

The Relationship Between Drug Concentration, Mixing Time, Blending Order and Ternary Dry Powder Inhalation Performance

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Summary

Some studies have shown that the order in which the drug, fines and coarse carrier of a ternary dry powder inhaler formulation are mixed affects performance, whereas others have seen no difference. This discrepancy was investigated by examining the influence of salbutamol sulphate concentration, mixing time and blending order (drug and lactose carrier first, followed by the addition of lactose fines, or fines and carrier first, followed by the addition of drug) on *in vitro* performance. The results showed that these three variables interact with each other in complex ways in determining fine particle fraction. As well as explaining the discrepancy in the literature, this complexity suggests it is extremely difficult to reach conclusions from blending order studies about the mechanism behind the effects of fines.

Introduction

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 formulation¹. The mechanism which results in this effect is not definitively known, however two mechanisms have been widely proposed. 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^{2,3}. 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 particles⁴⁻⁷.

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 found 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^{2,3,8,9}. 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 they are added to the formulation, resulting in greater fine particle delivery. Other investigations, however, have found that the fine particle delivery of ternary formulations produced by both blending orders is equal^{4,7,10}. This discrepancy is 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) is compared^{2,7-9}. When these studies are compared in detail, it is noticeable that those which found an influence of blending order on formulation performance used a lower drug concentration (1.5% w/w)^{2,3,8,9} than studies which found no effect (2.0 and 5.0% w/w)^{4,7,10}.

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. In order to allow comparison with previous work, the materials and 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^{2,8}.

Methods

An α -lactose monohydrate carrier (*Lactohale*) was sieved to obtain the 63-90 μ m fraction and air-jet sieved to remove intrinsic fines. Lactose fines ($d_{50} = 5.1 \mu$ m) were produced by air-jet milling the <63 μ m size fraction. Ternary DPI formulations containing different concentrations of micronised salbutamol sulphate (0.5, 1.5, 2.5, 3.5 and 4.5% w/w), fines (5.8% w/w) and carrier were prepared using a Turbula mixer at 46 rpm. Each formulation was prepared using two different blending orders – either drug and carrier first or fines and carrier first. The third component was added in a second blend. Each combination of drug concentration and mixing order was prepared using three different mixing times for each blend, 15, 30 and 60 minutes. The *in vitro* fine particle fraction (FPF) of each blend was determined by aerosolisation from a *Rotahaler* into a twin stage impinger at 60 l.min⁻¹ (n = 5).

Results

Inspection of Figure 1 reveals that the FPF of formulations produced by first blending the drug and coarse carrier for 15 and 30 minutes did not vary with salbutamol sulphate concentration in the original mixture. However, this blending order did result in FPF variation with changing drug concentration when 60 minutes mixing was used (ANOVA, $p < 0.001$).

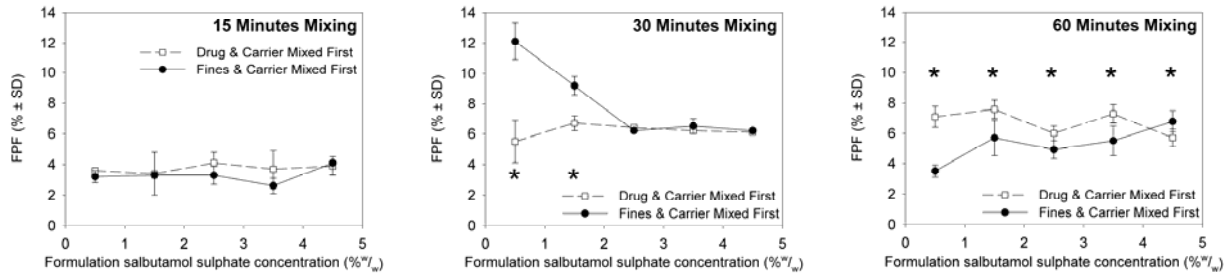


Figure 1: The relationship between *in vitro* FPF and formulation salbutamol sulphate concentration for each blending order and mixing time (n = 5). * = significant difference (t-test, $p < 0.05$) between FPFs for that particular blending order.

A different pattern of results was seen for the formulations produced by first blending the fines and coarse carrier (Figure 1). When a mixing time of 15 minutes was used, the FPF of the 4.5% w/w salbutamol sulphate formulation was significantly greater than the FPFs of the 0.5% w/w and 3.5% w/w formulations (ANOVA, $p = 0.001$). With 30 minutes mixing, the 0.5% w/w formulation had a significantly greater FPF 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 minutes, the 0.5% w/w formulation produced a significantly smaller FPF than the 1.5, 3.5 and 4.5% w/w formulations (ANOVA, $p < 0.001$).

Figure 2 demonstrates that the mixing time also affected formulation FPF. The drug and carrier first blending order resulted in significant FPF differences with mixing time for all drug concentrations (ANOVA, $p < 0.001$). With 0.5% w/w drug, each mixing time resulted in a significantly different FPF, whereas with the remaining salbutamol sulphate concentrations, 15 minutes mixing produced a significantly smaller FPF than either 30 or 60 minutes.

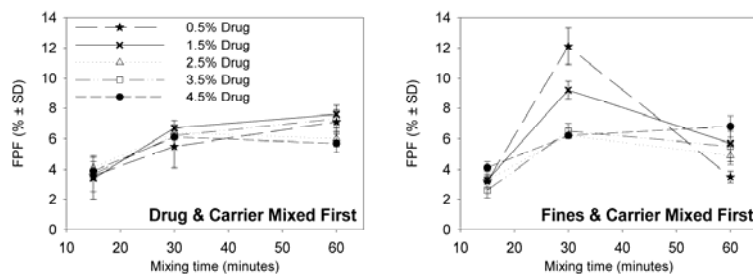


Figure 2: The relationship between *in vitro* FPF and formulation mixing time for each blending order and drug concentration (n = 5).

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 (Figure 2). For formulations containing 0.5% w/w salbutamol sulphate, 30 minutes mixing resulted in a greater FPF than either 15 or 60 minutes. Each mixing time produced a significantly different FPF 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 minutes mixing time resulted in a significantly smaller FPF than either 30 or 60 minutes.

There were no striking differences observable in scanning electron micrographs of formulations with varying *in vitro* performance due to blending order or salbutamol sulphate concentration. However, it was noticeable that formulations mixed for 30 minutes 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 (Figure 3). Such structures were not seen in the micrographs of formulations mixed for 15 or 60 minutes per blend. Based on micrographs of the individual formulation components, it is thought that the ~10 µm particles were probably lactose fines, whilst the finer particles were probably salbutamol sulphate.

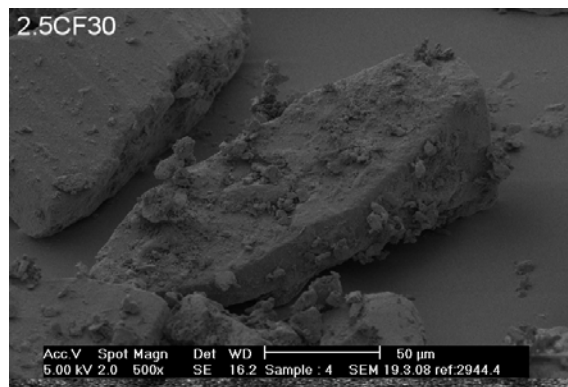


Figure 3: Scanning electron micrograph of the 2.5%^w/_w salbutamol sulphate, carrier and fines mixed first for 30 minutes formulation.

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. Figure 1 clearly demonstrates that when a mixing time of 30 minutes 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 carrier first mixing order resulted in the greatest FPF, whilst at higher concentrations, the fine particle delivery of both blending orders was equal. Therefore, it is likely that the discrepancy between the results of literature blending order studies (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 minutes^{2,3,8,9}.

However, the interaction between drug concentration and blending order only followed this pattern when a mixing time of 30 minutes was used. These formulations also 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 minutes mixing time formulations. Therefore, the unique interaction between drug concentration and blending order for the 30 minutes 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 structures⁴⁻⁷.

The relationship between salbutamol sulphate concentration, blending order and FPF was different for each mixing time. There was a general trend for more performance variation with changing drug concentration after longer mixing times. With a mixing time of 15 minutes, the general trend was for no change in FPF with different salbutamol sulphate concentrations, whereas the fines and carrier first formulations showed FPF variation with drug concentration after a mixing time of 30 minutes. After 60 minutes mixing, both blending orders resulted in variation with increasing salbutamol sulphate concentration, the drug and carrier first formulations showing decreasing FPF and the fines and carrier first formulations showing increasing fine particle delivery. In addition, when the mixing time was 15 minutes, the FPFs of the two blending orders were equal at all drug concentrations, whilst with a mixing time of 60 minutes, the performance of the two blending orders was significantly different at every drug concentration. These findings are counterintuitive, as longer blending times might be expected to "smooth out" differences in performance between different blending orders and drug concentrations. Indeed, this was the theory proposed in a previous investigation of blending order which produced contrasting findings to this study³. It formulated 1.5%^w/_w beclometasone dipropionate with a lactose carrier and 2.5 or 5%^w/_w lactose fines using a Turbula mixer rotating at 90-95 rpm. A mixing time of 15 minutes resulted in a significant difference between the FPF of formulations produced using each blending order, whereas with a mixing time of 60 minutes, no differences were seen³. The contrasting and counterintuitive findings of this study highlight the complexity of the relationship between blending methods, formulation variables and ternary DPI performance, as it would seem that a change of drug, fines concentration or mixing speed can result in the reversal of the trend seen with increasing mixing time.

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, it seems likely that these two variables are the cause of the discrepancy (discussed above) between previously published research. However, 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 been suggested that the formation of drug-fines agglomerates is dependant upon the mixing time.

This level of complexity suggests that it is extremely difficult to reach conclusions from blending order studies about the reason for the increase in FPF seen on the addition of fines to a ternary formulation. It is also 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, as 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.

Acknowledgements

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