produced on a relatively large scale from natural sources. The processing and production of such organic materials will invariably lead to particles containing regions which exhibit different roughness parameters (peaks and troughs). Furthermore, since DPI carrier based systems are based on organic crystalline materials there may also be specific crystal faces with different surface free energies present (Muster and Prestidge, 2002). In addition, production and processing methods may also result in the presence of surface macroscopic and/or microscopic amorphous regions.

Clearly, these possible inter–intra batch variations in physico-chemical properties in the surface of a carrier material may lead to differences in apparent adhesion properties of drug particles. Furthermore, during the dynamic process of mixing, the adherence of drug particles to the more adhesive areas of the carrier surface is likely to occur. Indeed, Hersey (1975) proposed that the surfaces of larger particles consisted of distinct regions containing so-called 'active sites'. It was further suggested that when the number fine carrier particles in the mixture is below the saturation limit of the large particles' adhesive potential, the fine particles will preferentially bind to these active sites. When these active sites have been completely occupied with fine particles a binary carrier system would then exist, i.e., carrier with strongly bound fine particles, and free or weakly bound fine particles.

This presence of active sites has obvious implications for DPI drug delivery, since retention of drug particles on these relatively high-energy sites during processing and aerosolisation would result in a decrease in apparent respirable drug fraction as suggested by Staniforth (1996). The possible detrimental effect of active sites may not be an issue for the majority of marketed DPIs since they are designed to deliver relatively high drug doses (e.g. Ventolin Accuhaler^{TM}, Ventodisks^{TM}, Pulvinal^{TM}, and Cyclohaler$^{TM} \geq 200\,\mu g$) More recently, the development of DPIs which can deliver lower doses of drugs has received attention. In this case, the drug has a higher potency and consequently requires a lower dose (e.g. formoterol fumarate 6–12 μg). This may lead to variations in drug delivery as a consequence of drug retention in high-energy sites resulting in severe deviations from target doses and label claims.

Current methods for overcoming such issues include 'filling' the potential active sited by increasing the fine particle content present on the carrier surface (Zeng et al., 1999) or pacifying the effects of active sites by the addition of so-called 'force control agents' such as magnesium stearate (Young et al., 2002). However, this approach does not answer questions concerning the role of active sites in DPI science.

As part of an ongoing study, the influence of drug dose (carrier/drug ratios 100:1–5000:1) on the aerosolisation performance of a model DPI system has been investigated. The use of such a system may give a valuable incite into the role of active sites in the performance of lactose based DPI systems.

2. Materials and methods

2.1. Materials

Micronised salbutamol sulphate was supplied by Aventis Pharma (Holmes Chapel, UK). α-Lactose monohydrate was supplied by Borculo Domo (Netherlands) and was sieved to produce a 63–90 μm size fraction. Water used was purified by reverse osmosis (MilliQ, Millipore, France). All solvents used throughout the study were supplied by BDH (Poole, Dorset, UK) and were of at least analytical grade.

2.2. Particle size analysis

The particle size distribution of the sieve fractioned lactose and micronised salbutamol sulphate was determined by laser light scattering (Malvern Mastersizer X, Malvern, Worcs, UK). Approximately 100 mg of sample was suspended in a 0.1% (w/v) lecithin–cyclohexane solution and ultra-sonicated at 25 °C prior to analysis (experimentally determined sufficient for de-agglomeration). A small volume, circulating cell was used equipped either with a 100 mm (salbutamol sulphate) or 300 mm lens (lactose).

2.3. Scanning electron microscopy

Morphology of the lactose blends was examined by scanning electron microscopy (SEM) (Jeol 6310, Jeol, Japan) at 10 keV. Samples were gold-coated prior to analysis (Edwards Sputter Coater, UK).

2.4. Drug content determination

Quantification of salbutamol sulphate content uniformity and in vitro deposition was by high performance liquid chromatography (HPLC). The HPLC system consisted of an AS950 intelligent sampler, PU-980 intelligent HPLC pump, 975 UV/VIS detector (all Jasco, Japan) and Spherisorb 15 cm, 5 μm ODS1 column.

The mobile phase used throughout the investigation was methanol/water (60:40) and acetic acid 0.1% (v/v). Settings were as follows: detection wavelength 276 nm; flow rate 1.25 ml min^{-1}; pressure approximately 400 kg m^2; injection volume 100 μl; analysis time 4 min; approximate retention time 2 min.

Linearity was confirmed between 0.1 μg ml^{-1} and 10 μg ml^{-1} ($R^2 = 0.99$). Lactose did not interfere with the salbutamol sulphate response. Sample injections were performed in duplicate using a bracket standard method containing standards prepared from separate stock solutions.

2.5. Preparation of blends

Powder formulations containing different levels of salbutamol sulphate were prepared by varying the ratio of lactose carrier to salbutamol sulphate. Eight blends with ratios ranging from approximately 100:1 to 5000:1 were prepared. Each formulation was designed so that 50 mg of the powder blend would contain the desired dose. The volume of the powder in the mixing cell was kept constant to reduce the effect of friction.

Salbutamol sulphate was blended with the lactose geometrically. Briefly, an amount of lactose, equivalent to about twice the total mass of salbutamol sulphate was used to 'sandwich' the drug in the blend. This was mixed for 1 min using a Whirlimixer (Fisons, UK). Lactose was then added in geometric quantities, mixing with a Whirlimixer for 1 min after each addition. The final blend was mixed in a Turbula (Bachofen, Basel, Switzerand) at 46 rev min^{-1} for 30 min.

The doses formulated were approximately 10 μg, 30 μg, 40 μg, 90 μg, 135 μg, 190 μg, 350 μg and 450 μg of drug.

Content uniformity (50 mg samples) across each blend indicated a coefficient of variation less than 5% ($n = 10$).

Each blend was stored in tightly sealed containers with a saturated solution of potassium carbonate (44% RH) for a minimum of 48 hours prior to analysis.

2.6. In vitro analysis

The influence of drug–lactose ratio on the aerosolisation performance was investigated using the twin stage impinger (TSI) (Copley Scientific, Nottingham, UK). Methodology followed that of the British Pharmacopoeia. Briefly the TSI contained 7 ml of mobile phase in stage one and 30 ml mobile phase in stage two, which at 60 l min^{-1} produces a cut off mass median aerodynamic diameter of 6.4 μm between the two stages. The flow through the TSI apparatus was controlled using a GAST rotary vein pump and solenoid

valve timer (Copley Scientific, Nottingham, UK). Flow rate was tested prior to operation using a calibrated flow meter.

A filled (49.90 ± 0.20 mg) size 3 gelatine capsule was placed into the dosage chamber of the Cyclohaler®. The mouthpiece was attached to the TSI apparatus and the Cyclohaler® inserted until the end met the inner edge of the rubber mouthpiece. The pump was turned on and allowed to stabilise for 10 s before starting the valve-timer for a 5 s period. The device was removed carefully, the empty capsule placed into a beaker, and the device was primed and tested with a second capsule. This procedure was repeated until five actuations had been conducted.

The TSI apparatus was then dismantled and each stage was washed with mobile phase into volumetric flasks. In addition, the device and capsules were washed into a separate volumetric flask. Appropriate sample dilutions were made prior to testing by HPLC. All values were divided by five to approximate a single dose.

The TSI aerosolisation studies were conducted in triplicate for each formulation and were randomised for dose.

3. Results

3.1. Particle size analysis

The particle size distributions for both lactose and salbutamol sulphate are shown in Fig. 1A and B, respectively. The size distribution for the lactose sample (volume median diameter) indicated the majority of particles to be between 60 μm and 200 μm in diameter. It is interesting to note that the upper size was higher than the sieve fraction value (90 μm) and was most likely due to the tomahawk geometry of the lactose particles. Furthermore, approximately 6% of the particles exhibited diameters of less than 20 μm with 2% less than 5 μm. These can be related to the presence of fine lactose particles attached to the large carrier particles which were clearly visible using SEM. This demonstrates that simple sieving does not provide sufficient energy to remove adhered lactose fines from larger carrier particles and that lactose fractions produced by sieving consist of a pseudo-ordered mix which has obvious formulation implications. Analysis of the particle size distribution of the micronised

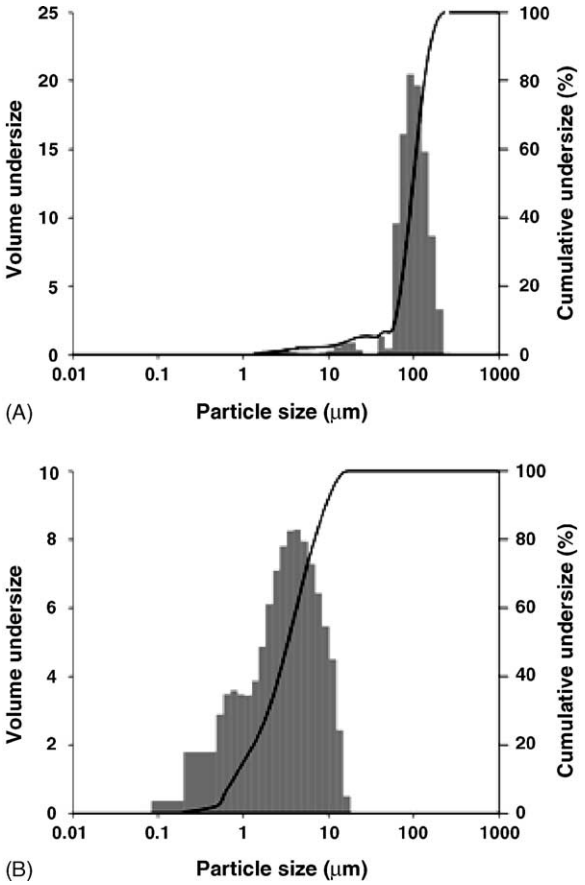

Fig. 1. Particle size distribution of 63–90 μm sieve fractioned lactose (A) and the micronised salbutamol sulphate (B).

salbutamol sulphate suggested 90% of the particulates exhibited a diameter less than 9.4 μm and 50% less than 3.4 μm.

3.2. Scanning electron microscopy

Representative SEM images of 12 μg, 135 μg and 450 μg dose blends are shown in Fig. 2A–C, respectively. Clear variation in the degree of fine particulates (<5 μm) was observed when comparing the blends. In general, a rank-order in particulate number matched that of the blend dose.

Unformulated lactose SEM images showed large carrier particles with some smaller adhering lactose particles which is in agreement with the previously described particle size data.

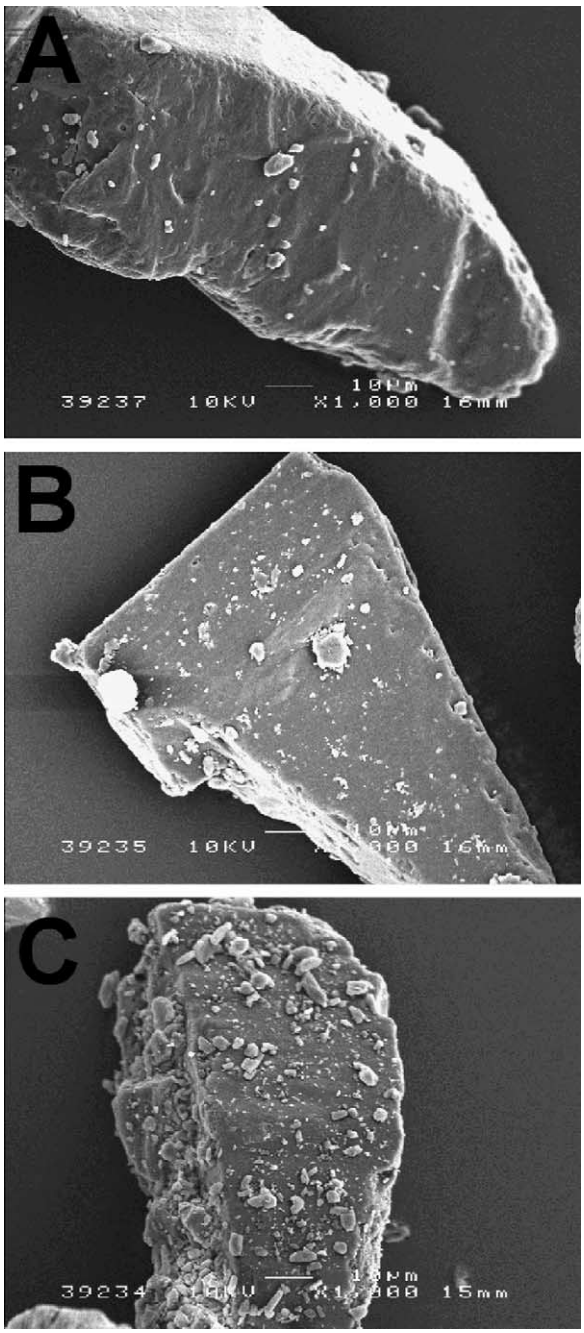

Fig. 2. Scanning electron microscope images of (A) 12 μg, (B) 135 μg and (C) 450 μg blends.

3.3. In vitro analysis

The aerosolisation efficiency of salbutamol sulphate from lactose carriers was investigated using the TSI. Specifically, the influence of dose on the fine particle aerosolisation was investigated since this is a good indicator of respiratory delivery. Data were processed and represented as follows: loaded dose (total recovered from all stages and device/capsules); emitted dose (total recovered from TSI stages); fine particle fraction (dose recovered in stage 2/loaded dose × 100); fine particle dose (dose recovered in stage 2).

The relationship between the emitted dose and loaded dose is shown in Fig. 3 which suggests a linear response ($R^2 = 0.998$) with a device removal efficiency of 89 ± 4% across all doses. Since the percentage drug loss in the device was apparently independent of dose, it is reasonable to assume that such losses are due to drug adhered to retained lactose, since the formulation mass remained constant.

The relationship between fine particle fraction and loaded dose is shown in Fig. 4. It can be seen from Fig. 4 that the loaded dose has a significant effect on performance (ANOVA $p < 0.05$). In general, a linear decrease ($R^2 = 0.977$) in aerosolisation (i.e., fine particle fraction) was observed on increasing the dose from 11 μg to 135 μg followed by an increase when the dose was increased from 135 μg to 450 μg.

In comparison, the relationship between fine particle dose and loaded dose, shown in Fig. 5, suggested that the dose level only had a statistically significant effect over the range 135–450 μg (ANOVA, Fishers pairwise,

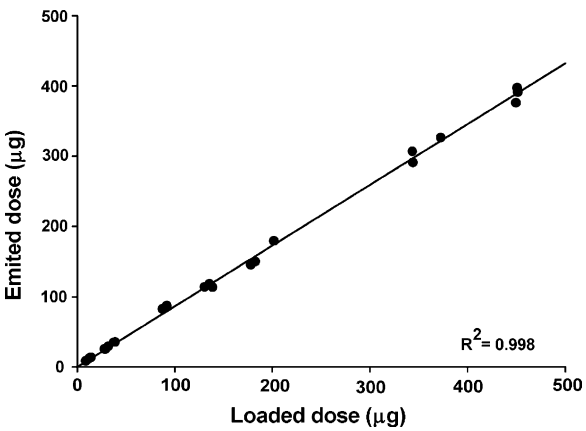

Fig. 3. Influence of loaded dose on the emitted dose.

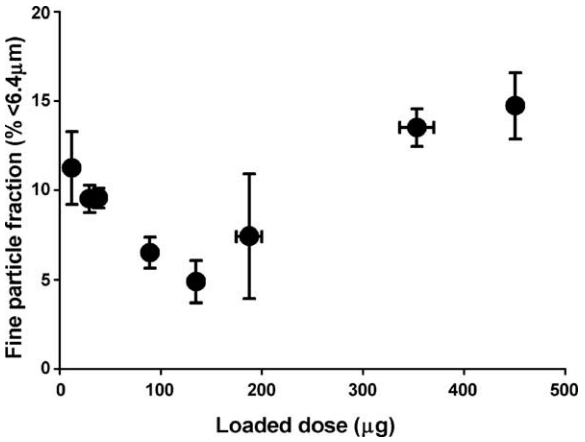

Fig. 4. Influence of loaded dose on the fine particle fraction.

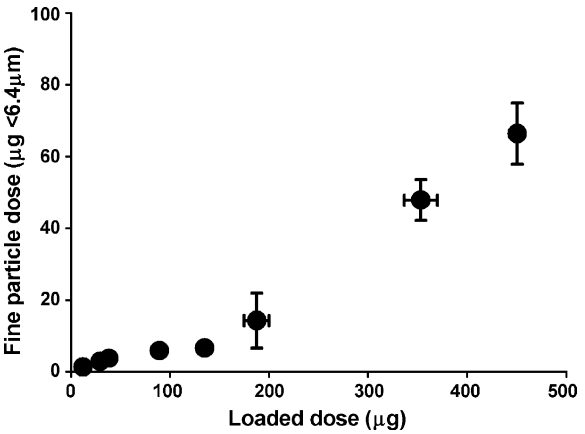

Fig. 5. Influence of loaded dose on the fine particle dose.

$p < 0.05$). No significant increase in fine particle dose was observed between 10 μg and 135 μg dose.

4. Discussion

The term active site may refer to variations in morphology and surface free energy (which will directly influence the thermodynamic work of adhesion). In terms of morphological effects, there are many possible topographical features that a drug particle may encounter on a lactose surface as represented in Fig. 6A. It is envisaged that sites with 'high energy' (area 1 in Fig. 6A) in the carrier surface would be preferentially occupied compared to sites with low energy (area 2 in Fig. 6A) as

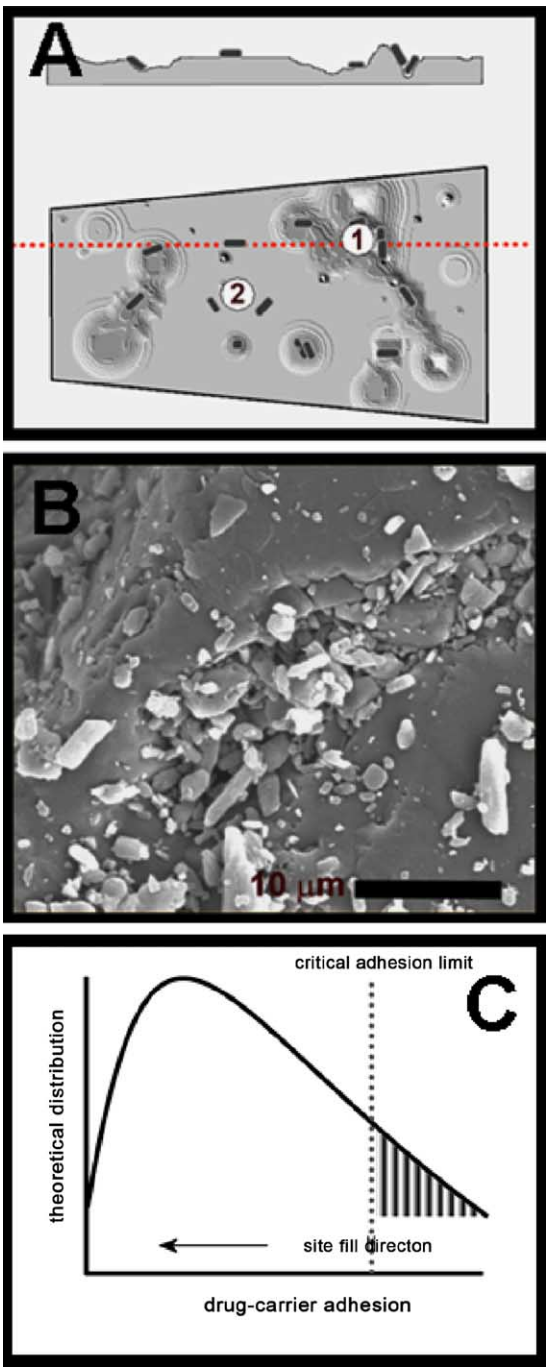

Fig. 6. (A) Schematic of regions on a carrier surface containing potential (1) high energy and (2) low energy 'active' sites. (B) SEM of a crevice on a lactose carrier surface containing many micron-sized particulates. (C) Theoretical distribution and process of active site filling.

a consequence of a combination of increased contact area, high surface free energy and simple geometric constraints.

A possible example of this is shown in Fig. 6B, where micron sized particulates have accumulated in a recess in the surface of a large lactose carrier particle.

Furthermore, it is suggested that the active sites present on the surface of the carrier will have a specific energy distribution (Fig. 6C) with a critical, average adhesion point below which particles, drug or lactose, could be removed. This concept correlates with previous studies, which have suggested that surface roughness and carrier material directly influence aerosolisation of drug from lactose carriers.

Analysis of variation in FPD with loaded dose (shown in Fig. 5) indicates that such a critical point exists, since no statistical variation in FPD was observed across the dose range 10–135 μg. As expected, a concurrent linear decrease in fine particle fraction was observed, since the FPD remained constant as total dose was increased linearly. Increase in dose from 135 μg to 450 μg resulted in an increase in both FPD and FPF, suggesting that the critical adhesion limit had been overcome. However, the relationship between loaded dose and FPF across the dose range 135–450 μg was not linear. This is to be expected, however, since such a system would still contain a certain distribution of active sites below the critical adhesion limit and therefore would result in a non-uniform performance response (i.e., the probability of drug–particle removal will dependent on the filling of differing energy sites). Furthermore, the inherent particle size distribution of the micronised drug would set an upper fine particle fraction limit.

Another point to consider is the potential for formation of drug or drug–lactose fines agglomerates. Previous studies have reported the presence of fines to increase the fine particle fraction through the formation of agglomerates or multiplets (Lucas et al., 1998). However, recent studies have suggested that the agglomeration or individual drug–carrier formation of a blend will be related to the balance of adhesion and cohesion in the system (Begat et al., 2004). It was suggested that for a salbutamol sulphate–lactose system, adhesion would dominate, thus reducing the potential for agglomeration (Begat et al., 2004). Such observations correlate well with the SEM images of the blends in this study, which suggested many of the micronised particulates in the salbutamol lactose system to be distributed as discrete entities. However, it is important to remember that the formulation mechanism will be dependent on the drug and carrier properties.

Indeed, recent studies by Louey et al. (2003) suggest that increased lactose fines result in an agglomerate based system. This is likely, since the potential free carrier space would be reduced. Furthermore, it is envisaged that the fine particle fraction would eventually plateau and decrease due to multilayer or aggregate formation and formulation segregation.

However, observations across the dose range 10–450 μg suggested the aerosolisation performance to be dominated by the active site theory. Clearly many variables would influence this relationship and are worth considering for future investigation. These include quantifying the influence of inherent fines and directly relating the influence of modified carrier surfaces to fine particle adhesion.

5. Conclusions

Clear variations in the FPF and FPD were observed as a function of dose or drug/lactose ratio (∼10–450 μg in 50 mg formulation). Furthermore, the relationship between drug/lactose ratio and aerosolisation performance was related to the possibility of active sites present on the lactose carrier surface.

Although the investigation here suggests the presence of such regions, a fundamental understanding of the mechanisms and mechanics of such a dynamic process is required further to circumvent the empirical formulation approaches used today. However, it is important to note, this study was conducted using one device, lactose and flow rate. In future, it would be interesting to study the influence of different carrier morphologies, devices (with different sheer and de-agglomeration mechanisms) and varied flow rates.

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