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Research Article

Treating tuberculosis with Chitosan microparticles loaded with rifampicin as respirable powder for pulmonary delivery

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ABSTRACT

Microparticles containing antitubercular agent, Rifampicin, was prepared and evaluated for its suitability as respirable dry powder formulation for lung delivery**.** Chitosan solution containing rifampicin, lactose and leucine in different ratio was spray dried. Ascorbic acid was used in concentration of 200µg/ml as antioxidant. The other physical properties of prepared microparticles like Particle size, morphology, densities and aerodynamic diameter were determined. Solid state of microparticles was characterized by Differential scanning calorimetry (DSC) and X-ray diffraction studies. The fine particle fraction and emitted dose required for a good aerosol performance was determined by Andersen Cascade Impactor. Microparticle with maximum drug loading of 98.31and particle size in range 5-7µm was obtained with % yield of ≤ 43 %. Satisfactory aerosol properties like FPF (47.29 %), emitted dose up to 92 % and theoretical aerodynamic diameter less than 3.62 µm were obtained. The in vitro drug release from chitosan microparticles was found to be slow and up to 68 to 84 % in 6 hrs. When drug release data were plotted in Ritger and Peppas model, all formulations except controlled formulation showed fickian diffusion kinetic. The present study revealed that the rifampicin loaded chitosan microparticles produced by spray drying showed good physicochemical properties deemed suitable for inhalation therapy.

INTRODUCTION

Pulmonary drug delivery system is gaining popularity amongst the research arena due to its effective ways of delivering drugs for the treatment of asthma, chronic obstructive pulmonary disease and other respiratory diseases. It is possible to achieve local and systemic delivery of drug with lung targeting. Recently, sustained release microspheres have been proposed for pulmonary delivery, which major advantage is the formulation parameters targeted to the deep lung, therefore can be used as targeted and sustained drug release carriers [1, 2].

Recently, biodegradable microspheres are becoming increasingly popular in the design of pulmonary drug delivery systems. However, as inhaled microspheres, biocompatibility is an important index to evaluate whether the microspheres can be applied in the pulmonary drug delivery systems because the inferior biocompatibility which are known to be an obstacle especially for pulmonary delivery systems [3].

Tuberculosis (TB) remains a major cause of mortality. Approximately more than 2 billion people, equal to one-third of the world's population, are infected with TB bacilli, Mycobacterium tuberculosis (MTB). Both the high prevalence of TB in patients infected with the human immunodeficiency virus (HIV) and the alarming increase in drug resistance have made tuberculosis (TB) an emerging threat [4]. Directly observed therapy (short course) or DOTS, recommended for patients with pulmonary TB[5] is reported to cure approximately 80% of Indian[6] and 95% of Chinese [7] patients completing the full course. These observations suggest that 5 to 20% of Asian patients are not cured by DOTS. One reason for treatment failure could be that drug concentrations achieved in the cytosol of target cells through oral administration are not

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sufficient to kill bacteria residing within. The failure of antitubercular chemotherapy is mainly due to patient non compliance [8] which is attributed to the requirement of multidrug administration daily or several times a week for at least 6 months.

In recent years, one of the best ways to achieve higher drug levels in the lungs has been the development of new formulations (nanoparticle-based) that are directly delivered to the lungs via the aerosol route [9].Targeting drug to the alveolar macrophage may improve efficacy and potentially reduce systemic toxicity. In addition, the high frequency of pulmonary tuberculosis demands the development of novel drug delivery approaches that enhance the bioavailability of drugs at the level of lungs. The dry powder inhaler (DPIs) has become widely known as a very attractive platform for drug delivery. Many patients have traditionally used DPIs to treat asthma and chronic obstructive pulmonary disease. The current market for DPIs has over 20 devices presently in use, and many devices under development for delivering a variety of therapeutic agents. DPIs are recognized as suitable alternatives to pressurized metered dose inhalers for some patients, but the performance of DPI devices may vary according to a given patient's physiological condition[10]. Rifampicin (RIF) and isoniazid are both first-line drugs for use in the therapy of tuberculosis and are included in the list of recommended drug regimens for treatment of latent *M. tuberculosis* infection in adults [11].

Chitosan a natural cationic polysaccharide, has many applications in pharmaceutical and biological fields, which have been extensively studied and investigated.[12], due to its favorable characteristics such as nontoxicity, biocompatibility, biodegradability and properties such as bioadhesion. In past few years chitosan microspheres were investigated for delivery of conventional drugs as well as protein and DNA [13]. The study of chitosan microsphere formation by the spray-drying method is justified by interesting results presented in the literature. Chitosan microspheres obtained by spray drying method are characterized by high sphericity and specific surface area which are important for inhalable powder formation for pulmonary drug delivery systems.

A high localized concentration of the drug can be given in cases where the organisms are resistant to the therapeutic oral dose [14, 15]. Microsphere technology has been used to develop formulations of rifampicin for targeted delivery to host macrophages [16]. Reports suggested that treatment of MTB-infected monocyte cell lines with RIF-loaded microspheres resulted in a significant decrease in the number of viable bacteria at 7 days following initial infection [17]. Thus, a microparticle drug delivery system targeting to the alveolar macrophages might contribute to improved chemotherapy of TB. In addition, encapsulated drugs had marginal side effects compared to the free drug. The present study was aimed to prepare chitosan microparticles loaded with rifampicin for lung delivery of drug. The physical characteristics of prepared microparticles were investigated for its aerosolization behavior.

MATERIAL AND METHOD

Rifampicin (RIF) was obtained as gift sample from Strides Acrolab, chitosan was generously provided by Indian institute of fisheries, Cochin. α, monolactose was obtained from Meggle, Wasserburg Gmbh and Co., Germany as a gift sample. Ascorbic acid was purchased from S D fine chemicals, Baroda, India. L leucine was purchased from Loba Chemicals, India. All Other chemicals and solvents used were of analytical grade.

Preparation of chitosan microparticles

Microparticles were prepared by spray dried technique. Chitosan (0.75 % was dissolved in 300ml of 1% acetic acid solution at 50°C by mechanical stirring), rifampicin (in polymer to drug ratio 8:1, 4:1 and 2:1) was prepared by dissolving rifampicin in 10 ml of methanol and mixed with previously prepared and filtered chitosan solution. When leucine and lactose were added; RIF, Chitosan, lactose and leucine was in the ratio of 4:1:1:1, 4:1:1:2, 4:1:1:3. Ascorbic acid was added in concentration of 200µg/ml, as antioxidant,. The solution prepared was then spray dried from standard 0.7mm nozzle. The spray drying conditions such as inlet temperature, outlet temperature, pump prate, pressure and aspirator setting were set as 160°C, 80-85°C, 5 ml/min, 2.5 kg/cm^2 and 45 m^3/h respectively. The dried microparticles were collected from the port of cyclone separator.

Drug loading

The yield of spray dried product was identified as the percentage of anticipated yields. The rifampicin content of powder was measured in triplicate, analyzing by UV spectroscopy and expressed as the percentage of nominal load.

The drug loading capacity for each drug (expressed as mg of drug/mg of microparticles) was calculated by the formula: amount of drug (mg) released from the lysed microparticles/amount of microparticles (g) put for lysis.

Scanning Electron Microscopy

Surface morphology of spray dried chitosan microspheres were obtained by using scanning electron microscope (ESEM TMP with EDAX, Philips, Holland). Spray dried powders were mounted onto separate, adhesive coated 12.5 mm diameter aluminium stubs. Excess powder was removed by tapping the stubs sharply and then gently blowing a jet of particle free compressed gas across each. The specimen was examined using EDAX SEM. The SEM was operated at high vacuum with accelerate voltage of 5KV and specimen working distance of 12mm.

Differential scanning calorimetric analysis

Differential scanning calorimetry (DSC-PYRIS-1, Perkin Elmer,USA) were used to investigate the effect of incorporation of drug on chitosan matrix. Pure drug, physical mixture and spray dried powder were heated from 30 to 300 ° *C at* scanning rate of 10° C /min. A physical mixture of the blank microspheres and the pure drug was used as control.

X ray diffraction (XRD) studies

The physical state of the model drug (INH) and in the spray dried chitosan-TPP microspheres were assessed by XRD studies. X ray diffraction patterns of pure drug, physical mixture, chitosan microspheres loaded with drug were obtained using XRD Diffractometer (powder) Philips Xpert MPD Range (2θ): 3º to 136º.

Particle Size analysis

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. The data were expressed as the volume weighted mean particle size.

In vitro drug release studies

Dissolution testing was performed as described by Amit Misra et al[18]. Briefly, Dissolution test was performed on a USP Type II tablet dissolution test apparatus (VEEGO) at a stirring speed of 150 rpm. A dialysis membrane (Himedia, LA 401) was cut into equal pieces of about 5 cm x 3 cm and pre-treated with phosphate buffer 7.4. Microspheres (50 mg) were accurately weighed out on the pre-treated dialysis membrane and sealed with clips. The pouch thus formed was attached to the paddles of the apparatus using cotton threads over the clips. 900 ml of phosphate-buffer, pH 7.4, was used as a dissolution medium to ensure sink conditions. Samples were withdrawn for analysis at specified time points, and assessed for rifampicin content by UV spectroscopy (Shimadzu UV-1700, Japan) at 475 nm. Each dissolution experiment was performed in triplicate.

Determination of Powder density and theoretical aerodynamic diameter

The poured density of the spray dried powder was determined by pouring known mass of powder (approximately 0.5g) under gravity into a calibrated measuring cylinder and recording the volume occupied by the powder. The tapped densities of the spray dried powders were determined by volume measurement of tapped mass until no further change a in the powder volume was observed. Measurement was performed in triplicate (n = 3).

Theoretical estimation of the particle primarily aerodynamic diameter $_{Dae}$ were derived from the sizing (d) and tapped density data (*p*) as follows [19].

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

Characterization of Aerosol performance of microparticles

The in vitro aerosol performance of Rifampicin loaded chitosan microparticles was evaluated using Andersen cascade Impactor.The cascade impactor consists of throat, a preseparator and eight stages (0-7). A hydroxypropyl methyl cellulose capsules was filled with 15mg of rifampicin or rifampicin microparticles. The inhalation test was performed at an inhalation rate of 60L/min for 10 secs [20]. The particles deposited and retained on capsule, device, throat, preseparator and at each stage of ACI was rinsed with 0.1 N HCl containing 200µg/ml and drug content were determined after serial dilution at 264nm using UV spectroscopy

(Shimadzu 1700). The fine particle fraction, which is total percentage deposited at stage2-7 of the cascade impactor was used to evaluate aerosol performance. A higher fine particle fraction deposited is thought to indicate a higher in vitro aerosol performance [21].

RESULTS

Particle size analysis and microscopy

The SEM micrograph of chitosan microparticles loaded with rifampicin is shown in Fig 1. The microparticles produced were of irregularly spherical in shape with wrinkles on surface. The morphological examination depicts the porous microparticles were generated on spray drying of feed solution. Porous structure formation was indicative of the larger surface area with low density particles. The microparticles were seen as individual entity rather than the agglomerates. The particle diameter obtained by the particle size analyzer was in the range of 4.85 to 7.13 µm as the D_{50} values.

Differential scanning calorimetric analysis

The differential calorimetric studies showed endothermic peak of rifampicin pure powder at 189.25º C in the thermogram which is shown in the Fig 2. The thermograms were obtained by scanning the sample at rate of 10ºC per min. The thermogram of D3, drug loaded microparticles, showed a peak at 125ºC and a peak at valley nearly at 240-260 ºC which indicated complexation and incorporation of rifampicin in chitosan matrix. The initial peak at around 125ºC may be due to the presence of moisture in microparticles.

X ray diffraction study

Fig. 3 shows X-ray diffraction patterns of rifampicin pure, controlled and spray dried RIF loaded chitosan microparticles. The peaks on x ray diffractogram indicate the crystalline form of RIF pure form, but these peaks were not observed in chitosan microparticles. The X-ray diffraction pattern indicated the amorphous nature of rifampicin present and /or less perfect and smaller crystals in the microparticles. There are chances of amorphous form of rifampicin getting solubilization in chitosan matrix.

Yield and Drug content

The yield of resultant powder of spray dried material depends on the process parameters and solid content of the liquid feed system. The spray dried powder obtained showed % yield in the range of 23 to 43 percentile. When the proportion of drug to polymer was increased the % yield was found to be increased. Addition of lactose and leucine as bulking agent and dispersing agent showed increase in yield that may be due to the increase in total solid content of the feed system. The effective drug loading from 90.81 to 98.31 % of the anticipated amount (Table 1) was observed. The drug loading was slightly decreasing when chitosan content was increased.

In vitro Drug release studies

For drug release studies dissolution studies were carried out in phosphate buffer at pH 7.4, dissolution medium, for 6 hours. It was observed that the chitosan microparticles swelled in short time period and faster drug release of 40 to 60 % in first hour was observed. The slow drug release was seen with the microparticles prepared with chitosan to drug ratio of 4:1 as compared to other formulations. The drug release was found to be slower after immediate release in all the batches prepared (Fig 4). Total drug release of 67.6 to 79.81 % was seen in 6 hrs in pH 7.4 phosphate buffer from the different formulations. The addition of lactose and leucine was not found to affect the drug release from chitosan microparticles. The $T_{50\%}$ and $T_{70\%}$ of the three formulations having different chitosan to drug ratio is shown in the Table 3.

Determination of powder density and theoretical aerodynamic diameter

The spray dried powder was evaluated for bulk densities and tapped densities. The tapped density of powders was in the range of 0.150 to 0.344 g/ml, as shown in table 2. The tapped density was increased when the lactose concentration was high (lactose: leucine = 1:1). There tapped density of controlled formulation was much lesser than the other formulations containing polymer and additives. The aerodynamic diameter ($_{Dae}$) of microparticles was calculated in the bases of the particle diameter and the tapped density data obtained. The aerodynamic diameter was in the range of 1.85 to 3.62 µm.

Table 1: Formulation component for preparation of chitosan microparticles

Formulation	Composition Chitosan:Rifampicin	lactose: leucine	Theoretical drug content (%)	Actual Drug loading (%)
F0	0:1	-	----	---
F1	8:1	-	11.11	10.09
F2	4:1	-	20.00	19.21
F3	2:1	-	33.33	32.77
F4	4:1	1:1	20	20.12
F5	4:1	1:2	20	19.11
F6	4:1	1:3	20	18.65

Table 2: Aerosolization properties of prepared chitosan microparticles

Formulation	Tapped density	Theoretical Aerodynamic diameter (D_{ae})	Particle size (µm)	% FPF
F0	0.150	1.87	4.85	20.04
F1	0.258	3.62	7.13	29.65
F2	0.265	3.14	6.10	35.44
F3	0.227	2.74	5.76	43.18
F4	0.344	3.04	5.19	45.61
F5	0.290	2.88	5.35	47.29
F6	0.218	2.55	5.48	46.12

Table 3: The kinetic constants (k), Diffusional exponent (n), Correlative coefficient (r) and time taken for 50% drug release

Formulation	r value	n value	K value	$T_{50\%\ (min)}$	$T_{70\%\ (min)}$
F1	0.9032	0.3253	1.1253	47	107
F2	0.9087	0. 3055	1.1009	65	~360
F3	0. 8872	0. 2405	1.2709	42	320

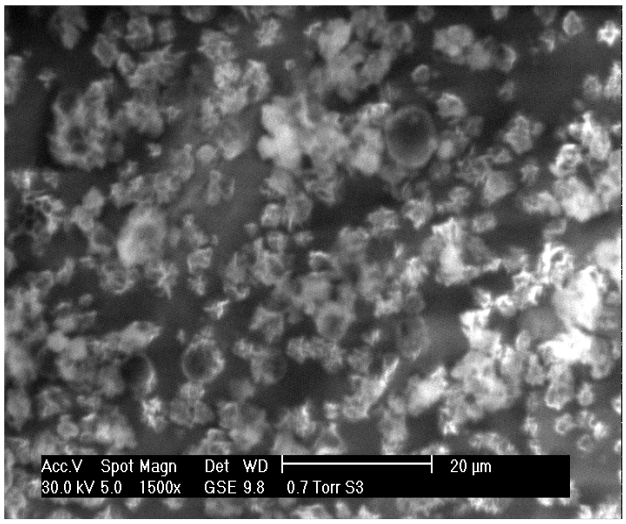

Figure 1: SEM photomicrograph of chitosan loaded chitosan microparticles

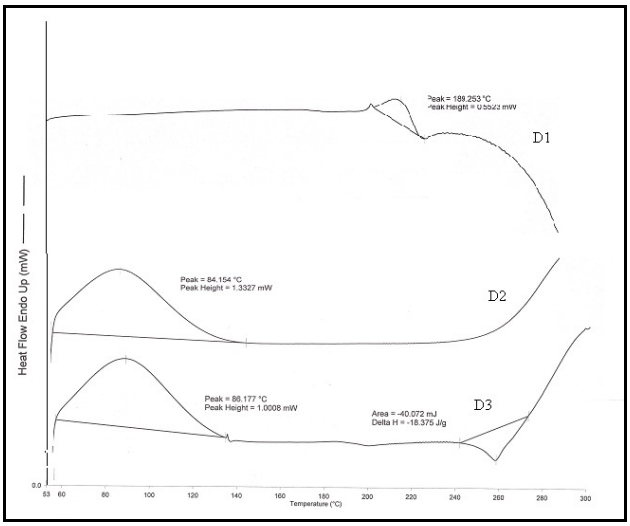

Figure 2: DSC thermogram of pure rifampicin powder (D1), The physical blend of Chitosan: Drug (D2) and Chitosan microparticles loaded with rifampicin.

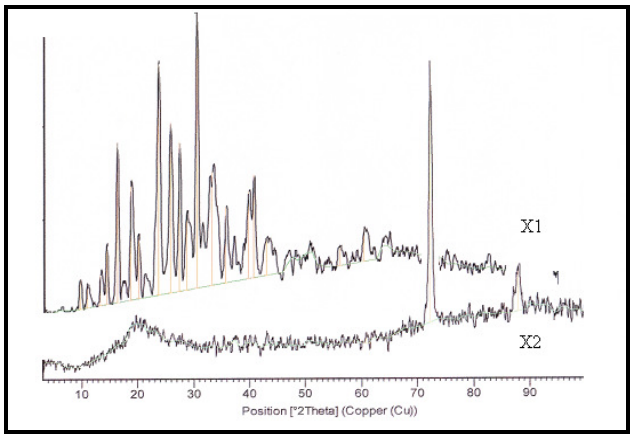

Figure 3: X-ray diffractogram of pure chitosan [X1] and chitosan microparticles [X2]

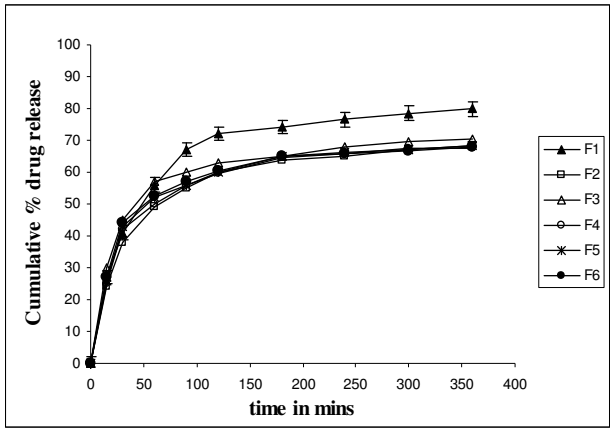

Figure 4: *In vitro* drug release profile from rifampicin chitosan microparticles of different batches (F1-F6)

Aerosol performance of chitosan microparticles

The emitted dose from the capsule was found in the range of 85-90% of the total respirable dose. Addition of leucine was found to improve the release of powder from capsule by increasing dispersibility. There was little difference observed when chitosan concentration was changed from 8 parts to 2 parts per one part of drug. An average 30 percentile of drug was found to be retained in preseparator and mouth piece. The fine particle fraction FPF) of drug deposited on the stages from 2-7 in Andersen cascade impactor is shown in table 2. The FPF was found in the range of 29-48% and deposition at last stages (6-7) was negligible.

DISCUSSION

Rifampicin loaded chitosan microparticles in the form of dry powder formulation for inhalation to lungs were prepared by spray drying technology. Instead of preparation of blend of drug-carrier, the components of formulation were spray dried together to produce final product. Different chitosan to drug ratio were experimented for preparation of chitosan microparticles in addition with lactose and leucine as bulking and dispersing agent respectively. Although several methods are available for preparation of microparticles, spray drying was especially used to produce respirable dry powder. Chitosan is a polysaccharide and natural polymer and being available abundantly used as carrier and a sustained release polymer for slow pulmonary delivery of rifampicin.

The chitosan microparticles loaded with rifampicin were obtained by spraying the aqueous chitosan solution containing rifampicin and additives from 0.7 mm nozzle to a drying chamber. The droplets coming out from the nozzle were immediately dried in drying chamber circulated with hot air; and the product in the form of dry powder was collected in cyclone separator. The % yield obtained was in the range of 23 to 43 % of the total amount anticipated. The increase in % yield was seen on addition of bulking agent and dispersing agent. The increase in yield was the reflection of increase in total solid content of the feed solution. There was an improvement seen in bulk densities and tapped densities with respect to addition of lactose and leucine in different proportion. The tapped density was best achieved when lactose and leucine was in ratio of 1:3 with chitosan to drug concentration of 4:1. Although the controlled rifampicin formulation showed least tapped density of 0.150g/ml, was the exception. Scanning electron microscopy was used to study morphological characteristics of prepared microparticles like the particle diameter, structural and surface morphology of the spray-dried powders (Fig. 1). The SEM micrograph revealed the porous nature of the chitosan microparticle obtained by spray drying. The controlled rifampicin formulation also showed the same surface properties. It was indicated that the porous nature of microparticles was more influence by the rifampicin itself. The porous and uneven surface of microparticles found to be contributed towards the low tapped densities. Due to these, the theoretical aerodynamic diameter calculated based on tapped density was below the 5µm which was a good indication for the aerosol behavior of the particles especially when it is to be utilized as dry powder form. The tapped density was higher in formulation when Lactose to leucine ratio was 1:1 and found least when the ratio was 1:3. The mass median aerodynamic particles must be less than 10 µm or less than or equal to 5µm for effective deposition of microparticles in deep lung. The theoretical aerodynamic diameters were found in the range of 1.87 to 3.62 µm. The particle size ($D_{50\%}$) of the prepared microparticles was in the range of 5 to 7 µm with narrow particle size distribution.

The drug release studies were carried out in USP dissolution type II apparatus as lack of specific apparatus to study drug release in lung environment. The initial study released that the chitosan microparticles swelled very fast due to small size and porous nature of microparticles. The faster adsorption and retention of water resulted in initial burst release followed by a subsequent slower release. The swelling and release of drug from the chitosan microparticles depend on the molecular weight and matrix formation as well as the diffusion path of the chitosan matrix [22]. All the formulation showed initial release of up to 60% in first hour followed by slow release of up to 70-80 % in next five hours. The surface adhered rifampicin released quickly within first hour. The slow release from the chitosan matrix was later governed by the diffusion ability (followed Fick's first law of diffusion) that was confirmed when drug release data were evaluated by Ritger and Peppas model. Here, logarithm of the cumulative drug release was plotted as a function of time. In each batch linear relationship was observed. The mechanism parameter n value, the correlation coefficient [r value], rate of release [k value, obtained from the intercept on the y axis] and the time taken to for 50%&70% drug release are presented in table 3. As reported by Ritger-Peppas, the n value is an empirical value which characterizes the drug release mechanism. On the basis of diffusion exponent, n value below 0.45 indicates the drug release mechanism approaches that of Fickian diffusion controlled release. An n value of 0.45 to 0.89 represents a drug release mechanism for non Fickian diffusion or chain relaxation controlled release. While n value above 0.89 indicates that the drug release approaches zero order release [23].

In vitro deposition study of rifampicin chitosan microparticle was carried out in Andersen Cascade Impactor (ACI). Lung deposition is the most important parameter to measure the efficacy of a pulmonary drug delivery system. Important information of an inhalation delivery system is obtained by evaluating the emitted dose. Emitted dose was found to be in the range of 92 to 81 % of total dose released. Formulation F5 and F6 showed less drug retention in mouthpiece and preseparator among the different formulations. An average of ~30 percentage of drug was found to be deposited in mouthpiece and preseparator. The higher drug deposition at mouthpiece and preseparator may be result of inter particle aggregation because of existing cohesive forces between particles or presence of larger particles. Number of investigators has reported that the microsphere prepared with spray drying technique shows

adhesion and cohesion due to fine particle formation. The FPF for different formulation is shown in table 2. The highest FPF was observed with formulation containing lactose and leucine in the ratio of 1:3. The controlled formulation where rifampicin alone was spray dried showed highest (44.6%) amount of drug retention in capsule and mouth piece compared to other spray dried formulations. The emitted dose was increased when lactose and leucine was added in microparticle formation. The addition of leucine improved the densities and ultimately emitted dose and FPF.

CONCLUSION

The study demonstrated that the dry powder formulation with chitosan as carrier could be effectively produced by spray drying technology. It was observed that the chitosan concentration influenced particle size and drug release behavior of prepared microparticles. When Leucine and lactose were added in the formulation, they were found to increase the yield as well as the aerosol properties in the form of higher fine particle fraction (FPF) which is very important for any inhalable formulation. Microparticles generated through spray drying technology were remained as individual entities with less aggregation. The characterization of microparticles in terms of size and shape showed that spray drying technique can be useful for preparation of respirable powder. Being a natural polymer chitosan can be effectively utilized by selecting different grades for preparation of an economical and sustained release pulmonary drug delivery system.

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