Formulation and Evaluation of Combination Dry Powder for Inhalation: Influence of Crystalline Excipient

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ABSTRACT
The present study is based on the study of influence of crystalline mannitol as an excipient on physical characteristics of combination dry powder inhaler formulation and comparison with traditional combination Dry Powder for Inhalation. Formulation contains salmeterol xinafoate (SX) and fluticasone propionate (FP). The formulation was prepared by Spray drying of suspensions obtained by Antisolvent method. The excipients used were D-mannitol and Poloxamer 188. The powders generated were of an expected size for inhalation with satisfactory yield. Close agreement among the spectra of all spray dried formulations and APIs was found by FTIR study. Effect of excipients was further investigated by different physical characters of spray-dried formulation s. Dissolution study gave immediate drug release profiles. In vitro aerosolisation showed better degree of FPF as compared to marketed formulation containing lactose. The stability study indicated that all the formulations were quite stable at accelerated storage conditions. The results obtained from all observations denote that in presence of poloxamer.188; mannitol was found to be superior over traditional combination dry powder inhaler formulation containing Salmeterol xinafoate and Fluticasone propionate as API and lactose as carrier.

Keywords: Pulmonary Drug Delivery, Dry Powder for Inhalation, Combination Inhalation Therapy.

INTRODUCTION
Pulmonary drug delivery has attracted tremendous scientific and biomedical interest in recent years and has progressed considerably within the context of local treatment for lung diseases, by virtue of enhanced local targeting and reduced systemic side effects with the administration of minute drug dosages. Furthermore, with the high surface area and permeability of the lung, the 21st century has seen a paradigm shift to inhaled therapy for systemic use. The inhalation delivery of therapeutic agents has been known, though poorly understood, for many years. But the pulmonary tract tends to be considered as very promising and attractive route for the administration of active substances intended to treat local pulmonary (e.g., asthma, chronic obstructive pulmonary disease (COPD), microbial infections) as well as systemic diseases (e.g., diabetes). The formulation typically contains structural components of the particle as well as agents allowing the release of the drug over an extended period of time. These include lipids, proteins, sugars or synthetic polymers such as poly (vinyl alcohol) or polyesters. Spray-drying technology also offers the potential to incorporate a range of excipients into the formulation. Combination inhalation therapy (e.g. a long acting β-2 agonist with inhaled corticosteroid) provides convenience to the patient along with synergistic pharmacological actions, leading to better compliance and therapeutic outcomes in the management of life threatening pulmonary disease.

Currently, lactose is the most commonly used excipient in marketed DPIs (Beclophar®, Flixotide®, Relenza®, Seretide®, Spiriva®, Symbicort®). In contrast to oral administration, lactose swallowed at the levels present in inhaled preparations (up to 25 mg) is unlikely to present problems even in patients with lactose intolerance. Nearly all DPI products already on the market rely on lactose as a carrier material. But lactose may not be the carrier of choice due to its sugar-associated reducing function that may interact with functional groups of
drugs such as formoterol or peptides and proteins.
Mannitol, which is a sugar alcohol previously inhaled for diagnosis of bronchial hyperresponsiveness, has emerged as a promising carrier. In addition, mannitol was shown to stimulate the reserve capacity of the mucociliary system and to enhance clearance of mucus. Different studies on the influence of the presence of Mannitol and lactose during spray drying have been published with contradictory results. In fact, mannitol has been shown to increase the Fine Particle Fraction of spray-dried powders, although a large amount of mannitol results in a decrease in aerosolization performance.

It has been demonstrated that the presence of Poloxamer 188 in the feed dispersion results in particles with improved dispersibility and a higher fine particle fraction. Poloxamer was also used to produce large, light respirable powders prepared by spray drying, which contain leucine and tobramycin sulfate or thymopenton. It is interesting to note that 2% of Poloxamer 188 significantly improved powder flowability.

Spray drying is a one-step constructive process that provides greater control over particle size, particle morphology and powder density. It offers an alternative approach to the generation of dry, potentially respirable powders for local pulmonary drug delivery.

Hence, the present research work is focused on the influence of excipients like D-mannitol and Poloxamer 188 on different physical properties and evaluatory parameters of Dry Powder for inhalation for pulmonary delivery; so as to prove them one of the efficient excipients combinations for production of DPI.

MATERIALS AND METHODS
Micronised Salmeterol xinafoate and Fluticasone propionate were donated by Vamsi Labs Ltd., Solapur and Sun pharm. Ind. Ltd., Mumbai respectively. D-mannitol and Poloxamer 188 were obtained from Research Lab., Mumbai. Potassium dihydrogen phosphate (KH2PO4) and Sodium hydroxide (NaOH) were purchased from Finar chemicals Ltd., Ahmadabad, KBr was obtained from Loba chemicals, Mumbai. Ethanol was supplied by Fisher Scientific LTD. (Loughborough, UK).

2 FORMULATION AND OPTIMIZATION
2.1 Formulation of suspension by anti-solvent method
Co-precipitation or co-spray drying a solution containing two APIs is a potential alternative to produce particles of uniform drug composition. However, this process usually results in amorphous powders which are cohesive and physically unstable. The low FPF was attributed to low flowability and high adhesiveness of the amorphous powder. Thus, blending with carriers will introduce additional process variables. These shortfalls can potentially be avoided by incorporating a crystalline excipient into the co-spray drying solution. Suspensions were prepared by liquid anti-solvent precipitation technique.

Solvent: Ethanol (95%), Antisolvent: Distilled Water, Solvent: Antisolvent ratio: 30:70.

Briefly, the fine drugs were dissolved in ethanol (95%) (S) and sonicated to get the diffusing phase. Excipients were dissolved in distilled water (non-solvent) to obtain dispersing phase. For every batch, different concentrations of excipients were added as shown in table 1. Diffusing phase was then added to the dispersing phase i.e. to non-solvent by means of a 18 G 11/2 TW syringe positioned with the needle directly in the medium under continuous stirring for 1 hr at 1200-1500 rpm by using Lab stirrer (Remi-Motors).

2.2 Conversion of dispersion (suspension by Antisolvent method) into Dry powder for inhalation by spray drying
Spray drying using a, LABULTIMA Lab Spray Dryer(LU-222 Advanced Spray Dryer: LABULTIMA, Mumbai) with a co-current 0.7 mm, two fluid nozzle equipped with autojet deblocking system, was applied in order to retrieve respirable powders in dried state from suspension described above. Suspensions were spray dried with constant stirring with the help of...
magnetic stirring. The conditions used during spray drying were as mentioned in the table 2. The resultant dry powder was blown through cyclone separator and collected in container.

3. Characterization of dry powder for inhalation (DPI)

3.1 Particle size analysis, Percentage yield (%) and drug content

The particle size and polydispersity of the particles in dried state were determined by (Beckman coulter N4 plus submicron particle size analyzer). Samples were dispersed in water as dispersant medium. Average size and polydispersity index (PDI) is determined. The yields of preparation were determined by the weight of the products, spray dried powders, with respect to the weight of the initial drugs and excipients used. The drug content of spray dried powders was determined using UV spectrophotometry. Samples from each batch of spray dried formulation were dissolved in phosphate buffer (pH-7.4): ethanol (95%) in 90:10 proportions and the actual drug content was determined by first-derivative UV spectrophotometric method. Drug content was calculated from the ratio of actual drug content to total weight of spray dried powders taken for analysis and expressed as a percentage.

2.3.2 Fourier transform infrared spectrometry (FTIR) and Scanning electron microscopy (SEM)

Fourier transform infrared spectrometry (FTIR) spectra were recorded to evaluate the molecular states of pure drugs, excipients, physical mixture and all spray dried formulations. Fourier transform infrared spectrometry (FTIR) of pure drugs: SX and FP; excipients: Mannitol and Poloxamer 188 was carried out using a JASCO FTIR-410, JAPAN FTIR Spectrophotometer .The spectra were scanned over wavelength region of 400 to 4000 cm⁻¹, resolution of 4 cm⁻¹ and accumulation of 20 scans were used in order to obtain good quality spectra by making a pellet of the sample with KBr. The Surface appearance and shape of the spray dried powders were investigated by scanning electron microscopy. Drug, excipients and all spray dried formulations were mounted onto separate, adhesive coated aluminium pin stubs. Excess powder was removed by tapping the stubs sharply and then gently blowing a jet of particle-free compressed gas across each. The specimen stubs were sputter coated with a thin layer of gold in a JEC-550 Twin coating unit at 10 mA for 4 min using an argon gas purge. The specimens were examined using a scanning electron microscope (SEM, JEOL-JSM-5400)11.

2.3.3 Differential scanning calorimetry (DSC) and X-Ray powder diffraction study (XRD)

The phase transition of the pure drug, excipients, physical mixture, and all spray dried formulation batches were studied by thermogram obtained by using Differential scanning calorimeter (Dupont 2000, model SDT- 2960, USA). An empty aluminum pan was used as reference. DSC measurements were performed at the heating rate of 10 °C/min from 25 to 350 °C using aluminum sealed pan. Sample weight was kept between 5- 10 mg. During the measurement, the sample cell was purged with nitrogen gas. The crystalline nature of pure drug and all spray dried formulation batches were examined by studying its X-Ray diffraction patterns by using powder X-Ray diffractometer (PW- 3710 BASED). The operating parameters for instrument were Cu filtered K (α) radiations, a voltage of 40 kV, current of 25 mA and receiving slit of 0.2 In. The instrument was operated over 2θ scale. The angular range was 5 to 50° and counts were accumulated for 0.8 second at each step.

2.3.4 Powder density

The powder density of all spray dried formulations was determined by pouring a known mass of powder under gravity into a calibrated measuring cylinder and recording the volume occupied by the powder. The tapped density of the spray dried powders was determined by tapped density measurements on the same samples until no further change in the
powder volume was observed. Measurements were performed in triplicate. Carr’s Index values for each spray-dried powder were derived from poured density and tapped density data, according to given formula. The Carr’s Index value gives an indication of powder flow; a value less than 25 % indicates a fluid powder, whereas a value greater than 25 % indicates cohesive powder.

2.3.5 Formulation development and in vitro powder aerosolization
The in vitro aerosolization and deposition properties of DPI blended with different concentrations of excipients are determined using an Andersen cascade impactor (ACI) (Copley Scientific Limited, Nottingham, UK) with a Rotahaler® (Glaxo, UK) device. Experiment is carried out with a preseparator at air flow rates of 30 and 60 L/min. In each experiment, 8mL of the extracting solvent is poured inside the preseparator. A coating of 1% (w/v) solution of silicon oil in hexane is used on the impaction plates to prevent particle bounce and re-entrainment. Depending upon actual drug content, the representative powder from each batch was filled into hard gelatin capsule (size 3, Capsugel, Germany) manually so that each capsule contained 50 µg SX and 100 µg FP. About 25 mg of L samples of powders were weighed and loaded into size 3 hard gelatin capsule, which were individually installed in a Rotahaler® device. Rotahaler® is used as the inhaler to aerosolize the powder inside the ACI. An actuation time of 4 and 8 s is used for flow rates of 60 and 30 L/min, respectively, for each capsule to completely disperse all the particles. Experimental runs are conducted in triplicate. Particles remaining in the capsule, inhaler and different parts of the ACI are extracted using the same solvent used for the blend homogeneity test mentioned in the earlier section. The solutions are also assayed in a similar way.

Each batch was analyzed in triplicate to find out the emitted dose (ED); the sum of drug collected from all parts of the ACI, The fine particle dose (FPD); the amount of drug deposited in the lower stages of the ACI, The fine particle fraction (FPF) or respirable fraction (RF) calculated as the amount deposited in the lower stage as a percentage of the emitted dose (amount emitted into upper and lower stages excluding the amount remaining in the device). In vitro drug release
The in-vitro drug release of all the spray dried formulations was investigated by dissolution study. An accurately weighed amount of DPI equivalent to 50 µg of SX and 100 µg was added to 700 ml of dissolution medium; Phosphate buffer pH 7.4: Ethanol (95%), in 90:10 proportion and drug release was investigated using the USP rotating paddle dissolution apparatus (Lab India 2000) at 100 rpm and 37 °C. A percent release study was continued from 5 min. to 3 hrs. The final volume in all cases was 700 ml. The samples were withdrawn from the dissolution medium at various time intervals. 5 ml of sample was diluted to 10 ml with dissolution medium and subjected to UV Spectrophotometric analysis at 214 nm (λmax of SX) and 246 nm (λmax of FP). All the samples were analyzed in triplicate.

2.3.7 Stability study
After the characterizations of physical properties of spray dried powders and drug content, all the formulations batches were kept for 1 month at accelerated stability conditions of temperature and relative humidity 40 ± 2°C and 75 ± 5% RH. The choice of appropriate storage condition during accelerated stability study is necessary to predict the long term stability of SX and FP respirable particles. The humidity during storage is also extremely important considering the stability of formulation. Therefore, for the present study, accelerated temperature and relative humidity 40 ± 2°C and 75 ± 5% RH were selected during stability. Samples were withdrawn after one month and characterized for drug content and stability was predicted.
3. RESULT AND DISCUSSION

3.1 Particle size, Percentage yield and Drug content

The particle size of all spray dried formulations was found in the range of 1.53 to 2.22 µm (Table 3). The polydispersity index (PDI) is also an important parameter as it gives an indication about the width of particle size distribution as well as the long-term stability of dispersion. A PDI value of 0.1–0.25 indicates a narrow size distribution whereas a PDI value greater than 0.5 indicates a very broad distribution. The PDI value obtained for all batches of DPI was found to have broad distribution for M series (Table 3).

From the observations it was concluded that the formulations prepared by using Mannitol and Poloxamer 188 as an excipients gives average particle size. Based upon the observations from Table 3; poloxamer concentration affects the particle size; as in mannitol series which indicates that poloxamer 188 acts as a stabilizer in certain amount which will give small particle size. Also, particle size of M series formulation suggests that incorporation of stabilizer such as poloxamer 188 but in optimum concentration improves the particle size reduction.

Percentage yield and actual drug content are mentioned in the Table 4, which showed as the poloxamer concentration was increased, the yield of the product increased. Presence of poloxamer was found to increase the flow properties of M series which is beneficial during the collection of spray dried particle from cyclone and collector, to increase the percent yield of respirable DPI.

The method used for preparation of suspension was solvent displacement (Antisolvent) method which enables the production of small particles in the micrometer range acceptable for pulmonary drug delivery. All the formulations showed satisfactory drug loading from 19 mg to 31.5 mg so as to get the appropriate dose containing 50µg of SX and 100µg of FP from the individual respirable DPI.

As shown in Fig. 1 & 2, close agreement between the spectra of all spray dried formulations with FTIR of pure drugs and physical mixture suggested that there were no changes in the structure of SX and FP induced by solvent displacement technique as well as spray drying.

3.2 FTIR spectrometry and Scanning Electron Microscopy (SEM)

The scanning electron micrograph of pure SX (Fig.3-a) showed the powder to be of a crystalline flat material, needle like in structure. Many irregular particles with much fragmentation were observed. The scanning electron micrograph of pure FP (Fig. 3-b) showed the powder to be typical aggregate of amorphous material. Many irregular particles with cluster were observed.

The SEM images of DPI containing SX, FP, mannitol and poloxamer 188 are shown in Fig. 4 indicated that the powders formed after spray drying were amorphous in nature; but shows broad distribution in clusters. SEM micrograph showed aggregation which indicates that powder will be of poor and cohesive flow. The possible reason is due to different precipitation behavior of drug in presence of mannitol and poloxamer 188 during the course of solvent removal and resulting in the deformation of microparticles.

The SEM images shown in Fig. 4 suggested that the powders formed after spray drying were amorphous, partial crystalline in nature; as this was expected, as powders generated through spray-drying are known to be predominantly amorphous in nature. This observation was further confirmed by differential scanning calorimetry and X-ray diffraction study.

3.3 DSC analysis and XRD measurements

DSC analysis of the SX, FP, Physical mixture and spray dried formulations were performed in order to characterize the physical state of the drug and excipients before and after spray drying are shown in Fig. 5 & 6 which indicates shows DSC scan for SX; an endothermic peak for SX was observed at 127.49°C. Fig. 5 (b) shows DSC thermogram of FP indicating endothermic peak 308.55°C which is quite more than its melting range, as it melts
with decomposition. Thermogram of Physical mixture M shows thermogram of physical mixture M that indicates poloxamer 188 at 62.62°C and no sharp Fig. 6 (a-c) shows thermogram of M series formulations, presenting indicative but no sharp peaks of SX and Mannitol. As it contains little amount of Poloxamer 188, peak could not be detected. All are indicating broad peak of Poloxamer 188 but melting endotherm of SX was found to be shifted towards right side. Also, absence of sharpness of peak indicates the amorphous nature of drug after spray drying.

From the observations of all thermograms, DSC measurements revealed a small melting peak of SX in the precipitate, whereas a FP melting peak could not be detected because it melts under decomposition and therefore, creates no interpretable signal. Also, the lack of endotherm can be concluded that drugs were dispersed inside the matrix of excipients as a solid solution. Since no single DSC curve showed sharp endotherms indicative of melting of crystalline material, the SX, FP and excipients co-precipitate exist as glass solution. Furthermore, flattened, a broad curve indicates amorphous nature of drug after spray drying. Hence, DSC data lead to assumption that coprecipitate is formed (Westmeier and Steckel, 2008)

The crystalline nature of pure drug and all spray dried formulation batches were examined by studying its X-Ray diffraction patterns. Fig. 7(a) shows high intensity peaks of SX at 17.3°, 22.2° and 24.6° which confirm that drug is crystalline in nature. As shown in Fig. 7 (b), XRD pattern of fluticasone indicates the high intensity peaks at 10° and 13.1° which confirm that drug is crystalline in nature, but it seems to be little amorphous or less crystalline than salmeterol in figure 7(a).

As it clear that degree of crystallinity of pure drugs; SX and FP is further reduced than individual drugs and it is shifted towards amorphous nature. So, the M1 formulation tends towards amorphous but still partial crystalline. It indicates the endothermic peak at 172.62°C which suggest that interaction between SX and Mannitol difference in excipient behavior as M series contains mannitol as an excipient.

XRD pattern of M2 formulation shown in figure suggests further increase in amorphous nature of pure drugs, as peak intensity in M2 is less than that of M1. This indicates the effect of stabilizer in the formulation, as batch M1 is with only Mannitol as an excipient while M2 contains Mannitol along with poloxamer 188.

The M3 formulation was found to be partial crystalline with peak intensity of M3 is less than M1 and more than M2. From this observation it gives an idea regarding the optimum concentration of stabilizer in the formulation, as concentration of Poloxamer is less in M3 than in M2 batch which may help to get stable formulation.

All spray dried formulations showed less intensive peak confirming that drugs are converted in amorphous nature. Results showed that as the drug to excipient ratio increases upto certain level, crystallinity decreases. Also, it reveals that presence of mannitol gives more amorphous formulation. Above discussed XRD pattern is due to proper dispersion of drug particle into the excipient matrix. This is in good agreement with previous DSC results. It has been known that transforming the crystalline state to the amorphous state leads to a high energy state and high disorder, resulting in enhancing solubility and dissolution rate.

### 3.4 Powder density

Powder flow is important in dry powder aerosol formulation for both the filling of gelatin capsules or devices and for subsequent release of drug from the dry powder inhaler. Tapped density of a formulation is associated with good aerosolization; as more porous particles hold better aerodynamic property over solid particles of the same dimensions. Table 5 shows the values for Carr’s Index which is used as an indication of powder flow properties; a value less than 25% indicates a fluid flowing powder, whereas
a value greater than 25% indicates cohesive powder characteristics.

From the observations given in Table 5, tapped densities for M1-M2 among M series were consistent. The difference in powder flow characteristics was because of excipient nature in the formulation.

3.5 In vitro powder aerosolization

The aerodynamic behavior of the microparticle (DPI) was estimated with Anderson cascade impactor (ACI) making it possible to study the in vitro deposition profile of the representative spray dried formulations. The emitted dose (ED), fine particle dose (FPD), fine particle fraction (FPF) or respirable fraction (RF) of the spray dried powders are displayed in Table 6.

The amount of drug deposited in the inhaler device and throat regions were 3.29% for L1 spray dried systems. Co-precipitates of fluticasone propionate and salmeterol xinafoate showed a FPF of 22% when formulated with lubricant and 36% with lactose carrier. For the commercial product Seretide®, which is a tertiary mixture of APIs with lactose; FPF of about 20% was found. It is due to low flowability and high adhesiveness of the powder.

Surface roughness of M series formulation particles is likely to have contributed to the cohesiveness and high dispersibility with poloxamer 188. Another contributing factor may be the crystallinity of the mannitol and possibly of the ICSs (as confirmed by XRD). M series formulation provides an innovative approach for combination formulations at appropriate doses without the need of physical blending. The powders with mannitol as an excipient showed high aerosol performance and uniform deposition of the two drugs.

3.6 In vitro drug release

In vitro dissolution study was carried out using USP rotating paddle dissolution apparatus (Lab India 2000). The dissolution medium used was Phosphate buffer (pH 7.4); Ethanol (95%) 18, 19. In formulation M series drug release in the first 45 min was in the range of 92.91% to 95.47 % (Figure 9). An initial burst effect was observed due to the drug located on or near the surface of the microspheres. The pores formed during rapid evaporation of the solvent may also lead to the rapid release of the drug.

The rate of drug release from the formulation depended on the drug to excipient binding while processing, as adhesive force between drug-excipient becomes more than cohesive force between drug molecules themselves. This can be explained by a decreased amount of drug present close to the surface and also by the fact that the amount of uncoated drug decreases with higher excipient concentration. Furthermore, smaller microspheres have a larger surface area exposed to dissolution medium, giving rise to faster drug release. The initial rapid drug release can be attributed to the formation of solid dispersion of the drug where the drug would have higher solubility and hence dissolution rates.

3.7 STABILITY STUDY

The results from drug content study provide an important information regarding stability of spray dried formulation containing SX and FP particles. The result of accelerated stability studies as shown in Table 7 indicated that the selected formulations did not show any physical changes during the study period and the drug content was found to have close agreement with the drug content of formulation before stability study. This indicates that all formulations were quite stable at accelerated storage conditions.

CONCLUSION

Based upon the evaluation of different physical characteristics of DPI, it demonstrates that mannitol has given a unique combination particle with much better respirable fraction in comparison with marketed formulation. Ethanol was found to have good behavior for both API's among different solvents; which gave particle size in respirable range. Also, the crystalline nature of excipients contributed to optimize the flow property and efficiency of spray drying process concluded to change in morphological characters of formulations from crystalline
to amorphous side. Dissolution study proved that DPI containing mannitol and Poloxamer 188 were found to have immediate release in dissolution media. Also, all the formulations were quite stable at accelerated storage conditions. Thus, it concludes co-spray drying of salmeterol xinafoate and fluticasone along with mannitol-poloxamer 188 proved to be a simple alternative to traditional combination DPI with lactose for effective implementation of combination formulations for inhalation.

ACKNOWLEDGEMENT
Authors are thankful to Prof. D. D. Chougale, Principal, A. B. College of Pharmacy, Sangli for providing necessary facilities. The authors are also thankful to Vamsi Labs Ltd., Solapur and Sun Pharmaceutical Industries Ltd., Mumbai for providing the gift samples of pure drug of Salmeterol xinafoate and Fluticasone propionate respectively.

Table 1: Formulation components for Batch M series

<table>
<thead>
<tr>
<th>Formulation code</th>
<th>SX:FP</th>
<th>Mannitol</th>
<th>Poloxamer 188 (% w/v)</th>
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<tbody>
<tr>
<td>M1</td>
<td>1:2</td>
<td>0.5 gm</td>
<td>-</td>
</tr>
<tr>
<td>M2</td>
<td>1:2</td>
<td>1 gm</td>
<td>0.1</td>
</tr>
<tr>
<td>M3</td>
<td>1:2</td>
<td>1 gm</td>
<td>0.05</td>
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Table 2: Spray drying parameters

<table>
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<tr>
<th>Parameter</th>
<th>Optimized conditions</th>
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<tbody>
<tr>
<td>Inlet Temperature</td>
<td>120°C</td>
</tr>
<tr>
<td>Outlet Temperature</td>
<td>60°C</td>
</tr>
<tr>
<td>Aspirator Speed</td>
<td>80 %</td>
</tr>
<tr>
<td>Feed Pump Speed</td>
<td>10 ml</td>
</tr>
<tr>
<td>Atomization pressure</td>
<td>20-30 psi</td>
</tr>
<tr>
<td>Vacuum (mmWc)</td>
<td>-80 mmWc</td>
</tr>
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</table>

Table 3: Particle size of DPI

<table>
<thead>
<tr>
<th>Batch code</th>
<th>Mean Diameter ± S.D. (µm)</th>
<th>PDI ± S.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td>M1</td>
<td>2.227 ± 0.3538</td>
<td>0.672 ± 0.0372</td>
</tr>
<tr>
<td>M2</td>
<td>1.534 ± 0.2964</td>
<td>0.549 ± 0.0249</td>
</tr>
<tr>
<td>M3</td>
<td>1.660 ± 0.3134</td>
<td>0.648 ± 0.0324</td>
</tr>
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Table 4: Percentage yield (%) and drug content of spray powder powders

<table>
<thead>
<tr>
<th>Batch Code</th>
<th>% Yield</th>
<th>Theoretical drug Content % (w/w)</th>
<th>Actual drug content % (w/w)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>SX</td>
<td>FP</td>
<td>SX ± SD</td>
</tr>
<tr>
<td>M1</td>
<td>44.42</td>
<td>2.31</td>
<td>4.63</td>
</tr>
<tr>
<td>M2</td>
<td>40.97</td>
<td>2.11</td>
<td>4.22</td>
</tr>
<tr>
<td>M3</td>
<td>49.46</td>
<td>2.20</td>
<td>4.41</td>
</tr>
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</table>

S.D. - Standard deviation (n=3)
Table 5: Tapped density, Carr's index and Flowability of spray dried powders

<table>
<thead>
<tr>
<th>Batch code</th>
<th>Poured density (g/cm$^3$)</th>
<th>Tapped density (g/cm$^3$)</th>
<th>Carr's Index (%)</th>
<th>Flowability</th>
</tr>
</thead>
<tbody>
<tr>
<td>M1</td>
<td>0.0915</td>
<td>0.1282 ± 0.02</td>
<td>28.01</td>
<td>Poor, cohesive</td>
</tr>
<tr>
<td>M2</td>
<td>0.0886</td>
<td>0.1108 ± 0.00</td>
<td>22.75</td>
<td>Poor, fluid</td>
</tr>
<tr>
<td>M3</td>
<td>0.1320</td>
<td>0.3300 ± 0.01</td>
<td>27.28</td>
<td>Poor, cohesive</td>
</tr>
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Table 6: In vitro powder aerosolization properties of DPI

<table>
<thead>
<tr>
<th>Batch code</th>
<th>ED (%)</th>
<th>FPD (µg)</th>
<th>FPF (%)</th>
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<tr>
<td>M2</td>
<td>78.12</td>
<td>63.48</td>
<td>43.76</td>
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Table 7: drug content (%w/w) after one month short term stability study

<table>
<thead>
<tr>
<th>Batch code</th>
<th>SX</th>
<th>FP</th>
<th>SX</th>
<th>FP</th>
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<tbody>
<tr>
<td>Before stability study</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M1</td>
<td>2.1588</td>
<td>4.4648</td>
<td>2.1540</td>
<td>4.4616</td>
</tr>
<tr>
<td>M2</td>
<td>1.9925</td>
<td>4.1148</td>
<td>1.9913</td>
<td>4.1108</td>
</tr>
<tr>
<td>M3</td>
<td>2.0074</td>
<td>4.3332</td>
<td>2.0061</td>
<td>4.3313</td>
</tr>
<tr>
<td>After stability study</td>
<td></td>
<td></td>
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<td></td>
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</table>

Fig. 1: FTIR spectra of SX, FP, Mannitol, Poloxamer 188 and physical mixture
Fig. 2: FTIR spectra of SX, FP, physical mixture and M series formulation

Fig. 3: SEM micrograph of: (a) Salmeterol xinafoate (b) Fluticasone propionate

Fig. 4: SEM micrograph of M1 and M2 formulations.
Fig. 4: SEM micrographs of M series formulations

Fig. 5: DSC thermogram of: (a) Salmeterol xinafoate  
(b) Fluticasone propionate  
(c) DSC thermogram of physical mixture of M series
Fig. 6: DSC thermogram of: (a) M1 formulation (b) M2 formulation (c) M3 formulation

Fig. 7: XRD pattern of: (a) salmeterol xinafoate (b) Fluticasone propionate
Fig. 8: XRD pattern of M series formulations

Fig. 9: Release profile of M series formulation

REFERENCES


