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Research Paper

Xyloglucan based Microspheres for Pulmonary Delivery of Rifabutin Dry Powder Inhaler

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ABSTRACT

Pulmonary delivery of anti-tubercular drugs can be an effective treatment for tuberculosis. The main objective of the present work was to prepare rifabutin loaded xyloglucan microspheres as dry powder inhaler for pulmonary delivery. Xyloglucan is natural polysaccharide with mucoadhesive property and temperature responsive gelling ability allows its application in microspheres development. Xyloglucan microspheres were prepared by using single step spray drying process using lactose. All microsphere formulations were evaluated for various physical properties such as density and flow. On the basis of results of evaluation parameters such as entrapment efficiency, mucoadhesion, swelling and *in vitro* drug release, microspheres with 2% xyloglucan and 1% lactose monohydrate were found to be most favorable. They possessed morphology and particle size distribution suitable for pulmonary administration. XRD studies reveal amorphous nature of microspheres. *In vitro* DPI performance demonstrates suitability of xyloglucan based microspheres for pulmonary delivery. In conclusion, it is suggested that this natural polymer based microspheres containing rifabutin DPI formulation could be used as a significant enhanced treatment for TB.

KEYWORDS: Xyloglucan; Microspheres; Pulmonary delivery; Dry Powder Inhalation.

Introduction

Considerable research is being directed towards developing polymeric particles for drug delivery (Panyam and Labhasetwar, 2003; Shenoy and Amiji, 2005). Xyloglucan is a natural polysaccharide isolated from the seed kernel of Tamarindus indica. It possesses properties like high viscosity, broad pH tolerance and adhesivity. This leads to its application as stabilizer, thickener, gelling agent in food and a binder in pharmaceutical industries (Nishinari et al., 2000; Glicksman, 1986). In addition to these other important properties of xyloglucan have been identified recently. They include non carcinogenicity, mucoadhesivity, biocompatibility, high drug holding capacity and high thermal stability. This lead to its application as excipients in hydrophilic drug delivery system (Shirakawa et al., 1998; Avachat et al., 2013; Ludwig, 2005). Thus, an attempt was made in this investigation to use xyloglucan as a mucoadhesive polymer for preparation of microspheres.

Rifabutin (RIFB) is a bactericidal antibiotic drug primarily used in treatment of tuberculosis (TB). The drug is a semi-synthetic derivative of rifamycin. Its effect is based on blocking DNA-dependent RNA-polymerase of bacteria. It is effective against Gram-positive and some Gram-negative bacteria, but also against the highly resistant Mycobacteria, such as *Mycobacterium tuberculosis, M. leprae,* and *M. avium intracellulare.*

Moreover, lung is the primary, portal of entry for mycobacteria that cause TB. It has therefore been of interest since 1950s to deliver drugs used in the management of TB via same route (Berishvilli, 1954). Pulmonary delivery has several unique advantages over other delivery routes, such as oral or injection. It circumvents first-pass hepatic metabolism, and thus reduces dose and side effects. Pulmonary delivery also enables the local delivery of therapeutics targeting respiratory diseases such as asthma, COPD, and cystic fibrosis (Labiris and Dolovich, 2003; Patton and Byron, 2007).

Several researchers, including Hickey and colleagues (O'Hara et al., 2000; Suarez et al., 2001). Khuller's groups have proposed the use of inhalable or respirable particulate delivery systems for chemotherapy of TB (Pandey and Khuller, 2005).

Dry powders for inhalation are currently of most interest in the field of pulmonary delivery research for advantages (e.g., higher stability, passive breath activation, high-dose delivery) they offer in comparison with Nebulizers and pMDIs. DPI relies on an indrawn breath of the patient to pull in dry powder. Most (DPI) formulations consist of micronized drug blended with larger carrier particles, which enhance flow, reduce aggregation and aid in dispersion. Moreover, high pulmonary concentrations of medication can be attained without high systemic levels, providing for efficient and effective therapies that spare patients from unwanted side effects (Chan, 2006; Edwrds et al., 1998). In this

context, application of RIFB dry powder inhaler has been proposed for the treatment of tuberculosis.

In this study, we report the successful development of spray-dried powders composed of xyloglucan based microspheres for the lung delivery of RIFB.

Materials and Methods

Materials

Rifabutin (RIFB) was gifted by Lupin Pharmaceutical Pvt. Ltd., Aurangabad, India. Xyloglucan (XYG) (45% galactose removal ratio) was provided by DSP Gokyo Food & Chemicals, Japan. Lactose monohydrate was purchased from Merck Pvt. Ltd., Mumbai, India. All other chemicals and solvents were of the highest analytical grade commercially available.

Preparation of Microspheres

XYG microspheres containing RIFB were prepared using a spray-drying technique. Briefly, a XYG solution (1 - 3%) heated to 50°C- 60°C with continuous stirring at 1000-1200 RPM for 3hrs. RIFB (1%) and lactose monohydrate (0.5-1.5%) was added to the above polymer solution and continue stirring for 30 min, was spray-dried in a spray drier (Spray drier LU222, Labultima, India) using 0.7mm nozzle under the following operating conditions: inlet temperature, 110ºC; outlet temperature, 80ºC; aspirator speed, 40%; feed pump rate, 5 mL/min; atomization pressure, 1.2 bars; and, vacuum, -110 mmWC. A cyclone separator was used to collect the spray- dried product. Particles were collected only from the collection jar and first cyclone.

Evaluation of Microspheres

Physical properties of microspheres: Bulk and tapped density were measured using a tap density tester (ETD 1020, Electrolab, Mumbai, India). Bulk density was determined by filling the powder into 10 ml measuring cylinder and tapped density was measured by tap density apparatus following 1000 taps. Bulk and tapped density values allow the determination of the Carr's compressibility index using the formula:

$$\text{Carr's Index } (\%) = t - b\ /t \times 100 \quad \text{.....(1)}$$

Where t is tapped density and b is bulk density.

Flow property of microspheres was depicted by determination of angle of repose calculated by using the following equation:

$$\tan\ \theta = h/r \quad \text{.....(2)}$$

Production Yield

The production yield of microspheres of various batches was calculated using the weight of the final product after drying with respect to the initial total weight of the drug and polymer used for preparation of microspheres and percent production yields were calculated as per the formula (3) mentioned below (Kellaway and Abd Ei-Hameed, 1997).

Production yield =

$$\frac{\text{Practical mass (microspheres)}}{\text{Theoretical mass (polymer + drug)}} \times 100 \quad \text{.....(3)}$$

Entrapment efficiency: The weighed amount of microspheres was dissolved in 0.1N HCl and kept overnight, and drug content was measured spectrophotometrically (UV 1700, Shimadzu, Japan) at 280.5 NM for rifabutin. The entrapment efficiency (%) was calculated by using the following equations.

$$\textit{Entrapment efficiency (\%)} = \frac{\mathrm{M_{actual}}}{\mathrm{M_{theoritical}}} \times 100 \quad \text{.....(4)}$$

Where, M_{actual} is the actual rifabutin content in weighed quantity of powder of microspheres and $M_{\text{theoretical}}$ is the theoretical amount of rifabutin in microspheres calculated from quantity added in the spray-drying process (Fu-De et al., 2003).

Swelling Behavior

The swelling ability of microspheres was determined by allowing them to swell to their equilibrium in simulated lung fluid. Accurately weighed amount of microspheres were placed on millipore filter (NY 11 0.22 μm) using a Franz diffusion cell (11 mL) with phosphate buffer (pH 7.4) kept for 5 min (Juan et al., 2001).

Degree of swelling was calculated as

$$\alpha = W_s - W_0/W_s \quad \text{.....(5)}$$

Where, α = Degree of swelling

W_o = Initial weight of microspheres and

W_s = Weight of microspheres after swelling

In vitro mucoadhesion study: *In vitro* mucoadhesion test performed using falling liquid film technique was used to determine mucoadhesive property of microspheres (Saraparn et al., 2006). A freshly cut piece, 5 cm long, of sheep lung mucosa obtained from a local abattoir within 1 hour of killing an animal was cleaned by washing with isotonic saline solution. An accurate weight (100 mg) of microspheres was placed on mucosal surface, which was attached over a polyethylene plate that fixed in an angle of 45° relative to the horizontal plane, and pH 7.4 phosphate buffers at 37°C was peristaltically pumped at a rate of 5 mL/min over tissue. After 1 hr, concentration of the drug in the collected perfusate was spectrophotometrically determined.

$$\%\ \text{Mucoadhesion} = \frac{\mathrm{A - B}}{\mathrm{A}} \times 100 \quad \text{.....(6)}$$

Where, A = Actual amount of drug in applied microspheres

B = Amount of drug in wash out liquid

In vitro Drug Release Study

A USP Type II dissolution test apparatus (Electrolab TDT- 08L plus, Dissolution tester USP Mumbai, India) was used at stirring speed of 100 rpm for these studies. A dialysis membrane (Sigma, molecular weight cut-off >12 kDa) was cut into equal pieces of about 6 cm × 2.5 cm and pre-treated with media for 1 h (Shah et al., 2011). Microspheres equivalent to 25 mg were accurately weighed out on pre-treated dialysis membrane and sealed with clips. The pouch thus formed was attached to paddles of apparatus using rubber bands wound over the clips. Seven hundred milliliters of phosphate buffer pH 7.4 was employed as a media to investigate drug release.

The samples were withdrawn from medium at various time intervals and subjected to UV spectrophotometric analysis (UV Shimadzu 1700).

Characterization of Selected Xyloglucan Microspheres

Particle size analysis: The mean particle diameter of xyloglucan microspheres was determined by photon correlation spectroscopy using particle size analyzer (Zeta sizer, ZS -90, Malvern Instruments, UK) at room temperature.

Zeta potential measurement: The microspheres sample was dispersed in deionised water. This dispersion was then filled in zeta cell and placed in the Zeta Sizer (Nano ZS, Malvern Instruments, UK).

Scanning electron microscopy (SEM): The morphology of microsphere powder was examined by scanning electron microscopy (JSM 6390, Japan). Samples of microspheres were dusted on double-sided tape on an aluminum stub and coated with gold using a cold sputter coater to thickness of 400°A, and then imaged using a 20 KV electron beam.

X-ray Diffraction (XRD) Study

The X-ray diffraction (XRD) patterns i.e. crystallinity of plane drug; blank microsphere and drug loaded microsphere were recorded on X-ray diffractometer (Brucker Axs, D8 Advance, Germany).The samples were irradiated with monochromatized Cu Kα radiation and analyzed between 3 and 80^0 (2θ). The voltage and current used were 30 kV and 30 mA, respectively (Jain et al., 2009).

In vitro DPI performance measurement

Assemble the Anderson Cascade impactor (SS 316, Copley Scientific Ltd, UK) with pre-separator and 1 µm glass fiber filter in place. Ensure that the system is airtight. The pre-separator contain 10 mL diluent. Connect the apparatus to a flow system. Draw 4 liters of air from the mouthpiece of device and through the apparatus. Connect a flow meter to induction port. Use a flow meter calibrated for the volumetric flow leaving the meter (60 ± 5% liter/min). Adjust the flow control valve to achieve steady flow through the system at required rate 60 ± 5% liter/min. switch off the pump. The dry powder was aerosolized using a dry powder inhalation device (rotahaler) containing 25mg/ capsule of RIFB. After the rotahaler was connected to mouthpiece of the cascade impactor, a capsule was placed in a holder of rotahaler, which was pinned to pierce the capsule. An air stream of 60 L/min was produced throughout the system by attaching out-let of cascade impactor to vacuum pump for 5s. The drugs in stages 0 to filter were collected by rinsing with fresh solvent (methanol) and were diluted to appropriate volumes and drug contents were determined by using spectrophotometer (UV-1700, Shimadzu, Japan) at 280.5nm. Repeat the discharge sequence for 9 more times and calculate the mean mass aerodynamic diameter (MMAD), geometric standard diameter (GSD), fine particle dose (FPD), % fine particle fraction (% FPF) and percentage mass balance (Muttil et al., 2007; Jensen et al., 2010).

Results and Discussions

The spray drying method described here appeared to be a suitable and simple technique to prepare xyloglucan microspheres loaded with rifabutin. It is one step process, easy and rapid, as it combines drying of the feed and embedding of the drug into a one step operation.

Evaluation of Prepared Microspheres

Physical properties: Dry powder blend (microspheres) was evaluated for various physical parameters such as angle of repose, loose bulk density (LBD), tapped bulk density (TBD), compressibility index and results were found satisfactory due the application of dry powder inhaler carrier lactose monohydrate which efficiently improves the physical properties of blend (Table 1).

Production yield: Yield of production was found in between 28.94 to 31.75 % for the xyloglucan microspheres. These values can be justified by the low quantity of feed used for the preparation of each batch and by the structure of the spray drier, which lacked a trap to capture the smallest and lightest particles.

Entrapment efficiency: Entrapment efficiency was found to be very high as prepared by spray drying technique. It was found that with increasing the ratio of drug to polymer, the entrapment efficiency was also increased (Table1) this may be due to more availability of polymer to encapsulate the drug.

Swelling behavior: Degree of swelling ranges from 0.8 to 1. Swelling capacity of the microspheres was mostly determined by polymer content in preparation, since mucoadhesive polymers were the only component in the spray dried system with swelling abilities. The amount of polymer significantly affected the swelling of microsphere. It has been observed that as the amount of XYG increases swelling property also increases.

In vitro mucoadhesion study: Mucoadhesion studies were carried out to ensure adhesion of formulation to mucosa for a prolonged period of time at the site of absorption. The results of *in vitro* mucoadhesion (Table 1) showed that all batches of microspheres had satisfactory mucoadhesive properties ranging from 73.98 - 91.08 % so could adequately adhere on lung mucosa. The results also showed that, with increasing polymer ratio, higher percentages of mucoadhesion were obtained. This could be attributed to availability of a higher amount of polymer for interaction with mucus.

In vitro drug release study: *In vitro* drug release from all formulation batches (F1-F9) were performed using USP rotating paddle dissolution test apparatus with dialysis membrane. The release pattern of optimized formulations revealed that drug release was sustained initially depending upon polymer concentration followed by rapid release. Percent cumulative drug release from microspheres shown in (Fig. 1).

TABLE 1
Formulation composition and physical properties of xyloglucan microspheres.

Formulation code	Xyloglucan (%)	Lactose monohydrate (%)	Angle of Repose[a] (θ)	Tapped Bulk Density[a] (gm/cm^3)	Loose Bulk Density[a] (gm/cm^3)	Compressibility index[a] (%)	Entrapment Efficiency[a] (%)	Mucoadhesion[a] (%)
F1	3	1.5	29.06±1.2	0.37±0.02	0.33±0.03	10.8±1.31	86.9 ± 1.23	89.99 ± 1.39
F2	1	1	33.98±1.7	0.47±0.02	0.40±0.04	14.8±1.01	82.6 ± 1.34	74.23 ± 1.24
F3	2	1	28.69±1.4	0.41±0.03	0.38±0.01	7.8±0.53	90.2 ± 1.21	86.46 ± 0.86
F4	2	0.5	38.51±1.6	0.51±0.05	0.39±0.07	23.5±1.63	89.1 ± 0.98	86.93 ± 0.98
F5	1	0.5	29.16±1.5	0.44±0.06	0.40±0.12	9.0±1.04	82.8 ± 1.43	74.84 ± 1.54
F6	1	1.5	38.66±2.3	0.48±0.09	0.36±0.09	25.0±1.84	87.2 ± 1.37	73.98 ± 1.03
F7	2	1.5	34.23±1.1	0.45±0.13	0.38±0.06	15.5±1.3	88.8 ± 1.64	85.94 ± 0.95
F8	3	0.5	39.71±1.3	0.54±0.27	0.43±0.13	20.3±1.1	89.7 ± 1.27	91.08 ± 1.38
F9	3	1	35.17±1.5	0.47±0.08	0.38±0.11	19.1±1.28	87.8 ± 1.19	90.63 ± 1.30

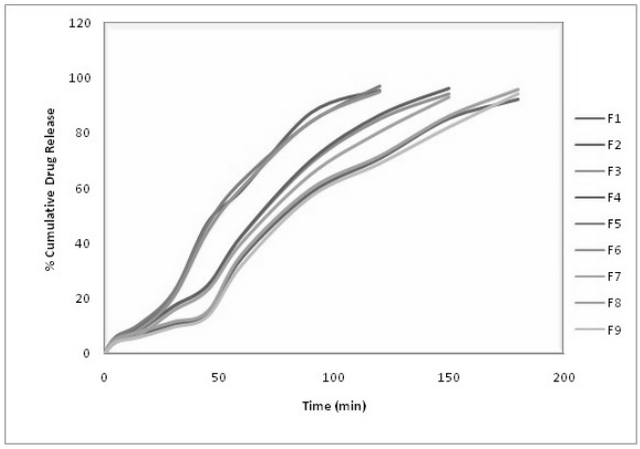
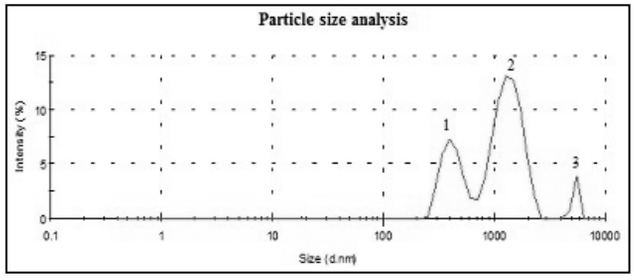


Fig. 1. Drug release profile form xyloglucan based microspheres.

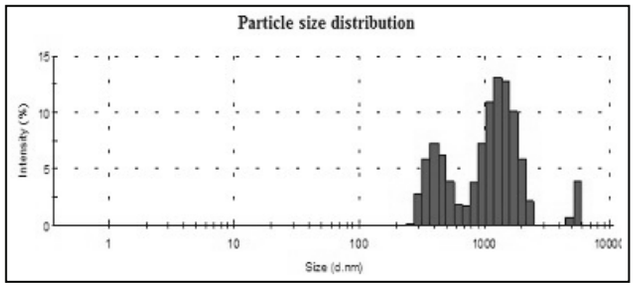
Characterization of Suitable Microspheres

Particle Size Analysis

Average particle size and its distribution of XYG microspheres indicated 3 peaks which demonstrated particle size of peak 1, 437.6 d.nm (29.2%), peak 2, 1377.0 d.nm (66.4%) and peak 3, 5460.0 d.nm (4.4%) respectively (Fig. 2). Such particle size distributions was considered to be appropriate for deep lung deposition with high FPF (Kinnarinen et al., 2003).



(a)



(b)

Fig. 2. Particle size analysis (a) and size distribution curve (b).

Zeta Potential Measurement

The zeta potential of drug loaded microspheres was -18.9 mV. XYG microspheres were negatively charged; indicating presence of XYG at the surface of all microspheres. The studies have shown that polymers with charge density can serve as good mucoadhesive agents. It has also been reported that polyanion polymers are more effective mucoadhesive than poly cationic or non-ionic polymers.

Scanning Electron Microscopy (SEM)

The morphology and surface structure of formulation was analyzed by SEM. Microspheres are spherical in shape and possessed a smooth surface and had no hole or rupture on the surface (Fig. 3), such morphology would result in slow clearance and good deposition pattern in lung.

X-ray Diffraction Study (XRD)

X-ray diffraction of pure drug, blank microspheres and optimized F3 batch are presented in (Fig. 4). X-ray diffractogram of RIFB illustrates slight crystalline nature of drug, with low intensity. In contrast, XYG and optimized F3 batch produces broader peaks with low intensity indicates almost amorphous state of these polymer and microsphere formulation. XRD spectra of

formulation show absence of drug peaks due to conversion of slight crystalline to the amorphous form of drug.

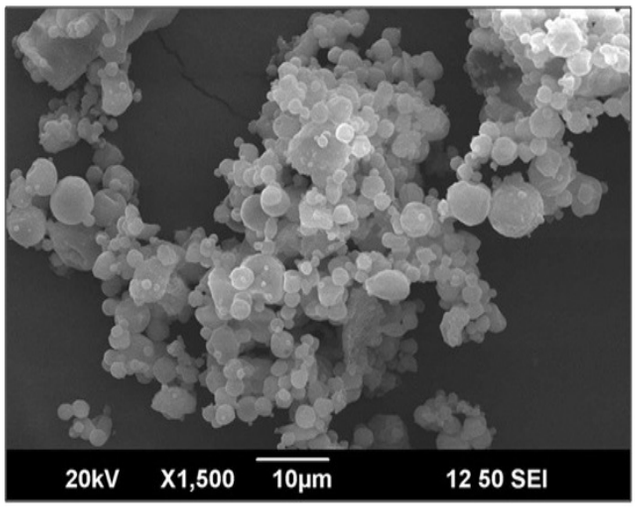


Fig. 3. SEM image of drug loaded xyloglucan microspheres.

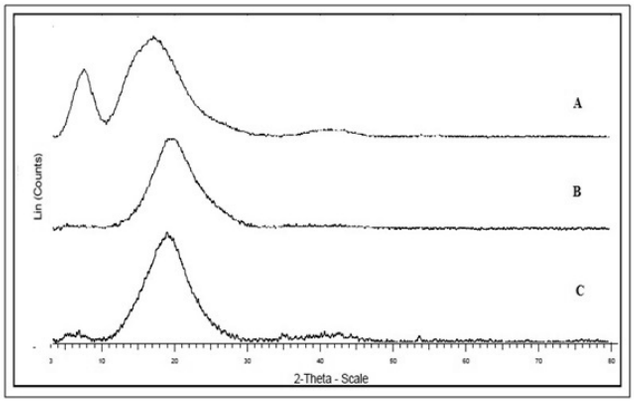


Fig. 4. X-ray diffractogram of drug (A), blank microspheres (B) and drug loaded microspheres (C).

In vitro DPI Performance Measurement

The deposition of particles in bronchus or respiratory system is influenced by aerosolisation performance of particles after inhalation. Thus, aerosolisation performance was evaluated *in vitro* using Anderson Cascade impactor (SS 316, Copley Scientific Ltd). Although Anderson Cascade Impactor is widely accepted *in-vitro* model of pulmonary deposition of particulate matter, *in vitro* results may not be totally predictive of in-vivo behavior. This experiment revealed that 7-8% drug was retained in capsule and device that has given indication of good dispersibility of formulated DPI. About 92.44% of the loaded RIFB was emitted from device (i.e., emitted dose), and 42.04% of loaded RIFB reached stage II (i.e., fine particle dose). Absence of particles in stage II suggests preferential delivery of RIFB to the deep lung tissue or alveoli, demonstrating the advantage of using lactose as the carrier for inhalation. The mass median aerodynamic diameter (MMAD) of the microsphere was less than 5 µm is prerequisite to have an inhalation of powders into lower region of the lung and it was found to be 1.63 µm, geometric standard diameter (GSD) was found to be 2.07 which was depend on aerodynamic behavior and % FPF was 42.04%. MMAD, GSD and % FPF values indicative of particles behaving as individual particle on aspiration. All these characteristics of microspheres are suitable for pulmonary deposition throughout the respiratory zone (Byron, 1986).

Conclusions

In the present study, xyloglucan microspheres containing rifabutin were successfully prepared by a spray-drying method. This technology has been widely used in pharmaceutical industry, but is seldom used in commercial production of respirable particles. The results of present study clearly indicated promising potential of xyloglucan containing microspheres for delivering drug to deep lung and could be viewed as alternative to conventional dosage form. In conclusion the formulated mucoadhesive microspheres as DPI is a promising approach for delivering antitubercular agents through pulmonary route.

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