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REVIEW

Development of natural polymers-based inhaled microspheres for tuberculosis

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Abstract

Background: Tuberculosis (TB) is a contagious disease caused by *Mycobacterium tuberculosis* (Mtb) that mainly affects the lungs (pulmonary TB). Treatment involves a 6-month regimen of four first-line anti-TB drugs: isoniazid (INH), rifampicin (RIF), ethambutol (ETH), and pyrazinamide (PZA). However, the effectiveness of this regimen is limited due to the protection of Mtb bacteria in lung lesions. An alternative approach involves delivering the drugs directly to the lungs through inhalation using innovative methods of microspheres, which can greatly enhance treatment efficacy. **Objective:** This review focuses on inhaled microspheres that use natural polymers for anti-tubercular drugs. **Method:** A comprehensive literature survey was pulled from databases (PubMed, Scopus, Google Scholar, and ScienceDirect) from 2012 to 2022. **Result:** The characterisation studies, formulation technique, and efficacy using in vitro and in vivo studies of anti-tuberculosis drugs inhaled microspheres. **Conclusion:** Microspheres have substantial potential as an inhaled drug delivery system and are likely to have significant clinical contributions in the future.

Introduction

Tuberculosis (TB) is a contagious disease that is becoming a global cause of death. During the COVID-19 pandemic, TB was the conducting cause of death from a solitary infectious agent, higher than HIV/AIDS (Bagcchi, 2023). According to the Global Tuberculosis Report 2022 by WHO, approximately 10.6 million people fell ill with TB in 2021, an increase of 4.5% from 10.1 million in 2020. The TB incidence rate (new cases per 100,000 population per year) rose 3.6% between 2020 and 2021.

TB is caused by *Mycobacterium tuberculosis* (Mtb), which is spread by infected persons who cough, sneeze, or even talk while expelling bacteria into the air. The disease, called pulmonary TB, frequently harms the lungs and other sites. Mtb survives and replicates in bad-perfused areas of the body, such as tubercles, granulomas, and alveolar macrophages

defined by a long chronic infection and progressive pathology (Sibum *et al.*, 2019).

TB therapy is recommended with regimens of four anti-TB first-line drugs for six months, which are isoniazid (INH), rifampin (RIF), ethambutol (ETH), and pyrazinamide (PZA) (Khadka *et al.*, 2022). They are prescribed during the first two months of treatment and continued for the next four months with rifampicin and isoniazid only. However, this drug regimen has sub-optimal therapeutic effects when used orally or intravenously. This is because Mtb bacteria are protected in lung lesions without blood vessels that prevent drugs from penetrating fibrous tissue. Thus, the drug concentrations in plasma and lungs are below the recommended therapeutic range (Khadka *et al.*, 2018). As a result, high doses of anti-TB drugs and lengthy treatment regimens are needed to reach bactericidal concentrations at the infected site, which requires high adherence and leads to drug

resistance. Therefore, a targeted drug delivery strategy can be a solution to TB treatment barriers.

An alternative route for targeted drug delivery is directly into the lung by the inhalation route. This non-invasive route targets drugs to the lungs for local or systemic effects, allowing the rapid onset of action and avoiding first-pass metabolism (Marreti *et al.*, 2014). This route makes it possible to achieve higher concentrations of anti-TB drugs in the lungs with smaller drug doses. It provided lower systemic exposure and reduced systemic side effects. Thus, reduces doses and frequencies of anti-TB drug intake to increase the patient's compliance. However, the inhalation route requires the drug size between 1-5

μm . Drugs with a diameter of $>5 \mu\text{m}$ tend to remain suspended in the upper respiratory tract, while $<1 \mu\text{m}$ are typically cleared during expiration due to their minimal inertia (Omar *et al.*, 2019). So they reached the deepest lungs and induced the alveolar macrophage endocytosis process for excellent clinical response. However, pure drugs are usually given a burst release and rapid unspecific distribution (Miranda *et al.*, 2018). An efficient solution is achieved by formulating the pure drugs into novel delivery systems such as nanoparticles, microparticles, micelles, liposomes, or dendrimers. One of the drug delivery systems that are suitable for the inhalation route is microspheres. The illustration of the systems is presented in Figure 1.

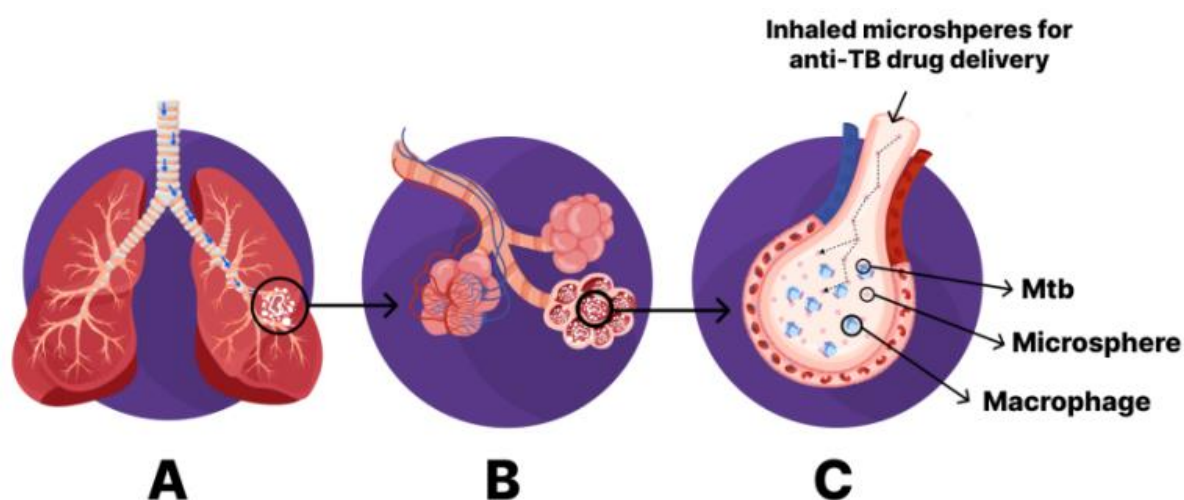


Figure 1: (A) Lung infected with Mtb; (B) Lung alveolar invasion by Mtb surrounded by blood vessels; (C) Inhalable microspheres delivering anti-TB drugs to alveolar macrophages for TB treatment

Methods

This manuscript consists of several extensively surveyed publications from databases, for example, PubMed, Scopus, Google Scholar, and ScienceDirect, within the last ten years, between 2012 and 2022. Publications were pulled from a search engine with keywords such as natural polymers, inhalation, microspheres, and tuberculosis. The data that is qualified were pulled manually and considered for incorporation within the manuscript. This review focuses on formulations and the techniques, characteristics, and in vitro and in vivo studies.

Results

Several articles encompassing original research and review articles were thoroughly investigated. These sources shed light on the development of anti-TB drugs, particularly for first-line therapy for inhaled microsphere delivery systems. The articles reviewed covered aspects such as formulation, formulation techniques, and characteristics of anti-TB inhaled microspheres made from various natural polymers. You can refer to the details presented in Table I for a comprehensive overview. Furthermore, Table II and Table III provide descriptions of in vitro and in vivo studies conducted in this study.

Table I: The Characteristic studies of anti-TB inhaled microspheres of various natural polymers

Drugs	Polymers	Formulation technique	Characteristics studies	Reference
INH	Sodium alginate	Ionotropic gelation - Spray drying	SEM: Smooth, spherical; Aerodynamic diameter: $1.23 \pm 0.07 \mu\text{m}$ Carr's index: $17.18 \pm 0.09\%$; Hausner's ratio: 1.18 ± 0.01 Yield: $26.45 \pm 1.54\%$; MC: $4.78 \pm 0.53\%$ EE: $53.34 \pm 4.28\%$	Garg et al., 2015
INH	Guar gum	Precipitation - Spray drying	SEM: spherical, homogeneous, and uniform Aerodynamic diameter: $3.53 \mu\text{m}$; Carr's index: 16.6 ± 0.7 Hausner's ratio: 1.19 ± 0.02 ; Yield: $30.3 \pm 1.5\%$ MC: $4.51 \pm 0.34\%$; EE: $51.3 \pm 2.17\%$	Kaur et al., 2016
ETH	Chitosan	Spray drying	SEM: spherical with a dimpled surface Adhesion force: $122-993 \mu\text{N}$; DL: 99–107%	Ahmad et al., 2015
INH-Rifabutin (RFB)	Carrageenan/starch	Spray drying	Yield: $71.0 \pm 1.7\%$; Volume diameter: $7.0 \pm 0.7 \mu\text{m}$ EE: $96.3 \pm 3.4\%$ (INH), $74.4 \pm 11.9\%$ (RFB) DL: $8.4 \pm 0.3\%$ (INH), $3.2 \pm 0.5\%$ (RFB)	Rodrigues et al., 2020
RIF- INH	Gelatin	Spray drying	Yield: 48.8%; Particle size: $4.89 \pm 0.25 \mu\text{m}$ EE% RIF: $51 \pm 6\%$; INH: $22 \pm 1\%$; Zeta potential: $13 \pm 2 \text{ mV}$ SEM: a rough surface, irregular shape	Manca et al., 2013

Table II: In vitro studies of anti-TB inhaled microspheres of various natural polymers

Drugs	Polymers	In vitro methods	Characteristics studies	Reference
INH	Sodium alginate	Drug release	The drug showed two phases: an initial burst release of 30-40% in the first 4 hours, followed by a sustained 90% release up to 60 hours.	Garg et al., 2015
INH	Guar gum	Drug release	The initial burst release of RIF is 20%.	Kaur et al., 2016
ETH	Chitosan	Cytotoxicity	A549 cells had >80% viability, Calu-3 cells showed 85-99%, and NR8383 cells ranged 81-100%. Macrophages ingested formulation within 30 mins.	Ahmad et al., 2015
		Anti-TB	Pure ETH had a MIC of 2 mg/mL, while ETH formulations < 1 mg/mL against <i>M. bovis</i> .	
		Permeability	After 2 hours, pure ETH permeability was 48.7%, while ETH formulations increased to 71%.	
INH- RFB	Carrageenan/starch	Aerodynamic behaviour	Powder aerosolisation showed 91% dose emission, MMAD of 3.3-3.9 μm for RFB and INH, indicating potential for deep lung co-deposition.	Rodrigues et al., 2020
		Drug release	INH released faster (60 mins vs. RFB's 240 mins), with 80% released in the initial 60 minutes, suggesting limited sustained release.	
		Anti-TB	The process did not affect the drug's efficacy.	
		Internalisation by macrophage	Showed macrophage interaction, inducing moderate activation.	
RIF- INH	Gelatin	Toxicity test	A549 cells: Microparticles less toxic than RFP (60% mortality at 48 hours). Laser microscopy confirmed probe uptake via microparticles.	Manca et al., 2013
		Impinger device's deposition	FPD: 1229 μg FPF: 12% NE: 59%; NER: 42%	

Table III: In vivo studies of anti-TB inhaled microspheres of various natural polymers

Drugs	Polymers	Administration method	Animal model	Results of in vivo studies	Reference
INH	Sodium alginate	Insufflation	Mice	Microspheres are released for 8 hours in the lungs, with higher lung uptake than the liver, spleen, and kidney. Microspheres significantly improved pharmacokinetics vs. plain drugs	Garg et al., 2015
INH	Guar gum	Pulmonary	Rats	Formulation maintained 15.21% vs. plain drug's 0% in lungs after 24 h, longer than plain drugs.	Kaur et al., 2016
RIF	Sodium alginate	Intratracheally instillation	Rats	RIF microspheres vs. oral pure drug: RIF lasted 4-72 hours in plasma (both routes), while free drug vanished in 24 hours. Pulmonary microspheres had better pharmacokinetics.	Patil et al., 2015
INH-RFB	Locust bean gum	Passive (nose only)	Mice	Safety assessment found no significant tissue differences in formulation. M.Tb infection model confirmed infection via CFU values in the lung, spleen, and liver.	Grenha et al., 2019

Discussion

Microspheres

Microspheres are microscopic spherical particles that have diameters of 1 µm to 1000 µm (Lengyel et al., 2019). Microspheres are characterised as a matrix system made up of a polymer in which the drug is homogeneously dispersed, either dissolved or suspended. The drug release mechanism results from various phenomena and mechanisms, such as diffusion, osmotically driven release, erosion, and swelling (Lengyel et al., 2019). Despite that, the particle size ranges from 1-5 µm for the inhalation route. The shape of the particles must also be spherical due to good flow properties, allowing the drug to reach the respiratory tract. Microspheres are one of the future solutions for targeted pulmonary drug delivery systems through their drug encapsulation capability, high bioavailability, biocompatibility, and stability, which also control the drug release to be sustained or prolonged release (Hariyadi et al., 2021; Rosita et al., 2022).

Formulations of inhaled microsphere

Microspheres enhance drug bioavailability and sustain release when incorporated with polymeric materials, protecting and controlling active ingredient release through surface degradation for targeted therapy (Hariyadi et al., 2018). Microspheres as a drug delivery system of anti-tuberculosis drugs are influenced by several factors, such as the variant and concentration of the polymer and crosslinker and formulation technique. The therapeutic goals to be achieved depend on the formulation, especially the selection of the polymer.

Polymers are classified into two types, which are mainly used for the preparation of microspheres: natural and synthetic polymers. Natural polymers avoid the

toxicological problems associated with the use of synthetic polymers (Kaur et al., 2012). Natural polymers are typically less expensive than synthetic polymers. They have better stability under harsh storage conditions, are capable of potentially biodegradable chemical modifications with few exceptions, and are biocompatible (Bangar et al., 2014).

Natural polymers are acquired from different sources like carbohydrates, chemically modified carbohydrates, and proteins (Choudhury et al., 2019). Carrageenan, chitosan, guar gum, and sodium alginate are examples of carbohydrates. Whereas proteins are albumin and gelatin. These polymers have been extensively investigated as carriers for inhalable microspheres.

Formulations affect the characteristics of inhaled microspheres as well as particle size and distribution, morphology by Scanning Electron Microscope (SEM), Moisture Content (MC), Entrapment Efficiency (EE), Drug Loading (DL), etc. Several anti-TB drugs have been researched for their inhalable formulation using in vitro and in vivo studies. These studies inform that inhaled drugs for TB treatment assure therapeutic benefits by delivering drugs directly to the lungs at higher concentrations (Khadka et al., 2022).

Characterisation

Characterising microspheres involves a range of techniques that systematically uncover the complex interweaving of their physical and chemical characteristics. Garg et al. (2015) and Kaur et al. (2016) both prepared INH-microspheres but with different polymers and techniques. Garg's sodium alginate-based microspheres had a lower aerodynamic diameter, higher entrapment efficiency, good morphology, and fair-good flow properties. Despite not achieving exceptionally high yields, the precipitation-

spray drying technique used by Kaur *et al.* appears to be preferable.

Overall, the characterisation of microspheres is an important step in their development and optimisation for drug delivery applications. It allows researchers to understand the physicochemical properties and optimise their design for specific applications.

***In vitro* studies**

In vitro microsphere studies involve lab experiments outside living organisms, examining drug release profiles and interactions with cells or biological molecules. For instance, these studies help assess drug release speed from microspheres and its modulation by factors like size, composition, and more.

The use of polymers plays an important role in this release ability. For example, INH-microsphere with sodium alginate showed sustained release for up to 60 hours (Garg *et al.*, 2015), whereas carrageenan achieves limited release with 80% released in the initial 60 minutes despite its better characteristics (Rodrigues *et al.*, 2020).

***In vivo* studies**

Patil *et al.* (2015) conducted *in vivo* studies using Rifampicin-alginate-microspheres on rats, comparing them to the free oral drug. The microspheres were administered through inhalation, while the free drug was given orally. When comparing Rifampicin-alginate-microspheres to the oral drug, RIF remained detectable in plasma for 72 hours for both routes, whereas the free drug was eliminated within 24 hours. Notably, the pulmonary administration of microspheres significantly enhanced pharmacokinetic parameters, resulting in C_{max} values of 85 ppm and 280 ppm, T_{max} values of 6 hours and 48 hours, and AUC values of 516 and 17670 ppm/mL, respectively.

In vivo studies are essential for clinical translation, highlighting improved TB treatment through inhaled drugs with lower doses and reduced toxicity. Selecting the right animal species and inhaled formulation is crucial for this study.

Conclusion

Inhalable microsphere delivery systems offer a promising approach to tuberculosis treatment, providing rapid action, lower doses, and reduced side effects. These microspheres enhance drug bioavailability and stability while allowing controlled release. Key factors for efficacy include polymer choice, concentration, size, and shape. They can potentially

improve drug targeting and personalised medicine through tailored formulations.

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