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Original Article

Chitosan-Based Linezolid Dry Powder Inhalers: A Novel Approach for Targeted Pulmonary Delivery in Tuberculosis Treatment

Mallamma Thippeswamy[1], Girish Meravanige[2,*], Predeepkumar Narayanappa Shiroorkar[2], Prakash Goudanavar[1,*], Nimbagal Raghavendra Naveen[1], Nagaraja Sreeharsha[3]

[1]Department of Pharmaceutics, Sri Adichunchanagiri College of Pharmacy, Adichunchanagiri University, B. G. Nagar, Karnataka, INDIA.
[2]Department of Biomedical Sciences, College of Medicine, King Faisal University, Al-Ahsa, SAUDI ARABIA.
[3]Department of Pharmaceutical Sciences, College of Clinical Pharmacy, King Faisal University, Al-Hofuf, Al-Ahsa, Kingdom of SAUDI ARABIA.

ABSTRACT

Introduction: Tuberculosis (TB) remains one of the leading causes of infectious deaths worldwide, ranking second only to COVID-19. The rise of Multidrug-Resistant (MDR) and Extensively Drug-Resistant (XDR) TB strains highlights the critical need for novel and effective treatment approaches. **Methodology:** This research explores a targeted pulmonary drug delivery system using Dry Powder Inhalers (DPI) to administer the antibiotic Linezolid (Lzd) directly to the lungs. Biodegradable Microparticles (MPs) of Linezolid were synthesized using chitosan polymer via spray drying, with Critical Process Parameters (CPPs) such as inlet temperature, aspiration rate, and feed rate optimized to achieve desired Particle Size (PS) and Entrapment Efficiency (%EE). Comprehensive evaluations were conducted, including *in vivo* studies, stability testing, H37 RV strain sensitivity, particle size distribution, crystallinity, flow properties, and drug-polymer compatibility. **Results and Discussion:** The optimized batch of Linezolid (Lzd) MPs exhibited an impressive 89.57% entrapment efficiency with a particle size 3.9 µm. Physically, the MPs were a free-flowing powder with a bulk density of 0.171 g/cm^3, tapped density of 0.2287 g/cm^3, Carr's index of 25%, and Hausner's ratio of 0.95. These spherical particles demonstrated sustained drug release for up to 12 hr, with a process yield of 75.91% and a moisture content of 1.58%. Importantly, the MPs showed significant inhibitory effects against the H37 RV strain of Mycobacterium tuberculosis across various concentrations. *In vivo* studies revealed a 55.2% increase in bioavailability with the Lzd DPI formulation, which was 1.25 times higher than the oral tablet. **Conclusion:** This novel inhalation system holds the potential to reduce dosing frequency, minimize side effects, and improve patient adherence, offering a promising alternative for effective TB management.

Keywords: Linezolid, Multi-Drug Resistant Tuberculosis (MDR-TB), *Mycobacterium tuberculosis*, Chitosan Microparticles, Pulmonary delivery.

Correspondence:
Dr. Prakash Goudanavar
Professor and Head, Department of Pharmaceutics, Sri Adichunchanagiri College of Pharmacy, Adichunchanagiri University, B. G. Nagar, Karnataka, INDIA.
Email: pgoudanavar01@gmail.com

Dr. Girish Meravanige
Department of Biomedical Sciences, College of Medicine, King Faisal University, Al-Ahsa, SAUDI ARABIA.
Email: gmeravanige@kfu.edu.sa

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INTRODUCTION

According to statistics from the WHO, in 2022, Tuberculosis (TB) led to the deaths of 1.3 million people, of which 167 000 were affected by HIV/AIDS. Despite COVID-19 having potential and becoming a pandemic, TB is still the second most deadly contagious disease in the world. That same year, there were 10.6 million new cases of TB, including 5.8 million men, 3.5 million women, and 1.3 million children.[1] There were many new cases amongst children indicating papable impact of the disease on all age ranges. Drug resistant TB is present throughout the world, but only approximately 40% of people with drug resistant TB received the sufficient treatment against that strain in the year 2022.[2] TB has burdened humans for many years and in past it has been established that the disease is more likely to spread as the population increases and living standards decrease. Treatment for bacterial infections has sure improved over time such as Chest X rays and sputum culture to Nucleic acid amplification tests which shorten the duration of diagnosis and are somewhat more straight forward. Yet, the development of resistant tuberculosis, like multi-drug resistance tuberculosis, makes it hard to treat TB with resistance partially caused by efflux systems.

Current treatment options for Drug-Resistant Tuberculosis (DR-TB) have significantly evolved in recent years. Novel and repurposed drugs like bedaquiline, delamanid, and pretomanid have revolutionized DR-TB management.[3,4] These drugs have

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shown superior efficacy, improved treatment success rates, better tolerability, and reduced treatment duration compared to older regimens.[5] The introduction of all-oral six-month regimens containing bedaquiline, pretomanid, and Linezolid (Lzd) has further enhanced treatment outcomes, leading to significant changes in WHO guidance.[6] Despite these advancements, challenges such as safety concerns, emergent resistance, and the need for continuous efforts to improve treatment approaches and monitoring tools persist. Among all these, Lzd was selected for TB due to its high sensitivity (88.9%) against drug-resistant strains, making it practical for M/XDR-TB and newer regimens like BPaL.[7]

Linezolid (Lzd) is chosen for drug-resistant tuberculosis due to its potent activity against M. tuberculosis despite its narrow therapeutic index, necessitating optimal dosing strategies to minimize toxicity.[8] Despite the emergence of resistance mechanisms in mycobacteria, it effectively combats multidrug-resistant mycobacteria by inhibiting protein synthesis through ribosomal binding.[9] It is a synthetic antibacterial agent composed of 1,3-oxazolidin-2-one and an acetamidomethyl group. It prevents bacterial protein synthesis by targeting a specific site on the 23S ribosomal RNA, thereby hindering the assembly of a functional 70S initiation complex.[10] Chitosan Microparticles (MPs), loaded with Lzd, have demonstrated efficacy in enhancing anti-mycobacterial immune responses, thereby enhancing the survival of TB bacilli, including drug-resistant strains.[11] Sha *et al.* (2022) formulated Chitosan nanoparticles loaded with Lzd. They showed sustained drug release, mucus penetrability, macrophage targetability, and superior safety, making them a promising option for inhalation therapy in TB treatment.[12] Lzd-loaded chitosan MPs are effective against tuberculosis due to enhanced chemotherapeutic efficacy against Mycobacterium and improved drug availability in the lungs, spleen, and liver post-nebulization.[14] The study suggests that chitosan-based delivery systems can improve the efficacy and safety of anti-TB treatments like Lzd due to their nontoxic, bioadhesive, and biodegradable properties and enhanced uptake and bioavailability.[13]

To reach the highest drug concentration of antibiotics in the pulmonary tissue, the method of dry powder delivery has been specifically developed. This approach has proven successful in treating 80% of tuberculosis cases.[14] The use of non-sterile formulations for inhaled anti-TB drugs can reduce dose, prevent first-pass metabolisms, and target infection sites. This reduces systemic toxicity by releasing drugs through alveolar macrophages or lung surfactants.[15] The research done by Smita N. *et al.* in 2020 reported that Dry Powder Inhalers (DPIs) rely heavily on PS, which affects aerodynamic performance and medication delivery efficiency.

Nora Y. K. *et al.* (2002) ensured that drugs could be delivered to the lungs efficiently. DPI formulations with a size in between 1 to 5 µm, ideal shape, minimal surface energy, and rapid deposition rates.[16] Maintaining stability and optimizing aerodynamic performance are becoming more challenging as DPI formulation strategies shift towards carrier-free systems, nanoparticle formulations, and biomacromolecules.[17] One attractive strategy uses particle engineering tools like spray dryers and supercritical fluid particle design. The study explores the use of spray drying technology to improve DPI efficiency and pulmonary deposition by transforming solutions, suspensions, or emulsions into dry particles, thereby enhancing stability and aerodynamic performance.[18] Lzd has been identified as a promising agent for the treatment of tuberculosis in numerous studies. Nevertheless, the utilization of Lzd-loaded formulations as a novel drug delivery strategy for the treatment of tuberculosis has not yet been examined. Therefore, the objective of the present investigation is to devise formulations that contain Lzd and assess their efficacy in the treatment of tuberculosis, with an emphasis on the bioavailability and stability indicators. The drug's therapeutic effectiveness will be improved through the precise delivery of the pharmaceutical agent to the intended target site, as demonstrated by both *in vitro* and *in vivo* evaluations involving Lzd-inhalable MPs (Figure 1).

MATERIALS AND METHODS

Lzd was acquired as a gift from Symed Laboratories, Hyderabad, India. Chitosan was acquired from Himedia Laboratories, India. Dimethyl Sulfoxide (DMSO), Poloxamer, and TPP were acquired from Sigma Aldrich, India. We obtained L6 cell lines from Thermo Fisher Scientific.

Formulation development

First, chitosan was dissolved in 1% (v/v) acetic acid with a weight-to-volume ratio of 1-2% using Ionic-gelation method. The fluid was thoroughly mixed using a magnetic stirrer to dissolve the chitosan. A 1% TPP solution was made by dissolving TPP in distilled water. After TPP dissolved, the solution was stirred. Drops of TPP solution were added to the chitosan solution while swirling to generate a complex. For optimal crosslinking, the chitosan-TPP ratio was maintained between 5:1 and 10:1. Chitosan-TPP MPs were produced by reacting the two substances for an hour at room temperature. Optimized formulation parameters are displayed in Table 1.

Spray Drying method

The spray dryer was configured by establishing the inlet temperature within the range of 150-170°C. The aspirator was set to its highest power level and the flow rate to 600 L/hr. The Chitosan-TPP mixture was introduced into the spray drier at a flow rate of 3-5 mL/min to keep the outlet temperature within the range of 70-90°C. The atomization pressure was maintained within the range of 1.5 to 2.5MPa.[19] The dehydrated MPs obtained from the cyclone separator were gathered and placed in a desiccator to avoid moisture absorption. The chitosan solutions

were transferred into glass bottles with screw caps and kept in an incubator set at 35°C for storage.[20]

QBD experimental design

The correlations between the factors and the responses were also examined using this method. An optimization technique called the Box-Behnken Design (BBD) was utilized. Every numeric factor is set to three levels. These designs have a lower number of runs compared to 3-Level Factorials. The response variables chosen were PS and %EE, while the independent variables were inlet temperature, aspiration rate, and feed rate.[21] The study examined the impact of three independent variables (feed rate, atomization pressure, and aspirator) on various responses (dependent factors), such as %EE (Y1) and PS (Y2). The concentration levels were categorized into low, medium, and high, symbolized by (-1, 0, 1), respectively. A polynomial model was selected after thoroughly evaluating several statistical metrics, including the multiple correlation coefficient, the adjusted multiple correlation coefficient, and the predicted residual sum of squares, utilizing Design-Expert® software (version 12.0.0.7). Experimental trials were carried out with defined independent variables, and the outcomes for the 13 prepared formulations were carefully documented and analyzed. The study analyzed the impact of various excipients on %EE and PS.[22]

Characterization

PS and Zeta potential (ZP)

The PS and ZP were validated by Malvern Zetasizer 3000 HSA devices. MP sizes in spray-dried materials were assessed with the Malvern Zetasizer. 10 mg of produced MPs were dispersed in 20 mL of water and added to the sample dispersion unit probe. Using dynamic light scattering, PS distribution was calculated from particle velocity in a dispersing medium. Precision and dependability were achieved by analyzing each sample in a triplicate.[23]

Qualitative estimation and %EE

The mobile phase (acetonitrile: acetic acid) was used for High-Performance Liquid Chromatography (HPLC) system analysis.[24] Lzd-loaded MPs were injected via injection and then the Retention time (Rt) was analyzed by comparing it to a standard reference. Lzd quantity was estimated by analyzing its distinct retention time and validating at different parameters as

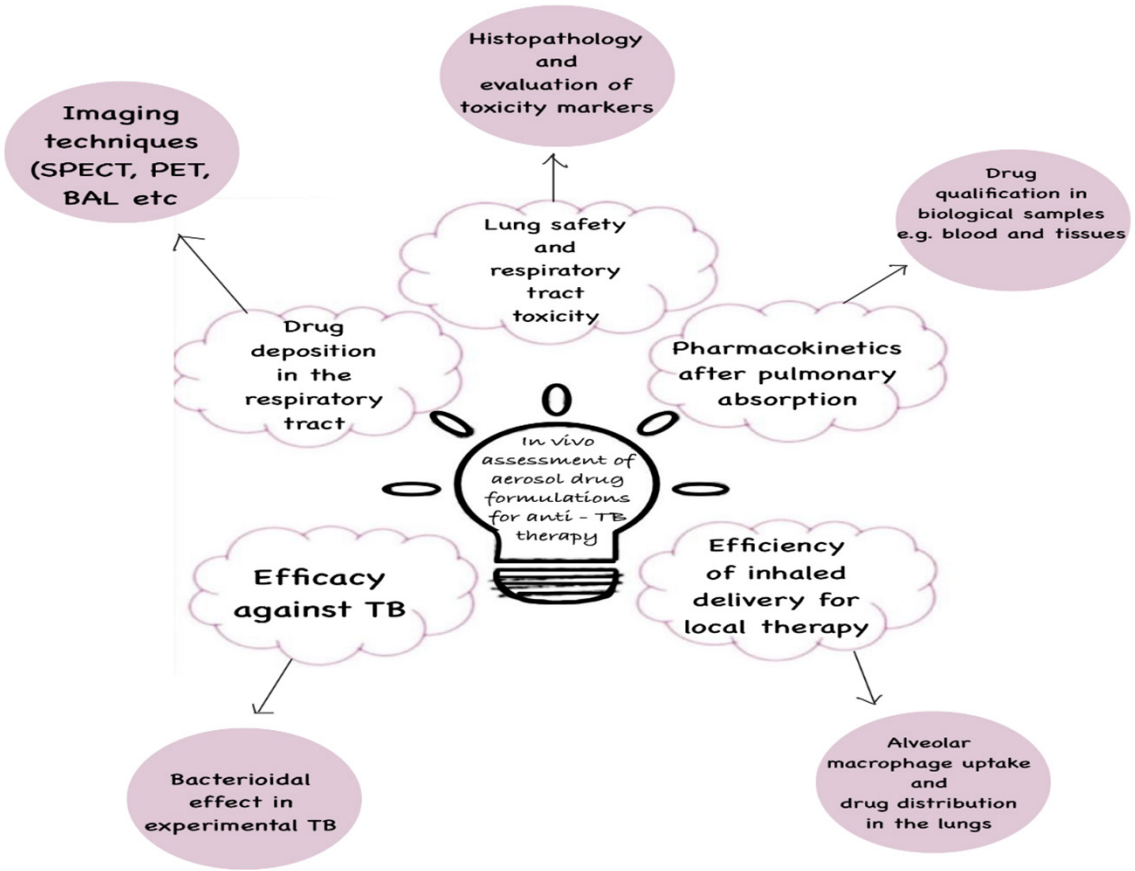

Figure 1: *In vivo* assessment of aerosol drug formulations for Anti-TB Therapy.

Table 1: Standardized formulation parameters.

Sl. No.	Ingredients	Quantity
1	Linezolid	40 mg
2	Chitosan	0.5% W/V
3	Poloxamer	0.1 %W/V
4	Methanol	50ml
5	Sodium Taurocholate	1.5%
6	Parabens	0.01%
7	TPP	0.75 %W/V

Table 2: Method development parameters and chromatographic conditions.

Parameters	Test results
Mobile phase	ACN: Acetic acid (50:50)
Flow rate	1.2 mL/mL
Detection wavelength	254 nm
Run time	10 min
Column temperature	Ambient temperature
Column pressure	160 kg/cm^2
Injection volume	10 mL
Retention time	3 min

per ICH guidelines. The wavelength was detected at 254 nm.[25] The experimental conditions are depicted in Table 2.

To measure %EE, 10 mg of precisely measured drug-loaded MPs were introduced into 100 mL of methanol. Through a process of ultracentrifugation at 18000 rpm for 40 min at 4°C. The quantity of drug that was not trapped (referred to as free drug) was determined by measuring the clear supernatant using HPLC spectrophotometry at a specific wavelength of 254 nm. EE (%) was determined using a mathematical equation.[26]

$$Drug\ Entrapment\ Efficiency = \frac{Practical\ Drug\ Content}{Theoretical\ Drug\ Content} * 100$$

Checkpoint analysis

The Design-expert software was used to determine the best formulation variables for synthesizing optimized MPs. The Lzd MPs checkpoint batch was then synthesized with these optimal variable values to confirm the effectiveness of the optimization technique. The size of particles and the efficacy of entrapment were tested. The percentage of relative error between the experimental and predicted values was calculated for the optimized MPs.[27]

$$\%\ \text{Relative Error} = \frac{\text{Predicted value} - \text{Experimental value}}{\text{Predicted value}} * 100$$

FTIR and XRD

The FTIR spectra of pure chitosan, linezolid (Lzd), and their physical mixture were recorded using Fourier Transform Infrared (FTIR) spectroscopy from Thermo Scientific, USA. Samples were prepared by blending with KBr and compressing into self-supporting discs. The KBr press applied a compression force of 5 tonnes for 5 min. Scans were conducted over the spectral range of 4000 to 400 cm^{-1}. The crystalline structure of spray-dried powders incorporating various excipients was analyzed using XRPD equipment, including BRUKER D8 or Rigaku Miniflex 300/600 systems. Powder samples were loaded on a planar quartz glass slide with an engraved square and assessed using a D/tex Ultra2 slit detector source.[28]

Formulated DPI's Flow Characteristics

The angle of repose, Bulk density, Carr's index, Hausner ratio, and % Porosity

The flow properties of the formulations and commercial DPI were evaluated using Carr's index, Hausner's ratio, and percentage porosity. The following formulas were used for the respective properties.

The angle of repose,

$$\text{Tan}\theta = \frac{\text{Height}}{Radius}$$

$$Bulk\ density\ (g/cc) = \frac{(Weight\ of\ powder\ (gm))}{Volume\ of\ powder\ (cc)}$$

Carr's Index,

$$(\text{Ci}) = \frac{(\text{tapped density} - \text{bulk density})}{\text{tapped density}} * 100$$

$$\text{Hausner ratio} = \frac{\text{bulk density}}{\text{tapped density}}$$

$$\%\text{porosity (E)} = 1 - \frac{\text{Pb}}{\text{Pt}} * 100$$

Where Pb and Pt are bulk and tapped density of DPI.[29]

SEM and TEM

Surface morphology was investigated using a Scanning Electron Microscope (SEM, Hitachi S4700; Tokyo, Japan, Hitachi Scientific Ltd) at a voltage of 1.00 kV and a Transmission Electron Microscope (TEM) 2100, manufactured by JEO in Tokyo, Japan, operating at a voltage of 160 kV.[30]

Anti-TB activity

The MABA (Microplate Alamar Blue Assay) assessed the anti-M. tuberculosis activity of the compounds. This approach uses a non-toxic, thermally stable reagent and demonstrated a strong correlation with proportional and BACTEC radiometric assays. To minimize evaporation during incubation, 200 µL of sterile deionized water was added to the peripheral wells of sterile 96-well plates.

Each well was filled with 100 µL of Middlebrook 7H9 broth, and the compounds were serially diluted on the plate to achieve final concentrations ranging from 100 to 0.2 µg/mL. The plates were sealed with parafilm and incubated at 37°C for 5 days. After

Table 3: Dosage regime for Wistar albino rats.

Group name	Treatment	No. of animals	Total no. of animals
I: Normal Control	Normal saline.	6	18
II: Drug formulation	25 mg Linezolid MPs orally for 16 hr.	6	
III: Standard drug	Zivox 600 mg orally for 16 hr.	6	

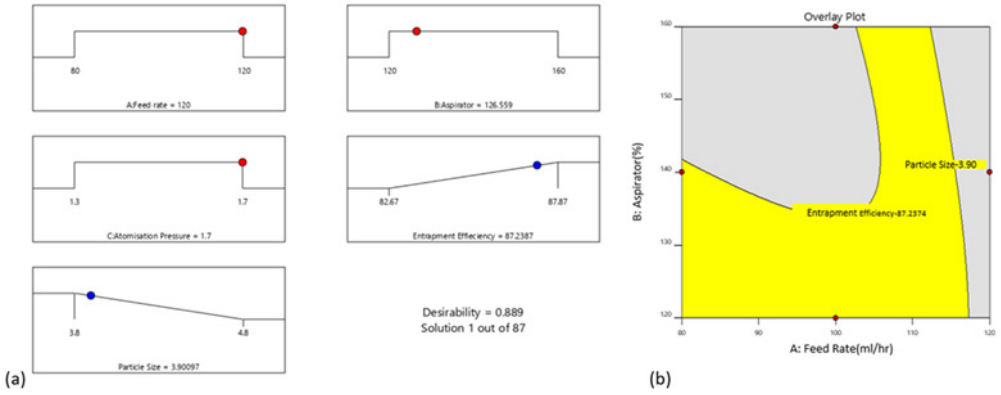

Figure 2: Desirability Graph (a) and overlay plot (b) of optimized formulation.

incubation, 25 µL of a freshly prepared 1:1 mixture of Alamar Blue reagent and 10% Tween 80 was added to the wells and incubated for another 24 hr. A colour change indicated bacterial growth: pink wells signified growth, whereas blue wells indicated inhibition. The Minimum Inhibitory Concentration (MIC) was the lowest concentration preventing a pink colour change.

The assay utilized the vaccine strain *Mycobacterium tuberculosis* H37Rv (ATCC No. 27294). Standard anti-TB drugs tested included Isoniazid (1.6 µg/mL), Ethambutol (1.6 µg/mL), Rifampicin (0.8 µg/mL), and Streptomycin (0.8 µg/mL).[31]

In vitro drug release and *in vitro* lung deposition study

The *in vitro* drug release of spray-dried linezolid microparticles was assessed in simulated lung fluid using modified dissolution equipment (USP, Type I, LABINDIA DS 8000, Mumbai). In summary, microparticles of Lzd, which had the same effect as 10 mg of the formulation, were evenly distributed in 25 mL of a solution with a pH of 7.2 and swirled at a speed of 100 revolutions per minute. At predetermined intervals of 1, 2, 3, 4, 5, 6, 7, 8, and 24 hr, 2 mL samples of the dissolution medium were collected and filtered through a 0.22-µm nylon syringe filter. Each time, the removed volume was replaced with an equivalent amount of fresh medium to maintain consistency. The concentration of the dissolved drug in the sampled medium was quantified using a spectrophotometer at a wavelength of 254 nm. The analysis relied on the regression equation derived from a calibration curve established in the same medium, covering the concentration range of 0-10 µg/mL. A drug release profile was generated by calculating and plotting the cumulative proportion of Lzd released at each time point.[32]

For the Lung deposition study, the optimized formulation was assessed for lung deposition *in vitro* using the Anderson Cascade Impactor (API) (Thermo Fischer Scientific, Waltham, Massachusetts, USA) at a flow rate of 28 L/min. A precisely measured amount of LZ NPs was combined with micronized lactose in a ratio of 1:3 (weight to weight) and administered using a 08-stage nonviable API. After administering the prescribed doses, various plates were gathered, and the concentration of Lzd was determined. The MMAD (Mass Median Aerodynamic Diameter) was determined by inputting the deposition data. Which is obtained by the ACI.[33]

In vivo animal study

The Indian government-registered Institutional Animal Ethics Committee (IAEC) authorized the animal experiment protocol (RPCP/IAEC/2012-2013/R-12). In the present research work male Wistar albino rats, weighing between 150 and 300 g. These rats were kept in cages with a 12-hr light/dark cycle, provided with free access to pellet food, and maintained in an environment with a temperature of 22±2°C and a relative humidity of 50-60%. The study assessed the effects of an acute oral dose on healthy male albino Wistar rats, following OECD rules (standard 423). This study determined the Lethal Dose 50 (LD_{50}). After a 7-day acclimatization period in the laboratory, rats were randomly assigned to 3 groups. This study tested various experimental conditions, including a normal control receiving only saline, a second group receiving 25 mg Lzd MPs orally for 16 hr and a 3rd

group with a standard drug. The dosage regime is provided in Table 3.[34]

Each rat in the group was anaesthetized by inhalation using diethyl ether. The tongue of the animal was carefully extended using blunt, plastic-tipped forceps, and an otoscope was employed to view the tracheal opening for the insertion of a 20-gauge cannula tube. Drug particles were blown into animals' lungs from a plastic syringe with varied air volumes. The same volume of air was given three times at 5-min intervals. Blood samples (0.5 mL) were drawn from the tail vein of each rat at various time intervals: 0 min, 15 min, 30 min, 1 hr, 2 hr, 4 hr, 8 hr, and 16 hr. This version keeps the same information but rephrases the sentence for better flow.

The samples were then diluted with 2 mL of PBS and centrifuged at 3000 rpm, 4°C for 10 min, after which the separated serum was stored for further pharmacokinetic analysis. The samples were filtered through a 0.22 µm membrane filter, and the HPLC system quantified the drug concentration. In microcentrifuge tubes, 10 µL of each standard solution was mixed with 90 µL of blank rat plasma to create plasma standards of Lzd at concentrations of 10, 20, 50, 100, 500, 1000, and 10,000 ng/mL. The pharmacokinetic parameters of Lzd, such as the peak plasma Concentration (C_{max}), half-life ($t_{1/2}$), and Area Under the Concentration-time curve (AUC), were determined using a non-compartmental analysis approach with GraphPad Prism 7.0.[35]

Histopathology assay

Histopathology is the process of examining tissue under a microscope to study the characteristics of illness. Histopathology investigations were conducted on male albino Wistar after administering the tested mixture. The lung tissue, which was separated from other tissues, was immersed in 5 mL of a solution containing 10% neutral buffered formalin to preserve it. The lungs were fixed and sectioned horizontally. They were then stained with hematoxylin and eosin and examined under an optical microscope to assess any damage to epithelial cells and inflammatory response.[36]

Evaluation of *in vitro* biodegradation behavior

A study was carried out to assess the degradation rate of the prepared samples. Approximately 3 mg of Chitosan Microparticles (CS-MPs) containing Tripolyphosphate (TPP) were weighed and recorded as the initial weight ('Wo'). These samples were then placed in a 1xPBS solution with lysozyme at a concentration of 10,000 µg/L and incubated at 37°C for various time intervals (24, 48, and 72 hr). After the specified incubation times, the samples were thoroughly rinsed with deionized water to eliminate residual ions. The drying process involved the use of filter paper to ensure

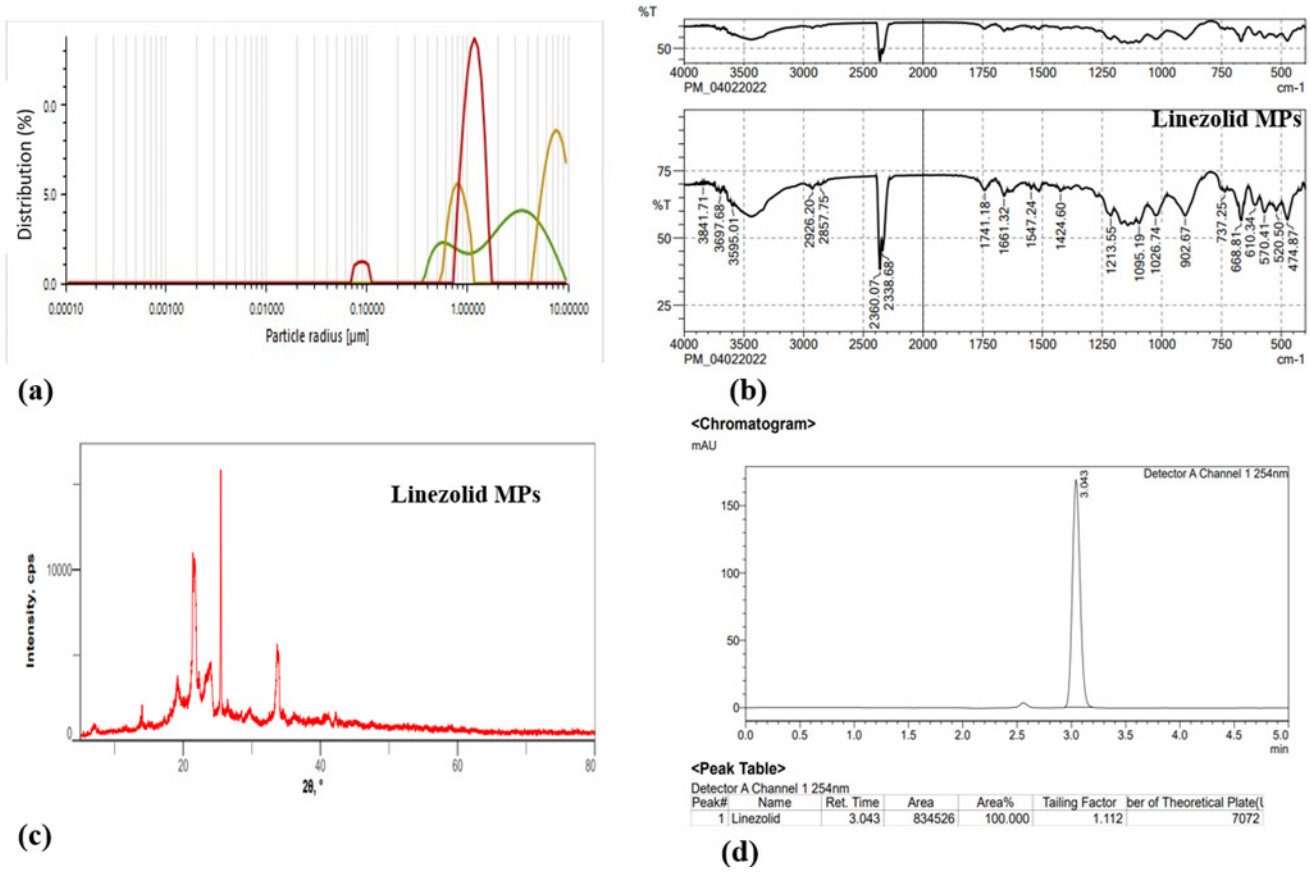

Figure 3: Particle size distribution (a), FTIR spectra (b), XRD pattern (c) and HPLC chromatogram (d) of optimized Linezolid MPs.

Table 4: Box-Behnken Experimental runs for Optimized MPs.

Run	Factor 1	Factor 2	Factor 3	Response 1	Response 2
	A: Feed rate (ml/hr)	B: Aspirator (%)	C: Atomisation Pressure Kg/m³	Entrapment Efficiency (%)	Particle Size (µm)
1	100	160	1.3	86.34	3.9
2	120	140	1.3	86.53	4.3
3	80	120	1.5	82.67	4.4
4	80	140	1.7	87.64	4.6
5	100	140	1.5	84.52	4.5
6	120	120	1.5	85.23	4.8
7	100	140	1.5	84.97	4.6
8	120	120	1.7	89.23	3.9
9	100	160	1.7	87.87	4.5
10	80	140	1.3	85.26	3.9
11	100	120	1.3	86.76	4.6
12	100	140	1.5	87.26	4.8
13	120	160	1.5	82.99	3.9

Table 5: ANOVA table for Response 1 and Response 2.

Responses	SD	R^2	Adjusted R^2	Predicted R^2	Press	*p*-value	Remark	Suggested Model
Particle size	0.1177	0.9470	0.8788	0.7199	9.9685	0.0087	Significant	Quadratic
%EE	0.8799	0.8517	0.6611	0.5440	8.1743	0.0123	Significant	

thorough drying. The Weights of the samples at specific time intervals (Wt) were recorded.[37]

The equation below was used to calculate the degradation.

$$\text{Degradation } \% = \frac{(\text{Wo} - \text{Wt})}{\text{Wo}} \text{x}100$$

RESULTS AND DISCUSSION

Optimization of Lzd microparticles

Utilizing Design-Expert Software (Trial Version 11.1.2.0, Stat-Ease Inc., MN), a batch was conducted to explore the desirability function and composition for optimized Lzd formulation. Table 4 presents the factor combinations and performance parameters of the formulation batches. It was noted that the aspirator and atmospheric pressure have a notable impact on the %EE, which was determined to be between 82.67% and 87.97%. Overlaying contours is a technique commonly used to optimize multiple responses. Figure 2 displays the Desirability graph and overlay of the contour plots for PS and EE. The desirability function enables the combination of all responses into a single measurement, allowing for the prediction of optimal levels for independent variables. A desirability function was utilized to calculate the most optimal batch outcome. Batch F8 was determined to be the best batch due to its high overall desirability of 0.889. Its independent variable values were found to be within the optimal range for MPs preparation. The optimized spray dryer parameters are as follows: a feed rate of 120±4 mL/min, an aspirator of 1260±200, and an atomization pressure of 1.7 kg/m3. The chosen formulation demonstrated a high %EE ranging from 82.67% to 87.97% and a small PS between 3.9 µm and 4.8 µm.

Effect of Aspirator, Atomization Pressure, Feed rate of spray dryer effect on PS and %EE

A study conducted by Baldelli and Vehring revealed that the particle formation process and morphology can be greatly influenced by various initial conditions, such as the composition of solutes and drying temperatures. These MPs have diameters ranging from 4 to 10 µm and densities that vary from 1250 to 1950 mg/mL.[38] In their study, Ruprecht and Kohlus emphasized the impact of atomizing air pressure on the mean residence time and PS distribution. They found that higher pressures led to narrower distributions, which can be attributed to the aspirator effect.[39] The feed rate has a significant impact on the %EE, as observed in various studies that have shown how different feed rates can result in differences in moisture content, dispersibility, and bulk density of the dried products. Ensuring the feed rate is carefully adjusted according to the material being processed is essential to achieve the desired PS distribution and EE in

spray drying processes. Modifying the feed rate has an impact on the average PS.[40] It is essential to optimize the spray drying parameters such as feed rate, aspirator, and atomization pressure to achieve the desired PS distribution, shape, and EE. This will ensure that the particles have the appropriate aerodynamic properties for effective delivery to the lungs.[41] Table 5 displays the ANOVA table.

Particle Size, Zeta Potential

Micro particulates (DPIs) have a narrow range of PSs, which is beneficial for pulmonary DPI applications as it allows for accurate targeting of specific areas in the lungs. As a result, this the feature encourages a significant accumulation of drug particles. A higher concentration of TPP led to a larger PS. All batches consistently maintained particle sizes below 5 µm, which is essential for ensuring effective inhalation administration. Particles that measure less than 5 µm are classified as respirable fractions, indicating the portion of particles that can reach the deepest parts of the lungs known as the alveolar regions. The average PS, as shown in Figure 3a, was found to be 3.9 µm, which falls within the specified range and is considered satisfactory. DPI MPs possess a precise PS distribution, offering significant benefits for pulmonary DPIs by allowing the particles to effectively target a specific region of the lungs. The formulation showed ZP of +27 mV, subjecting to their amine groups of the chitosan, and also which is crucial for stability and drug release kinetics.

FTIR, XRD

The FTIR spectra of the optimized formulation exhibited all the anticipated characteristic peaks, with little alterations observed for chitosan and TPP, indicating the absence of interaction between Lzd with these excipients. Figure 3b shows that Lzd's distinctive peaks were present in the spectrum, confirming that the drug and polymer's chemical integrity did not alter. XRPD patterns were acquired using a powder diffractometer with a CuKa source (λ1=1.54060 A and λ2=1.54439) in ambient circumstances. The Lzd and excipients have no XRD peaks, indicating no crystallinity (Figure 3c).

HPLC validation and Drug encapsulation

The approach demonstrated a linear relationship within the concentration range of 100-140ppm, with a high coefficient of

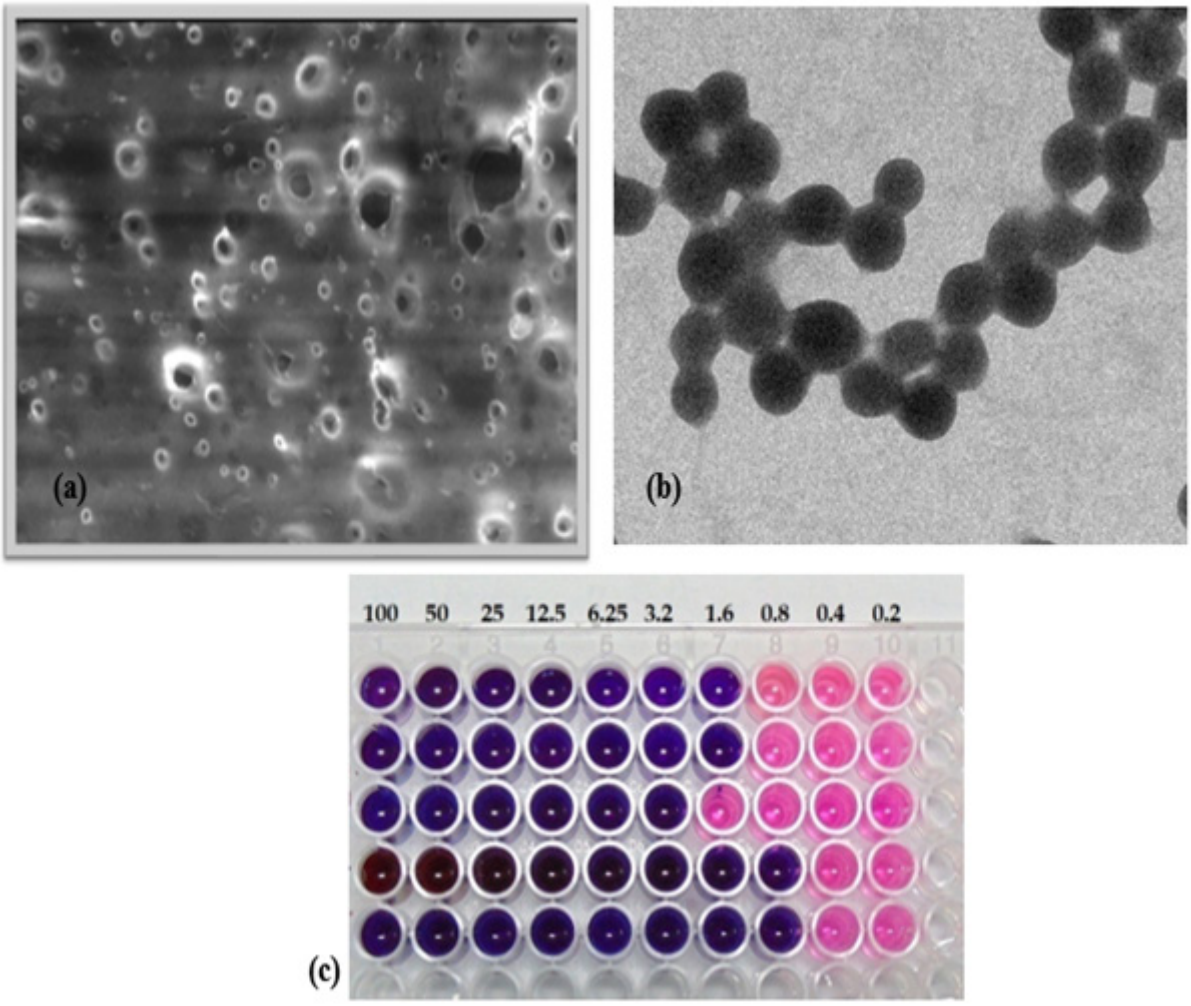

Figure 4: SEM (a), TEM (b) and Anti-TB activity (c) of Linezolid loaded Dry powder MPs.

determination (R2) value of 0.9986. The limits of detection and quantification for Linezolid MPs were 0.02 µg/mL and 0.05 µg/mL, respectively Additionally, the validation process confirmed that the data demonstrated a current, efficient, dependable, and precise approach. We observed a satisfactory percentage of recovery of 98-100% in the drug-loaded MPs suggesting that this method can be utilized to quantify Lzd in both bulk and MPs. Chitosan of a higher molecular weight typically exhibits a higher density when compared to chitosan of a lower molecular weight. The increase in viscosity may lead to a more efficient encapsulation of LZD. Likewise, a greater amount of chitosan can lead to a rise in viscosity and an increased number of polymers to capture Lzd. The increased molecular weight and concentration of chitosan resulted in an enhancement of viscosity. This, in turn, led to a notable increase in the encapsulation efficiency, which was measured at 89.37%. It was observed that the drug content in the MPs exhibited a substantial increase (p>0.05) with an increase in the drug/chitosan weight ratio up to 50%. The loading efficiency was found to be 49.16% (Figure 3d).

SEM and TEM

The surface properties of Lzd DPI were confirmed using SEM analysis, which revealed the presence of irregularly shaped spherical particles with rough surfaces and a uniform size distribution at specific magnifications. This modification enhances the spread of the substance in the body, enhances its ability to target specific areas and detect even small amounts, and decreases any harmful effects on the body caused by its use. The surface's exceptional adsorption capabilities are attributable to the use of tri-polyphosphate in combination with chitosan nanoparticles through the inotropic gelation technique. This approach offers greater benefits compared to utilizing chitosan alone (Figure 4a, 4b).

MIC study

The pink indicator dye in positive control wells indicated bacterial growth. The negative control wells had blue dye, indicating no bacterial culture. Additionally, Lzd dilutions from 0.2 to 0.8 µg/mL showed a pink color, indicating bacterial growth. The dye color did not change at a dilution concentration of 1.6 µg/mL for Lzd, suggesting the absence of bacterial growth. Therefore, it was determined that the Minimum Inhibitory Concentration (MIC) for Lzd was 1.6 µg/mL. The Lzd MPs demonstrate susceptibility to the H37 RV strain at all concentrations, comparable to the reaction reported with conventional medicines (Figure 4c).

Formulated DPIs flow characteristics

Chitosan can cover the DPI thinly to increase spherical particle production. The formulated DPI's flow can be enhanced. The specified DPI aerosolization efficiency depended on flow characteristics. The angle of repose, Carr's index, bulk density, tapped density, and Hausner's ratio for F8 formulations are depicted in Table 6 showing better flow properties than the commercial drug available. Improved Lzd DPI had a higher angle of repose and Carr's index than commercial DPI and other formulations. All spray-dried formulations satisfied appropriate powder flow requirements. Higher moisture content may explain Chitosan formulations' greater angle of repose. This batch had more moisture, probably due to molecular weight. Previous studies have shown that tapped density affects particle aerodynamics.[42] Carr's Index values below 25 indicate good powder flow, whereas values above 40 indicate poor flow. The compressibility index of High Carr's reveals particles' cohesiveness. The results show that feed composition and spray dryer parameters can be adjusted to achieve particle flowability.

In vitro drug release and *in vitro* lung disposition

Following the experimental design, the *in vitro* dissolution studies of the formulations were carried out. It was observed that almost all of the formulations-maintained drug release for a period of up to 24 hr. As the amount of chitosan increased, the polymer density increased, which resulted in a reduction in the mobility of the macromolecular chain, which most likely resulted in a decrease in drug release. The controlled drug release behavior in chitosan batches is linked to its polyelectrolyte nature. Chitosan maintains its non-protonated state in the alkaline pH of the chosen release medium.[43] In addition, the cross-linking of chitosan limits the flow of the dissolution medium, resulting in a controlled diffusion of the encapsulated drugs. The release studies for all of the formulations are displayed in Figure 5a. The Lzd and commercial DPI MMAD were measured at 4.2 µm and 22.8 µm, respectively, which is within the permissible range (0.5 to 5

Table 6: Dry linezolid powder Inhaler flow and Linezolid loaded MPs characteristics.

Linezolid loaded DPI's flow properties					
Formulation batch	**Angle of Repose (Degrees)**	**Bulk density (g/cc)**	**Tapped density (g/cc)**	**Carr's index (%)**	**Hausner's ratio**
F8	28±0.08°	0.171	0.128	25.0	0.95±0.08
Linezolid loaded MPs characterization					
	Particle Size (µm)	**Zeta potential (mV)**	**EE (%)**	**LE(a)**	**Moisture Content (%)**
F8	3.9	-42.0	89.37	49.16	1.58

μm) for lung deposition. The formulated DPI showed a fivefold decrease in MMAD compared to the commercial product due to improved aerodynamic behavior, including disaggregation due to its spherical nature, negative surface charge, and lower tapped density (0.128±0.02), affecting lung deposition. Lower MMAD was observed in fine particle fraction, respirable fraction, and emitted dosage, with achieved DPI achieving 67.58±0.03%, respirable fraction of 70.139, and emitted dose of 0.984, and commercial DPI of 29.03±0.09%. Probability paper helps to calculate PS concentration degree and average diameters. With the abscissa representing PS and the ordinate representing cumulative distribution frequency, PS distribution normality is depicted in Figure 5b.

In vivo animal study

Pharmacokinetic characteristics of DPI and oral tablets of Lzd in rats are measured, and their profiles of concentration in the bloodstream over time are noted (Figure 6a). Following the injection of the formulated drug, it was observed that it entered the bloodstream after 30 min in all the animals that were tested. The drug reached its highest concentration at 50±5 min. However, when the drug was administered in the form of an oral tablet, it took 15 min for it to emerge in the bloodstream, but only in two-thirds of the tested animals. The drug reached its maximum concentration at 110±8 min in this case. This suggests that the DPI formulation of Lzd can lead to a more rapid initiation of its effects compared to its oral tablets. The Lzd DPI formulation is of great importance, as indicated by the acquired p values

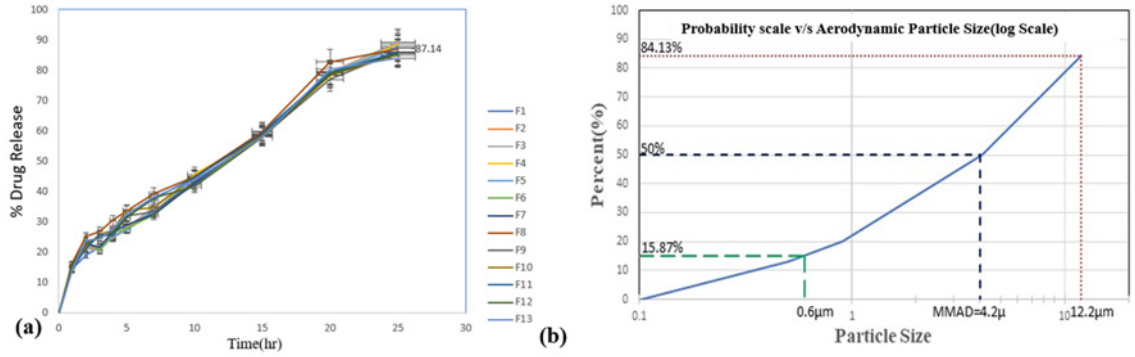

Figure 5: *In vitro* drug release profile (a) and *in vitro* Lung-deposition analysis (b) of Linezolid loaded inhalable MPs.

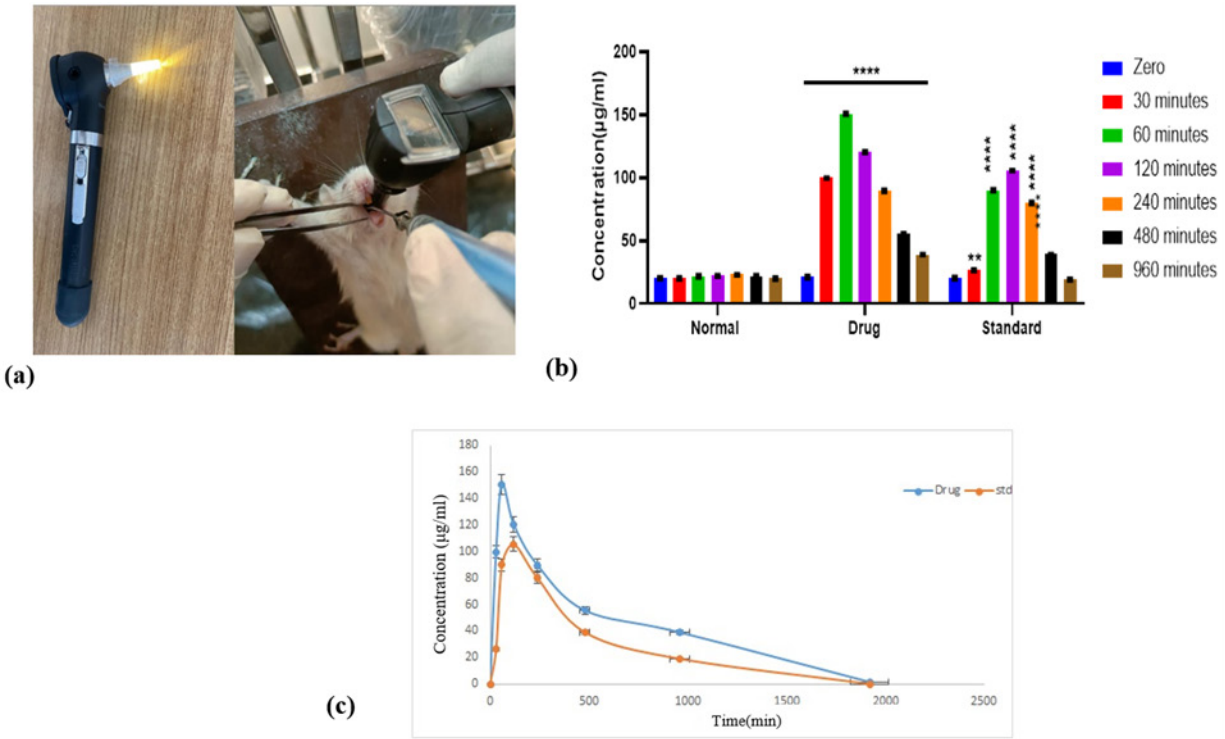

Figure 6: Insertion of the 20 G cannula tube, inflating the lung by blowing air with a plastic syringe (a), Two-way ANOVA followed by Tukey's multiple comparisons test (b), the values are presented as the mean±SEM *n*=3; The obtained p values analyzed data were: ***p*<0.01; *****p*<0.0001 and (c) pharmacokinetic parameters of Lzd loaded inhalable MPs.

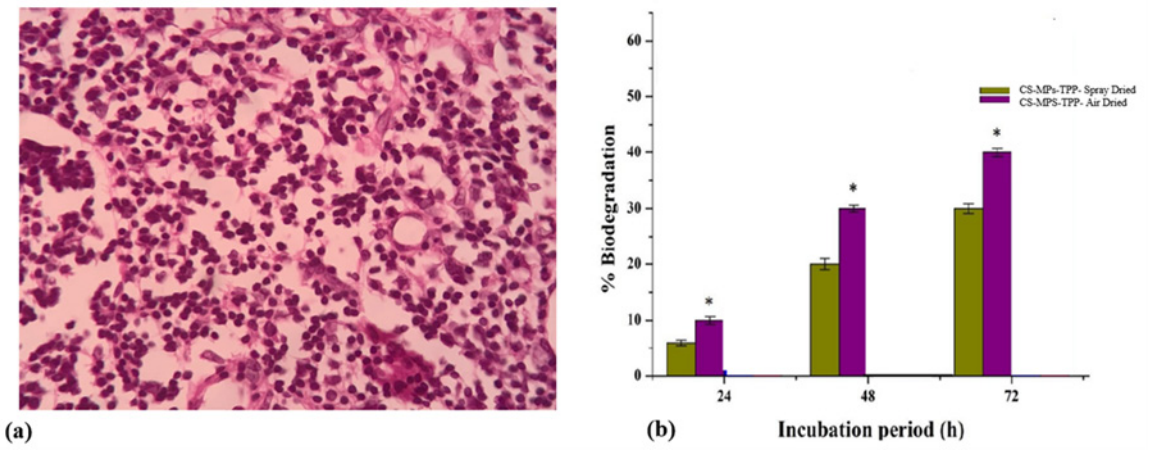

Figure 7: Histopathology of the Lzd loaded inhalable MPs treated lung's transverse section (a) and Biodegradation study developed spray dried Lzd loaded MPs (b).

Table 7: Optimized formulation Pharmacokinetic study.

Pharmacokinetic parameter	Treatment drug	Standard drug
C_{max} (ng/mL)	1730.75±26.3	1435.93±218.5
T_{max} (hr)	6.01±0.00	2.00±0.00
$T_{1/2}$ (hr)	9.42±1.94	4.26±0.34
Kel	0.223±31	0.0044±0.0003
AUC0-8 hr (ng/mL·hr)]	111947.1406±471.41	7517.8291±401.79
Area of the last part (ng/mL·hr)	1582.69±05.63	3664.81±267.24
$MRT_{0\text{-}inf}$ (hr)	17.92±1.35	5.69±0.11
Vd (L/Kg)	0.2233	0.537

displayed in Figure 6b (***p*<0.01; *****p*<0.0001). The higher bioavailability of Lzd DPI compared to its oral tablets suggests that the formulation is capable of delivering the drug to the lower airways and bronchioles. These areas have thin linings, a large surface area, and a rich blood supply, which allows for rapid absorption of the medication. The lungs are an excellent route for aerosolized medications intended for systemic absorption, as they have low metabolic activity and avoid the first-pass effect in the liver. The Lzd DPI formulation had a relative bioavailability of 55.2%, which was 1.25 times greater than that of the oral tablet. The improved absorption rate of the Lzd powder was achieved through the utilization of the spray dryer techniques to produce MPs. Pharmacokinetic parameters are displayed in Table 7. The rapid achievement of peak plasma drug Concentrations (C_{max}) was observed in the case of the developed formulation, thanks to its controlled release mechanism, as compared to the free drug. The developed formulations demonstrated higher AUC values, indicating that the drug concentrations remained within the pharmacologically effective range for an extended duration (Figure 6c).

Histopathological study

Figure 7a displays the microscopic images of histopathology samples. The formulation loaded with Lzd exhibited a minor presence of inflammatory cells, likely caused by the rapid release of Lzd. Furthermore, a thin layer of connective tissue and numerous capillaries lined with simple squamous epithelium were observed, suggesting minimal inflammation and toxicity to healthy lung cells.

Biodegradability study

Biodegradability is essential for biological material usage. Biodegradation affects the metabolic result of materials in the body, especially drug-delivery polymers. Humans break down chitosan chemically and enzymatically. Vertebrates break down this polymer mostly with lysozyme and bacterial enzymes. Lysozyme, leucine aminopeptidase, pectinase isozyme, and rat fecal and colonic bacteria enzymes break down chitosan *in vitro*. *In vitro* studies show that chitosan MPs containing TPP biodegrade in 1xPBS with lysozyme (10,000 µg/L) at 37°C for 24, 48, and 72 hr. Figure 7b shows the degradation rate. The enzyme biodegrades 10% of CS-MPs-TPP-Air and 6% of spray-dried chitosan MPs within 24 hr, rising over time. Biocompatibility is shown by 30% and 50% MP degradation after 72 hr. Air-dried MPs degrade faster than spray-dried ones.

Statistical analysis

The statistical tests were done in Graph Pad Prism. To compare the control and treated groups' pharmacokinetic data, Tukey's *t*-test and ANOVA were used. Differences with *p*-values below 0.05 were statistically significant.

CONCLUSION

This study investigated the potential of natural polysaccharides as drug vectors for lung infection. These polysaccharides possess mucoadhesiveness, biodegradability, and ligand

specificity, making them excellent candidates for this purpose. Based on the Results and Discussion, it was determined that the optimized formulations demonstrated a controlled and sustained drug release for an extended period. The inhalable Lzd MPs formulation is a highly effective treatment option for TB. By utilizing a combination of ionic gelation and spray drying techniques, along with meticulous optimization and evaluation via BBD design, a remarkably efficient inhalable formulation of Lzd has been developed. This formulation showed great promise in improving the treatment of TB. The formulation process guarantees the stability and effectiveness of Lzd, which possesses desirable qualities for pulmonary delivery. This inhalable formulation has a highly improved pharmacokinetic profile and bioavailability, along with strong antitubercular activity. It has the potential to greatly enhance therapeutic outcomes for patients with TB. Future studies should prioritize clinical trials to validate these findings and further investigate the therapeutic potential of this innovative inhalable formulation in human subjects.

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AUTHOR CONTRIBUTIONS

The final manuscript was prepared with the help of all contributors. Compliance with Ethical Standards.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

TB: Tuberculosis; **Lzd:** Linezolid; **MDR:** Multidrug-Resistant; **DPI:** Dry Powder Inhalers; **MPs:** Microparticles; **PS:** Particle Size; **%EE:** Entrapment Efficiency.

SUMMARY

This study investigates a targeted pulmonary drug delivery system using Dry Powder Inhalers (DPI) for the antibiotic Linezolid (Lzd) to treat tuberculosis (TB). Linezolid-loaded biodegradable microparticles (MPs) were synthesized using chitosan via spray drying. The optimized formulation achieved 89.57% entrapment efficiency, with a particle size of 3.9 µm, and demonstrated sustained drug release for up to 12 hr. *In vivo* studies showed a 55.2% increase in bioavailability compared to oral tablets. This novel DPI system could enhance TB treatment by improving drug delivery, reducing side effects, and increasing patient adherence.

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