



Pharmacokinetic Study of Dry Powder Inhaler Formulations of Antiasthmatic Drugs

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ABSTRACT

The objective of this study was to develop a dry powder inhaler (DPI) of drug loaded poly (DL-lactide) and poly (DL-lactide/glycolide copolymer) microparticles and evaluate the selected formulations from in vitro studies by pulmonary drug release study. 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 salbutamol sulphate and budesonide were prepared using mannitol and sucrose as carriers. All formulations selected from in vitro drug deposition studies, micromeritics, in vitro drug release studies and stability studies were subjected for pulmonary drug release study. MRT of plain SBS was found to be 4.312h while MRT of formulation was 17.358h. Similarly MRT of plain BUD was 5.158h while MRT of formulations was 21.937h. There was an increase in AUC for formulation compared to the plain drug. AUC of plain drugs were found to be 106.59 and 92.79 for SBS and BUD respectively while AUC of SLM and BLM were found to be 516.59 and 382.24 respectively. From the AUC values, Site Exposure Enhancement Factor was calculated for both the drugs. For SLM, this value was found to be 4.846 while for BLM it was 4.119. Across- the- board enhancement of AUC, MRT and SEEF confirms that sustained drug release was provided by the formulations of both the drugs in comparison to plain drugs. Drug concentrations were maintained in the PH for longer durations and disappeared from the BAL less rapidly demonstrating the superiority of formulations compared to the plain drugs. Enhancement of AUC, MRT and SEEF confirms that sustained drug release was provided by the formulations of both the drugs in comparison to plain drugs. The entrapment of anti-asthmatic 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 treatment of asthma.

Keywords: Asthma, microparticles, salbutamol sulphate, budesonide, poly (DL-lactide), poly (DL-lactide/glycolide copolymer), Mass Median Aerodynamic Diameter (MMAD), Dry Powder Inhaler (DPI).

INTRODUCTION

Asthma is a disease of diffuse airway inflammation caused by a variety of triggering stimuli resulting in partially or completely reversible bronchoconstriction. Treatment involves controlling triggering factors and drug therapy, most commonly with an inhaled corticosteroid. Prognosis is good with treatment¹. World Health Organization (WHO) reports, asthma is a chronic respiratory disease that affects 5-20% of the world population and can cause significant morbidity and mortality. People of all ages especially children are affected by asthma. It can be severe and sometimes fatal diseases².

The development of controlled release formulations for inhalable drugs has been widely investigated for several years. Nonetheless, no controlled release product for pulmonary application is currently on the market. The reduction of dosing frequency is of great concern for a number of pulmonary disorders including asthma and chronic obstructive pulmonary disease (COPD). In particular, short-acting β_2 -adrenergic receptor agonists used for the relief of asthma and COPD related bronchospasms have a relatively short plasma half life that constrain the patient to an administration of the drug every 4-6 hours. A controlled release formulation

leading to a prolonged duration of action of more than 8 hours would prevent nocturnal exacerbation in bronchial asthma³.

For these reasons, several types of novel drug delivery devices have been proposed and characterized for pulmonary administration, in order to maximize the therapeutic and minimize the toxicity and side effects for these drugs^{4, 5}. Polymeric drug carriers are included among 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. The 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, 7, 8, 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^{10, 11} it was determined that spray drying would be an important technology to investigate for producing SBS and BUD loaded polymeric microparticles. 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, 13, 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 SBS and BUD. Liposomes of BUD¹⁷ and microparticles of chitosan, alginate for targeting SBS and BUD have been prepared and characterized for in vitro drug release previously by researchers but work relating to the 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^{18,19}.

In vitro drug delivery data for pulmonary and nasal drug products have great importance, particularly for quality control and product release. However, in vitro tests which measure emitted dose and particle size distribution of both pulmonary and nasal aerosols, as well as the spray pattern and plume geometry of nasal sprays, do not take into account the interactions between the aerosol particles and the airways when inhalers are used by patients. While agreement between in vitro and in vivo data is sometimes good, in vitro data may not predict in vivo drug delivery adequately in some situations. In vivo tests of drug delivery, using either radionuclide imaging or appropriate pharmacokinetic methods, may be a useful adjunct to in vitro testing in early product development²⁰.

The pulmonary route has been used with success for the treatment of both lung (asthma) and systemic diseases (diabetes). The fate of an inhaled drug (absorption and deposition) within human lungs has great importance, particularly in drug development and quality control. In vivo methods provide direct information about the concentration and the location of inhaled drug within lung²¹. The drugs can be administered by pulmonary route utilizing two techniques: aerosol inhalation (also used in intranasal applications) and intratracheal instillation. By applying aerosol technique, we could achieve more uniform distribution with greater extent of penetration into the peripheral or the alveolar region of the lung, but this costs more and also faced with difficulty in measuring the exact dose inside the lungs. In contrary to this, instillation process is much simple, not expensive and has non-uniform distribution of drugs²².

Instillation has certain advantages over inhalation, as discussed in detail by Brain et al (1976). Briefly, with instillation, the actual dose delivered to the lungs of each animal can be essentially assured. The technique is

simpler than inhalation exposure procedures and minimizes risks to laboratory workers from highly toxic, carcinogenic, or radioactive materials. Intratracheal instillation permits the introduction of a range of doses to the lungs within a short time, and avoids exposure to the skin and pelt that can occur with inhalation exposure. In larger animals, when using certain types of test material, highly localized exposures to specific lobes of the lung can be performed, with one lobe serving as a control for another²³.

From a financial and a global health care perspective, finding new ways to administer the anti-asthmatic drugs and delivering the multiple doses, long-term therapy in inexpensive, potent forms with improved bioavailability is needed. Development of dry powder inhaler of anti-asthmatic drugs delivering the drug directly to the site of action 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.

MATERIALS AND METHODS

Materials

Salbutamol Sulphate (RLCC, Mumbai), Budesonide (Glenmark Pharmaceuticals, Mumbai), 75:25 Poly (DL-lactide/glycolide copolymer) (PLGA) of IV 0.2dl/g (Purasorb PDLG 7502 received gift sample from Purac Biochem, Netherlands), poly (DL-lactide) of IV 0.2 dl/g (Purasorb PDL 02 received gift sample from Purac Biochem, Netherlands), Lactose- Pharmatose 325M (Vedant Pharmaceuticals), pellet feed, injection of ketamine, injection of diazepam, PE50 tubing, injection of ampicillin and diclofenac sodium, 3 way stopcock (Medicon, Delhi), glass syringe (Eutech, Mumbai), USP Phosphate buffer (pH 6.8), cellulose nitrate membrane filter 0.45µm, 47mm (Whatman Schleicher & Schuell), sodium dihydrogen phosphate (A. R. grade, Qualigens, Mumbai), acetonitrile (HPLC grade, Qualigens, Mumbai), Teflon coated magnetic beads (Kasbalanaka, Mumbai)

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 SBS-PLGA and SBS-PLA batches, dichloromethane (DCM) was used as solvent for polymer while acetone was used as solvent for the drug. For BUD-PLGA and BUD-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 5 μ m, higher entrapment efficiency and high % yield were selected for both the drugs and subjected for further studies.

2. Preparation of Dry Powder Inhaler Formulations

A fixed the dose of both SBS and BUD for DPI at 200 μ g. Powder formulations containing drug dose of 200 μ g were prepared by varying the ratio of carrier to drug. Two blends with ratio ranging from approximately 50:1 to 100:1 were prepared. Drugs were blended with carrier geometrically. An amount of carrier equivalent to about twice the total mass of drug was first used to sandwich the drug in the blend. This was mixed for 1 min using a vortex mixer (Remi Instruments, Mumbai). Carrier was then added in geometric quantities, mixing with a vortex mixer for 1 min after each addition. The formulations of drug/microparticles along with carriers in different ratios were prepared as mentioned in the table. All blends were filled into hard gelatin capsules (size 2) manually so that each capsule contained 200 μ g of drug as a nominal dose.

3. Pulmonary Drug Release Studies

Formulations selected from in vitro drug deposition studies, micromeritics, in vitro drug release studies and stability studies were subjected for pulmonary drug release study.

Animals and Treatment

The experimental design and the protocol were prepared as per the prescribed format of CPCSEA [Committee for the Purpose of Control and Supervision of Experimentation on Animals] and were approved by the IAEC [Institutional Animal Ethics Committee].

In vivo studies of promising DPIs were performed using intratracheal instillations technique as per earlier reports

(Joshi and Misra ²⁴; Yiwei Qi et al ²⁵; John Lay et al ²⁶; Hiroshi Sunaga et al ²⁷; F. J. Jacono et al ²⁸; Naazneen Surti et al ²⁹; Mahavir Chougule et al ³⁰).

Albino Rats having body weight 200-250gm were selected for the study.

Dose was maintained equivalent to 100 μ g. The SBS and BUD loaded microparticle formulations were administered to rat with drug content equivalent to a dose of 100 μ g.

Intratracheal instillation studies

To study the in vivo effects of microparticles and their retention in the lungs, rats were intratracheally instilled with plain drug and drug loaded microparticles formulation. At different time intervals the rats were sacrificed to collect the biological samples like Bronchoalveolar lavage (BAL) and Pulmonary Homogenate (PH). The Intratracheal instillation of drug solution and formulation was carried out according to a well adapted method described by Mahavir Chougule et al ³⁰ and Naazneen Surti et al ²⁹.

Intra peritoneal administration of Ketamine (50mg/kg) and intramuscular injection of diazepam (5mg/kg) was used to anesthetize rats. Anaesthetized animals were placed in supine position on a 45^o slanted support. A small middle incision was made over the trachea.

The trachea was exposed by blunt dissection of the sternohyoideus muscle and a small midline incision was made over the trachea.

A small hole was made in the trachea between the fifth and the sixth tracheal rings using a 20 gauge needle. The trachea was cannulated with a PE50 tubing (8 to 10 cm) with the tip positioned approximately at the tracheal bifurcation.

Doses equivalent to 100 μ g of drug as a solution or microparticles suspended in sterile saline (1ml) were slowly instilled over a 1 min period followed by 100 μ l of saline using glass syringe attached to PE50 tubing.

Following instillation, the tubing was withdrawn and a small drop of cyanoacrylate adhesive was placed over the hole to seal the opening. The skin was clothed with 3-0 Dexon sutures. Intraperitoneal Ampicillin (10 mg/kg) and Diclofenac Sodium (1 mg/kg) were administered to the rats to combat infection and pain of the animals. Following anesthesia, the animals were allowed to recreate under the heating lamp. After recovery, animals were housed in individual plastic cages with free access to food and water for the remainder of the study. At the end of each time point biological samples were collected and the animals were sacrificed.

Biological sampling

Bronchoalveolar lavage (BAL)

Bronchoalveolar lavage (BAL) was performed on anaesthetized animals with PBS, pre warmed to 37^o C. At



this, the glass syringe connected to the PE50 tubing was replaced by a 3-way stopcock attached to two 20ml syringes. The tubing was reinserted through the cannula and advanced till the tracheal bifurcation. Fluid (PBS) was slowly injected in to the lung via one syringe and then BAL withdrawn by gentle aspiration via the other one.

This BAL yielded between 7 to 10ml liquid. Microparticles in this liquid were dissolved in phosphate buffer pH 6.8 by stirring on magnetic stirrer. This liquid was centrifuged at 250 RCF (1060 rpm) for 5 min. The supernatant was then extracted and assayed by HPLC to determine unabsorbed drug.

Pulmonary Homogenate (PH)

Homogenization means to prepare uniform dilution of a known quantity of tissue suspended within a known quantity of a suitable diluents and uniformly macerate or crush the tissue in such a manner as to disperse minute fragments of tissue evenly throughout the mixture³¹.

Lung homogenates were prepared by slight modification of method as mentioned by Bakhle et al³².

The lungs and the portions of trachea below the instillation site were excised, chopped and homogenized (PH) in PBS. Entire separated organ was placed in a 25ml glass homogenization tube and chilled PBS was added to the tube. An appropriate sized pestle was placed in homogenization tube containing the organ and PBS. Organ was homogenized using the tissue homogenizer set at a speed of approximately 1300 rpm. The tissue was homogenized by going up and down the tube with the pestle twice only so as to keep the state of homogenization of each organ uniform from sample to sample. The crude homogenate was filtered through gauze to remove any remaining large fragments and the filtrate was subjected for next testing.

The released drug was analyzed by HPLC method.

HPLC method

The above deproteinized samples were analyzed by HPLC method (as described by Amit Misra et al³³ and R.M.H. Leite³⁵ for SBS and as described by A. K. Hemanth Kumar et al³⁴ and R.M.H. Leite³⁵ for BUD) and compared with calibration graphs generated obtained by analyzing known amounts of drug to obtain the drug concentration versus time profile.

Statistical Analysis^{29, 30}

The statistical analysis of the data was carried out using ANOVA and unpaired students t test. Differences greater than $p < 0.05$ were considered significant.

The various pharmacokinetic parameters calculated for comparing are defined below:

C_{max}: Maximum concentration of the drug attained in the lung during the study. The drug concentration in the lung is the drug estimated in the PH.

T_{max}: The time point at which maximum drug concentration is attained in the PH.

AUC: The area under the curve of the drug concentration in PH versus time, over the period of study.

Site Exposure Enhancement Factor (SEEF)

For drug targeting or drug delivery in general it is important to be able to quantitatively assess the site targeting effectiveness. A site enhancement factor was calculated as follows:

$$\text{Site Exposure Enhancement Factor (SEEF)} = \frac{\text{AUC (drug delivery system)}}{\text{AUC (drug alone)}}$$

----- Equation 1

RESULTS AND DISCUSSION

Preparation and characterization of drug microparticles

Microparticles of SBS and BUD 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 as shown in table no. 1, while a detailed evaluation of the dominant variable effects and interactions was accomplished.

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 3 μ m.

Inlet temperature of drying air is an important parameter for both particle size and % yield. The % yields ranged from 13% to 23%. 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 13-23% was achieved.

For SBS microparticles, percentage yield varied from 17.12 to 21.04%, while for BUD microparticles, percentage yield was between 18.83 to 21.73%. SBS microparticles showed drug entrapment in the range of 60.43% to 77.45% while BUD microparticles showed drug entrapment in the range of 63.14% to 79.49%.

SBS microparticles showed particle size (D_{90}) in the range of 3.038 μm to 4.656 μm , while BUD microparticles showed 3.057 μm to 4.395 μm . Depending on the particle two formulation of either of the drug was selected as shown in table no. 2. All the microparticle batches of SBS and BUD were subjected to FTIR and DSC. DSC and FTIR studies confirmed that no significant interaction occurred between drug and polymer during spray drying process. Microphotographic image analysis (Motic digital microscope DMB1 with image 2000 software) showed that the prepared microparticles were spherical in shape.

Preparation of Dry Powder Inhaler Formulations

DPI formulations of microparticles of SBS and BUD were prepared using mannitol and sucrose as carrier in two different drug: carrier ratios as 1: 50, and 1: 100. 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.

Pulmonary Drug Release Studies

The in vivo evaluation was carried out by the estimation of the percent drug in BAL and PH after administration of plain drugs and formulation. The drug dose of 100 μg was intratracheally instilled. The amount of drug present in the BAL was considered as the drug unabsorbed into the lung tissue but still retained in the bronchial spaces and amount of drug present in the PH was considered as the drug absorbed and available for pharmacological response. The drug present in the BAL represents the reservoir of drug that eventually would be absorbed by the lung tissue.

Following a single intratracheal administration of drug loaded microparticles to rat, drug concentration were maintained in PH for 60h for SBS and BUD microparticles. On the other hand, free drugs administered were not detected in PH beyond 12h of administration for both the drugs.

After instillation of SLM, 42.45% of the administered SBS was recovered in BAL during the first 8 h, eventually decreasing to 10.46% by 24h as shown in table no. 3. Similarly in case of BLM formulation, 40.244% of the administered BUD was recovered in BAL during the first 8 h, eventually decreasing to 9.553% by 24h as shown in table no. 4. The total drug balance between the percent drug released and percent drug present in the

microparticles (estimated in BAL) was initially found to be close to 100%. It was assumed that the amount of drug which could not be accounted in later stage may have either metabolized or systemically absorbed or both.

Table no. 5 shows that the MRT of plain SBS was found to be 4.312h while MRT of formulation was 17.358h. Similarly MRT of plain BUD was 5.158h while MRT of formulations was 21.937h. There was an increase in AUC for formulation compared to the plain drug. AUC of plain drugs were found to be 106.59 and 92.79 for SBS and BUD respectively while AUC of SLM and BLM were found to be 516.59 and 382.24 respectively. From the AUC values, Site Exposure Enhancement Factor was calculated for both the drugs. For SLM, this value was found to be 4.846 while for BLM it was 4.119.

C_{max} and T_{max} were not calculated by integrating the values over the entire period of study. The integration was not done due to the constraints of having different sets of animals at each sampling point making the integration a lesser valid approach.

Across- the- board enhancement of AUC, MRT and SEEF confirms that sustained drug release was provided by the formulations of both the drugs in comparison to plain drugs.

Different portions of bronchopulmonary tree possess different characteristics, it is possible that drug release from microparticles is affected by its distribution within lung and later altered by mucociliary transport and other mechanisms. Animal studies reported till date has utilized instillation of liquid formulations in order to obtain accurate dosimetry. Such results depend upon the spreading of the instilled dose within the lung. The distribution and absorption of inhaled aerosols in the lungs and airways may be different from those of instilled liquids and it is possible that release kinetics of aerosol formulations in humans may differ considerably from release kinetics of instilled formulations in animals.

Findings of the study give only comparative performance of plain drug and microparticle formulations without any predictive value on performance in human. Though the drug concentrations maintained in the PH and less rapid disappearance from the BAL demonstrated the superiority of microparticles compared to the plain drug.

Lung offers a large surface area for drug absorption (approximately 75 m^2). In addition, the alveolar epithelium is very thin (approximately 0.1-0.5 μm thick), thereby permitting rapid drug absorption. The alveoli can be effectively targeted for drug absorption by delivering the drug as an aerosol, with MMAD less than 5 μm . also, the first pass metabolism of the GIT is avoided. Although metabolic enzymes can be found in the lungs, the metabolic activities and pathways may be different from those observed in GIT³⁶.

The respiratory system works with the circulatory system to deliver oxygen from the lungs to the cells and remove carbon dioxide, and return it to the lungs to be exhaled.



The exchange of oxygen and carbon dioxide between the air, blood and body tissues is known as aspiration. Healthy lungs take in about 1 pint of air about 12-15 times each minute. All of the blood in the body is passed through the lungs every minute³⁷.

The potential advantage of direct delivery of the anti-asthmatic drug to the lungs include the possibility of

reduced systemic toxicity, higher drug concentration at the main site, more rapid absorption into systemic circulation and higher bioavailability than with other non-invasive methods of administration. Moreover, in contrast to the oral route of administration, inhaled drugs are not subjected to first pass metabolism.

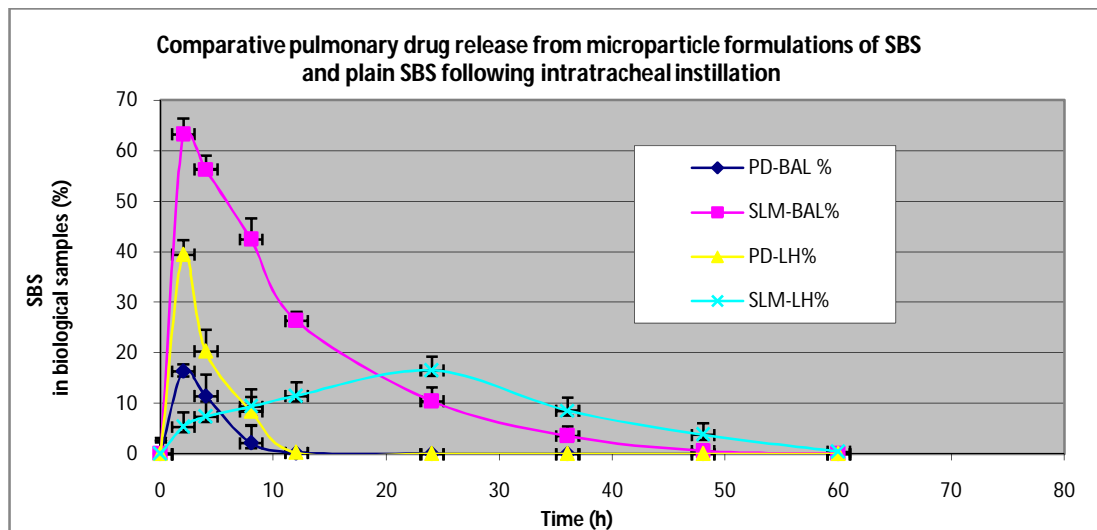


Figure 1: SBS level in biological samples following instillation of PD- plain drug and formulation (SLL)

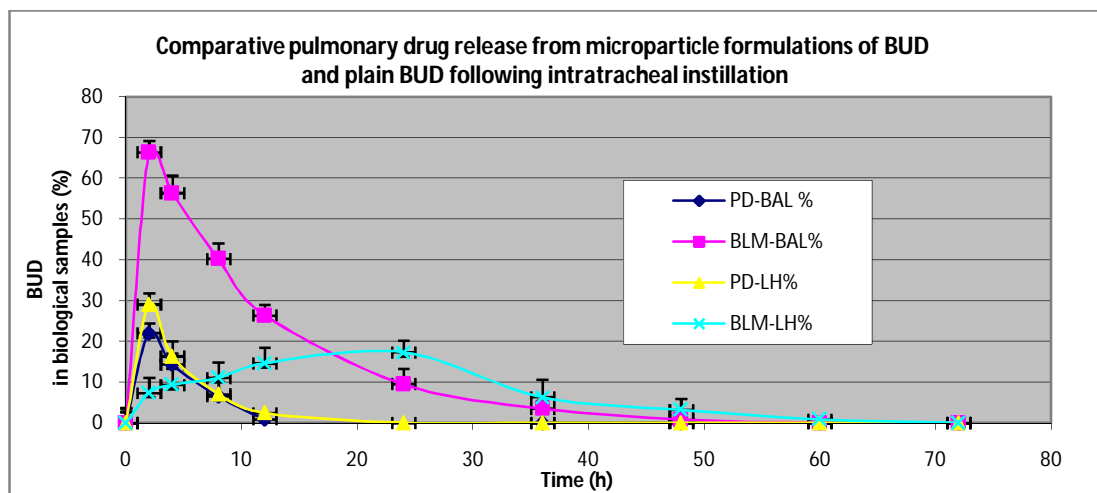


Figure 2: BUD level in biological samples following instillation of PD- plain drug and formulation (BLL)

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

Run No.	Atomization Pressure (bar)	Inlet Temp ^o C	Particle Size d ₉₀ (μ)	% Yield
1	1.5	35	6.452	13.56
2	1.5	40	4.964	15.81
3	1.5	45	4.542	17.88
4	2	35	5.567	15.08
5	2	40	4.601	17.78
6	2	45	3.890	19.43
7	2.5	35	3.860	18.48
8	2.5	40	3.424	19.69
9	2.5	45	3.315	21.24

Table 2: Selected formulations for further studies

Details of the formulation	Mean Particle Size (μm)/ d_{90}	% Entrapment	% Yield
SBS			
SBS-PLGA 60:40	3.362	70.27	21.04
SBS-PLA 50:50	3.038	67.05	19.34
BUD			
BUD -PLGA 60:40	3.129	69.27	21.73
BUD -PLA 50:50	3.057	71.57	20.92

Table 3: SBS level in biological samples following instillation of plain drug and formulation (SLM)

SBS in biological samples (%) (mean \pm SD, n=5)				
Time (h)	Bronchoalveolar Lavage (BAL)		Pulmonary Homogenate (PH)	
	PD %	SLM%	PD%	SLM%
2	16.35 (2.65)	63.26 (2.34)	39.46 (3.18)	5.356 (2.67)
4	11.46 (1.36)	56.34 (3.16)	20.34 (2.84)	7.366 (2.88)
8	2.14 (4.23)	42.45 (2.74)	8.452 (4.23)	9.353 (3.98)
12	0.178 (3.53)	26.35 (4.14)	0.275 (2.85)	11.45 (3.42)
24	0	10.46 (1.74)	0	16.52 (2.78)
36	0	3.56 (2.69)	0	8.58 (2.77)
48	0	0.56 (1.87)	0	3.78 (2.56)
60	0	0	0	0.43 (2.29)
72	0	0	0	0

Table 4: BUD level in biological samples following instillation of plain drug and formulation (BUD)

BUD in biological samples (%) (mean \pm SD, n=5)				
Time (h)	Bronchoalveolar Lavage (BAL)		Pulmonary Homogenate (PH)	
	PD %	BLM%	PD%	BLM%
2	22.013 (3.61)	66.356 (3.78)	29.053 (2.74)	7.346 (2.78)
4	14.355 (2.45)	56.355 (2.85)	16.346 (2.88)	9.345 (3.74)
8	6.574 (2.74)	40.244 (4.25)	7.066 (3.68)	11.056 (4.08)
12	0.895 (3.63)	26.35 (3.74)	2.463 (4.07)	14.63 (3.75)
24	0	9.553 (2.65)	0	17.324 (3.85)
36	0	3.546 (3.77)	0	6.345 (2.89)
48	0	0.784 (1.85)	0	3.256 (4.26)
60	0	0	0	0.893 (2.67)
72	0	0	0	0

Table 5: Mean pharmacokinetic parameters of plain drugs and formulations

Parameter	Plain SBS	SLM	Plain BUD	BLM
AUC ^{60h} ₀ ($\mu\text{g/ml}\cdot\text{h}$)	106.59	516.59	92.79	382.24
AUMC ^{60h} ₀ ($\mu\text{g/ml}\cdot(\text{h})^2$)	459.68	8967.52	478.68	8385.49
MRT (h)	4.312	17.358	5.158	21.937
C _{max} (μg)	39.46	16.52	29.053	17.324
T _{max} (h)	2	24	2	24
SEEF	-	4.846	-	4.119

Note: T_{max} and C_{max} are not determined by integrating the values over the entire time period of study as the study had different set of animals used at each sampling point. Only static values obtained in the study are taken as such.

CONCLUSION

Drug concentrations were maintained in the PH for longer durations and disappeared from the BAL less rapidly demonstrating the superiority of formulations compared to the plain drugs. Enhancement of AUC, MRT and SEEF confirms that sustained drug release was provided by the formulations of both the drugs in comparison to plain drugs. However, prior to clinical application, the presented DPI formulation need more further thorough and extensive pharmacological and toxicological studies in animals.

Inhalable microparticles containing high payload of anti-asthmatic drugs can be prepared as a dry powder inhalation targeting alveolar macrophages as well as for systemic circulation. These may be expected to control drug release in macrophages as well as in systemic circulation. Microparticle residence and biodegradation in vivo in macrophages need to be established.

The entrapment of antiasthmatic 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 asthma.

Abbreviations:

Salbutamol sulphate (SBS), budesonide (BUD), poly (DL-lactide) (PLA), poly (DL-lactide/glycolide copolymer) (PLGA), Dry Powder Inhaler (DPI), area under the curve (AUC), site exposure enhancement factor (SEEF), pulmonary homogenate (PH), bronchoalveolar lavage (BAL), mean residence time (MRT).

REFERENCES

- Frieden, T.R., Sterling, T.R., Munsiff, S.S., Watt, C.J., Dye, C., Tuberculosis, *The Lancet*, 362(9398), 2003, 1858-1859.
- World Health Organization – Global surveillance, prevention and control of chronic respiratory disease: a comprehensive approach, 2007.
- Singh, S., Tuberculosis. *Current Anaesthesia & Critical Care (Focus on: Tropical Diseases)*, 15(3), 2004, 165-171.
- Katzung, B. G., "In; *Basic & Clinical Pharmacology*", 8th Edn., McGraw-Hill, San Francisco, U.S.A., 2001, 33-43.
- Williams, D. A., Lemke, T. L., "In; *Foye's Principles of Medicinal Chemistry*", 5th Edn., Lippincott, Williams and Wilkins, Philadelphia, U.S.A., 2002, 304.
- Gavini, E., Rassa, G., Muzzarelli, C., Cossu, M., and Giunchedi, P., Spray-dried microspheres based on methylpyrrolidinone chitosan as new carrier for administration of metoclopramide, *Eur. J. Pharm. Biopharm.*, 68(2), 2008, 245-253.
- Nettey, H., Haswani, D., Oettinger, C.W., and D'Souza, M.J., Formulation and testing of vancomycin loaded albumin microspheres prepared by spray-drying, *J. Microencapsul.*, 23(6), 2006, 632-642.
- Oster, C.G., and T. Kissel, T., Comparative study of DNA encapsulation into PLGA microparticles using modified double emulsion methods and spray drying techniques, *J. Microencapsul.*, 22(3), 2005, 235-244.
- Youan, B.B., Microencapsulation of superoxide dismutase into biodegradable microparticles by spray-drying, *Drug Delivery*, 11(3), 2004, 209-214.
- Naikwade, S., Bajaj, A., Preparation and in vitro evaluation of budesonide spray dried microparticles for pulmonary delivery, *Sci. Pharm.*, 77, 2009, 419-441.
- Clarke, N., O'Connor, K., and Ramtoola, Z., Influence of formulation variables on the morphology of biodegradable microparticles prepared by spray drying, *Drug Dev. Ind. Pharm.*, 24 (2), 1998, 169-174.
- Khilnani, G., and Banga, A., Aerosol therapy, *J. Indian Acad Clin Med.*, 5, 2004, 114-123.
- Newman, S.P., and Busse, W.W., Evolution of dry powder inhaler design, formulation and performance, *Respir Med.*, 96, 2002, 293-304.
- Telko, M.J., and Hickey, A.J., Dry powder inhaler formulation, *Respir Care.*, 50, 2005, 1209-1227.
- Csaba, N., Gonzalez, L., Sanchez, A., and Alonso, M.J., Design and characterisation of new nanoparticulate polymer blends for drug delivery, *J. Biomat. Scienc. Polymer*, 15(9), 2004, 1137-1151.
- Bramwell, V.W., and Perrie, Y., Particulate delivery systems for vaccines, *Crit Rev Ther Drug Carrier Syst.*, 22(2), 2005, 151-214.
- Lohade, A. A., Singh, D. J., Parmar, J. J., Hegde, D. D., Menon, M. D., Soni, P. S., Samad, A., and Gaikwad, R. V., Albumin microspheres of fluticasone propionate, *Indian J. Pharm. Sci.*, 69 (5), 2007, 707-709.
- Parikh, B.V., Upadrashta, S.M., Neau, S.H., Oestrone loaded poly (l-lactic acid) microspheres: preparation, evaluation and in vitro release kinetics, *J. Microencapsul.*, 10, 1993, 141-153.
- Tozuka, Y., Takeuchi, H., Fine particle design and preparations for pulmonary drug delivery, *Drug Delivery System.*, 23 (4), 2008, 467-473.
- Stephen, P. N., Gary, R. P., In Vitro/In Vivo Correlations for Respiratory Drug Delivery, *Clin. Res. Regul. Aff.*, 22(1), 2005, 15-30.
- Chiraz, J., Veronique, A., Abdelhamid, E., Hatem, F., Assessment methods of inhaled aerosols: technical aspects and applications. *Expert Opin. Drug Deliv.*, 6 (9), 2009, 941- 959.
- www.drugdel.com/Pulm_review.pdf.
- Kevin, E. D., Daniel, L. C., Gary, H., Rogene, H., Gunter, O., Harry, S., Richard, B. S., Intratracheal Instillation as an Exposure Technique for the Evaluation of Respiratory Tract Toxicity: Uses and Limitations, *Toxicol. Sci.*, 55(1), 2000, 24-35
- Joshi, M., Misra, A. N., Pulmonary disposition of budesonide from liposomal dry powder inhaler, *Methods Find Exp. Clin. Pharmacol.*, 23(10), 2001, 531-536.
- Yiwei, Q., Ganlin, Z., Dongfang, L., Zachary, S., Mallik, S., Shiladitya, S., Ganesh, V., Robert, L., Ram, S., Delivery of therapeutic levels of heparin and low-molecular weight heparin through a pulmonary route, *Proc. Natl. Acad. Sci. U S A*, 101(26), 2004, 9867-9872.



26. John, C. L., William, D. B., Chong, S. K., Robert, B. D., Philip, Retention and Intracellular Distribution of Instilled Iron Oxide Particles in Human Alveolar Macrophages, A. B., Am. J. Respir. Cell Mol. Biol., 18, 1998, 687-695.
27. Hiroshi, S., Masahisa, K.,Yoshikiyo, A., The efficacy of intratracheal administration of vecuronium in rats, compared with intravenous and intramuscular administration, A & A., 103(3), 2006, 601-607.
28. Jacono,F. J., Peng, Y. J., Nethery, D., Faress, J. A., Lee, Z., Kern J. A., Prabhakar, N. R., Acute lung injury augments hypoxic ventilatory response in the absence of systemic hypoxemia, J. Appl. Physiol., 101, 2006, 1795-1802.
29. Surti, N., Naik, S., Misra, A., Pharmacokinetic Evaluation of Wheat Germ Agglutinin-Grafted Nanoparticles of Mometasone Furoate, Sci. Pharm., 77, 2009, 123-133.
30. Chougule, M., Padhi, B., Misra, A., Nano-liposomal dry powder inhaler of tacrolimus: preparation, characterization, and pulmonary pharmacokinetics, Int. J. Nanomedicine, 2(4), 2007, 675-688.
31. Standard operating procedure for animal tissue homogenization, New animal models for invasive Aspergillosis (IA), NIH-NIAIN-N01-30041.
32. Bakhle, Y. S., Benharari, R. R., Metabolism of angiotensin and bradykinin in rat isolated lungs during the oestrous cycle [proceedings], J. Physiol., 291, 1979, 11-17.
33. Verma, R. K., Kaur, J., Kumar, K., Yadav, A. B., Misra, A., Intracellular time course, pharmacokinetics, and biodistribution of isoniazid and rifabutin following pulmonary delivery of inhalable microparticles to mice, Antimicrob. Agents Chemother., 52 (9), 2008, 3195-3201.
34. Edsbäcker, S., Andersson, K.E., Ryrfeldt, A., Nasal bioavailability and systemic effects of the glucocorticoid budesonide in man, Eup. J. Clin. Pharmacol., 29(4), 1985, 477-481
35. Hindle, M., Chrystyn,H., Determination of the relative bioavailability of salbutamol to the lung following inhalation, Br J Clin Pharmacol., 34(4), 1992, 311–315.
36. Remigius, U. A., Michael, I. U., Michoel, A., Renaat, K., Norbert, V., The lung as a route of systemic delivery of therapeutic protein and peptides, Respir. Res., 2, 2001, 198-207.
37. Malgorzata, S., Vandamme, T., Sokolowski, A., Nanocarriers as pulmonary drug delivery systems to treat and to diagnose respiratory and non respiratory diseases Int. J. Nanomedicine, 3(1), 2008, 1-19.

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