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A review on recent advances in lipid-based drug delivery systems for tuberculosis

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ABSTRACT

Tuberculosis (TB) remains a global health challenge, as current therapeutic strategies, albeit effective, require prolonged treatment durations and strict patient adherence. This often results in treatment failure and contributes to the growing issue of antibiotic resistance. To address these challenges, extensive research has focused on innovative drug delivery systems to improve bioavailability, enhance site-specific targeting, and overcome the limitations of conventional TB treatment. In this review, we summarise recent advancements in solid lipid nanoparticles, nanostructured lipid carriers (NLCs), and functionalized lipid nanoparticles for TB treatment. A literature review was conducted focusing on the pathophysiology of TB, *in vitro* and *in vivo* efficacy, and toxicity of lipid nanoparticles, and recent advancements in lipid nanoparticles for anti-TB drug delivery to the lungs. Studies demonstrated lipid nanoparticles significantly improve the solubility, stability, and targeted delivery of anti-TB drugs to infected macrophages. Rifampicin-loaded NLCs exhibited over 90% drug release sustained over 7 days and remained physically stable for up to 6 months. Mannose-functionalized NLCs showed around 70% macrophage uptake, doubling the rate of non-functionalized systems. *In vivo* studies on isoniazid-loaded SLNs reported a 3-fold increase in LD_{50} and a> 26-fold enhancement in bioavailability. Mannosylated clofazimine-NLCs exhibited prolonged lung retention and significantly reduced hepatic and renal toxicity. These quantitative improvements highlight the potential of lipid-based systems to outperform conventional formulations in both efficacy and safety. These advancements demonstrate strong potential for clinical translation, offering a more effective and patient-friendly approach to TB treatment.

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Introduction

Tuberculosis (TB) is a deadly airborne disease that causes millions of deaths annually, making it the second leading cause of death in the world, surpassing HIV/AIDS (1, 2). Approximately 4,910,000 cases of TB and 583,000 deaths were reported in Southeast Asia in 2023 (3). TB is caused by *Mycobacterium tuberculosis* (MTB), a contagious, intracellular bacterium measuring 0.2–0.6 µm. The survival of MTB is highly dependent on the host's immune system. MTB exhibits an exceptionally slow cell division rate, enabling it to evade and persist within the host's immune system for prolonged periods (4). Besides, the high concentration of lipids and mycolic acids in its cell wall enhances its resistance to a hostile environment, further challenging its eradication (4, 5).

To address the global burden of TB, the WHO introduced the End TB Strategy, aiming to reduce TB incidence by 90% and decrease TB-related deaths by 95% (6). While this is ambitious, it is supported by the high treatment success rate of approximately 95% among TB patients receiving conventional antibiotic treatment (7). The current treatment involves a combination of four antibiotics administered for 2 to 8 months. However, the prolonged duration and complexity of the regimen pose significant challenges to patient compliance. With the introduction of nanotechnology, there is a growing potential to overcome these challenges and improve TB outcomes (Figure 1).

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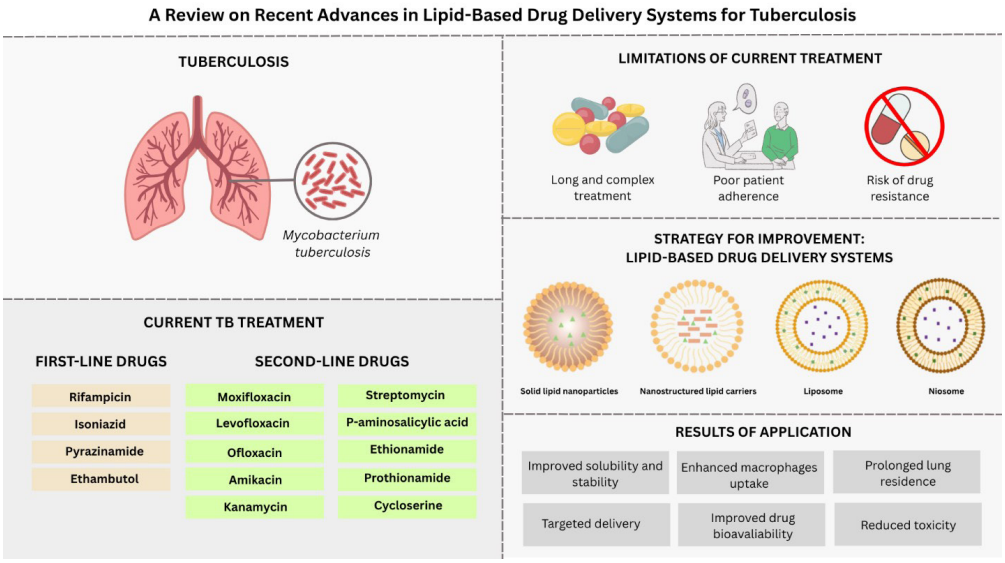

Figure 1. Overview of current tuberculosis treatment, its limitations, and the impact of lipid-based drug delivery strategies on therapeutic outcomes

Nanotechnology-based drug delivery systems, such as lipid-based carriers, offer several advantages, including enhancing antimycobacterial activity through targeted delivery to alveolar macrophages, enhanced uptake via endocytosis, resulting in increased bioavailability, and reduced toxicity (8, 9) (Figure 2). Their effectiveness has also been demonstrated in other infectious and chronic diseases, including oral amphotericin B nanoformulations for fungal infections and liposomal systems for hepatic fibrosis, both of which improved drug performance and safety profiles (10, 11). Thus, we review the recent developments in TB treatment, focusing on lipid-based drug delivery systems.

Pathogenesis of TB

The TB infection begins when MTB is inhaled in respiratory droplets and exhaled by people with active pulmonary TB (12). Once inhaled, MTB bacilli are deposited in the alveoli, initiating an infection (13). This process triggers an early innate immune response, wherein alveolar macrophages engulf the MTB through phagocytosis. However, rather than effectively eliminating the bacterial population, the macrophages provide an intracellular niche where MTB can survive and replicate. Following phagocytosis, the MTB inhibits phagosomes-lysosomes fusion, a critical defence mechanism that would otherwise facilitate bacterial destruction (14).

The host immune response to MTB infection leads to granulomas – cellular structures composed of infected macrophages surrounded by immune cells (15). While granulomas contain the infection and prevent further bacterial dissemination, they create a protective environment that allows MTB to persist in a dormant state, resulting in latent TB infection (16). In some cases, MTB bacilli evade the initial immune response, enabling dissemination through lymphatic or circulatory systems to secondary sites such as the kidneys, brain, and bones (17, 18).

TB infection is classified into two types: latent and active TB. Individuals with latent TB do not show any symptoms and have a lower amount of MTB load (19). In contrast, active TB is characterized by clinical symptoms, the potential for disease transmission, and its infectious state. While latent TB remains asymptomatic, patients have a 5-10 % lifetime risk of developing active TB, with a higher likelihood in immunocompromised patients (20).

Current therapeutic approaches to TB

Active TB treatment involves two phases: initiation and continuation (Table 1). The initiation phase consists of 40 to 56 doses of first-line antibiotics —rifampicin (RIF), isoniazid (INH), pyrazinamide (PZA), and ethambutol (EMB) —daily for 2 months. This intensive treatment regimen primarily targets MTB in its high-replication

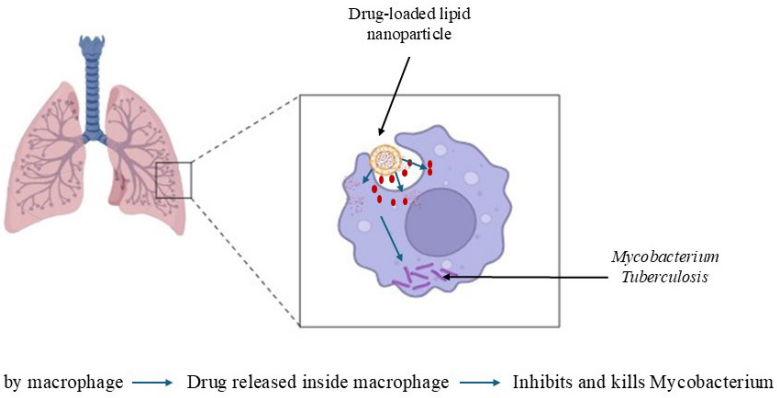

Figure 2. Uptake and release of drugs from lipid-based nanocarriers in tuberculosis treatment

Table 1. Summary of current tuberculosis treatment in terms of dose, frequency, and duration

Treatment phase	Drugs	Dose (mg)	Frequency	Duration (months)
First-line (Initiation)	Isoniazid	300	Once daily	2
	Rifampin	600		
	Pyrazinamide	1500–2000		
	Ethambutol	800–1200		
First-line (Continuation)	Isoniazid	300		4–7
	Rifampin	600		
Second-line	Moxifloxacin	400		6–12
	Levofloxacin	750–1000		
	Ofloxacin	800		
	Amikacin			
	Kanamycin	15		
	Streptomycin			
	p-aminosalicylic acid	8000–12000	Twice/thrice daily	
	Ethionamide			
	Prothionamide	500–750	Once/twice daily	
	Cycloserine			

state (21). Following this, the continuation phase lasts an additional 4 to 7 months, during which RIF and INH are used to eliminate semi-dormant and slow-growing MTB (4, 21). Overall, TB treatment typically requires 6 to 9 months to complete (7).

RIF, INH, PZA, and EMB each have a distinctive mechanism of action for targeting MTB. RIF inhibits bacterial RNA synthesis by binding to the β subunit of RNA polymerase, thereby blocking transcription and replication in MTB (22, 23). Approximately 12% of TB patients receiving RIF report gastrointestinal symptoms such as nausea, vomiting, and diarrhoea, while around 5% experience mild dermatological reactions (24). INH, once activated, acts as a bactericidal agent by preventing mycolic acid synthesis, an essential component of the MTB cell wall. It forms a covalent adduct with the nicotinamide adenine dinucleotide cofactor, which subsequently inhibits the enoyl-acyl carrier protein reductase enzyme (25). INH treatment carries risks of acute and chronic toxicity, leading to neurological symptoms, peripheral neuropathy, and hepatotoxicity (25, 26).

PZA, upon conversion to pyrazinoic acid, disrupts MTB's intracellular pH balance, creating an unfavourable environment for bacterial survival and growth (27). Additionally, pyrazinoic acid interferes with mycolic acid biosynthesis by inhibiting fatty acid synthase I, disrupts membrane potential, and impairs membrane transport (27,28). PZA use results in red urine discharge in 98% of patients, while 50% report nausea, reduced appetite, and joint pain; additional side effects, such as fever, abdominal discomfort, headache, and skin discolouration, occur in fewer than 30% of cases (29). EMB, on the other hand, targets the MTB cell wall by inhibiting arabinosyl transferase, an enzyme responsible for arabinogalactan synthesis – a polysaccharide in the MTB cell wall (30). This inhibition disrupts cell wall structure and impairs bacterial growth and replication (31).

While the first-line anti-TB drugs are effective, their prolonged treatment duration, high pill burden, and side effects often lead to low patient compliance, resulting in early treatment discontinuation (32). Thus, the second-line regimens, including fluoroquinolones (FLQs), aminoglycosides, p-aminosalicylic acid, ethionamide, prothionamide, and cycloserine, have been developed (Table 1) (30). FLQs such as levofloxacin and moxifloxacin target the DNA gyrase topoisomerase IV enzyme, inhibiting bacterial DNA replication and protein synthesis (33). FLQs are associated with side effects like musculoskeletal and neurological complications, while moxifloxacin may lead to cardiac dysrhythmias (34).

Aminoglycosides, including capreomycin, amikacin, viomycin, and kanamycin, primarily inhibit bacterial protein synthesis by binding to the A-site on the 16S rRNA of the 30S ribosomal subunit (30,35). Approximately 20% of patients on aminoglycosides experience gastrointestinal disturbances such as nausea, vomiting, diarrhoea, and abdominal pain, while around 5% develop psychiatric disorders. Additionally, aminoglycosides can cause ototoxicity, leading to hearing loss, tinnitus, and vertigo (36, 37).

Thioamides, such as ethionamides, share a similar mechanism of action as INH, whereby they target mycolic acid biosynthesis (22). Depending on the drug concentration, ethionamide may exhibit bacteriostatic or bactericidal effects. Cycloserine functions as a bacteriostatic agent by inhibiting MTB cell wall synthesis. Acting as an analog of D-alanine, it competitively inhibits two essential enzymes involved in early cell wall formation: L-alanine racemase, which converts L-alanine to D-alanine, and D-alanyl alanine synthetase, which combines D-alanine into the pentapeptide chain. By blocking these enzymes, cycloserine disrupts cell wall synthesis and weakens the bacterial structure (22). However, cycloserine is associated with significant central nervous system side effects, such as depression and psychosis (38, 39).

Developing innovative drug delivery systems encapsulating anti-TB drugs presents a promising strategy to enhance targeted drug delivery, reducing the required dose, dosing frequency, and systemic exposure. This targeted approach not only minimises adverse effects but also improves patient adherence, ultimately enhancing treatment efficacy.

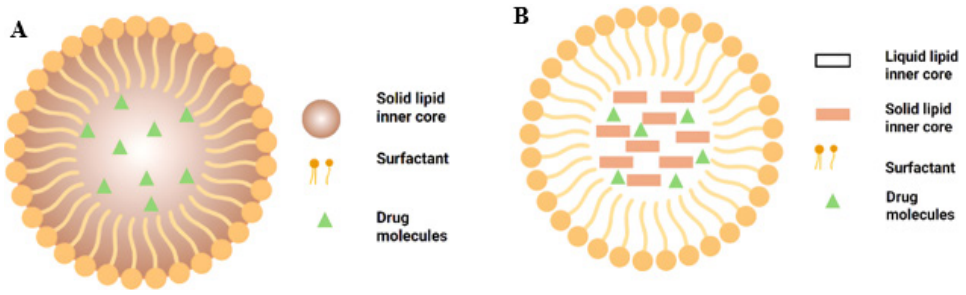

Figure 3. Illustrations of A) SLN and B) NLC formulations
NLC: Nanostructured lipid carriers; SLN: Solid lipid nanoparticles

Lipid-based drug delivery system in TB treatment

Lipid-based delivery systems have garnered significant attention due to their good biocompatibility and biodegradability (40). Taking advantage of their physicochemical characteristics, they have been extensively explored for anti-TB drug delivery, particularly solid lipid nanoparticles (SLNs), and nanostructured lipid carriers (NLCs) (Figure 3) (41).

SLNs in TB treatment

SLNs are commonly composed of steroids, triglycerides, glyceride mixtures, or waxes, and they remain solid at both room and body temperatures (42). SLNs are typically spherical, with diameters of 50-1000 nm, and can incorporate both lipophilic and hydrophilic drugs into the lipid matrix (42, 43).

Table 2 provides an overview of studies involving SLNs in TB treatment. Khatak *et al.* (2020) developed SLNs loaded with RIF, INH, and PZA intended for oral administration using the microemulsion technique. The optimised formulation demonstrated a particle size of 187.9 ± 10.73 nm, a negative zeta potential of -47.4 mV, and an encapsulation efficiency (EE) of over 85% for all three drugs. However, the formulation exhibited a polydispersity index (PDI) of more than 0.3, indicating poor homogeneity. Drug-loaded SLNs showed lower minimum inhibitory concentration (MIC) as compared to pure drugs and drug-lipid physical mixture: by three-fold and two-fold, respectively, for RIF (44).

Obinu *et al.* (2020) formulated their SLNs using Witepsol and Gelucire lipids and encapsulated SS13, a novel anti-TB compound, using the solvent emulsification technique. The SLNs showed particle sizes of 247.1 ± 19.8 nm and were negatively charged. The MIC value for the SS13 compound in the MTB H37Rv strain was not reported; however, other findings showed that when SS13 was used with the conventional RIF, it yielded lower MIC values of 0.25 and

Table 2. Overview of studies involving solid lipid nanoparticles (SLNs) for anti-tuberculosis drug delivery

Drugs	Type of solid lipid	Route of administration	Physicochemical characteristics	Antimycobacterial assays	Ref.
INH, PZA, and RIF	Stearic acid, Compritol® 888 ATO	Oral	PS: 187.9 ± 10.73 nm PDI: 0.568 ZP: – 47.4 mV EE: RIF: 85.21%; INH: 92.47%; PZA: 88.30%	MTB strains: *Mycobacterium marinum* Assays: REMA assay MIC values: RIF (2.16 µg/ml), INH (2.55 µg/ ml) and PZA (5.04 µg/ml)	(44)
SS13	Witepsol and/or Gelucire	Oral	PS: 247.1 ± 19.8 nm PDI: 0.772 ± 0.051 ZP: 13.82 ± 2.44 mV EE: 86.84 ± 2.49%	MTB strains: *M. tuberculosis* H37Rv strain (ATCC 27294) Assays: REMA MIC values: RIF+SS13 (0.06 µg/ml); INH + SS13 (0.125 µg/ml)	(9)
RIF	Palmitic acid, cholesteryl myristate	Intratracheal	PS: 559 ± 113 nm PDI: 0.68 ± 0.10 ZP: – 43.1 + 1.6 mV DL: 9.6 ± 0.2%	No *in vitro / in vivo* studies specific to antimycobacterial assays	(46)
EMB	Compritol	Inhalation	PS: 57.65 ± 0.23 nm PDI: 0.253 ± 0.01 DL: 29.71 ± 0.19% EE: 99.04 ± 0.41%	No *in vitro / in vivo* studies specific to antimycobacterial assays	(45)
RIF	Cetyl palmitate	Pulmonary	PS: 524 ± 39 nm PDI:0.219±0.029 ZP:30.85±2.7mV EE: 90.2 ± 1.3% DL: 4.5 ±0.1%	No *in vitro / in vivo* studies specific to antimycobacterial assays	(47)

DL: Drug loading; EE: Encapsulation efficiency; EMB: Ethambutol; INH: Isoniazid; PDI: Polydispersity index; PS: Particle size; MIC: Minimum inhibitory concentration; PZA: Pyrazinamide; REMA: Resazurin microtiter assay; RIF: Rifampicin; ZP: Zeta potential.

0.06 µg/mL, respectively. For INH, EMB, and streptomycin, there were no significant differences in MIC values when combined with SS13 (9).

Nemati *et al.* (2019) developed an SLN-based dry powder inhaler loaded with EMB using a combination of homogenization and ultrasonication. The developed SLNs had particle sizes below 100 nm and an EE of over 99%. Compared to free EMB, SLNs demonstrated a slower and controlled release, with approximately 34% of the EMB released within 8 hr, whereas free EMB released 47% within the same period (45). Thus, the sustained release profile suggests the potential of SLNs to reduce dosing frequency. Truzzi *et al.* (2019) explored the potential of mannose-coated SLNs in improving lung targeting and retention of RIF. The SLNs have an average size of 559 ± 113 nm with a zeta potential of -43.1 mV. In simulated lung fluid, SLNs demonstrated a slow and sustained release of RIF. Unlike the free RIF, which undergoes a rapid release, only 30% of the encapsulated drug was released at the end of the study (46). This slow release from the SLNs helps ensure a sufficient concentration of drugs remains in the lung environment for an adequate duration. In another study, Vieira *et al.* (2021) developed chitosan-coated SLNs for RIF delivery to alveolar macrophages. The formulated SLNs had an average size of 524 ± 39 nm with a positive zeta potential of +38.5 mV. It showed an EE of 90.2 ± 1.3% and a drug loading of 4.5%. Thus, the SLNs demonstrated high potential for delivering anti-TB drugs, exhibiting optimal physicochemical characteristics and high EE (47). However, further studies focusing on the efficacy and safety aspects of SLNs are much needed for clinical translation.

NLCs in TB treatment

NLCs were developed in the early 2000s as a second-generation lipid-based nanocarrier system. NLCs consist of a mixture of solid and liquid lipids stabilized by surfactants, with sizes ranging from 50 to 1000 nm (8). Compared to the SLNs, the presence of liquid lipids within the NLCs created structural disorder within the solid lipids, resulting in improved drug loading with lower drug expulsion during storage (48). Moreover, NLCs can exhibit more controlled release profiles and improve drug solubility in lipid matrices (49). Due to their versatile nature, they can be administered via oral, topical, intravenous, and ocular routes (50). The optimised NLCs formulation typically consists of solid-to-liquid lipids ratios of 70:30 to 99.9:0.1, with surfactant concentrations of 1.5 to 5% w/v (Table 3) (4, 51). The common method of preparation is through a combination

Table 3. Overview of studies involving nanostructured lipid carriers for anti-tuberculosis delivery

Drug	Type of lipids	Route of administration	Physicochemical characteristics	Antimycobacterial assays	Ref.
RIF	Purified Oecithin, Medium Chain Triglyceride, Octadecylamine	Intravenous	PS: 157.4 ± 0.7 nm PDI: 0.244 ± 0.008 ZP: +34.3 ± 1.3 mV EE: 91.6 ± 2.3%	No *in vitro* / *in vivo* studies specific to antimycobacterial assays	(52)
RIF and INH	Glyceryl distearate, soybean phospholipids	Oral	PS:121.3±11.07nm PDI: Not reported ZP: −28.1 ⊥ 1.77 mV EE:87.98±1.89% (RIF); 78.08 ±2.09% (INH)	No *in vitro* / *in vivo* studies specific to antimycobacterial assays	(53)
RFB	Precirol® ATO 5, Polysorbate 60, Miglyol-812	Pulmonary	PS: 213 ± 2 nm PDI: 0.12 ± 0.02 ZP: +37.82 ± 1.0 mV EE: 90 ± 4 %	No *in vitro* / *in vivo* studies specific to antimycobacterial assays	(54)
RIF	Precirol® ATO 5, Polysorbate 60, Miglyol-812	Pulmonary	PS: 302 ± 23 nm PDI: 0.16 ± 0.03 ZP: +36 ± 7 mV EE: 95 ± 2 %	No *in vitro* / *in vivo* studies specific to antimycobacterial assays	(47)
Cfz	CompritolVR 888 ATO	Inhalation	PS: 295 ± 7 nm PDI: 0.299 ± 0.010 ZP: -38.99 ± 1.60 mV EE:71.53±0.70%	No *in vitro* / *in vivo* studies specific to antimycobacterial assays	(32)
Ruthenium (II) Compounds	Cholesterol, Soy phosphatidylcholine (SPC) and Eumulgin® (Castor oil polyoxyl-40-Hydrogenated)		PS: 188.0 ± 1 nm PDI: 0.211 ± 0.02 ZP: Not reported EE: Not reported	MTB strains: *Mycobacterium tuberculosis* H37Rv ATCC-27194 Assays: REMA MIC values: 2.9–6.1 µg/ml	(60)

Cfz: Clofazimine; DL: Drug loading; EE: Encapsulation efficiency; INH: Isoniazid; PDI: Polydispersity index; PS: Particle size; REMA: Resazurin microtiter assay; RIF: Rifampin; RFB: Rifabutin; ZP: Zeta potential; NLC: Nanostructured lipid carriers

of homogenization and ultrasonication (47,52–54). Notably, most studies focused on the development of NLC-based formulations aimed at improving drug delivery efficiency and safety (55–57)

Table 3 shows an overview of studies involving NLCs for the delivery of anti-TB drugs. Song *et al.* (2015) developed RIF-loaded mannosylated NLC for targeted delivery to alveolar macrophages in the lungs. They showed a particle size of 157.4 ± 0.7 nm, PDI of 0.244 ± 0.008, zeta potential of +34.3 ± 1.3 mV, and EE of 91.6 ± 2.3% (52). Vieira *et al.* (2021) further developed RIF-loaded mannosylated NLC for TB treatment, reporting a particle size of 302 ± 23 nm, a PDI of 0.16 ± 0.03, and an EE of more than 90%. Their findings showed that RIF-loaded mannosylated NLCs have greater uptake by bone marrow-derived macrophages than non-mannosylated NLCs, with 68.4% and 37.6%, respectively (47). Baranyai *et al.* (2021) highlighted that smaller-sized particles have greater lung deposition as they avoid upper airway sedimentation, impaction, mucociliary clearance, and exhalation loss (58). Silva *et al.* (2016) developed NLCs to evaluate the antimycobacterial activities of copper (II) complexes and demonstrated that copper (II) complex NLCs significantly improve inhibitory action against MTB, achieving up to 52-fold increased activity (59).

A study by Banerjee *et al.* (2020) developed both SLNs and NLCs loaded with RIF and INH. NLCs exhibited a smaller particle size of 121.3 ± 11.07 nm, higher EE of RIF and INH, showing 87.98 ± 1.89 and 78.08 ± 2.09 %, respectively, as compared to SLNs. Additionally, NLCs provided a faster yet sustained release profile, demonstrating RIF release of more than 90% over 7 days. Animal studies further confirmed that NLCs significantly enhanced the bioavailability of RIF and INH compared to SLNs. The higher area under the curve (AUC) values observed in NLCs indicate more efficient drug absorption and prolonged circulation. Overall, NLCs are a more promising delivery mechanism for increasing the efficacy of oral anti-tubercular therapy since they performed better than SLNs in drug encapsulation, release, bioavailability, and cellular uptake (53).

Pinheiro *et al.* (2016) developed mannose-coated NLCs to enhance rifabutin (RFB) delivery to alveolar macrophages. NLCs had particle sizes of 175–213 nm and EE values exceeding 80%. Mannosylation further increases EE to 90%, compared with 83% in non-mannosylated NLCs. This suggests that mannosylation enhances the drug loading. The release profile of RFB from mannose-coated NLCs was slower than that from non-mannosylated NLCs, particularly at pH 5.0 and 6.2, mimicking the macrophage phagolysosomal environment. The NLC formulations reported showed potential as effective delivery systems for anti-TB drugs, demonstrating promising physicochemical characteristics and EE (54). This warrants further investigations into their therapeutic and toxicity outcomes.

Toxicity studies on SLN and NLC in TB

In a drug delivery system, evaluating drug and formulation toxicity is crucial to protecting patient safety. The evaluation involves *in vitro* and *in vivo* assays that provide insights into drug metabolism, enzyme interactions, and potential toxicity (61). *In vitro* techniques are preferred due to ethical concerns, but animal models remain widely used as they offer comprehensive physiological, biochemical, and histological insights (62). Toxicity is a significant concern in TB treatment due to the prolonged treatment and associated side effects (63). Adverse effects arise when anti-TB drugs accumulate in non-target tissues, leading to organ damage, particularly the liver and kidneys (64). Cytotoxicity assessments in TB-relevant models are crucial to evaluating the safety and therapeutic efficacy of SLN and NLC systems, as summarized in Figure 4.

In vitro toxicity studies

In vitro studies are performed in a controlled environment outside living organisms, such as cells or tissues, and provide insights into cellular and biochemical mechanisms (65).

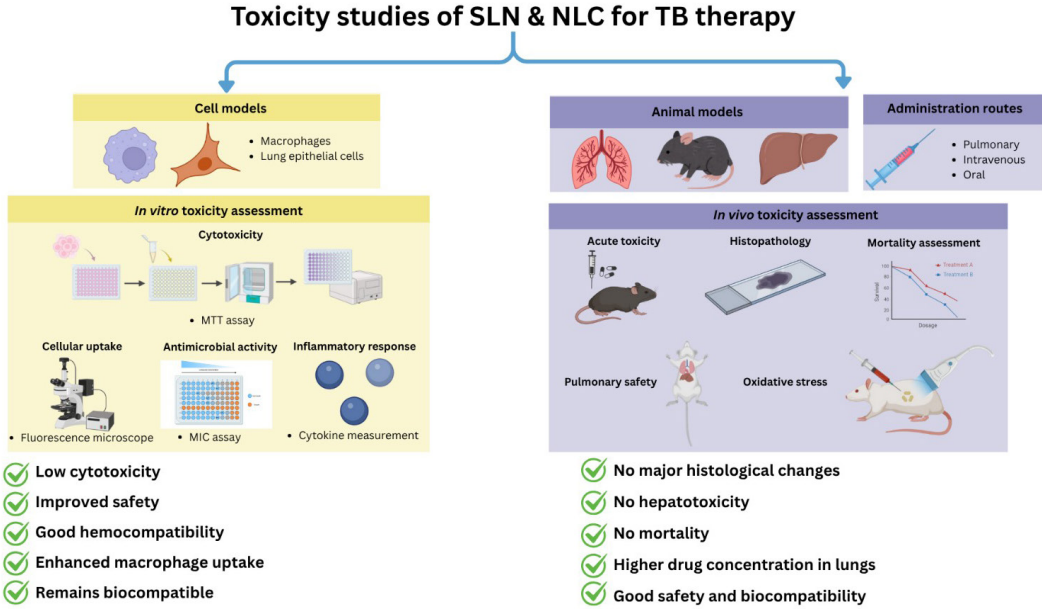

Figure 4. Overview of *in vitro* and *in vivo* toxicity studies of SLN and NLC on tuberculosis
NLC: Nanostructured lipid carriers

These methods efficiently assess cytotoxicity, genotoxicity, and other cellular responses. A well-designed cytotoxicity assessment in relevant cell models is crucial in optimising formulations for TB therapy, ensuring both safety and therapeutic efficacy before advancing to *in vivo* studies. Table 4 summarizes the representative *in vitro* and *in vivo* toxicity studies of SLN and NLC formulations.

For the *in vitro* study, Bhandari *et al.* (2021) evaluated the toxicity of INH-SLNs and found a significant improvement in safety profiles compared to free INH. Their analysis revealed that INH-SLNs exhibited threefold higher LD_{50} (2000 mg/kg) than free INH (650 mg/kg), suggesting lower acute toxicity (66). These findings highlight the potential of SLNs as a promising approach to enhance TB treatment by lowering systemic toxicity. A study by Makled *et al.* (2021) demonstrated that RFB-loaded SLNs showed no signs of acute cytotoxicity as compared to free RFB, suggesting an improved safety profile (67).

Patil *et al.* (2021) evaluated the safety and biocompatibility of mannosylated-clofazimine-loaded NLCs for lung targeting. The hemocompatibility test showed minimal hemolysis (0.23%), indicating good blood compatibility—an essential factor in reducing systemic side effects. Cytotoxicity was evaluated using an MTT assay, revealing that mannosylated clofazimine-loaded NLCs exhibited low toxicity and maintained high cell viability (>90%) in J774 macrophage cells over a concentration range of 2.5–25 µg/ml (32). These findings support their use for lung-targeted applications; however, further studies are required to evaluate macrophage uptake and long-term toxicity in a TB-mimicking environment. Obinu *et al.* (2020) assessed the cytotoxicity of SS13-loaded NLCs in Caco-2 cells, demonstrating a good safety profile, with effects depending on the administered nanoparticle concentration. The study found that RIF-loaded mannosylated NLCs showed significantly higher cellular uptake in alveolar macrophages than non-mannosylated NLCs, highlighting their targeting potential (9). Even at higher concentrations, the formulations showed low cytotoxicity in L929 mouse fibroblast cells (52). Cytotoxicity studies on mannose-coated NLC in A549 alveolar lung carcinoma, Calu-3 lung adenocarcinoma, and RAW 264.7 macrophage cell lines demonstrated low toxicity (54).

Truzzi *et al.* (2020) investigated the uptake and toxicity of respirable mannosylated-coated RIF-loaded SLNs in macrophage cell lines, including J774 and MH-S. The mannosylated-coated RIF-loaded SLNs demonstrated enhanced uptake and were non-toxic to macrophage cell lines, compared to non-functionalized SLNs (46). This indicates good biocompatibility, essential for TB treatment. The cytotoxicity of rifabutin-loaded SLNs was evaluated in A549 and Calu-3 cell lines using MTT and propidium iodide assays (68). The SLN formulation showed low toxicity with more than 80% cell viability.

Hence, based on the above *in vitro* studies, both SLN and NLC showed promising safety profiles, demonstrating reduced cytotoxicity as compared to conventional treatment. This underscores the potential of lipid formulations as safe and viable delivery systems.

In vivo toxicity studies

As studies on SLN and NLC formulations have demonstrated their potential to reduce toxicity in *in vitro* models, further research has been conducted using animal models, which provide a more comprehensive representation of human physiology (69). Animal studies are commonly used to evaluate the pharmacokinetics, biodistribution, and toxicity of formulations, ensuring their safety and efficacy before clinical translation.

Table 4. Overview of *in vitro* and *in vivo* toxicity assays in SLN and NLCs for TB treatment

Treatment phase	Drugs	Dose (mg)	Frequency	Duration (months)
First-line (Initiation)	Isoniazid	300		2
	Rifampin	600		
	Pyrazinamide	1500–2000		
	Ethambutol	800–1200		
First-line (Continuation)	Isoniazid	300	Once daily	4–7
	Rifampin	600		
Second-line	Moxifloxacin	400		6–12
	Levofloxacin	750–1000		
	Ofloxacin	800		
	Amikacin			
	Kanamycin	15		
	Streptomycin			
	p-aminosalicylic acid	8000–12000	Twice/thrice daily	
	Ethionamide			
	Prothionamide	500–750	Once/twice daily	
	Cycloserine			

Cfz: Clofazimine; INH: Isoniazid; NLC: Nanostructured lipid nanocarriers; SLN: Solid lipid nanoparticles

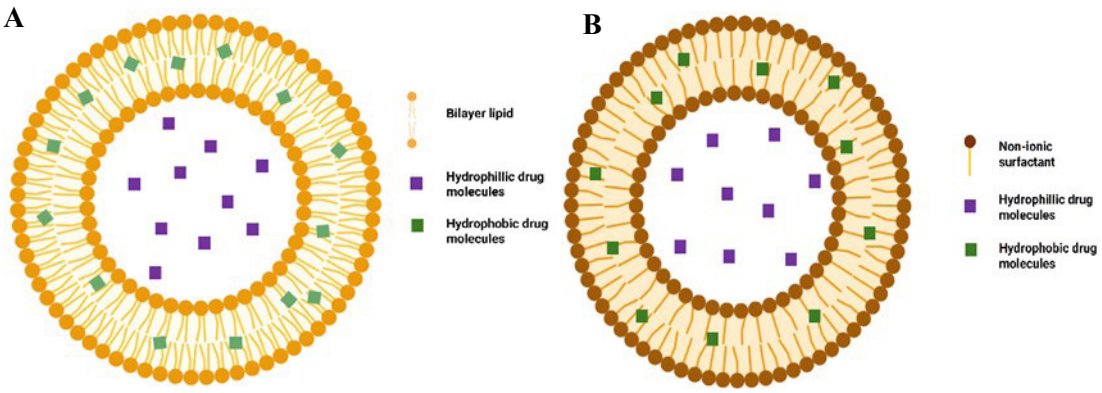

Figure 5. Illustrations of A) liposomes and B) niosomes

Bhandari *et al.* (2021) investigated acute and repeated dose toxicity assays in INH-SLNs. Histological examinations revealed fewer pathological changes in liver and sciatic nerve tissues in INH-SLN-treated animals than in those receiving free INH, indicating improved biocompatibility. Notably, repeated-dose toxicity studies demonstrated that oral administration of INH-SLNs at high doses, 500–1000 mg/kg, did not induce hepatotoxicity, a major concern with conventional INH therapy (66).

Patil *et al.* (2021) evaluated the toxicity of mannosylated-coated clofazimine-loaded NLCs in Wistar rats via intratracheal administration. No fatalities, negative behavioural or physiological impacts were reported during the 14-day observation period. Histopathological analysis of the liver, spleen, and lungs showed no abnormal tissue changes, further confirming the formulation's safety profile. The mannosylated-coated clofazimine-loaded NLC formulation showed a higher drug concentration in lung tissues (35.44 µg/g) at 48 hr than in other organs, such as the liver and spleen (32). These findings suggest that the NLC formulation could provide a safe and lung-targeted approach for CLF delivery.

Recent developments in lipid-based drug delivery systems in TB treatment

Recent advancements in lipid-based carriers, particularly liposomes and niosomes (Figure 5), have significantly enhanced their therapeutic potential for infectious diseases such as TB. Lipid-based nanoparticles are a cutting-edge technology for sustained drug delivery, enabling drugs to remain in the bloodstream for extended periods. As a result, this method significantly lowers dosing frequency and improves patient compliance.

Liposomes

Liposomes are lipid-based nanocarriers used for delivering therapeutic and diagnostic agents. These spherical vesicles are stabilised *in vitro* and *in vivo* by incorporating cholesterol into phospholipids, which possess both hydrophilic and hydrophobic properties. In aqueous environments, phospholipids naturally form bilayers, allowing hydrophobic molecules to be encapsulated within the lipid layer while hydrophilic compounds remain in the aqueous core (Figure 5A) (70). Liposomes are classified into various types, including giant unilamellar vesicles (GUVs), oligolamellar vesicles (OGVs), small unilamellar vesicles (SUVs), and large unilamellar vesicles (LUVs), distinguished by size and structural complexity (Figure 6). Unilamellar vesicles consist of a single bilayer, with small ones measuring under 100 nm and larger ones exceeding 100 nm (Figure 6) (71). Multilamellar vesicles, containing multiple layers, are ideal for lipophilic drugs, whereas unilamellar vesicles are more suitable for hydrophilic drugs. Liposome-based drug delivery enhances the effectiveness of treatments, particularly in targeting lung infections, improving the therapeutic potential of anti-TB medications (72).

Extensive research has explored liposomes as carriers for anti-TB drugs, demonstrating their safety and effectiveness

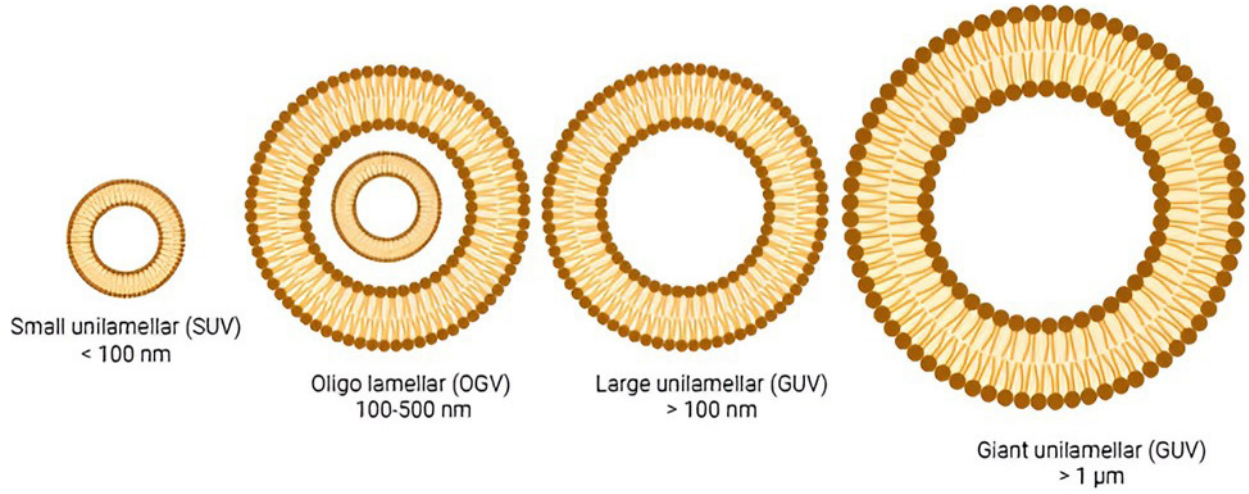

Figure 6. Illustrations of types of liposomes

for pulmonary drug delivery. Their phospholipid composition makes them biocompatible and similar to lung surfactants, minimising local irritation. Liposomes are efficiently absorbed by macrophages, making them ideal for targeting intracellular infections like MTB. Studies have also focused on enhancing the pharmacokinetics and efficacy of anti-TB drugs through liposomal encapsulation (73). For instance, RIF-loaded inhalable liposomal particles were developed using thin film hydration and freeze-drying methods. A formulation with a 1:5 drug-to-lecithin ratio and a 3:2 soy lecithin-to-cholesterol mass ratio achieved 79.25% EE. In rat models, oral and intratracheal administration of these liposomes improved drug release profiles compared to the free drug, ensuring more controlled and sustained delivery (74). Liposomes represent a promising drug delivery system to enhance the efficacy of anti-TB drugs and their pharmacokinetic profiles. However, further research is needed before clinical translation.

Niosomes

Niosomes share structural similarities with liposomes but differ in composition (Figure 5B). Instead of phospholipids, niosomes form bilayers using non-ionic surfactants, such as polysorbates (Tweens®), sorbitan esters (Spans®), and polyethoxy ethers (Brijs®), combined with hydrated cholesterol. Like liposomes, cholesterol enhances the stability and rigidity of the bilayer (75). The inclusion of non-ionic surfactants increases vesicle size, improving drug encapsulation efficiency. Hydrophilic drugs are stored in the aqueous core, while lipophilic compounds are incorporated into the bilayer. Functionally, niosomes enhance drug solubility and bioavailability, but they offer superior chemical stability, lower production costs, and greater penetration ability compared to conventional liposomes (76).

Niosomes, lipid-based nanocarriers, have been extensively studied for optimising the pharmacokinetics and biodistribution of anti-TB drugs. One study tested RIF-loaded niosomes using Span® 85 and cholesterol in varying molar ratios. *In vivo* distribution tests on rats revealed that approximately 65% of the encapsulated RIF accumulated in the lungs after administration via the caudal vein (77). Another study explored INH-loaded niosomes, produced through reverse phase evaporation, which exhibited sustained drug release for up to 30 hr. Additionally, these niosomes showed strong macrophage penetration (around 62%), making them highly effective for targeting mycobacterial infections (78).

Research on niosome technology for TB has explored how surface properties affect particle activity. A study examined PZA-loaded niosomes composed of cholesterol and Span® 60 or 85, with dicetyl phosphate (DCP) or stearyl amine added to create negative or positive charges. The formulation with the highest drug entrapment contained cholesterol and Span® 60 in a 4:2 molar ratio. Negatively charged niosomes demonstrated the best EE, followed by neutral ones. *In vivo* studies on MTB-infected guinea pigs revealed that PZA was more effective when delivered via niosomes (79).

A study explored the co-encapsulation of first-line anti-TB drugs—RIF, INH, and PZA—into highly stable niosomes composed of Triton X 100, PEG 2000, water, and Span® 80. The formulation demonstrated excellent drug compatibility, stability, and EE. Another study utilised the biocompatible surfactant tyloxapol to encapsulate the same drugs, achieving similarly high EE and stable formulations (80). Overall, studies demonstrated that niosomes can effectively encapsulate anti-TB drugs with promising *in vivo* results as compared to free drugs.

Nucleic acids

Lipid-based nanocarriers have been explored as carriers for nucleic acids such as messenger RNA (mRNA) and small interfering RNA (siRNA) (81). However, in the context of mRNA, most studies have focused on vaccine development rather than treatment options for TB (82,83,84). As for siRNA, Yang *et al.* (2022) explored the co-encapsulation of transforming growth factor (TGF)-β1-specific siRNA and anti-TB drugs (INH, RIF, and PZA) within cationic liposomes targeting spinal TB. The liposomes were around 168.1 ± 0.5 nm and slightly positively charged, +4.03 ± 1.32 mV. The EEs of INH, RIF, and PZA were 90, 88, and 37%, respectively. For the *in vitro* cytotoxicity assay, the formulation illustrated an IC_{50} of 37.47 mg/ml in macrophages. However, no controls were reported (85). This study demonstrates preliminary proof-of-concept on the use of siRNA in addition to standard TB treatment, but further *in vivo* and stability studies are required before clinical translation.

Conclusion

Significant advancements in TB treatment have been achieved by improving the solubility, stability, and bioavailability of the drugs. Lipid-based drug delivery systems have enhanced drug targeting, controlled release, and the ability to overcome the biological barriers associated with TB treatment. Surface modification techniques helped to improve the targeting, biodistribution, and reduce side effects. Despite promising developments, challenges such as formulation complexity, scalability, and clinical validation remain. Future research should focus on addressing these challenges, exploring new drug combinations, and evaluating the efficacy of these systems in diverse patient populations. The ongoing progress in nanotechnology and surface modification strategies holds the promise of revolutionizing TB treatment, offering more effective, safer, and patient-compliant therapeutic options. Ultimately, lipid-based drug delivery systems present a crucial step forward in the fight against TB, potentially transforming the standard of care and offering new hope for patients, particularly in regions where drug-resistant strains pose a significant threat.

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Authors' Contributions

N MH, R SN, and JSL T conceptualised the review topic. FN Z conducted the literature search, analysed

and synthesized the findings, and prepared the first draft of the manuscript. A R reviewed and designed graphics in the manuscript. PV N and S N contributed to guiding the methodological approach. INA S and NA O provided critical input during the writing process. JSL T, N MH, and R SN supervised the overall project, refined the review scope, and contributed to the interpretation of the literature. All authors reviewed, edited, and approved the final version of the manuscript. The authors have not used any AI tools or technologies to prepare this manuscript.

Conflicts of Interest

No competing interests to declare.

Declaration

We did not use any AI tools or technologies to prepare this manuscript.

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