2. Literature Review

Tuberculosis Diseases continue to be a threat and pose many obstacles in the development of mankind. Several types of diseases are prevalent which either affect a particular part of body or the whole body itself. For some, there is permanent cure but for others the cure is temporary with continuous advancement in science and technology and constant research. Ways are evolving out for the treatment of diseases otherwise considered as incurable`(Folkers, et al., 2001). Yet another strike of this constant Endeavor is towards the betterment of existing therapy in terms of enhancement of efficiency of treatment, cutting short the duration of therapy and curtailing the side effect of drug. Tuberculosis is a dreaded disease, which has affected the population in many parts of the world including India (Sachs, 2008).

Over the previous decades the outline and synthesis of particles that can impact intracellular trafficking of medications have expanded. While huge advance has been made in detached tissue focusing on utilizing, Lipid particulate bearers in Novel Drug Delivery System, which exploits the alleged permeation and retention of drug to particular cell compartment this feature has been exploits in the design of system that facilitate the delivery of active compound to the cytoplasm. In this way a perfect strategy for treating tuberculosis would be one that not exclusively can security delivery of drug systematically for long time; yet in addition would have the capacity to target drug to intracellular condition in which the tubercular bacilli are found. (i.e. macrophage) (Dolman, 2011).

2.1 Approaches to Drug Targeting

The term 'drug targeting' infers to the technique, which exist for the restricting of helpful therapeutic agents on the cells that are really required for treatment. The targeted delivery system has been depicted, who imaged drug delivery as a “magic bullet” a drug is a transporter preselected target cell type (Hoffman, 2008). Some researcher’s describes the novel drug delivery system as “old drug in new cloth” An ideal targeted drug delivery approach would not only increase the therapeutic efficacy of drugs but also decrease the toxicity associated with them. A vast array of methods, which can be classified into two key approaches-active and passive have been explored for targeting drugs by means of designing innovative nanosystems (Berti, et al., 1995).

Table 2.1: Drug Targeting Approaches

PASSIVE TARGETING	ACTIVE TARGETING
1. Pathophysiological factors	1. Biochemical targets
• Inflammation/infection	• Organs
• EPR effect	• Cellular
2. Physicochemical factors	• Organelles
• Size	• Intracellular
• Molecular weight	2. Physical/External stimuli
3. Anatomical opportunities	• Ultrasound
• Catheterization	• Magnetic field
• Direct injection	3. Pretargeting/Sandwich Targeting
4. Chemical approaches	4. Promoter/Transcriptional targeting
• Prodrugs	
• Chemical delivery system	

2.1.1 Objective of Drug Targeting

Site specific drug delivery includes various objectives:

- Exclusive delivery to specific compartment.
- Alter the body distribution of drug with a view to reduce the toxicity and unwanted deposition of existing drug.
- Delivery of drug to previously inaccessible site.
- Reduction in the amount of active principle employed.
- Protection of drug from different untoward reaction (Vasir, et al., 2005).

2.2 Strategies for Drug Targeting

2.2.1 Slow Release Targeting

Through a relationship with an appropriate carrier, the drug is delivered to circulating or fixed macrophages. The drug is step by step discharged from the carrier and diffused out of the cells, prompting an expanded residence time in the body (Sengupta, et al., 2005).

2.2.2 Passive (Side Effect Avoidance) Targeting

Through the association of the drug with a suitable carrier it is prevent from distribution to site of toxicity. e.g. sequestration of colloidal carriers by liver and spleen.

2.2.3 Active Targeting

Through coupling of macromolecular, cell specific carrier the drug reaches higher therapeutic concentrations at the site of virus replication. Consequently, the dose can be reduce and side effect can be minimized (Byrne, et al., 2008).

(1) First Order Targeting (Organ Compartmentalization)

e.g. targeting to lymphatic, peritoneal cavity cerebral ventricles, lungs join, eyes, plural cavity.

(2) Second Order-Targeting (Cellular Targeting)

e.g. targeting to tuner cell, kupffer cells.

(3) Third Order Targeting (Intracellular Targeting)

e.g. receptor mediated entry of a drug complex into a cell by endocytosis, followed by lysosomal degradation of carrier intracellular release of drug (Bordería, et al., 2007).

2.3 Components of Drug Targeting

For designing the drug delivery system some component should be considered which include (Kreuter, 1994).

Table 2.2: Components of Targeting and Its Descriptions

Components of targeting	Description
A. Target	A cell or group of cell in minority which are in the need of treatment. Different type of targets are: 1. Cells in vitro for genome grafting or manipulation of DNA 2.Accessible anatomical compartments i.e. peritoneal cavity, lungs 3. Macrophage and other phagocytic cells 4. Non phagocytic cells of RES 5. Lymphocyte and APC
B. Carriers (drug carrier system)	The vectors which sequester transport and retain drug 'enroute' while delivering it in to the vicinity of target in controlling manner by either of two approaches. 1. By interacting selectively with biological target 2. By using synthetic or bioengineering approaches to release drug in he vicinity of target cells.
C. Ligand	These are surface appended group(s), which can selectively pilot the carrier to the pre-specified cellular linings equipped with desired unites e.g. antibodies, polypeptides, oligosaccharides, viral proteins etc.
D. Receptor Unit	It refers to those cell components or cytoportals with which carrier system binds through its surface appended ligands. And the loaded drug is release at the receptor and shows its pharmacological activity.

2.4 Absorption Mechanism of Drugs

The principal mechanisms for transport of drug molecules across the cell membrane in order of their importance are:

(i) Passive diffusion (ii) Pore transport (iii) Carrier mediated transport
(iv) Ion pair transport (v) Endocytosis

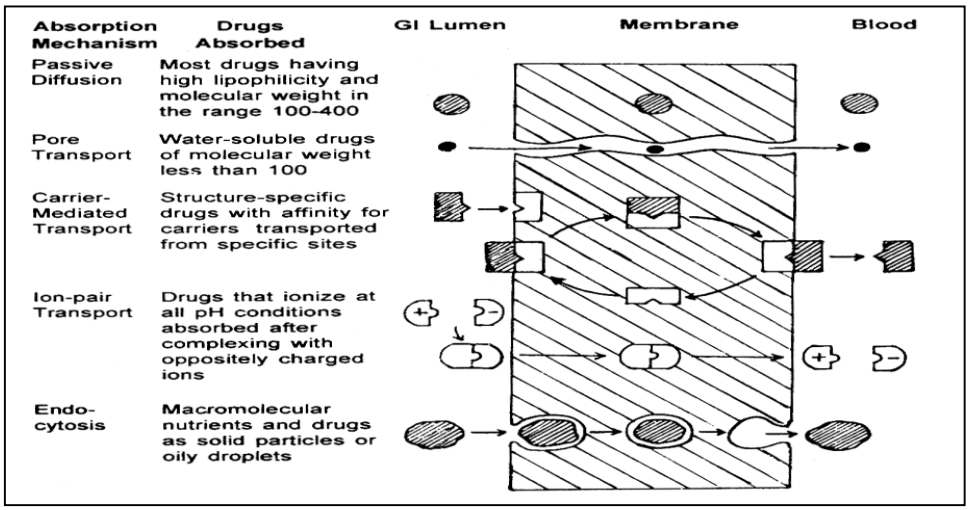

Fig. 2.1: Summary of important transport process and drugs absorbed through them

2.5 Intracellular Targeting

Most intracellular contamination are difficult to eradicate because bacteria are protected from antibiotics inside lysosomes infected cells, may also constitute a reservoir for microorganism which are released from time to time causing recurrence of systemic infection and most of the available antibiotics have following limitations (Rosqvist, et al., 1991).

- Poor intracellular diffusion or reduce activity at the acidic pH of the phagosome and lysosomes.
- Most intracellular infection is difficult to eradicate because bacteria inside phagosome are protected from antibiotics.
- Multi drug resistance due to functional P- glycoprotein pumps.

The nature of antibiotics is also determining their fate in vivo. Antibiotics with basic characters, aminoglycoside lead to lysosomal activity in acidic environment (Pundir, et al., 2009). Acidic antibiotics, β- lactam do not diffuse through the lysosomal membrane because their ionic character at neutral extra cellular or cytoplasmic pH. Certain antibiotics which penetrate the cell more rapidly and to the larger extent e.g. clidamycin are poorly retained in the cell as they efflux fast. The need for antibiotics with greater intracellular efficacy lead to

development endocytosable drug carrier like Liposome, Nano Lipid Carrier which mimic the entry path of bacteria by penetrating the cell into phagosomes and lysosomes (Singh, et al., 2010).

2.6 Barriers to Drug Targeting

Targeting of drug offers enormous advantage yet is similarly challenging. A better understanding of the physiological hindrances, which a drug needs to overcome, should enable the pharmaceutical researchers to create effective design of targeted drug delivery system. Main obstacles to drug targeting on incorporate physiological barriers, biochemical challenges to recognize and validate the molecular targets and the pharmaceutical challenges to devise suitable strategies of conjugating targeting legends to the Nanosystem (Liu, et al., 2014).

The challenge in drug targeting is not only the targeting of drug to a specific site but also retaining it for the desired duration to elicit pharmacological action. For a nanosystem administered intravenously, the first and foremost barrier is that of the vascular endothelium and the basement membrane. Presences of the Plasma proteins are another parameter, which have the ability to affect the biodistribution of drug carrier systems or nanosystem introduced in the blood stream. The in vivo distribution as well as opsonization of nanosystems in blood circulation is governed by their size, surface characteristics as well as molecular weight of drug molecules (Vasir, et al., 2005).

The retardation of opsonization and subsequent uptake by the phagocytic cells as a major step to enhance the blood circulation time of drug & carrier system. Another barrier is that of the extracellular matrix, which should be crossed to access the target cells in a tissue. If the whole tissue constitutes a target then the uniform distribution of drug throughout the tissue is another problem (Vasir, et al., 2005).

For those drugs whose targets are located in the cytoplasm/nucleus of a cell, further barrier needs to be crossed to allow internalization of nanosystems into specific cells. The barriers not end here, a number of endocytic pathways have been described for the cellular entry of nanosystems. Drugs/nanosystems need to through the viscous cytosol to access the particular cytoplasmic targets where site of action is located. Nuclear membrane poses another formidable barrier for drugs such as oligonucleotides, plasmid DNA and other low molecular weight drugs whose site of action is located in the nucleus of a cell (Nori, et al., 2005). Although a number of cellular and molecular targets are emerging, the real problem lies with the poor accessibility of drugs/ nanosystems to the target tissue. The presence of such

barriers leads to a poor in-vitro/in-vivo correlation when the targeted delivery systems are tested in receptor bearing cells in-vitro and fail in-vivo (Mozafari, 2006).

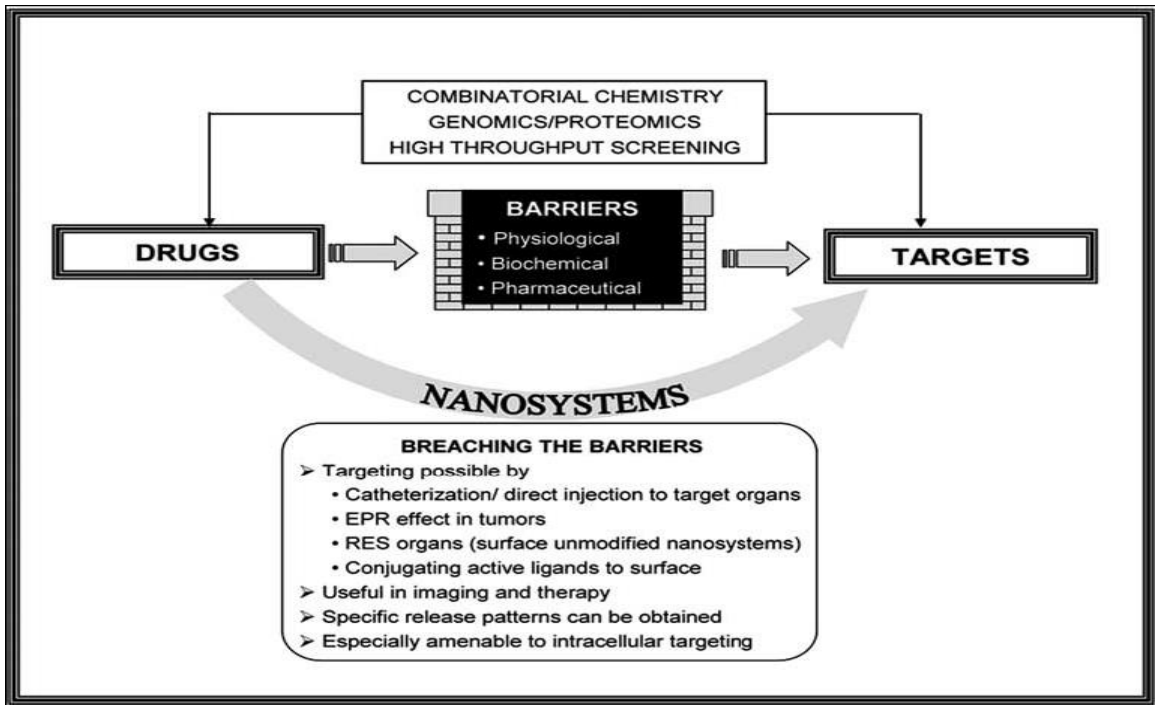

Fig. 2.2: Schematic describing barriers to drug targeting and the role of nanosystems in overcoming these barriers (Labhasetwar)

Thus, to harness the potential of new targets in imaging and therapy, one would need to develop targeted systems which can successfully overcome the physiological barriers, and for drug therapy, deliver the pharmacological agent to its site of action at therapeutically relevant drug levels for a time sufficient to allow therapeutic action. Conjugation of targeting ligands to drugs or drug carrier nanosystems is the most popular way of directing them to their target sites where targeting ligand are the moieties which are recognized by the cells of the barrier and are preferentially internalized through either active or passive mechanism carrying with them (Vasir, et al., 2005).

The whole drug bearing delivery system to which they are attached resulting in delivery of drug ligands particularly is attached to the delivery system through conjugation by virtue of the groups present in both of ligand as well as the system. Various techniques have been devised, including covalent and non-covalent conjugation (Chen, et al., 2002). The emphasis is that the ligand must be attached stably and accessibly to the drug carrier, so that the ligand is presented in its right orientation for binding to the target receptors. Some factors must be consider at the time of conjugation, that is the coupling reaction must not affect the

biological activity of ligand and should not adversely affect the structure of drug delivery nanosystems (Mozafari, 2006).

2.7 Current Antituberculosis Chemotherapy

Since the control measures for TB such as Bacillus Calmette-Guérin (BCG) vaccination and chemoprophylaxis appear to be unsatisfactory, treatment with anti-tubercular (anti-TB) drugs becomes the only option available. The goals of treatment are to ensure cure without relapse, to prevent death, to impede transmission, and to prevent the emergence of drug resistance. Long-term treatment with a combination of drugs is required. Treatment of active TB with a single drug should never be attempted, and a single drug should never be added to a failing regimen, the result being development of MDR TB (Zumla, et al., 2013). As suggested by WHO, treatment of TB and drug resistant cases requires Multi-Drug Therapy, comprising:

An initial intensive phase of rifampicin (RIF), isoniazid (INH), pyrazinamide (PYZ), and ethambutol (ETB) daily for 2 months.

A continuation phase of RIF and INH for a further 4 months, either daily or 3 times per week, to be administered as advised. INH eradicates most of the rapidly replicating bacilli in the first 2 weeks of treatment, together with streptomycin and ETB (Stec, et al., 2015). Thereafter, RIF and PYZ have an important role in the sterilization of lesions by eradicating organisms; these two drugs are crucial for successful 6-month treatment regimens. RIF kills low or non-replicating organisms and the high sterilizing effect of PYZ serves to act on semidormant bacilli not affected by any other anti-TB agents in sites hostile to the penetration and action of the other drugs. INH and RIF, the two most potent anti-TB drugs, kill more than 99% of tubercular bacilli within 2 months of initiation of therapy (Du Toit, et al., 2006). Using these drugs in conjunction with each other reduces anti-TB therapy from 18 months to 6 months. The emergence of strains resistant to either of these drugs causes major concern, as treatment is then deferred to drugs that are less effective, have more toxic side effects, and result in higher death rates, especially among HIV-infected persons. The current armamentarium of drugs available for the treatment of TB, their mechanism of action, and activity, has been reviewed by numerous authors (Du Toit, et al., 2006).

TB is treated with a Multi-Drug Regimen, and is thus exceptionally vulnerable to incidences of side effects, unsatisfactory patient compliance and slow improvement of patients. Therefore, despite the availability of these highly effective treatments for TB, cure rates remain low, as commercial anti-TB formulations are inconvenient to administer and patients

do not take the prescribed medications with sufficient regularity and duration to achieve a cure. Patients have to consume a large number of tablets (up to eight at one time), which is a common cause for non-compliance (Pomerantz, et al., 2001). It can be anticipated that non-optimal application of these short course regimens will result in the deterioration of their therapeutic potential, an escalation in the mortality rate and increased risk of developing acquired drug resistance. Resistance of *M. Tuberculosis* to anti-TB agents is a worldwide problem in both immunocompetent and HIV-infected populations (Control, et al., 2006).

Table 2.3: **Recommended treatment regimens for each diagnostic category by WHO**

T.B Diagnostic category	T.B. Patients	T.B. Treatment regimen	
		Initial Phase	**Continues Phase**
I	New smear positive patients. New smear negative patients. Parenchymal involvement, concomitant HIV disease or severe form of extra pulmonary TB (PTB).	Preferred 2 hrs Optional 2 $(hrs)^3$	Preferred 4 hrs Optional 4 $(hrs)^3$
II	Previously treated sputum smear positive - relapse - treatment after adult	Preferred 2 hrs/1 hrs Optional $2(hrs)^3/1(hrs)^3$	Preferred 5 hrs Optional 5 $(hrs)^3$
III	New smear positive (other than category I) and less severe from extra pulmonary T.B.	Preferred 2 hrs Optional 2 $(hrs)^3$ Or 2 hrs	Preferred 4 hrs Optional 4 $(hrs)^3$ or 6 hrs
IV	Chronic / still sputum positive after supervised retreatment proven or suspected MDR-TB	Specially designed standardize and individualized regimen	

Number preceding regimens indicate length of treatment (months) subscript following regimen indicate frequency of administration (days per week). When no subscripts are given regimen is daily.

2.8 Novel Drug Delivery System in Tuberculosis

Tuberculosis (TB) continues to be a major infectious burden worldwide. even though the availability of powerful Antitubercular drugs (ATD) such as rifampicin (RIF), isoniazid

(INH) and pyrazinamide (PZA) makes TB a curable disease, the latter is far from eradication, the main reason being that multiple ATD need to be a administered for 6-9 months. Chemotherapy of TB is complicated by the need of Multi-Drug Regimens that need to be administered over long periods. Poor patient compliance is the single most common reason for chemotherapy failure in TB (Na, et al., 2003; Singh, et al., 2010). To minimize toxicity and improve patient compliance, extensive progressive efforts have been made to develop various implant-microparticulates, and various other carrier-based drug delivery systems to either target the site of *M. tuberculosis* infection or reduce the dosing frequency, which forms an important therapeutic strategy to improve patient outcomes. The systems under discussion employ either biodegradable polymers or systems requiring removal after use, and can release the drug either by membrane or matrix-controlled diffusion (Gelperina, et al., 2005).

Recent trends in novel and controlled drug delivery have seen microencapsulation of pharmaceutical substances in biodegradable polymers as an emerging technology. Carrier or delivery systems such as Liposomes and Microspheres have been developed for the sustained delivery of anti-TB drugs and have demonstrated better chemotherapeutic efficacy when investigated in animal models (e.g. mice). Anti-TB drugs have been successfully entrapped and delivered in biodegradable polymers such as poly (DL-lactide-co-glycolide) (PLG), which are biocompatible and release drug in a controlled manner at therapeutic levels (Griffiths, et al., 2010).

Dutt and Khuller, have entrapped INH and RIF in PLG polymers. When injected subcutaneously as a single dose, the Microparticles, having a diameter ranging from 11.75 μm to 71.95 μm, provided sustained release of drugs over 6-7 weeks when tested in mice. Formulation of three frontline anti-TB drugs, i.e. RIF, INH and PYZ encapsulated in PLG nanoparticles (Dutt & Khuller, et al., 2011). On oral administration of drug-loaded nanoparticles to *M. tuberculosis*-infected mice at every 10^{th} day, no tubercle bacilli could be detected in the tissues after 5 oral doses of treatment. Therefore, oral Nanoparticle-based anti-TB drug therapy can allow for a reduction in dosing frequency for better management of TB (Pandey, et al., 2003).

Further attempts to solve the problems inherent in Multi-Drug Therapy have included the development of biodegradable polymeric micro- or nanoparticulate carrier systems to target alveolar macrophages that harbour *M. tuberculosis*. In the case of pulmonary TB, delivering the drug directly to the site of infection through inhalation of an aerosolized delivery system

has the inherent advantages of bypassing first-pass metabolism and maintaining local therapeutically effective concentrations with decreased systemic side effects(Zhou, et al., 2005). Because *M. tuberculosis* is known to infect alveolar macrophages and affect the pathogenesis of TB, there have been renewed interests in targeting of anti-TB drugs to these cells. Despite the success of these systems in targeting and providing sustained release of anti-TB drugs to alveolar macrophages, the methods used to generate particles in these studies vary in their capability for the production of reproducible particles with the optimal size for inhalation therapy (i.e. <5 μm)(Saravanan, et al., 2016). Encapsulated INH and RIF into hardened PLG Microparticles by a double emulsification solvent evaporation procedure, and these had a resultant volume mean diameter of 11.75 μm for INH Microparticles and 11.64 μm for RIF Microparticles. These are currently undergoing Phase I trials. incorporated both INH and RIF into PLG Microspheres using a combination of solvent extraction and evaporation, but these particles had a mean diameter of 6.214 μm and only 38% of the Microspheres fell in the size range of 0.5–3 μm (Pandey, et al., 2006).

Liposomes have long been known to serve as drug carrier. They are composed to a lipid shell surrounding an aqueous core containing the drug of interest. RIF/INH encapsulated Liposome out of physiological lipids. These Liposomes were modified so as to reduce their non specific uptake by the reticuloendothelial system and to make them lung specific**.** The preparation was stable, non toxic and a single intravenous (i.v.) administration produced therapeutic plasma/tissue drug level for 5-7 days. Further, weekly therapy for six weeks led to a significant reduction in mycobacterial counts in M.tuberculosis (Derycke, et al., 2004). The administration of ATD via the respiratory rout is an exciting possibility. Beside Liposomes work has been carried out with subcutaneous (s.c.) polymeric [poly DL- lactide-co-glycolide. PLG] Microparticle based ATD delivery systems. PLG is biocompatible, biodegradable and a single s.c. injection not only maintained therapeutic drug level for 6-7 weeks conceivably, PLG forms a depote at the s.c. injection site from where the drug is slowly released over a prolong time period. It is an ideal therapeutic system because the problems associated with drug stability in the intestine. Interference with food and absorption etc. area at once eliminated from the patients point of view, the only concerned with Liposome / PLG was whether patients would accepted injection over oral preparations. The discomfort associated with parental routs lead us to attempt development of PLG based oral drug delivery systems indeed (Dave, et al., 2016). It was shown that is was possible to dose and that a single oral administration of PLG- encapsulated drug (RIF+ INH+PZA)

could maintain therapeutic drug level for 5-9 days Moreover, with a few modification in the preparation methodology, We succeed in reducing the particle size (i.e., PLG Microparticle to PLG Nanoparticles). The Nanoparticle were remarkable advantageous in term of a high drug encapsulation efficiency. Higher oral bioavailability and last but not the least, complete bacterial clearance in infected mice following just five shots orally every 10 days. Interestingly, nanoparticles shared an important property with Liposomes and NLC that of nebulisation and this aspect is being further explored. NLC following intravenous administration rapidly accumulates in liver and spleen which is the main organ of Reticulo Endothelial System associated infection (Blasi, et al., 2007).

2.9 Nano Lipid Carrier's (NLCs)

Polymeric nanoparticles have been intensely investigated since their introduction by Speiser and co-workers in the mid-seventies. Despite their interesting properties, not many products made it to market because of the presence of solvent residues left over from production, the cytotoxicity of the polymers, and the lack of low-cost, qualified large scale production units yielding a product of a quality acceptable by the regulatory authorities (Müller, et al., 2007).
As an alternative, solid lipid nanoparticles (SLNs) were developed in 1991, a technology now owned by the drug delivery company SkyePharma. The matrix is a blend of solid lipids. SLN formulations have been developed, for example, for the oral delivery of cyclosporine and the intravenous delivery of Paclitaxel. Vector pharma/Trieste followed a similar route by developing SLNs from microemulsions. A new generation of nanostructured lipid carriers (NLCs) consisting of a lipid matrix with a special nanostructure has been developed. This nanostructure improves drug loading and firmly incorporates the drug during storage. These NLCs can be produced by high-pressure homogenization and the process can be modified to yield lipid particle dispersions with solid contents from 30–80% (MuÈller, et al., 2000).

2.10 Salient Features of NLC (Nano Lipid Carrier)

- NLC can entrap solutes in a manner analogous to Nanoparticles.
- NLC are particulate active and stable.
- NLC possess an infra structure consisting of hydrophobic and hydrophilic mostly together and so also accommodate the drug molecules with a wide range of solubility's.
- NLC exhibits flexibility in their structural characteristics (composition, fluidity and size) and can be designed according to the desired situation.
- NLC can improve the performance of the drug molecules by:

- Delayed clearance from the circulation.
- Better availability to the particular site, just by protecting the drug from biological environment.
- Controlled delivery of drug at a particular site.
- No special conditions are required for handling and storage of NLCs.
- NLCs solid and liquid lipids are biodegradable, biocompatible and non-immunogenic (Kulkarni, et al., 2017).

2.10.1 Types of NLCs

It is well known from the study of suppositories that highly ordered crystalline lipid matrices will lead to drug expulsion. Lipid nanoparticles and microparticles made from blends of solid lipids can experience this, especially when nanoparticles are prepared from highly purified lipids, for example, tristearin. The formation of highly ordered βi or β-modifications, particularly during storage, leaves little space for drug molecules, and the expulsion of drugs leads to drug crystals in suspensions and solid dosage forms. To avoid this problem, the particles should have a controlled nanostructure that offers enough space to accommodate the drug (Doktorovova, et al., 2017).

Four different approaches were taken for an optimized nanostructure of NLCs. In type I, solid lipids and liquid lipids (oils) are blended. The difference in the structures of the lipids and special requirements in the crystallization process leads to a highly disordered, imperfect lipid matrix structure offering space for drug molecules and amorphous clusters of drugs (I).

In general, drug solubility is higher in liquid lipids than in solid lipids. Based on this, particles were produced with a high content of liquid lipids (oils). During the production process, the liquid lipid particles (nanoemulsions) are cooled from the molten state to room temperature to crystallize and form solid particles (Pasinetti, et al., 2017).

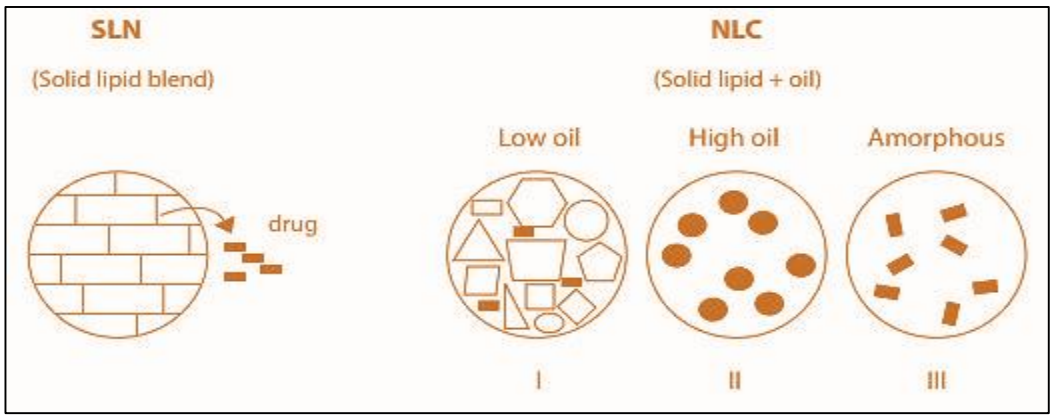

Fig:2.3 - Schematic diagram of Nano Lipid Carriers

At high oil concentrations a miscibility gap of the two lipids (solid lipid plus oil) occurs during the cooling phase, leading to phase separation, that means precipitation of tiny oily nanocompartments (II). In this multiple oil/fat/water, type II drug can be accommodated in the solid, but at increased solubility in the oily parts of the lipid matrix(Li, et al., 2017).

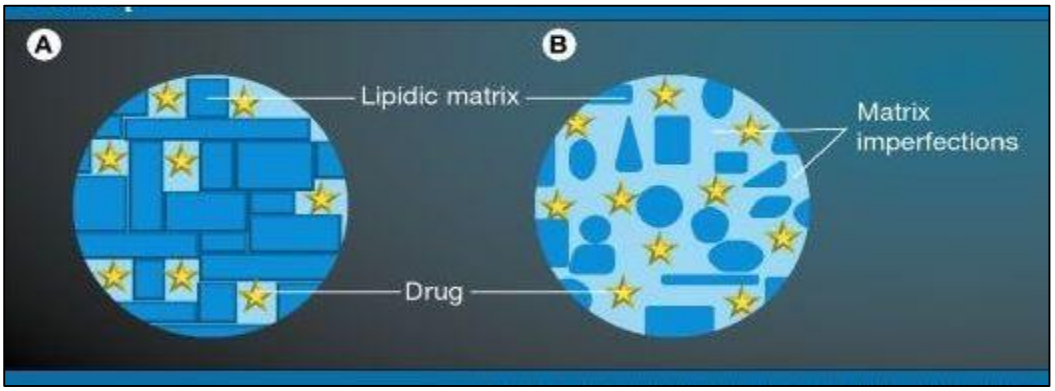

Fig:2.4 - (A) NLC with Lipid Matrix (B) NLC with Lipid Matrix Imperfections

In type III, lipids are mixed in a way that prevents them from crystallizing. The lipid matrix is solid, but in an amorphous state (III). The absence of crystallization avoids drug expulsion by crystallization. Lipid particles are preferentially suited to incorporate lipophillic drugs; hydrophilic drugs can only be incorporated at a low percentage (however, this is still sufficient for highly potent peptides and proteins). In a further variation of the lipid matrix, water-soluble drugs were conjugated with a lipid, thus forming a water-insoluble lipidic conjugate. The lipid conjugate powder was melted and processed in the same way as the other types to yield a lipid drug conjugate (LDC) nanoparticle(Wissing, et al., 2004).

Depending on the conjugate, this lipidic conjugate has a drug loading of up to 30–50% for water-soluble drugs. Conjugation is performed by salt formation or covalent linkage. Modulation of Drug Release Drug release from lipid particles occurs by diffusion and simultaneously by lipid particle degradation in the body. In some cases it might be desirable to have a controlled fast release going beyond diffusion and degradation. Ideally this release should be triggered by an impulse when the particles are administered(Selvamuthukumar, et al., 2012).

NLCs accommodate the drug because of their highly unordered lipid structures. By applying the trigger impulse to the matrix to convert in a more ordered structure, such a desired burst drug release can be initiated. NLCs of certain structures can be triggered this way; for example, when applying the particles to the skin incorporated in a cream.

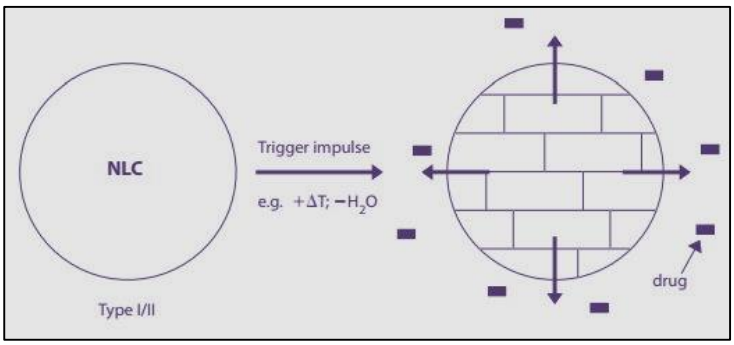

Fig:2.5- Diagrammatic presentation of drug release from NLC type I/II

Increase in temperature and water evaporation leads to an increase in drug release rate. Based on these cyclosporine-lipid particles are under development to treat psoriasis. The cream itself is saturated with cyclosporine, as well as a cyclosporine-loaded NLC contained in the cream. After application to the skin, accelerated release from the lipid particles should lead to a supersaturated system (similar to microemulsions, but without high surfactant concentration) leading to an improved penetration of cyclosporine into the skin(Sánchez-López, et al., 2017).

2.10.2 Long Term Stability

During long-term storage of dispersions, particle aggregation can occur. Aggregation and shell formation were reported for SLNs. Single particles diffuse in the dispersion medium; collision of particles can lead to perikinetic flocculation (Figure a). In the highly concentrated NLC dispersions the particles form a 'pearl-like network', thus the particles are in a fixed position and cannot undergo collision and perikinetic flocculation(Obeidat, et al., 2010).

After administration of the particles and dilution with fluids (gastrointestinal fluids, for example) the network is destroyed releasing single non-aggregated particles. Lipid particle dispersions were produced at identical surfactant concentration, but with low lipid content (below 30%, outside patent coverage) and with 35% lipid. The low particle dispersion aggregated during storage time, the gel-like NLC dispersion remained stable during storage and, after dilution, single particles were obtained showing no size increase(Yostawonkul, et al., 2017).

2.10.3 Preparation of NLCs

NLCs can be produced by various traditional dispersion techniques. The preferred production method is high-pressure homogenization. Up to approximately 60% solid content,

high-pressure homogenization can be applied alone to achieve solid contents of, for example, 80% when the multistep process is applied (Garg, et al., 2017).

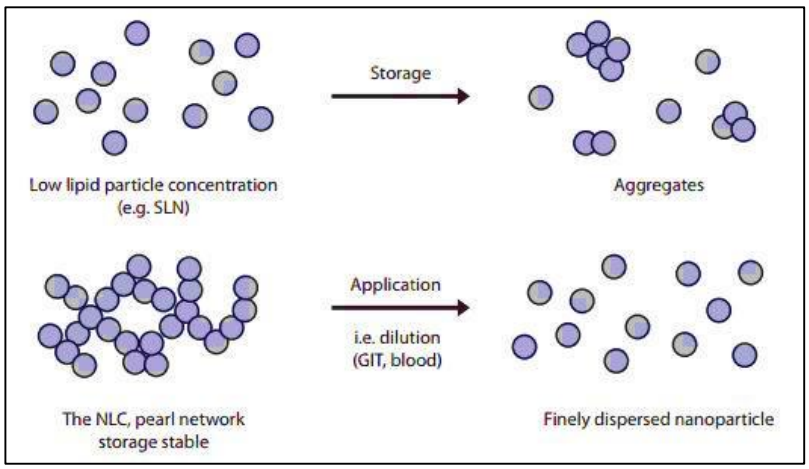

Fig:2.6- Storage and Application condition of SLN and NLC

First the lipid phase is melted. Then the drug is dissolved in the molten lipid thereby, preparing a drug containing lipid melt. This melt is then dispersed in an aqueous surfactant solution heated at the same temperature, using a high-speed stirring. The obtained pre-emulsion is then homogenized using a piston-gap homogenizer. Small lab-scale batches of 40 mL are produced using a discontinuous LAB 40 (APV Homogenizer GmbH, Germany). Larger batches up to 0.5 L dispersion are produced using a modified continuous LAB 40 homogenizer tower and the two product vessels possess temperature control jackets. The highly concentrated NLC dispersions are highly viscous, gel-like or pasty. These systems have no flowability the difference to conventional emulsions and SLN dispersions (Wang, et al., 2017).

To produce an 80% NLC dispersion, a multistep production process is applied. First, a 50% SLN dispersion is produced by high-pressure homogenization. One hundred grams of such dispersion contains 50 g of lipid and 50 g of water. In the next step, 10 g of lipid is added, which is dispersed by high-speed stirring in the remaining 50 g of water. This results in 110 g of dispersion containing 60 g of lipid (55%) and again 50 g of water. In the next step, another 10 g of lipid is dispersed in this 50 g of water phase and so on until a lipid content of 80% is reached. Large-scale production of NLC is easily possible. High-pressure homogenizers are available to process one ton and more per hour.

There are also no regulatory hurdles because these machines are accepted in production lines for parenterals, in parenteral emulsions for nutrition (Jain, et al., 2017).

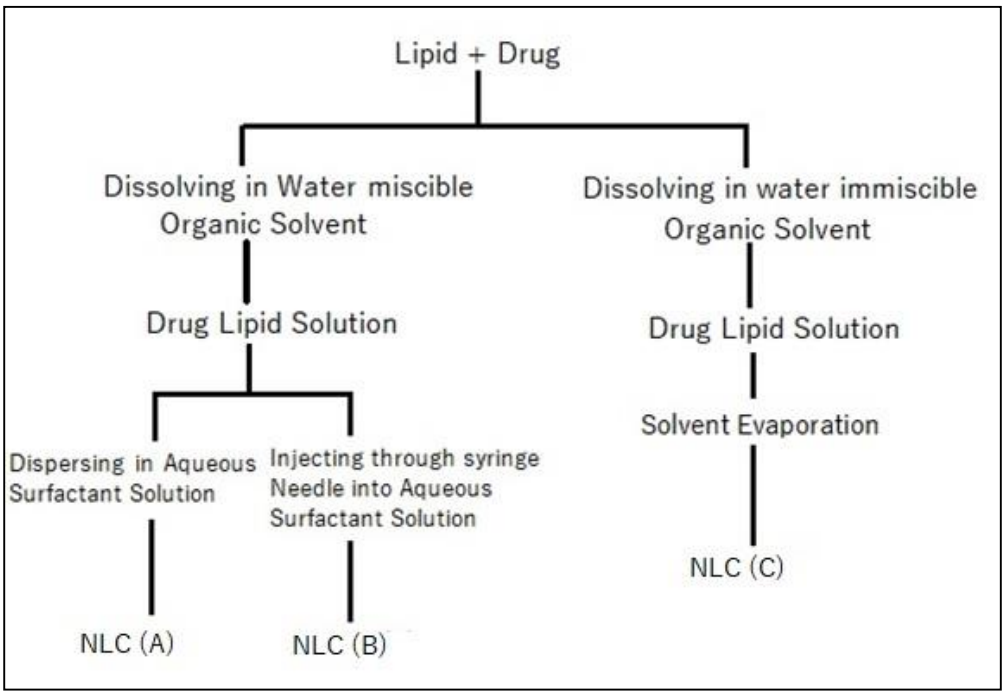

Fig:2.7- Line diagram of NLC Preparations

2.10.4 Application Areas

Oral administration of NLCs is definitely a very interesting and easy-to-realize area. The basic usefulness of lipid particles for oral delivery has been shown by the SLN-cyclosporine patents-NLCs has the potential to do an even better job. In addition, lipids promote the absorption of a range of drugs, also supporting the use of lipid particles for oral delivery (Jain, et al., 2017). Of special interest for oral delivery are lipid–drug conjugate (LDC) nanoparticles providing high loading capacities for hydrophilic drugs. Primary drugs of interest are compounds undergoing chemical degradation in the gastrointestinal tract. NLCs can be incorporated into traditional dosage forms such as tablets and pellets using the NLC dispersion as granulation fluid or wetting liquid for the pellet mass. NLCs produced in oil or polyethylene glycol (PEG) 400 can be filled directly into soft gelatin capsules (Gokce, et al., 2010). The second easy-to-realize area is topical application. All the lipids and surfactants used in traditional pharmaceutical creams can be employed, thus leaving little regulatory hurdles. Data are available showing delivery advantages of lipid particles compared to normal creams and ointments. Because of the high consistency of NLC dispersions, they can be used as topical dosage forms without further processing. First lines in parenteral delivery are controlled release forms (subcutaneous or intramuscular, for example) and the intravenous route. LDC nanoparticles have proved particularly useful for targeting water-soluble drugs to the brain (Kiparissides, et al., 2013).

2.11 In Vitro Characterization of NLCs

2.11.1 Particle Size

There are number of methods to determine size and distribution of the particle using electron microscopy, laser light scattering (quasi-elastic laser light scattering) or any other similar technique. These particles have been characterized by small angle X-ray scattering; freeze fracture electron microscopy and dynamic light scattering. On addition of dicetyl phosphate there is an introduction of change in the bilayer, which might increase the outer layer curvature and a slight decrease in the size of particle (Murdoch, et al., 2011).

2.11.2 Separation of Unentrapped Drug

This method is used to separate unentrapped drug from NLCs and is analogous to that used in 'Liposome technology'. The most commonly used methods are Gel filtration, dialysis and centrifugation (Souto, et al., 2007).

2.11.3 In vitro Drug Release

The release of drug from NLCs can be monitored by dialyzing a dilute NLCs Suspension/ Emulsion against a buffer at a definite temperature and determining the drug content of dialysate (Jaafar-Maalej, et al., 2011).

2.11.4 Stability and Toxicity of NLCs

Compared to Liposomes and vesicular systems against NLCs where NLCs are relatively stable structures some concern has been expressed regarding the stability of NLCs in vitro and their toxicity in vivo. Solid/ Liquid Lipids are used in the preparation of NLCs, which may be a cause of toxicity. However, there are virtually no reports available on the in vivo toxicity of NLCs linked with the concentration of ether or esters and Solid/ Liquid Lipids used in the preparation of NLC particle (Doktorovova, et al., 2017).

2.12 Endocytosis and Intracellular Digestion of NLC

Lipid molecule taken into the cell by receptor mediated endocytosis by the process of transferring the lipids are transferred to early endosomes where receptors are removed and returned to the cell surface. The lipid molecule are then moved to late endosomes and finally to lysosomes where digestion occurs.

Macrophages are phagocytic cells present either in circulating plasma or in endothelial lining and the reticular spaces of the connective tissue of mammals. They have a pivotal role in protecting the host against a wide variety of invading microorganism and developing

neoplasm through initialization of humoral and cell mediated immune response as well as intracellular oxidative or hydrolytic activity (Ferrari, et al., 2017).

2.12.1 Uptake of NLC at the cell surface

Phagocytosis - the ingestion of large particles by cells. The particles are taken into phagosomes or food vacuoles. This occurs primarily in specialized cells, e.g. macrophages, or protists such as *Amoeba* or *Paramecium*.

Pinocytosis - the ingestion of fluid and macromolecules via small particle < 150 nm in diameter.

Endocytosed macromolecules are sorted in Early Endosomes. Early Endosomes are relatively small particle that receive membrane and cargo from endocytotic particles. Early Endosomes are main sorting sites on the endocytotic pathway, as the Trans Golgi network serves this function in the secretory pathway. Endosomes receive a number of different types of receptors along with their cargo. Early endosomes have an acidic internal environment due to proton pumps in their membrane. The acidic environment causes many receptors or carrier lipid molecule to release their cargo of bound macromolecules(Walkey, et al., 2012). Macromolecules that are removed from their receptors are transferred to lysosomes for digestion. Most of the receptors are returned to the same plasma membrane domain (e.g. basal or apical) from which they came, others are passed to lysosomes and are destroyed. Macromolecules from early endosomes that are destined for degradation in lysosomes are passed in particle to Late Endosomes**.** Late endosomes also receive particle containing lysosomal enzymes from the trans Golgi network (Homman-Loudiyi, et al., 2003).

2.12.2 NLC as carrier for anticancer drugs

NLC encapsulation of vincristine enhanced the antitumoer activity against ehrlich's ascites and sarchoma- 180 models. Multiple dose of NLCs vincristine and methotrexate increase the survival rate of mice. Tumour volume of S-180 tumours increased rate of proliferation of sarchoma. Subsequent to macrophage activation using encapsulated muramyl dipeptide a more quantitative delivery of anticancer drugs to the tumour site could be achieved. Tumour drug level increase after macrophage activation. Subsequent to i.v. administration, the NLC encapsulated anticancer drugs were cleared from the plasma much more slowly than the free drug. A markedly enhance plasma drug concentration was archived in mice when it was administered in NLC (Narvekar, et al., 2014).

2.12.3 NLC as Carrier for Leishmaniasis Treatment

NLC which desirable release profile and stability characteristic can be prepared by tailoring surfactant chemically or via bilayer composition modification. However, the real therapeutic potentialities of a system can only be evaluate and established through in vivo experiment in animals followed by clinical trials (Das, et al., 2017).

2.12.4 NLC as Carrier for Immunological Adjuvant

The ability of NLCs to enhance antibody production in response to bovine serum alumina (BSA) was compared with freund's complete adjuvant (FCA) in the BALB/c mouse. The administration of NLCs bovine serum alumina BSA as two subcutaneous inoculum induced antibody levels comparable to those produced by FCA by subcutaneous or intraperitonial rout of inoculation. Intraperitonial administration however did not generate a stronger antibody response. The adjuvant activity of NLCs was found to be dependent on the BSA entrapped within the perform particle.

Tuberculosis is a leading killer of young adults worldwide and the global scourge of multi-drug resistant tuberculosis is reaching epidemic proportions. The tubercule bacillus is facultative parasite tuberculosis prominent in lungs but it is also present in liver cell, these intracellular mycobacteria are also largely protected against drugs. Consequently it is difficult to destroy the pathogen while leaving the host cell intact. The bacilli secrete the molecule that prevent phagosome lysosome fusion, moreover due to very hydrophobic waxy cell wall, bacilli are resistant to digestion by lysosomal enzyme and hence resist to killing effect of macrophage. The intracellular infection are difficult to eradicate with the use of conventional drug delivery system are found to be infected in achieving the therapeutic concentration in the cytosolic and it frequently resulted in dose related toxic side effect (Minz, et al., 2017).

2.13 Transmission of Tuberculosis

TB is transmitted almost exclusively by people with active pulmonary or laryngeal forms of disease, who expectorate bacilli in droplet nuclei as they cough, sneeze or talk. In poorly ventilated, enclosed environments bacilli can remain air born for several hours.

The Tuberculosis Bacillus is an intracellular pathogen. Transmission requires inhaled bacilli to reach the alveoli in the lung periphery and to be ingested by alveolar macrophage. Each macrophage then rapidly transports bacilli by the lymphatic system to the hilar lymph nodes and if, replication is not checked, infection can reached almost any other organ. Actively

replicating bacilli destroy their host cell and are liberated into the blood and lymph to invade other macrophages (Pearson, et al., 1992).

2.14 Clinical Presentation

TB can affect almost any organ (most commonly is lymph nodes), bones and joints, and the CNS, gastrointestinal and genitourinary systems. For this reason, the clinical Presentation of active disease can take many forms depending on the site (or sites) affected, the severity of the infection and the immune status of the host (Walters, et al., 2008).

Pulmonary disease is the most common clinical manifestation. Accounting for around 80 percent of cases. Only a proportion of these cases are infectious. Classically, patients with pulmonary TB presents with the following symptoms:-

- A cough of more than two weeks duration
- Weight loss
- Fever

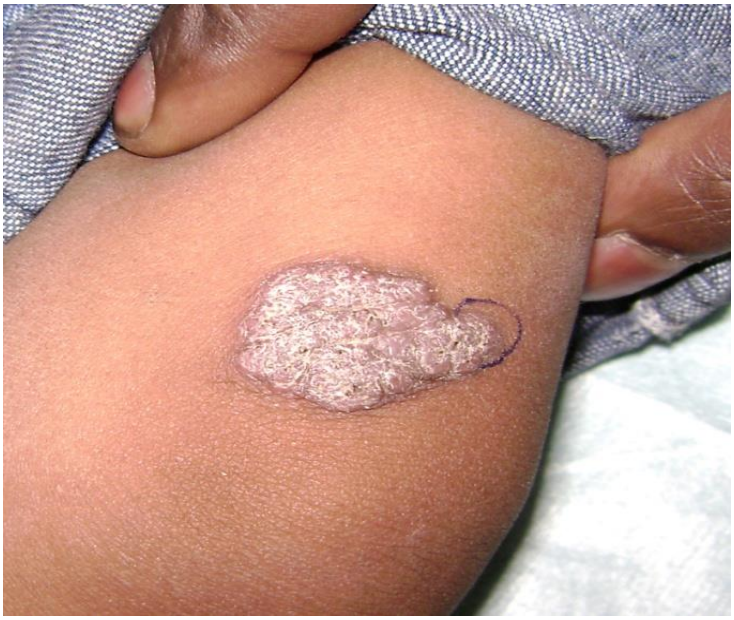

Fig: 2.8- Clinical Presentation of Skin Tuberculosis

http://www.pulmonologyadvisor.com/dermatology/tuberculosis-cutaneous/article/657836/

- Night sweats
- Fatigue
- Shortness of breath
- Chest pain
- Haemoptysis (coughing up blood) in later stages.

Tissue distraction can result in scarring and fibrosis and, if the infection is untreated or unchecked, erosion through blood vessels can occur leading to massive Haemoptysis. Although the availability of effective chemotherapy meant that surgical intervention become

a rarity, surgical excision is again being restored to, spastically in patients with multi- drug – resistant forms of the disease, because the bioavailability of Antitubercular drug with in cavities is poor (Ajmal, et al., 2017).

Children with TB can be asymptomatic and, more commonly, other organs are involved, particularly those of the CNS. If not treated promptly tuberculosis meningitis carries a high mortality and a high incidence of serious neurological squeal. Military TB is most common in infants and young children and occurs if the bacilli become blood born and disease becomes established in the lungs and other sites simultaneously. The term "military" is derived from the Latin word milium meaning millet seed and describes the characteristic multiple small lesions. Military diseases can occurs shortly after initial infection or as a result of post primary disease many years later. Often, the onset of military disease is insidious with a gradual development of weight loss, malaise, anorexia and low grade fever (Kuok, et al., 2017).

2.15 Co-infection with HIV

Approximately half the world's population is infected with Tuberculli bacilli. Nearly three forth of all case of active tuberculosis is in the developing countries on account of population size and frequent break down of CMI is due to HIV infection due to socio-economic reasons. This pattern of tuberculosis is distribution is expended to be altered with the advent and spread pattern of AIDS when the CMI breakdown is due to HIV infection. A clearer picture in this regard is yet to emerge. HIV infection is on the risk in groups having permissive sexual practice, i.v. drug abuse, high prevalence of tuberculosis e.g. the degree of HIV induce immune suppression required to reactive healed tuberculosis lesions required appear to be less than that needed to be more opportunistic pathogens to get a foothold. Diagnosis of Pulmonary Tuberculosis/extra Pulmonary Tuberculosis, if found associate with a positive serological test (ELISA) for HIV infection, is now regarded sufficient proof of the patient having AIDS (Gandhi, et al., 2010).

MAIC is the most common opportunistic mycobacterial infection in HIV seropositive person in a developed country. This may be because of very low prevalence of tubercular infection in these countries. Such infection, therefore may lead to the diagnosis of AIDS in those communities, perhaps next only to the appearance of active pulmonary tuberculosis. People with advance HIV infections are also at increased risk of disseminated disease involving multiple organs and similarly, present a significant diagnostic challenge. It is endemic in most developing countries and resurgent in developed and developing countries with high

rates of human immunodeficiency virus (HIV) infection. With particular reference to Africa, the increase in TB incidence is strongly associated with the prevalence of HIV infection: rates of HIV infection among TB patients are correspondingly high (Khan, et al., 2010).

2.16 Drug and Excipients specific review

2.16.1 Rifabutin

In the perview of Description it's a broad-spectrum antibiotic that is being used as prophylaxis against disseminated Mycobacterium avium complex infection in HIV-positive patients (Khachi, et al., 2009).

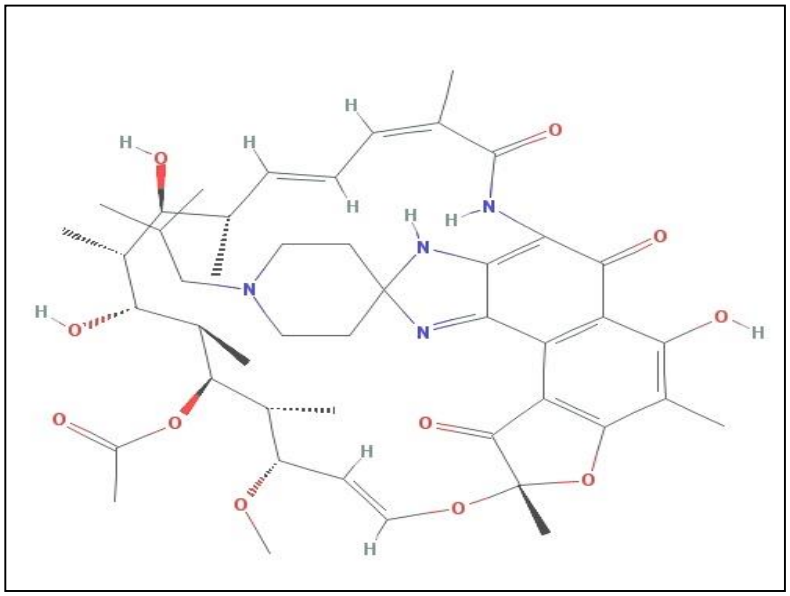

Fig:2.9- Structure of Rifabutin

Mechanism of action

Rifabutin acts via the inhibition of DNA-dependent RNA polymerase in gram-positive and some gram-negative bacteria, leading to a suppression of RNA synthesis and cell deathc (Sousa, et al., 2008).

Affected organisms

Mainly effected organisms by Rifabutin are as given below:-

1. Enteric bacteria and other eubacteria,
2. Mycobacterium tuberculosis
3. Mycobacterium leprae
4. Mycobacterium avium

Absorption

Rifabutin is readily absorbed from the gastrointestinal tract, with an absolute bioavailability averaging 20% (Nirbhavane, et al., 2017).

Metabolism

Through Hepatic metabolism the five metabolites that have been identified, 25-O-desacetyl and 31-hydroxy are the most predominant. The former metabolite has an activity equal to the parent drug and contributes up to 10% to the total antimicrobial activity (Sekar, et al., 2010).

Table: 2.4 - Physiochemical properties of Rifabutin

S. No	PROPERTY	VALUE
1	Protein binding	85%
2	Chemical Formula	$C_{46}H_{62}N_4O_{11}$
3	Half life	45 (± 17) hours
4	Clearance	0.69 +/- 0.32 L/hr/kg
5	Toxicity	LD50 = 4.8 g/kg (mouse, male)
6	Water Solubility	0.017 mg/mL
7	Melting Point	169-171^{0}C
7	log P (As Per IP)	4.25
8	log P (Actual)	4.19
9	log S	-4.7
10	pKa (Strongest Acidic)	7.93
11	pKa (Strongest Basic)	8.62
12	Physiological Charge	1
13	Hydrogen Acceptor Count	13
14	Hydrogen Donor Count	5
15	Polar Surface Area	205.55 Å^2
16	Rotatable Bond Count	5
17	Refractivity	232.64 m^3·mol^{-1}
18	Polarizability	90.72 Å^3
19	Number of Rings	6

Route of Elimination

A mass-balance study in three healthy adult volunteers with 14C-labeled rifabutin showed that 53% of the oral dose was excreted in the urine, primarily as metabolites. About 30% of the dose is excreted in the feces (Winter, et al., 2004).

Food Interactions

High-fat meals slow the rate of absorption. Take with food to reduce irritation.

Table:2.5-Market Preparation of Rifabutin

S.No	Dosage Form	Dose	Route of Administration	Manufacturer
1	Capsule	150 mg/1	Oral	Lupin Pharmaceuticals Pvt. Ltd.
2	Capsule	150 mg/1	Oral	Simpx Pharma Pvt. Ltd.
3	Capsule	150 mg/1	Oral	Omax Pharmaceuticals Pvt. Ltd
4	Capsule	150 mg/1	Oral	Pfizer, Ltd
5	Capsule	150 mg/1	Oral	Greenstone, Ltd

2.16.2 Precirol® ATO 5

Synonyms

Glycerin palmitostearate; glycerol palmitostearate; 2-[(1-oxohexadecyl)-oxy]-1,3-propanediyl dioctadecanoate and 1,2,3-propane triol; Precirol® ATO 5.

Chemical Name and CAS Registry Number

Octadecanoic acid, 2,3 di-hydroxy propyl ester mixed with 3- hydroxy-2-[(1-oxohexadecyl)-oxy] propyl octadecanoate [8067- 32-1]

Empirical Formula and Molecular Weight

Glyceryl palmitostearate is a mixture of mono-, di-, and triglycerides of C16 and C18 fatty acids.

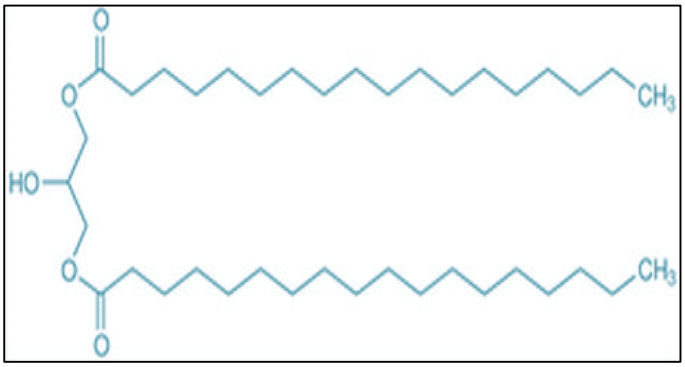

Fig:2.10 - Chemical Structure of Precirol ATO 5

Functional Category

Biodegradable material; coating agent; gelling agent; release modifying agent; sustained-release agent; tablet and capsule diluent; tablet and capsule lubricant; taste-masking agent.

Applications in Pharmaceutical Formulation or Technology

Glyceryl palmitostearate is used in oral solid-dosage pharmaceutical formulations as a lubricant. Disintegration times increase and tablet strength decreases with increase in mixing time. It is used as a lipophilic matrix for sustained-release tablet and capsule formulations (Sznitowska, et al., 2017). Tablet formulations may be prepared by either granulation or a hot-melt technique, the former producing tablets that have the faster release profile. Release rate decreases with increased glyceryl palmitostearate content. Glyceryl palmitostearate is used to form microspheres, which may be used in capsules or compressed to form tablets, pellets, coated beads, and biodegradable gels. It is also used for taste-masking.

Table: 2.6- Uses of glyceryl palmitostearate

S. No.	Use	Concentration %
1	Matrix for Sustained Release	10.0-25.0
2	Taste Masking	2.0-6.0
3	Tablet Lubricant	1.0-3.0

Incompatibilities

Glyceryl palmitostearate is incompatible with ketoprofen and naproxen.

LD50 (rat, oral): > 6 g/kg

Safety

Glyceryl palmitostearate is used in oral pharmaceutical formulations and is generally regarded as an essentially nontoxic and nonirritant material.

Handling Precautions

Observe normal handling precautions appropriate to the circumstances and quantity of material handled(Sabzichi, et al., 2017).

Regulatory Status

GRAS listed. Included in the FDA. Inactive Ingredients Database (oral Suspension/ Emulsion, oral tablet). Included in nonparenteral preparations licensed in Europe. Included in the Canadian List of Acceptable Non-medicinal Ingredients.

Description: Glyceryl palmitostearate occurs as a fine white powder with a faint odor.

Table: 2.7- Physiochemical Properties of Glyceryl palmitostearate

S. No.	Properties	Value
1	Acid value	<6.0
2	Boiling point	200^0C
3	Colour	<3 (Gardner scale)
4	Free glycerin content	<1.0%
5	Heavy metals	<10 ppm
6	Iodine value	<3
7	Melting point	52–55ºC
8	1-Monoglycerides content	8.0–17.0%
9	Solubility	Freely soluble in chloroform and dichloromethane practically insoluble in ethanol (95%), mineral oil, and
10	Unsaponifiable matter	<1.0%
11	Water content	<1.0%

2.16.3 Poloxamer 188

Chemical Nature: The Lutrol L and F- block co polymers are synthetic co-polymers of ethylene oxide and propylene oxide represented by the following chemical structure.

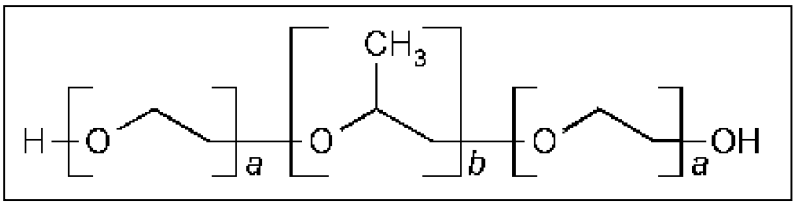

Fig:2.11- Basic chemical structure of Poloxamer

Discription: Lutrol L44 is milky white paste of liquid. The product contains d,l-alpha tocopherol as an antioxidant (Curry, et al., 2004).

Table:2.8- Representation of a and b blocks with its values

S. No	Lutrol	Poloxamer	a	b
1	L 44	124	12	20
2	F 68	188	80	27
3	F 87	237	64	37
4	F 108	338	141	44
5	F 127	407	101	56

The lutrol F-grades are white, coarse-grained powders with a waxy consistancy. They contain a appropriate quality of the antioxidant BHT.

Applications

Poloxamers are nonionic polyoxyethylene–polyoxypropylene copolymers used primarily in pharmaceutical formulations as emulsifying or solubilizing agents. The polyoxyethylene segment is hydrophilic while the polyoxypropylene segment is hydrophobic.

All of the poloxamers are chemically similar in composition, differing only in the relative amounts of propylene and ethylene oxides added during manufacture. Their physical and surface-active properties vary over a wide range and a number of different types are commercially available (Tagami, et al., 2015).

Poloxamers are used as emulsifying agents in intravenous fat emulsions, and as solubilizing and stabilizing agents to maintain the clarity of elixirs and syrups. Poloxamers may also be used as wetting agents; in ointments, suppository bases, and gels; and as tablet binders and coatings. (Serbest, et al., 2005) Poloxamers may also be used therapeutically as wetting agents in eye-drop formulations, in the treatment of kidney stones, and as skin-wound cleansers. Poloxamer 338 and 407 are used in solutions for contact lens care (Peng, et al., 2014).

2.16.4 Capmul® MCM C8 (Glyceryl Caprylate)

Capmul® MCM C8 is composed of mono and diglycerides of medium chain fatty acids (mainly caprylic). It is an excellent solvent for many organic compounds, including steroids. It is also a useful emulsifier for water-oil systems. It can develop from vegetable-based lipids and multifunctional specialty ingredients for Personal Care. Derived from renewable resources and materials are biodegradable.

2.16.5 Capmul® MCM EP

These products are specifically designed for meeting the solubility challenges of the pharmaceutical industry. These products can be used alone or in conjunction with one another to formulate a Self-Emulsifying Drug Delivery System (SEDDS). These are highly-functional, highly reproducible medium and long chain monoglycerides (MCM). The CAPMUL can be used alone as a primary solubilizer or in conjunction, as an emulsifier, with other ingredients.