Original Research Paper

Novel potential for optimization of antitubercular therapy: Pulmonary delivery of rifampicin lipospheres

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ABSTRACT

The aim of the present work is to develop rifampicin loaded phospholipid lipospheres containing sulfobutyl ether β-cyclodextrin and Vitamin C for inhalation to test their potential for deep lung delivery. The findings of the solid state characterization revealed the amorphous nature of the lipospheres. These exhibited a better flowability, an aerodynamic diameter in the range of 1.76 to 3.99 μm. Moreover, the fine particle fraction and emitted dose was found in the range of 68.84–83.73% and 80–93%, respectively. Moreover, lipospheres exhibited enhanced/equivalent efficacy in vitro in H37Rv strain. Hence, the results show the potential of lipospheres for pulmonary delivery of rifampicin.

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Phospholipid
Cyclodextrin
Vitamin C
Inhalation
Lipospheres

1. Introduction

Tuberculosis (TB) is a chronic infectious disease caused by Mycobacterium tuberculosis (MTB). According to World Health Organization (WHO), 1.5 million people died from TB, including 360,000 among people who were HIV-positive (WHO Global Tuberculosis Report 2014) [1]. A major paradigm shift in the treatment of TB occurred with the introduction of rifampicin (RMP), the last landmark drug introduced for TB treatment [2]. RMP is the semi-synthetic hydrazine derivative of rifampicin B [3]. In addition to bactericidal effect on MTB, it exhibits excellent late
sterilizing action on semi-dormant organisms undergoing short bursts of metabolic activity. However, some of the challenges that this potent antibiotic faces include poor water solubility, low oral bioavailability, relatively short biological half-life (1.5–5 h) [3], induction of a prehepatic first-pass metabolism, hepatotoxicity and adverse effects due to multiple doses [4]. Hence, conventional oral treatment of TB is not only long-term [5] but also associated with disadvantages of side effects and systemic toxic effects due to high doses [6]. Vitamin C (Ascorbic acid) (AA) is a water soluble vitamin which is a known antioxidant [7,8] and free radical scavenger [9]. AA has been reported to modulate RMP-induced hepatotoxicity in vivo [10] and of other drug molecules as well [11,12]. Moreover, due to the presence of high iron concentration, reactive oxygen species production and DNA damage, AA has sterilizing action on MTB in vitro [13]. Cyclodextrins (CDs) are macrocyclic oligosaccharides composed of (α-1, 4)-linked α-D-glucopyranose units [14–16] with a hollow hydrophobic interior and a hydrophilic outer surface. Application of CD in pharmaceutical, food and cosmetic industry is extensive because of its inexpensive and nontoxic nature. In pharmaceutical industry, they have been employed to enhance the drug aqueous solubility and thereby enhance the oral bioavailability of the encapsulated drug [17–21]. However, natural CDs are associated with problems of limited solubility and toxicity related issues. To address this problem, alkyl moieties such as hydroxyalkyl or methyl on free hydroxyl groups of CD were introduced [15,22]. Sulphobutyl ether β-cyclodextrin (SBE-β-CD) is a chemically modified β-CD, cyclic hydrophilic oligosaccharide. Also, the solubility in water for SBE-β-CD is significantly higher than the parent β-CD. Furthermore, SBE-β-CD does not exhibit the nephrotoxicity associated with β-cyclodextrin [8]. Moreover, no cytotoxic effects of SBE-β-CD have been reported [23]. Pulmonary delivery via inhalation is a common technique of drug administration to patients with a variety of lung diseases [24,25]. This is the favoured route of administration of drugs over both parenteral and oral route of drug administration. The principal advantages include reduced systemic side effects and higher dose levels of the applicable medication at the site of drug action. Unlike the oral route of drug administration, pulmonary inhalation is not subject to first-pass metabolism. Administration of drugs to the lungs via the inhaled route offers rapid onset of action, high local concentration by direct delivery to the airways and hence high therapeutic ratio [26,27]. However, the airway geometry of the lung poses a challenge for delivery into the alveoli. For effective delivery deep into the lung, the particle size should be between 1 and 5 μm [28], with mass median aerodynamic diameter (MMAD) and geometric standard deviation (GSD) of aerosolized particles. Particles above 5 μm are likely to be deposited in the upper respiratory tract while particles below 1 μm could be exhaled during expiration [29]. Therefore, the aim of this study was to evaluate RMP-loaded dry powder formulation containing sulphobutylether β-cyclodextrin (SDRPL-CD), Vitamin C (SDRPL-AA) and combination of both SBECDD and AA (SDRPL-Comb). RMP has previously shown interesting potential for treating pulmonary TB infection via inhalation [4,30,31] and was chosen in this report as a model molecule. The reports are not available for inhaled phospholipid based lipospheres of RMP containing sulphobutylether β-cyclodextrin and Vitamin C along with the in vitro antimycobacterial efficacy and aerosol performance studies.

2. Materials and methods

2.1. Materials

RMP was obtained as a kind gift by Sandoz, Mumbai. The phospholipid (Lipoid S-75) sample was kindly provided by Lipoid Ludwigshafen, Germany. HPLC grade methanol was purchased from Fisher Scientific, UK. Dichloromethane and other chemicals were obtained from Loba Chemie, Mumbai, India. Vitamin C was purchased from HiMedia, Mumbai. SBECDD was obtained as a kind gift sample from Cydex Pharmaceuticals USA. All other chemicals were of analytical grade.

2.2. Methods

2.2.1. Preparation of spray dry powders

The spray drying open cycle system process was performed using the BUCHI Mini Spray Dryer B-191 attached with high-performance cyclone (BUCHI Labortechnik G, Flawil, Switzerland). Co-spray dried particles (SDRPLs) were obtained by spray drying organic solvent (dichloromethane) and aqueous solutions RMP, PL and SBECDD and AA, respectively. The feed solution (3.5% w/w) was passed through a stainless steel 0.7 mm diameter atomizing nozzle via a peristaltic pump at a flow rate of 4 ml/min (pump rate 12%) employing atmospheric gas for drying at a flow rate between 30 and 40 kg/h. A set inlet temperature of 80 °C ± 2 °C (primary drying step) resulted in outlet of 55 °C ± 3 °C (secondary drying step) with an aspirator rate of 90%. The resultant dry powder particles were blown through a high-performance cyclone separator and collected in the sample container. Dry powder formulation was stored in glass vials sealed with parafilm at room temperature.

2.2.2. Fourier transform infra red spectrometry

Fourier transform infra red spectrometry (FTIR) spectra of samples were recorded with a FTIR spectrometer (Nicolet, Impact 410, USA) employed with a denuded triglycine sulphate detector. The spectra were scanned in the region 450–4000 cm⁻¹ derived from 11 single average scans. Potassium bromide pellet method was employed for the analysis. The data were collected and analyzed using Omnic 5.1a software (Thermo Nicolet, USA). These are similar conditions to those previously reported [5].

2.2.3. Thermal analysis

Thermograms were obtained on a Mettler Stare System (Mettler Toledo, DSC 821e, Switzerland) using similar conditions previously reported in the literature [5]. Approximately 2–8 mg of powder was carefully weighed into hermetic anodized aluminium DSC pans. An empty, hermetically sealed, anodized aluminium pan was used as reference. Samples were heated at a rate of 20 °C/min over a temperature range of 25 °C to 300 °C. Nitrogen gas at purge rate of 100 ml/min was used as the purging gas.
2.2.4. Crystallinity determination
The crystalline nature and non-crystallinity of samples were examined using X-ray powder diffraction (XRPD) (Bruker axs System, D8, Germany) with a slit detector Cu Kα radiation (35 kV, 20 mA) source. The scan range was 3 to 40° 2θ with a scan rate of 10°/min at room temperature in a continuous scan mode. The samples were placed on a horizontal quartz glass sample holder plate.

2.2.5. Hot stage microscopy under cross polarizer
Hot stage microscopy (HSM) studies were performed under Leica DMPL polarized microscope (Leica Microsystems Wetzlar GmbH, Wetzlar Germany) equipped with Linkam LTS 350 hot stage. Photomicrographs were captured using JVS colour video camera and analyzed using Linksys32 software. The powder samples were placed on glass slides with cover glass and heated at the rate of 10 °C/min from 25 °C to 250 °C.

2.2.6. Morphology evaluation
Scanning electron microscopy (SEM) of the samples was evaluated using SEM, equipped with a Hitachi S-3400 microscope (Hitachi Ltd, Tokyo, Japan). Samples were placed on double sided adhesive carbon tabs (Ted Pella, Inc., Redding, CA, USA), which were adhered to aluminum stubs (Ted Pella, Inc.), and were coated with gold thin film using a Hummer VI sputtering system from Hitachi. The coating process was operated at 25 mA discharge current for 20 s.

2.2.7. Drug content and entrapment efficiency
RMP content in the spray dried powders was determined using LCMS (Thermo Scientific, LTQ XL, Germany). For this analysis, methanol and ammonium acetate buffer (10 mM, pH 3.4) system was used in the ratio of 70:30. The column used for chromatographic separation was Phenomenex C18 column (250 mm × 4.6 mm, 5 μm) and flow rate was 1 ml/min at 4 °C column temperature. The detection wavelength was 333 nm and injection volume was 20 μl.

Entrapment efficiency (% EE) was calculated using the equation shown below:

\[
\% \text{ Drug content} = \frac{\text{Amount of drug entrapped}}{\text{Mass of microparticles}}
\]

\[
\% \text{ EE} = \frac{\text{Actual drug loading}}{\text{Theoretical drug loading}} \times 100
\]

2.2.8. Bulk and tap density
For this, a known mass of powder was poured into a graduated cylinder, which is then tapped for defined number of times. For calculation of tap and bulk densities, we noted the initial volume and final volume (after tapping). Owing to the sample size constraint, a 10 ml graduated cylinder was filled with 1000 mg of dry powder for testing and 1250 taps were applied for this measurement. The static powder flow was determined by Carr’s compressibility index (CI). This was determined by the following equation:

\[
\text{CI} = \frac{\text{Tapped density} – \text{Bulk density}}{\text{Tapped density}} \times 100
\]

2.2.9. Powder flowability
For assessment of powder flowability, measurement of angle of repose is the most frequently adopted method. Powder was poured through a funnel to form a cone-shaped pile with an angle, \(\alpha\), to the horizontal. The value of \(\alpha\) was calculated by measuring the height and radius of the pile formed. Powder flowability is inversely proportional to the angle of repose i.e. a large angle of repose is indicative of poor flow properties while a small angle of repose indicates a free flowing powder [32]. Apart from angle of repose properties, Hausner ratio was also determined.

\[
\text{Tapped density} = \frac{\text{Height}}{0.5 \times \text{Base}}
\]

\[
\text{H. R.} = \frac{\text{Tapped density}}{\text{Bulk density}}
\]

2.2.10. Specific surface area
To determine the surface area, approximately 100 mg of each formulation were dried at 50 °C for 30 min under vacuum and dead volume of sample cell was measured at room temperature. Nitrogen adsorption and desorption measurements were performed using sample cells and empty reference cell immersed in liquid nitrogen [33]. Each measurement was repeated twice to obtain the average surface area calculated by BET equation (Brunauer–Emmett–Teller) method.

\[
\text{Vm} = \frac{\text{Va}}{\left(\frac{\text{PD}}{\text{F}} – 1\right) + \frac{\text{C}}{1 + \frac{\text{C}}{\text{P}}}}
\]

\[
\text{S} = \frac{\text{VmNa}}{m \times 22,400}
\]

2.2.11. In vitro antimycobacterial activity by BACTEC method
The antimycobacterial activity of SDRPL-CD, SDRPL-AA and SDRPL-Comb formulation and RMP was tested on MTB H37Rv strain (Tuberculosis Research Centre, Chennai, India). Briefly, the bacteria were cultured in Middlebrook 7H9 liquid medium (HI Media, India) supplemented with 10% albumin, dextrose, and catalase (ADC; HiMedia, India) to mid-log phase and then frozen sterilized through 0.2 μm DMSO safe filters (PALL Life Sciences) before testing. The BACTEC 460 TB system (Becton Dickinson, USA) was employed to determine a growth index (GI) of the MTB. GI is the quantitative measure of 14CO2 liberated by metabolism of 14C-labelled substrate in a medium and expressed in numbers on a scale from 0 to 999. Briefly, 0.1 ml of samples were transferred to 12B BACTEC vials, in duplicate for each sample/drug concentration, unless mentioned otherwise, and incubated at 37 °C in 5% CO2 atmosphere. GI was calculated daily under aerobic condition until in 1:100 controls; a value greater than 30 was obtained. In order to determine the per cent inhibition, undiluted control reading was used. Appropriate positive and negative controls were also included in the calculation. The growth inhibition was expressed as a ratio of GI of drug to the respective control vial.
The per cent growth inhibition was calculated for each drug concentration [34,35].

2.2.12. In vitro pulmonary deposition studies
Aerosol performance of SDRPL-CD, SDRPL-AA and SDRPL-Comb formulation was assessed using an eight stage, nonviable Anderson Cascade Impactor (Graseby-Anderson, Atlanta, USA) operating at an airflow of 28.3 l/min [36]. To overcome the particle bounce and re-entrainment phenomenon, the impaction plates were coated with 1.5%w/v of HPMC (4000 cps) gel in water. Five hydroxypropyl methylcellulose hard capsules (Size 3; Quali-V®, Qualicap® Inc, Whitsett, NC, USA) were loaded with 20 mg of powder per capsule followed by actuation time of 10 s. The drug content deposited in individual impaction plates was rinsed with methanol and subjected to HPLC analysis. From the drug deposition data the emitted dose (ED), fine particle fraction (FPF), mean median aerodynamic diameter (MMAD) and geometric standard deviation (GSD) were calculated [37] as follows:

\[
\text{Emitted dose (ED)\%} = \left( 1 - \frac{\text{Final mass remaining in capsules}}{\text{Initial mass in capsules}} \right) \times 100% \tag{8}
\]

\[
\text{Fine particle fraction (FPF)\%} = \left( 1 - \frac{\text{Mass deposited on stage 2 through 7}}{\text{Initial particle mass loaded into capsules}} \right) \times 100% \tag{9}
\]

2.2.13. Statistical analysis
The values are reported as the mean ± SD. The minimum level of significance was set at \( P < 0.05 \). All statistical analyses were performed using the GraphPad Prism 5.0 software (Graph pad software [CA, USA]).

3. Result and discussion

3.1. FTIR
The FTIR spectra of RMP, PL, PM, SBECD, AA, SDRPL-CD, SDRPL-AA and SDRPL-Comb formulations are shown in Fig. 1 (upper legend). The characteristic absorption peaks of RMP present at 1734 cm\(^{-1}\) (\( \nu_{C=O} \) acetyl stretching) and 1566 cm\(^{-1}\) (\( \nu_{C=\text{N}} \) stretching) in the physical mixture indicate the additive effect (Fig. 1C).

In the spectra of AA (Fig. 1D) and SBECD (Fig. 1E), the characteristic peaks were observed at 3412 (\( \nu_{O-H} \) bond) and 3221 cm\(^{-1}\) (\( \nu_{C-H} \) bond) and 3394 (\( \nu_{O-H} \) bond) and 1650 cm\(^{-1}\) (bending vibration of \( \nu_{C=\text{C}} \), respectively. However, in the spectra of the SDRPL formulations (Fig. 1F–H), the intensity of four characteristic absorption peaks of RMP at 1655 cm\(^{-1}\) (\( \nu_{\text{asymmetric stretching}} \), 1566 cm\(^{-1}\) (\( \nu_{\text{symmetric stretching}} \), 1252 cm\(^{-1}\) (\( \nu_{O-O-C-\text{ether group}} \) and 1430 cm\(^{-1}\) (\( \nu_{\text{N stretching}} \)) was changed. These findings suggested possible inter-molecular interactions between the components in the dry powder.

3.2. DSC
The thermograms of RMP, PL, SBECD, AA, PM, SDRPL-CD, SDRPL-AA and SDRPL-Comb formulations are shown in Fig. 1 (lower legend). The AA (Fig. 2A) and RMP (Fig. 1B) thermograms show an endothermic peak at 195 °C and 187–193 °C, which represents the melting point of AA and RMP samples, respectively. There are two endothermic peaks in the thermogram of PL (Fig. 1C) at 153 °C and 210 °C. The thermogram of PM (Fig. 1D) shows that the endothermic peak corresponding to the melting point of the AA is present but reduced. However, there was no melting endothermic peak present in the SDRPL-CD, SDRPL-AA and SDRPL-Comb formulations (Fig. 1F–H). Absence of these characteristic endothermic peaks is an indication of the amorphous nature of the formulations [38,39].

3.3. PXRD
PXRD is the direct method for determination of basic structure information of a material. Crystalline behaviour of free RMP, SDRPL-CD, SDRPL-AA and SDRPL-Comb formulations were examined by studying its PXRD. As shown in Fig. 2, the PXRD diffractogram pattern of RMP (Fig. 2A) and AA (Fig. 2H) shows distinct sharp diffraction peaks (i.e. the presence of long range molecular order), indicating its crystalline nature. Fig. 2B and D presents the diffractogram pattern of PL which displayed peaks at position 2\(^{\circ}\) and 8\(^{\circ}\) and halo pattern of SBECD, indicating its amorphous nature while in diffractogram pattern of PM (Fig. 2C), there are still reduced diffraction peaks. This could be due to the presence of RMP and AA in the sample. However, no such diffraction peaks were observed in the diffractogram pattern of SDRPL formulations as displayed in Fig. 2E–G, indicative of its non-crystallinity. Hence, based on the PXRD results it is evident that SDRPL formulations are amorphous in nature and are in agreement with DSC findings.

3.4. HSM
Photomicrographs of RMP, PL, PM, SDRPL-CD, SDRPL-AA and SDRPL-Comb formulations are shown in Figs 3–8. The RMP micrographs exhibited the birefringency (blue arrow) pattern (Fig. 3A–E). Gradually, it diminishes as the temperature is increased. This kind of pattern confirms the crystalline nature of the RMP. In Fig. 4A–E, melting of the crystals (black arrow) of RMP was observed by deformation of the crystals. On the contrary, the micrographs of SDRPL-CD (Fig. 5A–E), SDRPL-AA (Fig. 6A–E) and SDRPL-Comb (Fig. 7A–E) showed softening (black arrow) of the particles. This is probably due to the amorphous nature of the SDRPL formulations following spray drying process. Representative micrograph of SDRPL-CD is presented under the cross-polarizer light in Fig. 8A–E wherein completely dark images that lack birefringency are present. The softening and lack of birefringency pattern in the SDRPL formulations is evident of their amorphous nature [5]. Overall, HSM findings confirmed the amorphous nature of the formulations: These results are in agreement with DSC and PXRD data.

3.5. SEM
Dry powder particles were successfully produced by using spray drying technology. The shape and surface morphology of all samples were visualized via SEM and displayed in Figs 9 and 10. The image shown in Fig. 9A confirms the irregular size and morphology of RMP crystal, which is in agreement with PXRD
analysis that showed the crystalline nature of RMP. Fig. 9B displayed the shrunk and irregular shape of PL. Moreover, in Fig. 9C and D a spherical shape and cubic crystals are observed of SBECD and AA, respectively. The PM (Fig. 10A) revealed presence of RMP crystal along with SBECD and AA. SDRPL-CD, SDRPL-AA and SDRPL-Comb (Fig. 10B–D) presented the formation of fine particles, indicated by the spherical morphology within the size range of 1 μm to 5 μm, which shows the potential for delivery to the lungs [32,40]. In conclusion, the particle size exhibited by all the powder formulations was in the respirable range.

3.6 Powder characterization

The tapped density of spray dried formulations was similar for all powders, and ranged between $0.26 \pm 0.003 \text{ gcm}^{-3}$ and $0.33 \pm 0.005 \text{ gcm}^{-3}$ (data not presented). The better aerosolization of the formulation is associated with the lower powder densities [41,42]. Carr’s Index, Angle of Repose and Hausner ratio were used to provide a measure of the flow properties. In Carr’s Index, a value less than 25% indicates good flowing powder, whereas value greater than 25% indicates cohesive flowing powder [43]. Carr’s Index values in the SDRPL-CD, SDRPL-AA and SDRPL-Comb formulations varied from 11.7% (excellent flowability) to 13.5% (good flowability). Moreover, Angle of Repose and Hausner ratio of the spray dried formulations were in the range of $31.8 \pm 4$ to $35.1 \pm 3$ and $1.03 \pm 0.02$ to $1.21 \pm 0.05$, respectively. Results of the powder characterization were not significant ($P > 0.05$) with pure SDRPL formulation without CD and AA (data not presented). Findings of these parameters further proved the good flowing behaviour of the
powders. Hence, it can be concluded that the developed SDRPL formulations were found to have potential for the pulmonary delivery of poor-aqueous soluble drugs. Powder characterization data are compiled in Table 1.

3.7. In vitro pulmonary deposition studies

In vitro pulmonary deposition study results of SDRPL-CD, SDRPL-AA and SDRPL-Comb formulations after 28.3 l/min using the eight

Fig. 2 – PXRD diffractogram pattern of (A) Rifampicin, (B) Phospholipid, (C) Physical mixture, (D) Sulphobutylether-β-cyclodextrin, (E) SDRPL-CD, (F) SDRPL-AA, (G) SDRPL-Comb and (H) Ascorbic acid.

Fig. 3 – HSM micrographs of Rifampicin under cross polarizer.
Fig. 4 – HSM micrographs of Rifampicin under ordinary light.

Fig. 5 – HSM micrographs of SDRPL-CD under ordinary light.
stage Anderson Cascade Impactor are shown in Table 2 and Fig. 11. To investigate in vitro aerosol powder performance, we used Size 3 HPMC capsules. Aerodynamic studies by Andersen Cascade Impactor were then conducted on these preparations that met the delivered dose specifications (>75%) and showed a respirable fraction higher than 50%. Efficiency of inhalation delivery system depends on particle size and particle size distribution which are considered as the critical factors.

Fig. 6 – HSM micrographs of SDRPL-AA under ordinary light.

Fig. 7 – HSM micrographs of SDRPL-Comb under ordinary light.
for deposition of particles in the alveolar region. An aerodynamic particle size of 1–3 is required for optimal delivery to the lung.

The efficiency of powder recovery was found to be >80%. This indicated that the maximum amount of the drug was delivered by the device and small amount of the drug was retained in the inhaler device. The per cent mass fraction of the aerosolized particles from DPIs on the different stages of cascade impactor is graphically presented in Fig. 11. Each bar represents the powder of certain sizes deposited on the stages of the Anderson Cascade Impactor. Maximum FPF of 83.73 ± 2% was observed with SDRPL-CD as compared to SDRPL-AA of

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Fig. 8 – HSM micrographs of SDRPL-CD under polarized light.

Fig. 9 – Representative SEM image of (A) Rifampicin, (B) Phospholipid, (C) Sulphobutylether-β-cyclodextrin and (D) Ascorbic acid.
68.84 ± 4% and SDRPL-Comb of 69.01 ± 3% (Table 2). No significant difference was observed between SDRPL-AA and SDRPL-Comb. The per cent emitted dose was ranged from 80 ± 4% of SDRPL-AA to 93 ± 1.5% for SDRPL-CD (Table 2). For efficient delivery of formulation to the lung, a combination of lower MMAD and higher FPF is required [44]. Therefore, higher the FPF greater will be the deposition in the deeper lung resulting in enhanced efficacy. SDRPL-CD was found to have a maximum FPF compared to SDRPL-AA and SDRPL-Comb (Table 2). This could be due to its less cohesive nature with lower surface area and vander Walls forces per unit mass of particles. In addition to this, these particles tend to separate easily and provide maximum fines. The mass median aerodynamic diameter (MMAD) and geometric standard deviation (GSD) of SDRPL-CD, SDRPL-AA and SDRPL-Comb were in the range of 1.76 ± 0.23 to 3.99 ± 0.13 and 2.11 ± 0.09 to 3.28 ± 0.1, respectively. Findings of the study also revealed that SDRPL-CD displayed the best aerosol powder performance among the various formulations in terms of ED, FPF and MMAD. Deposition of SDRPL-AA formulation in the cascade impactor was in the lower stages (stages 4 and 5) which is most relevant for deep lung penetration [45], while in case of SDRPL-CD and SDRPL-Comb, it was on stages 5 and 6 and stages 3 and 5 of impactor, respectively. Thus, these results demonstrated that the RMP loaded phospholipid lipospheres incorporated with SBEC and AA formulations have potential for inhalation delivery.

3.8. In vitro antimycobacterial activity by BACTEC method

All the three SDRPL-CD, SDRPL-AA and SDRPL-Comb formulations were tested for antimycobacterial activity. All the formulations exhibited enhanced/equivalent efficacy in vitro in MTB H37Rv strain. DMSO was employed as the solubilizing agent and its possible inhibitory effect on MTB growth was considered as well. The mechanism of action of RMP is to arrest DNA-directed RNA synthesis of MTB by interacting with the subunit of RNA polymerase [46]. The MIC values of RMP and SDRPL formulations against MTB were found to be 0.05 and

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**Table 1 – Powder properties characterization.**

<table>
<thead>
<tr>
<th>Samples</th>
<th>Angle of Repose (°)</th>
<th>Carr’s Index</th>
<th>Hausner ratio</th>
<th>Specific surface area</th>
</tr>
</thead>
<tbody>
<tr>
<td>SDRPL-CD</td>
<td>35.1</td>
<td>13.5</td>
<td>1.21</td>
<td>5.23</td>
</tr>
<tr>
<td>SDRPL-AA</td>
<td>32.4</td>
<td>12.3</td>
<td>1.11</td>
<td>6.11</td>
</tr>
<tr>
<td>SDRPL-Comb</td>
<td>31.8</td>
<td>11.7</td>
<td>1.03</td>
<td>6.21</td>
</tr>
</tbody>
</table>


*b* Carr’s Index: 5–12%, Excellent; 12–18%, good; 18–21%, fair; 21–25%, poor, fluid; 25–32%, poor, cohesive; 32–38%, very poor; >40%, extremely poor.

*c* Hausner ratio: 1.00–1.11, Excellent; 1.12–1.18, good; 1.19–1.25, fair; 1.26–1.34, passable; 1.35–1.45, poor; 1.46–1.59, very poor; >1.60, very-very poor.

**Table 2 – In vitro aerosol performance data of SDRPL formulations.**

<table>
<thead>
<tr>
<th>Formulations</th>
<th>%FPF</th>
<th>%ED</th>
<th>MMAD</th>
<th>GSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>SDRPL-CD</td>
<td>83.73</td>
<td>93</td>
<td>1.76</td>
<td>2.11</td>
</tr>
<tr>
<td>SDRPL-AA</td>
<td>68.84</td>
<td>80</td>
<td>2.16</td>
<td>2.11</td>
</tr>
<tr>
<td>SDRPL-Comb</td>
<td>69.01</td>
<td>84</td>
<td>3.99</td>
<td>2.54</td>
</tr>
</tbody>
</table>

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Fig. 10 – Representative SEM image of (A) Physical mixture, (B) SDRPL-CD, (C) SDRPL-AA and (D) SDRPL-Comb.
0.005, 0.024, 0.028 μg/ml, respectively. Thus, SDRPL-CD formulation exhibited significant (P < 0.001) lowest MIC value among all the formulations. Also, in comparison to pure SDRPL lipospheres, it exhibited two folds (0.005 μg/ml vs 0.01 μg/ml) improved in vitro efficacy. Probably, this may be due to the cyclodextrin–cholesterol complex formation and subsequently, removal of cholesterol from the membrane (Fig. 12). It has been reported that MTB degrades the cholesterol to derive

Fig. 11 – In vitro aerosol performance as per cent deposition on each stage of Anderson Cascade Impactor (ACI).

Fig. 12 – Possible mechanism of cyclodextrin–cholesterol complex formation.
the carbon and energy from this molecule for its growth [47–51]. As a result of the cholesterol removal of membrane, fewer nutrients will be available for MTB and unable to survive in the cells. On the other hand, SDRPL-AA has also shown improved/equivalent efficacy in vitro but not as remarkable as SDRP-CD formulation. It is reported that sterilizing effect of the AA on the MTB culture is due to its pro-oxidant effect which results in increase in the ferrous ion concentration leading to ROS generation, alteration in lipid, imbalance in redox reaction and DNA damage (Fig. 13). To our knowledge, this is the first time that activity of a RMP loaded lipospheres formulation containing SBEC and AA has been reported.

4. Conclusion

In the present study, a novel DPI system of rifampicin for inhalation therapy was developed with the use of cyclodextrin and vitamin C. Micronized spray dry powders prepared with a spray drying technique showed suitable physicochemical and powder flow properties for the inhalable dry powder. SDRPL-CD formulation presented excellent in vitro antimycobacterial efficacy and aerosol performance. The in vitro deposition study using the cascade impactor also highlighted that spray dry powders consistently deposited in deep lung regions. Overall, the data from the study suggest an alternative option for inhalation delivery of an antitubercular drug directly into the lungs, which may enhance therapeutic efficacy and greatly reduce adverse effects. The study establishes that rifampicin can be successfully loaded into phospholipid lipospheres for pulmonary delivery. Hence, it can be concluded that SDRPL formulations have potential for better management in the tuberculosis therapy.

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