AMERICAN JOURNAL OF PHARMTECH RESEARCH

Journal home page: http://www.ajptr.com/

Preparation of Microparticles Containing Rifampicin as Dry Powder Formulation: *In Vitro* Studies on Aerosol Performance

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ABSTRACT

The Aim of this study was preparation of dry powder formulation of rifampicin loaded polymeric microparticles as dry powder formulation for inhalation in effective tuberculosis treatment.The microparticles containing rifampicin (RIF) were prepared by spray drying method using different biocompatible polymers like chitosan and hydroxyl propyl methyl cellulose (HPMC) The microparticles and microparticle blend with coarse carrier 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 wrinkle surfaced porous microparticles under the size range of 10µm. Mass median aerodynamic diameter obtained for all formulation ranged in 2.68 µm to 3.73 µm and Fine particle fraction in between 51.58 ± 5.36 to 72.74 ± 3.18. The lowest tapped density value obtained was 0.102 g/cm^2 belong to formulation coded M1. In vitro deposition studies using cascade impactor showed emitted dose of > 90% for all batches. The polymeric microparticles produced by spray drying technique showed promising particle characteristics suitable for inhalation with Fine particle fraction (72.74 ± 3.18) 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: Rifampicin, Dry Powder Inhalation, Chitosan, HPMC, Interactive blend.

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Received 25 May 2012, Accepted 14 June 2012

Please cite this article in press as: Kundawala AJ *et al.*, Preparation of Microparticles Containing Rifampicin as Dry Powder Formulation: In Vitro Studies on Aerosol Performance. American Journal of PharmTech Research 2012.

INTRODUCTION

Inhalation therapy is employed to deliver drugs to the respiratory epithelium, predominately for the local delivery of drugs in the treatment of Asthma, COPD, lung infections and in Cystic fibrosis. Dry powder inhalers (DPIs) for respiratory drug delivery is more sophisticated and have an important role in delivering medicinal aerosols. The majority of the dry powder inhalers available in market are utilizing the energy of the patient's inspiration to generate an effective drug delivery to the lung. A large number of DPI device and technologies have been patented and published over the last few decades. The modern dry powder inhaler was first introduced in 1960's with the fisons Spinhaler and Allen and Handburys Rotahaler [1]. The particles were dispersed through the first piercing the gelatin capsule and then by turbulence generated by spinning blades or plastic grid at the time of inhalation. The success of Advir Diskus has directed DPIs to new research and investigation in asthma therapy [2].

DPI performance varies widely by device and inhalation flow rates through the device. The clinical efficiency of lung deposition is mainly influenced by the inhalation flow rate, inspiration time, inhaled volume and initial rate of inspiration. The drug deposition in lung from the emitted dose is dependent on powder dispersibility which controlled by cohesive forces [3]. The dry powder formulations are prepared generally by micronization or jet-milling to satisfy the particle size need of small particles. The micronization also produces flat surface that is also one of the cause of increase in cohesive forces between particles [4].

DPI formulations generally incorporate at least one or more component, as a carrier to facilitate effective aerosolization of active pharmaceutical agents. The carrier particles are incorporated in formulation to improve drug particle flowability, dosing accuracy and minimize variability during manufacturing processes [5]. Large coarse particles as carrier (lactose, mannitol, trehalose, amino acids, etc,) are most often incorporated with microparticles to make the powder less cohesive and free flowing. Apart from easy handling and accurate dosing during manufacturing process, these blends also helps in to improves the release of API from delivery system [6].

The thermodynamic instability produced by jet milling causes the crystalline state to change and decreases the glass transition temperature [7]. The spray drying technology is an alternative 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].

In this study, we choose Rifampicin (RIF) as model drug to prepare polymeric microparticles for effective delivery of drug into lung provided with sustained release properties. Rifampicin is first line antitubercular drug given orally in combination with isoniazid to treat TB. The delivery of rifampicin to lung in controlled manner would facilitate the reduction of dose, dose frequency and toxicity; most importantly targeting mycobacterium residing in macrophages. The oral dose of rifampicin has difficulty in reaching deep lung where the mycobacterium residing. Since the rifampicin reaches the target tissue through systemic circulation, delivering the drug directly to lung via inhalations at low dose seems advantageous with batter therapeutic efficacy [13].

Chitosan is a natural cationic polysaccharide and has many applications in pharmaceutical and biological fields, which have been extensively studied and investigated. With nontoxicity, biocompatibility, biodegradability and bioadhesion properties chitosan is a promising polymer for drug delivery [14]. Chitosan microspheres were prepared and investigated for delivery of many conventional drugs as well as DNA, proteins and peptides [15, 16, 17]. For example, spray-dried microspheres composed of hydroxypropyl methyl cellulose have been used for sustained drug release[18].

In this research work microparticles loaded using rifampicin 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.

MATERIAL AND METHOD

Material

Rifampicin (RIF) was obtained as gift sample from Strides Acrolab, 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.

Preparation of microparticles

Rifampicin containing microparticles were prepared using spray drying method. In brief, the organic phase was prepared by dissolving Rifampicin in 10 ml of ethanol. The organic phase was emulsified with aqueous phase containing 0.5 % polymer concentration (when polymer is chitosan, it is prepared in acetic acid solution at pH 5) and mixed by magnetic stirrer. The chitosan solution was prepared by dissolving chitosan in 1 % v/v acetic acid while HPMC solution was prepared by dissolving it in boiling water and immediately cooled to get clear solution. Ascorbic acid in concentration of 200µg/ml was added in feed liquid as antioxidant. The prepared suspension was then spray dried using 0.7 mm standard nozzle at 150º C, 5 ml/min feed rate and 2.5 kg/cm^2 of pressure. The dried powder product was then collected from cyclone separator and kept in desiccator until further use.

Morphology of microparticles

The surface morphology and shape of microparticles were investigated by electron microscopy. For the sample preparation the microparticles were mounted on the metal stud using double sided adhesive tape. The microparticles were examined by SEM operated at 15 KV acceleration of voltage. The microparticles were also studied under optical microscopy, manually.

Particle size Determination of microparticles

The particle size of the spray dried powder was measured by laser diffraction (HELOS particle size analyzer VIBRO/RODOS dry dispersion system: Sympatec Gmbh system partikel technik, Clausthal Zelerfeld, Germany). Approximately 100 mg of each powder was used to achieve the required obscuration of 5%, and each sample was measured in triplicate.

Preparation of powder blend

The coarse carrier, lactose (Inhalac 230) was geometrically blended with rifampicin microparticles to provide the final ratio of (lactose: microparticles) of 5:1 ratio. All the formulation blends were then stored in tightly sealed amber colored glass vials. The mixture of microparticles prepared and coarse carrier were coded as MB1, MB2, MB3 and MB4 for microparticles M1,M2,M3 and M4 respectively.

Drug content and determination of homogeneity of dry powder formulations

The rifampicin content in microparticles was determined by dissolving 50 mg of microparticle formulations and dissolving it in 0.1N HCl containing 200µg/ml ascorbic acid. After making

suitable dilutions with same solvent drug content was determined by UV spectroscopy at wavelength of 475nm.

The Blend of Microparticles with Inhalac 230 was prepared in ratio of 1:5 respectively, where each capsule contains 3 mg of RIF. The homogeneity of rifampicin microparticle-Inhalac 230 blends was determined by detecting drug content of each dry powder formulation blend (MB1-MB4). Three powder samples were selected randomly for the purpose. 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 475 nm.

Determination of powder densities and primary aerodynamic diameter

The powder density was evaluated by tapped density measurement. Densities of microparticles alone and in the blend were determined with 10 ml measuring cylinder by filling the known mass of powder under gravity and recording 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).

Theoretical aerodynamic diameter was determined by the following equation using tapped density (p) values [19].

$$d_{ae} = d\sqrt{\frac{\rho}{\rho_1}} \quad \text{Where } \rho_1 = 1 \text{ g cm}^{-3}$$

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

Aerosol performance and aerodynamic diameter

The actual aerodynamic diameter and the aerosol performance of the formulations were tested by eight stage Andersen cascade Impactor (ACI). The ACI is consisting of induction port, preseparator, seven stages and a final filter. The preseparator was attached to impactor to prevent large particle aggregation. After assembling the ACI 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 off rifampicin. 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 6o 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 475nm. 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 [20].

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.

RESULT AND DISCUSSION

Microparticles were prepared under consideration to overcome the patient's non compliance of frequent daily dosing of rifampicin and targeting to deep lung as a novel approach for antitubercular therapy using biocompatible polymers as carrier. Considering the advantages of DPI over the other inhalation system rifampicin microparticles were prepared as dry powder formulation. The microparticles were prepared using chitosan, a natural polysaccharide, and Hydroxypropyl methyl cellulose as biocompatible matrix forming polymers which are generally accepted as safe for oral drug delivery by US FDA. The microparticle were prepared by spray drying process which is one step manufacturing process and uses least amount of organic solvent as well as gives feasibility of getting optimum particle size.

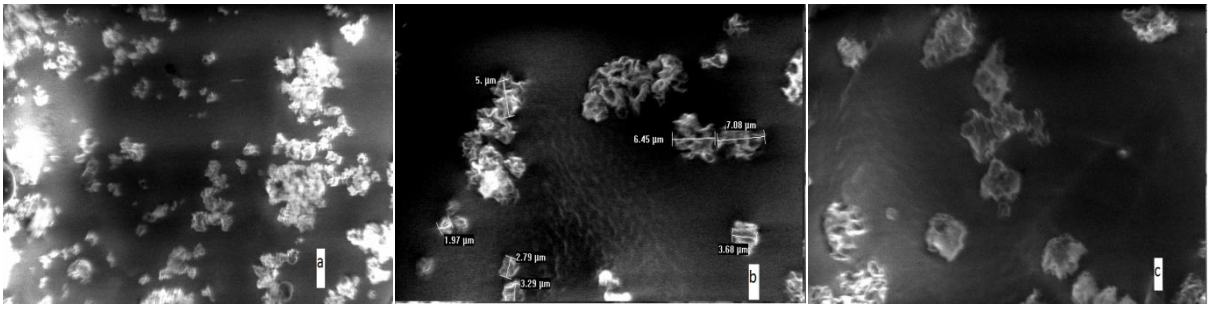
The effective drug deposition in lung via Dry powder inhalers is dependent on powder dispersibility which is controlled by cohesive forces exist because of fine particles size. The cohesive force may exist because of particulate interaction which may be result of number of concurrent forces like Van Der Waals, electrostatic, capillary or mechanical interlocking. Strong interparticulate forces result in poor flow properties as well as poor deposition in lung [3]. To overcome above mentioned problems interactive blends of microparticles were prepared with lactose as coarse carrier. While selecting a carrier for dry powder formulation, it should be kept in mind that a carrier of choice must not produce side effects at lung site like irritation, cough and hoarseness. In DPI development attention is often paid to analyze dose reproducibility, fine particle fraction and emitted dose[8].

The continued threat of tuberculosis and patient non compliance related issues encourage investigation for the improved disease management with better therapy options that could reduce

dosing frequency shortens treatment period and side effects. Rifampicin and other antitubercular drugs have been investigated in polymeric particle form for inhalation as both microparticles [21, 22] and nanoparticles [23, 24]. These studies showed that insufflations of particles containing antibiotics were effective in reducing bacterial counts with low dose.

In this present study, we prepared microparticles loaded with 50 % w/w rifampicin with similar physical and aerosol characteristic using different carrier such as chitosan and HPMC (two grades as mentioned above). The comparative evaluation was carried out among the different encapsulating polymers by keeping the concentration of drug and polymer constant for all formulation.

The volume median diameters of the prepared microparticles were in the range of 4µm to 7.5µm, as determined by laser diffraction. The larger Volume mean diameter may be result of particle shape and aggregation of particles which was observed by optical microscopy and Scanning electron microscopy, figure 1. Scanning electron microscopy studies showed microparticles with similar structure and surface properties. SEM images of different formulation were indicative of thin wall, wrinkled surface, porous and irregular sphere like structure, Figure 1. The particle aggregates observed were more in case of microparticles prepared with chitosan.



a) b) c)

Figure 1: Scanning electron microscopic images of Rifampicin loaded microparticles. a) M1 b) M2 c) M3

The drug loading capacities were found in the range of 65 to 99 %. The HMPC E 50 found to load lesser amount of RIF that may be due to the lower matrix forming capacity, while the chitosan grades were able to incorporate more amounts of RIF in the polymer matrix. The derived properties like tapped density is an important physical property of dry powders. The tapped density provides significant information about the flow properties of the microparticles from the inhaler device, the porosity of the particles and particle size distribution. The tapped density is also the indicator of the interparticulate cohesive and adhesive forces [25]. For better aerosolization lower tapped density is required [26, 27]. In this study the tapped densities of microparticles alone were found below 0.150 g/ml while the tapped densities of blends were

much higher ranged between 0.786 g/ml to 0.617 g/ml. The lowest tapped density was obtained with formulation M1 containing chitosan (viscosity 10cp). The low tapped density was the result of porous nature and aggregation of microparticles.

To overcome the particle aggregation and dispersion, coarse carriers are generally employed, so that flow characteristics can be improved. During inhalation the drug particles are dispersed from the surface of the coarse carrier particles by the energy of the inspired air flow. The larger carrier particles impact in the upper air ways where as the small drug particles should penetrate into lungs. In this study Inhalac 230 was blended geometrically with rifampicin microparticles to provide a final ratio (Inhalac 230: microparticles) of 5:1 w/w. The content uniformity of each blend was determined for drug content uniformity as shown in Table 1. The physical mixture of lactose and microparticles caused the density values to increase, that is because of the higher tapped density (0.98 g/ml) of Inhalac 230. The Carr's index and Hausner ratio of microparticles and microparticle blend are shown in table 2. The Carr's index and Hausner ratio are considered indirect of measure of powder flowability. Carr's index values found were of higher values that indicated the existence of cohesive-adhesive forces between the particles. The Carr's index values were in the range of 30 to 38 % indicating of poor flow. Making of blend could not leave much impact on the Carr's Index values as shown in Table 2.

Table 1: Composition and physical Characterization of microparticle formulations and microparticle blend with coarse carrier lactose (Inhalac 230)

Formula code	Type of polymer (0.5 % w/v)	Drug: polymer ratio	Mean particle size (µm)	Theoretical mass median aerodynamic diameter (d_{ae})	Drug content (%±SD)	Content uniformity of microparticle blend	
						Formulation code	Drug content (%±S.D)
M1	Chitosan (10 Cp)	1:2	4.61 ± 0.27	1.64 µm	93.22 ± 2.60	MB1 (M1+ Inhalac 230)	93.95 ± 1.64
M2	Chitosan (100 Cp)	1:2	5.55 ± 0.19	2.23 µm	98.72 ± 3.06	MB2 (M2+ Inhalac 230)	98.43 ± 1.09
M3	HPMC K100M	1:2	6.49 ± 0.34	2.27 µm	69.89 ± 2.94	MB3 (M3+ Inhalac 230)	91.05 ± 2.65
M4	HPMC E50	1:2	7.50 ± 0.48	2.73 µm	64.65 ± 2.25	MB4 (M4+ Inhalac 230)	94.13 ± 2.39

*MB1-MB4 indicates microparticle blend with coarse carrier (Inhalac 230) for Microparticle batch M1,M2,M3 and M4 respectively.

Table 2: Powder Properties of microparticle formulations alone and in blend

Formulation code	Bulk density (g/ml)	Tapped Density(g/ml)	% Carr's Index	Hausner's ratio
M1	0.070 ±0.002	0.102 ±0.010	30.00	1.43
M2	0.098 ±0.004	0.151 ±0.010	35.10	1.54
M3	0.072 ±0.001	0.105 ±0.002	31.43	1.46
M4	0.080 ±0.004	0.120 ±0.003	33.17	1.50
MB1	0.489 ±0.017	0.688±0.006	33.39	1.50
MB2	0.458 ±0.003	0.786±0.005	37.82	1.61
MB3	0.423 ±0.002	0.617±0.003	30.77	1.44
MB4	0.480±0.001	0.727±0.005	34.06	1.52

The theoretical primary aerodynamic diameter (d_{ae}) of each formulation was calculated from geometrical particle diameter and tapped density that ranged between 1.49 µm to 2.60µm, indicating particles having suitable 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 >90 % 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 M3, M4 and MB3. 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 d_{ae} values calculated theoretically, as shown in table 2 and 3. The largest value of d_{ae} 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 (2.68 ± 0.38 µm) was seen with the formulation coded MB2.

The fine particle dose was defined as the amount of the drug recovered from the lower stages of ACI. % mass deposition of rifampicin is shown in Figure 2 and 3. Majority of the particles are found to deposit on stages between stages 1-3 representing the aerodynamic diameter in the range of 3-6.5 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 5µm. The Fine Particle Fraction of the spray dried microparticles ranged 51.58 ± 5.36 to 72.74 ± 3.18 of the total loaded dose; details are shown in table 3. The most significant increase in FPF was found with the formulation coded MB2; lactose added to this formulation as carrier caused the FPF value rise up to 73 % from 58 %. 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 [28, 29]. In this study, the calculated GSD values for microparticle formulation were found in the range of 1.92 to 2.07 as shown in Table 3.

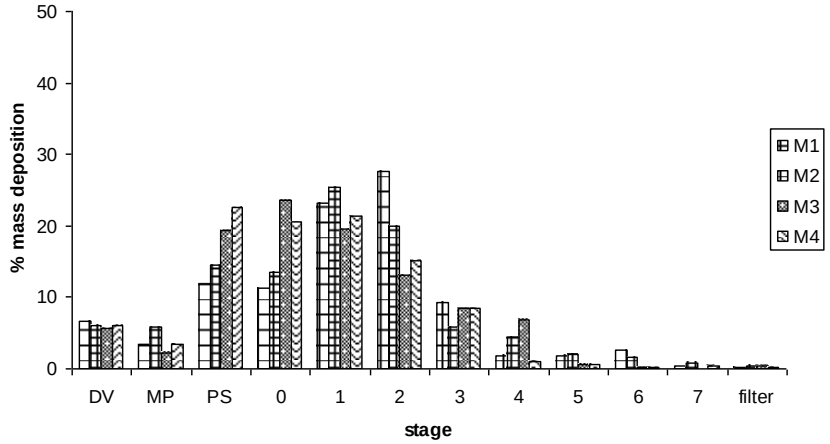


Figure 2: Comparative percentage mass distribution of formulations containing Rifampicin microparticles in cascade impactor.

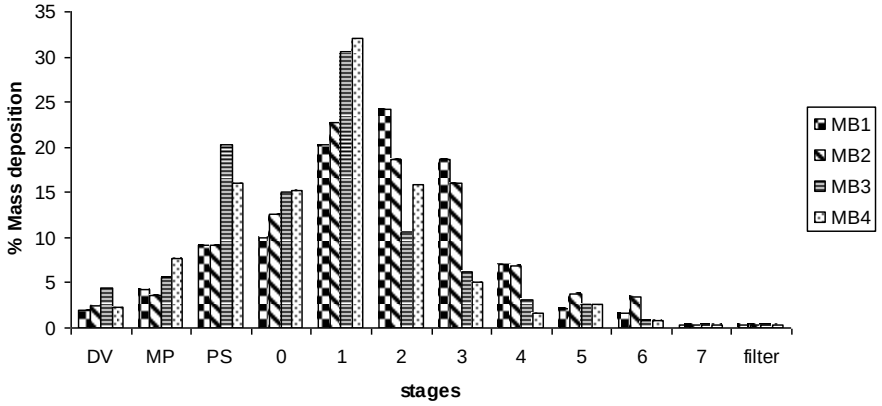


Figure 3: Comparative percentage mass depositions of formulations of Rifampicin microparticle blend with lactose in cascade impactor.

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

Formulation	%Emitted dose	MMAD	GSD	FPF
M1	95.04 ± 3.65	2.80 ± 0.44	2.12	54.96 ± 2.14
M2	96.19 ± 2.07	2.98 ± 0.39	2.07	57.52 ± 2.97
M3	95.89 ± 5.19	3.45 ± 0.56	1.92	51.58 ± 5.36
M4	96.41 ± 4.22	3.73 ± 0.28	2.15	57.01 ± 4.22
MB1	97.92 ± 2.10	2.72 ± 0.28	2.08	62.42 ± 2.02
MB2	97.68 ± 1.55	2.68 ± 0.38	2.10	72.74 ± 3.18
MB3	96.76 ± 2.06	3.41 ± 0.42	2.25	53.98 ± 2.56
MB4	96.99 ± 2.33	3.61 ± 0.51	2.30	59.52 ± 3.12

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

CONCLUSION

We have shown that rifampicin microparticles using biocompatible polymers with optimum size can be prepared 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 formulation (with blend as well) indicated that the formulation of microparticles were of a suitable size for deposition in the alveolar region of the lung. The addition of carrier influenced the dispersibility which was confirmed by the FPF values obtained when blends were prepared. 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, although the in vivo pharmacokinetic studies yet to be performed for establishment of *in vitro in vivo* correlation.

ACKNOWLEDGEMENT

The authors are grateful to Director, Sophisticated Instrumentation Center Applied Research and Testing (SICART), Anand, for providing analytical facilities.

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