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REVIEW ARTICLE

Nanomedicines as Drug Delivery Carriers of Anti-Tubercular Drugs: From Pathogenesis to Infection Control

Afzal Hussain[1], Sima Singh[2], Sabya Sachi Das[2], Keshireddy Anjireddy[3], Subramanian Karpagam[3] and Faiyaz Shakeel[4,*]

[1]*Faculty of Pharmacy, S. Sinha College, Aurangabad-824101, Bihar, India;* [2]*Department of Pharmaceutical Sciences and technology, Birla Institute of Technology, Mesra, Ranchi 835215, Jharkhand, India;* [3]*Department of Chemistry, VIT University, Vellore 632014, Tamilnadu, India;* [4]*Department of Pharmaceutics, College of Pharmacy, King Saud University, P.O. Box 2457, Riyadh 11451, Saudi Arabia*

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Abstract: In spite of advances in tuberculosis (TB) chemotherapy, TB is still airborne deadly disorder as a major issue of health concern worldwide today. Extensive researches have been focused to develop novel drug delivery systems to shorten the lengthy therapy approaches, prevention of relapses, reducing dose-related toxicities and to rectify technologically related drawbacks of anti-tubercular drugs. Moreover, the rapid emergence of drug resistance, poor patient compliance due to negative therapeutic outcomes and intracellular survival of *Mycobacterium* highlighted to develop carrier with optimum effectiveness of the anti-tubercular drugs. This could be achieved by targeting and concentrating the drug on the infection reservoir of *Mycobacterium*. In this article, we briefly compiled the general aspects of *Mycobacterium* pathogenesis, disease treatment along with progressive updates in novel drug delivery carrier system to enhance therapeutic effects of drug and the high level of patient compliance. Recently developed several vaccines might be shortly available as reported by WHO.

Keywords: Clinical therapy, diagnosis, nanomedicine, pathogenesis, review, *Tuberculosis*.

1. INTRODUCTION

World Health Organization (WHO) has recently published that tuberculosis (TB) incidence rate is falling about at 2 % annually which needs to be falling at 4-5% per year by 2020 as per report [1]. In 2017, TB caused around 1.3 million deaths among HIV-negative and an estimated 0.3 million deaths among HIV-positive patients. Globally, WHO estimated 10.0 million new cases of TB (range, 9.0–11.1 million) equivalent to 133 cases (range 120–148) per 100 000 population in 2017 [2]. BRICS (Brazil, Russia, India, China and South Africa) countries accounted for more than 40% cases of the global TB burden [2]. The severity of national epidemics varies at a larger extent among different countries. There were less than 10 new cases of TB per 100,000 populations in most high-income countries, 150-400 in most of the high TB-burden counties and above 500 in a few countries in 2017. DOTs (directly observed therapy, short duration) strategy and its successor (the stop TB strategy) successfully cured a cumulative total of 5.6 million people between 1995 and 2012. Thus, the strategy saved about 22 million lives. The target of "The Stop TB Strategy" was expected to decline the prevalence of deaths up to 2015 owing to tuberculosis by 50% compared with a baseline of 1990 and by 2050 less than one case per million per year [3]. Now, the 2030 targets set in the end TB Strategy are a 90% reduction in TB deaths and an 80% reduction in TB incidence, compared with levels in 2015 [2].

TB is the most deadly and airborne disease due to *M. tuberculosis* as the causative agent among global infectious disorder. In spite of having potential treatment of TB, it presents a global health threat and challenges for complete cure worldwide. Multidrug resistance (MDR) strains are identified as *Mycobacterium* resistance to two powerful first-line anti-tubercular drugs (Isoniazid and rifampicin). Extensive drug resistance (XDR) strain is defined as MDR strain including additional resistance to fluoroquinolones and at least two injectable second-line anti-tubercular drugs. Both have been identified by the WHO as a major challenge for the treatment of TB. For effective TB treatment of new cases, six-month regimen of first-line anti-TB drugs (Rifampicin, Ethambutol, Isoniazid, and Pyrazinamide) is recommended as initial phase and then continued with the continuous phase of treatment. Both MDR-TB and XDR-TB have become a challenge for effective TB treatment on the global front due to relapses and frequent emerging resistance to ATDs drugs. Currently available potential TB therapies are partially effective owing to inherent least permeable nature of the cell wall of *Mycobacterium* and the chance of the *Mycobacterium* to develop resis-

*Address correspondence to this author at the Department of Pharmaceutics, College of Pharmacy, King Saud University, Riyadh, Saudi Arabia; Tel: +966-14673139; E-mail: faiyazs@fastmail.fm

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tance against used anti-TB drugs by gene mutation [4]. Furthermore, the tendency of *M. tuberculosis* to survive within host cell for a longer period of time and then disseminated to another uninfected cell is another issue for the treatment of TB. This is an airborne pathogen, surviving within host cell intracellularly for longer time and relapse of the infection depends on the host immune system [5]. For the first time in last four decades, WHO documented some newer anti-TB drugs (Table **1**) and vaccines (Table **2**) which have started to emerge from the pipeline and are in clinical trial phase I, II and III [6, 7]. There are around 20 pipeline anti-TB drugs in phase I, II or III trials which have been found more potent and efficacious than already existing ant-TB drugs [2]. Out of 20 pipeline drugs, there are 11 new compounds which include contezolid, delpazolid, GSK-3036656, macozinone, OPC-167832, pretomanid, Q203, SQ109, sutezolid, TBA-7371 and TBI-166 (Table **1**). Two new anti-TB drugs namely bedaquiline and delamanid have already received conditional regulatory approval based on the results of IIb clinical trials [2]. In 2018, WHO reported two vaccines reached to clinical trial phase III as revealed in Table **2**. Recently developed several vaccines might be shortly available as reported by WHO. Hence, in this review, we briefly compiled the general aspects of *Mycobacterium* pathogenesis, disease treatment along with progressive updates in novel drug delivery carrier system in order to enhance therapeutic effects of drug and the high level of patient compliance.

2. *MYCOBACTERIUM TUBERCULOSIS*, CLASSIFICATION, PATHOGENESIS AND DIAGNOSIS

2.1. *Mycobacterium tuberculosis*

This gram positive bacterium was first identified by Robert Koch in 1881 by culturing crushed granuloma. *Mycobacterium* is characterized as filamentous, non-motile, acid fast and gram positive bacteria distinguished by complex lipid composition of the cell wall. The *M. tuberculosis* (Mtb) is free from any flagella and capsule. However, the cell wall architecture is composed of waxy and complex mycolic acid making the bacteria acid fast strain. The bacterium replicates very slowly with size about 0.5µm in diameter and 1-4µm in length intracellularly in aerobic condition [8]. The shape of the bacterium is cylindrical as shown in Fig. (**1**). Human strain causing tuberculosis varies in their phenotype and virulence. *M. tuberculosis* H37 Rv strain was obtained from 19 years old TB patient (pulmonary) in 1905. Later, the strain was differentiated as virulent strain (H37 Rv) based on virulence and an avirulent (H37 Ra) in guinea pigs [9]. Both strains can be cultured in a suitable growing medium [10]. Various clinical isolates, BCG and the H37R are mostly taken to study in different *in vitro* and *in vivo* pathogenesis of *Mycobacterium*. *M. smegmatis* is mostly used strain to be taken in the laboratory as model strain being non-pathogenic in nature. The uniqueness of the cell wall (Fig. **2**) composition of Mtb consists of long chain fatty

Table 1. The development pipelines of newer anti-tubercular drugs from August 2015 to August 2018.

Development Phases					
Discovery in 2015	**Preclinical Development (PD) in 2015**		**Drugs in Clinical Phases (I, II, III) in 2018**		
Lead Optimization	**PD**	**GLPT**	**Phase I**	**Phase II**	**Phase III**
• Cyclopeptides • Diarylquinolines • DprE inhibitors • Inh inhibitor, Indazoles • LeuRS inhibitors, Ureas • Macrolides, Azaindoles • Mycobacterial Gyrase Inhibitors • Pyrazinamides Analogs • Ruthenium (II) Complexes • Spectinamides SPR-113 • Translocase I inhibitors	• TBI-I66	• [φ]PBTZ 169	• Contezolid (MRX-1) • GSK-303656 • Macozinone (PBTZ169) • OPC-167832 • Q-203 • TBA-7371 • TBI-166	• Delpazolid (LCB01-0371) • SQ-109 • Sutezolid (PNU-100480) • Linezolid dose ranging • Nitazoxanide	• Bedaquiline (TMC-207) • Delamanid (OPC-67683) • Pretomanid (PA-824) • Clofazimine • High dose rifampicin for treatment of DS-TB
	• CPZEN-45	• Q203		• High dose rifampicin for DS-TB (Panacea)	• [†]Rifapentine for treatment of DS-TB
	• SQ-609			• Bedaquiline and pretomanid with existing and re-purposed anti-TB drugs for MDR-TB	• Bedaquiline-pretomanid-linezolid (ZeNix trial)
	• SQ-641 • [‡]DC-159a			• Delamanid, linezolid, levofloxacin and pyrazinamide for quinolone sensitive MDR-TB (MDR-END trial) • Levofloxacine with OBR for MDR TB (OPTI-Q)	• Bedaquiline with two optimized background regimens (09 months for oral and 06 months for injections) (STREAM trial) • Bedaquiline-linezolid-levofloxacin with OBR for MDR-TB • Bedaquiline and delamanid with various existing regimens for MDR-TB and XDR-TB (end TB trail) • Pretomanid-Moxifloxacin-pyrazinamide regimen (STAND trial) • Rifapentine-moxifloxacin for treatment of DS-TB (TB trial consortium study)

Abbreviation: GLPT: Good laboratory practice toxicity; TB: Tuberculosis; ϕ: chemically benzothiazinone; OPC: Optimized background regimen; MDR: Multidrug resistant; XDR: Extended drug resistance; Ref: WHO, Global Tuberculosis Report 2015; 2018.

Table 2. New anti-tubercular vaccines in pipelines of clinical trials phase and sponsor, August 2018.

New Vaccines	Clinical Trial Phases	Immunity Type	Sponsors
AEC/BC02	Phase-I	IT	Anhui Zhifei Longcom
MTBVAC	Phase-I	P	TBVI, Zaragoza, Biofabri
ID 93+GLA-SE	Phase-IIa	B	Infectious Disease Research Institute (IDRI), Wellcome Trust, Aeras
ChAdOx185A/MVA85A	Phase-I	P, B	Crucell, University of Oxford, Aeras
VPM 1002	Phase-III	P, B	Max Planck, VPM, TBVI, Serum Institute
RUTI,	Phase-IIa	B, PI, IT	Archivel Fharma, S.L
H56:IC31	Phase-IIa	P, B, PI	SSI, Velneva, Aeras
Ad5Ag85A	Phase-I	IT	Mc Master, CanSino
M72+AS01	Phase-IIb	B, PI	GSK Aeras
M. Vaccae	Phase-III	IT	Anhui Zhifei Longcom
DAR-901 booster	Phase-IIb	IT	Dartmouth, Aeras
TB-FLU-04L	Phase-IIa	B	RIBSP

Abbreviation: P: Prime; B: Boost; PI: Post infection; IT: Immunotherapy (Ref: WHO, Global Tuberculosis Report, 2018).

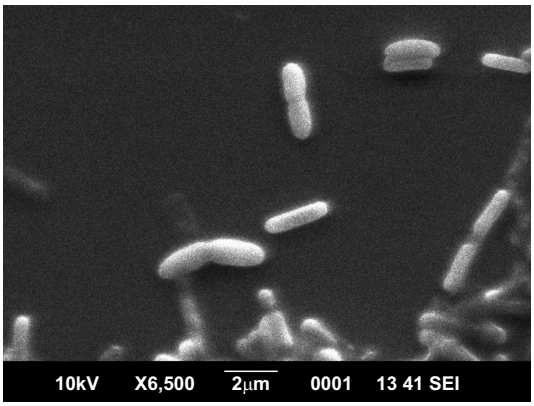

Fig. (1). Scanning electron microscopy of *M. smegmatis* showing cylindrical shape of *Mycobacterium.*

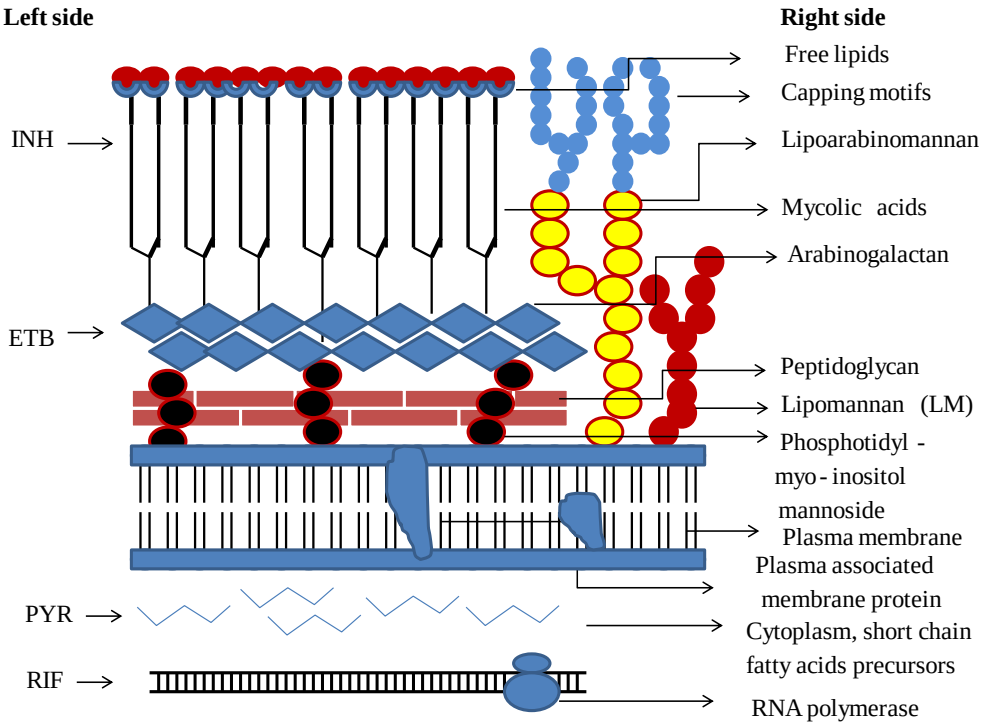

Fig. (2). Schematic representation of complex Mtb cell wall composition on the right side and the site of action of anti-tubercular drugs like isoniazide (INH), ethambutol (ETB), pyrazinamide (PZA) and rifampicin (RIF) on the left side of the figure.

acids (especially mycolic acid) bonded to arabinogalactan which is further attached with peptidoglycan. Additionally, this contains various lipoglycans like lipidarabinomannan (LAM), its precursor lipomannan (LM) and phosphotidyl myo-inositol-mannosides (PIM). Therefore, these LAM, LM and PIM are non-covalently linked to the plasma membrane and then extended to the exterior of the cell wall [8, 11]. LAM is the major virulence factor and consists of phosphotidyl-myo-inositol anchor, a D-mannan polymer attached to the inositol ring D-arabinose chain and capping motifs at the ends of the arabinose residues [12]. It inhibits macrophage function by inhibiting phagosomal maturation including all inflammatory signaling responses. Virulent *Mycobacterium* harbor mannose capped LAM in their cell wall whereas non-virulent fast growing *Mycobacterium* harbors non- capped Ara-LAM or PILAM (phosphor-myo-inositol LAM capped). Capping of different type is essential for virulent nature of *mycobacterium* [13]. Plasma membrane-associated protein (19Kda), LAM and Ara-LAM of fast growing *Mycobacterium* trigger inflammatory responses in the host body by binding to the Toll like receptors (TLR) present on the host infected cell surfaces [14].

2.2. Classification of *Mycobacterium* Species

These bacilli can be broadly classified as pathogenic and non-pathogenic species of *Mycobacterium*. Among these, they are classified as slow growing and fast growing species of *Mycobacterium* as shown in Table **3**. All mycobacterae belong to the genus *Mycobacterium* in the order Actinomycetals of the family Mycobacteriacae. However, more than 70 species have been recognized which mostly belong to non-pathogenic *Mycobacterium* [15, 16]. A thorough understanding of the chemistry of the cell wall composition is required for the scientist engaged in new anti-tubercular drug designing for the treatment of *tuberculosis*. The cell wall of these bacteria contains quantum amounts of Lipoarabinomannan (LAM), Lipomannan (LM) and phosphotidyl-myo-inositol mannosides (PIM). All of these complex molecules are interspersed throughout the cell wall of *Mycobacterium* [17-19]. LAM is the most studied complex compound of the cell wall and classified into three families based on capping motifs present on the arabinosyl side chains. The first family where the arabinan termini are capped with mannose residues and designated as Man-LAM results into pathogenic/virulent strain [20-22]. In the second family, arabinan termini are capped with inositol-phosphate (PI-LAM) like fast growing non-pathogenic *M. smegmatis* [22]. The *M. megmatis* is filamentous and non-virulent as revealed in transmission electron microscopy (TEM) in Fig. (**1**). Recently, a third family is recognized with the lack of both capping molecules as observed in *M. chelonae* [23].

Moreover, *M. smegmatis* are frequently used for the molecular analysis among non-virulent species [5]. This saprophytic *Mycobacterium* is used as a suitable model to study the pathogenesis of *Mycobacterium*. In addition, this species provides some very important and useful comparative biological studies to define the particular characteristics that make *MTb* to be very cautiously interpreted. This does not survive within the professional phagocytic cell and does not enter the epithelial cells. So, these reasons have disregarded for the study of *Mycobacterium* pathogenicity. However, within a short generation time (3-4 h), it is a good option to study *Mycobacterium* in general [24]. Particularly, the *M. smegmatis* mc^2155 strain can be used as a model because of the high rate of transformation and survives in phagocytic cells only for few days [25, 26]. It has been reported that intracellular persistence of the *M. smegmatis* depends on the permeability nature of the cell wall. The distribution of glycolipids and phospholipids within the outer cell wall determines outer membrane surface properties, cell wall permeability nature and bacterial phenotype [27, 28].

2.3. Pathogenesis and Host Cell Signaling to *Mycobacterium*

Mtb are inhaled from contaminated droplets released from infected individuals. The ingested *Mycobacterium*s are taken up by alveolar macrophage cells and other immune cells in order to phagocytose the infected pathogen. In order to present *Mycobacterium* by antigen presenting cells like alveolar macrophage, a series of sequential events take place including invagination, budding and physical fusion between cells using intracellular adhesion molecules (ICAM)-3 grabbing nonintegrin (DC-SIGN). Biological understanding of granulomas of *M. tuberculosis* remains only partially understood and believed as structured clusters along with *Myco-*

Table 3. Classification of *Mycobacterium species.*

Pathogenic Mycobacteriums		Non Pathogenic Mycobacteriums	
Fast Growers	**Slow Growers**	**Fast Growers**	**Slow Growers**
M. abscessus	*M. tuberculosis*	*M. kansasii*	*M. gastri*
M. fortuitum	*M. africanum*	*M. brumae*	*M. cooki*
	M. avium	*M. aurum*	*M. gordonae*
	M. bovis	*M. chitae*	
	M. intracellulare	*M. gilvum*	
-	*M. kansasii*	*M. phlei*	-
	M. leprae	*M. smegmatis*	
	M. marinum	*M.vaccae*	
	M. ulcerans	-	

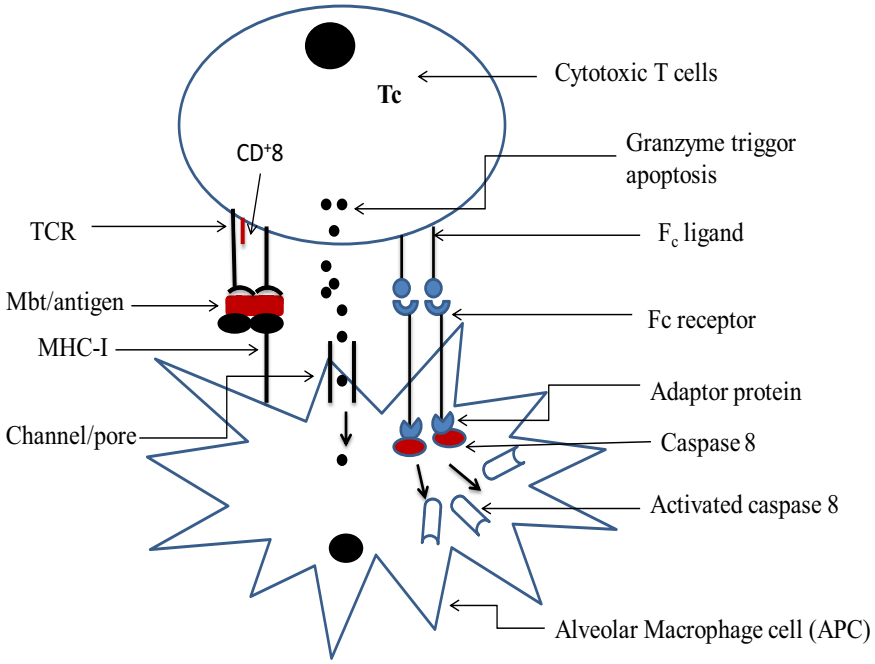

Fig. (3). Presentation and processing of *Mycobacterium tuberculosis* or antigen by antigen presenting cells like alveolar macrophage cell (APC).

bacterium infected cells at the center, surrounded by various type of immune cells especially alveolar macrophage cells and T lymphocyte cells [29]. The interior of the granulomas contains both activated and non-activated macrophage cells where activated macrophage cells (particularly alveolar macrophage cells) are present (Fig. **3**). Furthermore, these *Mycobacterium* antigens are eliminated when activated T-lymphocyte cells trigger the release of several immune molecules such as cytokines and chemokines. These released cytokines and chemokines chemical mediators keep the macrophage cell in the activated state and ensure to recruit other immune cells at the site of infection [30, 31]. Several molecules of IL (interleukin, IL-2, IL-4, IL-5) exhibit cell differentiation, proliferation, apoptosis using cascpase factor, inhibition of proliferation, metabolic inhibition and chemotaxis process at the target site.

A number of complementary receptors (CR1, CR2, CR3, CR4), F_{ac} receptor (death receptor), mannose receptor, Tc surface glycoprotein $CD8^+$and $CD4^+$, MHC-I, MHC-II and different apoptosis cascade protein are involved in apoptosis mechanism. A brief antigen presenting mechanism involving T-lymphocyte cell (Tc) and macrophage cell has been shown in Fig. (**3**). As different stages of TB advance, various immune cells are involved to develop equilibrium between virulent granuloma and host immune system of the infected individual. If individual's immunity is sufficiently effective, then there is no chance of development of adverse reaction of *M. tuberculosis* on the health of the host even up to life term [32]. Initiation (initial stage) of *tuberculosis* is characterized by replication of the *Mycobacterium* strain when the host's adaptive immune system gets weakened. Eventually, adaptive immune system gets initiated and $CD4^+$ and $CD8^+$ effectors T lymphocyte cells are produced against infected cell to terminate *Mycobacterium* growth [33].

In this process, a number of pro-inflammatory and inflammatory cytokines (previously described), monocytes cells, dendritic cells, neutrophils cells and apoptotic cascades are synthesized. However, the survival strategies of *Mycobacterium* inside the host cell are achieved through a series of sequential events that are discussed shortly here.

The successful survival of ingested pathogenic *Mycobacterium* inside the macrophage cells involves inhibition of the host cell process enabling them to reside inside the host cell. Various processes after infections are (i) phagosomes's fusion with lysosomes, (ii) Presentation of antigen, (iii) initiation of apoptosis program and (iv) initiation of bactericidal responses. Cell wall glycolipids (19Kda) and LAM molecules are potential virulence factor to initiate pathogenesis. Non pathogenic *Mycobacterium* is rich with Ara-LAM whereas pathogenic strain contains a large quantity of Man-LAM. There are several survival strategies adopted by Mtb, some of them are highlighted as follows:

1. Entry of the Mtb into the macrophage cells takes place through complement receptors, mannose receptors and Fcγ receptors. Among these, complement receptors play important roles in its survival strategies. However, this mechanism is considered a minor way.

2. Phagosome maturation occurs with a sequential series of fusion process during the course of the maturation process. Interestingly, dead, attenuated and heat killed *Mycobacterium* follow differently than pathogenic Mtb while phagosomal maturation. Pathogenic *Mycobacterium* modifies signaling pathway that allows them to infect and survive inside the host cell by inhibiting phagosome maturation, apoptosis process and suppressing the host immune system. On the other hand, dead or non-pathogenic *Mycobacterium* stimulates the immune

system and thus promotes phagosomal maturation. Pathogenic *Mycobacterium* limits the MAPK (Mitogen Activated Protein Kinase) activation pathway inside macrophage cells impairing the bactericidal immune responses whereas attenuated *Mycobacterium* stimulates the MAPK. Moreover, Man-LAM inhibits phagosomal maturation by preventing calcium release from stimulation of calmodulin dependent kinase II (CaMKII). Heat killed *Mycobacterium* activates sphingomysin kinase which get phosphorylated to sphingomysin 1-phosphate resulting into enhanced calcium release from CaMKII. Insight story of phagosomal maturation involves several other complex molecules like small GTP Rab5, Rab7, EEA1 (early endosome antigen1), phosphotidylinositol 3-kinase (PI3K) and phosphotidylinositol 3-phosphate (PI3P). In non-pathogenic *Mycobacterium*, Rab5 is replaced with Rab7 during phagosome maturation at intermediate endosome fusion stages. Late endosome gets fused with phagosome and form phagolysosome containing several hydrolytic enzymes and acidic pH [34]. Man-LAM *Mycobacterium* inhibits calcium mediated endosome fusion, thereby preventing phagosome maturation inside the host cell. In this way, non-virulent *Mycobacterium* is subjected to phagosome that further fuses to the lysosome and later on undergoes to acidic exposure due to the presence of proton ATPase from the membrane of vacuole for lysis. On the other hand, Man-LAM *Mycobacterium* phagosomes are not directed to fuse with lysosome and subsequently fail to acidification process. Thus, reduced level of acidic intracellular condition allows the *Mycobacterium* to survive [35].

3. TACO (Tryptophan Aspartate containing Coating Protein) and certain small GTP binding protein are characteristics of the *Mycobacterium*, recruited and present on the *Mycobacterium* phagosome membrane containing M. bovis bacille-Calmette-Guerin (BCG). Thus the stable association of this protein with the live and pathogenic *Mycobacterium* in phagosome inhibits the fusion with lysosome and further acidification. However, killed *Mycobacterium*, heat killed *Mycobacterium*, attenuated *Mycobacterium* and even newly formed endosome from uninfected cell lack potent TACO and GTP. This is why these non-pathogenic and heat killed *Mycobacterium* get harbored through general phagolysosome maturation pathway [34].

4. *Mycobacterium* interferes with the phosphotidylinositol-3 kinase (PI3K) signaling pathway by reducing EEAI level recruited by PI3K. Furthermore recruitment of PI3K depends on its interaction with Ca^{2+}-bound calmodulin. Man-LAM prevents cytoplasmic Ca^{2+}/calmodulin concentration increment and blocks the cytosolic Ca^{2+}/calmodulin association with PI3K, thereby inhibiting recruitment of EEA1 to phagosome [12, 36].

5. *Mycobacterium* interfer with the host lipid signaling pathway by inducing actin molecules assembling around the phagosome. Some ceremide lipid like sphingosin-1-phospate (S1P) might play this role by developing actin associated endosome, lysosme and guide their movement during vesicular fusion with phagosome [37]. Hence disruption of this actin bridge prevents the fusion of endosome with lysosome. It must be acknowledged here that pathogenic *Mycobacterium* fails to assemble actin molecules and further crucial fusion process whereas killed or non-pathogenic *Mycobacterium* readily induces actin formation and successful fusion process is achieved for lysis of phagolysososme.

6. Apoptosis is a program cell death involved in harboring antigen, pathogenic cells and cancerous cells. *Mycobacterium* induced apoptosis is a very complicated phenomenon. Virulent strain induces a very low level of apoptosis cascade mechanism than it does infection with the attenuated strain. Man-LAM antagonizes *Mycobacterium* induced apoptosis by blocking the rise in cytoplasmic Ca^{2+} concentration being believed as to facilitate apoptosis [38, 39].

7. Mtb also inhibits macrophage apoptosis by inducing immunosuppressive cytokines interleukin 10 supposed to block tissue necrosis factor alpha (TNFα) synthesis. TNFα is apoptosis inducer in infected macrophage cells.

8. *Mycobacterium* interferes with host MAPK and JAK/STAT (Janus kinase/signal transducer and activator of transcription) pathways. Both (MAPK and JAK/STAT) pathways result to produce several pro-inflammatory cytokines and induce innate immune responses while infections. Virulent Mtb suppress these signaling transduction cascade pathaways in order to survive in the host macrophage cells.

9. Modulation of signaling in host dendritic cells: TLR (Toll Like Receptor) and C-type lectin protein are expressed on the DCs surface during infections. TLR are conserved receptor on the cell surface that recognizes pathogen associated molecular pattern to establish innate immunity. Other C-type lectin protein recognizes a wide variety of pathogen to be internalized and presented for processing to T cells. Like phagososme, DCs phagocytose *Mycobacterium* and undergoes to differentiate and activate T cells to produce helper T cells (T_H1) and T_H2. Helper T cell 1 releases INFγ responsible for killing intracellular pathogen whereas Helper T cell 2 secretes IL-4 responsible for killing extracellular *Mycobacterium*. In patient with insufficient immune system, increased Man-LAM released from infected macrophage or DCs subverts the functions of DCs [34].

2.4. Diagnosis of Tuberculosis

Common symptoms of TB patient are chronic coughs (sometimes with blood), sweating at night and loss of weight. However, any organs/tissues of the body are affected by the spread of *Mycobacterium* through lymphatic system leading to extra-pulmonary TB. Latent TB is characterized by developing granuloma in which bacilli are present and remain for decades or wait to be active in favorable condition or in suppressed immune condition. There are several diagnostic methods to diagnose TB *viz* the chest X-rays, sputum smear microscopy, immunological memory based test and Mtb specific PCR in addition with a less specific tuberculin skin test. But INFγ release assay is a more specific test. Phage amplification assay, solid culture and automated liq-

uid culture test are also performed. However, the utmost need of test is to find rapid testing, cost-effective testing to confirm tuberculosis cases and to find drug-resistant cases [40]. About 5.7 million individuals were recently diagnosed in 2012 and 0.4 million were previously diagnosed TB patients whose treatment regimen was changed. India and China accounted for 39 % of notified TB cases worldwide in 2012 [6].

2.5. Diagnostics and Laboratory Strengthening by World Health Organization (WHO)

Sputum smear microscopy and *Mycobacterium* growth culture are the conventional laboratory tests for the diagnosis of TB. Cultures specimen is used as the standard reference for diagnosis. Drug susceptibility testing (DST) on cultures is used to detect resistance to both first-line as well as second-line anti-tubercular drugs. In 2010, first time rapid molecular test to find out pulmonary tuberculosis and rifampicin resistance simultaneously was endorsed by WHO using Xpert ® MTB/RIF. Rapid molecular test is recommended for use in children and to diagnose specific extrapulmonary tuberculosis with more accuracy than smear sputum test method [2]. This specific test in term of the sensitivity of the test was comparable to solid culture test but much better than smear sputum microscopy method. A review of the 2010 policy was started in 2013 for its application and status of Xpert® MTB/RIF to diagnose pulmonary, extra-pulmonary and pediatric TB and updated guidance was expected in 2014. Several countries frequently adopted Xpert® MTB/RIF. Up to end of June 2013, 1402 Gene Xpert machines and 2.3 million Xpert® MTB/RIF cartridges (1.1 million in June, 2012) had been procured by 88 (67 in June, 2012) of the 145 countries eligible for the concessional price [6]. It is noteworthy that WHO reported a new development in newer cartridges (Xpert omni and Xpert ultra) as new generation vaccines which are expected to replace the described diagnostic cartridges [7].

3. OVERVIEW OF NEW ANTI-TB DRUGS

Recently, WHO documented some newer anti-TB drugs and their overview is tabulated in Table **1**. The total number of these drugs is around 20 [2]. Bedaquiline has been recommended for the treatment of MDR-TB in adults based on phase IIb clinical trials. In some countries such as Philippines, Russian Federation and South Africa, it has also been used in the treatment of MDR-TB in children. Contezolid (MRX-1) has been found to effective against Gram-positive pathogens. Delamanid has also been found in the treatment of MDR-TB in adults based on phase IIb clinical trials. Delpazoid (LCB01-0371) had entered to phase II trials in the Republic of Korea in 2017. GSK-3036656 had entered to phase I clinical trials in March 2017. Macozinone had completed phase I trials and entered to phase II trials in Russian Federation. OPC-167832 has been found to be effective against both growing and intracellular bacilli. A single dose study of this compound has been completed. A multiple dose and early bactericidal activity study of OPC-167832 alone and in combination with delamanid has been planned. Pretomanid is currently being evaluated as part of combination regimens in the treatment of both drug-susceptible and DR TB. Q203 has been tested for phase I trials and its phase II trials have been planned. SQ109 had completed phase IIb/III trials in which drug was added to a standard regimen for the treatment of MDR-TB. Sutezolid (PNU-100480), an analogue of linezolid has been found a good bactericidal agent in early bactericidal activity studies. License agreement for the clinical development of sutezolid has already been completed. TBA-7371 has been proposed as an inhibitor of enzyme DPrE1 which is essential for the synthesis of the components of mycobacterial cell walls. It has been found to be active against strains of *M. tuberculosis* resitant to known TB drugs. TBI-166 has been found to be effective in improving physicochemical and pharmacokinetic properties and has efficacy similar to clofazimine. A Phase I trial for TBI-166 has been started in January 2018 in China [2].

4. OVERVIEW OF NEW ANTI-TB VACCINES

The status of the pipeline for new anti-TB vaccines is tabulated in Table **2**. The total number of these vaccines is 12 which are under phase I, II or III trials [2]. Four vaccines namely Ad5 Ag85A, AEC/BC02, ChAdOx185A-MVA85A and MTBVAC are under phase I trials. Ad5 Ag85A has been proposed as an adenovirus serotype 5 vector expressing Ag85A which is evaluated for safety and immunogenicity in both BCG-naive and previously BCG-immunized healthy volunteers in Canada. AEC/BC02 has been developed as a freeze-dried recombinant vaccine expressing Ag85B and fusion protein ESAT6-CFP10. Its safety and immunogenicity is underway in China under phase I trilas. Intramuscular administration of ChAdOx185A in BCG-vaccinated volunteers in UK, both alone and as part of a prime-boost strategy with MVA85A has been completed under phase I trials. Aerosol administration of ChAdOx185A in BCG-vaccinated volunteers was scheduled in the third quarter of 2018 under phase I trials. Two studies of aerosol administration of MVA85A in BCG-vaccinated volunteers have already been completed and a further study in volunteers with LTBI is being implemented. MTBVAC has been proposed as a live strain of *M. tuberculosis*. The primary target population is neonates; the secondary target population is adolescents and adults.

There are eight vaccines such as DAR-901, H56:IC31, ID93 + GLA-SE, M72/AS01E, RUTI®, TB/FLU-04L, Vaccae™ and VPM1002 in Phase II or Phase III trials. DAR-901 has been proposed as a whole-cell, heat-inactivated, nontuberculous vaccine booster. The trial for DAR-901 has been scheduled for completion in 2019. H56:IC31 has been proposed as an adjuvanted subunit vaccine which combines three *M. tuberculosis* antigens (Ag85B, ESAT-6 and Rv2660c) with the IC31© adjuvant. Three phase I or I/IIa trials of safety and immunogenicity for this vaccine have been completed. ID93 + GLA-SE vaccine contains four *M. tuberculosis* antigens associated with virulence (Rv2608, Rv3619 and Rv3620) or latency (Rv1813), and the adjuvant GLASE. M72/AS01E has been proposed as a subunit vaccine which pairs two *M. tuberculosis* antigens (32A and 39A) with an adjuvant (AS01E). It has been evaluated in a phase IIb efficacy trial in HIVnegative volunteers in Kenya, South Africa and Zambia. RUTI® is a non-live, polyantigenic vaccine which is based on cell wall fragmented *M. tuberculosis* bacteria. A phase I study in healthy subjects and a phase II study in volunteers with LTBI have

suggested a good safety profile. TB/FLU-04L has been proposed as a mucosal-vectored vaccine which is based on an attenuated replication-deficient influenza virus vector expressing antigens Ag85A and ESAT-6. A phase IIa trial in volunteers with LTBI has been implemented. Vaccae™ has been proposed as a specified lysate which has been licensed by the China FDA. A phase III trial with LTBI has been completed and data analysis is underway. VPM1002 has been proposed as a live recombinant vaccine. A phase II trial is being implemented. A phase II/III trial in volunteers is being implemented in India [2].

5. CURRENT DRUG DELIVERY AND ITS LIMITATIONS IN ANTIMYCOBACTERIAL THERAPY

The prime goal of *tuberculosis* treatment includes cure without the emergence of relapses, without the emergence of drug resistance, therapy without failure, reduced patient mortality, and prevention from transmission and to eliminate rapidly growing bacilli completely from host's cell/tissue. In order to achieve these goals, multiple drug therapy with high dose regimens is required for a sufficiently long period of time as fixed dose combination therapy to cure TB and has very less likelihood of relapse. The rationale of using fixed dose combination is to eradicate TB of all category falling under category-I (bacilli surviving extracellularly in sufficient oxygen), category-II [bacilli growing in acidic macrophage where only pyrazinamide (PZA) is effective], category-III (bacilli growing in solid caseous mass under low oxygen tension where only Rifampicin is effective), and category-IV (bacilli growing in dormant phase which are not probably susceptible to any anti-tubercular drugs). Category II and Category III are termed as persister which might be reactivated in host body when the host immunity is ineffective. Only rifampicin and pyrazinamide are effective against persisters, so called as sterilizing agents [41]. A number of potentially recommended drugs are listed in Table **4** [1]. Currently recommended *tuberculosis* chemotherapy (DOTS, directly observed therapy, short duration) mainly includes six-month therapy with co-administered drug comprising as below:

(1) **An initial intensive phase**: First four powerful and potential drugs are recommended on daily basis for 2 months. This regimen consists of rifampicin (RIF), isoniazid (INH), pyrazinamide (PZA) and ethambutol (ETB).

(2) **A continuous phase**: This phase of treatment comprised of 4 months, recommended with RIF and INH either daily or three times per week on alternate days.

On the other hand, WHO recommends DOTS plus for the treatment of multidrug resistance *tuberculosis* where the above regimen is prescribed followed by second-line anti-tubercular drugs [42]. The WHO specifically discourages programs using dose twice weekly, the reason being that there is a lesser margin of safety if a dose or dose are missed. To strengthen TB treatment, DOTS provides five essential components. These include government regular TB control program, regular supply of anti-tubercular drugs, DOTs for atleast 2 months of initial treatment, case detection by positive smear microscopy and a standardized case recording and reporting system.

Tuberculosis is cured using multiple dosage regimen, high dose content, and lengthy (usually 6 to 9 months) current protocol of treatment leading to develop propensity of drug resistance, unsatisfactory patient compliance and incidence of adverse drug reactions. Thus in spite of the availability of potential and effective treatment for TB, cure success still remains low. The lengthy treatment protocol has to be shortened. Therefore, present efforts have been focused on shortening the duration of tuberculosis chemotherapy to improve treatment either by using innovative drug delivery approaches or alternative route of drug administration which may improve the efficacy of the drug to target the infectious site and with high patient compliance. Major causes of failure of TB treatment includes non-adherence to DOTS therapy and dosage regimen, failure of health care, malabsorption of the drug, drug resistance or drug interaction with food while absorption, extreme biological variation, laboratory error and others are shown in Fig. (**4**).

A number of authors have explored novel drug delivery approaches and carrier systems to deliver the anti-tubercular drugs with significantly high bioavailability, reduced side effects, reduced dose frequency and high patient compliance. These carriers include nanoparticulate materials (nanoparticles and microparticles), vesicular system (liposome and niosome), nanoemulsion and dry powder inhalations. In this article, a comprehensive account on recent advances in novel drug delivery carriers and their targeting to the site of action to minimize dose related side effects and to improve patient compliance in the treatment of tuberculosis. For efficacious drug delivery design, formulation scientists need a throughout knowledge of drug's characteristics for successful delivery to the target site and in a sustained delivery manner as shown in Table **4** and Table **5** [1].

5.1. Novel Drug Delivery Carriers for the Treatment of TB

Conventional drug delivery for anti-tubercular drugs presents a lot of limitations including negative therapeutic outcome, poor patient compliance and technological demerits of the presently used therapeutic agents which might be resolved by new drug delivery approaches. Common limitations of conventional drug delivery and advantages of new drug delivery have been shown in Fig. (**5**). In the present review article, we have compiled general aspects of the pathology of *Mycobacterium* (earlier) and the current research updates using the novel nano carrier based drug delivery in the treatment of tuberculosis which results to increase therapeutic efficacies and good patient compliance. In this review, we also focused to glean the information regarding attempts made to achieve optimum effectiveness of the anti-tubercular drug(s) by drug targeting to the infection vicinity.

Novel drug delivery systems employed various carriers, different alternative route of administration and drug targeting as the strategies of processes or designed devices to enhance the efficacy of the drugs through modified or controlled release drug pattern. All these approaches may significantly improve drug bioavailability, therapeutic index and high patient compliance. The lack of patient's attention on the prescription and patient's failure to take dose result in significant mortality and morbidity. Therefore, the researches on novel

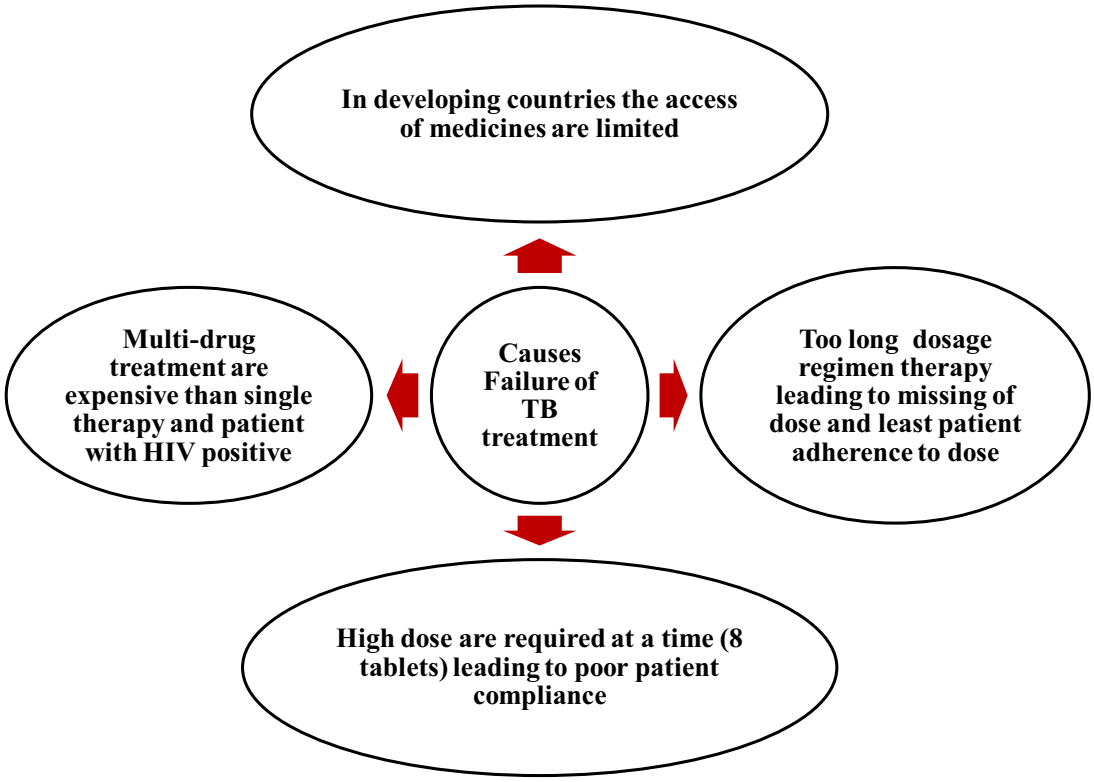

Fig. (4). Major factors causing failure of tuberculosis treatment.

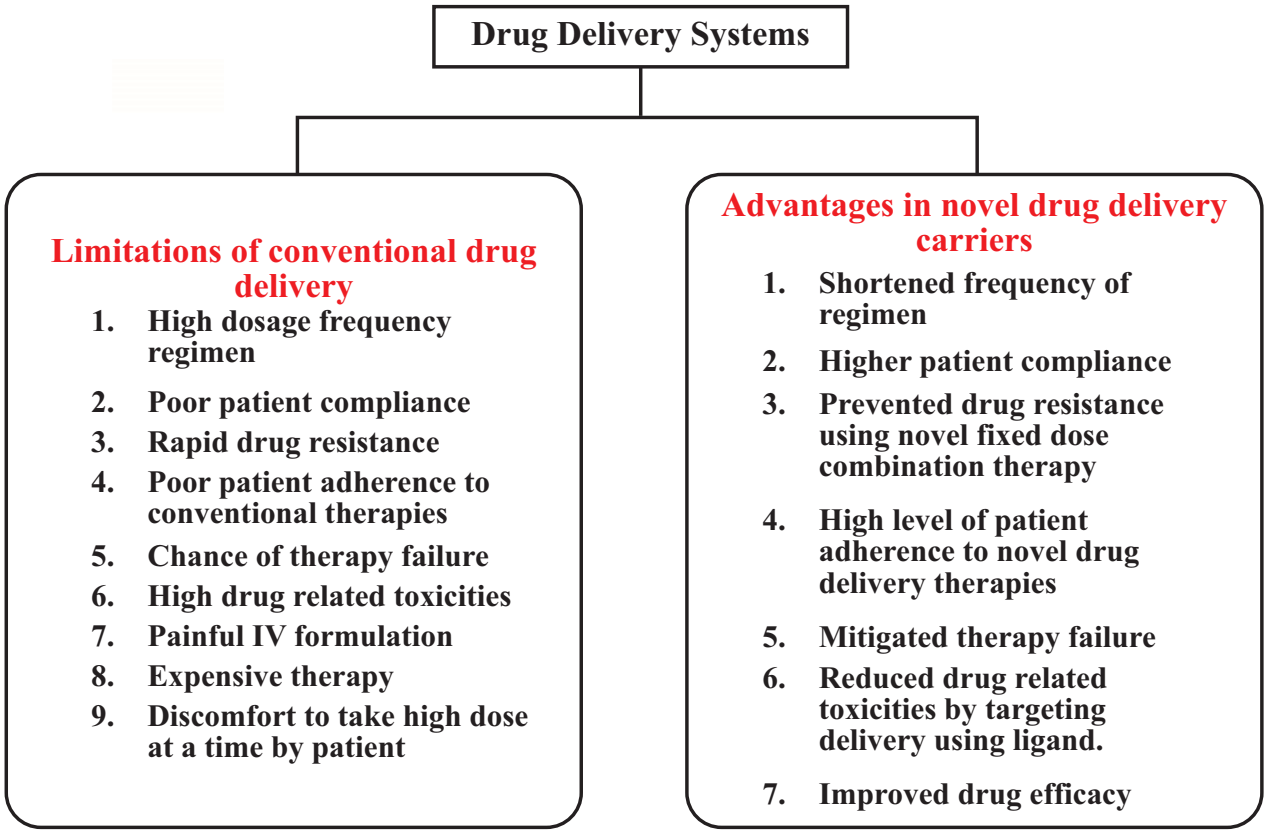

Fig. (5). Common limitations of conventional formulation and advantages of novel drug delivery.

Table 4. Anti-Tubercular drugs with their dosage form, route of administration (ROA), mechanism of action (MOA) and their adverse reaction.

ATD Name	Brand Name	Dose/Dosage Form	ROA	MOA	Adverse Drug Reaction
First-line drugs					
Rifampicin	Abrifam(Abbott)	10 mg/kg daily/Tablet or IV	Oral/IV	Rifampicin polymerase inhibitor	Flu like syndrome, hypotension, shortness of breath, organic brain syndrome, orange red coloration of all body fluid, hypersensitivity reaction, thrombocytopenia etc.
Isoniazid	Cotinazid (Pfizer)	5mg /kg for adult and 10-20 mg/kg for children	Oral	ACP reductase inhibitor involved in fatty acid synthesis	Peripheral neuropathy, hepatotoxicity, GIT disturbances
Pyrazinamide	Pyraldina (Bracco)	20-25 mg/kg daily tablet	Oral	Fatty acid synthesis *via* pyrazinamic acid	Adversely affect on liver, blood clotting mechanism, causes gout, hypersensitivity reaction
Ethambutol	Diambutol	30/kg three times /week-tablet	Oral	Inhibits arabinosyl transferases involved in cell wall systhesis	Optic neuropathy, reversible or irreversible eye blindness, hepatotoxicitypruritus, joint pain
Streptomycin	Streptobrettin (Norbrook)	1 g daily, IM/IV	IM/IV	Inhibits protein synthesis by binding tightly to the A site of 16S rRNA in 30S of RNA	Neurotoxicity, optic nerve dysfunctioning, peripheral neuritis, nephrotoxicity and paraesthesia
Rifabutin	Ansamycin	300mg daily tablet, 150 capsule twice	Oral	Same as Rifampicin	Same as Rifampicin
Second-line drugs					
Ciprofloxacin	CIPRO	250-500 mg Tablet/suspension	Oral	Inhibits ATP dependent DNA gyrase inhibitor (topoisomerase II and IV)	GIT and CNS disturbances
Ofloxacin	Floxin	800 mg daily/tablet	Oral	Inhibits ATP dependent DNA gyrase inhibitor (topoisomerase II and IV)	GIT and CNS disturbances
Sparfloxacin	Zagam	200 mg tablet	Oral	Inhibits ATP dependent DNA gyrase inhibitor (topoisomerase II and IV)	GIT and CNS disturbances
Levofloxacin	Cravit (Daiichi)	500-1000mg daily/tablet	Oral	Inhibits ATP dependent DNA gyrase inhibitor (topoisomerase II and IV)	GIT and CNS disturbances, hypersensitivity, tendon disorders
Moxifloxacin	Actimax (sankyo)	400 mg tablet daily	Oral /IV	Inhibits ATP dependent DNA gyrase inhibitor (topoisomerase II and IV)	GIT and CNS disturbances, prolong QT interval, blood disorder, arthralgea,skin, urogenital disorder
Gatifloxacin	Zymar (allergan)	400 mg daily/tablet	Oral	Inhibits ATP dependent DNA gyrase inhibitor (topoisomerase II and IV)	GIT and CNS disturbances but less than other quinolones
Amikacin	Lukadin (San carlo)	1 g daily	IV/IM	Inhibits protein synthesis by binding to A site of 16S rRNA in the 30 S ribosomal subunit	Hearing loss, nephrotoxicity, neurotoxicity, headache etc
Kanamycin	Klebsil	1 g vial daily	IV/IM	Inhibits protein synthesis by binding to A site of 16S rRNA in the 30 S ribosomal subunit	Hearing loss, nephrotoxicity, neurotoxicity, headache etc

Table (4) contd….

ATD Name	Brand Name	Dose/Dosage Form	ROA	MOA	Adverse Drug Reaction
Capreomycin	Capastat	1 g vial daily	IV/IM	Inhibits protein synthesis by binding to A site of 16S rRNA in the 30 S ribosomal subunit	Hearing loss, nephrotoxicity, neurotoxicity, headcah etc
Ethionamide	Tractor (wyeth)	750 mg daily/tablet	Oral	Enoyl ACP reductase inhibitor	Gastric and nausea vomitting
Cycloserin	Farmaserina (Farmatalia)	500-750 mg daily-tablet	Oral	Inhibits alanine racemase resulting in cell wall synthesis inhibition	Neurotoxicity, convulsion and slurred speech and paralysis
P-aminosalicylic acid	PASER (Jacobus)	8-12 g daily/tablet	Oral	Inhibits dihydropteroate synthase involved in protein sysnthesis	Contraindicated in renal disorder, thyroid disfunctioning, dermatological side effects and lymphadenopathy
Thiacetazone	Thiazina	150 mg daily	Oral	Mycolic acid synthesis inhibition	Cutaneous reactions
Third-line drugs					
Linezolid	Zyvoxid (Pfizer)	600 mg drug every 12 hrs	Oral / IV	Inhibits protein synthesis by binding to 23S RNAs and preventing the proper binding of formyl methionone tRNA	GIT headache and nausea
Clofazimine	Lampren (Novartis)	50 mg	1-2 tablet daily	Inhibits cell wall synthesis by interfering electron transport system	Gastrointestinal toxicity, reddish black skin reversible skin discoloration, eye pigmentation, CNS depression
Clarithromycin	Biaxin (Abbott)	250-1000 mg daily	Tablet or suspension	Inhibits protein synthesis by binding to 50S ribosomal subunit	Gastro-intestinal disturbances
Amoxicillin	Amoxil	250-500 mg thrice a day tablet	Oral	Inhibits cross linking of peptidoglycan in cell wall synthesis of bacteria	Gastro-intestinal disturbances, Reddish black reversible skin discoloration, eye pigmentation, urine discoloration, eye irritation
Linezolid	Zyvoxid (Pfizer)	600 mg every 12 hr/ tablet or injection	Oral / IV	Inhibition of protein synthesis	Diarrhoea, nausea and vomiting
Miscellaneous drugs					
LL-3858	Sudoterb (lupin)	No data	-	Not known	Not known
OPC 67683	No data	Patent filed by Otsuka in 2003	No data	Inhibition of cell wall lipid and mycolic acid syntheses	Not known
PA 824	No data	Not approved for human use	No data	Inhibits protein and cell wall synthesis *via* generating free radical as toxic to *mycobacterium*	Not known
Prothionamide	No data	500-750 mg daily/tablet	Oral	Enoyl ACP reductase inhibitor	Dose related GIT disturbances, anorexia, CNS disturbances, peripheral and optic neuropathy, hepatitis
Rifapentin	Priftin (Aventis)	600 mg twice weekly	Oral	Same as Rifampicin	Hepatitis, CVS, CNS, GIT and respiratory problems
Rifalazil	KRM 1648	Not data	NA	Same as Rifampicin	Flu like syndrome and same as Rifampicin
SQ 109	IPR Sequella	Not data	NA	Inhibition of cell wall synthesis	Not known
TMC 207	NA	Oral solution	Oral	Inhibition of ATP synthesis	Not known

Reference: Global Alliance for TB Drug Development. Handbook of Anti-Tuberculosis Agents Tuberculosis 88 (2): (2008) 85-170.

Table 5. Review of anti-tubercular drugs with their informative parameters.

Drugs	Microbial Activity	MIC[††] (µg/ml)	Solubility[‡] (mg/ml)	Log P	pKa
First-line drugs					
Rifampicin	Bactericidal	0.25	1-2	3.719	1.7 and 7.9[a]
Isoniazid	Both[†]	0.025	125	-0.64	1.8 at 20°C
Pyrazinamide	Bactericidal	6-50	14	-1.884	0.5
Ethambutol	Both[*]	0.5	100	-0.3	6.6 and 9.5
Streptomycin	Bacteriostatic	1.0	> 20	-6.4	10.88
Rifabutin	Bactericidal	0.015	0.19	4.1	7.93
Second-line drugs					
Ciprofloxacin	Bactericidal	0.5	Water soluble	0.28	6.09
Ofloxacin	Bactericidal	0.71	28.3	-0.39	5.45
Sparfoxacin	Bactericidal	NA	0.113	2.5	5.75
Levofloxacin	Bactericidal	0.5	Sparingly soluble	2.1	5.45
Moxifloxacin	Bactericidal	0.5	60 at pH 4	2.9	5.6 (acidic)
Gatifloxacin	Bactericidal	0.25	40-60 at pH 2-5	2.6	5.6 (acidic)
Amikacin	Bactericidal	0.5-1.0	Water soluble	-7.4	12.1 (acidic)
Kanamycin	Bactericidal	2.0	Water soluble	-6.3	7.2
Capreomycin	Bactericidal	2.0	Water soluble	-9.609	6.2-13
Ethionamide	Bactericidal	0.25	0.1	0.5	11.89 (acidic)
Cycloserin	Both	25.0	100	-2.3	4.5
Terizidone	-	NA	Water soluble	-0.85	-
P-aminosalicylic acid	-	0.3-1	1690	0.89	2.06
Thioacetazone	Bacteriostatic	-	-	0.9	-
Linezolid	-	0.25	3	0.9	0.61
Clofazimine	Bacteriostatic	0.1	0.01	7.66	8.51
Clarithromycin	Bactericidal	8	0.000333	3.16	8.99 (25°C)
Amoxicillin	-	NA	3430	0.89	2.4 (carboxy)
New drugs					
LL-3858	Bactericidal	0.12-0.025	NA	NA	NA
OPC 67683	Bactericidal	0.12	NA	NA	NA
PA 824	Bactericidal	0.15-0.3	NA	3.393	NA
Prothionamide	Bactericidal	6.25-12.5	Insoluble	NA	NA
Rifapentin	Bactericidal	NA	NA	4	7.01
Rifalazil	Bactericidal	0.015	>2g/ml at pH 2	6.3	8.99
SQ 109	Bactericidal	NA	-	6.45	NA
TMC 207	Bactericidal	0.5	NA	NA	NA

[*]Bacteriostatic at low dose but bactericidal at high dose (25 mg/kg), [†]Bacteriostatic for resting and bactericidal for rapidly dividing bacilli, [††]MIC *in vitro* against H37Rv [‡]Aqueous solubility, [a]1.7 for the 4-hydroxy and pKa 7.9 for the 3-piperazine nitrogen.

drug delivery have been invovled into anti-TB drugs drug delivery to improve therapeutic effectiveness by predicting, monitoring and controlling the drug delivery. The oral route of administration provides great comfort and compliance to the patient in dosing. Finding new ways to deliver anti-tubercular drugs in oral forms, delivering the multiple doses, inexpensive long term therapy, potent and form with improved bioavailability are still challengeable for formulation scientist.

As discussed earlier that conventional chemotherapy is complicated with the need of multi-drug regimen, lengthy treatment protocol likely to cause several side effect, poor patient compliance and poor improvement in patient condition particularly in term of parenteral drug administration and patient adherence to dosage regimen. To minimize drug related toxicities and enhance patient compliance, several extensive efforts have been made to develop novel drug delivery carriers like nanoparticles, microparticles, vesicular systems (liposome and niosome), inhalation dry microparticles, solid lipid nanoparticles and nanoemulsion using safe polymers and lipid. These systems have been developed to

either target the replicative niche of *MTb* or minimizing dose frequency which forms the basis for therapeutic strategies to improve patient compliance [43, 44]. Ligand (using mannose as a ligand) based carrier modification for targeting the alveolar macrophage had been reported to achieve high drug concentration in the infected phagosome [45]. Conventional therapy results in severe dose-related toxicities owing to multiple dosing. Hence, these novel nanocarrier systems have emerged as an alternative to the convention drug delivery system to solve these issues. Nanotechnology rationale based drug delivery might improve drug efficacy and attain high levels of patient adherence by resolving technical limitations like poor bioavailability, cellular and anatomical structural barrier and drug resistance [46].

On reviewing drug interaction on cellular level, newly developed antibiotics are still facing difficulty to enter into the infected cell in order to attain high drug concentration within the infected phagosome [47]. Thus, problems in delivering free drugs within the cell have led to investigations of improved drug carriers for treating intracellular residing pathogen loaded with antibiotics into vesicular liposomes, polymeric nanoparticles, nanocomplexs, solid lipid nanoparticles and polymeric microparticles [48]. Lipid and polymer based drug delivery are more suitable as carrier for the delivery of anti-tubercular drugs because of providing high drug entrapment efficiency, higher drug loading capacity, means of sustained and controlled drug delivery, safe and effective carrier systems. In addition, these carriers also save the therapeutic agents from endogenous enzymatic drug degradation and synergize with cellular bactericidal mechanisms to improve the intracellular drug efficacy. Absence of new anti-tubercular drug in the market till date explains the acute scarcity of *in vivo* effcicacy data for large scale industrial production. However, the development of new drug delivery may be representative of the feasibility of route of administration, efficacious and promising alternative in TB chemotherapy. The ultimate goal of this review is to highlight the recent advances and updates in novel anti-tubercular drug delivery approaches in the prevention tuberculosis which may enhance therapeutic efficacy and patient compliances.

5.2. Vesicular Carrier Systems: Liposomes and Niosomes

Liposomes and niososmes as vesicular systems have been reportd to deliver ATDs by several authors. Both systems are composed of lipid bilayer and are capable of entrapping hydrophilic as well as lipophilic drugs successfully with maximum entrapment efficiency. Niosome is slightly different from liposome being composed of non-ionic surfactant and phospholipid. Vesicular systems as carrier have great potential ability for drug delivery owing to its biocompatibility and biodegradability. However, the novel liposomes systems suffer with the lack of reproducibility of the preparation and the limited stability during storage due to changes by drug-carrier complex [49]. In Table **7**, we have explained briefly the major findings of several ATDs that have been developed for the modified release and have also been demonstrated for improved chemotherapeutic efficacy after investigating in animal models (*e.g*. mice) [50]. Liposomal streptomycin and gentamycin were investigated in liposomal injection with significantly reduced acute toxicity in mic. Moreover, bacterium counts were profoundly reduced in the organs (lungs, spleen and liver) in infected mice when treated with the liposomal formulation [50, 51]. Leitzke *et al*. incorporated amikacin into lectin coated liposome to treat infected mice on weekly basis that could lead to significant antibacterial activity by reducing bacterium count in tissue and enhanced survival time [52]. Similarly, capreomycin was studied by two different authors using same DSPC, DPPC and HPC liposomal composition. They investigated that DSPC based liposome were found to be within narrow size distribution even less than 200 nm that offered suitability for inhalation formulation. Another authors explored that freeze thaw and surface response methodology was more effective and promising methodology to improve the drug content [53, 54]. Sparfloxacin (Flouroquinolone) was investigated for reduction in growth rate index using liposome loaded sparfloxacin. There were reduced growth index by 25 and 30% that of free drug and encapsulated respectively when 6mg/ml dose was administered [55]. DPPC was modeled as lung surfactant (monolayer) to design liposome for RIF and INH delivery. INH and DPPC in 1:1 ratio by weight significantly reduced surface tension to zero and improved adsorption properties. Similarly, drugs combination of these three (RIF, INH, PZA) with the DPPC weight by weight ratio improved similar effects. Moreover, enhanced stability (at 4°C) and accumulation into macrophage as dual role of the formulation were investigated because of antiatelectatic effect of surfact [56, 57]. PZA was tried using DPPC and significantly high reduction in *Mycobacterium* load was attained when the infected mice were treated with liposomal formulation twice per week in seven doses as compared to free drug solution in saline buffer dosed six days weekly in total of 18 days (25mg/kg) [58]. RIF loaded in niosomes was localized in the lungs up to 65% using lipophilic surfactant span 85 and intrathoracic administration showed 145 folds higher RIF accumulation in the lungs as compared to free drugs. In addition, IV administration led to attain substantially higher concentration in the lungs, liver and spleen [59, 60]. High drug (RIF, INH and PZA) entrapment, enhanced *in vitro* drug release and high drug recovery from the site of action by surface coating/modification of liposome with mannan proved as suitable for DPI formulation [61]. Recently, one author delineated the use of biocompatible surfactant Tyloxapol in niosome preparation for RIF, INH and PZA to achieve low size range (150 nm), highly stable, significantly improved dissolution rate and high drug entrapment efficiency without having any drug carrier interaction [62].

Active targeting of liposome to the site of action to minimize drug associated side effects and reduce dose have also been investigated using various ligands. Lung specific stealth liposome was earlier used to target specific drug delivery of anti-tubercular drugs with liposome containing o-stereoylamylopectin (O-SAP) and monosialogangliosides/ distereoylphosphotidyethanolamine-polyethylene glycol [DSPE-PEG] as targeting molecules for site specific delivery of RIF and INH with significantly reduced hepatotoxicty as compared to free drugs. It was found that 31% nanocarrier accumulation was obtained than conventional liposome (only 3.1%) with reduced *in vivo* toxicity in infected mice in 30 min. Furthermore, Sub-therapeutic doses (4mg/kg for INH and 3mg/kg body weight for RIF, respectively) led to reduced CFU value indicating significant anti-tubercular activ-

ity of liposome prepared with surface modified ligand [63]. Thus, therapeutic efficacy of free as well as INH and RIF loaded formulation were evaluated in both therapeutic and sub-therapeutic dose. Later, sufficient reduction in CFU value was observed in both the cases [64]. Vyas *et al*. evaluated selective targeting of RIF loaded aerosolized liposome to alveolar macrophages by adding negative charge (using Dicetyphosphate, DCP) or coated with alveolar macrophage specific ligand (O-SAP) and maleylated bovine serum albumin (MBSA). *In vitro* airway penetration efficiency was attained 1.5 to 1.8 fold higher than free drugs. Moreover, the *in vitro* viability of *M. smegmatis* inside macrophage was only 7-11% with ligand attached liposome aerosol formulation whereas 45.7% and 31.6% were found with free drugs and plain liposome carrier respectively. Both negatively charged and anchored liposome found to have high drug accumulation in the lungs after 24 hrs. After 6 hrs, drug localization index was calculated about 1.4 and 3.5 times in both anchored formulation as compared to negatively charged and plain liposome formulation respectively. This result indicated that anchored liposomes are highly effective and rapidly deposited in the lungs followed with maintained high drug amount over prolonged period of time in the lungs [65]. Another second-line anti-tubercular drug ciprofloxacin (CIP) was delivered by surface modification to target the alveolar macrophage and mannosylated CIP-liposomes with 4-minophenyl-a-D-mannopyranoside were prepared and pulmonary inhalation was diagnosed in rats. The targeting efficiency of CIP to alveolar macrophage of mannosylated CIP-liposomes was significantly greater than that of unmodified CIP-liposomes [45]. Drug loaded ligand appended with mannan coating on the liposome loaded with three frontline ATDs and their dry powder inhalation (DPI) were evaluated for better entrapment efficiency, flow property and drug release from formulation and drug recovered from site of target. This study justified for improved drug delivery of anti-tubercular drugs [61]. Additional reports tabulated in Table **6** explain very briefly the major findings of the study [66-73].

Based on the above results of the studies, it can be concluded that vesicular carriers (liposome, anchored liposome and niosome) were more significantly effective in reducing *Mycobacterium* load in the lungs and other major organs than the free drugs or combination of free drugs ininhalation form. Drug induced toxicity was reduced during the treatment and high drug concentration was maintained in the alveolar macrophage over prolonged period of time. Ultimately, the vesicular mediated TB treatment might be a promising approach as safe, cost effective, efficient drug efficacy and with high level of patient compliance.

5.3. Nanoparticles and Microparticles as Carrier for ATDs

Nanoparticles are in the size range of 1 to 100 nm. Both polymeric and non-polymeric nanoparticle (NP) and microparticles (MP) have been used in delivering ATDs. NP and MP based drug delivery have considerable role in TB treatment. Several natural polymer carriers such as biocompatible hydrophilic gelatin, alginate, hyaluronic acid, carboxymethylcellulose and biodegradable PLGA have been exploited as nanocarrier to deliver drugs with high drug loading capacity, stability, with feasibility of incorporating drugs and the feasibility of variables route of administration (IV or inhalation). Polymer based drug delivery can be designed for slow, sustained and controlled release of drug from the matrix. Several authors have formulated nanoparticles and microparticle using polymer as shown in Table **6**. These systems are rapidly recognized by host immune system and are quickly removed from the host body by opsonization and cell phagocytosis process. To avoid recognization by host immune system, surface modification using hydrophilic moity (*e.g*. polyethylene glycol) on nanoparticles carrier system are carried out. This prolongs the systemic circulation time of the NPs. Hence, these systems have been most extensively used in the delivery of the anti-tubercular drugs.

Anisimova *et al*. investigated encapsulation of INH, Streptomycin and RIF in PBCA Poly(butylcyanoacrylate) and PIBCA Poly(isobutylcyanoacrylate) with 4-8, 7 and 22-25 times higher accumulation intracellularlly than extracellularly as compared to free drugs in human blood monocyte *in vitro* working as depot. Moreover, intracellular activity was found to have lesser than extracellular [74]. Similarly, ciprofloxacin for increased pharmacokinetics parameters (AUC, $T_{1/2}$, and V_d) was studied [75]. Moxifloxacin was entrapped in PIBCA and PBCA polymer. After injecting into macrophage, moxifloxacin led to three folds accumulation of nanoparticle into the macrophage efficiently and six times longer detected as compared to free drug. In addition, 0.1μg/ml formulation inhibited bacterial growth and same effect was obtained with 1μg/ml of free drug [76]. It is also reported that moxifloxacin NP was more toxic with 2-3 folds enhanced cellular uptake and IV administration into the mice showed significant antimycobacterium activity [75, 77, 78]. Microencapsulation of pharmaceutical anti-tubercular drugs in biodegradable polymer PLGA was used to deliver in sustained manner of INH from a single dose injectable PLGA microparticle as therapeutic approach to treat TB with controlled release of drug at therapeutic levels [79]. Again, INH and RIF were successfully encapsulated in PLGA polymers and administered subcutaneously to provide sustain release of drug over 6-7 weeks in tested mice having particle size ranging from 11.75μm to 71.95μm [80, 81]. Previously same author studied that subcutaneous injection of the same formulation in size range of $^{>}$ 10μm was found to retain at the site of injection and worked as depot. Slow, sustained and controlled release of the drug from the microparticles by diffusion mechanism was found over extended several months degrading the entire polymeric microparticles. Doan *et al*. reported that when RIF was complexed with hydroxypropyl β-cyclodextrin and RIF loaded PLGA microsphere showed slow and fast release microsphere were prepared. After IV administration of RIF and RIF hydroxylpropyl β-cyclodextrin nebulization, plasma profile and lung Epithelium Lining Fluid (ELF) profile were superimposed respectively indicating that RIF diffused almost instantaneously through broncho-alveolar barrier. PK model predicted that ELF fluid contains RIF concentration much higher after microsphere administration than IV or nebulization over prolonged period of time [82]. However, injecting subcutaneously, IV administration, the pain and the discomfort allied with this polymeric microparticle are not compliant to the patient. Therefore, there is need to develop patient friendly carrier system.

As concerns with these issues, Ain *et al*. reported oral administarotion of PLG based INH significantly retained the drug in plasma up to 72 h. The encapsulated microparticle contained drug higher than its MIC value (0.1µg/ml) followed with improved pharmacokinetics parameters such as Cmax, AUC and $T_{1/2}$ [83]. Sharma *et al*. studied that by incorporating three front-line anti-tubercular drugs INH, RIF and PZA in PLG based nanoparticles. Oral administration to *MTb* infected guinea pigs at every 10^{th} day and after 5 oral doses of treatment, no MTb bacilli were found in the tissue as similar effect as of did by 46 days adiminstration of conventional drug form. Moreover, a single oral sub-therapeutic dose (2/3th of therapeutic dose) resulted in sustained release in the plasma up to 7-12 days and 11-14 days in the organs with pronounced mean residence time and significant improvement in bioavailability [84]. Oral administration of all four ATDs (RIF, INH, PZA and ETB) encapsulated in PLGA at every 10^{th} day (5 doses) completely cleared bacilli from meninges after delivery to the brain. The author also reported that oral administration of same four drugs in same carrier led to maintained drug level in the various tissues up to 9-10 days without revealing any adverse reaction even after administration of several folds higher than therapeutic doses. But conventional dosage form at equivalent drug concentration could lead to severe side effects. These combination reduced frequency of dosing and shortened the duration of treatment [85, 86]. Again four drugs were investigated in alginate based nanoparticle and oral administration significantly retained the drug in the plasma up to 7, 9, 11 and 11 days for ETB, RIF, INH and PZA drug respectively [87]. Gelatin is the natural, safe, biodegradable and biocompatible nanocarrier system as mentioned earlier in this article. Hence, several authors tried to deliver RIF (gelatin B, 52-83% EE) and INH in gelatin (mannose anchored targeted delivery) based drug delivery with high encapsulation efficiency and targeted drug delivery [88, 89]. Method was entrapped in PLGA nanoparticle for oral delivery. Two different doses of NP after oral administration to mice led to sustained release of the drug in the plasma for 6 days of NP and 6h for free drugs. In addition, the drug was detected in the lungs, liver and spleen up to 5-7 days in case of NP and only 12 h for free drug. Significant improvement in pharmacokinetic parameters were exhibited and dose proportional increase in PK values showed that the work had a great potential in reducing dosing frequency and toxicities [90]. A combination PLGA based microsphere in alginate in situ gel was prepared by complexation to get combination drug delivery. The *in vitro* drug release from combination microsphere (microsphere in alginate *in situ* gel) was slower than microsphere alone. The percent cumulative drug release was found to be 91.83±1.2% for combination delivery system and 97.36±3.41% of rifampicin from microsphere. *In vivo* fluorescence microscopy suggested that gel adhered to lungs within 24 h and microsphere remained atleast for 504 h (21 days). After 21 days, local concentration was above minimum inhibitory concentration of rifampicin [91]. Hence, novel combinational drug delivery was supposed as significant for sustained drug delivery of drug in the treatment of tuberculosis. This suggests us that oral administration of PLG nanoparticle was helpful in reduction of dosing frequency and better patient compliance.

Despite the potential utilization of oral drug delivery, certain limitations need to be addressed such as first pass metabolism and low drug deposition into the lungs. Therefore, another inhalation approach of the anti-tubercular drug delivery could be considered as an emerging new drug delivery system for high drug effectiveness by targeting the infection reservoir in the lungs. RIF, INH and PZA encapsulated in PLG nanoparticle was administered after nebulization and detected in the plasma after 6 h and the therapeutic concentration was observed up to 6 days for RIF and 8 days for other two drugs. After nebulization at every 10^{th} day (5 doses) with the developed nanoparticles to the infected guinea pigs cleared bacilli from the lungs which required total 46 days of treatment using conventional formulation with equivalent dose [92]. Suarez *et al*. reported the RIF loading into the biodegradable PLG to treat animal model. Single and double insufflations of drug reduced significantly viable count of bacilli from tissues. Additionally, it was found that excellent reduction inflammation was observed after 28 days when treated with nanoparticle as compared to free drugs [93]. A comparative study was conducted on PLG based NP loaded with RIF, INH and PZA to evaluate the efficacy of NP. NP was administered in aerosol form at every 10^{th} day versus daily administration of free drug (aerosol) in *M. tuberculosis* infected guinea pigs that improved histopathological condition and substantially reduced bacteria counts [94]. *In vivo* uptake of PLG microsphere loaded with RIF in mannitol get enhanced in the lungs after inhalation of drug entrapped microparticle by alveolar macrophage. Furthermore, RIF and INH microparticle showed increased bactericidal activity [95, 96]. Second-line ofloxacin was studied by formulating a complex with palladium and then encapsulated into the PLGA microparticle. It was evaluated for better size distribution, aerodynamic diameter (2.5 µm) and entrapment efficiency (30%). Slow and sustained *in vitro* drug release from complexed PLG microparticle in pH 7.4 suggested a potential delivery to the alveolar macrophage in the lungs [97]. Other recent updates are tabulated in Table **6** and Table **7** [98-154].

5.4. Solid Lipid Nanoparticles (SLN) for Delivery of ATDs

SLN has a promising role to deliver ATDs by employing solid lipid being solid at room temperature. The carrier combines the virtue of conventional polymeric nanoparticles and eliminating their demerits. High drug loading, more stable and significant bioavailability attracted the formulation scientists to deliver these challengeable drugs. Pandey *et al*. investigated to find the potential effect of solid lipid particle (stearic acid) loaded with RIF, INH and PZA after oral delivery in the TB management. Single oral dose to guinea pigs showed that therapeutic drug concentration in plasma was found up to 8 days, 10 days in organs (lungs, spleen and liver) and whereas drug solution was eliminated within 1-2 days. Furthermore, pharmacokinetic parameters like mean residence time and drug bioavailability were increased several fold higher than free drugs. Nebulization of solid lipid particle to *MTb* infected guinea pigs at every 7^{th} day, was found that no bacilli were observed after 7 doses of treatment in the lungs and spleen whereas 46 daily doses of drug

Table 6. Novel carriers employed for delivery of anti-tubercular drugs.

A Synopsis on Novel Drug Delivery System Used in the Treatment of Tuberculosis					
A Synopsis of Novel Drug Delivery System Loaded with ATD					
Carriers	**Polymer/Lipids**	**Drugs**	**Major Findings**	**Year**	**Refs.**
Liposomes	Lecithin	Streptomycin	Reduced *Mycobacterium* count in spleen of infected mice when injected with liposomal streptomycin and prolonged mouse survival with low acute toxicity.	1982	[50]
	PC	Gentamycin	Significant reduction of bacterium count in both spleen and liver. Moreover, dose related reduction were also achieved without sterilization.	1990	[51]
	PC and PG	Sparfloxacn	Reported reduction in growth index to 25 and 30% of that of untreated control when treated with free and encapsulated drug respectively (6 mg/ml).	1996	[55]
	PC and Diacetylphos-phate	INH, RIF	On ligand binding to the liposome surface, drug targeting significantly reduced hepatotoxicity. Interestingly, 31% nanocarrier with PEGylated system containing O-SAP get accumulated in the lung as compared to conventional liposomes (3.1%).	1997	[63]
	PC, CHOL, Diacetyphos-phate	INH, RIF	Sub-therapeutic doses (4mg/kg for INH and 3mg/kg body weight, respectively) led to reduced CFU value indicating significant anti-tubercular activity of liposome prepared with surface modified ligand.	1997	[64]
	Lecithin	Amikacin	Significant reduction in bacterial count in tissues even after treatment with once weekly or once monthly basis of liposomal amikacin in mice with extended survival time.	1998	[52]
	PC	Clofazimine	Reduced *in vitro* and *in vivo* toxicity of clofazimine encapsulated in liposome. In addition enhanced anti-tubercular activity in both acute and chronic model. Also, total clearance of bacteria from chronically infected mice has been achieved on treating with encapsulated formulation (particularly from liver and spleen).	1999	[66]
	PC, DPSE, PEG	INH, RIF	Significant reduction in *Mycobacterium* load in lung, liver and spleen were obtained from infected mice when treated with liposomal drug delivery of INH and RIF together on once weekly basis for six weeks.	2002	[67]
	DSPC, DPPC, HPC	Capreomycin	DSPC (Distereoyl phosphotidylcholine)based liposome was within narrow size distribution even less than 200 nm offered suitability for inhalation formulation.	2003	[53]
	PC	RIF	O-SAP coated liposome were found to be preferentially more effective and accumulated in alveolar macrophage cell than MBSA ligand anchored liposome.	2004	[65]
	PC, CHOL	RIF, INH	Aerodynamic characterization on a 7 stage Andersen Cascade Impactor showed that 94% of the generated aerosols were in the respirable range (≤6 μm) with a mass median aerodynamic diameter of 0.96 ± 0.06 μ and geometric standard deviation of 2.3 ± 0.4 μ, suitable for bronchoalveolar drug delivery in guinea pigs.	2004	[68]
	DPPC	INH, RIF, ETB	Evaluation of effects of anti-tubercular drug (s) on exogenous lung surfactant (modeled DPPC monolayer) for its surface properties and acts as pulmonary drug delivery agent. INH and DPPC in 1:1 ratio by weight significantly reduced surface tension to zero and improved adsorption properties. Similarly, drugs combination of these three with the DPPC weight by weight ratio improved similar effects.	2005	[57]
	DSPC, DPPC, HPC	Capreomycin	Freeze thaw and surface response methodology were used to improve the drug content.	2006	[54]
	DPPC, CHOL,DPC	PZA	Significantly high reduction in *Mycobacterium* load was attained when the infected mice were treated with liposomal formulation twice per week in seven doses as compared to free drug solution in saline buffer dosed six days weekly in total of 18 days (25mg/kg).	2007	[58]
	PC, DPPC	RIF, INH, PZA, ETB	Formulation was biocompatible and stable even after storage for a month at 4°C. anti-tubercular drug loaded liposome served as dual role as alveolar stabilization due to surfactant action and better accumulation of these due to antiatelectatic effect of the surfactant.	2007	[56]

Table (6) contd….

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Carriers	**Polymer/Lipids**	**Drugs**	**Major Findings**	**Year**	**Refs.**
	HSPC, DOPE	4-aminophenylα-d annopyranoside	Mannosylated liposome was administered through pulmonary rout in the rat and a profound increase in uptake by lung was obtained as targeted drug delivery.	2007	[69]
	DPPC, DPPG	RIB	RIF free drug and encapsulated in DPPC and DPPG made liposome were explored in non-infected mice for its biodistribution. Over all result suggested that more efficient drug delivery to liver, lung and spleen were obtained with liposome prepared with phospholipid with high Tc. Additionally more effective delivery of DPPC: DPPG liposome were found to these organs.	2008	[70]
	HSPC, DOPC	Ciprofloxacin	Drug targeting with surface modified liposome with mannosylated ligand caused significant increase in accumulation by alveolar macrophage cells than unmodified. In addition, PK/PD data suggested that these modified formulation are more potent antibacterial activity than unmodified against several strain.	2008	[45]
	Mannan , PC, Cholesterol	RIF, INH, PYZ	Drug loaded ligand appended liposome and their dry powder inhalation (**DPI**) were evaluated for better entrapment efficiency, flow property and drug release from formulation and drug recovered from site of target. This study justified for improved drug delivery of anti-tubercular drugs.	2013	[61]
Niosome	Span 85	RIF	Drug was localized in the lungs up to 65% just by adjusting size of the niosome formulation.	1995	[59]
	-	RIF	A substantially higher drug concentration was attained in lung, liver, kidney and blood plasma when administered through **IV route**. Interestingly 145 times higher accumulation of RIF loaded niosome were obtained in the lungs as compared to free drugs after intrathoracic administration.	2004	[60]
	Span (20-80, 85)	RIF	Significantly higher concentration of drug was attained in thoracic lymph nodes.	2006	[71]
	Span 20, span 80	RIF	Niosome was prepared by reverse phase evaporation method and charged by dicetyl phosphate. They investigated that it was remained in the site of action for prolonged period of time and was capable of maintaining the drug concentration in the therapeutic concentration.	2010	[72]
	Tyloxapol	RIF, INH, PZA	Non ionic liposome was prepared using biocompatible tyloxapol surfactant to encapsulate ATDs (RIF, INH and PZA). The vesicle size was optimum (150nm) and high drug loading efficiency above 90% that might be a promising delivery system fot TB treatment.	2013	[62]
Microparticles and nanoparticles	PLG	RIF	Bioassay assessment of cell culture supernatants from monocyte cell line showed extended drug release over period of seven days. The released drug level was within monocyte was more effective than free drug solution.	1998	[73]
	PIBCA	Ciprofloxacin	Increased values of AUC, $T_{1/2}$ and Vd were achieved by formulating in nanoparticle formulation of ciprofloxacin than free drugs and enhanced activity was obtained against M. avium complex.	1998	[75]
	PBCA, PIBCA	RIF, INH, Streptomycin	Encapsulated INH, Streptomycin and RIF reported enhanced 4-8, 7 and 22-25 times higher accumulation intracellularlly than extracellularly as compared to free drugs. Moreover, intrcellular activity were found lesser than extracellular.	2000	[74]
	PLG	RIF, INH, PZA	Biodegradable polymer based nanoparticle prepared for nebulization showed plasma detectable concentration after 6 hrs and therapeutic drug concentrations were detected until 6 days for RIF and days 8 for both INH and PZA. Infected guinea pigs were nebulized with nanoparticles at every 10^{th} days and no tubercle bacilli were detected in the lung after only five doses of treatment whereas 46 daily doses are required to get the same therapeutic concentration.	2000	[92]

Table (6) contd….

A Synopsis on Novel Drug Delivery System Used in the Treatment of Tuberculosis					
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Carriers	**Polymer/Lipids**	**Drugs**	**Major Findings**	**Year**	**Refs.**
	PLG	INH	A comparative study between porous, nonporous and hardened microparticle suggested that drug release from hardened PLG microparticle was sustained release profile up to 7 weeks than porous and nonporous (up to 2 days only in plasma).	2001a	[79]
	PLGA	RIF	Reported animal treated with biodegradable PLGA based microparticle loaded with RIF. It was found that animal treated with formulation led significant reduction in bacteria viable count when treated with single and double dose (**insufflation**) as compared with free RIF. Additionally reduction in inflammation and lung damage were observed than free drug after 28 days post infection.	2001	[93]
	PLG,	RIF, INH	PLG microparticle of RIF and INH formulation were administered in experimental murine model of TB as single dose that led to sustained release of both drugs up to 7 and 6 weeks respectively. Furthermore combination both free drugs **was injected** in same dose that was detected *in vivo* up to 24 hr only. Single dose was enough to clear bacteria more effectively from lungs and liver in the same model.	2001b	[80]
	PLG	INH, RIF	Intracellular concentration resulting from particle **inhalation** were found to be significantly higher than vesicular delivery of soluble drugs.	2001	[98]
	PLGA	INH, RIF, PZA,ETB	Drugs remained in the blood circulation up to 72 hr after **oral administration**. Moreover, INH levels encapsulated in PLG microparticles were found to be higher than its MIC value (0.1μg/ml). Encapsulation also increased pharmacokinetics parameters (Cmax, AUC, $T_{1/2}$).	2002	[83]
	PLGA	INH,RIF, PZA	These three drugs were encapsulated into the PLGA nanoparticle that led to sustained release of the drugs up to 9 days and therapeutic concentration in tissues were detected up to 9-11 days after **oral administration**. 50 days administration of these formulation to mice substantially cleared bacteria from different organs.	2003b	[99]
	PLG	RIF, INH, PZA	Only a single **subcutaneous dose** of microparticle was sufficient to maintain drug plasma, lungs and spleen over more than one month. In addition chemotherapeutic efficacy was found to be more efficient than daily dose of free drugs leading to clearance of bacterial count from several organs.	2004	[100]
	PLG	RIF, INH, PZA	PLG microparticle ligant bound with wheat germ agglutinin loaded with these three drugs and three doses were administered fore-nightly for 45 days which was able to produce sterilizing effects in lungs and spleen.	2004	[84]
	PLG	RIF, INH, PZA	Studied to evaluate chemotherapeutic efficacy of these three anti-tubercular drugs loaded in PLG NP at sub-therapeutic dose ($2/3^{rd}$ of therapeutic dose) by administering **orally** to guinea pigs. Single dose given resulted into sustained release of the drug for 7-12 days in plasma and 11-14 days in the organs with pronounced improvement in mean residence time and bioavailability. Oral administration of this every 10 day (5 doses) was able to clear bacilli from major organs from *M. tuberculosis* infected guinea pigs with similar effect of as did by 46 day treatment with conventional formulation.	2004	[101]
	Stearic acid	RIF, INH, PZA	Three drugs incorporated in stearic acid based microparticle and a single dose of this formulation was **orally** administered and therapeutic concentration in plasma was observed up to 8 days, 10 days in liver, spleen and lungs whereas free drugs get cleared within 1-2 days.	2005	[102]
	PLG	INH, RIF, PZA	A comparative study suggested that the efficacy of NP capsulated drugs administered every 10 days versus that of daily free drugs in aerosol form against *M. tuberculosis* infected guinea pigs improved histopathological condition and substantially reduced bacterial counts.	2005	[94]
	Alginate	INH, RIF, PZA	Inhalational delivery of these drugs showed drug concentration above MIC value in the spleen, lung and liver up to 15 days as compared to free drugs (one day only).	2005	[103]

Table (6) contd….

A Synopsis on Novel Drug Delivery System Used in the Treatment of Tuberculosis					
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Carriers	**Polymer/Lipids**	**Drugs**	**Major Findings**	**Year**	**Refs.**
	PLGA	INH, RIF, PZA, ETB	It has been reported that after **oral** administration of formulation the therapeutic level of the drug was maintained 4-5 days in blood and 9 days in plasma. One administration in every 10^{th} days (5 doses) cleared bacteria from meninges.	2006	[85]
	PLGA	INH, RIF, PZA, ETB	These combination reduced the dosing frequency and shortened the duration of the treatment.	2006	[86]
	Alginate	INH, RIF, PZA, ETB	Encapsulation of ETB, RIF, INH and PZA in formulation released drug and detected in plasma up to 7, 9 11 and 11 days after **oral administration** respectively and up to 15 days in tissues.	2006	[87]
	PLGA	RIF	Daily administration of RIF solution over 20 days had a positive effect on pulmonary and splenic inflammation but not on the viable number of bacteria from lungs whereas a single dose of nanoparticle or 20 dose of free drug equally reduced the bacterial count from spleen. Moreover, PLGA MPs increased the drug residence time.	2006	[104]
	PLGA	RIF,INH, PZA,ETB	A single **oral** dose of four drugs in PLGA nanoparticle maintained drug level in various tissues for 9-10 days without showing any adverse effects even after administering several folds higher than therapeutic doses. On the other hand, conventional free drugs at equivalent dose produce lethal effects.	2006	[105]
	PBCA	Moxifloxacin	To improve effect of moxifloxacin against *M. tuberculosis* was achieved by formulating poly (butylcyanoacrylate) (PBCA) based nanoparticle and injected into macrophage that led to three folds accumulation of nanoparticle into the macrophage efficiently and six times longer detected as compared to free drug. In addition, 0.1µg/ml formulation inhibited bacterial growth and same effect was obtained with 1µg/ml of free drug.	2007	[78]
	PBCA	Moxifloxacin	Reported that encapsulated moxifloxacin *in vitro* drug release pattern was initially with burst release then sustained release with 65% drug release at the end of 48 hrs. moxifloxacin NP was more toxic with 2-3 folds enhanced cellular uptake and **IV administration** into the mice showed significant anti-*Mycobacterium* activity.	2008	[77]
	PLA	INH, RIB	Intracellualr concentration of the drugs encapsulated respirable MPs was found to be 4 fold higher than the drug solution.	2007	[106]
	Albumin, Gelatin, Chitosan and SLN	Ciprofloxacin	Five different drugs to carrier ratios formulation developed and evaluated for *in vitro* drug release and sustained release profile. Maximum drug encapsulation was obtained with albumin (48.2±3.1%) with extended release time (120 hrs) as compared to free drug without burst effect.	2007	[107]
	Alginate	RIF, INH, PZA	Alginate nanoparticle loaded with all ATD and econazole were evaluated. All ATDs were found up to 15 days whereas econazole till day 8 in major organs (lungs, liver and spleen) and free drugs was detected up to 12-24 hrs. Thus alginate NP reduced dosing frequency 15 folds than free drugs.	2007	[108]
	PEGylated Dendrimer	RIF	New architecture design of PEGylated dendrimer was loaded with RIF and evaluated for *in vitro* drug release and hemolytic potential. It was found as sustained release carrier and safe delivery for RIF.	2007	[109]
	PLGA	RIF	Studied about the effect of lung surfactant on the release rate of RIF from monodisperse PLGA microsphere due to adsorption phenomenon. It was faster release from pulmonary surfactant adsorbed PLGA microsphere at pH 7.4 buffer as compared to pH 4 buffer.	2007	[110]
	PLGA	RIF	Biodegradable PLGA NP was prepared using solvent evaporation and diffusion method to load RIF and optimized based on particle size, entrapment efficiency, internal phase volume and composition. PLGA NP was considered as efficient anti-bacterial activity.	2007	[111]
	PLA	ECO, MOX, RIF	Only 8 doses of individual NP were enough to clear bacterial count from infected mice instead of 56 daily dose of moxifloxacin and 112 doses twice a day of econazole. Interestingly addition of third drug RIF to this combination cleared completely bacterial count within 8 weeks.	2008	[76]

Table (6) contd….

A Synopsis on Novel Drug Delivery System Used in the Treatment of Tuberculosis					
A Synopsis of Novel Drug Delivery System Loaded with ATD					
Carriers	**Polymer/Lipids**	**Drugs**	**Major Findings**	**Year**	**Refs.**
	Hyaluronan	Ofloxacin	Ofloxacin loaded in hyaluronan based nanoparticle was administered intratracheal route led to lower 50% reduction in plasma bioavailability as compared to intravenously given or orally given. This result suggest that inhaled microparticle may reduce the systemic side effects but also suggest that it may not be addressed alone.	2008	[112]
	PLGA, Chitosan	RIF	Different formulation of RIF was formulated using PLGA, Chitosan and combination of these two in order to get stabilized product. Entrapment efficiency and ability of MP (microparticle) to be nebulized were assessed. PLGA based was better than chitosan but cytotoxicity was a challenge.	2008	[113]
	PHBV	RIF	RIF was loaded (0.035mg/g) in biodegradable microparticle poly(3-hydroxybutyrate-co-3-hydroxyvalerate) PHBV within size range of 20-60μm and 14% encapsulation efficiency.	2008	[114]
	PLGA	RIF	Encapsulated microsphere in mannitol improved *in vivo* uptake of the drug by alveolar macrophage in the rat lungs as compared to PLGA nanoparticle and mannitol micropshere.	2009	[95]
	Microparticle	RIB, INH	Microparticle prepared of these two drugs was compared with soluble individual drugs for accumulation of microparticle by alveolar macrophage cells. It was observed that sustained level of microparticle was retained in cytoplasm of macrophage and for prolonged time followed with enhanced bactericidal activity.	2009	[96]
	Gelatin B	RIF	Gelatin B based microsphere of RIF for **oral administration** was prepared using gelation method to circumvent the high dose of conventional formulation of RIF. Entrapment efficiency was in range of 52-83% and detected in intestine for 24 hr post oral administration.	2009	[88]
	Gelatin	RIF	Gelatin NP (**DPI)** of RIF was prepared by two step desolvation technique. Drug release showed biphasic pattern initially burst then sustained release. *In vitro* cytotoxicity study revealed safe and biocompatible as compared to free drugs followed with improvement in pharmacokinetic parameters like AUC, MRT and significant reduction in bacterial count in the lungs and spleen of infected mice.	2010	[115]
	PLGA	RIF	Particles were taken up very efficiently by alveolar macrophage by inducing potential bactericidal effects. RIF loaded microsphere of PLGA does not induce any toxic humoral factors. Phagocytosis does not affect the viability of the AMs.	2010	[116]
	Isoxyl	Isoxyl	Isoxyl microparticle (**DPI**) was prepared by anti-solvent precipitation and simultaneous spray drying method. Control was prepared in DMSO within cell culture media. Both isoxyl microparticle and control were found to be non-toxic to macrophage cell up to concentration of 100μl/ml as revealed in MTT (Methy thiazol tetrazolium) and LDH (Lactose dehydrogenase) assay. Furthermore, both were mycobactericidal at concentration of 5μl/ml.	2010	[117]
	PLGA	RIF	Developed biocompatible PLGA microsphere to load RIF by spray drying technique. Investigated cellular uptake of microsphere by rat alveolar macrophage cell-line NR8383 in an energy dependent manner using fluorescent microscopy.	2010	[118]
	Gelatin	INH	Mannose ligand designed nanoparticle was suggested as the most safe and efficient targeted drug delivery for TBs.	2011	[89]
	Span 85, Tween 85 and DMAA (N,N'- Dimethylacrylamide)	RIF, INH	Microsphere loaded with RIF and INH prepared by radical copolymerization method. *In vitro* drug release of RIF was performed in pH 7.4 and pH 5.2 (alveolar macrophage cytoplasm). Same amount of the drug INH was released at pH 7.4 but small quantity was released at pH 5.2. *In vitro* activity against *M. tuberculosis* activity was comparable to RIF.	2011	[119]
	Mesoporous silica, β-TCP Scaffold	RIF, INH	Mesoporous silica based NP of bicomponent (RIF and INH) formulation showed high performance, excellent biocompatibility and extremely more sustained release of drug for the treatment of osteoarticular *tuberculosis.* Drugs concentration were enough higher than their effective values to eradicate *M. tuberculosis* for as long as 42 days. In addition hepatic and test indicated no long term lesions.	2011	[120]

Table (6) contd….

A Synopsis on Novel Drug Delivery System Used in the Treatment of Tuberculosis					
A Synopsis of Novel Drug Delivery System Loaded with ATD					
Carriers	**Polymer/Lipids**	**Drugs**	**Major Findings**	**Year**	**Refs.**
	PLGA	Ethionamide	PLGA NP of second-line Method ATDs were prepared with high drug encapsulation and loading efficiency. **Oral administration** of the formulation at two different doses to mice and control group receiving drug free formulation led to sustained release of drug for 6 days in plasma and 6 hr for free drug. Also, drug was detected up to 5-7 days in lungs, liver and spleen for NP whereas only 12 hr in case of free drugs. Significant improvement in pharmacokinetics parameters were exhibited by encapsulated drug and dose proportional increase in $AUC_{0-\infty}$ values. Thus, the work had a great potential in reducing dose frequency.	2011	[90]
	Soy PC and HSPC	RIF	Investigated to evaluate the effect of lipid amount and composition of liposome on physico-chemical properties, aerosol performance and toxicity. High lipid content caused a better lipid bilayer packing and subsequent nebulization stability. Entrapment efficiency was more with oleic acid containing liposome than others but *in vitro* cell-line study reported its toxicity issue.	2012	[121]
	Mesoporous silica	RIF, INH	ATDs were targeted to alveolar macrophage intracellularly *via* functionalized mesoporous silica NP loaded with INH and RIF leading to enhanced therapeutic efficacy and reduced systemic toxicity. Mesoporous silica NP (MSNP) was employed drug delivery either equipped with polyethyleneimine (PEI) to release RIF or equipped with cyclodextrin to release INH in acidic medium of macrophage cell infected with the *M. tuberculosis.*	2012	[122]
	PLGA	RIF, INH	Prepared polymeric NP using modified double emulsion solvent evaporation method to encapsulate both INH and RIF. *In vitro* drug release study at endosomal macrophage ph 5.2 and physiological ph 7.4 was carried out. *In vivo* targeting potential was evaluated in peritoneal macrophage by confocal laser scanning microscopy and fluorescent microscopy.	2012	[123]
	PLGA	RIF	PLGA based microsphere was prepared and then complexed with alginate in situ gel to get combination drug delivery. The *in vitro* drug release from combination microsphere was slower than microsphere. The cumulative release percent of drug was 91.83±1.2% for combination delivery system and 97.36±3.41% of rifampicin from microsphere. *In vivo* fluorescence microscopy suggested that gel adhered to lungs within 24 hr and microsphere remained at-least for 504 hr (21 days). After 21 days, local concentration was above minimum inhibitory concentration of rifampicin. Hence novel combinational drug delivery was supposed as significant for sustained drug delivery of drug in the treatment of *tuberculosis*.	2012	[91]
	PLGA, Sucrose palmitate	RIF	Microsphere of PLGA was prepared using biocompatible sucrose palmitate surfactant (replaced in place of PVA) to encapsulate RIF and characterized for size, morphology, drug entrapment efficiency and *in vitro* drug release profile. Optimized microsphere was subjected for cellular uptake by alveolar macrophage and showed efficient internalization with microsphere. Intracellular levels of drug were found to have 7 folds higher than equivalent amount of free drug in alveolar macrophage.	2012	[124]
	PLA	Ofloxacin	Ofx palladium complex was prepared in 1:1 stoichiometry and then encapsulated in PLA microparticles. Optimized microparticle was evaluated for size, aerodynamic diameter (2.5µm), morphology and drug content (30%w/w). The optimized microparticle showed very slow drug release at pH 7.4 than spray dried Ofx loaded microparticle. Thus spray dried ofloxacin was found to have potential **inhalation** effect to target alveolar macrophage.	2013	[97]
	PLGA, Hydroxyprpyl β cycldextrin	RIF	RIF was complexed with hydroxypropyl β-cyclodextrin and two RIF loaded PLGA microsphere with slow and fast release microsphere were prepared. After **IV administration** of RIF and RIF hydroxylpropyl β-cyclodextrin nebulization, plasma profile and lung epithelium lining fluid (ELF) profile were superimposed respectively indicating that RIF diffused almost instantaneously through broncho-alveolar barrier. PK model predicted that ELF fluid contains RIF concentration much higher after microsphere administration than IV or nebulization over prolonged period of time.	2013	[82]

Table (6) contd….

A Synopsis on Novel Drug Delivery System Used in the Treatment of Tuberculosis					
A Synopsis of Novel Drug Delivery System Loaded with ATD					
Carriers	**Polymer/Lipids**	**Drugs**	**Major Findings**	**Year**	**Refs.**
	PLGA	INH	Isoniazid loaded core shell nanoparticles derived from PLGA–PEG–PLGA tri-block copolymers was prepared by double emulsification method. Drug entrapment efficiency and loading were found to be 12.8-18.67% and 6.4-8.9% respectively.	2013	[125]
	PLGA,PEG	INH, RIF	PLGA based nanoparticles were prepared by double emulsion solvent evaporation spray drying technique followed with surface coating with the PEG. Encapsulated RIF and INH in NP were administered **orally** to *M. tuberculosis* infected mice that led to distribution of RIF and INH into liver and lungs up to 10 days respectively. *In vitro* study was also performed against same *mycobacteroium.*	2013	[126]
	PLA, Chitosan, PEG and Gelatin	RIF	Chitosan PLA based nanoparticle prepared was coated with PEG gelatin to controlled release and targeted drug delivery of rifampicin. The formulation was characterized for all *in vitro* parameters such particle size and size distribution, shape (SEM study), *in vitro* drug release study using dialysis membrane.	2013	[127]
	HPMC, Folate, cross linking agent	RIF	The study had focused to sustain (over 25 days) the delivery of RIF by fabricating the liquid crystalline folate nanoparticle using HPMC polymer and folate. The results showed improved drug efficacy and pharmacokinetics profile as compared to free RIF suspension. Moreover, the study reported more efficient drug loading as well as least drug loss (~20 to 30%). In addition, cytotoxicity study was performed and demonstrated lower cytotoxicity by the developed nanoparticles in alveolar macrophages.	2015	[128]
	Magnetic NP		Microparticles (MPs) were prepared by a cast technique using calcium carbonate ($CaCO_3$) sacrificial templates and integrated superparamagnetic iron oxide nanoparticles (SPION) to concentrate MPs in alveoli and permit P3 release upon actuation of an external alternate magnetic field (AMF). The MPs showed appropriate delivery of P3 to the lower airways and for alveolar macrophage phagocytosis.	2018	[129]
	Ag-NP and Zn-NP	RIF	Developed biodegradable multi-metallic microparticles (MMPs), comprising of silver nanoparticles (Ag-NPs) and zinc oxide nanoparticles (ZnO-NPs), for the pulmonary delivery of some antituberculous drugs to the endosomal system of Mycobacterium tuberculosis *(M.tb)* infected macrophages. Efficient uptake of MMPs by *M.tb*-infected THP1 cells was established using an *in vitro* macrophage infection model, with straight interface between MMPs and *M.tb* envisaged with the usage of electron FIB-SEM tomography. The release of Ag-NPs and ZnO-NPs within the macrophage endosomal system improved the effectiveness of the model antibiotic rifampicin (RIF) by 76%.	2018	[130]
Solid lipid nanoparticle (SLN)	Stearic acid	RIF, INH PZA	Investigated to evaluate the potential effect of oral administration of solid lipid particle loaded with anti-tubercular drugs in the TB management. Single oral dose to guinea pigs showed that therapeutic drug concentration in plasma was found up to 8 days, 10 days in organs (lungs, liver and spleen) and whereas free drug was cleared within 1-2 days. Furthermore, pharmacokinetic parameters like mean residence time and drug bioavailability was increased several fold higher than free drugs. Nebulization of solid lipid particle to *M. tuberculosis* infected guinea pigs at every 7^{th} day, no bacilli were detected after 7 doses of treatment in the lungs and spleen whereas 46 daily dose of free drugs were required to get equivalent drug concentration. No evidence of hepatic toxicity was observed.	2005	[131]
	Phospholipon 90 H	INH	Study was performed to circumvent isoniazid associated toxicity by formulating SLN of isoniazid and single **oral administration** (25 mg/kg BW) of formulation to rat showed 6 times higher plasma concentration and 4 folds in brain as compared to free drug at the same dose. Thus significant improvement in relative bioavailability, reduced hepatic toxicity and 3 fold higher LD_{50} value were obtained.	2012	[132]

Table (6) contd….

A Synopsis on Novel Drug Delivery System Used in the Treatment of Tuberculosis					
A Synopsis of Novel Drug Delivery System Loaded with ATD					
Carriers	**Polymer/Lipids**	**Drugs**	**Major Findings**	**Year**	**Refs.**
		RIF, INH	A drug-drug interaction was seriously studied at stomach pH (pH 1.2). Authors studied degradation of RIF and INH alone as well as in presence of each other. Degradation of RIF and INH were 26.5% and 1.43% in gastric pH at 37°C after 4 hrs. Degradation of RIF in presence of INH was 84.48% which was mitigated to 20% in SLN formulation and to nearly 9% when RIF was prepared alone in SLN drug delivery carrier. The degradation of INH in combination with RIF also reduced significantly from 13.2% to 2.7% when both the drugs were encapsulated individually within SLNs.	2013	[133]
	Compritol 888 ATO, Lecithin, Tween 80	RIF	SLN was prepared with 50% loading efficiency and 67 % EE to enhance poor oral BA. Authors reported optimum particle size (~130 nm) and negative surface charge alongwith about 70% drug release within 9 days indicating slow and sustained drug delivery in PBS solution (pH 6.8). *In vivo* pharmacokinetics studies in wistar rats reported that the oral BA was improved with 8.14 folds hogher than the free drug solution upto five days which further corroborated the sustained delivery of drug drug from the developed formulation. Moreover, the pharmacokinetic and pharmacodynamic parameters were improved as compared to free RIF. More intriguing aspect was safety assessment which was studied when 50 times the human dose was repeatedly administered for 50 days leading to no significant adverse effects in rat models. Thus, the developed RIF-SLN was supposed to be safe and sustained drug delivery for the treatment of tuberculosis.	2015	[134]
	Stearic acid and taurocholate	RIF	The objective of the study was based on design of **breathable** solid lipid microparticle loaded with RIF to make conventional anti-tubercular treatment more efficacious by targeting alveolar macrophages. *In vitro* study on J774 cell line demonstrated non-cytotoxicity and ability to taken up by cells.	2014	[135]
Micelle forming carrier	Derivative	INH	A slow acting isoniazid derivative was synthesized for prophylactic use or chemotherapeutic application. The micelle forming co-polymer of poly(ethylene glycol)-poly(aspartic acid) prodrug with isoniazid was synthesized. Thus obtained derivative was potentially active against *M. tuberculosis* with 5.6 times less MIC values than isoniazid.	2001	[136]
	Derivative	PZA	A micelle forming prodrug of PZA was obtained by condensing with poly(ethylene glycol)-poly(aspartic acid) copolymer that was further characterized for CMC value and micelle diameter (78.2 nm).anti- *Mycobacterium* activity was found significant than original drug.	2006	[137]
	Derivative	INH, RIF, PZA	Prodrug with three ATDs were synthesized by condensation hydroxymethylpyrazinamide, isoniazid and rifampicin with free carboxylic group on the copolymer poly(ethylene glycol)-poly(aspartic acid). Small size distribution, low CMC value, stable micelle and stronger anti-tubercular activity than the original drugs were achieved.	2007	[138]
	Enantiomeric	RIF	RIF was delivered as stereocomplex micelle and tested for controlled of drug. Drug loading and encapsulation efficiency with stereocomplex micelle were found to be higher than single copolymer prepared from poly(L-lactide)-poly(ethylene glycol) by ring opening method. Stereocomplex polymer was prepared using equimolar concentration of PLA-PEG copolymer in water.	2007	[139]
DPI(dry powder inhaler)	PBCA	RIF, INH, PZA	Three antibiotics together formulated into **dry powder inhalation** for pulmonary delivery in the treatment of TB. Individual spray dried powder was physically unstable and these excipient free triple antibiotic dry powder inhalation were characterized for aerodynamic diameter (3.4±0.1μm) and fine particle fraction for excellent aerosol performance.	2012	[140]
	Mannan	RIF, INH, PZA	High drug entrapment, enhanced *in vitro* drug release and high drug recovery from the site of action proved as suitable for **DPI** formulation.	2013	[61]

Table (6) contd….

A Synopsis on Novel Drug Delivery System Used in the Treatment of Tuberculosis					
A Synopsis of Novel Drug Delivery System Loaded with ATD					
Carriers	**Polymer/Lipids**	**Drugs**	**Major Findings**	**Year**	**Refs.**
Nanoemul-sion	Sefsol 218 oil	RIF	Sefsol 218 based o/w nanoemulsion was prepared by aqueous phase titration method and characterized for size and size distribution, viscosity, entrapment efficiency, homogeneity and pH value. Best selected were subjected for stability study. Thus RIF loaded nanoemusion was prepared for intravenous delivery for TB treatment.	2008	[141]
Miscellane-ous type		RIF	In order to improve lung deposition of RIF, it was transformed into flake like crystal hydrate. Control was prepared by spray drying method as amorphous RIF. RIF crystal dehydrate was characterized for size, aerodynamic diameter (2.2µm) and stability (more stable than amorphous form). Moreover, least chemical degradation and maximum potency was achieved with dehydrate form leading to drug deposition directly to the site of action.	2011	[142]
Phospholipid complex	Lipoids-S-75	RIF	To enhance the oral BA of RIF, a phospholipid-RIF complex was prepared by solvent evaporation method. The complex was result of hydrogen bond and electrostatic force with improved solubility and stability even in pres-ence of INH. *In vivo* investigation suggested that the Cmax (from 48.5 to 54.3 µg/ml), AUC (from 147.71 to 472.4 µg.hr/ml) and T1/2 (from 1.5 to 8.5 hrs) were improved as compared to free drug solution.	2014	[143]
Implants		RIF	RIF was investigated as **implant** depot delivery in guinea pig.	1985	[144]
	PLGA	INH	INH was incorporated into PLGA **implant** for controlled delivery and found delivery up to 63 days in rabbit and to achieve good patient compliance.	1994	[145]

Table 7. Some recent patents on delivering of ATDs in nanostructures formulations.

Patent No.	Drug (s)	Carriers	Major highlights	References
US Patent 20100310662A1	RIF, INH, EMB, Econa-zole, PZA	PLG nanoparti-cles	• The inventors prepared oral PLG nanoparticle to load individual drug (RIF, Moxifloxacin and Econazole) by multiple emulsion and solvent evaporation technique for improved BA and retention time. • Disclosed average particle size of 217 nm-250 nm. • Single oral dose administration maintained therapeutic plasma concentraton for several days as compared to free drug. • Total bacterial clearance observed within eight weeks equivalent to four first-line ATDs in conventional form. • All the for ATDs were dosed daily while encapsulated RIF, econazole and moxifloxacin combination every 10^{th} day except EMB (administered weekly). • The claimed combinations are highly potent and active against multiplying, non-dividing, latent and persistent bacilli due to presence of moxifloxacin and econazole. • The therapy is the most sterilizing, rapid (reduced time) and circumventing the pain of conventional ways.	[81]
US Patent 6054133	RIF, INH, PZA, Trans-ferrin, PLG, Low density protein	Drug con-jugation	• The inventors conjugated the ATDs with transferring, ligand and low density protein for targeting to intracellular pathogen *M. tuberculosis* to clear the in-fected endosomes. • Prepared three formulation (a) PLG NP to load RIF, INH, PZA, (b) PLG NP to load RIF and INH (c) PLG NP to load EMB. • The invention disclosed that MIC was attained when EMB was administered separately not in combination. • The patent critically proposed efficient delivery of ATDs to maximize thera-peutic efficacy and minize the drug interaction.	[146]
US Patent 07018657	ATDs and surfactants	Colloidal micelle	• The inventors proposed particulate composition of the ATDs comprising of continuous ohase and micelles. • Micelle was compoased of surfactants. • Dispersed phase was quenched to solid particulate. • The NP was intented for delivery through aerosol to the lungs.	[147]

Table (7) contd….

Patent No.	Drug (s)	Carriers	Major highlights	References
US Patent 20060222716A1	RIF, STR, Moxifloxacin and stabilizer	SLN	• The inventors claimed to prepare SLN to load STR (131 nm) and SLN to load RIF-moxifloxacin (100-450 nm) by using 10-20% aqueous phase to molten lipid-surfactant blend at 70-80°C. • A homogeneous SLN formulations was prepared.	[148]
US Patent 5858410	RIF, EMB	Nanosuspension	• The inventors reported to prepare nanosuspension using air jet grouding in aqueous surfactant solution and further homogenization by the piston-gap homogenizer. • The pharmaceutical prepration avoided to use organic solvent.	[149]
US Patent 6994862	RIF, emulsifier, organic solvents, monoglycerides	Liquid composition	• The inventors invented and reported liquid composition of RIF using monoglycerides and emulsifier followed with evaporation of organic solvents. • The prepration of particle size 321.3 nm was obtained and lyophilized with cryoprotectant which exhibited slow and sustained drug release (80% in 5 hrs) as compared to free RIF.	[150]
WO Patent 2009002227A1	RIB, milk, glycol acid copolymer, d-mannitol, Polysorbate-80, DMSO	Suspension	• The inventors claimed to produce novel generation controlled activity antimicrobial nanosuspension containing RIB. • A predetermined ratio of RIB, milk, co-polymer, mannitol, polysorbate and DMSO were heated at 50-60°C till homogeneous mixture ad then allowed to cool to room temperature. • Thus, prepared nanosuspension exhibited broad spectrum antimicrobial activity and non-toxic.	[151]
WO Patent 2006/109317	ATDs, EMB, PLG	PLG NP	• The inventors prepared the ATDs loaded NP using PLG. • Single administration of EMB PLG NP attained MIC in mice when dosed alone (1.5µg/ml in plasma). • The invention disclosed that drug level was attained elow MIC when EMB was co-administered with other three ATDs. • Furthermore, the oral BA of EMB is considered to be 1 but it was improved by 10.6 folds and 5.1 folds higher than free drug when administered using EMB-PLG-NP and EMB-PLG-Combination-NP respectively. • The patent reported that EMB is unstable in presence of INH and should be avaoided in combination therapy.	[152]
Indian Patent Application 3093/DEL/2012	STR	SLN	• The inventors prepared STR loaded SLN with 60% EE and140.2 nm particle size. • The patent reported that nanocolloidal carrier would be suitable suitable for oral, nasal and topical delivery effectively. • Moreover, the carrier could enhanced BA to the blood and brain of STR.	[153]
PCT/IN2012/000154	INH	SLN	• The inventors prepared INH loaded SLN with 69% EE and48.8 nm particle size. • The patent reported that SLN nanocolloidal carrier improved oral BA by 6 folds which couls be due to firstpass hepatic avaoidance.	[154]

solution were required to get equivalent drug concentration. No evidence of hepatic toxicity was observed [102]. Further study was performed to culminate isoniazid related toxicity by formulating SLN (using phosphotidylcholine H90) and single oral administration (25 mg/kg body weight) of formulation into the rat showed six folds increase in plasma drug concentration and four folds in the brain as compared to free drug at the same dose. Additionally, improvement in relative bioavailability, reduced hepatic toxicity and 3 fold higher LD_{50} value were also obtained [132]. Currently, RIF was investigated in SLN formulation for pulmonary delivery using stearic acid and taurocholate that might be capable of delivering the drug to the target site of the lungs alveolar macrophage. The *in vitro* study on J774 cell line demonstrated non-cytotoxicity on cells [135]. The micelles drug delivery has been reviewed and shortly tabulated in Table **6**.

5.5. Dry Powder Inhalation for Delivery of ATDs

Pulmonary drug delivery has been exploited to deliver a number of drugs for the treatment of local lungs infection in addition to TB that requires aerosol vehicle consisting droplets with drugs or drug powder particles with an appropriate particle size for lungs delivery. However, dry powder delivery to the lungs is challenging due to particle aggregation behavior to the size above optimum diameter. In the last decade, an increasing attention has been paid on the treatment of pulmonary infections owing to offering an attractive way to deliver high drug concentration directly to the lungs. Important factors of clinical outcomes would be the concentration or performance of the drug at the site of infection together with the penetration of the drug into the lungs lining or lungs mucosal layer. Chan *et al*. investigated the effectiveness of

DPI prepared by spray dry method using RIF, INH and PZA (anti-tubercular drugs). These three first-line ATDs together were formulated into dry powder inhalation for pulmonary delivery in the treatment of TB. Individual spray dried powder was physically unstable and these excipient free triple antibiotic dry powder inhalation were characterized for aerodynamic diameter (3.4±0.1μm) and fine particle fraction for excellent aerosol performance [140].

Some authors reported RIF delivery using sefsol 218 based nanoemulsion for enhanced stability and RIF flake like crystal dehydrate to improve drug deposition into the lungs due to polymorphic behavior [141, 142].

5.6. Implants Used to Deliver ATDs

Mathur *et al*. reported the development of subdermal implant using microencapsulated RIF biodegradable polymer with two 45 and 100 mg implants in experimental *tuberculosis* guinea pigs. The rationale was based on to culminate adverse effect of RIF after oral administration. There was constant delivery of the drug from implant in the therapeutic concentration up to 30 and 50 days until complete dissolution of the implant carrier [144]. Poor patient compliance and low level of patient adherence to the treatment are the major cause of TB treatment failure. To improve the poor compliance, isoniazid was formulated into the implant using PLGA polymer [145]. Authors claimed that earlier study of INH formulated in biodegradable PLGA implant showed sustained delivery of drug for up to 8 weeks. Similar study had been repeated into the rabbit. Concentration of the isoniazid and acetylisoniazid in the serum as well as urine were determined using HPLC method. Initially, there was no any abnormal drug release. Isoniazid concentration ≥ 0.2μg/ml was found to be in the serum and the urine up to 63 days. This finding confirmed the usefulness of the depot drug delivery to avoid patient compliance in the treatment of *tuberculosis*.

6. FUTURE PROSPECTIVE

Despite of several potential benefits of nanotechnology mediated drug delivery of ATDs, TB has been emerged as leading cause death worldwide. Therefore, there is still need of convincing and awareness required for implementing at commercial scale in the developing countries. It can also be expected that the research underway will focus to achieve new drug delivery approaches with clinical efficacy, affordability, accessability, cost effectiveness, patient friendly and non-toxic. After going through the several studies, the utmost need is that the attention of researcher should have to be focused on the serious shortage of biosafety data of nanocarrier used in healthy as well as diseased individual. No any *in vitro* method has been established till date to ensure safety. Additionally, on large scale production or on industrial scale production, it is very expensive and its delivery other than oral route is not patient friendly especially in developing countries where the economic and social dire straits are major determinants in the failure of TB therapy.

CONCLUSION

Tuberculosis is an airborne deadly disorder in the world. Several nanotechnological approaches have been well employed to fabricate nanocarrier mediated drug delivery systems without or mitigated premature release of the drug before reaching to the replicative niche of the *Mycobacterium*. As discussed earlier in this article, various novel drug delivery systems have been used to deliver ATDs effectively to the site of infection such as liposome, niosome, nanoparticles, microparticles, micelles, solid lipid nanoparticle and implant. All of them offered significant merits such as improved drug bioavailability, reduced drug related toxicities, shortened frequency of dosing, attained high level patient compliance and targeted the drug to the reservoir of infection. Furthermore, nanoscale or microscale together with alternate route of drug administration has presented advantages over conventional dosage form. However, the success of these nanocarrier based drug delivery systems depends on biocompatibility of the carrier, high drug loading efficiency, improved bioavailability, active targeting to the infection site (intracellular residing *Mycobacterium*) and more patient compliance.

In fact, feasibility of patient friendly oral drug delivery can be better translated for use on global scale by establishing clinical efficacy, affordability, accessibility and exploiting new technology based approaches. Nevertheless, there are several challenges and barrier in the TB therapies such as emergence of drug resistance, lengthy treatment protocol, expensive treatment, high dose and dosing frequency, drug related toxicities as well as to translate from lab to industrial scale such as cost of production, cost pf material and regulatory issues, market potential, fabrication of nanocarrier on large scale, investment on advanced technical instrument, reproducible characteristics, *in vivo* efficacy on large scale production and significant variation in pharmacokinetics among human population. Thus these are major determinants for commercialization of nanocarrier based drug delivery.

LIST OF ABBREVIATIONS

CHOL	=	Cholesterol
DOPC	=	Dioleoyl phosphatidylcholine
DPPC	=	Dipalmitoylphosphotidylglycerol
DSPE	=	Distearoylphosphotidylethanolamine
ECO	=	Econazole
HPC	=	Hydroxygenated phosphatidylcholine
HSPC	=	Hydrogenated stearoyl phosphatidylcholine
MOX	=	Moxifloxacin
PBCA	=	Polybutylcyanoacrylate
PC	=	Phosphatidylcholine
PEG	=	Polyethylene glycol-400
PG	=	Propylene glycol
PHBV	=	Poly(3-hydroxy butyrate-co-3-hydroxy-valerate)
PIBCA	=	Poly isobutylcyanoacrylate
PLA	=	Poly(lactide-co-glycolate)
PLGA	=	Poly(lactide-co-glycolic) acid
RIB	=	Rifabutine
WHO	=	World Health Organization

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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