

Targeted Macrophages Delivery of Antitubercular Agent Through Solid Lipid Nanoparticles

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Abstract: The objective of this paper is to summarize the current aspects related to nanoparticle targeted delivery via; lipid nanoparticulate drug carrier system. *M. tuberculosis* ranks IInd most fatal disease after AIDS, and thus WHO declared it as “A Global Health Emergency”. *Mycobacterium* species source of tuberculosis infection; infects the lungs primarily and some other part of the body after infection is thoroughly spread. Antitubercular agents are classified according to their therapeutic action and safety. In this, an overview is provided on WHO-recommended treatment regimens. The study summarizes antitubercular delivery to the targeted site, i.e., macrophages via; nanoparticulate drug delivery system. Thus, the study was conducted to provide concise knowledge about nanoparticulate, solid lipid nanoparticles: introduction, advantages, components, types, preparation methods, models, parameters, characteristics, mechanism of drug release, and applications in the antitubercular delivery field. It also provides an overview of the information about macrophages, their biological role, their mechanism of action, and a few studies conducted. Macrophages act as a host body for some intracellular pathogens such as *Mycobacterium* species; thus, they act as an active targeted site for target-specific action. The antitubercular delivery to macrophages can reduce dosage frequency, increase solubility/permeability, bioavailability, enhance therapeutic effects, decrease toxicity, and increase patient compliance.

Keywords: tuberculosis; antitubercular; macrophages; solid lipid nanoparticles; simulated fluid.

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1. Introduction

According to World Health Organization (WHO), approximately 1/3rd of the total world's population has been infected by *M. tuberculosis* (Mtb), infecting more than 9 million new cases and 2 million deaths occurs due to Mtb annually, and the remaining population is asymptomatic [1]. Tuberculosis, also called TB, is known as 2nd most fatal infectious disease after AIDS, caused by *Mycobacterium tuberculosis* (Mtb). In 1993, WHO declared tuberculosis “a global health emergency”. *Mycobacterium* firstly infects the lungs and also infects other organs and body parts like the nervous system (meningitis), lymphatic system, kidney central, circulatory system (miliary tuberculosis), genitourinary system, joints, and bone, etc. [2]. Extended treatment regimens, higher dosage/dosing frequency, lesser patient compliance, and strong administration schedules are a few factors that lead to the emergence of MDR and XDR tuberculosis. Recently, except BCG vaccine, there is no other potential immune protective vaccine, i.e., ineffective against adult pulmonary TB, the most active and widespread form of TB.

An attractive target site for active pharmaceutical ingredients (APIs) administration is the pulmonary site (Lungs) for various drug delivery systems due to non-invasive administration via inhalation aerosols. Compared with conventional oral administration, this route provides various advantages; high surface area and rapid absorption due to more vascularization and lack of the first-pass effect. This feature provides targeted drug delivery, thus reducing the side effects. Drug delivery systems carrying nanoparticles as carrier systems offer new approaches by improvising the particles' physical properties such as increased drug solubility, high encapsulation efficacy, and surface modifications to extend the drug release profiles and obtain maximum therapeutic effects. To determine the safe therapeutic dose, it is necessary to carry out toxicological testing in various cell culture models: *in vitro*, *ex vivo*, and *in vivo* models; But it is not necessarily completely signifying similar *in vivo* situation of a patient; whereas to reduce the risk of adverse reactions or toxic effects the testing of the nanoparticulate system follows such models [3]. Lungs directly deliver the drug to the site of action to treat respiratory disorders, which provides a huge surface area for local drug action/systemic absorption [4].

Nanoparticles, due to their smaller size (<200nm), may circumvent endogenous mucosal and immune defenses in the distal lung via rapid penetration through respiratory secretions and the surfactant lining layer and escaping detection and phagocytosis by alveolar macrophages [5]. Nanocarrier systems in pulmonary drug delivery offer many advantages [6]. The main characteristics of SLN include good physical stability, protection of incorporated drugs from degradation, controlled drug release, and good tolerability [7]. Solid lipid nanoparticles (SLNs) are made from solid lipids (i.e., lipids solid at room temperature), surfactants, and water. Since the beginning of the 1990s, the SLNs have been focused on an alternative to polymeric nanoparticles. The advantages of drug release from SLNs in the lung are control of the release profile, achievement of a prolonged release, and faster *in vivo* degradation than particles made from PLA or PLGA [8].

Tuberculosis is an airborne disease which are inhaled infectious bacilli as droplets from the atmosphere phagocytoses the bacteria via; alveolar macrophages in the lungs. Tuberculosis (TB) is a highly infectious disease. *M. tuberculosis* species are also known as tubercle bacilli [9,10].

TB infection covers $\frac{1}{3}$ of the world's total population, and in which 5%–10% may develop active TB in its lifetime. TB is an infectious disease that results in 1.4 million deaths each year [11]. In 2015, the World Health Organization (WHO) reported global mortality of 1.5 million people due to TB infection. In the same year, 9.6 million people were diagnosed with this infection. Anti-tuberculosis therapy's main goals are to kill actively proliferating bacteria quickly, prevent developed drug resistance, and sterilize infected host tissues to prevent clinical relapse. To attain these goals, official guidelines prescribe a minimum of 6 months of combined antibiotic therapy. [12]. Multidrug-resistant TB (MDR-TB) is defined as resistance to the first-line drugs isoniazid and rifampicin, and extensively drug-resistant TB (XDR-TB) is defined as resistance to fluoroquinolones and at least one of the injectable second-line drugs (capreomycin, kanamycin, and amikacin) with MDR-TB. These resistances pose formidable challenges to global TB control efforts. [13,14].

2. Solid Lipid Nanoparticles (SLN)

Nanoparticles are particulate dispersions or solid particles between 10 and 1000 nanometers. A Nanoparticles matrix is dissolved, entrapped, encapsulated, or linked to the medication [15]. Nanospheres or nanocapsules, depending on the technique of preparation. Nanocapsules are matrix systems in which the drug is physically and uniformly spread. In contrast, nanospheres are matrix systems in which the drug is restricted to a hollow enclosed by a unique polymer membrane [16]. Polymers, which can be natural (e.g., gelatin and albumin), manufactured (e.g., polylactides and poly alkyl cyanoacrylates), or solid lipids, are examples of biocompatible and biodegradable materials (SLN and NLC). Nanoparticles are more efficiently absorbed by cells than bigger molecules, making them a feasible mode of transport and delivery. These carriers are formed to enable controlled, slow, and persistent drug release from the matrix. SLNs are made of a solid lipid core with a monolayer phospholipid shell. The lipophilic moiety of phospholipids is embedded in the lipid matrix [17].

Nanoparticles were solid colloidal particles ranging from 10 – 1000 nm [18]. These are polymeric or solid lipidic particles. These are composed of solid lipid material, solid at room temperature and body temperature. SLN is stable in aqueous solvents with emulsifiers [19].

Solid Lipid Nanoparticles (SLNs) had been introduced in 1991 to act as an alternative and better carrier system compared to traditional colloidal carriers: emulsions, liposomes, polymeric micro, and Nanoparticles [20]. Solid Lipid Nanoparticles (SLNs) are categorized as a new generation of lipid nanoparticles constituting a solid lipid matrix. At the beginning of the 1990s, SLNs as Nano colloids were developed by Schwarz *et al.* [21]. SLNs constitute a solid lipid core with a monolayer phospholipids shell. The lipophilic moiety of phospholipids is embedded in the lipid matrix. Many drugs or diagnostics materials can be entrapped by SLNs for better delivery to the site of action. The benefit of SLNs for oral administration is a promising approach for enhancing, targeting, and sustaining drug delivery. The solid state of the nanoparticulate matrix provides shielding to chemically labile drugs and extension of drug release [22]. SLNs comprise spherical nanometer particles dispersed in water/aqueous surfactant solution. Lesser toxic than polymeric nanoparticles due to the biodegradable and biocompatible nature of SLNs, and it also overshadows some disadvantages of the traditional colloidal drug carrier system. These constitute a solid hydrophobic core with a monolayer of phospholipids coating. The medication is dissolved or disseminated in a solid high melting fat matrix in the solid core. Phospholipid hydrophobic chains are incorporated in the fat matrix. SLN combines the benefits of several colloidal carriers while eliminating some of their drawbacks, such as physical instability, protection of integrated labile medicines from degradation, regulated release, and high tolerability. Nanomedicine facilitates the delivery efficacy of orally administered drugs [23-25]. SLNs were categorized as a new generation of lipid nanoparticles comprising a complete solid lipid matrix. SLNs used for oral administration have many advantages over conventional formulations: enhanced solubility, enhanced stability, increased epithelium permeability and bioavailability, prolonged half-life, site-specific targeted delivery, and minimized side effects. The nontoxic excipients and delicate material production of SLNs produce the controllable physicochemical properties of the nanoparticles for GI penetration [25,26].

3. Methods of Preparation of Solid Lipid Nanoparticles

3.1. High-pressure homogenization.

It is an appropriate technique for the production of SLNs. High-pressure homogenization passes a liquid with high pressure (100-2000 bar) through a narrow gap with force. The fluid accelerates in a very short distance with a very high velocity (over 100 km/hr.). High shear stress and gravitational forces wreck the particles down to the sub-micron size range. Generally, 5-10% lipid content is used, and in some, 40% lipid content has also been reported [27].

3.1.1. Hot homogenization.

Lipids melt above their melting point range. Homogenized with the aqueous phase, the drug mixed in lipid / aqueous phase based on the drug nature during pre-emulsion. Increasing homogenization pressure and centrifugation of obtained emulsion; SLNs were obtained [28].

3.1.2. Cold homogenization.

The drug-containing lipid melt is brought to the lowest temperature range; then, the solid lipid converts to lipid microparticles/dispersed in a cold surfactant solution during pre-suspension. Pre-suspension is then homogenized at/below room temperature. Gravitational force breaks the lipid microparticles, SLNs obtained.

3.2. Ultrasonication/high-speed homogenization.

Drug and lipid were mixed in an organic solvent (methanol/ethanol). Then, it mixed with the aqueous phase in high-speed homogenization at 70°C during pre-emulsion for 15 minutes. Cooled with continuous stirring, SLNs were formed.

3.3. Solvent evaporation method.

Lipophilic material dissolved in a water-immiscible organic solvent (e.g., cyclohexane); homogenization occurs. Then, this emulsified with the aqueous phase at reduced pressure. Evaporation of the solvent followed with precipitation of nanoparticles dispersion [29].

3.4. Solvent emulsification-diffusion method.

Lipid dissolved in the organic phase in the water bath at 50°C with an acidic aqueous phase. Then, separation through centrifugation. SLN suspension produced [30].

3.5. Supercritical fluid method.

By using particles from the gas-saturated solutions (PGSS). Solvent-less processing occurs. It requires mild pressure and temperature. The dried powder must obtain—a rapid expansion of supercritical carbon dioxide (alternative for solvent).

3.6. Microemulsion-based method.

Lipid melts at the same temperature as the aqueous phase. The aqueous phase and surfactant were mixed with continuous stirring at a magnetic stirrer at 70°C. Mixed in cold aqueous phase with stirring; SLNs obtained.

3.7. Spray drying method.

It is an alternative to the lyophilization method. It transforms an aqueous SLN dispersion into a drug product; causes particle aggregation due to a rise in temperature (more than 70°C) / shear forces / partial melting of the particles.

3.8. Double emulsion method.

To prevent partitioning in the exterior water phase during solvent evaporation (w/o/w double emulsion), the medication is encapsulated with a stabilizer. W/O microemulsion can be prepared by adding an aqueous solution containing the drug in the mixture of melted lipid. Then, the formed w/o microemulsion is added to a mixture of water/surfactant / co-surfactant, clear w/o/w system. SLNs were obtained on dispersing the warm micro double emulsion in the cold. Then, it is washed with an ultrafiltration system by dispersion medium [31].

3.9. Precipitation method.

Glycerides are dissolved in an organic solvent (e.g., Chloroform). The solution is then emulsified in an aqueous phase. Lipid precipitates during evaporation of the organic solvent, forming nanoparticles [32].

3.10. Film ultrasound dispersion.

Lipid/drug was mixed incompatible organic solutions after decompression, rotation, and evaporation of that solution, a lipid film forms. Then, the aqueous solution containing the emulsion was added using the ultrasound with the probe to the diffuser at the end. SLN with the small and uniform size particles are formed [33].

3.11. Membrane contractor method.

The lipid phase is pressed at a temperature above the melting point of the lipid. Then, the liquid to pass through the membrane pores; small droplets are formed. The aqueous phase circulates inside the membrane module. The droplets form at the pore outlets are swiped away. Cooling of the preparation to room temperature; SLNs are formed. Process parameter depends on the SLN size: aqueous phase and lipid phase temperature, aqueous phase cross-flow velocity and lipid phase pressure, membrane pore size [34-36].

4. Macrophages as a Drug Carrier

Macrophages play various roles in mammalian biology: development, homeostasis, repair, and innate immunity. Macrophages are the main hosts of intracellular pathogens in chronic infectious diseases and, thus, conducted as a therapeutic target for intracellular delivery of antibiotics. These infections result from bacteria residing in host cells, causing them to replicate, survive, and resulting in damage to the host. Macrophages accord an immune-

privileged vocation and act as target for these intracellular pathogens [37]. Enhanced co-localization of antibiotics and intracellular pathogens could be achieved through targeted drug delivery on macrophages, significantly increasing therapeutic effects [38].

To lessen the reverberations effects on macrophages, to attenuate drug release, they are first loaded in nanoparticles (NPs). It attaches to macrophages via non-covalent adsorption, ligand-receptor interactions, or covalent coupling, or is internalized into the macrophages before administration [39]. Drug delivery systems for intracellular delivery to macrophages. Microparticles/NPs have an increased interest as drug carriers for macrophage-targeted therapy. Particulate drug delivery systems for macrophage-targeting have several benefits in delivering drugs to macrophages in the RES organs [40]. Particulate drug delivery can protect drugs from degradation in the course of circulation, readily reach the RES organs, and phagocytoses into macrophages. This includes liposomes, polymeric particles, dendrimers, and nanogels, preferably designed for macrophage-targeted drug delivery [41].

4.1. Liposomes.

Liposomes are spherical vesicles comprised of phospholipid bilayer surrounding an aqueous space, mostly used in cosmetic and pharmaceutical industries [42]. Liposomes are preferably used for macrophage-targeted therapy due to declined toxicity, flexibility in surface morphology, and preferential uptake by the RES organs [43]. Some studies conducted on liposomes targeted macrophages.

4.1.1. Gentamicin liposomes.

Exhibits Lowered morbidity in mice with Salmonella Dublin infection (*in vivo*) [44].

4.1.2. Rifampicin liposomes (MBSA or O-SAP coated).

Enhanced rifampicin lung retention in rats (*in vivo*); pre-treated with liposome aerosols have increased intracellular antimicrobial activity in alveolar macrophages isolated from rats (*in vitro/in vivo*) [45].

4.1.3. Ciprofloxacin Liposomes prepared from HSPC/DOPC/DCP.

Increased drug retention in alveolar macrophages of rats infected from pneumonia (*in vivo*) [46].

4.1.4. Vancomycin liposomes prepared from DSPC/Chol.

Effective against MRSA in THP-1 macrophages (*in vitro*).

4.2. Polymeric nanoparticles.

Natural/synthetic polymers are used to prepare polymeric NPs (colloid systems), have an average diameter in the range of 10–1000 nm. This drug may be in the form of the matrix (nanospheres) or drug in the form of a drug core encapsulated in a polymeric shell.

4.2.1. Poly (lactic-co-glycolic acid).

PLGA is one of the predominantly used synthetic polymers due to its biodegradability and biocompatibility. PLGA is most probably used for the targeted delivery of antibiotics to macrophages for the therapy of intracellular infection. Chitosan, derived from the exoskeleton of crustaceans, is a natural polysaccharide; it has been widely used for mucoadhesive drug delivery and gene transfection.

4.2.2. Gentamicin (PLGA NPs).

Enhanced antibacterial effects against *Listeria monocytogenes* in J774a.1 macrophages (*in vitro*) and *Brucella melitensis* infection in mice (*in vivo*) [47,48].

4.2.3. Isoniazid analog (PLGA NPs).

Enhanced intracellular antibacterial effect against intracellular *Mycobacterium tuberculosis* in BMMs (murine bone marrow-derived macrophages) (*in vitro*) [49].

4.2.4. Ceftriaxone chitosan (NPs).

Killed intracellular *Salmonella typhimurium* in J774.2 macrophages (*in vitro*) [50].

4.2.5. Ciprofloxacin chitosan (NPs).

Equivalent to free ciprofloxacin in killing *Salmonella* in Raw 264.7 and Intestine 407 cells despite attenuated drug release (*in vitro*); reduced dosage and frequency for effective killing of *Salmonella* in diseased mice (*in vivo*) [51].

4.2.6. Gentamicin Core-shell (NPs) from block copolymers.

Enhanced antibacterial effects against *Salmonella* in J774.1 cells (*in vitro*) and AJ646 mice (*in vivo*) [52].

4.3. Dendrimers.

Dendrimers have developed an interest as a carrier of drugs, genes, and imaging agents due to their unique properties: uniform size, high control over the molecular structure, versatile surface functionality, and internal cavities accessible for encapsulation of their payloads [52]. The dendrimeric formulation signifies improved pharmacokinetics and bio-distribution with more assenting toxicity profiles [53].

4.3.1. PAMAM-erythromycin dendrimeric nanoparticles (NPs).

Suppressed the nitric oxide level in LPS activated RAW 264.7 macrophages (*in vitro*) [54].

4.3.2. Amphotericin B Mannose-PPI dendrimeric NPs.

Enhanced cellular uptake of mannose-PPI dendrimeric NPs in J774a.1 macrophages (*in vitro*); improved parasite killing in mice infected with *Leishmania donovani* amastigotes (*in vivo*).

4.4. Nanogels.

Nanogels are hydrogels that are in nanoscale composed of cross-linked polymer networks. It is prevalence as a drug carrier stems from the stimuli-responsive potential for controlled drug release, design flexibility, and good biocompatibility [55]. Several natural polysaccharides: hyaluronic acid (HA), chitosan, pullulan / their combinations, used as a base for nanogels. Drugs are emblematically loaded via electrostatic interactions with polysaccharides/hydrophobic interactions with hydrophobic moieties harbinger to the polysaccharides. Some nanogels studies conducted:

4.4.1. Chlorin HA-decorated chitosan nanogels.

Selectively taken up by RAW 264.7 macrophages as compared with L929 or NIH-3T3 fibroblasts (*in vitro*); Prolonged retention of Chlorin e6 in arthritic knees of mice (*in vivo*) [56].

4.4.2. Tetracycline O-carboxymethyl chitosan nanogels.

Improved intracellular antimicrobial efficacy (*in vitro*) [57].

4.4.3. Distyrylbenzene derivative Thermoresponsive HA nanogels.

Enhanced cellular uptake of distyrylbenzene in RAW 264.7 cells (*in vitro*) [58].

4.4.4. Antitumor vaccine Cholesteryl pullulan nanogels.

Preferentially engulfed by medullary macrophages (*in vitro*); Inhibited *in vivo* tumor growth in mice [59].

4.4.5. Chloramphenicol Dextran sulfate nanogels.

The Enhanced intracellular killing of Salmonella paratyphi in RAW 264.7 macrophages (*in vitro*) [60].

5. Barriers in Macrophages Drug Delivery

In treating intracellular bacterial infection of macrophages, macrophages have promising techniques. Nonspecific biodistribution, ineffective cellular absorption, poor intracellular trafficking, inadequate drug release, and the development of drug resistance are all obstacles to overcome. 5.1. Pharmacokinetics and biodistribution.

Liver and spleen monolayer phagocytes the circulating NPs. RES is not considered as an intended target. “Stealth” polymers are coated on the surface for recognition during circulation in NPs targeting other organs and tissues. But in case applications targeting macrophages in the liver or spleen (e.g., intracellular infections), NP uptake by the RES is advantageous. Current approaches aim to deliver NPs to macrophages in target tissues. NPs can first target blood-borne macrophages/precursors to maximize their motility and tumor tropism. Some studies demonstrate that circulating monocyte /macrophages propose additional means to anchorage passive eructation and deliver NPs into target tissues.

5.2. *Macrophages cellular uptake.*

The first step for target delivery is that the drug carrier arrives at the target tissue, then secondly to transport the drug from the extracellular to the intracellular of the target cell. The critical role of carriers is to cross the cell membrane due to the polarity or the size. The universality of NPs/microparticles in macrophage-targeted drug delivery depends on the fact that they can enter macrophages via specialized endocytosis mechanisms: micropinocytosis, phagocytosis, clathrin-mediated endocytosis/caveolae-mediated endocytosis. Distinct physicochemical properties determine the efficiency of macrophage uptake of the particles.

5.2.1. Particle size.

The particle size ranged preferably for macrophage uptake varied with the type of particles and tested cell lines [61,62].

5.2.2. Surface charge.

Charged particles are attracted more by macrophages than neutral particles and enhanced cellular uptake with an increase in net charge. There is no resultant difference observed between cationic/anionic particles with the same net charges [63,64].

5.2.3. Particle shape.

The extent of cellular uptake of macrophages' drug delivery can be controlled significantly through particle shape. Particle shape influences the attachment and internalization independently: the attachment of particles to macrophages was ranked in the sequence of prolate ellipsoids, oblate ellipsoids, spheres; but in case of internalization, oblate ellipsoids were preferred than spheres or prolate ellipsoids [65]. Its role can be explained by its effect on the actin-remodeling process during phagocytosis.

5.2.4. Cell-interactive ligands.

Macrophages display a variety of receptors such as mannose receptors, folic acid receptors, Fc-receptors, and fibronectin lipoproteins [66]. Particle surfaces are covered with ligands for the receptors to enhance delivery efficiency to macrophages.

5.3. *Intracellular trafficking and drug release.*

Internal drug carriers are transported to the target organelles and released drugs timely to provide the therapeutic effect.

When a foreign substance is internalized in the cells, the endocytic vesicles are transported to early endosome and phagosomes, which fuses with sorting endosome, where the fate of the internalized material is determined [66]. Particles are typically captive to late endosome (pH 5-6) and later to lysosomes (pH 4-5)/degradative enzymes. Three distinct features of the intracellular environment are maneuvers for controlling the intracellular activity of drug carriers: acidic pH of intracellular organelles / lysosomal enzymes / relatively high reductive potential [67].

6. Conclusion

Nano/micro-particulate drug carriers have shown a significant effect on intracellular delivery of antibiotics to macrophages. The particulate carriers having optimum physicochemical properties are found in the RES, which minimizes systemic toxicities, and facilitates cellular uptake (polar antibiotics). The macrophages delivery *invitro* can be achieved for further studies in an artificial environment through simulated lung fluids.

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Conflicts of Interest

The authors declare no conflict of interest.

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