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The relationship between drug concentration, mixing time, blending order and ternary dry powder inhalation performance

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ABSTRACT

Some studies have shown that the mixing order of drug, fines and coarse carrier in a ternary dry powder inhaler (DPI) formulation affects fine particle delivery; others have seen no difference. This was investigated by examining the influence of salbutamol sulphate concentration (0.5–4.5%$^{w}/_{w}$), mixing time and blending order (drug and lactose carrier first, then lactose fines; versus fines and carrier first, then drug) on formulation *in vitro* fine particle delivery. With 15 min of mixing, there was no effect of drug concentration or blending order on fine particle fraction (FPF). With 30 min of mixing, lower drug concentrations produced larger FPFs with the fines and carrier first blending order. Higher drug concentrations resulted in equal performance between the blending orders. With 60 min of mixing, the drug and carrier first blending order resulted in larger a FPF at 0.5%$^{w}/_{w}$ salbutamol sulphate. The previous conflicting studies used a mixing time of 30 min; these results suggest that their conflicting results may have been due to the use of different drug concentrations. The complexity in the whole dataset suggests that blending order studies are of limited use for the investigation of the mechanism behind the effects of fines.

1. Introduction

Despite almost 40 years of research and development, the performance of conventional passive dry powder inhalers (DPI) remains poor, with the maximum reported *in vivo* lung deposition of drug being ~30% (Frijlink and de Boer, 2004). One strategy aimed at improving the performance of carrier-based DPI formulations is the inclusion of fine excipient particles (fines) in the blend of fine drug particles and coarse excipient carrier particles (typically α-lactose monohydrate), to produce what is known as a ternary formulation. This approach has recently been extensively reviewed (Jones and Price, 2006), but in summary, reducing the intrinsic fines content of a carrier is known to reduce the fine particle delivery (and thus performance) of a formulation, while the addition of 5–10 μm diameter excipient fines results in an increase in performance (Islam et al., 2004; Zeng et al., 1999). Various materials have been used as fines, such as erythritol, glucose, lactose, mannitol, sorbitol and trehalose (Adi et al., 2007; Jones et al., 2008b), while factors such as fines concentration, size and shape may also affect fine particle delivery (Adi et al., 2006; Lucas et al., 1998a; Zeng et al., 2001).

However, the mechanism (or mechanisms) which results in these effects has not been definitively proven. Two mechanisms have been widely proposed (Jones and Price, 2006). The "active sites" hypothesis suggests that there are areas of the carrier surface which are more "adhesive" than others. These areas are known as active sites. It is thought that fines preferentially adhere to these sites, blocking them to drug particles, which are, therefore, more easily detached during aerosolisation, increasing the amount of drug available for inhalation (Zeng et al., 1999, 2000). Under the agglomerates hypothesis, it is proposed that fine drug and excipient particles adhere to each other in the formulation, forming structures that are better aerosolised and dispersed than single drug particles (Adi et al., 2008; Jones et al., 2008b; Louey and Stewart, 2002; Lucas et al., 1998a).

Recently, a third mechanism was proposed, which suggests that fines increase the tensile strength of the bulk formulation, which increases the aerodynamic drag force required to fluidise the powder during inhalation. This increased force, combined with a greater frequency of particle–particle and particle–wall collisions in the presence of fines, may result in increased performance (Jones et al., 2008b; Shur et al., 2008).

A lot of the evidence which supports the active sites hypothesis is derived from investigations of the influence of the blending order of the drug, fines and coarse carrier on formulation performance. Various studies have demonstrated that formulations produced by blending the coarse carrier and fines before the addition of the drug gave greater fine particle delivery than formulations produced by blending the coarse carrier and drug first (Zeng et al., 1996a,b, 1999, 2000). It was suggested that when coarse carrier and fines are blended first, the fines have the first opportunity to adhere to active sites, thus drug particles adhere to less "adhesive" sites when

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they are added to the formulation, resulting in greater fine particle delivery. The opposite situation was suggested to apply when coarse carrier and drug are mixed first.

However, other investigations have demonstrated that the fine particle delivery of ternary formulations produced by both blending orders is equal (Louey and Stewart, 2002; Lucas et al., 1998a,b). Some of these studies employed different drugs to those used in the investigations discussed above. This may account in part for their differing conclusions, as variation in interparticulate adhesion caused by the use of different drugs has been shown to affect ternary formulation behaviour (Jones et al., 2008b). However, the discrepancy between the results of blending order studies was also observed even when the aerosolisation of the same drug (salbutamol sulphate) from the same inhaler (*Rotahaler*) at the same flow rate (60 l min^{-1}) was compared (Louey and Stewart, 2002; Zeng et al., 1996a,b, 1999). When these studies are compared in detail, it is noticeable that those which indicated an influence of blending order on formulation performance used a lower drug concentration (1.5%$^{w}/_{w}$) (Zeng et al., 1996a,b, 1999, 2000) than studies in which no effect was evident (2.0 and 5.0%$^{w}/_{w}$) (Louey and Stewart, 2002; Lucas et al., 1998a,b).

The aim of this study was, therefore, to investigate if the concentration of drug in a ternary DPI formulation determines whether its blending order has a significant effect on fine particle delivery, a possibility that has not been previously investigated in a systematic manner. Such a study is necessary in order to attempt to resolve the discrepancy between the results of different blending order studies and thus better assess the strength of the existing evidence in support of the active sites hypothesis. Furthermore, although the effect of dose on the performance of binary DPI formulations has been previously studied (Young et al., 2005), there has been no similar study utilising ternary formulations.

In order to allow comparison with previous work, the materials, formulation preparation and *in vitro* testing methods used in this investigation were taken from studies in which blending order was found to affect formulation performance (Zeng et al., 1996a,1999).

2. Materials and methods

2.1. Materials

Micronised salbutamol sulphate was obtained from GlaxoSmithKline Research and Development (Ware, UK). Coarse carrier α-lactose monohydrate (*Lactohale*) was donated by Friesland Foods Domo - Pharma (Zwolle, The Netherlands). Water was prepared by reverse osmosis (PURELAB Option, Elga LabWater, Marlow, UK).

2.2. Methods

2.2.1. Preparation of coarse α-lactose monohydrate carrier

The carrier α-lactose monohydrate received from the manufacturer was sieved to obtain the 63–90 μm size fraction using stainless steel sieves (Endecotts Limited, London, UK) and an AS 200 digit sieve shaker (Retsch UK Ltd., Leeds, UK) set to amplitude of 1 mm. This size fraction was then air-jet sieved for 75 min on a 45 μm stainless steel sieve (Haver and Boecker, Oelde, Germany) in batches of ~10 g using an Alpine (Augsburg, Germany) air-jet sieve 200 (laboratory type), in order to remove intrinsic fines.

2.2.2. Preparation of fine α-lactose monohydrate

α-Lactose monohydrate fines were prepared by air-jet milling the <63 μm *Lactohale* size fraction using a Trost Gem-T mill (Plastomer Technologies, Newtown, PA, USA) with feed and grind pressures set to 100 psi.

2.2.3. Particle size analysis

Particle size analysis was carried out in the dry state. Samples were dispersed with compressed air at 3 bar through a RODOS dry disperser fed by an ASPIROS micro-dosing unit before sizing with a HELOS laser diffraction sensor (all from Sympatec GmbH, Clausthal-Zellerfeld, Germany). The particle size analysis was performed using WINDOX 5 software (Sympatec GmbH, Clausthal-Zellerfeld, Germany). Values presented are the mean ± standard deviation of five determinations.

2.2.4. Preparation of formulations

Before blending, the micronised salbutamol sulphate was passed through a 500 μm stainless steel sieve (Endecotts Limited, London, UK) to remove large agglomerates of particles. Several weeks were then allowed to elapse before further use, in order to allow time for electrostatic charge to decay. A number of binary (no fines) and ternary (5.8%$^{w}/_{w}$ fines) carrier-based formulations were prepared in 4 g batches containing different salbutamol sulphate concentrations and using different blending orders and mixing times, as described in Table 1. Each blend used the same method—the appropriate period of mixing in a 15 ml glass tube using a Turbula shaker-mixer (Willy A Bachofen AG, Basel, Switzerland) at 46 rpm.

Following blending, the salbutamol sulphate content uniformity of all the formulations was assessed. Formulations were spread evenly over a clean surface and ten samples of 33 ± 1 mg taken from random positions. Each sample was dissolved in water to an appropriate volume and drug concentration assessed using a spectrofluorimetric assay (see below). The proportion of drug in each sample was calculated and the content uniformity expressed as the relative standard deviation (RSD) of these values.

Each formulation was then stored in a sealed container containing a saturated solution of potassium carbonate, providing a relative humidity of 44% (O'Brien, 1948). After 24 h, formulations were manually filled into size 3 gelatin capsules (donated by Capsugel, Bornem, Belgium) with a fill weight of 33 ± 1 mg. Filled capsules were then stored for a further 24 h at 44% RH prior to analysis.

2.2.5. Determination of in vitro fine particle delivery

The *in vitro* fine particle delivery of the various formulations was investigated using a twin stage impinger (TSI) (Radleys, Saffron Walden, UK) following the methodology described in the British Pharmacopoeia 2008. 7 and 30 ml of water was introduced into stages 1 and 2 of the TSI, respectively. Air flow through the TSI was controlled using a custom built solenoid valve timer and set to 60 l min^{-1} prior to operation using a flow meter connected to the mouthpiece of the TSI. Under these conditions, the cut off aerodynamic diameter between the two stages was 6.4 μm. For each test, a *Rotahaler* (GlaxoWelcome, Ware, UK) was connected to the TSI using a silicone rubber mouthpiece. The contents of five capsules (twenty capsules for 0.5%$^{w}/_{w}$ salbutamol formulations) were then aerosolised sequentially from this inhaler for 5 s each (controlled by the valve timer). The apparatus was then dismantled and the inhaler and capsules, mouthpiece and throat, stage 1 and stage 2 washed out with known volumes of water. Each formulation was tested five times in this way.

The salbutamol sulphate content of the washings was determined using the spectrofluorimetric assay, from which the mass of drug deposited in each part of the apparatus could then be determined. The recovered dose (RD, mass of drug recovered from all parts of the TSI and inhaler), emitted dose (ED, mass of drug recovered from all parts of the TSI) and fine particle dose (FPD, the mass of drug deposited on stage 2) were calculated. This then enabled calculation of two types of fine particle fraction: the FPF_{ED} (FPD expressed as a percentage of the ED) and the FPF_{RD} (FPD expressed as a percentage of the RD).

Table 1
Name and preparation details for the various formulations tested.

Formulation	Type	Drug concentration (%,$^{w}/_{w}$)	Mixing time per blend (min)	First blend	Second blend
0.5_{CD15}	Ternary	0.5	15	Carrier + drug	Carrier + drug + fines
0.5_{CD30}			30		
0.5_{CD60}			60		
0.5_{CF15}	Ternary	0.5	15	Carrier + fines	Carrier + fines + drug
0.5_{CF30}			30		
0.5_{CF60}			60		
1.5_{CD15}	Ternary	1.5	15	Carrier + drug	Carrier + drug + fines
1.5_{CD30}			30		
1.5_{CD60}			60		
1.5_{CF15}	Ternary	1.5	15	Carrier + fines	Carrier + fines + drug
1.5_{CF30}			30		
1.5_{CF60}			60		
2.5_{CD15}	Ternary	2.5	15	Carrier + drug	Carrier + drug + fines
2.5_{CD30}			30		
2.5_{CD60}			60		
2.5_{CF15}	Ternary	2.5	15	Carrier + fines	Carrier + fines + drug
2.5_{CF30}			30		
2.5_{CF60}			60		
3.5_{CD15}	Ternary	3.5	15	Carrier + drug	Carrier + drug + fines
3.5_{CD30}			30		
3.5_{CD60}			60		
3.5_{CF15}	Ternary	3.5	15	Carrier + fines	Carrier + fines + drug
3.5_{CF30}			30		
3.5_{CF60}			60		
4.5_{CD15}	Ternary	4.5	15	Carrier + drug	Carrier + drug + fines
4.5_{CD30}			30		
4.5_{CD60}			60		
4.5_{CF15}	Ternary	4.5	15	Carrier + fines	Carrier + fines + drug
4.5_{CF30}			30		
4.5_{CF60}			60		
BIN_{15}	Binary	0.5	15	Carrier + drug	–
BIN_{30}	Binary	0.5	30	Carrier + drug	–
BIN_{60}	Binary	0.5	60	Carrier + drug	–
BIN_{120}	Binary	0.5	120	Carrier + drug	–

2.2.6. Spectrofluorimetric assay

Salbutamol sulphate concentration in aqueous solution was determined using a PerkinElmer LS 50 B spectrofluorimeter, with an excitation wavelength of 274 nm and emission wavelength of 305 nm. The fluorescence intensity of salbutamol sulphate was found to be linear ($R^2 > 0.999$) over the range 0.5–10 μg ml^{-1}. The limit of detection of the assay (defined as three times the standard deviation of the fluorescence signal obtained from pure water ($n = 10$) (Thompson et al., 2002)) was 0.06 μg ml^{-1}. The fluorescence signal obtained from a 6.6 mg ml^{-1} solution of α-lactose monohydrate (the maximum concentration that could theoretically be encountered during the TSI experiment) was less than the limit of detection, so the presence of this excipient did not interfere with assay for salbutamol sulphate.

2.2.7. Scanning electron microscopy

Scanning electron microscopy (SEM) was used to investigate the particle morphology and powder structure of both the raw components and final formulations. Samples were sprinkled onto sticky carbon tabs mounted on aluminium stubs, excess powder was removed with a gentle tap before they were coated with gold using a K550 sputter coater (Emitech, Ashford, Kent, UK) and examined using an XL30 TMP scanning electron microscope (FEI (Philips), Hillsboro, OR, USA) at 5–10 keV.

2.2.8. Light microscopy

Light microscopy (LM) was used during the development and validation of the SEM methodology. It enabled the examination of powder structure with minimal preparation (unlike SEM) and comparison of LM and SEM images provided some reassurance that the latter technique was not producing major artefacts.

Samples were gently scattered onto glass slides and observed using a Nikon Microphot FXA optical microscope equipped with a digital image capture system.

2.2.9. Angle of repose

It has recently been suggested that fines might increase fine particle delivery due to their effects on bulk powder properties (Shur et al., 2008). The flowability of some of the formulations was, therefore, investigated. This was achieved through measurement of the angle of repose, as more advanced techniques, such as ring shear testing (Tuley et al., 2008) or powder rheometry (Shur et al., 2008), incorporate a preshear or conditioning stage, which might have obliterated any structural differences between formulations produced using different blending orders. In addition, these techniques would have required the manufacture of a greater amount of formulation than was permitted by the available salbutamol sulphate supply.

Angle of repose was determined following the procedure outlined in the British Pharmacopoeia 2008. A cone-like pile of powder was formed on a 12 mm diameter circular steel base (with retaining lip) by dropping the formulation from a short height through a funnel. The maximum height of the cone was measured and the angle of repose (α) calculated using the following equation (British Pharmacopoeia, 2008):

$$\tan\alpha = \frac{\text{height}}{0.5 \times \text{base}} \quad (1)$$

Table 2
Summary particle size statistics for the study materials ($n = 5$).

	d_{10} (μm ± SD)	d_{50} (μm ± SD)	d_{90} (μm ± SD)	% <5 μm (±SD)	% <10 μm (±SD)
Salbutamol sulphate	0.67 ± 0.00	1.50 ± 0.01	3.31 ± 0.01	98.8 ± 0.1	100.0 ± 0.0
Air-jet sieved lactose	57.56 ± 0.53	98.47 ± 0.94	154.18 ± 1.91	1.6 ± 0.1	2.2 ± 0.1
Micronised lactose	1.04 ± 0.01	5.12 ± 0.10	18.50 ± 0.65	49.1 ± 0.7	75.7 ± 0.8

For each formulation tested, the angle of repose was determined three times and the mean and standard deviation were calculated.

2.2.10. Statistical analysis

Statistical analysis was carried out using Minitab 15 software (Minitab Inc., State College, PA, USA). Comparison of two mean values was carried out using a two-tailed student's *t*-test. Comparison of three or more mean values was carried out by analysis of variance (ANOVA) followed by Tukey's Honestly Significant Difference test. In all cases, a significance level of 5% was used with Bonferroni correction applied in the case of simultaneous *t*-tests.

3. Results

3.1. Particle size analysis

Table 2 shows summary particle size statistics for the study materials. These show that the vast majority of the salbutamol sulphate particles were <5 μm diameter, suggesting they were suitable for DPI formulation (Frijlink and de Boer, 2004). The two types of lactose appear to have similar particle size distributions to those used in the studies on which the methods described here are based (Zeng et al., 1996a, 1999).

3.2. Formulation in vitro fine particle delivery

A drug content relative standard deviation of <6% is commonly taken as an indication that a DPI formulation is sufficiently uniform. This was not the case for every formulation used in this study and this was shown to be reproducible between replicate blends. However, as blending variables were the subject of this investigation, using a method deliberately chosen from previous studies for the purposes of direct comparison (Zeng et al., 1996a, 1999), it was not possible to improve the formulation content uniformity. There were no consistent relationships between content uniformity and drug concentration, mixing time or blending order.

When using a TSI at 60 l min^{-1}, the drug deposited in stage 2 (<6.4 μm aerodynamic diameter) forms the respirable fraction or fine particle dose (FPD). As might be expected, this parameter increased with the concentration of drug in the formulation for all mixing orders and times (see Fig. 1 for representative data relating to 30 min mixing time). Therefore, it is clear that in order to allow valid comparisons between the performance of different formulations, the *in vitro* deposition data need to be expressed as percentages to take into account drug concentration in the formulation. Table 3 shows these data. Virtually identical trends and patterns of statistically significant difference were seen with the FPF_{ED} data and the FPF_{RD} data. Therefore, the following discussion considers only the FPF_{ED}, as this is the most commonly used method for calculating FPF. Figs. 2 and 3 show how this parameter varied with drug concentration, mixing time and mixing order.

Inspection of Fig. 2 reveals that the FPF_{ED} of formulations produced by first blending the drug and coarse carrier for 15 and 30 min did not vary with salbutamol sulphate concentration in the original mixture. This was confirmed by ANOVA, which found no significant differences in each of these sets of FPF_{ED} values ($p = 0.71$ and 0.13, respectively). However, this blending order did result in FPF_{ED} variation with changing drug concentration when 60 min mixing was used. The FPF_{ED} of the 4.5%$^{w}/_{w}$ salbutamol sulphate formulation was significantly less than the FPFs of the 0.5, 1.5 and 3.5%$^{w}/_{w}$ formulations and the FPF_{ED} of the 2.5%$^{w}/_{w}$ formulation was significantly less than that of the 1.5%$^{w}/_{w}$ and 3.5%$^{w}/_{w}$ formulations (ANOVA, $p < 0.001$).

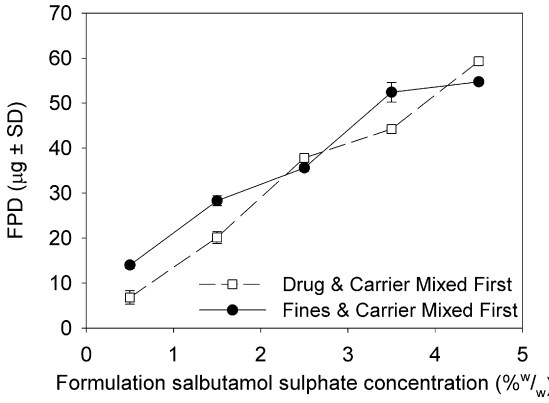

Fig. 1. Representative data for 30 min mixing time showing the relationship between *in vitro* FPD and formulation salbutamol sulphate concentration ($n = 5$).

Table 3
Summary *in vitro* deposition data for each formulation following aerosolisation from a *Rotahaler* into a TSI at 60 l min^{-1} ($n = 5$).

Formulation	ED ± SD (%of RD)	FPF_{ED} ± SD (%)	FPF_{RD} ± SD (%)
0.5_{CD15}	75.9 ± 2.0	3.6 ± 0.2	2.7 ± 0.2
0.5_{CD30}	82.2 ± 2.9	5.5 ± 1.4	4.5 ± 1.4
0.5_{CD60}	81.6 ± 2.9	7.1 ± 0.7	5.8 ± 0.7
0.5_{CF15}	75.4 ± 1.4	3.2 ± 0.4	2.4 ± 0.3
0.5_{CF30}	80.8 ± 2.2	12.1 ± 1.2	9.8 ± 0.9
0.5_{CF60}	77.8 ± 3.0	3.5 ± 0.4	2.7 ± 0.3
1.5_{CD15}	77.7 ± 8.5	3.4 ± 1.4	2.7 ± 1.3
1.5_{CD30}	69.8 ± 2.1	6.7 ± 0.5	4.6 ± 0.3
1.5_{CD60}	78.2 ± 3.8	7.6 ± 0.6	5.9 ± 0.6
1.5_{CF15}	77.7 ± 3.7	3.3 ± 0.2	2.6 ± 0.2
1.5_{CF30}	74.0 ± 1.8	9.2 ± 0.6	6.8 ± 0.4
1.5_{CF60}	79.2 ± 2.9	5.7 ± 1.2	4.5 ± 1.1
2.5_{CD15}	77.4 ± 2.2	4.1 ± 0.7	3.2 ± 0.6
2.5_{CD30}	87.8 ± 6.8	6.4 ± 0.2	5.4 ± 0.2
2.5_{CD60}	79.0 ± 2.4	6.0 ± 0.5	4.7 ± 0.3
2.5_{CF15}	68.2 ± 8.8	3.3 ± 0.6	2.3 ± 0.7
2.5_{CF30}	82.1 ± 0.9	6.2 ± 0.1	5.1 ± 0.1
2.5_{CF60}	76.1 ± 0.8	4.9 ± 0.6	3.8 ± 0.5
3.5_{CD15}	73.4 ± 3.3	3.7 ± 1.2	2.8 ± 0.9
3.5_{CD30}	79.6 ± 0.3	6.2 ± 0.2	4.9 ± 0.2
3.5_{CD60}	84.1 ± 1.6	7.3 ± 0.6	6.1 ± 0.5
3.5_{CF15}	69.9 ± 4.1	2.6 ± 0.5	1.8 ± 0.3
3.5_{CF30}	81.3 ± 1.8	6.5 ± 0.5	5.3 ± 0.3
3.5_{CF60}	79.5 ± 3.3	5.6 ± 0.9	4.4 ± 0.7
4.5_{CD15}	73.1 ± 4.0	3.9 ± 0.6	2.9 ± 0.5
4.5_{CD30}	76.0 ± 1.4	6.1 ± 0.2	4.7 ± 0.1
4.5_{CD60}	78.5 ± 4.0	5.9 ± 0.5	4.7 ± 0.4
4.5_{CF15}	76.4 ± 3.8	4.1 ± 0.4	3.2 ± 0.4
4.5_{CF30}	76.0 ± 1.1	6.2 ± 0.1	4.7 ± 0.1
4.5_{CF60}	77.7 ± 5.4	6.8 ± 0.7	5.3 ± 0.7
BIN_{15}	86.4 ± 3.3	2.4 ± 0.4	2.0 ± 0.3
BIN_{30}	75.0 ± 5.8	3.3 ± 0.7	2.5 ± 0.3
BIN_{60}	78.6 ± 2.6	4.3 ± 0.7	3.4 ± 0.6
BIN_{120}	83.9 ± 1.8	2.3 ± 0.3	1.9 ± 0.2

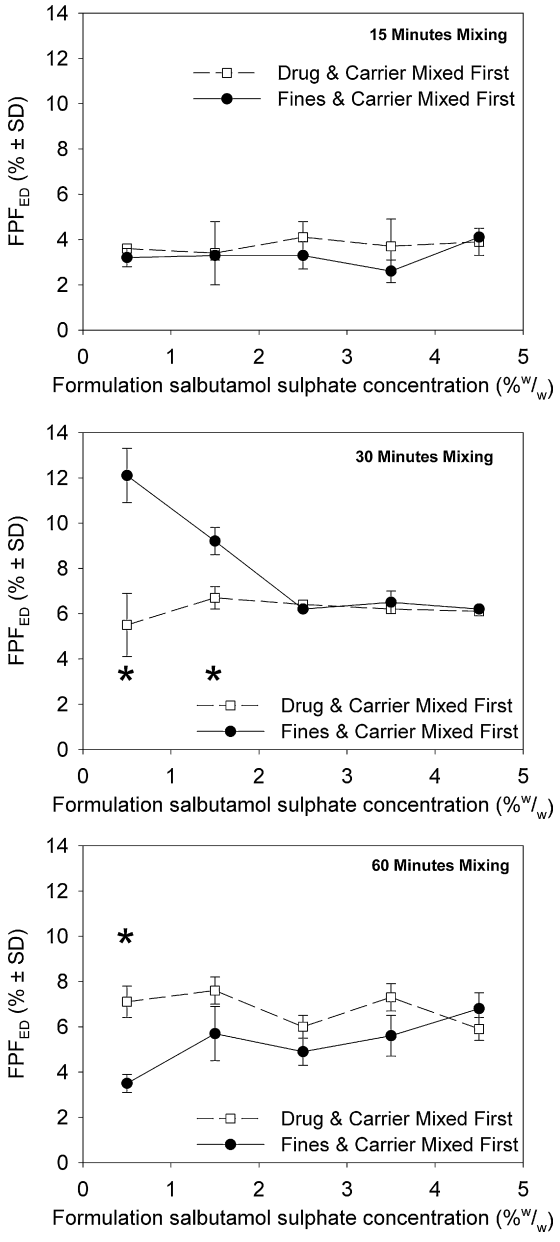

Fig. 2. The relationship between *in vitro* FPF_{ED} and formulation salbutamol sulphate concentration for each blending order and mixing time ($n = 5$). *Significant difference (t-test, $p < 0.0033$) between FPFs for that particular blending order.

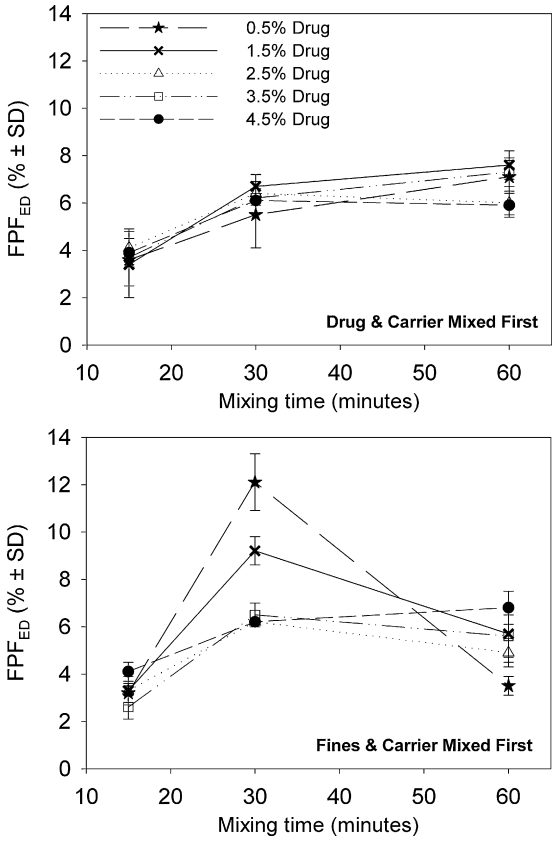

Fig. 3. The relationship between *in vitro* FPF_{ED} and formulation mixing time for each blending order and drug concentration ($n = 5$).

A different pattern of results was seen for the formulations produced by first blending the fines and coarse carrier (Fig. 2). When a mixing time of 15 min was used, the FPF_{ED} of the 4.5%$^{w}/_{w}$ salbutamol sulphate formulation was significantly greater than the FPF_{ED}s of the 0.5%$^{w}/_{w}$ and 3.5%$^{w}/_{w}$ formulations (ANOVA, $p = 0.001$). With 30 min mixing, the 0.5%$^{w}/_{w}$ formulation had a significantly greater FPF_{ED} than all the other drug concentrations and the 1.5%$^{w}/_{w}$ formulation had a significantly greater FPF than the three formulations with a higher drug concentration (ANOVA, $p < 0.001$). Finally, when each blend lasted 60 min, the 0.5%$^{w}/_{w}$ formulation produced a significantly smaller FPF than the 1.5, 3.5 and 4.5%$^{w}/_{w}$ formulations, and the 2.5%$^{w}/_{w}$ formulation produced a significantly smaller FPF than the 4.5%$^{w}/_{w}$ formulation (ANOVA, $p < 0.001$).

To further investigate these patterns, a series of t-tests was employed to compare the FPF_{ED} of the two blending orders for each salbutamol sulphate concentration and mixing time. The significant differences ($p < 0.0033$ after Bonferroni correction) located in this way are indicated in Fig. 2. With a mixing time of 15 min, there were no significant differences between the FPF_{ED}s of the two blending orders at any salbutamol sulphate concentration. When 30-min mixing time was used, the fines and carrier first blending order resulted in significantly greater FPF_{ED}s at 0.5 and 1.5%$^{w}/_{w}$ drug. However, with 60-min mixing, the drug and carrier first blending order resulted in a significantly larger FPF when the salbutamol sulphate concentration was 0.5%$^{w}/_{w}$.

Fig. 3 demonstrates that the mixing time also affected formulation FPF_{ED}. The drug and carrier first blending order resulted in significant FPF_{ED} differences with mixing time for all drug concentrations (ANOVA, $p \leq 0.004$). With 0.5%$^{w}/_{w}$ drug, each mixing time resulted in a significantly different FPF_{ED}, whereas with the remaining salbutamol sulphate concentrations, 15-min mixing produced a significantly smaller FPF than either 30 or 60 min.

Once again, a different pattern of significant differences (ANOVA, $p < 0.001$ in each case) was seen with the fines and carrier first mixing order (Fig. 3). For formulations containing 0.5%$^{w}/_{w}$ salbutamol sulphate, 30 min mixing resulted in a greater FPF_{ED} than either 15 or 60 min. Each mixing time produced a significantly different FPF_{ED} to the other mixing times for formulations containing either 1.5 or 2.5%$^{w}/_{w}$ drug. Finally, for formulations containing either 3.5 or 4.5%$^{w}/_{w}$ salbutamol sulphate, 15-min mixing time resulted in a significantly smaller FPF_{ED} than either 30 or 60 min.

Fig. 4 shows the FPF_{ED} of the four binary formulations. As indicated in this figure, the FPF_{ED}s of the 15 and 120 min mixing formulations were significantly different (ANOVA, $p < 0.001$) from that of the 60 min mixing formulation. The mixing times of the various binary formulations were chosen to reflect the time for which salbutamol sulphate was mixed in the ternary formulations. For example, in the 0.5_{CD60} formulation, salbutamol sulphate particles were mixed for 120 min in total: 60 min during the first blend and

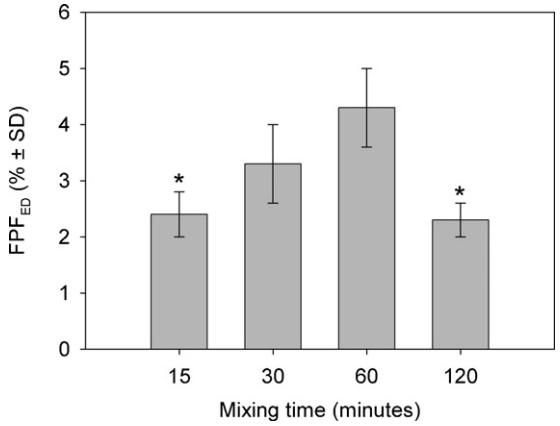

Fig. 4. The FPF_{ED} of the four 0.5% ($^{w}/_{w}$) salbutamol sulphate binary formulations ($n = 5$). *Significantly different (ANOVA, $p < 0.001$) from formulation mixed for 60 min.

60 min following the addition of fines. However, in the 0.5_{CF60} formulation, the drug was only mixed for 60 min, as it was added in the second blend, following the first mix of coarse carrier and fines. Therefore, the FPF_{ED} of each of the 0.5%$^{w}/_{w}$ salbutamol sulphate ternary formulations was compared with the FPF_{ED} of the appropriate binary formulation using a *t*-test with a significance level of $p < 0.00833$ after Bonferroni correction. These showed no significant differences after 15 or 60 min total drug mixing time. After 30 min total drug mixing time, the carrier and fines first blending order resulted in greater ternary formulation FPF_{ED} than the binary formulation ($p < 0.001$). Finally, the 0.5_{CD60} formulation resulted in a significantly greater FPF_{ED} than the BIN_{120} formulation ($p < 0.001$). It should be noted that a 0.5_{CF120} formulation was not produced for comparison.

3.3. Microscopy

Figs. 5–8 show scanning electron micrographs of representative formulations. The selected formulations were chosen as they produced significantly different FPF_{ED}s during *in vitro* testing and allow the reader to study the effect of drug concentration, mixing time or blending order on powder structure whilst other variables remained constant. However, similar patterns to those described below were seen for all imaged formulations.

There were no striking differences observable in the blend structure of formulations with varying *in vitro* performance due to blending order (e.g. 0.5_{CD30} versus 0.5_{CF30}) or salbutamol sulphate concentration (e.g. 0.5_{CF30} versus 2.5_{CF30} and 4.5_{CF30}). However, it was noticeable that formulations mixed for 30 min per blend included a considerable number of particles of ~10 μm diameter adhered to the coarse lactose carrier particles and forming complex multiparticulate structures containing much finer particles (e.g. the micrographs of formulation 2.5_{CF30}). Based on micrographs of the individual formulation components (no shown), it is thought that the ~10 μm particles were probably lactose fines, while the finer particles were probably salbutamol sulphate. Such an interpretation is also supported by the particle size data (Table 2) and micrographs of the binary formulations (Fig. 8), which showed only the presence of very fine particles adhered to the coarse carrier and very few ~10 μm particles. Such multiparticulate agglomerates were not seen in the micrographs of formulations mixed for 15 or 60 min per blend, which were dominated by very small particles adhered directly to the coarse carrier particles.

The light micrographs of formulation 0.5_{CD30} shown in Fig. 9 also show the presence of multiparticulate agglomerates, suggesting that these structures were not produced by the SEM vacuum or sample preparation technique. However, these micrographs also demonstrate the limited magnification and depth of field that limits the use of light microscopy in this context.

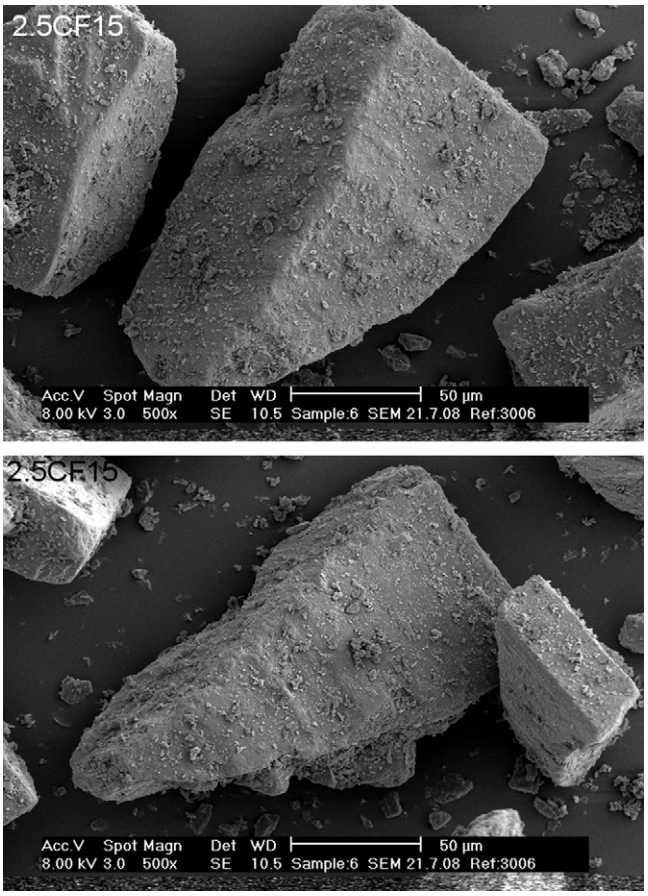

Fig. 5. Scanning electron micrographs of representative ternary formulations produced with a mixing time of 15 min. The formulation is identified in the top left corner of each image. As described in the text, these micrographs are dominated by very small particles adhered directly to the coarse carrier particles.

It should be borne in mind that the preparation of DPI formulations for SEM analysis may introduce artefacts by altering the blend structure, although as discussed, the light micrographs in Fig. 9 are reassuring in this case. In addition, even if many micrographs are captured, only a tiny proportion of a single dose of formulation will have been observed.

3.4. Angle of repose

Fig. 10 shows the angle of repose of the various formulations tested. Powders with poorer flowability have a larger angle. Statistical analysis revealed a number of significant differences in these data (ANOVA, $p < 0.001$): the angle of repose of formulation 0.5_{CD30} was significantly greater than that of formulations BIN_{30} and BIN_{60}, and the angle of repose of formulation 0.5_{CF30} was significantly greater than that of formulations 1.5_{CF30}, BIN_{30} and BIN_{60}.

4. Discussion

The aim of this study was to investigate if the concentration of drug in a ternary DPI formulation determines whether its blending order has a significant effect on fine particle delivery. Fig. 2 clearly demonstrates that when a mixing time of 30 min was used, the effect of blending order did indeed depend on salbutamol sulphate concentration. At low concentrations ($\leq 1.5\%^{w}/_{w}$), the fines and car-

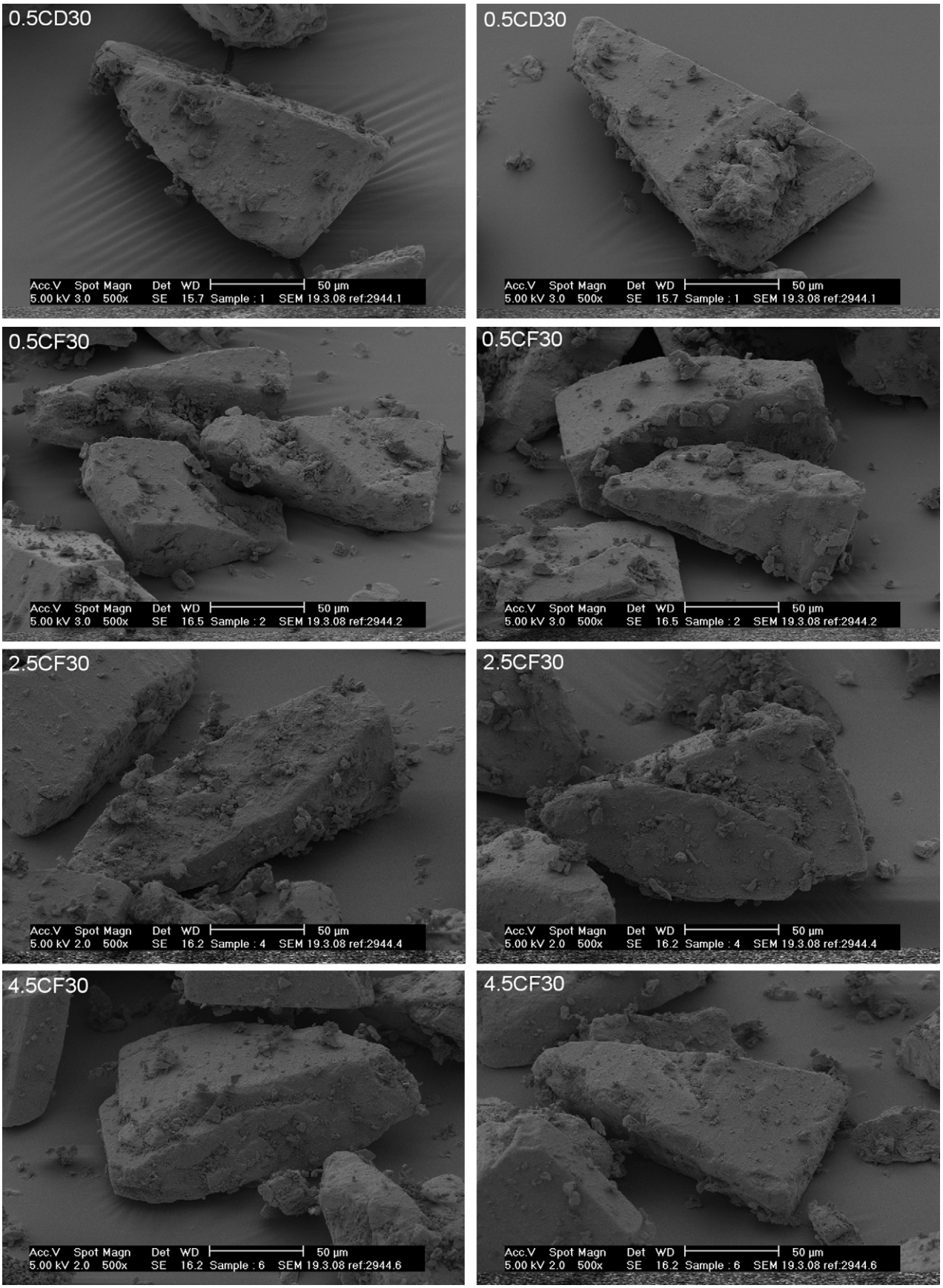

Fig. 6. Scanning electron micrographs of representative ternary formulations produced with a mixing time of 30 min. The formulation is identified in the top left corner of each image. As described in the text, these micrographs show the presence of a considerable number of multiparticulate agglomerates of drug and fines particles adhered to the coarse carrier lactose.

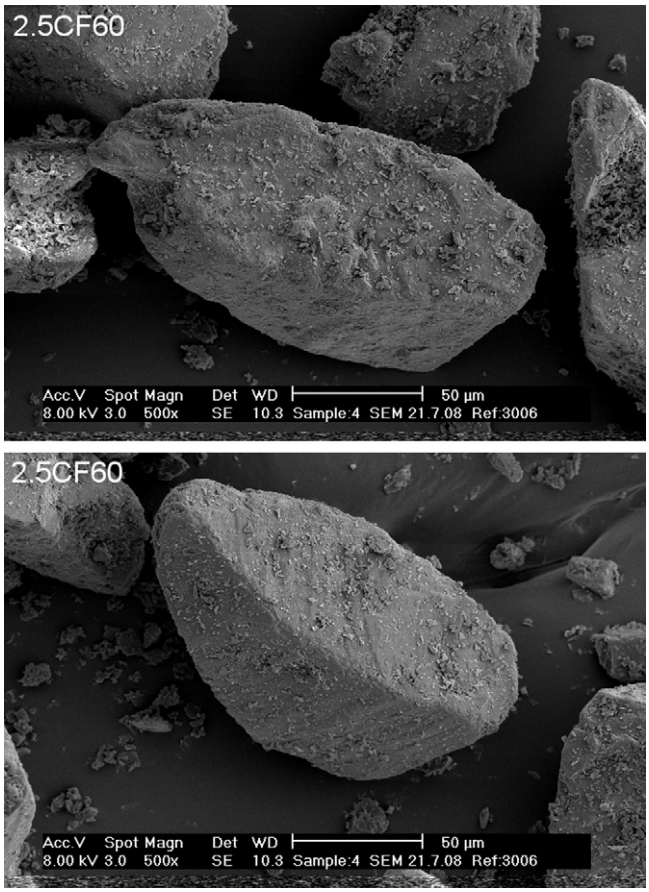

Fig. 7. Scanning electron micrographs of representative ternary formulations produced with a mixing time of 60 min. The formulation is identified in the top left corner of each image. As described in the text, these micrographs are dominated by very small particles adhered directly to the coarse carrier particles.

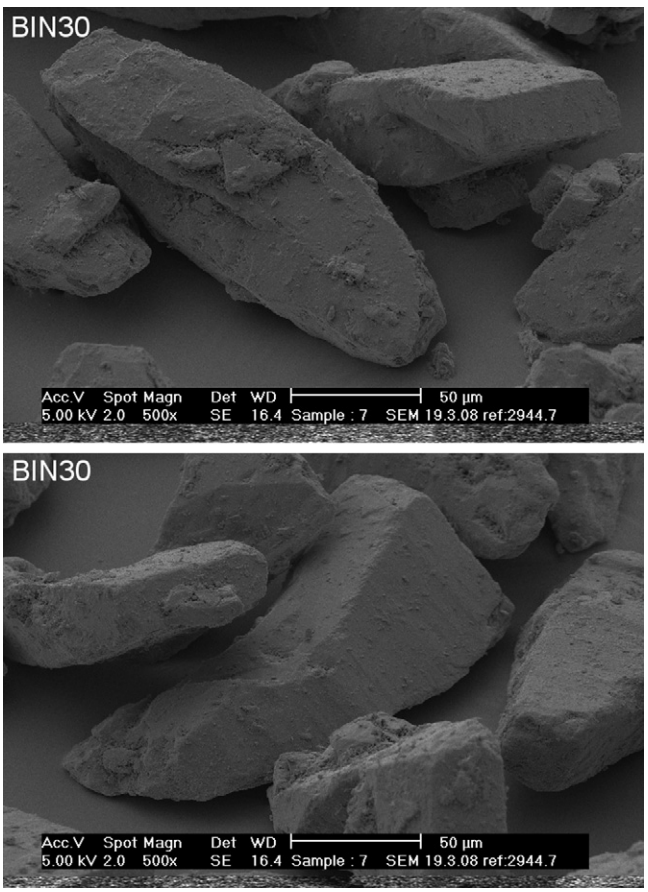

Fig. 8. Scanning electron micrographs of the binary formulation produced with 30 min of mixing.

rier first mixing order resulted in the greatest FPF, while at higher concentrations, the fine particle delivery of both blending orders was equal. Such a finding is similar to that of Hartmann and Steckel (2004), who found that blending order influenced the performance of formulations containing 0.1%$^{w}/_{w}$ salbutamol sulphate but not the performance of formulations containing 0.9%$^{w}/_{w}$ drug. Therefore, it is likely that the discrepancy between the results of literature blending order studies which used identical drug and aerosolisation conditions (discussed above) is attributable to the use of different drug concentrations, as those studies which found an effect of blending order also used a Turbula mixing time of 30 min (Zeng et al., 1996a,b, 1999, 2000). However, it should be noted that one of the studies that found no effect of blending order (Louey and Stewart, 2002) used a different mixing method: hand shaking in a test tube containing ceramic balls. Given that the use of different blenders has been shown to influence the outcome of blending order studies (Hartmann and Steckel, 2004), this difference may also account for the discrepancy with the results of Louey and Stewart's study.

However, the interaction between drug concentration and blending order only followed this pattern when a mixing time of 30 min was used. These formulations may also have exhibited a unique powder structure when examined using SEM, with complex drug-fines agglomerates adhered to the surface of the carrier particles. These were not seen in the 15 and 60 min mixing time formulations. Therefore, the unique interaction between drug concentration and blending order for the 30 min mixing time formulations might be related to the presence of drug-fines agglomerates, as it has previously been suggested that the effects of fines on the fine particle delivery of ternary formulations might be related to these structures (Adi et al., 2008; Jones et al., 2008b; Louey and Stewart, 2002; Lucas et al., 1998a).

Consideration therefore needs to be given to why drug-fines agglomerates may have formed with 30 min of mixing but not with 15 or 60 min of mixing time. Fine particles, such as those of the salbutamol sulphate used in this study, are known to spontaneously form agglomerates. Upon blending with larger excipient particles, such drug-only agglomerates are known to undergo a rapid initial deagglomeration, by impact with other particles and the mixing vessel walls (deVilliers, 1997). In a study of the mixing of 2.5 μm furosemide particles with 300–350 μm sodium chloride particles, this rapid initial deagglomeration was not complete after 10–20-min Turbula mixing, but was complete after 40 to 60 min (deVilliers, 1997). Following this initial deagglomeration, the process continues at a much slower rate, as the remaining (smaller) agglomerates are now attached to the excipient and are reduced in size by the abrasive removal of single particles, rather than by impact (deVilliers, 1997). In addition, it is known that agglomerates may also be formed during the mixing process and that powder structure reaches an equilibrium after a certain period of blending (Venables and Wells, 2001). Further mixing results in segregation (or demixing), due to differences in particle size, shape and density (Venables and Wells, 2001).

It is conceivable, therefore, that for the 15-min mixing time formulations examined in this study, there had been insufficient time to allow the deagglomeration of the initial salbutamol sulphate agglomerates and the subsequent formation of drug-fines agglomerates. Therefore, these latter structures were not seen in

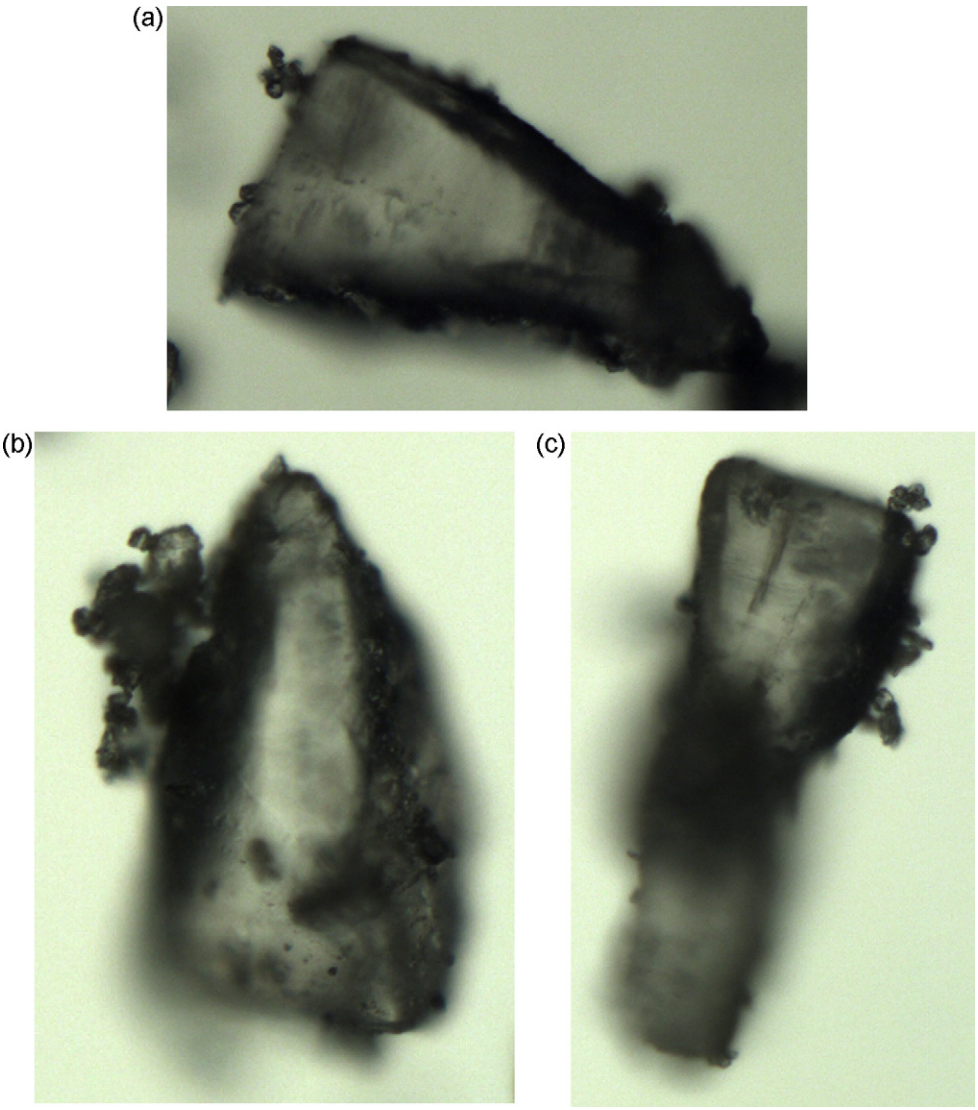

Fig. 9. Light micrographs of formulation 0.5_{CD30} captured using a 10× objective lens. As described in the text, these micrographs show the presence of multiparticulate agglomerates adhered to the coarse carrier lactose, similar to those observed using SEM for formulations with a mixing time of 30 min.

the 15 min mixing time formulations. It is further conceivable that 30 min mixing may have been close to the optimum time to allow for the formation of drug-fines agglomerates, whereas 60 min mixing may have been too long, resulting in the deagglomeration of drug-fines agglomerates through the processes of abrasion and/or segregation. Such theories, however, cannot yet be proved beyond doubt and as mentioned above, it is not possible to reach definitive conclusions from the SEMs regarding powder structure, due to the possible introduction of artefacts during the sample preparation and imaging process.

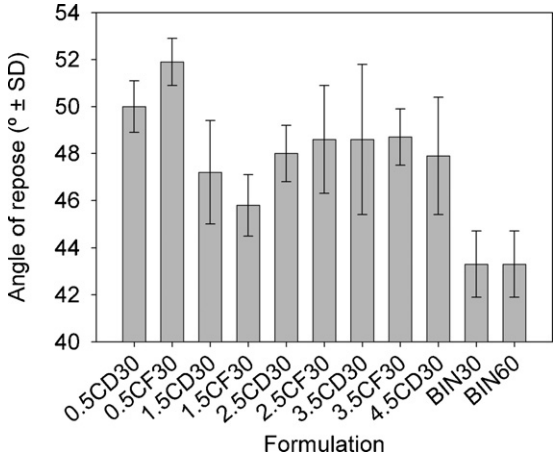

Fig. 10. Angle of repose of the various formulations tested ($n = 3$).

The general trend for both blending orders and all drug concentrations was for 30 and 60 min mixing time to result in a greater FPF than 15 min mixing (Fig. 3). The reason for this is unclear, but as discussed above, 15 min may not have been sufficient mixing time to complete the initial deagglomeration of salbutamol sulphate and the aerosolisation of large, strong agglomerates rather than single particles of drug might have reduced the FPF. This pattern fits with that displayed by the binary formulations (Fig. 4), where FPF_{ED} increased with mixing time up to 60 min. However, it should be recalled that for the drug and carrier first blending order ternary formulations, the actual time for which salbutamol sulphate was blended was twice the mixing time (i.e. 30, 60 or 120 min in total). The FPF_{ED} of the BIN_{120} formulation was significantly lower than that of the BIN_{60} formulation, a pattern not seen with the drug and carrier first blending order ternary formulations. This suggests that the presence of fines in a formulation can modify the relationship between mixing time and FPF_{ED} seen for binary formulations, as

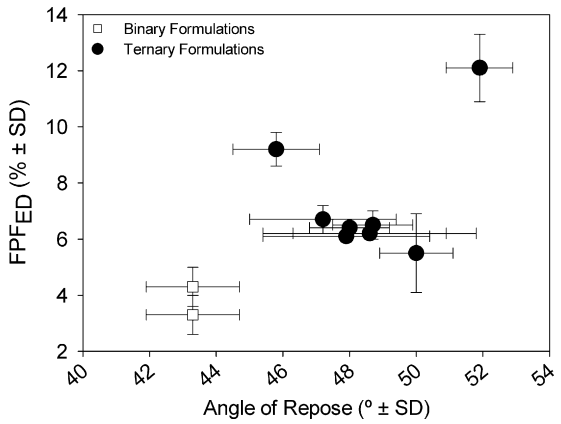

Fig. 11. The relationship between FPF_{ED} and angle of repose.

might be expected for a more complex system in which drug-fines agglomerates can form.

As mentioned above, it has recently been suggested that fines might increase fine particle delivery due to their effects on bulk powder properties (Shur et al., 2008). The data presented in Fig. 10 are consistent with this theory, as in addition to producing greater fine particle delivery, the two 0.5%$^{w}/_{w}$ ternary formulations (0.5_{CD30} and 0.5_{CF30}) had significantly greater angles of repose than the two 0.5%$^{w}/_{w}$ binary formulations (BIN_{30} and BIN_{60}). This suggests that these ternary formulations were less flowable than their binary equivalents, a property previously associated with increased FPF (Shur et al., 2008).

There was only one significant difference between the angles of repose of the ternary formulations, despite the many significant differences between their FPF_{ED}s (Fig. 2). However, Fig. 10 does show non-significant differences between these angles of repose. This is explored further in Fig. 11, which does not suggest a strong relationship between FPF_{ED} and angle of repose, although it is clear that the formulations with the lowest fine particle delivery (the binary formulations) were the most flowable and the formulation with the greatest performance was the least flowable.

Taken together, these figures suggest that a more sensitive and precise technique of flowability measurement might have located more significant differences and found a stronger relationship with FPF_{ED}. However, the angle of repose data for the binary formulations suggest that this technique is sufficiently sensitive to detect flowability differences between differently performing systems, so alternatively, the FPF_{ED} differences between the ternary formulations may not be attributable to differences in bulk powder properties. The use of more precise and advanced analytical techniques might allow a definitive conclusion to be reached, but as discussed above, such methods have other limitations.

As discussed, the effect of formulation drug concentration on the performance of binary DPI formulations has been studied previously (Young et al., 2005). This investigation studied ternary (rather than binary) formulations and utilised much higher drug concentrations than Young et al. (0.5–4.5%$^{w}/_{w}$ versus 0.02–0.9%$^{w}/_{w}$). In particular, all of the drug concentrations employed in this study were much greater than the critical point at which active site saturation occurred in the work of Young et al. (0.27%$^{w}/_{w}$). However, as Fig. 1 demonstrates, the fine particle delivery of the formulations tested during this study continued to follow the trend described by Young et al., with increasing drug concentration (once above the critical point) leading to increasing FPD (Young et al., 2005).

It should be noted that in order to allow comparison with previous work, this study utilised the *Rotahaler*, a device with a very low resistance known to produce lower fine particle delivery than other inhalers (Srichana et al., 1998). A different pattern of results may have been seen had a different inhaler device been used.

Future research should focus on two areas. Firstly, the quantitative investigation of the effect of mixing time on blend structure. A number of more advanced analytical techniques might be able to confirm or refute the formation of multiparticulate agglomerates with 30 min mixing time, but not with 15 or 60 min mixing time. These include automated image analysis of multiple electron micrographs, dynamic image analysis (Jones et al., 2008a) and laser diffraction particle size analysis of formulations either aerosolised directly from an inhaler device (Jones et al., 2008b) or using a pressure titration technique (Jones, 2006). Secondly, more advanced and precise techniques for the measurement of powder flowability, such as powder rheometry or shear testing, should be applied to study complete DPI formulations. To date, such techniques have only been applied to the study of excipient powders (Jones et al., 2008a; Shur et al., 2008; Tuley et al., 2008) and not to final DPI formulations. Such studies might give further insights into the question of how mixing time and blending order affect ternary DPI formulation performance.

5. Conclusions

The results of this study clearly demonstrate that the drug concentration and blending method of a ternary DPI formulation can influence the relationship between blending order and fine particle delivery. Therefore, these two variables are the likely causes of the discrepancy (discussed above) between previously published research using the same drug (salbutamol sulphate). As described in Section 1, much of the evidence in support of the active sites hypothesis comes from blending order studies. The results of this study imply that this evidence cannot be applied to all formulations and thus suggest that the agglomerates and tensile strength hypotheses are more likely to be at work, at least in certain formulations.

This study also highlights the complexity of the relationship between blending methods, formulation variables and ternary DPI performance, as it has been shown that the effect of a variable on FPF is not consistent between different levels of another variable. It is also suggested that the formation of drug-fines agglomerates is dependant upon the mixing time and that powder bulk properties might explain the differences in FPF between DPI systems with and without fines, but possibly not between different ternary formulations. It is therefore clear that the interactions between all the variables of a formulation and blending process need to be considered in order to produce DPI systems with the best performance.

Further work is required to investigate the complex interactions suggested by this study in more detail, using advanced techniques to examine the trends seen with other drugs, blending methods and aerosolisation conditions. A study of the behaviour of ternary formulations at very low drug concentrations would also be of interest, to enable direct comparison with the work of Young et al. (2005). A greater understanding of this subject should allow for the rapid optimisation of DPI performance during development and more insight into the mechanism(s) responsible for the effects of fines.

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