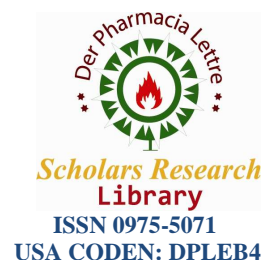




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Development and Characterization of Ethionamide loaded microparticles as Dry Powder Inhalers for Multi-drug Resistant Tuberculosis

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ABSTRACT

The aim of the study was to prepare dry powder formulation of Ethionamide loaded polymeric microparticles for pulmonary delivery in effective treatment of multi drug resistant tuberculosis (MDR-TB). The microparticles were prepared by spray drying method using chitosan and hydroxy propyl methyl cellulose (HPMC) as biocompatible polymers. The microparticles and microparticle blend with lactose (Inhalac 230) were investigated for its aerosolization properties like emitted dose, mass median aerodynamic diameter, fine particle fraction, geometric standard deviation. The spray drying method produced rough surfaced microparticles under the size range of 8.5 μ m. Mass median aerodynamic diameter obtained for all formulation ranged in 2.28 μ m to 3.33 μ m and fine particle fraction in between 54.58 \pm 5.16 to 75.74 \pm 3.48. The lowest tapped density value obtained was 0.096 g/cm² belong to formulation coded EM1. In vitro deposition studies using cascade impactor showed emitted dose of > 97% for all batches. The polymeric microparticles produced by spray drying technique showed promising particle characteristics suitable for inhalation with Fine particle fraction (75.74 \pm 3.48) of total emitted dose, after blending with lactose. The blending of the microparticles with Inhalac 230 allowed the fine particle fraction values to increase by increasing the dispersibility of powder on inspiration.

Key words: Ethionamide, Dry Powder Inhalation, Chitosan, HPMC, Interactive blend.

INTRODUCTION

Direct drug administration to the lungs via inhalation offers several theoretical advantages over systemic delivery, including the possibility of regional drug delivery to the lungs and airways with lower doses and fewer systemic side effects, avoiding first-pass metabolism of the drug in the liver and the use of a non-invasive “needle-free” delivery system. The alveolar surface also provides a large surface area for rapid systemic absorption of drugs [1-4].

Dry powder inhalers (DPIs) are more sophisticated form of dosage form for respiratory drug delivery and play an important role in delivering medicinal aerosols. Their most common application is the transport of drugs to the bronchioles. In the case of respiratory maladies such as asthma, COPD, Tuberculosis the benefits of delivering drugs to the lungs rather than by any other means are obvious; since the drug is targeted directly to the site of illness it can begin acting much quicker (clearly an important advantage during an asthma attack) and it avoids flooding the rest of the body with the drug, which is wasteful and potentially harmful [5].

The marketed DPI's are fabricated based on the concept of the energy produced by the patient during the inspiration process to generate an effective drug delivery to the lung. Many number of DPI's devices are available in market.

Dry powder inhalers deliver drugs to the lungs. Aerosols with a mass median aerodynamic diameter (MMAD) of 5–10 μ m are mainly deposited in the oropharynx and large conducting airways. Particles of 1–5 μ m diameter are

deposited in the small airways and alveoli with >50% of 3 μ m diameter particles being deposited in the alveolar region, therefore aerosol particle size are one of the most important determinants of aerosol dose and distribution in the lungs. However, the lung deposition for clinical efficiency is also determined by the inspiration time, inhalation flow rate, initial inspiration rate and inhaled volume. The performance of DPI depends on the device and inhalation flow rates through the device. [4]

Micronization or jet-milling is commonly used to produce DPI's as these methods provides smaller sized particles. While, the micronized product has high cohesive forces between particles because of the flat surfaced particles obtained in this process. [6]

Due to the thermodynamic change; reduction in glass transition temperature and change in crystalline state is seen in case of jet milled product [7]. The spray drying technique is an effective method to produce micron sized, spherical and amorphous particles for inhalation. The amorphous particles characterized by low area of contact, smaller and more homogenous particle distribution can result in higher particle fraction deposited into lung [8, 9]. The spray drying technique is advantageous in many ways to prepare microparticles for pharmaceutical application as it is reproducible, one step, rapid and easy to scale up. Spray drying technique can be used to produce dry powders, granules or agglomerates from drug-excipient solutions and suspensions. The particle size of the microparticles prepared by spray drying technique ranged from microns to several tens of microns and had a relatively narrow size distribution. Spray drying is successfully used by pharmaceutical industry to produce products of defined physical and chemical properties [10-12].

Pharmaceutical invention and research are increasingly focusing on delivery systems which enhance desirable therapeutic objectives while minimizing side effects.

Multi-drug resistant tuberculosis (MDR-TB) is caused due to the resistance of *Mycobacterium tuberculosis* (M.TB) strains to at least two first line drugs, isoniazid and rifampicin. Treatment of MDR-TB involves combination of second line drugs thioamides (ethionamide and prothionamide), aminoglycosides (streptomycin, kanamycin and amikacin), fluoroquinolones (ciprofloxacin, ofloxacin & levofloxacin) and pyrazinamide for the prolonged period of around 21 months. [13]

Mycobacterium tuberculosis harbor mainly in lung macrophages, which makes the pulmonary route, a viable option for therapy of MDR-TB. Inhaled therapy of MDR-TB drugs can reduce the amount of drug lost due to degradation before reaching the lungs and also reduce the systemic toxicity effects associated with drugs. [13]

Ethionamide has been chosen to prepare polymeric microparticles for effective delivery of drug into lung provided with sustained release properties. Ethionamide is second line anti tubercular drug given orally in combination with first line drugs to treat TB. The delivery of Ethionamide to lung in controlled manner would facilitate the reduction of dose, dose frequency and toxicity; most importantly targeting mycobacterium residing in macrophages. [14]

Chitosan, a natural linear biopolyaminosaccharide, is obtained by alkaline deacetylation of chitin, which is the second abundant polysaccharide next to cellulose. Chitosan has been shown to possess mucoadhesive properties. Chitosan microspheres are the most widely studied drug delivery systems for the controlled release of drugs, antibiotics, antihypertensive agents, anticancer agents, proteins, peptide drugs and vaccines. [15, 16]

Synthetic biodegradable polymers have gained more popularity than natural biodegradable polymers. The major advantages of synthetic polymers include high purity of the product, more predictable lot-to-lot uniformity, and free of concerns of immunogenicity. Hydroxy propyl methyl cellulose is most commonly used as drug carrier due to their excellent biocompatibility and biodegradability and mechanical strength. [17]

In the current study microparticles loaded with ethionamide was prepared with different polymers by spray drying method with the aim to generate low density and aerodynamically suitable particles for inhalation. The lactose (Inhalac 230), as a carrier material, was employed in the formulation to evaluate its influence on fine particle fraction for better aerodynamic performance.

MATERIALS AND METHODS

2.1 Materials

Ethionamide was obtained as gift sample from Micro labs ltd; chitosan (100cp) and chitosan (10cp) were generously provided by Indian institute of fisheries, Cochin and C E Roeper GmbH, Hamburg, and Germany respectively. Inhalac 230 was obtained from Meggle, Wasserburg GmbH and Co., Germany as a gift sample. HPMC K100M and

HPMC E 50 were obtained as gratis sample from colorcon, India. Ascorbic acid was purchased from S D fine chemicals, Baroda, India. All Other chemicals and solvents used were of analytical grade.

2.2 Preparation of microparticles

Ethionamide loaded microparticles were prepared using spray drying method. Ethionamide solution in 10 ml ethanol was emulsified with aqueous phase containing 0.5 % polymer with continuous stirring on a magnetic stirrer. Aqueous phase of HPMC was prepared by dissolving HPMC in boiling water and immediately cooled to get clear solution. Whereas; aqueous solution of chitosan was prepared by dissolving chitosan in 1 % v/v acetic acid at pH 5. Ascorbic acid in concentration of 200µg/ml was added in feed liquid as antioxidant. The mixtures were spray-dried from a 0.5mm nozzle at a feed rate of 6ml/min under 2.5Kg/cm² pressure. The inlet and outlet temperature were maintained at 150°C and 95°C, respectively. The spray dried product was collected by a cyclone separator.

2.3 Morphology of microparticles

The shape and surface morphology of obtained microparticles were investigated by both optical and scanning electron microscopy. The microparticles were mounted on to the metal stud using double sided adhesive tape. The microparticles were examined by SEM operated at 15 KV acceleration of voltage. The optical microscopy was used for the determination of the shape of Ethionamide microspheres. A small drop of microspheres suspension was placed on a clean glass slide and mounted on the stage of the microscope and observed.

2.4 Particle size Determination of microparticles

Prepared microparticles were analysed for particle size by laser diffraction. To obtain an obscuration of 5% around 75 mg of powder was used. Results are the means of triplicate experiments.

2.5 Powder blend analyses

The coarse carrier, lactose (Inhalac 230) was geometrically blended with Ethionamide microparticles to provide the final ratio of (lactose: microparticles) of 5:1 ratio. All the formulation blends were then stored in tightly sealed container. The mixture of microparticles prepared and coarse carrier were coded as EML1, EML2, EML3 and EML4 for microparticles EM1, EM2, EM3 and EM4 respectively.

2.6 Drug content and determination of homogeneity of dry powder formulations

The Ethionamide content in microparticles was determined by UV spectroscopy method. 50mg of EM was dissolved in 0.1N HCl containing 200µg/ml ascorbic acid. After suitable dilutions drug content was determined by UV spectroscopy at wavelength of 274nm.

The homogeneity of Ethionamide microparticle-Inhalac 230 blends(was determined by analysing the drug content of each dry powder formulation blend (EML1-EML4). Only 3 sample blends were taken randomly for analysis. The mixture was dissolved in 0.1 N HCl containing 200µg/ml of ascorbic acid. The drug content was determined by UV spectrophotometry at wavelength of 274 nm. The Blend of Microparticles with Inhalac 230 was prepared in ratio of 1:5 respectively, where each capsule contains 3 mg of Ethionamide.

2.7 Determination of powder densities and primary aerodynamic diameter

The density of Ethionamide microparticle (EM) and Ethionamide : Inhalac 230(EML) blend was measured by tapped density measurement. A known mass of EM and EML were filled into 10ml measuring cylinder and recorded the volume occupied by the powder. The tapped densities of all the formulation were determined by tapping the measuring cylinder from a constant height and volume of tapped mass was noted until no further change in the powder volume was observed. Measurement was performed in triplicate (n= 3). Using tapped density (p) values theoretical aerodynamic diameter values were determined. [18]

$$d_{ae} = d\sqrt{\rho/\rho_1}$$

The primary aerodynamic diameter d_{ae} was determined from particle size (d) and tapped density data (p).

2.8 Aerosol performance and aerodynamic diameter

By using Andersen cascade Impactor (CI) actual aerodynamic diameter and the aerosol performance of the formulations were tested. The CI consists of port, preseparator, seven stages and a final filter. The preseparator was attached to impactor to prevent large particle aggregation. After assembling the CI stages, the assembly was then attached to a vacuum pump, equipped with flow meter. The air flow was than adjusted for 60L/min. Capsules (HPMC size, 2) were filled with powder containing 5mg of Ethionamide. One capsule was placed into the sample compartment of the aerosolizer device attached to induction port. The capsule was pierced and vacuum was operated

for 10 sec with steady air flow rate of 60 L/min. In all cases, 10 capsules were subjected for discharge into apparatus per determination and each experiment was repeated in triplicate (n=3). The powder deposited on plate of each stage depending on the particle aerodynamic diameter. The powder was collected from each plate and analyzed for drug deposition in each stage. The collected powder was dissolved in 0.1 N HCl and drug content was determined by UV spectroscopy at wavelength of 274nm. The effective cut off diameter obtained for stages 0-6 are 6.5, 4.4, 3.2, 1.9, 1.2, 0.55 and 0.26 μm [19]

The fine particle fraction of the total dose of powder less than 5 μm was calculated by dividing the powder mass recovered from stages of apparatus by the total mass emitted. The cumulative mass of powder less than the stated size of each stage was calculated and plotted on a log probability scale as a % total mass recovered from the apparatus against the effective cut of diameter. Mass median aerodynamic diameter (MMAD) was derived from the graph of cumulative distribution as the particle size at which the line crosses the 50% mark.

RESULTS AND DISCUSSION

Ethionamide microparticles were prepared using biocompatible polymers as carriers to directly target the alveolar macrophages as a novel approach for anti tubercular therapy to overcome the frequent daily dosing non compliance of patient. Considering the advantages of DPI over the other delivery system Ethionamide microparticles were prepared as dry powder formulation.

The microparticle was prepared by spray drying method using chitosan, a natural polysaccharide, and Hydroxypropyl methyl cellulose as biocompatible matrix forming polymers. Spray drying was chosen as it is a one step manufacturing process which uses least amount of organic solvent and gives desired particle size.

The drug deposition in the lung is mainly determined by the powder dispersibility. The powder dispersibility of an good DPI is governed by cohesive forces such as Van Der waals, electrostatic, capillary or mechanical interlocking exist because of fine particle size. This increased force will result in poor flowability causing poor deposition in the lung. To avoid these forces of microparticle; lactose is blended with the microparticles as coarse carrier. The carrier selected should be inert and must not cause lung irritation (cough or hoarseness) to improve the lung deposition. The dose reproducibility, fine particle fraction and emitted dose further determine the efficient lung deposition. [8, 20]

The volume median diameters of the prepared microparticles were determined by laser diffraction and found to be in the range of 3.5 μm to 7.2 μm . As observed by optical microscopy and scanning microscopy the larger volume mean diameter was due to particle aggregation and the rough shape obtained, **Figure 1**.

By SEM it is evident that the microparticles obtained with similar structure and surface properties. It also showed that microparticles were porous, roughly sphere, coarser surfaced and thin walled structure, Figure 1. Microparticle prepared using chitosan formed more aggregates compared to microparticle prepared using HPMC.

The drug loading efficiency were determined and found to be in the range of 70% – 97%. Microparticle with chitosan as polymer resulted in high loading efficiency while that of HPMC E 50 which may be due to low matrix forming capacity of the polymer.

The flow property, porosity and particle size distribution of the inhalable microparticle can be derived from tapped density data. Tapped density is an important physical property of the DPI's and also an indicator of inter particulate cohesive and adhesive forces. The tapped density of microparticle alone was found to be below 0.136 g/ml, whereas the microparticle and Inhalac 230 (EML) blend was found to be at much higher range of 0.706g/ml to 0.545g/ml. The lowest tapped density was obtained with formulation EM1 containing chitosan (viscosity 10cp) which is due to porous nature and aggregates of microparticles. [21-23]

The flow characteristics can be improved by employing coarse carriers. This will overcome the particle aggregation and dispersion. The drug particles are dispersed from the surface of the coarse carrier particles by the energy of the inspired air flow during inhalation. The smaller microparticles penetrate deep in to the lung where as the larger carrier particles impact in the upper airway. A 5:1% w/w geometrical blend of Inhalac230: ethionamide microparticle was prepared and content uniformity of each blend was determined as shown in **Table 1**.

The tapped density values were increased due to the physical mixture of lactose and microparticle

The physical mixture of lactose and microparticles caused the density values to increase, that is because of the higher tapped density (0.95 g/ml) of Inhalac 230. The Carr's index and Hausner ratio are the indirect measure of

powder flowability. Carr's index values were found to be higher because of the cohesive – adhesive forces formed between the particles. The Carr's index values were in the range of 27 to 35 % indicating of poor flow. Blending of microparticle with lactose did not significantly affect the flowability as seen by the Carr's Index values as shown in **Table 2**.

The theoretical primary aerodynamic diameter (*dae*) of each formulation was calculated from geometrical particle diameter and tapped density that ranged between 1.78 μm to 2.90 μm , indicating particles having favourable size for lung deposition in alveolar region.

All powder formulation was subjected to in vitro lung deposition studies using Andersen Cascade Impactor. All the formulations showed higher values of dose emission during aerosolization. All the formulations batches showed emission of >97 % of total capsule content as shown in table 3. It was observed that there was higher collection of powder at preseparator in case of formulations coded EM3, EM4 and EML3. This may be attributed to the larger particles, shape and/or particle aggregations. It was also found that mixture of microparticles with lactose resulted in increase in emission as well as the % FPF values. The MMAD values found by the measurement performed in ACI were seen to be higher than the *dae* values calculated theoretically, as shown in table 2 and 3. The largest value of *dae* belongs to microparticles when processed with HPMC K100 M as carrier polymer. Blending of microparticles with Inhalac 230 resulted in improved dispersibility and reduction in MMAD values. Among the blends, lowest MMAD value ($2.28 \pm 0.31 \mu\text{m}$) was seen with the formulation coded EML2.

The fine particle dose was defined as the amount of the drug recovered from the lower stages of ACI. Majority of the particles are found to be deposited on stages between stages 2-4 representing the aerodynamic diameter in the range of 1.2 – 3.2 μm . However, the microparticles prepared with HPMC as carrier polymer was found to deposit more on preseparator and stage 0, representing the aerodynamic diameter greater than 5 μm . The Fine Particle Fraction of the spray dried microparticles ranged 54.58 ± 5.16 to 75.74 ± 3.48 of the total loaded dose; details are shown in table 3. The most significant increase in FPF was found with the formulation coded EML2; lactose added to this formulation as carrier caused the FPF value rise up to 75 % from 54 %. This increase indicated a good aerodynamic characteristic of the microparticles when blended with carrier. In general the GSD values for the aerosol particles are reported to be in the range of 1.30 to 3. [24,25] In this study, the calculated GSD values for microparticle formulation were found in the range of 1.87 to 2.20 as shown in **Table 3**.

Table 1: Formulation composition, particle size and drug content of microparticle and microparticle blends

Formulation	Polymer (0.5 % w/v)	Drug: polymer ratio	Mean particle size (μm)	Theoretical mass median aerodynamic diameter (<i>dae</i>)	Drug content (% \pm SD)	Content uniformity of microparticle blend	
						Blend Formulation	Drug content (mean \pm S.D)%
EM1	Chitosan	1:2	3.54 \pm 0.25	1.24 μm	92.89 \pm 2.67	EML1	94.76 \pm 1.79
EM2	Chitosan	1:2	4.87 \pm 0.19	1.84 μm	94.98 \pm 3.04	EML2	97.01 \pm 1.46
EM3	HPMC K100M	1:2	5.95 \pm 0.29	2.02 μm	74.95 \pm 2.83	EML3	92.45 \pm 2.65
EM4	HPMC E50	1:2	7.05 \pm 0.34	2.48 μm	70.12 \pm 2.12	EML4	96.58 \pm 2.15

Table 2: Powder characteristics of microparticle and microparticle blend

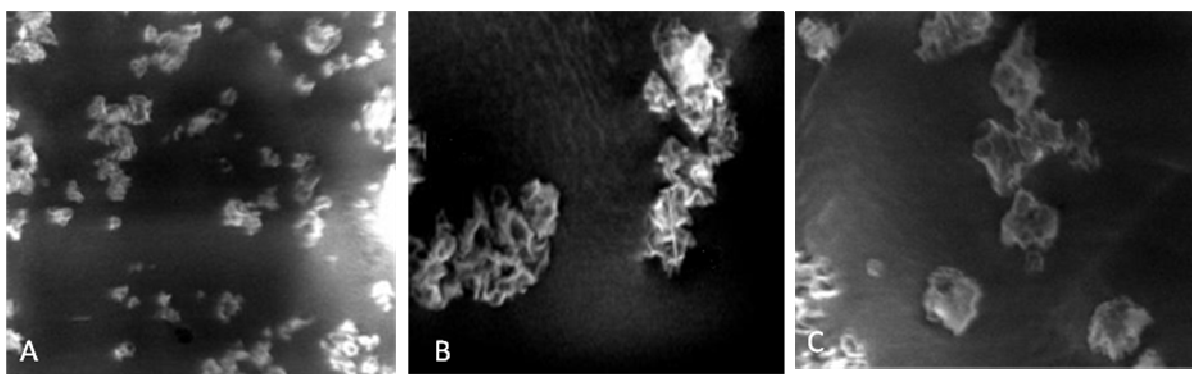
Formulation	Bulk density (g/ml)	Tapped Density(g/ml)	% Carr's Index	Hausner's ratio
EM1	0.07	0.096	27.08	1.37
EM2	0.089	0.136	34.55	1.53
EM3	0.068	0.097	29.89	1.43
EM4	0.079	0.117	32.47	1.48
EML1	0.468	0.706	33.71	1.51
EML2	0.414	0.637	35.00	1.54
EML3	0.389	0.545	28.62	1.40
EML4	0.44	0.643	31.57	1.46

Table 3: Aerosol performance of the microparticle formulations alone and in blend

Formulation	%Emitted dose	MMAD	GSD	FPF
EM1	97.04 \pm 3.65	2.40 \pm 0.39	1.98	57.96 \pm 2.41
EM2	98.19 \pm 2.07	2.58 \pm 0.27	1.87	60.52 \pm 2.67
EM3	97.89 \pm 5.19	3.05 \pm 0.48	1.72	54.58 \pm 5.16
EM4	98.41 \pm 4.22	3.33 \pm 0.35	1.95	60.01 \pm 3.98
EML1	99.92 \pm 2.10	2.32 \pm 0.34	1.88	65.42 \pm 3.02
EML2	99.68 \pm 1.55	2.28 \pm 0.31	1.90	75.74 \pm 3.48
EML3	98.76 \pm 2.06	3.01 \pm 0.39	2.00	56.98 \pm 3.56
EML4	98.99 \pm 2.33	3.21 \pm 0.43	2.20	62.52 \pm 3.75

(MMAD) Mass median aerodynamic diameter, (GSD) % Geometric Standard Deviation, (FPF) Fine Particle Fraction

Figure 1: Scanning electron microscopic images of Ethionamide loaded microparticles. a) EM1 b) EM2 c)EM3



CONCLUSION

The results reveal that ethionamide DPI microparticles can be prepared by using biocompatible polymers by Spray drying method. The spray drying technique can be useful to produce powders with a good narrow particle size distribution and aerodynamic properties. The MMAD values of each microparticle and microparticle blend indicated that the formulation of microparticles were of a favourable size for deposition in the alveolar region of the lung. The FPF values showed that addition of carrier influenced the dispersibility. This drug delivery holds therapeutic advantage to deliver drug for local as well as for systemic bioavailability for longer period with low dose. This can further lead to improvement in tuberculosis treatment by improving the patient compliance. A further in vivo pharmacokinetic study has to be performed for establishment of *in vitro in vivo* correlation.

Declaration of Interest

The authors report no conflicts of interest.

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