Research Article

PREPARATION AND *IN VITRO* EVALUATION OF SPRAY DRIED MICROPARTICLES OF ANTITUBERCULAR DRUGS FOR PULMONARY DELIVERY

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ABSTRACT

The objective of this study was to develop dry powder inhaler (DPI) of drug loaded poly (DL-lactide) and poly (DL-lactide/glycolide copolymer) microparticles. Microparticles were prepared by spray drying process. Spray drying was optimized by keeping inlet temperature, outlet temperature, feed rate and aspiration speed as process optimization parameters. DPI formulations of microparticles of isoniazid and rifampicin were prepared using mannitol and sucrose as carriers. All formulations were evaluated for *in vitro* drug deposition studies. Selected formulations were evaluated for micromeritics, in vitro drug release studies and stability studies. DPI formulations of PLA polymer with mannitol as carrier showed highest %FPF values and smallest MMAD values at the same time maintaining the uniformity of drug content within the pharmacopoeia limits with no significant variations in MMAD values and drug content on storage. Formulations having PLGA as polymer showed in vitro drug release up to 6 days while those having PLA as polymer showed release up to 7 days for both drugs. The entrapment of antitubercular drugs using biopolymers like PLA, its formulation using carrier like mannitol and administration using a DPI device like Rotahaler seems to be a promising therapeutic approach for the chemotherapy of pulmonary tuberculosis.

Keywords: Tuberculosis (TB), Microparticles, Isoniazid, Rifampicin, Mass Median Aerodynamic Diameter (MMAD), Dry Powder Inhaler (DPI).

INTRODUCTION

Tuberculosis (TB), an ubiquitous, highly contagious chronic granulomatous bacterial infection, is still a leading killer of young adults worldwide. TB has returned with a new face and the global scourge of multi-drug resistant TB (MDR TB) is reaching epidemic proportions. Nearly one-third of the global population – two billion people – is infected with *Mycobacterium tuberculosis* (*M. tuberculosis*), more than eight million people develop active TB every year, and approximately two million die annually (World Health Organization, 2003). TB is the world's second most common cause of death from infectious disease, after acquired immuno deficiency syndrome (AIDS).[1]

Isoniazid and rifampicin are the first choice drugs for tuberculosis infections, requires high-doses and prolonged treatment. Moreover, it is known that resistance develops[2], while several side effects have been reported in long term therapy. Patient failure to take the prescribed medications at the required intervals results in significant morbidity and mortality. In the case of pulmonary TB, delivering the drug directly to the site of infection through inhalation of an aerosolized delivery system has the inherent advantages of bypassing first-pass metabolism and maintaining local therapeutically effective concentrations with decreased systemic side effects.[3] Because *M. tuberculosis* is known to infect alveolar macrophages and affect the pathogenesis of TB, there has been renewed interest in targeting of anti-TB drugs to these cells. The need for research into anti-TB drug delivery system is thus warranted as the efficacy of the current regimen may be improved if the delivery rate, biodegradation, and site-specific targeting can be predicted, monitored, and controlled.

For these reasons, several types of novel drug delivery devices have been proposed and characterized for INH and RIF administration, in order to maximize the therapeutic and minimize the toxic and side effects for these drugs.[3-5] Polymeric drug carriers are included between the various types of drug delivery systems proposed. Although experience with natural and synthetic polymers is extensive and encouraging, more recently the trend has been to shift towards synthetic biodegradable polymers as polylactides and co polymers. Main advantages of these polymers are their biocompatibility, biodegradability, bioresorbability and compatibility with the encapsulation of a wide range of drugs, with minimal use of organic solvents. Furthermore, stability, safety and approval for human use by the US FDA are additional advantages.

Several groups have investigated the use of microparticles produced by spray drying for various drug delivery applications.[6-9] Spray drying is of interest as an encapsulation technology because it is a single step process that, unlike emulsion dispersion and ionic pregelation, yields dry particles. Based on the fundamental spray drying work of researchers like Naikwade and Clarke it was determined that spray drying

would be an important technology to investigate for producing INH and RIF loaded polymeric microparticles.[10, 11] Spray drying is one-step constructive process that provides greater control over particle size, particle morphology and powder density whereas micronization is a destructive technique. [12-14]

Biodegradable microparticles composed of poly lactic acid (PLA) and poly (lactide-co-glycolide) (PLGA) can be considered as a well established drug delivery system, having high potential to serve as carriers for drugs as well as vaccines.[15, 16] One such application is the alveolar delivery of INH and RIF. Liposomes, nanoparticles, microparticles of chitosan, alginate for targeting INH and RIF have been prepared and characterized for *in vitro* and in vivo drug release previously by researchers but work relating to development of dry powder formulations and in vitro drug release, *in vitro* drug deposition studies, stability studies and micrometric properties DPI formulations of PLA and PLGA polymers along with carriers like mannitol and sucrose is not yet done. [17-19]

From a financial and a global health care perspective, finding new ways to administer the anti-TB drugs and delivering the multiple doses, long-term therapy in inexpensive, potent forms with improved bioavailability is needed. Development of dry powder inhaler of antitubercular drugs delivering the drug directly to the site of infection bypassing first-pass metabolism and maintaining local therapeutically effective concentrations with decreased systemic side effects, lowering the required doses and improving the bioavailability and patient compliance is the solution to this need.

Microparticles developed in this investigation contains antitubercular drugs isoniazid (INH) and rifampicin (RIF) and are meant for 'adjunct therapy' of pulmonary TB, through uptake of alveolar macrophages harboring *Mycobacterium Tuberculosis* and/or related species. It is also speculated that following uptake of drug loaded microparticles, uninfected macrophages could traffic to other lung areas where infected cells congregate. Formulation design of antitubercular drugs as dry powder inhaler may improve the efficacy of pulmonary tuberculosis treatment because it delivers drugs directly to the lungs where the *Mycobacterium Tuberculosis* resides.

MATERIALS AND METHODS

Materials: Isoniazid (Loba Chemie, Mumbai), rifampicin (Hi-media Laboratories, Mumbai), 75:25 Poly (DL-lactide/glycolide copolymer) (PLGA) of IV 0.2dl/g (Purasorb PDLG 7502 received as gift sample from Purac Biochem, Netherlands), poly (DL-lactide) of IV 0.2 dl/g (Purasorb PDL 02 received as gift sample from Purac Biochem, Netherlands), sucrose (Merck Specialties Pvt. Ltd., Mumbai), Dialysis Sac-Cellulose dialysis bag (MWCO 12-14 Kda, Himedia, India). All other chemicals were analytical grade and used without further modifications.

Methods

1. Preparation and characterization of drug microparticles

Drug microparticles were prepared by spray drying method. For INH-PLGA and INH-PLA batches, dichloromethane (DCM) was used as solvent for polymer while acetone was used as solvent for the drug. For RIF-PLGA and RIF- PLA batches, DCM was used as solvent for polymer while Chloroform was used as solvent for the drug. Microparticles were prepared by spraying the respective solutions through spray dryer (Labultima, India).

Design of experiments (DOE) technique was used to provide an efficient means to optimize the spray drying process. A 2-factor 3-level factorial experimental design technique was employed to investigate the variables using the statistical software package Graphis (Kylebank Software). This technique was applied to quantify the influence of operating parameters on the production of microparticles during the spray drying operation in minimum number of experimental runs.

The factorial design created constituted 9 of the experiments in this study. Preliminary experiments were performed to confirm the operational phase range that would successfully yield spray-dried microparticles and to verify that the runs could be conducted at the operational units dictated by the factorial design. The goal of the experimental design was to find out, with the minimum number of experimental runs, which process variables have the biggest impact on the final product. Polymer concentration, outlet temperature, feed rate and aspiration rate were kept as fixed parameters while % yield and particle size were dependent variables.

Prepared microparticles were characterized for % yield, particle size analysis, entrapment efficiency, drug-polymer interaction studies and morphological characterization. Microparticles having mean particle size below 5µm, higher entrapment efficiency and high % yield were selected for both the drugs and subjected for further studies.

2. Preparation of Dry Powder Inhaler Formulations

One adult dose of isoniazid the is 5 to 10 mg/kg/day to a maximum of 300 mg, for a minimum of 6 months while that of rifampicin is 10 mg/kg, once daily, to a maximum of 600 mg. Generally from the literature review it has been shown that the dose for inhalation may be reduced to less than 50-80 times compared to the conventional oral formulation. Hence considering this we have fixed the dose of both isoniazid and rifampicin for DPI at 8mg. The formulations of plain micronised drugs or drug microparticles along with carriers- mannitol and sucrose in different ratios were prepared by mixing the mentioned respective quantities of drug/microparticles with carrier sugar in V blender at 100 rpm for 30 minutes. Prepared formulations of plain drugs as well as drug

microparticles with carriers were tested for drug- carrier interaction studies using FTIR and DSC.

3. *In-vitro* Drug Deposition Studies of DPI formulations

DPI formulations were subjected to in vitro drug deposition studies using Twin Stage Impingner (TSI). The selected formulations from TSI study were further subjected for Content Uniformity and in vitro drug deposition study using Anderson Cascade Impactor (ACI).

A. In vitro drug deposition study using Twin Stage Impinger (TSI):

Empty gelatin capsules (Size 2) were hand filled with the powder blends equivalent to 8 mg of drug. The capsules were closed tight and placed in the desiccators. The aerodynamic behavior of all formulations was estimated with Twin Stage Impinger (Apparatus A) official in British Pharmacopoeia (BP 2005)[20]. Apparatus A (TSI) was assembled as per BP 2005 with 7 ml and 30 ml of a suitable solvent for respective drugs into the upper and lower impingement chambers respectively. A Rotahaler device containing empty size 2 capsule was inserted into the throat via a custom formed silicone rubber adaptor and the pump set to draw air through the device at 60± 5 liters per minute. Vacuum pump was switched off after 5 seconds and the Rotahaler device was allowed to remain in TSI for further 30 seconds. Rotahaler was removed and weighed. Apparatus A was separated in to Throat, Stage 1 and Stage 2 units. The drug depositions on the Adaptor and Throat were rinsed into Stage 1 using the solvent for respective drugs and transferred to 50ml volumetric flask and volume was adjusted using phosphate buffer (pH 7.4). Similarly, the drug depositions in the Stage 2 were rinsed using solvent and transferred to 50ml volumetric flask and volume was adjusted using same phosphate buffer (pH 7.4). The drug residues remaining in the capsule and on the Rotahaler mouthpiece were rinsed using the solvents and transferred to 25ml volumetric flask. The Rotahaler body was tapped to collect any adhered powder and the collected powder was also transferred to 25ml volumetric flask and volume was adjusted using same phosphate buffer (pH 7.4). In case of microparticle formulations of both the drugs, the microparticles were first added to solvent, crushed, and the solution was filtered using 0.45µ membrane filter and then was subjected for volume make up and further analysis. The quantities of drug recovered from three samples were determined by UV Spectroscopy method already described. The results were calculated as Emitted Dose (ED), Fine Particle Dose (FPD) and Fine Particle Fraction (FPF). Experiment was carried out 6 times for each formulation.

B. Content Uniformity

DPI formulations with higher values of %ED and %FPF in TSI study were subjected for further studies. Each formulation was examined by sampling the dry formulation following the USP 1995 (USP 23 NF 18) acceptance. A total of 10 doses were collected, three doses at the top, four doses in the middle and three at the bottom of the powder blends.

In the formulation of INH, total powder blend for each drug: carrier ratio was weighed accurately and put into a 100ml volumetric flask. Phosphate buffer (pH 7.4) was added to dissolve the isoniazid and volume was made up to 100ml using phosphate buffer (pH 7.4). 1ml of this solution was transferred to a volumetric flask of 10ml and volume was made up to 10ml using phosphate buffer (pH 7.4). All the solutions such obtained were analyzed by UV-VIS Spectroscopy method. In case of RIF, the drug content uniformity in each powder blend was estimated by the method similar to that of isoniazid using methanol as solvent and phosphate buffer (pH 7.4) for further dilutions. In case of microparticle formulations of both the drugs, the microparticles were first added to respective solvents, crushed, and the solution was filtered using 0.45µ membrane filter and then was subjected for volume make up and further analysis.

Unless otherwise specified in the individual monograph, the requirements for dose uniformity are met if the amount of active ingredients from the weight variation or the content uniformity method lies within the range of 85% to 115% of label claim and no unit is outside the range of 75% to 125% of label claim and the relative standard deviation of the 10 dosage units is less than or equal to 6.0%.[21]

C. In vitro drug deposition study using Anderson Cascade Impactor (ACI):

The deposition of each dry powder formulation was assessed in vitro using an Andersen 1 ACFM Non-viable Ambient Particle Sizing Sampler. Instrument was recalibrated at 28.3l/min. The eight plates within the impactor were coated with a thin layer of 316 silicon grease to prevent fine particles from bouncing on the plates and becoming re-entered in the air stream, which could give an incorrect size distribution. A preseparator was attached to the top of the impactor to prevent large particles aggregating.

The powder was aerosolized in the Anderson Impactor using a dry powder inhaler Rotahaler. A molded mouthpiece adapter was attached to the end of the induction port to produce an airtight seal between the inhaler mouthpiece and the induction port of impactor. The impactor was fixed on the testing stand. The flow rate was maintained by a vacuum pump at a steady flow rate of 28.3l/min. Before each sampling run, continuous airflow through the impactor was allowed to equilibrate for 5 minutes. Once the inhaler was positioned, powder was discharged into the apparatus by activating the timer and opening two way solenoid valve for 10 seconds. Each test was repeated six times. Samples collected from each plate were subjected for estimation of drug content by UV VIS Spectroscopy. Mass of particles collected on each plate was calculated from the difference in the weight of the plates before and after dispersion. Mass Median Aerodynamic Diameter (MMAD), Geometric Standard

Deviation (GSD) and Fine Particle Fraction (%FPF) were calculated.

4. Micromeritic Studies of selected DPI formulations

Selected formulations from in vitro drug deposition studies were subjected for micromeritic studies to study the flow and packing properties of the DPI formulations. Formulations were subjected for determination of flow properties - Angle of Repose, Tapped Density, Bulk Density, Carr's Index, Hausner Ratio and Residual Water Content.

A. Angle of Repose: In order to determine the angle of repose of DPI formulations, a pile of the sample was carefully built up by dropping the material through a funnel till the formed pile touches the tip of the funnel, 2cm above the flat surface. The angle of repose was calculated by inverting the ratio of height and radius of the formed pile.[22]

Angle of repose of different formulations was measured according to fixed funnel standing method.

B. Tapped Density: Tapped density was determined by mechanically tapping a measuring cylinder containing 1gm of powder samples carefully placed in to a 10 ml graduated cylinder. After observing the initial volume, the cylinder was mechanically tapped and volume reading was taken until little to no change in volume is observed.[23] The plateau condition was obtained after 500 taps for all samples.

C. Bulk Density: Bulk density of a powder is obtained by dividing its mass by the bulk volume it occupies. The volume includes the spaces between particles as well as the envelope volumes of the particles themselves.

D. Compressibility Index or Carr's Index (CCI): The Carr's compressibility index (CCI) may be used to quantify the powder flow using two density terms and is defined as: [24]

$$\text{Carr}\,(\%) = \frac{(\text{Tapped density} - \text{Bulk density})}{\text{Tapped density}} \times 100 \qquad \text{Equation 1}$$

E. Hausner Ratio (HR): Hausner ratio is a measure of flow ability of formulation and is calculated using equation:

$$\text{Hausner ratio} = \frac{\text{Tapped Density}}{\text{Bulk Density}} \qquad \text{Equation 2}$$

5. *In-vitro* Drug Release Studies of DPI Formulations

In vitro drug release studies of DPI formulations were carried out by dialysis sac method using diffusion cell cum dialysis sac apparatus (Model mFDC 08, Orchid Scientifics, India). A 6cm long portion of the dialysis tubing was made into a dialysis sac by folding and tying up one end of the tubing with thread, taking care to ensure that there would be no leakage of the contents from inside the sac. The sac was then soaked overnight in phosphate buffer (pH 7.4). The wet sac gently opened and washed copiously with phosphate buffer (pH 7.4). Then it was filled up with phosphate buffer (pH 7.4) and examined the leaks. The sac then emptied and formulation to be investigated was accurately transferred into the sac which becomes the donor compartment. The sac was suspended in the glass beaker containing 100ml of dissolution medium which becomes the receptor compartment. The temperature of the dissolution medium was maintained at 37°C±0.5°C.

At predetermined time intervals, 1 ml aliquots were withdrawn from the receptor compartment and replacement was made each time with 1ml of fresh dissolution medium. All the samples were tested in triplicate. Samples were filtered through 0.22µm membrane filter (Pall filters) and filtrate was subjected for UV-VIS spectroscopy estimation method as described earlier for determining drug concentrations in each sample.

Cumulative percent drug released was found out at each point time. Values of $t_{50\%}$ (time for 50% dissolution), $t_{70\%}$ (time for 70% dissolution) and $t_{90\%}$ (time for 90% dissolution) was determined from graph. Dissolution data was given zero order, first order, Higuchi's square root and Korsmeyer-Peppas model kinetic treatment.

6. Stability Studies

The stability studies of the DPI formulations were carried out to study the effect of storage conditions on the formulation properties. Stability studies were carried out at 40°C±2°C/ 75±5%RH up to 3 months in stability chamber (Remi Instruments, India). The DPI formulations containing 8mg of drug were filled into gelatin capsule shells (Size 2). These capsules were packed in HDPE bottles and the bottle was sealed with aluminum foil. The bottles also contained silica bags as dehumactant and were resealed after each sampling. Six sets of 10 capsules from a batch were filled in the HDPE bottles for above mentioned condition. The stability studies were carried out for the time of 3 month. After storage for 3 months, the % drug contents, MMAD (µm) and in vitro drug release were determined as per method described previously.

RESULTS AND DISCUSSION

1. Preparation and characterization of drug microparticles

Microparticles of isoniazid and rifampicin using PLGA and PLA as biopolymers with different drug: polymer ratios were prepared by spray drying method. The spray drying process was optimized for all the microparticle batches by keeping inlet temperature, outlet temperature, feed rate and aspiration speed as process optimization parameters. The advantage of spray drying is that it is a one step method allowing fast processing of small batches with reasonable yields. Spray dried microparticles have suitable size and shape for inhalation applications. Factorial design proved to be a valuable technique for optimizing the production of spray-dried microparticles. Since spray-drying involved various parameters for study, the number of experiments was minimized while a

detailed evaluation of the dominant variable effects and interactions was accomplished.

Table 1: Experimental design grid and responses pertaining to microparticle production

Run No.	Atomization Pressure (bar)	Inlet Temp (°C)	Particle Size d_{90} (µ)	% Yield
1	1.5	35	6.848	14.56
2	1.5	40	5.1	16.73
3	1.5	45	4.611	18.85
4	2	35	5.88	16.56
5	2	40	4.575	18.69
6	2	45	3.759	21.1
7	2.5	35	3.962	19.18
8	2.5	40	3.504	21.6
9	2.5	45	3.409	23.75

Spraying air flow pressure (atomization pressure) was the important factor affecting particle size of resulting microparticles. A higher air spray flow through the nozzle increases the shear force between the gas and the liquid. This higher atomizing energy leads to smaller droplets and consequently to smaller solid particles. Increasing the spraying air flow rate from 1.5 to 2.5 bars reduced the mean particle size from 6 to 3µm.

Inlet temperature of drying air is an important parameter for both particle size and % yield (fig 1 and 2). The % yields ranged from 14% to 24%. Most of the runs resulted in low yields because of the difficulty of particle collection. Condensation inside the chamber led to a low yield as the particles tended to stick to the walls of the chamber and therefore could not be collected. The yield was expected to be greater at higher temperature due to the increased throughput of the polymer slurry and rapid evaporation of the solvent.

Ratio of drug: polymer and inlet air temperature were the important factors affecting drug entrapment efficiency. Drug entrapment efficiency was found to change as drug: polymer ratio changed from 70:30 (drug: polymer) to 40:60 (drug: polymer) but was not found to change when the ratio of drug: polymer was changed from 40:60 to 30:70. An average yield in the range of 14-24% was achieved. These values are comparable with results obtained by Gattani *et al.,* (23-31%)[25]. Most of the runs resulted in low yields because of the difficulties in particle collection.

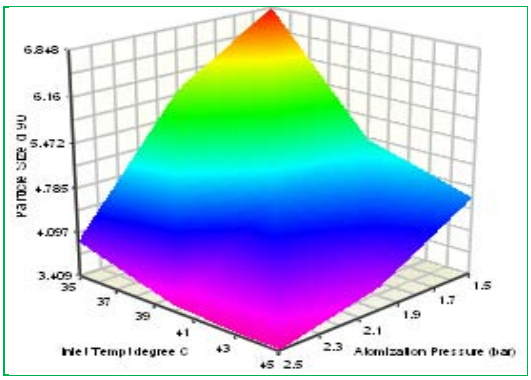

Figure 1: Effect of inlet temperature and atomization pressure on particle size d_{90}

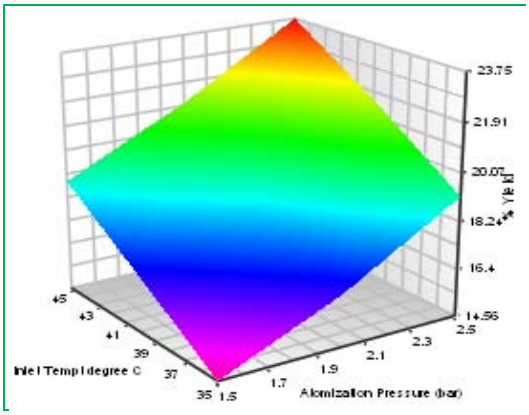

Figure 2: Effect of inlet temperature and atomization pressure on % yield

Table 2: $d_{90,}$ % entrapment and % yield of INH and RIF microparticles

Details of the formulation	Mean Particle Size (µm)/d_{90}	% Entrapment	% Yield
INH			
INH-PLGA 30:70	3.512	78.02	22.75
INH-PLGA 40:60	3.409	78.75	23.75
INH-PLGA 50:50	3.497	76.86	22.68
INH-PLGA 60:40	3.521	70.75	22.68
INH-PLGA 70:30	3.530	69.18	23.06
INH-PLA 30:70	3.456	70.80	23.12
INH-PLA 40:60	3.425	71.12	23.18
INH-PLA 50:50	3.622	66.47	22.06
INH-PLA 60:40	3.441	64.17	23.04
INH-PLA 70:30	3.818	59.02	21.98
RIF			
RIF-PLGA 30:70	3.508	70.12	20.55
RIF -PLGA 40:60	3.814	70.09	22.67
RIF –PLGA 50:50	3.516	68.04	21.94
RIF –PLGA 60:40	3.791	62.56	22.62
RIF –PLGA 70:30	3.796	60.19	23.06
RIF -PLA 30:70	3.563	68.36	21.58
RIF -PLA 40:60	3.550	68.34	22.67
RIF -PLA 50:50	3.622	66.08	23.12
RIF-PLA 60:40	3.608	60.12	22.75
RIF-PLA 70:30	3.693	58.12	22.34

For INH microparticles, percentage yield varied from 21.98 to 23.75%, while for RIF microparticles, percentage yield was between 20.55 to 23.12%. INH microparticles showed drug entrapment in the range of 59.02% to 78.85% with INH-PLGA 40:60 microparticles showing the highest drug entrapment of 78.85%. RIF microparticles showed drug entrapment in the range of 58.12% to 70.12% with RIF-PLGA 30:70 microparticles showing the highest drug entrapment of 70.12%.

INH microparticles showed particle size (D_{90}) in the range of 3.409µm to 3.818µm, while RIF microparticles showed 3.508µm to 3.814µm. All the microparticle batches of INH and RIF were subjected to FTIR and DSC. DSC and FTIR studies confirmed that no significant interaction occurred between drug and polymer during spray drying process. Microphotgraphic image analysis (Motic digital microscope DMB1with image 2000 software) showed that the prepared microparticles were spherical in shape.

Table 3: Drug depositions in the ACI after aerosolization of the different blends (mean ± SD, n=6)

Formulation code	%ED	%FPF	MMAD	GSD
INH				
IM	94.50±0.71	52.85±0.84	6.07±0.54	2.26±0.75
IS	92.25±0.43	42.75±0.63	7.26±0.39	2.30±0.81
IPM	98.60±1.04	71.94±0.26	4.54±1.13	2.20±0.36
IPS	97.57±0.93	56.20±0.17	5.51±0.85	2.27±0.61
ILM	98.82±0.43	75.89±0.53	4.00±0.54	2.24±0.85
ILS	98.04±0.62	69.26±0.74	4.59±0.67	2.23±0.66
RIF				
RM	96.65±0.97	54.12±0.71	6.30±0.82	2.43±0.62
RS	92.20±0.81	45.22±1.12	7.10±0.49	2.51±0.81
RPM	97.69±0.89	64.23±0.79	4.89±0.98	2.42±0.69
RPS	97.39±1.06	57.16±0.98	5.55±1.04	2.37±1.06
RLM	98.72±0.56	73.32±0.73	4.10±0.62	4.10±1.15
RLS	98.17±0.73	61.62±0.42	4.88±0.42	4.88±0.48

2. Preparation of Dry Powder Inhaler Formulations

DPI formulations of microparticles of INH and RIF were prepared using mannitol and sucrose as carrier in three different drug: carrier ratios as 1: 1, 1: 1.5 and 1: 2. These formulations were analyzed by FTIR and DSC to check the interaction between drug/microparticles and carriers. Drug/microparticles- carrier interactions were verified using FTIR and DSC and confirmed no significant interactions or alterations occurred.

3. *In-vitro* Drug Deposition Studies of DPI formulations

A. In vitro drug deposition study using Twin Stage Impinger (TSI)

DPI formulations of both carriers showed higher % FPF values as compared to that of micronised plain drug formulations of both the carriers. DPI formulations of PLA showed higher % fine particle fraction (%FPF) values as compared to the DPI formulations of PLGA. It was observed that all the DPI formulations showed increase in % FPF values as the proportion of carrier was increased. This may be attributed to less aggregation between carrier and microparticles imparting the formulation more flow ability.

B. Content Uniformity

Homogeneity of powder blends is required for DPIs that contain inactive excipient or active added substances. In all cases of isoniazid and rifampicin DPI formulations, blend uniformity so called "uniformity of dosage units" was obtained as per USP 1995.

C. In vitro drug deposition studies of DPI formulations by ACI

The DPI formulations of microparticles of both the drugs showed higher % emitted dose (%ED) and %FPF values as compared to the formulations of plain drugs. Formulations with mannitol as carrier showed higher %FPF values as compared to the formulations containing sucrose as carrier. The reason may be higher moisture in sucrose formulations as compared to mannitol formulations resulting in formation of aggregates of drug and carrier. DPI formulations of PLA polymers showed higher % FPF values as compared to DPI formulations of PLGA polymers. This may be attributed to higher glycolic content in PLGA polymer which resulted increase in hydrophilic properties facilitating formation of microparticle and carrier aggregates.

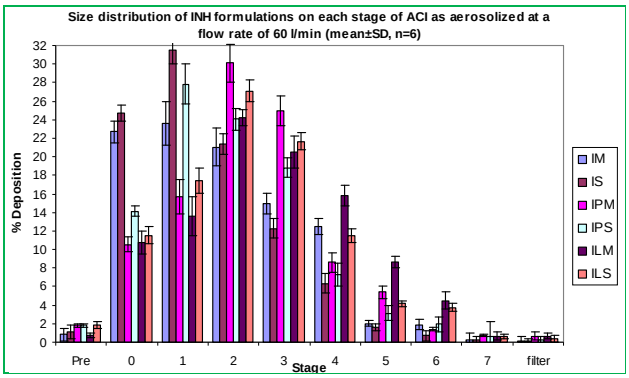

Figure 3: Size distribution of INH formulations on each stage of ACI

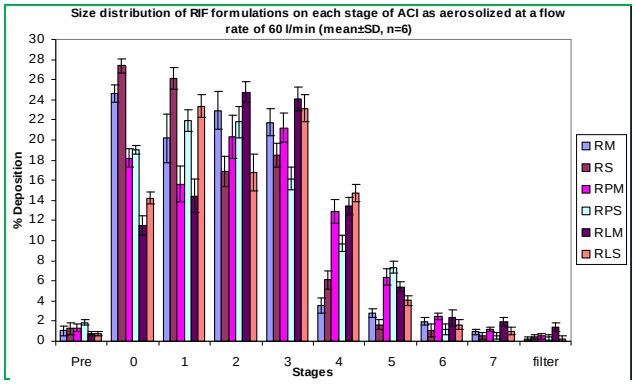

Figure 4: Size distribution of RIF formulations on each stage of ACI

The mass mean aerodynamic diameter (MMAD) varied from 4.00 to 7.26µm for INH formulations and 4.10 to 7.10µm for RIF formulations. Formulations IPM, ILM, ILS, RPM, RLM and RLS were selected for further studies as the MMAD values were lesser than 5µm. Formulations with MMAD values above 5µm were rejected as for the therapeutic lung delivery MMAD values must be below or equal to 5µm. All the formulations had the geometric standard deviation (GSD) values above 1.25 therefore the formulations were polydisperses.

4. Micromeritic Studies of selected DPI formulations

Formulations containing mannitol as carrier showed angle of repose values in the range of 31° to 35°, indicating good flow properties of the formulations, while formulations containing sucrose showed angle of repose values in the range of 40° to 41° indicating the higher interparticulate forces imparting the formulation poor flow properties in comparison to formulations containing mannitol.

Carr's index ranges from 22.966% to 32.682%. ILM and RLM formulations showed lowest CCI index, indicating low interparticulate forces and therefore excellent flow properties. Formulations containing mannitol as carrier showed lower CCI values in comparison to formulations

containing sucrose as carrier indicating the better flow properties of mannitol containing formulations. Microparticle formulations having PLA showed lower CCI values in comparison to formulations having PLGA as polymer.

In general from the data of CCI and HR it can be predicted that, formulations having mannitol as carrier showed better flow properties as compared to that of sucrose. This may be attributed to higher moisture content in sucrose formulations responsible for increasing the cohesiveness in the particles imparting poor flow properties to the formulation. Formulations with PLGA as polymer showed higher moisture content in comparison to formulations with PLA as polymer. This may be due to higher glycolic acid content, leading to increase in both the amorphous and hydrophilic properties.

Table 4: Flow properties of DPI formulations (Results ± SD, n=3)

Formulation Code	Angle of repose (θ)	Tapped Density (g/cm^3)	Bulk Density (g/cm^3)	% CI	Hausner ratio	Residual water content (%)
IPM	35.50±2.45	0.205±1.88	0.153±3.02	25.365±2.86	1.339±1.54	3.91± 1.75
ILM	31.65±2.93	0.209±1.64	0.161±2.64	22.966±2.66	1.298±3.05	3.07± 3.53
ILS	40.80±3.43	0.201±2.03	0.140±1.95	30.348±1.75	1.435±2.65	5.11± 2.65
RPM	35.64±3.06	0.208±1.80	0.149±1.86	28.365±2.80	1.395±3.35	6.58± 3.06
RLM	32.60±3.64	0.211±3.05	0.159±2.45	24.644±3.60	1.327±2.65	5.93± 3.26
RLS	41.54±4.22	0.205±2.76	0.138±1.65	32.682±2.55	1.485±2.54	7.18± 2.78

5. *In-vitro* Drug Release Studies of DPI Formulations

Isoniazid formulations ILM and ILS showed release up to 7 days while IPM formulation showed release up to 6 days. Similarly, Rifampicin formulations RLM and RLS showed release up to 7 days and RPM formulation showed release up to 6 days. The best fit was obtained with the Higuchi's square root kinetic treatment. Higuchi's square root kinetic treatment is applicable to the systems with drug dispersed in uniform swellable polymer matrix as in case of matrix tablets with water soluble drug.

Table 5: Mean (±S.D.) percent cumulative drug released from INH formulations

Time (h)	Percent cumulative drug release (±S.D)		
	Formulations		
	IPM	ILM	ILS
0	0	0	0
2	9.823(±0.524)	8.251(±0.378)	8.448(±0.535)
4	15.422(±0.430)	13.835(±0.486)	14.033(±0.689)
8	19.898(±0.680)	19.277(±0.325)	19.477(±0.458)
12	28.346±0.459)	25.361(±0.492)	24.974(±0.226)
24	41.002(±0.414)	33.862(±0.943)	33.472(±0.565)
48	51.031(±0.868)	44.411(±0.578)	44.016(±0.521)
72	65.086(±0.482)	55.847(±0.434)	55.448(±1.215)
96	77.509(±0.579)	67.000(±0.519)	67.187(±0.585)
120	92.406(±0.514)	76.884(±0.509)	76.483(±0.278)
144	99.783(±0.545)	90.297(±0.156)	89.703(±0.348)
168	--	99.408(±0.868)	98.891(±0.732)

It is reasonable to conclude that the release profiles of both the drugs from the microparticles in all cases showed 3 distinct phases: after a burst release of surface located and poorly encapsulated drug, a phase of lower release rates controlled by diffusion follows. Ultimately, increased diffusion rates corresponding to the polymer cleavage mark the third release period.

An initial burst release phase for all INH and RIF formulations occurred within the first day. Formulations with PLGA as polymer showed more burst release as compared to the formulations with PLA as polymer. Formulation IPM showed 41.002% cumulative release while formulations ILM and ILS showed 33.862% and 33.472% cumulative drug release respectively in one day. Formulation RPM showed 38.446% cumulative release while formulations RLM and RLS showed 33.165% and 33.919% cumulative drug release respectively in one day.

Table 6: Mean (±S.D.) percent cumulative drug released from RIF formulations

Time (h)	Percent cumulative drug release (±S.D)		
	Formulations		
	RPM	RLM	RLS
0	0	0	0
2	5.470(±0.463)	5.221(±0.784)	5.719(±0.644)
4	14.227(±0.378)	11.987(±0.663)	12.240(±0.459)
8	20.336(±0.682)	17.824(±0.741)	17.583(±0.425)
12	26.007(±0.531)	24.963(±0.474)	24.719(±0.461)
24	38.446(±0.356)	33.165(±0.505)	32.919(±0.632)
48	47.526(±0.804)	45.922(±0.824)	45.425(±0.635)
72	59.925(±0.451)	56.815(±0.452)	56.313(±0.792)
96	71.698(±0.469)	67.563(±0.602)	67.056(±0.843)
120	88.307(±0.592)	77.171(±0.362)	76.907(±0.783)
144	99.853(±0.835)	90.401(±0.560)	89.933(±0.823)
168	--	99.107(±0.824)	98.903(±0.904)

Initial burst in case of PLGA microparticle is higher in comparison to initial burst in PLA microparticle because the glycolic acid content in the PLGA polymer imparts it hydrophilic nature leading to rapid water intake by the PLGA microparticles surface region, which in turn results in swelling of chains close to the surface and drastically increases the diffusion of the drug molecules. The rate of water uptake of polymer particles increases with the hydrophilicity of polymer. Hence the initial burst is higher for more hydrophilic (PLGA) particles than less hydrophilic (PLA) particles. Initial bursts of 20% to 70% are reported in literature. In case of PLGA (50:50) the initial burst of 70%

has been reported for haloperidol- loaded PLGA nanoparticles.[26] We found the burst release in our experiments in the range of 38 to 41% as we have used PLGA polymer having lactide: glycolide proportion as 75:25. The burst release was followed by slow release period during which the drug is released at a steady slow rate. In this phase the release rates are controlled by diffusion. This steady slow release rate phase is followed by final fast release period. This deviation form steady slow drug releasing state to fast drug releasing state is due to the polymer degradation. In this phase, increased diffusion rates leads to the polymer cleavage. The polymer degradation is faster for PLGA particles than for PLA particles and hence the deviation is observed earlier for PLGA particles in comparison to PLA particles. The above discussion can also be confirmed by applying the dissolution data to Korsmeyer-Peppas model. The n value obtained from Korsmeyer-Peppas model was used to characterize different release mechanisms.

Anomalous transport means that the mechanism of drug release is dependent of the drug diffusion and the swelling of the polymer.[27] The n value for IPM formulation was higher in comparison to the n value of ILM and ILS formulations indicating the faster degradation of polymer. Similar was the case with rifampicin formulations. The n value for RPM formulation was higher in comparison to the n value of RLM and RLS formulations. The n values of isoniazid formulations were higher in comparison to the n values of Rifampicin formulations. This indicates the faster diffusion of isoniazid from microparticles in comparison to rifampicin. This may be due to the hydrophilic nature of isoniazid in comparison to rifampicin which is hydrophobic leading to a slow diffusion from microparticles.

Formulations of both the drugs of PLA polymers showed release up to 7 days while formulations of PLGA polymers showed release up to 6 days (fig 5 and 6). This may be due to the fact that with higher glycolic acid content, both the amorphous and hydrophilic properties increase and facilitate the faster release of loaded drug. Park *et al.*,[28] reported that hydration of PLGA matrices during in vitro release lowered the glass transition temperature. Hence, the polymer changed from the glassy to the rubbery state, which accelerated polymer degradation and release rates.

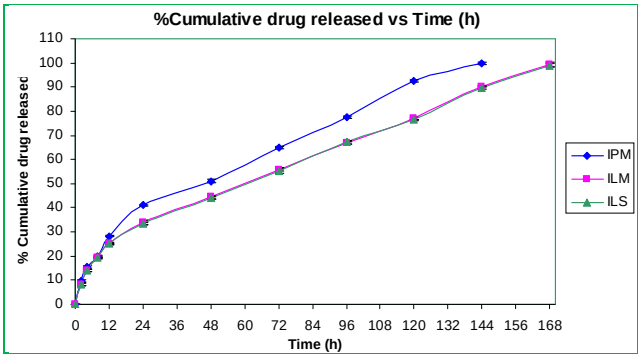

Figure 5: % cumulative drug released versus Time (h) for INH DPI formulations

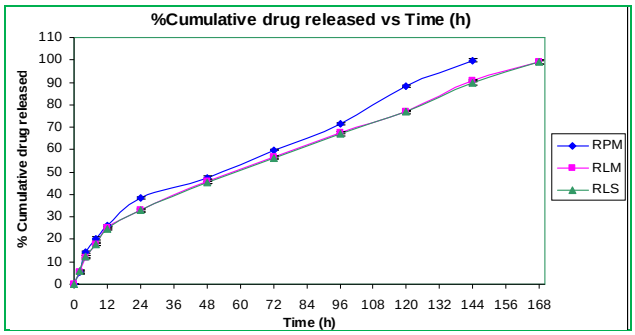

Figure 6: % cumulative drug released versus Time (h) for RIF DPI formulations

6. Stability Studies

The degradation of INH in all the formulations containing mannitol as carrier was nearly below 1% of the initial drug content. The degradation of INH in ILS was nearly 1.5%. This may be due to higher moisture content in ILS formulations as compared to other formulations leading to degradation of drug. Degradation of RIF in all formulations containing mannitol as carrier was less compared to formulations containing sucrose as carrier. The results suggest that the chemical stability of INH formulations is higher than that of RIF formulations. All the RIF formulations showed more degradation of drug content as compared to INH formulations. This may be due the fact that RIF was sensitive to moisture and light causing degradation.

The RIF in PLA microparticle formulations found to degrade at slower rate as compared to the RIF in PLGA microparticle formulations. PLA microparticle formulations for both the drugs containing mannitol as carrier showed very less variations in MMAD values after storage, while the MMAD values of PLGA microparticle formulations were higher than the initial MMAD values.

The formulations containing sucrose as carrier showed highest variations in MMAD values which is clearly due to higher moisture content. The significant increase in MMAD values after storage may probably due to the formation of particle aggregates. These aggregates were presumably responsible for the large fraction of these microparticles depositing in the preseparator and the higher stage of cascade impactor. Particles aggregation may occur by moisture absorption and electrostatic force. Therefore, these formulations had to be improved in the aggregation of particles. Aggregation of particles in the DPIs or upon their administration can influence the respirable fraction of the delivered dose. The molecular, electric and capillary forces are responsible for powder aggregations are influenced by particle size, surface morphology, surface charge and moisture absorption.[29]

Cumulative % drug release data for INH formulations showed very less variations from the initial values with IPS formulation showing highest variation in data in the category. Cumulative % drug release data for RIF formulations showed more variations form the initial values with RPS formulation showing highest variation from the initial cumulative % release data.

CONCLUSION

The entrapment of antitubercular drugs using biopolymers like PLA, its formulation using carrier like mannitol and administration using a DPI device like Rotahaler (Cipla Ltd.) can play promising role in alleviating problems associated with pulmonary tuberculosis treatment. However, prior to clinical application, the presented DPI formulation need more further thorough and extensive pharmacological and toxicological studies in animals.

ABBREVATIONS

Tuberculosis (TB), Isoniazid (INH), Rifampicin (RIF), poly (DL-lactide) (PLA), poly (DL-lactide/glycolide copolymer) (PLGA), Mass Median Aerodynamic Diameter (MMAD), Dry Powder Inhaler (DPI)

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