CHAPTER 1

INTRODUCTION

1.1 TUBERCULOSIS

1.1.1 General Description

In the changing scenario of world, the science and technology play big role for making better life of human being but there are several challenges in the field of human health, among those challenges tuberculosis is one of the biggest challenge throughout the world.

On 26th September 2018, the United Nations (UN) had organized the first high level of meeting on tuberculosis (TB). The title of the meeting was **"Unite to End TB: An Urgent Global Response to a Global Epidemic"**. This highlighted that immediate action is required to step up the progress towards the goal of ending the TB epidemic by 2030. This meeting showed the alarming situation and indicated that the morbidity and mortality is also too high and affected large population especially in developing countries.

WHO (World Health Organization) in 1993 declared tuberculosis (TB) as a global emergence disease due to high immune compromised risk. Worldwide Tuberculosis is considered as one of the top ten causes of death and is caused by single causative organism *mycobacterium tuberculosis*. In 2017, WHO reported 1.3 million deaths (range 1.2-1.4 million) among HIV negative people and additional 3 lakh death among HIV positive people. About 10 million people developed TB disease in 2017: 3.2 million women, 5.8 million men, 1.0 million children as reported by WHO in 2018

WHO has listed 30 countries with high TB burden rate. Among them three countries contributed almost half of the world's cases of MDR-TB India (24%), China (13%), Russian Federation (10%). About 1.7 billion i.e. 23% of world's population are estimated to have

latent TB infection and so are at the risk of developing TB disease during their life time (WHO report., 2018).

1.1.2 History and Pathogenesis

In ancient time around 600 BC tuberculosis disease was known as wasting disease (Rajayakshma) or kshaya (*Rama P. T. et al.,* 2005). It was considered as "the king of disease" as found in "sushruta sanhitha" (Gandy M.*et al.*, 2002*)*. The causes of disease were overstrain, suppression (due to grief, anxiety) and Proscuous diet. All these causes overburst the imbalancing of vata, pitta, kapha and flare up TB (Barnes D.S. *et al*., 2000).The classical Indian system of health and healing has provided treatment based on principles of Ayurveda, in which medicines and dietary prescriptions were detailed. Alcohol in moderate quantities, the flesh of birds and animals, goat milk were some of recommended items for treatment. Till the second half life of 19th century, the TB cases were very rare but since then TB incidences increased progressively due to increased growing population density caused by industrialization (Niharika J. *et al*., 2011).

The major challenges of TB control can be classified in five area: HIV co infection, inadequate diagnosis and treatment, more efforts in Directly Observed Therapy short course program and multidrug resistant tuberculosis (Sally M. *et al.,* 2006, Ramachandran R. *et al., 1999).*

Tuberculosis is highly contagious disease caused due to different strains of Mycobacteria usually *Mycobacterium tuberculosis*. The causative organism i.e. Tubercle bacilli are rod shaped, slender, aerobic, non-motile, acid fast positive bacilli (Shegokar R. *et al., 2011*). The bacillus has high lipid content in outer membrane and lacks cell wall. It divides with an extremely slow rate than other bacilli i.e. divide in 16-20 hrs. *Mycobacterium tuberculosis* was first identified and isolated by Robert Koch a German physician in 1882. He received noble prize for his great discovery.

The disease is highly contagious as it spreads due to expelled viable tubercular bacilli (coughing, sneezing, spitting, shouting or singing) by patients with active TB. The viable bacilli contaminate the air and infect the person who is exposed to it. During inhalation the

bacilli may enter in the body or may invade through epithelial surface (Dannenberg A.M *et al., 1993).* The inhaled viable bacilli get inoculated into the respiratory bronchioles, alveoli (apices of lungs) as these are the high oxygen pressure area (Daniel T.M *et al., 1994).* The entered pathogen multiplies at these sites and provoke the antigen antibody reaction as a result tubercle is formed by accumulation of macrophages at the infected site. Being a part of immune system the macrophages normally take up invaders (tubercle bacilli) by phagocytosis. The engulfed pathogen via phagosome fuses with lysosome and kill the foreign material by oxidative, non-oxidative killing mechanism favored by enzymes phagolysome. However, *Mycobacterium tuberculosis* overcome the macrophage digestion and reside in macrophage as a dormant bacilli. The dormant bacilli in macrophages are called latent bacilli (Shiratsuchi H *et al.,* 2000). These latent bacilli are carried by circulatory system to different parts of body and may turn to active form under weak host defence mechanism or when macrophages die. The transformation from latent to active bacilli may happen immediately, months or years later (Schmitt E *et al.,* 1977). The liberated active bacilli are fetched by blood vessels or lymphatic channels to distant tissues and organs which can also infect other persons.

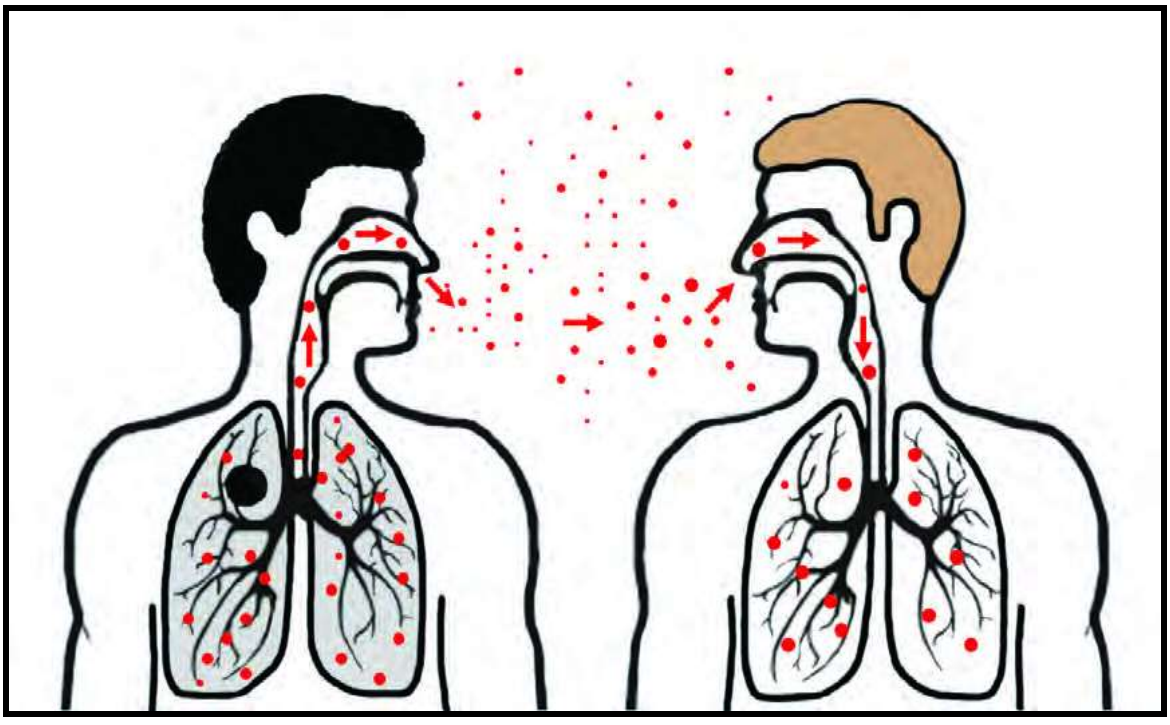


Fig 1.1 Transmission of Tuberculosis

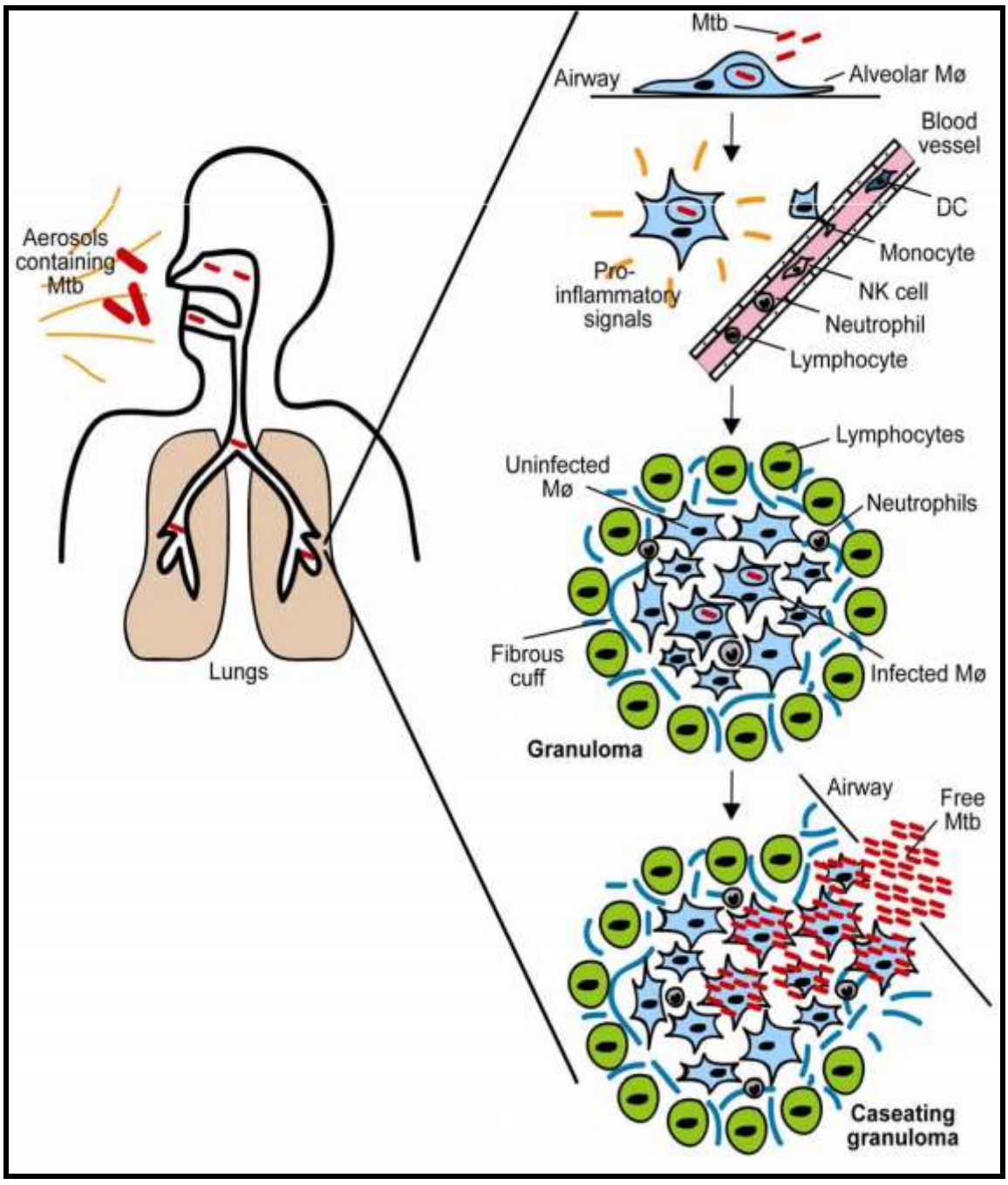


Fig 1.2 The inhalation of *Mycobacterium tuberculosis* (Mtb) in host causing formation of granuloma. Resident alveolar macrophages(Mϕ) phagocytose, the inhaled bacteria. This activates the immune system and lead to pro inflammatory response and formation of granuloma. The dormant bacilli within granuloma retain for long time, but if immune system weakens or fails, the bacilli proceeds replication, break granuloma core and Mtb spilled into the airways as active TB.

1.1.3 Characteristics of *Mycobacterium Tuberculosis* Bacteria

- Shape :Rod-shaped
- Size: 1-5 microns
- Nature: Aerobic
- Multiplication rate: Grows very slowly (divides once in every 15 to 20 hours)
- Special feature: The cell walls of Mycobacterium tuberculosis is composed of high lipid content. Therefore, it requires specific laboratory methods to identify TB bacteria in smear examinations (acid-fast staining) and in culture (mycobacterial culture versus routine bacterial culture).

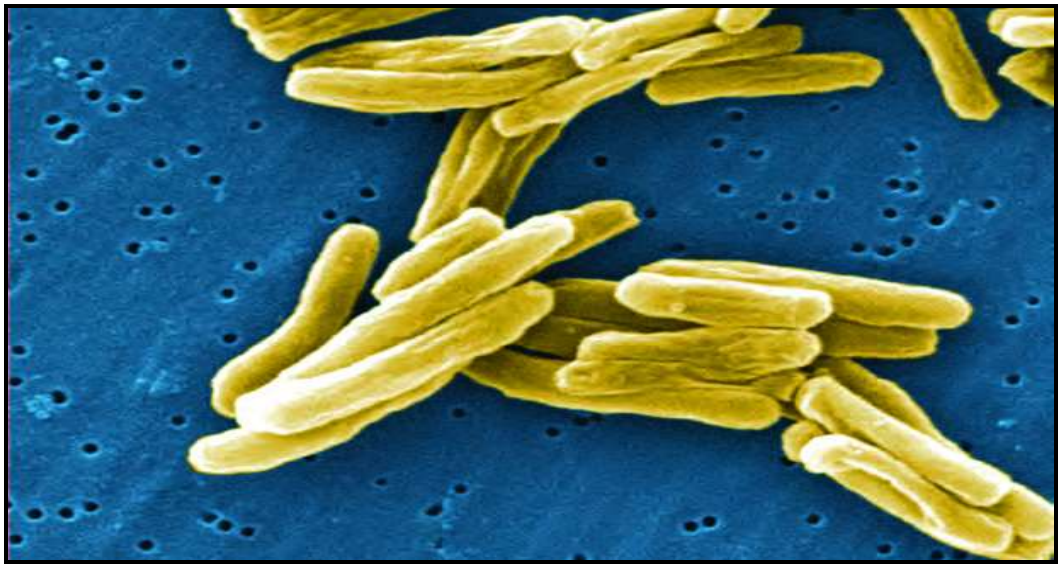


Fig 1.3 *Mycobacterium Tuberculosis*

1.1.4 Epidemiology

Tuberculosis generally affectes the human from the starting of their history and continues as one of the leading causes of death worldwide, despite of fruitful and affordable medicines more than 50-60 years ago (Holloway K.L *et al*., 2011, Cosmas L. *et al*., 2013). The overall prevalence of HIV infection in India is less than 1 percent, so India continues to be in the category of low prevalence countries (NACO). As per estimates in year 2003, about 5.1 million people were infected with HIV in India. The disease accounts for about 13 percent

death due to HIV co-infection in India. Rate of HIV infection among TB patients has been lowered (Padayatchi *et al., 2008*). Survey of India reported HIV positivity rate ranging between 0.5-20% (Paranjape R. S *et al*., 1997). Approximately 0.5 million patients die every year due to pulmonary TB disease. Scientists are trying to find out cause of death such as degenerative disease, oxidative stress, and antioxidant status.

In year 2000-2015, mortality rate has fallen down from 55 to 36 per 0.1 million populations per year. The estimated death in 2015 due to TB is approximately 480 thousand (www.dnaindia.com). Extra pulmonary TB accounts for 15-20% of overall TB case; skeletal TB comprises about 10% of these cases. TB spondylitis accounts for 50% of the skeletal TB cases. Hence overall osteoarticular TB represents 1-2% and TB spondylitis represents 0.5-1% of all TB cases (Polley P. *et al.,* 2009). The Skeletal TB is more likelihood to be in immunosuppressed persons. The World Health Organization (WHO) global report 2015 estimates that there were 480,000 pulmonary MDR-TB cases world wide and 15000 cases of MDR-TB in the eastern mediterranean region in 2014, but there is no mention of the incidence of extra pulmonary TB.

1.1.5 Types of TB

1.1.5.1 Latent TB: The latent TB means that causative organism is residing in body but the immune system stops them from spreading. Such condition doesn't show any symptoms but is contagious i.e. infection is alive in body and can one day become active. In some cases there are high risks of reactivation especially in immune-compromised persons.

1.1.5.2 Active TB: This means that causative organism replicates fast and make person symptomatic. Such symptomatic patients are susceptible to spread disease to others. The most of the active TB cases are originated from reactivation of latent TB infection.

1.1.6 Symptoms and Diagnosis

The classic symptoms are chronic cough with blood stained sputum, fever, night sweats, weight loss, anorexia and weakness. Infections to other organs cause wide range of

symptoms. Diagnosis involves radiology (chest X ray), Tuberculin test, blood test as well as microscopic examination and morphological culture of body fluids

Table 1.1 Different diagnostic approaches for Tuberculosis

	Diagnostic method	Advantages, Disadvantages
1	Chest radiology	Less sensitive method, need trained staff for imaging
2	Culture growth	Sensitive method, require weeks to diagnose & conclude, biosafety lab required as contagious disease.
3	Symptom based diagnosis	Done only in active bacilli, not possible with latent bacilli (symptoms not found)
4	Tuberculin skin test (Subcutaneous route)	Standard test for latent TB, inexpensive, measures immune response to PPD (purified protein derivatives) antigen, Individual previously exposed to pathogen (e.g. latent TB) exhibit immune response and induration in fore arm, require at least two visit to clinic. Drawback: a) False positive result in previously vaccinated person with BCG vaccine b) False negative results in patient co-infected with HIV low T cells
5	Blood based diagnosis	
A	IGRAs test (interferon gamma release Assay)	Highly specific, measurement of INF ϒ by T-cells on exposure to TB antigen
B	Elisa test / Quantiferon test(Enzyme linked immune assay)	Measures INF ϒ by T-cells on exposure to PPD (purified protein derivatives) antibody mixture.
C	Quantiferon Gold /Elisa test	Measures INF ϒ by T-cells on exposure to RDI antigens (CPF-10 and ESAT-6) superior to PPD antibodies due to specificity towards *M. tuberculosis.* Interferences not by BCG vaccination or patients co-infected with HIV

1.1.7 Antitubercular therapy and therapeutic failure

The control of TB stands between preventive measures i.e. vaccination or chemotherapy (antibiotics). Vaccination is considered as preventive measure because it has ability to enable the body to respond the invading microbes. Currently available vaccine for TB is BCG i.e. Bacillus Calmette Guerin. Many drawbacks are associated with vaccine: variable efficacy in different population, short term immunity, has limited success against Pulmonary TB, which accounts for most of the disease burden. On the other hand, chemotherapy also gives opportunity to treat disease. Very first effective treatment was found in 1940 with the introduction of streptomycin. Despite globalization, very few drugs have been introduced over the last four decades (Kuo M.R *et al.*, 2003). Existing drugs has many limitations like drugs need to be administered with high dose due to poor bioavailability, premature degradation, low solubility, intestinal malabsorption (sosnic A *et al.*, 2010) therefore tubercular therapy needs long term treatment with daily multiple antitubercular drug.

The patient compliance for long term treatment is very difficult as the drugs fail to effectively target pathogen, having high percentage of side effects (ototoxicity, neurotoxicity), changes in patient life style. All these causes low adherence and low compliance towards treatment regimen and brings therapeutic failure (Blumberg H.M *et al., 2005*). WHO adopted DOTS (Directly Observed Treatment and Short-course drug therapy) programmes where patients are observed when they take their medication to ensure compliance, as non-compliance is a major contributor to the development of antibiotic resistance. Even though strategy has not given the complete solution for patient noncompliance (Karakousis P *et al.*, 2012).

Therapeutic failure is main cause of MDR TB (multi drug resistant TB) and XDR TB (Extensively drug resistant TB). Management of drug resistant TB is really a challenge, treatment requires very expensive as well as highly toxic second line agents. Most of these injected intravenously for about 2 years but response of treatment is poor and mortality rate is high (Singh M M *et al.*,2002).Prevention of therapeutic failure is the key to control resistant cases such as MDR TB, XDR TB or new drugs and treatments are urgent need of MDR TB as

well as XDR TB as these strains are virtually untreatable which cause of high lethality in infected individual (Alladi Mohan *et al.,* 2013).

Table 1.2 Types of TB (Iseman M.D *et al.,* 1993)

Active TB	Symptomatic, bacteria multiply and spread in body and causes tissue damage
Latent TB	Usually Asymptomatic, bacteria exist in dormant phase, Phase can last even decades.
MDR TB	TB in which bacteria are resistant to at least two first line drugs INH and RMP. Primarily reason patient not taking their full regimen of antibiotics, which allow mutating and developing resistance to drugs.
XDR TB	XRD TB is resistant to isoniazid and rifampicin plus any fluoroquinolone and atleast one of three injectable second line drugs (i.e amikacin, kanamycin or capreomycin). XRD TB is resistant to first line and second line drugs, Patients are left with treatment options that are more toxic, more expensive and much less effective.

Table 1.3 List of first line antitubercular drugs (Vedha H.B.N. *et al;* 2010**)**

Drug	**Dose(mg)**	**BCS Class**
Isoniazid	300	III
Rifampacin	300	II
Pyrazinamide	500	III
Ethambutol HCl	400	III
Rifabutin	150	II

Table 1.4 List of second line anti tubercular drugs

Drug	Dose(mg)	BCS Class
Ethionamide	500	II
Clarithromycin	500	II
p-Aminosalicylic acid	500	Na
Cycloserine	500	IV/II
Amikacin	1000	III
Kanamycin A	1000	N/A (iv or I.m)
Capreomycin	1000	N/A (iv or I.m)
Levofloxacin	500	Na

Some approaches for complete eradication of tuberculosis are: (Barry C. E *et al.*, 2004)

1) To develop newer or derivatives of existing drugs in more potent compounds.

2) Screening of compounds active against replicating as well as latent bacilli.

3) Develop new drug delivery system as novel drug target.

4) Identifying and targeting the host pathogen via drug delivery approaches such as nanoparticles, liposomes, niosomes, dendrimers etc.

1.2 NANOPARTICLES

The term nano has originated from Latin word which means dwarf. The ideal size range offered by Nanotechnology mention one thousand millionth of particle unit i.e. nanometer is one thousand millionth of a meter ($1nm=10^{-9}m$). Nanotechnology is the branch of science that deals with matters that occur at molecular level and of nano scale size (Sovan L.P *et al.*, 2011). Recent exploration of nanotechnology in biomedical and pharmaceutical science resulted a fruitful improvement in conventional means of drug delivery system. It had

great potential impact in sub-fields of medicine like cardiology, endocrinology, immunology, ophthalmology etc. Along with this the technology is highly utilized in specialized areas like in targeting and gene delivery field.

Nanotechnology provides better safety profile against drugs which have high toxic potential. These nano forms can be effectively directed to target tissues by active or passive targeting. Although these nano-materials can easily bypass the biological membrane and reach to target site, but the potential of nanomaterial for intracellular targeting can be much improved by using multifunctional nano-material (ligand based nanocarriers). The nanoparticle based specific drug targeting and delivery problem reduce toxicity, other side effects and improve therapeutic index of targeted drug. This advanced technology provides the opportunity to overcome multidrug resistant problem. These nano sized targeted delivery approaches have following benefits:

- Increased drug targeting potential
- Increased patient compliance
- Reduced dose requirement
- Suitability for incorporation of both hydrophobic and hydrophilic drugs
- Increased surface area and rate of dissolution
- Increased oral bioavailability
- Reduced toxicity
- Reduced possibility of drug resistance

The range of acceptable nanoparticle size for optimized chemotherapy is highly dependent on construction material and development method. The nanoparticles exist from polymeric to inorganic to lipid based formulation (Stephanie T *et al*., 2017).

The advantage of using nanoparticles as a drug delivery system includes the following (Rajesh S *et al.*, 2009):

- Particle size and surface properties of nanoparticles can be easily manipulated
- Both active and passive targeting is possible
- Controlled or sustained release of drug
- The enhanced therapeutic efficacy and reduced toxic effects as it alters the distribution of drug and successively clearance of drug

- The drug release and particle degradation properties can be easily modified by option of suitable matrix constituent
- Drug loading is comparatively high. Drug incorporation into nanosystem without any chemical reaction is best way to preserve drug activity
- The attachment of target ligands on the surface of nanoparticles or use of magnetic guidance promotes site specific targeting
- Applicable for various routes of administration such as oral parenteral, nasal, transdermal, ocular etc
- Nanoparticles with targeting approach reduce drug toxicity and provide efficient drug distribution
- The resistance offered by different physiological membrane can be easily overwhelmed by nanoparticles. They can easily by pass the biological membrane and reach to target site

Regardless of several advantages, nanosystem possesses some limitations too (Sriharitha *et al*, 2016).The small particle size and large surface area favors particle-particle aggregation, along with this physical handling of nanoparticle is difficult in dry and liquid form. The small particle size also limits the drug loading and results in burst release (Carina S. *et al.,* 2016). Thus before commercialization and clinical use of these nanosystems the associated practical problems should be overcome (Brajesh K.*et al*., 2016).

1.2.1 Ideal properties desired for nanoparticulate drug delivery system (Siddhi P. *et al.,* 2015)

- Should be stable in blood
- Non-toxic
- Non immnogenic
- Non thrombogenic
- Non inflammatory
- Biodegradable
- Bypass reticuloendothelial system.
- Should be good carrier for all type of drug molecules
- Manufacturing should be easy, feasible and inexpensive

1.2.2 Classification of nanoparticles:

There are various approaches for classification of nanoparticles. Nanoparticles can be classified on the basis of their dimensions (Sovan L. P *et al*., 2011).

1) One dimension nanoparticles: One dimensional system thin films, manufactured surfaces or coatings has been used for many decades in various fields such as in electronics, chemistry, engineering. They are applied for purpose corrosion resistance, wired scratch resistance, hydrophobicity, self-cleaning, dirt repellant, catalytic activity etc.

2) Two dimension nanoparticles: Nanotubes, nanowires, nanofibres and nanopolymers are two dimensional nanoparticles.

Carbon nanotubes: Carbon nanotubes are unique form of carbon molecules. These are hollow cylinder with diameter of 0.7 nm (approx.) formed by hexagonal network of carbon atoms. The nanotubes exist in two forms i.e. as a single layer just like straw or many layers of coaxial cylinders. Depending upon layers in nanotubes the diameter varies in common axis. Nanotubes have remarkable physical, mechanical and electrical properties, which enhances its stability (Micheal K *et al*., 2004).

3) Three dimension nanoparticles

Fullerenes, dendrimers, quantam dots are three dimensional nanoparticles.

a) Fullerenes

The Fullerenes are hollow balls consisting of interconnected carbon hexagons and pentagon looking like a soccer ball. These spherical cages are formed from 28 to more than 100 carbon atoms. They possess unique physical properties as they regain its original shape even after exposure to extreme pressure.

b) Dendrimers

It is a novel discovery in nano-metric dimension. Dendrimers are composed of controlled structure polymers which extend from central core to outwards radially forming three dimensional macromolecule. They are mainly formed from branches upon branches structural designs. They normally range 2-10 nm in diameter, with approximately spherical shape. The structure of dendrimers consist of three apparent architectural zones 1) core or focal moiety 2) zone of branched repeated units extending from core 3) functional end groups on the outter layer of repeated units. They are recognized to be robust, covalently fixed, three dimensional

structure possessing both solvent filled interior core and exterior surface extremities (Svenson *et al*., 2005)

c) Quantum dots

These are special form of spherical nanocrystals from 2-10nm in diameter. They are synthesized by colloidal synthesis or electrochemistry method using various types of semiconductor materials. Quantum dots are semiconductor nano-crystals and core shell nanocrystals with interface between different semiconductor materials. Due to unique fascinating optical properties it gained popularity in biomedical field especially for multiplexed, quantitative and long term fluorescence imaging and detection (Smith A M *et al.*, 2006).

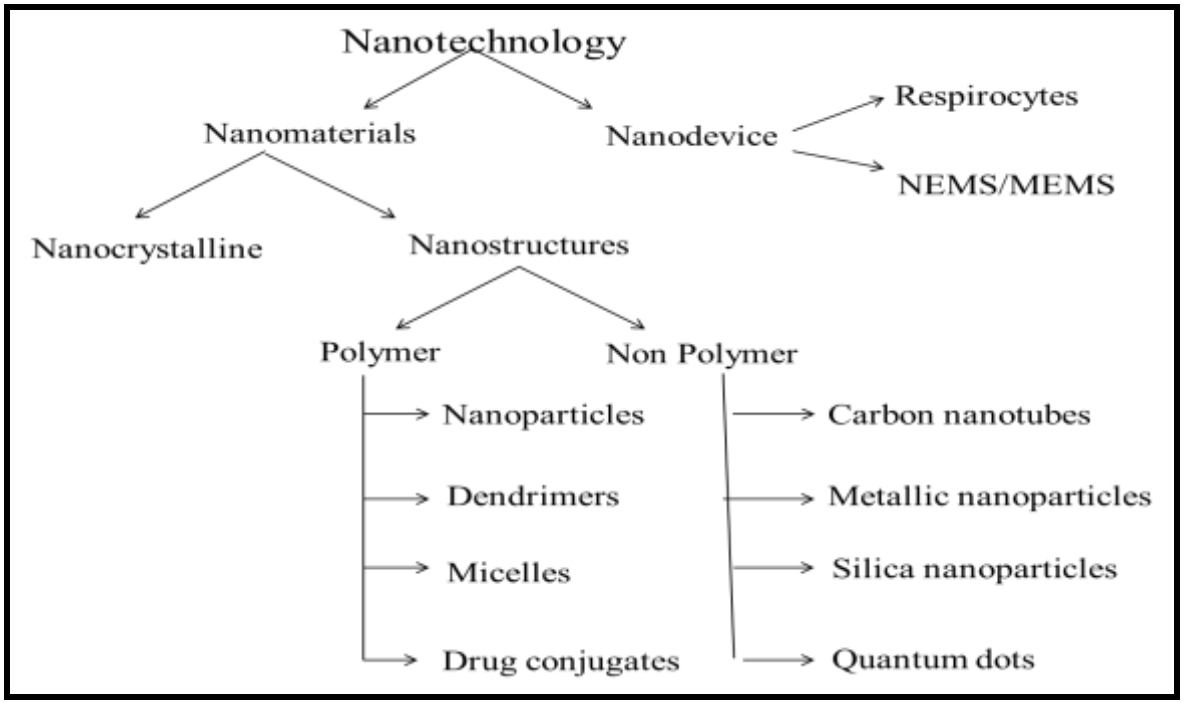


Fig 1.4 Various types of pharmaceutical nanosystems

1.5 Brief characterstics and applications of various types of Nanosystems (Nahar M. *et al.*, 2006).

Types of nanosystem	Size (nm)	Characterstics	Applications
Carbon nanotubes	0.5-3 diameter 20-1000 length	Third allotropic crystalline form of carbon sheets either single layer or multiple layers. These crystals have uncommon strength and unique electrical properties (conducting, semiconducting, insulating)	Functionalization enhanced solubility, penetration to cell cytoplasm and to nucleus, as carrier for gene delivery, peptide delivery.
Dendrimers	< 10	Highly branched, nearly monodisperse polymer system produced by controlled polymerization. Three main parts are Core, branch, surface.	Long circulatory, controlled delivery of bioactives, targeted delivery of bioactives to macrophages, liver targeting.
Liposomes	50-100	Phospholipid vesicles, biocompatible, versatile, good entrapment efficiency, offer ease in formulation.	Long circulatory, offer passive and active delivery of gene, protein, peptide and various other.
Metallic nanoparticles	< 100	Gold or silver colloids, very small size resulting in high surface area available for functionalization, stable.	Drug and gene delivery, highly sensitive diagnostic assays, thermal ablation and radiotherapy enhancement.
Nanocrystals Quantum dots	2-10	Semi conducting material synthesized with II-VI and III and V column element. Size between 10 -100 A^o; Bright fluorescence, narrow emission, Broad UV excitation and high photostability	Long term multiple color imaging of liver cell; DNA hybridization, immunoassay; receptor mediated endocytosis; labeling of breast cancer marker.
Polymeric micelles	10-100	Block amphiphilic copolymer micelles, high drug entrapment, pay load, biostability	Long circulatory, target specific, active and passive drug delivery, diagnostic value

continued on page no. 16

Polymeric nanoparticles	10-1000	Biodegradable, biocompatible, offers complete drug protection	Excellent carrier for controlled and sustained delivery or drugs .Stealth and surface modified nanoparticles can be used for active and passive delivery of bioactives

1.2.3 Preparation of nanoparticles

The choice of appropriate method for manufacturing of nanoparticles depends on active constituent to be loaded and physicochemical properties of polymers. The materials normally used in preparations of nanoparticles are proteins, polysaccharides and synthetic polymers. The selection of matrix material is very crucible during formulation as it affects various parameters.

- Antigenic property of final product
- Biocompatibility and toxicity
- Degree of biodegradability
- Drug release profile
- Inherent properties of the drug
- Size of nanoparticles
- Surface characteristics

Nanoparticles are usually prepared by three methods:

- Dispersion of polymers
- Coacervation / controlled ionic gelation of hydrophilic polymers
- Polymerization of monomers

1.2.3.1 Dispersion of polymers

The method involves the preparation of biodegradable nanoparticles by dispersion of biodegradable polymers (PLG-Poly (D, L lactide-co-glycocide); PCA-poly (Cyanoacrylate); PLA poly (lactic acid) in suitable solvent.

1.2.3.1.1 Solvent evaporation method

In this method the polymeric solutions are prepared in volatile organic solvents (chloroform and dichloromethane) and active moiety is dispersed in aqueous solvent. Emulsions are formulated by high speed homogenization or ultra-sonication of both phases. The solvent of polymeric emulsion is evaporated i.e. diffusion of continuous phase of emulsion in order to convert emulsion to nanoparticle suspension. The conventional method involves two steps for formation of emulsions. Preparation of single emulsion e.g. oil in water (o/w) or double emulsion e.g. (water in oil) in water (w/o/w). (Mohanraj V. J. *et al*., 2006).

1.2.3.1.2 Solvent displacement technique/ Nanoprecipitation

In solvent displacement technique the polymers are dissolved in organic, water miscible solvent and further added to aqueous phase with or without surfactant. This addition of organic solvent from the oil phase to aqueous phase causes incompatibility immediately with the precipitation of polymers and formation of nanospheres. The solvent diffusion towards the aqueous phase, generates nanoemulsions causes polymer to precipitate uniformly within nanoemulsion template. Due to immiscibility of solvent with aqueous phase the method is suitable for hydrophobic drugs. The method is not suitable for encapsulation of water soluble drugs.

1.2.3.1.3 Solvent diffusion method

In solvent diffusion method the matrix polymer in partially water soluble solvent saturated with water to ensure the initial thermodynamic equilibirium of both liquids. Further the saturated solvent phase is emulsified in an aqueous solution containing stabilizer. This leads to diffusion of solvent from external phase and causes the formation of nanospheres or nanocapsules. At last the solvent is removed by evaporation based on its boiling point.

1.2.3.1.4 Salting out

The method involves the separation of water miscible solvent from aqueous solution by salting out technique. The polymer and drug are dissolved in acetone (water immiscible

solvent), subsequently emulsified into aqueous gel containing salt out agent. It is considered as modified version of emulsification / solvent diffusion method.

1.2.3.1.5 Dialysis

The method offers the simple, effective and efficient method for the preparation of narrow ranged small polymeric nanoparticles. The method involves the solvation of drug and polymer in suitable organic solvent (Acetone or Dimetyl formamide). The resulting solution is placed in dialysis tube of appropriate molecular weight cut off and dialyzed against deionized water. Due to displacement of solvent inside the dialysis membrane there is loss in solubility of polymer which causes slow aggregation of polymer and formation of homogeneous suspension of nanoparticles. Fresh water is introduced at suitable intervals for dialysis. The method involves the use of physical barrier such as dialysis membrane or semipermeable membrane. These physical barriers permit the passive transport of solvents and exchanged solvent promote the aggregation of polymer in non-solvent.

1.2.3.2 Preparation of nanoparticles by polymerization of a monomer

1.2.3.2.1 Emulsion polymerization

Emulsion polymerization is one of the fastest and feasible method for preparation of nanoparticles. It is classified into two categories, based on the use of an organic and aqueous continuous phase. The continuous organic phase process involves the dispersion of monomer into an emulsion or micro emulsion or into a material in which polymer is not soluble (nonsolvent). This procedure is not popular as it requires toxic organic solvents, surfactants, monomers, initiators, which are subsequently removed after the formation of nanoparticles. The alternative aqueous continuous phase method is more acceptable. In this method the monomer is dissolved in continuous phase that is usually an aqueous solution without need of surfactants and emulsifiers. The polymerization can be initiated by various mechanisms. Initiation occurs when a monomer molecule is dissolved in the continuous phase colloids with an initiator molecule which might be an ion or free radical. Phase separation and formation of nanoparticles can take place before and after the termination of polymerization reaction (Amol T. R. *et al*., 2015)

1.2.3.2.2 Interfacial Polymerization

It is one of the well-recognized method for preparation of polymeric nanoparticles. The process involves the polymerization of two reactive monomers, which are dissolved in two phases, i.e. one in continuous phase and other in dispersed phase. The reaction takes place at the interface of two liquids. The interfacial reaction results in formation of nano-sized hollow polymeric particles. To promote nanocapsule formation the use of aprotic solvents are used such as acetone, acetonitrile. Protic solvents such as isopropanol, butanol, ethanol are found to induce nanosphere in addition to nanocapsules.

1.2.3.3 Ionic gelation or coacervation of hydrophilic polymers

The method involves the use of biodegradable polymers such as chitosan, sodium alginate, gelatin, pectin etc. In this process polymer containing two different aqueous phases are mixed which causes the interaction between positively charged group and negatively charged groups of polymers. The electrostatic interaction results in the formation of coacervates in the range of nanometer. The ionic interaction involves the material transition from liquid to gel due to ionic interaction at room temperature. Calvo and co-workers developed a method for preparing hydrophilic chitosan nanoparticles by ionic gelation method (Nagavarma B.V.N. *et al.*,2012)

1.2.4 Polymeric nanoparticles

The polymeric nanoparticles are highly prefered in drug delivery due to some inherent properties such as biocompatibility, non-immunogenicity, non-toxicity and biodegradability as well as ability to release drug in controlled/ sustained manner at tissues or subcellular level. The size of colloidal particles ranges between 10-1000 nm. Polymeric nanoparticles are broadly classified as vesicular system (nanocapsules) and matrix systems (nanospheres). The drug candidate is dissolved, entrapped, encapsulated or attached throughout or with in the polymer matrix. Based on method of preparation for nanoparticles, nanocapsule or nanosphere can be obtained (Amol T. R., 2015).

1.2.4.1 Types of polymeric nanoparticles

Nanocapsule: These are vesicular systems in which drug reservoir is confined to the cavity and surrounded by a polymer membrane (Christine V. *et al.,* 2008*).*

Nanosphere: These are matrix system in which drug is physically and uniformly dispersed (Christine V. *et al.*, 2008).

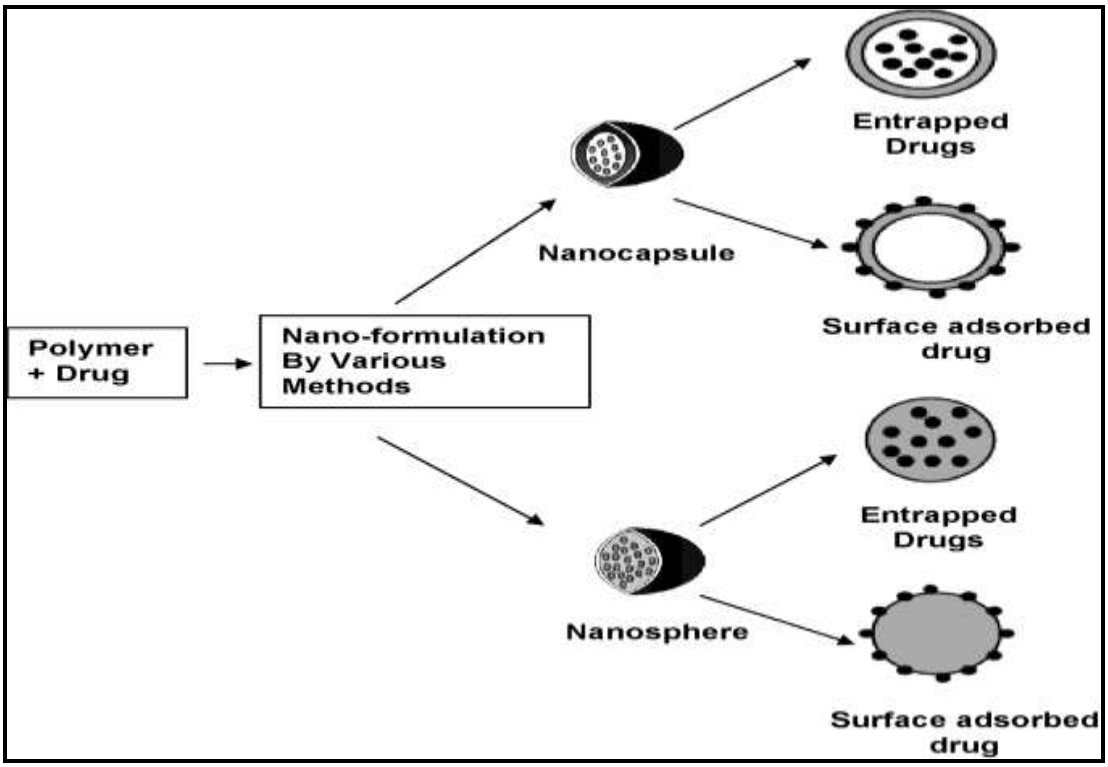


Fig 1.5 Types of nanoparticles

1.2.4.2 Polymers used in preparation of nanoparticles

The polymers used in preparation of nanoparticles should be non-toxic, non-immunogenic, biodegradable and biocompatible (Shirwaikar A *et al.*, 2008)**.**

Polymeric nanoparticles are classified as follows

a) **Natural polymer:** The most common natural polymers used in preparation of nanoparticles are

Polysaccharide based: Chitosan, Alginate, Pectin.

Protein based: Albumin, gelatin.

b) **Semisynthetic Polymers:** These polymers are obtained from natural polymers but modified slightly by simple chemical treatment to change the physical properties of natural polymers.

c) **Synthetic polymers:** These polymers are synthesized in laboratory by polymerization using simple chemical moieties. Poly(lactides)-PLA, Poly(glycolides)-PGA, Poly(lactide co glycolide)-PLGA, Poly(anhydrides),Poly (orthoesters), Poly (glutamic acids), Poly (caprolactone).

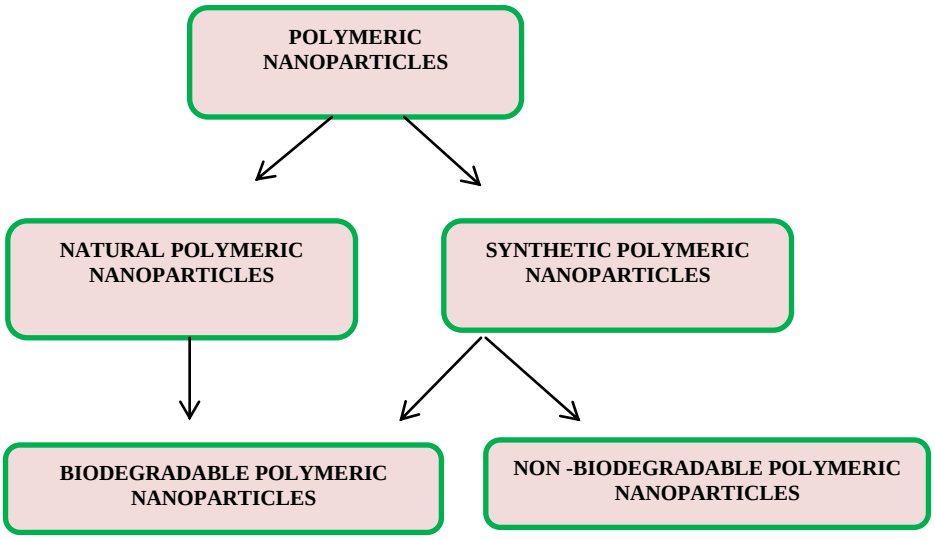


Fig 1.6 Various types of polymeric nanoparticles

1.2.4.3 Mechanism of drug release from biodegradable nanoparticles

The release of drug from polymeric nanoparticles is either controlled diffusion or erosion or both from the core matrix or across the polymeric membrane. The polymeric membrane acts as a barrier to release, hence the solubility and diffusivity of drug in polymer becomes a determining factor in release of drug. Along with this the ionic interaction between drug and additional auxiliary components in nanoparticle also affects the release rate. The interaction between drug and auxiliary ingredient may form a less water soluble complex which further makes drug release very slow or with no burst release effect.

1.2.5 Importance of natural polymers over synthetic polymers (Kusum K. et al., 2016)

Biodegradable: Naturally occurring polymers are produced by living organisms so they have no side effects on the environment and human being. Conversely synthetic polymers are prepared with the use of chemicals so have side effects on human being as well as on atmosphere.

Economic: Natural polymers are cheaper and their production cost is less than synthetic materials.

Biocompatible and non- toxic: Chemically the plants or animal materials are of carbohydrate or protein in nature which is composed of repeating units of either monosaccharide or amino acids. Hence natural polymers are safe and non- toxic compared to synthetic polymers.

Economic: Natural polymers are cheaper and their production cost is less than synthetic material.

Safe and devoid of side effects: The synthetic polymers are prepared by using chemical so they have side effects while natural polymer are found naturally so they have no side effects.

Ease of availability: Natural polymers are of either plant or animal origin. It is more economical than synthetic polymers (Girish K.J. *et al*., 2009).

1.2.6 Drawbacks of natural polymers (Kusum K *et al*., 2016):

Microbial contamination: During extraction they are exposed to external environments so there are chances of microbial contamination.

Batch to batch variation: Synthetic polymer production is carried out under controlled procedure with fixed quantities of ingredients while natural polymers are dependent on environment and various biotic and abiotic factors.

The uncontrolled rate of hydration: The natural materials are collected at different time moreover from different species, regions and climatic conditions. Therefore they generally vary with respect to percentage of chemical constituent in it.

Slow Process: The production rate of natural polymers is slow as the production rate is dependent upon the environment and many other factors.

Heavy metal contamination: There are chances of heavy metal contamination often associated with natural polymers.

The products obtained from natural sources are becoming an integral part of health care system. Natural polymers are assisting as an promising carrier system for most challenging conventional drugs which are used for treatment and management of many chronic diseases. Semisynthetic polymers have been used extensively for nanomaterial. Although the synthetic polymers shows more chemical stability but their unsatisfactory biocompatibility limits its potential clinical application. The natural polymers always display low toxicity, low immunogenicity, and good compatibility so they have gained more attention in drug delivery system (Krishna S. A. *et al.*, 2011). The potential hazards can be overcome by use of natural polymers (Shweta S. *et al.*, 2018). Recently, scientists have changed their attention on conforming chitosan and sodium alginate for use in nano-drug delivery system.

1.2.7 CHITOSAN

Chitosan has acquired notable attention in biomedical and pharmaceutical field due to its unique properties such as biocompatibility, biodegradability, non-toxicity, antimicrobial anti-tumour activities. Some of the chitosan based formulations widely used in biomedical and pharmaceutical field are: nanoparticles, microspheres, hydrogel films, fibres etc. (Islem Y *et al*., 2015).

It is naturally occurring polysaccharides, cationic, highly basic, mucoadhesive biocompatible polymer approved by FDA for drug delivery. Chitosan is obtained by partial N-deacetylation of chitin found in shells of crustacean such as from prawn, crabs as well as from cell wall of fungi. The deacetylation process is concentration and temperature dependent. The optimal yield is achieved at temperatures between 600^{o}C and 800^{o}C using 50% w/w alkali. The compound consists of glucosamine and N acetyl glucosamine linked by 1-4 glucosidic bonds. Chitosan is characterized on the basis of degree of de-acetylation which is determined by the proportion of D glucosamine and N-acetyl-glucosamine. The structural similarity

between cellulose and chitosan exist but cellulose is composed of glucose monomeric unit. Physicochemical properties of chitosan such as biodegradability, solubility, reactivity, adsorption of many substrate is dependent on the number of protonated amino groups in polymeric chain that is on acetylated and non-acetylated D glucosamine units. The acids with pKa less than 6.2 result in complete protonation of amino groups (pK_a 6.2-7.0) in chitosan, subsequently increases water solubility.

Chitosan is soluble in acids such as acetic, perchloric, nitric and phosphoric acid and insoluble in water, aqueous bases and organic solvents. The penetration enhancer property of chitosan is due to its ability to open tight juctions of epithelium hence it promotes the drug transportation through both paracellular and transcellular. It interacts with mucus to form complex by ionic, hydrogen bonding as well as hydrophobic interactions.

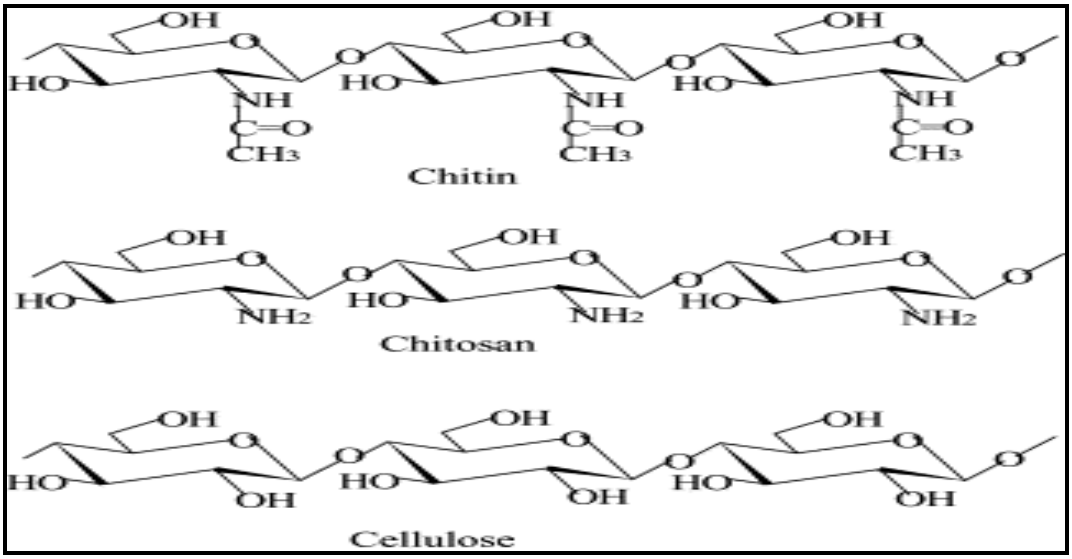


Fig 1.7 Stucture of chitin, chitosan and cellulose

1.2.7.1 Preparation of chitin and chitosan

The term chitosan does not refer to unique defined compound; it simply refers to a family of copolymers with various fractions of acetylated units. It consists of two types of monomers, Chitin monomer and chitosan monomers. Chitin is a linear polysaccharide consisting of (1-4)-linked 2 acetamido-2 deoxy-b-D glucopyranose.

Chitosan is linear polysaccharide consisting of (1-4) - linked 2 amino-2 deoxy-b-D-glucopyranose. Commercial chitin and chitosan consist of both types of monomers. Chitosan is found in nature, to a lesser extent than chitin. Chitin is found in cell walls of fungi and it is believed to be second most abundant biomaterial after cellulose. The estimated production of chitin is about 109-1011 tons. Chitin is extensively distributed in nature.

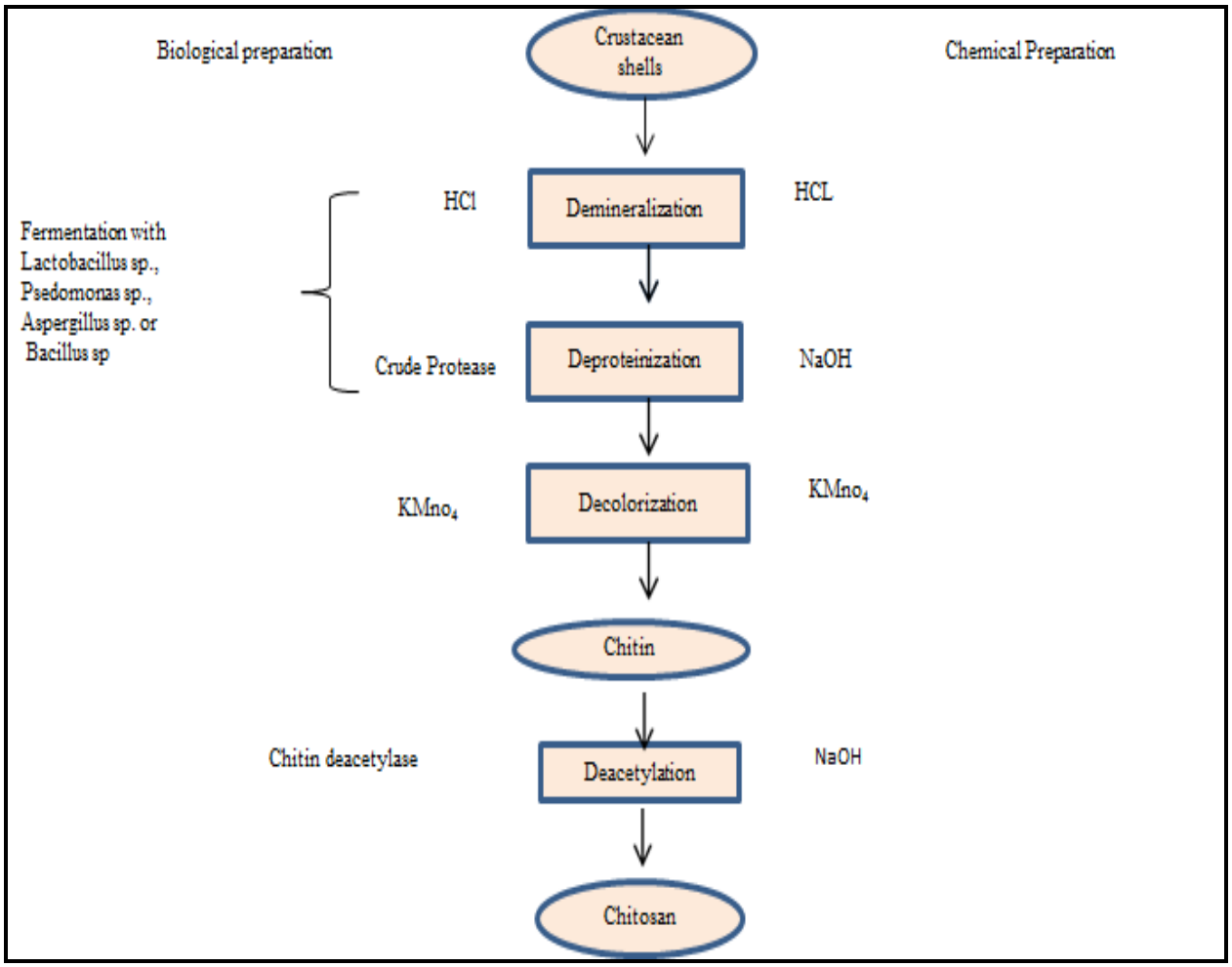


Fig 1.8 Extraction of chitin and preparation of chitosan

1.2.8 Sodium alginate

The recent trend points are increasing interest towards natural substances in food, drugs and cosmetics. The naturally occurring alginate polymer has great promise in drug delivery system due to its extensive application as food additive and lack of toxicity. The polymer possesses a number of characteristics that make it useful as formulation aid, both as a

conventional excipient and specifically as a tool in polymeric controlled drug delivery (Tonnesen H. *et al.*, 2002).The alginate was discovered by British Pharmacist, E.C.C Stanford and commercialized in year 1929 (Johnson F.A. *et al.*,1997*).*The annual production of alginates in the world is about 30,000 tones from which 30% is utilized by food industry and rest is being used in industrial ,Pharmaceutical and dental applications (Ertesvag H. *et al*., 1998).

Alginate is natural occurring anionic polysaccharide polymer, obtained from brown seaweed. It consist of α-L-gluronic acid (G) and β-D-mannuronic acid (M), linearly linked by 1-4 glycosidic linkage. The composition and sequence of G and M residues depends on the source of algae used which also affects the properties of alginate (Paques J.P. *et al*., 2014). Alginate can also be chemically modified to alter its properties and also extensively used in pharmaceutical industry due to its biocompatibility, low toxicity, economic and mild gelation by adding divalent cation such as Ca^{2+}. Alginate hydrogels can be prepared by various cross linking methods. The structural similarity to the extracellular matrices of living tissues make them applicable in wound healing, delivery of bioactive agents such as small chemical drugs and proteins

Commercially available alginate is extracted from brown algae. Alginic acid and its salts (calcium, magnesium, sodium potassium) are abundantly present in brown algae (phenophyta) of genera "*macrcystis, laminaria, Ascophyllum, Alario, Ecklonia, Eisenia, Necrocystis, sagasum , cystoseira and fucus*". The most important species of Laminaria is known as kelps or sea tangles and specimen of *Fucus* known as wracks. The bulk of alginate is obtained from two species, *Macrocystis porifera* and *Ascophyllum nodosum*. Phycocolloids are the primary component of both cell wall and extracellular matrix, function as a "skeleton" increasing the mechanical strength and flexibility to the tissues probably due to their ability to accumulate divalent metal ion and form gel of required mechanical strength with these ions. Acetylated alginates are also isolated from some bacteria genera *Pseudomonas* and *Acetobacter*. Red algae belonging to the family *coralenacease* also contain alginate (Nikhil S. *et al;* 2009).

1.2.8.1 Extraction and preparation of sodium alginate

The alginate occur in the form of insoluble due to calcium, magnesium, sodium and potassium salts contained in the algae cell walls and extracellular matrix. The method involved for extraction purification is usually ionic exchange technology. The alginate extraction protocol is consisting of five steps: acidification, alkaline extraction, solid liquid separation, precipitation and drying. To prepare alginate for commercial use, the algae is mechanically harvested and dried before further processing except for *M. pyrifera* which is processed wet. Alginates are extracted from dried and milled algal material by treatment with aqueous alkali solution i.e. sodium hydroxide. The extract is filtered and either sodium or calcium chloride is further added to filtrate in order to precipitate alginate i.e. alkaline cations are exchanged for H^+ ions. Alginate salt is further converted to alginic acid by treatment with dilute hydrochloric acid. Insoluble alginic acid is converted from insoluble protonated form to soluble sodium salt by addition of sodium carbonate at pH below 10 (Kuo M.R. *et al*, 2003). After extraction, the alginate can be further purified and then converted to either salt or acid.

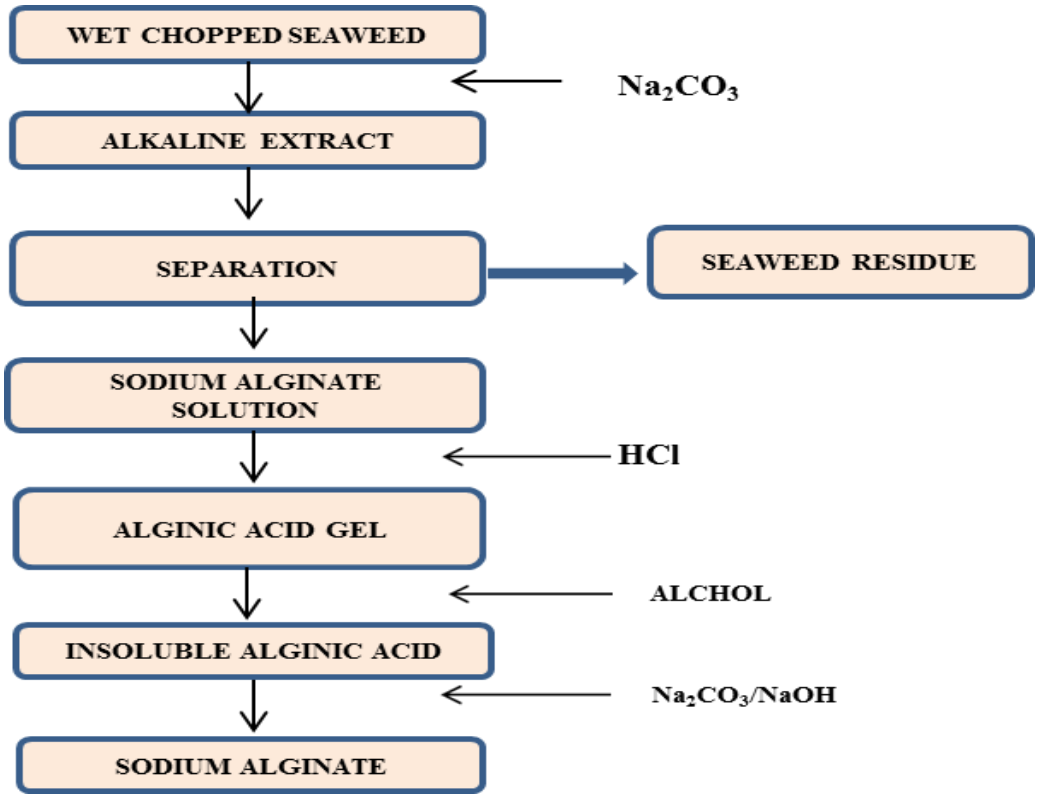


Fig 1.9 Sodium alginate production

Alginates belong to the family of linear copolymer containing blocks of (1, 4)-linked D mannuronate (M) and α L guluronate (G) residue. The ratio of guluronate to mannuronate varies depending on the natural source. The blocks are composed of consecutive G residues, consecutive M residues and alternating M and G residues. Alginates extracted from various sources differ in M and G ratio as well as in length of each block. In market about 200 different grades of alginates are available.

Only G blocks of alginate are believed to participate in intermolecular cross linking with divalent cations (e.g. Ca^{2+}) to form hydrogels. The M/G ratio, sequence, G blocks length, molecular weight are some critical factors that affect physical properties of alginate and formed gel. The mechanical properties of gel are improved by increasing length of G block and molecular weight. The stability of alginate, drug release rate, activity of entrapped molecule is significantly governed by physical properties of alginate.

1.2.8.2 Method of gelling

Alginate is typically used in the form of hydrogel in biomedicine. Some applications include wound healing, drug delivery, tissue engineering etc. Hydrogels are three dimensionally cross linked network of hydrophilic polymers with high water content. These hydrogels are biocompatible as they are structurally similar to the macromolecular based components in the body. Chemical and physical cross linking of polymers are typical approaches to form hydrogels and their physicochemical properties are highly dependent on the cross linking type and cross linking density, in addition to the molecular weight and chemical composition of the polymers (Kuen Y.L. *et al;*2012).

1.2.8.2.1 Ionic gelling

This is the most common method to form hydrogel. The process involves ionic cross linking between alginate and divalent cation (Ca^{2+}). The divalent cations bind solely to the glucouronate blocks of alginate chain. This phenomenon increases coordination and forms gel structure which is termed as egg box model crosslinking (Grant G. T. *et al*, 1973). Calcium chloride is most frequently used cross linking agent. The high solubility of calcium chloride results in fast and poorly controlled gelation with alginate. The use of alternatives such as

sodium hexa metaphosphate, calcium sulfate and calcium carbonate can be used for slow and controlled gelation due to limited solubility in water. One of the major advantage of this for human system is that ionically cross linked gels get dissolved due to exchange of divalent ion with monovalent ion in surrounding media (Al-Shamkhani A. *et al*, 1995).

1.2.8.2.2 Covalent cross linking

Covalent cross linking method is investigated to improve the physical properties of gel. In ionic gelation method the applied stress causes gel relaxation and crosslink dissociation with loss of water leading to plastic deformation. While in covalently cross linked gels water migration also occurs leading to stress relaxation but inability to dissociation and lead to significant elastic deformation. The cross linkers used for covalent crosslinking may be toxic, so it is necessary to remove unreacted chemicals from gels (Zhao X *et al*, 2010).

1.3 DRUG TARGETING

Since many decades, the medication of an acute disease and chronic illness was achieved through conventional dosage forms like tablet, capsule, ointment, liquids, aerosols, injectable etc. These conventional drug delivery system do not ensure maximum therapeutic responses. To achieve and to maintain drug concentration at site of action it is necessary to take conventional formulations several times a day. This results in fluctuating concentration, premature degradation, drug toxicity, patient non compliance, inability to attain effective concentration at site of action. In year 1981, Gregoriadis introduced the Drug targeting, a novel drug delivery system as “old drug in new clothes”. Targeted drug delivery causes accumulation of drug at desired site in therapeutic concentration. Subsequently restricts the entry to non-target cells, therefore targeted site get the higher and maximize benefits of targeted drug delivery (Jaya A *et al.*, 2011).

Targeted drug delivery can be achieved by carrier system. Carrier is a special molecule or system essentially required for effective transportation of loaded drug up to the preselected sites. The nanotechnology based drug delivery system has shown promising approach in treatment of TB. This approach can give best outcome in treatment of latent TB,

the most challenged phase for treatment. Patient with latent TB do not show any symptoms but causative organism reside inside the macrophage by subverting the immune system.

1.3.1 Components of targeted drug delivery

Targets: Target means specific organ or a cell or group of cells which in acute or chronic condition need treatment.

Carriers: Targeting can be achieved by use of carrier system. Carrier is a special molecule or system that is preferably required for effective transportation of loaded drug to the predetermined site. These engineered vectors retain drug inside or onto them and/or via ligand moiety transported into the area of target cells.

1.3.2 Methods of targeting

1.3.2.1 Passive targeting

These drug delivery system are targeted to the systemic circulation. In this technique drug targeting occurs because of the body's natural response to physiochemical characteristic of drug and drug carrier system. It is the ability of reticular endothelial system (RES) to take up the colloidal carriers such as by liver, spleen clears off colloidal carriers.

1.3.2.2 Inverse targeting

In this targeting approach attempts are made to avoid passive uptake of colloidal carrier by RES, so process is termed as inverse targeting. To achieve inverse targeting, the normal function of RES is suppressed by pre injecting large amount of blank colloidal carriers or macromolecules like dextran sulphate. This approach is suitable for targeting drug targets to non RES.

1.3.2.3 Active targeting: Active targeting can be further classified into ligand mediated and physical targeting.

a) Physical Targeting: In this targeting, some characteristics of environment changes such as pH, temperature, light intensity, electric field, ionic strength, and specific stimuli like glucose

concentration are used to localize the drug carrier to predetermined site (Jose M. M. *et al*., 2012).

Table 1.6 Physically targeted drug delivery systems

Physical Targeting	Formulation system	Mechanism of drug delivery
Heat	Liposomes	Change in permeability
Magnetic modulation	Magnetically responsive polymeric microspheres containing iron oxide.	Magnetic field can retard flow of particles
Ultrasound	Polymers	Change in permeability
Electric Pulse	Gels	Change in permeability
Light	Photo responsive hydrogels containing azo-derivatives.	Change in diffusion channels, activated at specific wavelength.

b) Ligand mediated targeting: Drug carrier system can be fuctionalized with the use of biological relevant ligand such as antibodies, polypeptides, fusogenic, lectins residues. These type of engineered carrier system selectively make the drug available to the cell or group of cells that is to targets. In ligand mediated active targeting the interaction between ligand to the corresponding receptor enhances the uptake of the entire drug carrier into the cell. An example of this approach is folate receptor targeting. Folate receptor is 38-KD glycosyl phosphatidylinositol anchored protein that binds the vitamin (Folic acid) with high affinity .The conjugation of folate with drug carrier system promotes receptor binding followed with internalization (phagocytosed) by macrophages (Reddy J. A. *et al.*, 1998).

1.4 MACROPHAGES

Macrophages are the major differentiating cell of mononuclear phagocyte system, which comprises of bone marrow monoblast and pro monoblast, peripheral monocytes and tissue macrophages. The precursors of macrophages are monocyte, promonocyte and monoblast. All these cells originate from a common progenitor called colony forming unit, granulocyte

macrophages. Monoblast, the least mature cell of the mononuclear phagocyte system, firstly differentiate into monocyte and remain in the bone marrow for 24 h and then they enter into the peripheral blood and from peripheral blood , monocyte migrate to extravascular tissue where they differentiate into macrophages. Macrophages colonize in the liver (kupffer cells), lungs (alveolar interstitial macrophages), spleen, lymph nodes, thymus, guts, gut, brain, marrow, connective tissue and serous tissue. They play important role in host defense against many infectious agents, including bacteria, viruses, protozoa, parasites. Macrophages are migrated to an infected focus following attraction by a variety of substances, including bacterial components and endotoxins, complement components, immune complexes and collagen fragments. Once they are at the infected site, the macrophages may phagocytose and kill infectious agents by variety of mechanisms. By taking protein antigens and generating immunogenic fragments from them macrophages play a significant role in induction and regulation of immune response.

Macrophages are known to secrete large number of substances involved in diverse functions. Some are involved in acute phase response, regulation of haematopoiesis, cleaning and healing of injured tissue. Macrophages are professional killers (phagocytes). They ingest a pathogen and entrap in the phagosome. Within Phagolysosome the enzymes and toxic peroxide digests the pathogen. However, some bacteria such as *Mycobacterium tuberculosis* are resistant to these methods of digestion. These intracellular parasites use macrophage as reservoir and safe heaven.

The nature of macrophages acts as constraint for the delivery of drugs. Scientists have discovered wide opportunities to deliver drugs within macrophages by exploiting their biological and morphological aspects.

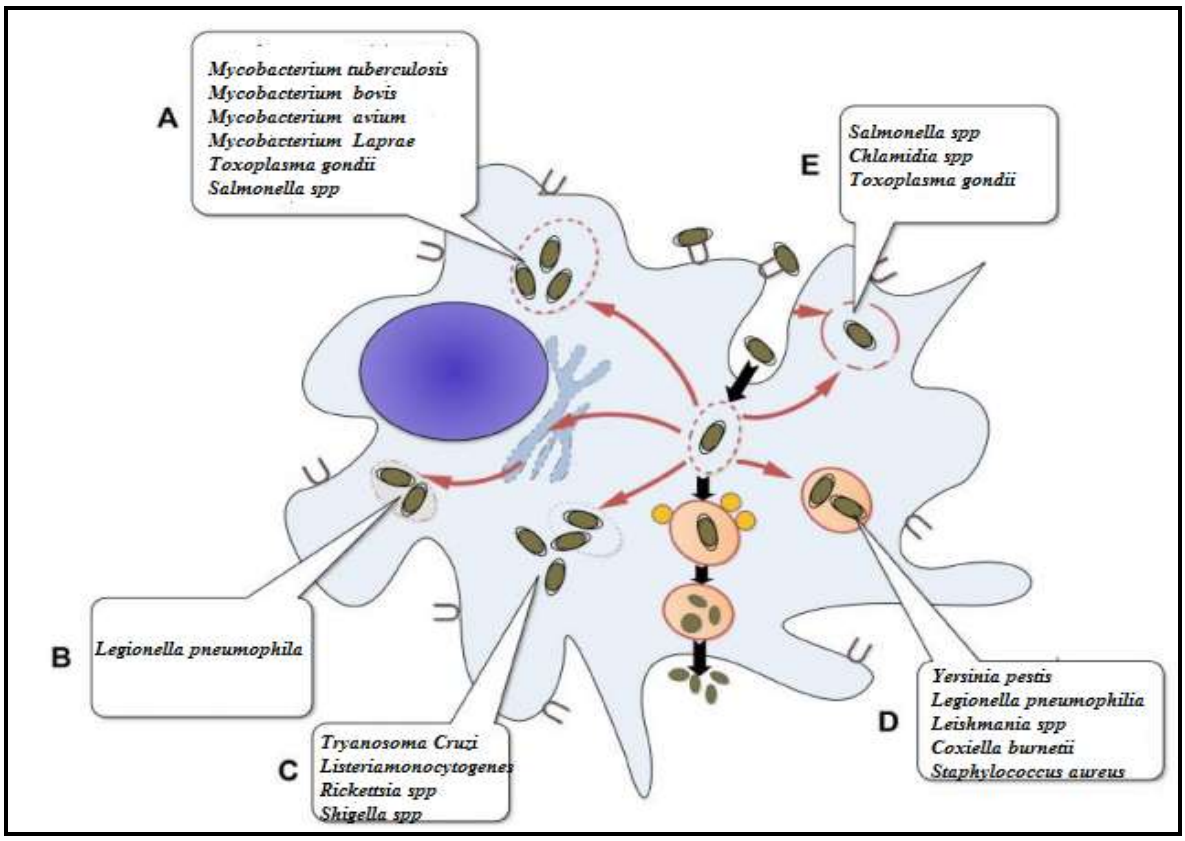


Fig 1.10 Infectious agents that manage to survive in macrophages

1.41 Receptors over macrophages

a) Fc receptors (Fc R): Fc Receptors (fragment, crytallizable) are members of the Ig super family of receptors, have main function to recognize and bind the Fc portion of immunoglobulin producing an antibody mediated phagocytosis.

b) Complement Receptors: Complement Receptors from the immune cell surface recognizes the deposited C3 fragments of the complement system which opsonize foreign particle. The CR include two distinct families of cell surface receptors-SCR family receptors (as CR1-CR35) and the integrin complement receptors CR3 and CR4 (CD18, CD11b/CD11c). CR3 mediated phagocytosis was observed by Takagi *et al* to be involved in uptake of oligomannose coated liposome

c) Mannose Receptor: The mannose receptor are highly expressed on macrophages and recognizes mannose and fucose glucoconjugates from the surfaces of a broad pathogen group

of microscopic pathogens, also involved in the intracellular transport of *Mycobacterium tuberculosis*. The mannose binding protein is composed of an extracellular fraction represented by lectin like carbohydrate binding groups and a cytoplasmic group critically involved in the cytoskeleton remodeling during endocytosis.

d) Scavenger Receptors: Scavenger receptors (SR-A and SR-B or CD36) are broad group of transmembrane receptors which recognizes the variety of structures as LDL, Phosphatidylserine, polyanionic ligands, unopsonized and negatively charged nanoparticles.

e) Integrins: Integrins are widely distributed cell receptors and are mostly considered in connection with cell adhesion and migration. These are receptors for recognition of apoptopic cells and opsonized pathogens. The CR3 and CR4 integrin receptors are involved in uptake of complement opsonized microorganisms but other integrins such as α5β1 and β1 are involved in a non-complement dependent phagocytosis. The last two receptors are especially involved in internalization of specific pathogens such as *Staphylococcus aureus*.

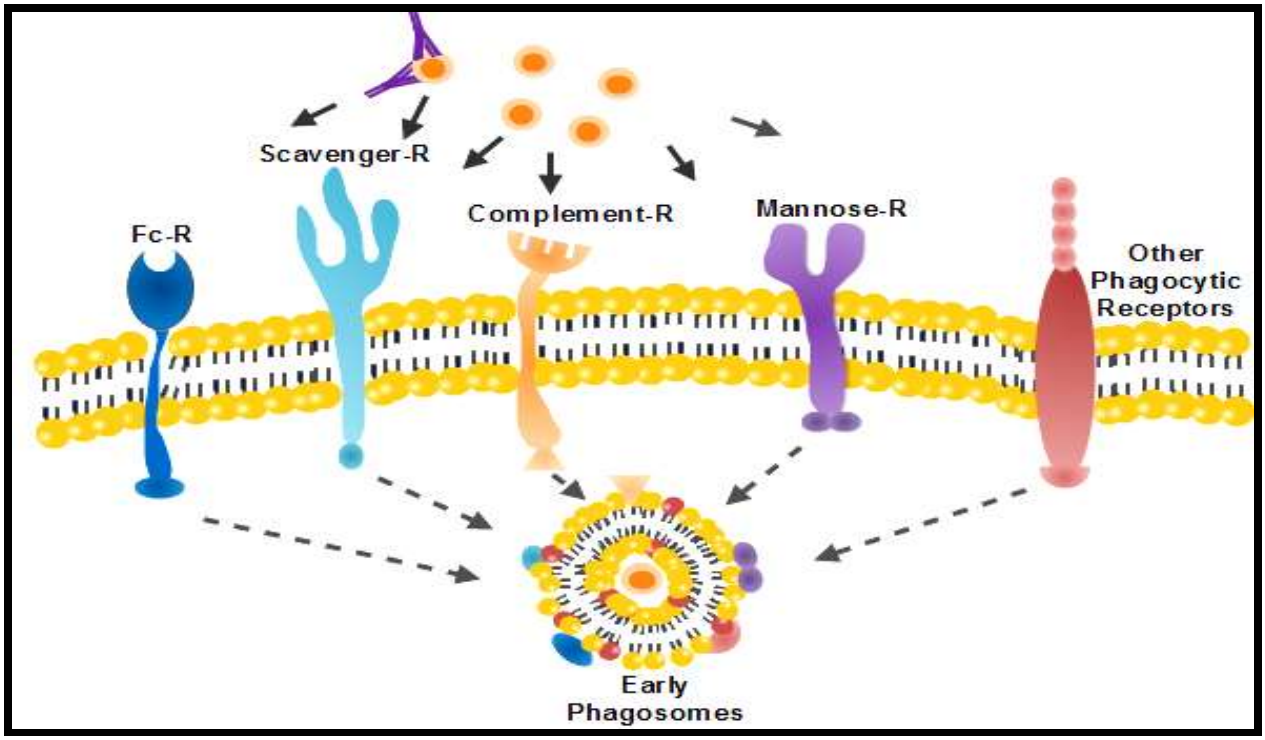


Fig 1.11 Various types of receptors over macrophages

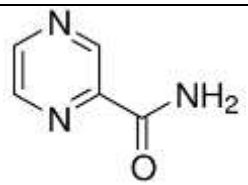
These large number of phagocytosis receptors located on the surface of macrophages are optimal structures for macrophage targeted therapy. These receptors reflect the physiological function of macrophages and are used to internalize the pathogens in macrophages. The therapeutic carrier targets same receptor and causes the accumulation of therapeutic moiety in the same intracellular compartment as those in which microorganism develops.

Hence ligand based polymeric nanocarrier opens a new perspective for the treatment of obligate intracellular parasite such as *Mycobacterium tuberculosis*

1.5 DRUG PROFILE

1.5.1 PYRAZINAMIDE

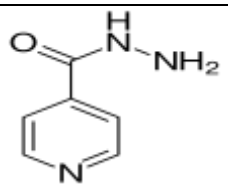
Table 1.7 Profile of Pyrazinamide

PROPERTIES	SPECIFICATIONS
Drug Name	**Pyrazinamide**
Chemical Name	Pyrazine-2-carboxamide
Chemical Structure	
Empirical Formula	$C_5H_5N_3O$
Drug Category	Anti-tubercular drug
Physical Properties	White, crystalline powder, odourless and slightly bitter taste.
Melting Point	192^oC
Protein Binding	Very Low (0-7%)
Half Life	9-10 hours
Bioavailability	> 90% orally
Route of Elimination	Approximately 70% of oral dose is excreted in the urine, mainly by glomerular filtration within 24 hours
Molecular Weight	123.11g/mol
Mode of Action	PYZ diffuses into *Mycobacterium tuberculosis,* where the enzyme pyrazinamidase converts pyrazinamide to the active form pyrazinoic acid. Under acidic conditions, the pyrazinoic

	acid that slowly leaks out, converts to the protonated conjugate acid, which is thought to diffuse easily back into the bacilli and accumulate
Dose	25-30 mg/kg (daily); 50-75 mg/kg (3 times a week)
Contraindication	Severe hepatic damage, acute gout in person who have shown hypersensitivity to it.
Drug Interactions	Cyclosporine, Pyrazinamide decreases the effect of cyclosporine.
Adverse Effects	Side effects include liver injury, arthralgias, anorexia, nausea and vomiting dysuria, malaise and fever, megaloplastic blastic anaemia, adverse effects on the blood clotting mechanism or vascular integrity and hypersensitivity reactions such as utricaria, pruritis and skin rashes

1.5.2 ISONIAZID PROFILE

Table 1.8 Profile of Isoniazid

PROPERTIES	SPECIFICATIONS
Drug Name	**Isoniazid**
Chemical Name	Pyridine-4-Cabohydrazide.
Chemical Structure	
Empirical formula	$C_6H_7N_3O$

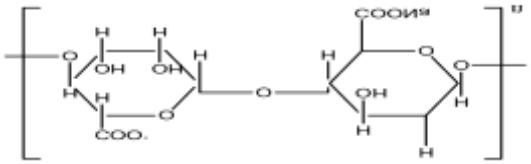
Drug Category	Anti-tubercular drug
Physical Properties	White, crystalline powder, Odourless and slightly bitter taste.
Melting Point	170-173^{o}C
Protein Binding	Very low (0-10%)
Half Life	2-5 hrs (Slow acetylators), 0.5-1.6 hrs (Fast acetylators)
Bioavailability	> 80%
Route of Elimination	From 50 %-70% of the dose of isoniazid is excreted in the urine within 24 hours
Molecular Weight	137.142 g/mol
Storage Conditions	To be stored at room temperature (59 ^{o} - 86 ^{o} C) in tightly closed, light resistant container.
Mode of Action	Isoniazid is a prodrug is activated by a bacterial catalase peroxidase enzyme that is in *M tuberculosis* called Kat-G which couples the isonicotinic acyl with NADH to form iso-nicotinic acyl NADH complex. This complex binds tightly to the enoyl-acyl carrier protein reductase. Thereby blocking the natural enoyl-acyl cmp substrate and the action of fatty acid synthase. This process inhibits the synthesis of mycolic acid, required for myco bacterial cell wall.
Dose	25-30 mg/kg(daily); 50-75 mg/kg (3 times a week)
Contraindication	Aluminium hydroxide inhibits INH absorption. INH inhibits phenytoin , carbamazepine, diazepam and warfarin metabolism may raise their blood levels. PAS inhibits INH metabolism and prolongs its half life.

Drug Interactions	Aluminium salts, Carbamazepine, Disulfiram, Hydantoin.
Adverse Effects	INH is well tolerated by most patients. Peripheral neuritis, hepatisis and variety of neurological manifestations are the most important dose dependent toxic effects.

1.6 EXCIPIENT PROFILE

1.6.1 SODIUM ALGINATE

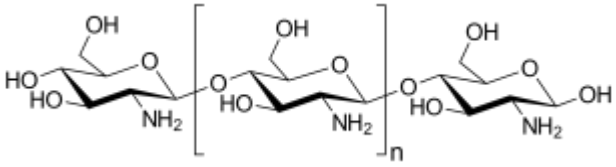
Table 1.9 Profile of Sodium Alginate

PROPERTIES	SPECIFICATIONS
Official Name	**Sodium alginate**
Molecular Structure	
Category	Natural biodegradable polymer
Empirical Formula	$NaC_6H_7O_6$
Composition	Blocks of (1-4)-linked β –D mannuronate (M) and its C-5 epimer α-L-glucuronate (G) residues, respectively, covalently linked together in different sequences or blocks.
Sources	Extracted from sea weed, including the giant kelp *Macrocystispyrifera*, *Ascophyllumnodosum* and various types of *Laminaria*. It is also produced by two bacterial genera *Pseudomonas* and *azotobacter*
Solubility	Capable of absorbing 200-300 times water of its own weight

Use in Drug Delivery	Alginate has also been extensively investigated as a drug delivery device where in the rate of drug release can be varied by varying the drug polymer interaction as well as by chemically immobilizing the drug to the polymer back bone using the reactive carboxylate groups.
Features	The high acid content allows alginic acid to undergo spontaneous and mild gelling in the presence of divalent cations such as ca^{2+} ions

1.6.2 CHITOSAN

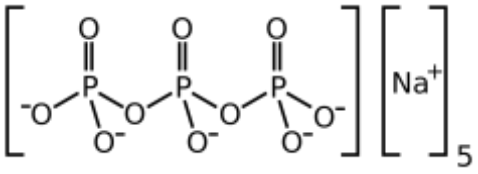
Table 1.10 Profile of Chitosan

PROPERTIES	SPECIFICATIONS
Official Name	Chitosan
Molecular Structure	
Category	Natural biodegradable polymer
Empirical Formula	$(C_6H_{11}NO_4)_n$
Composition	Randomly distributed β-(1-4)-linked D glucosamine (deacetylated unit) and N-acetyl-D- glucosamine (acetylated unit).
Sources	Chitosan is obtained by the thermochemical deacetylation of chitin in the presence of alkali and naturally it occurs only in certain fungi (Mucoraceae).
Solubility	Insoluble in water but can be dissolved by dilute acids, which

	would make it viscous.
Use in Drug delivery	Mucoadhesive, use for oral and transdermal drug delivery system: tablet capsules, microspheres, nanoparticles, beads, films and gel
Features	Cationic, insoluble at high pH, haemostatic, biodegradability, mucoadhesion and molecular weight.
Therapeutic uses	Chitosan hemostatic agents are often chitosan salts made from mixing chitosan with an organic acid (such as succinic acid and lactic acid) and anti-coagulant properties, antioxidant, antimicrobial, analgesics

1.6.3 SODIUM TRIPOLYPHOSPHATE

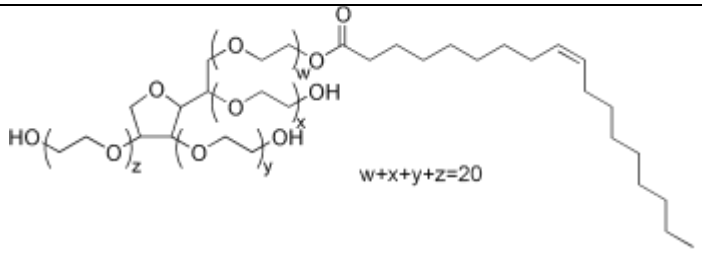
Table 1.11 Profile of Sodium Tripolyphosphate

PROPERTIES	SPECIFICATIONS
Official Name	Sodium Tripoly Phosphate
Molecular Structure	
Density	2.52 g/cm^3
Empirical Formula	$Na_5P_3O_{10}$
Molecular Weight	376.86 g/mol^3
pH	9.8
Solubility	NaTPP is easily soluble in water about 20g/100ml (200^{o}C). It may

	be clear to slightly hazy
Toxicity	The products are not considered to be toxic during the natural course of handling. The food grade Na.TPP has been used as food additive for many years.
Stability	The prolonged heating of sodium tripolyphosphate solution intends to revert to the ortho-phosphate. More stable than the higher i.e meta phosphates, but less stable than tetra sodium pyrophosphate.
Uses	In water softening (calcium and magnesium hardness is sequestered from solution without precipitation). Peptizing agent, emulsifier, dispensing agent. Ingredient of cleansers in drilling fluids to control mud

1.6.4 TWEEN 80

Table 1.12 Profile of Tween 80

PROPERTIES	SPECIFICATIONS
Official Name	**Tween 80**
Molecular Structure	
HLB	15.0
Empirical Formula	$C_{32}H_{60}O_{10}$

Molecular weight	604.81g/mol
Description	Tween 80 is yellow to orange colored, oily liquid or lemon to amber coloured, oily liquid, polyethylene sorbitol ester, with a calculated molecular weight of 1.31 daltons, assuming 20 ethylene oxide units, 1 sorbitol and 1 oleic acid as the primary fatty acid.
Brook field Viscosity	400-620 Centipoise ($25^{o}C$)
Solubility	Tween 80 is miscible in water (0.1 ml/ml) yielding a clear to slightly hazy faint yellow solution. It is reported to be miscible with alcohol, cottonseed oil, corn oil, ethyl acetate, methanol and toluene, but insoluble in mineral oil. Aqueous solution of polysorbate, the neat liquid will undergo auto oxidation over time.
Stability	For special applications, storage under argon or nitrogen may be preffered. The product is not sterile. Sterile filteration is more easily done if the liquid is warmed to about $40^{o}C$ and TWEEN 80 is poured through the 0.22 µm filter.

1.6.5 CALCIUM CHLORIDE

Table 1.13 Profile of Calcium Chloride

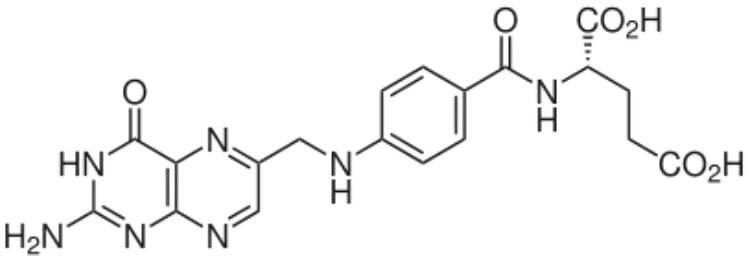
PROPERTIES	SPECIFICATIONS
Official Name	**Calcium Chloride**
Molecular Structure	Cl—Ca—Cl
Melting Point	$772^{o}C$ (anhydrous); 176 ^{o}C (dehydrate); $30^{o}C$ (Hexahydrate).

Empirical Formula	$CaCl_2$, $CaCl_2.2H_2O$, $CaCl_2.6H_2O$.
Molecular Weight	110.98 g/mol
Boiling point	> 1600^oC
Density	0.835 g/cm^3
Acidity/Alkalinity	pH= 4.5-9.2 (5% w/v aqueous solution).
Description	Calcium chloride occurs as a white colorless granules or crystalline mass, and is hygroscopic (deliquescent).
Functional Category	Antimicrobial, preservative, therapeutic agent, water absorbing agent.
Solubility	Freely soluble in water and ethanol (95%); insoluble in diethyl ether.
Pharmaceutical Application	The main applications of calcium chloride as an excipient relate to its dehydrating properties, it is also used as an antimicrobial preservative, as a desiccant and eye lotions. Therapeutically calcium chloride injections are used to treat hypocalcaemia

1.6.6 FOLIC ACID

Table 1.14 Profile of Folic Acid

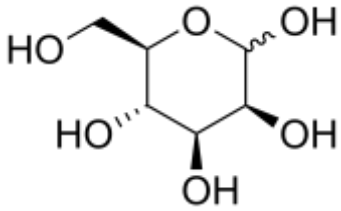
PROPERTIES	SPECIFICATIONS
Official Name	Folic Acid
Synonymn	PteGlu, Pteroyl-L glutamic acid, Vitamin M, Vitamin 9

Molecular Structure	
Empirical Formula	$C_{19}H_{19}N_7O_6$
Molecular Weight	441.40 g/mol
Functional Category	Vitamin Suppliment
Pharmaceutical Uses	Bioreagent, suitable for cell culture, plant culture
Sources	Certain cereals, breads, leafy greens (spinach, swiss chard, kale), lemon, oranges, grape fruits are sources of folic acid.
Over dose side effects	Nausea, bloating, poor appetite, trouble sleeping, feeling depressed or overly excited
Contraindications	Methothrexate, Diuretics, antibiotics, NSAIDs (naproxen, diclofenac) decreses the concentration of folic acid

1.6.7 D-MANNOSE

Table 1.15 Profile of D-Mannose

PROPERTIES	SPECIFICATIONS
Official Name	D-Mannose
Synonymn	Carubinose, D Manosa, Seminose, D-Mannopyranose

Molecular Structure	
Empirical Formula	$C_6H_{12}O_6$
Molecular Formula	180.156
Uses	A kind of sugar related to glucose. Used for preventing urinary tract infection, treating carbohydrate –deficient glycoprotein syndrome.
Side Effects	In high doses, might harm kidneys, loose stools or bloating problems**.**
Contraindications	Diabetes, Pregnancy, breast feeding**.**

1.7 REVIEW OF LITERATURE

Gong W. *et al*., 2018 investigated and reviewed the need of novel vaccine for TB prevention and control. The major cause of mortality and failure of existing BCG vaccine against TB have drawn attention of researcher for developing better drug delivery system. The result showed that the emergence of nanotechnology is likely to have a significant impact on drug delivery sector, acting just about in every route of administration from oral to injectable.

Mayur K. *et al*., 2018 reviewed the problem associated with the emergence of antimicrobial resistance. Nanotechnology approach presented a potential answer to antimicrobial resistance, which could stimulate innovation and create a new generation of antibiotic treatments for future medicines. The result concluded that nanotechnology based antimicrobial therapy could combat the growing threat of reistance to antibiotics displayed by pathogenic bacteria.

Mohammad Nasiruddin *et al*., 2018 reviewed and discussed about the need of an effective, robust system to reduce the emergence of MDR and XDR. The results showed that nanotechnology based therapies have convincing treatment and promising outcomes for chronic infectious diseases. The reduced dosing frequency, improved compliance, sustained and controlled release profile of drug are potential powerful benefits of nanocarriers therefore best be trialed in reducing the emergence of MDR and XDR case in tuberculosis.

Shivangi *et al*., 2018 investigated the potential of glucose polymer based nanoparticulate drug delivery system towards infected site of the body or in infected macrophages. The use of of natural polymers is continuously increasing in the field of targeting due to biodegradability and very slow immune response. The result suggested, the use of biomarkers (Beta 1, TGFb-1, IL-2, IL-13 SEC14L1, GUSB, BPI, and CCR7) as a ligand makes nanoparticles more specific to destination. Ligand based targeting reduces toxicities of antituberculer drugs to the other uninfected sites and gets operated only in the infected macrophages.

Sujit K. D. *et al*., 2018, formulated chitosan coated freeze dried Prothionamide to get DPI (Dry powder inhaler) with aerodynamic particle size 1.76µm. The *in-vitro* evaluation studies exhibited initial burst release followed by sustained release up to 96.91% in 24 h. The study revealed DPI maintained the PTH concentration above MIC for more than 12h after single

dose administration and increased the PTH residency in the lungs tissue more than 24h. Hence Animal reduction of dose in pulmonary administration will improve the management of tuberculosis

Susan S. *et al*., 2018 investigated and reviewed the utility of long-acting/extended release drug formulations in treatment of existing and latent TB. The complications in treatment of TB is continuously increasing due to earlier therapy discontinuation and treatment default. Hence administration of long-acting injection in a month could improve patient adherence and treatment outcomes. The review concluded that biomarkers along with long acting formulation not only help to reduce the high risk of disease progression, also it would be a potential tool accelerating progress towards TB elimination.

Zhaohui G. *et al*., 2018 prepared isoniazid, rifamacin (combined dug) loaded bovine serum albumin nanoparticles by modified self-emulsion solvent diffusion method. Formulated nanoparticles were evaluated for physicochemical properties, loading efficiency and dissolution release profile. Results revealed that obtained nanoparticles had average diameter 60.5±4.6 nm with an excellent drug loading, entrapment efficiency i.e. 19.8% and 87.8% for isoniazid, respectively, and 20.1% and 98.0% for rifampicin, respectively. Nanoparticulate approach slowed and sustained the drug release, showing 97.02% INH released at sixth day and full release of at rifampicin on sixth day of dissolution study.

Seoung-Ryoung C. *et al*; 2017 synthesized, characterized and tested different formulated batches of nanoparticles containing Ga(III) or rifampicin. The nanoparticles exhibited sustained drug release over a long time period and significantly inhibited the growth of virulent tuberculosis strain in infected macrophages. The result revealed that nanoparticles and ligand nanoparticles are promising tool for latent TB treatment.

Garg T. et *al*., 2016, fabricated spray dried chitosan nanoparticles (CHNPs) by ionic gelation method. The obtained CHNPs had smooth spherical shape with average size 230± 4.5nm, PDI 0.180 ± 0.021. The results revealed that incorporated drug was found in various organs lungs, liver, kidney, spleen until 24 h post nebulization. The drug loaded CHNPs have excellent chemotherapeutic efficacy on mycobacterium than free drug.

Harshad R. A. *et al*., 2016 fabricated CS-TPP nanoparticles at novel pH -6.2 and optimized the chitosan and tripolyphosphate in order to improve procees yield. The prepared formulations were characterized in term of particle size, zeta potential and percentage yield. The result revealed that TPP concentration was dominant factor in controlling CS-TPP nanoparticle size and process yield. Optimized formulation showed 91.5% yield with mean size 227 nm and zetapotential +24.13 mV.

Khan M.A. *et al*., 2016 investigated and developed curcumin loaded chitosan nanoparticles (CLCsNPs) by ionotropic gelation method. The results showed average size of CsNPs and CLCsNPs were approximately 189±11.8nm and 197±16.8nm, exhibited a zeta potential of +76±5.6mV and +71±6.4mV respectively and drug entrapment efficiency was 85%. *In vitro* studies revealed a fast release of 35% at pH 5 and 25% at pH 7.4 of the drug during the first 3h, followed by controlled release of curcumin over a period of 120h and sustained anti-proliferative activity.

Kusum K. *et al*., 2016 reviewed and described the vital role of polymers in drug delivery system. The selection of polymer plays an important role in physicochemical parameters of the dosage form. But selection is done carefully with regards to its toxicity, drug compatibility, stability, drug release pattern. The result concluded that natural polymers can be good susbsitute for the synthetic polymers. Many of the side effects of the synthetic polymers can be overcome by using natural polymers.

Liliana A *et al*., 2016 developed Bacille Calmette-Guerin (BCG)-loaded polymeric microparticles for mucosal immunization. Microparticulate preparation involved polyanionic complexation method. Particles obtained were in micrometer size with spherical morphology. The result suggested low molecular weight chitosan produced particle suspensions of lower size distribution and higher stability, allowing high BCG entrapment efficiency and biocompatibility. The stoichiometric proportion of alginate and chitosan in formulation improved the consistency of particle formulation.

Sriharitha *et al*., 2016 reviewed and described nanoparticles are used to alter or modify particle size of drug, its surface properties, thus reaching pharmacologically active drug

molecules to its specific site action with minimal dose and reduced dosing frequency. The result showed the ability of nanoparticles to improve pharmacokinetic and pharmacodynamics of drug by means of nanoparticle based targeted drug delivery system. The sustained, controlled releases via nanoparticles have made a breakthrough.

Balaji R.A. *et al*., 2015 revealed that lipophilic drug levofloxacin could be entrapped within chitosan/alginate (CS/ALG) nanoparticles using a very simple ionotropic pregelation technique as strong electrostatic interactions exist in the nanoparticles. The result showed nanoparticles obtained were with a diameter of 25-55nm in meta acid environment. Levofloxacin released from chitosan-alginate nanoparticles was 71% at pH 7.4 within 7 h. The release profile was characterized by an initial burst effect in phosphate buffer solution, followed by a continuous and controlled release.

Emilia S. *et al*., 2015 investigated that despite the great potential of use of chitosan in drug delivery or tissue engineering systems, its poor long-term stability is a big drawback. The result described various crucial parameters (internal and external) those affects the stability of chitosan formulations. Several stratagies were introduced in order to improve stability of chitosan formulations such as blending with hydrophilic polymer, addition of the stabilizing agent during the preparation process and use of ionic or chemical crosslinkers.

Hou D.Z. *et al*., 2015 investigated the potential of montmorillonite as a sustained carrier in the Betaxolol hydrochloride (BH) -loaded chitosan nanoparticles for prolonged ocular application. Nanoparticles were prepared by ionic gelation of chitosan with sodium tripolyphosphate (TPP) The result revealed the enhanced precorneal residence time that facilitated an effective sustained release and non-irritant, tolerable as determined by modified Draize test.

Natrajan D *et al*., 2015 developed and investigated the effect of various process parameters such as the effect of heat and the concentrations of AL and CS on chitosan alginate nanocarrier. The result suggested that 0.3 mg/mL AL and 0.6 mg/mL CS produced minimum-sized particles (<300 nm) with good stability. The obtained oil loaded nanocarrier had significant antiproliferative properties than the bare oil as determined by 3-(4, 5-

dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay in A549 cell lines along with hemocompatibility.

Patricia S. *et al.*, 2015 fabricated polymixin B sulphate (cationic) loaded solid lipid nanoparticle. Cationic natured drug was cross linked with sodium alginate and loaded into SLN produced using high pressure homogenization technique The Optimized batch of SLN with mean particle size 439.5± 20.42 nm, PDI 0.241 ± .050, zeta potential -34.8± 0.55 mV was exposed to HaCat and NIH/3T3 cell line to determine MIC in *Pseudomonas aureoginosa* strain. The result revealed SA/PLX loaded SLN were less toxic and enhanced MIC than free Polymixin B sulphate.

Shukla A. *et al.*, 2015 formulated Diphtheria loaded alginate coated chitosan microparticles (ACMs). The studies reflected alginate coating led to negative zetapotetial value -32.6 ± 4.2mV. The Confocal scanning microscopy revealed ACMs were effectively taken up by M cells and boosted significant immune response at serum IgG as well as mucosal IgG levels.

Siddhi P. *et al.*, 2015 reviewed and discussed conventional chemotherapeutic agents re distributed nonspecifically in the body and they affected all types of cells. The result concluded that nanoparticle based molecular targeted therapy evade the cytotoxicity by using active and passive targeting stratagies. These concepts enhance the intracellular concentration of drugs in cancerous cells via receptor mediated endocytosis.

Yolandy L. *et al.*, 2015 fabricated mycolic acid decorated isoniazid loaded Poly-lactic-co-glycolic acid (PLGA) nanoparticles by double emulsion solvent evaporation method. Further nanocarriers were exposed to macrophages, derived from bone marrow of infected mouse. The results showed the significant phagocytic uptake of nanoparticles by macrophages and phagalosome fusion events with Mycobacterium containing phagalosome.

Benson J *et al.*, 2014 formulated and evaluated long acting nano-formulation of Rifampicin and Isoniazid for MP (macrophage Phagocyte) particle uptake, retention, cell viability, antimicrobial efficacy. The result revealed that drug reached to 6μg/10^6 cells in human monocyte derived macrophages for nanoparticles compared with 0.1 μg/10^6 cells for native

drugs. Hence nanocarrier based target drug delivery facilitated *mycobacterial tuberculosis* selections to a mononuclear phagocyte.

Dheda K.*et al.*, 2014 investigated the emerging problem of functionally untreatable tuberculosis and the issues and challenges that it possesed to public health and clinical practice. The emergence and growth of highly resistant strains of tuberculosis promoted the development of new drugs and rapid diagnostics for tuberculosis. Government Funding had strengthened global control efforts, research and TB eradication programmes.

Kidenya R.B. *et al.*, 2014 investigated and reviewed the prevalence and molecular epidemiology of multi drug resistant TB in east Africa, including Burundi, Kenya, Rwanda, Tanzania and Uganda. They reported that the estimated MDR TB prevalence in east Africa ranged from 0.4 to 4.4 % in new patients and from 3.9 to 17.7 in recurrent TB patient. Therefore diagnostics and treatment of increased capacity are required.

Mojtaba S. *et al.*, 2014 discussed challenges in treatment of latent T.B. Till yet no antibiotic therapy has been reported to eliminate the most intracellular bacteria such as *Mycobacterium Tuberculosis.* The ideal nano carrier would possibly reduce drug dosage, improve drug absorption, delivery to right place in living system, increased local concentration of drug at favourite site and limited its side effects. The result revealed that mainly polymeric nanoparticles powerfully enhance phagocytosis and suitable for intracellular delivery of antibacterial agents.

Paques J. P. *et al.*, 2014 reviewed and explained different methods for preparation of alginate nanoparticles. Primarily alginate nanoparticles were formed by two methods: the complexation and w/o emulsification coupled with ionic gelation method. The result concluded alginate is promising biodegradable polymer for formation of nanoparticles for drug delivery. Formation of shell layer and functionalizing the particle surface with ligands are useful to obtain stability and functionality.

AttiaShafie M.A. *et al.*, 2013 formulated Betamethasone sodium phosphate loaded mucoadhesive chitosan-sodium alginate nanoresevoir by ionotropic gelation method and investigated the effect of various parameters (pH of chitosan solution, sodium alginate

concentration, calcium chloride concentration, chitosan concentration, drug concentration and tween 80) on physicochemical properties, release profile of drug. The results showed that mean particle size, zeta potential ranged from 16.8 to 692 nm and +18.49 to +29.83 mV. *In vitro* release studies of batches revealed an initial burst release of the drug followed by slow sustained release over 24, 48 or 72 hours depending on the formulation parameters.

Daisy S. *et al*., 2013 prepared methotrexate (BCS Class –III) loaded alginate nanoparticles by ionic gelation method using Box Behnken method. The objective of research was to study the effect of various parameters (conc. of chitosan, conc. of sodium alginate, amount of drug) on particle size, zeta Potential, entrapment efficiency and % release. It was concluded that all independent variables have significant effect on Particle size, zeta potential, entrapment efficiency and drug release (%). The *in-vitro* drug release profile showed the controlled release of methotrexate from sodium alginate-chitosan loaded nanoparticle .

Jadhav S.S. *et al*., 2013 formulated and characterized the Rizatriptan benzoate loaded mucoadhesive chitosan nanoparticles by ionic gelation of chitosan and tripolyphosphate anions. The optimized batch had particle size, entrapment efficiency and drug loading 248µ, 69.1% and 60.63% respectively. Spray dried nanoparticles were further evaluated for mucoadhesion efficacy and release behavior on goat nasal mucosa. The results concluded ionic gelation method is easy, reproducible led to efficient entrapment also revealed RZB loaded CS nanoparticles is most suitable for intranasal drug delivery.

Masalova O. *et al*., 2013 prepared and investigated the effect of stabilizers, polymer concentration, molecular weight stable aqueous colloids of alginate and chitosan developed by nanoemulsion method. The result revealed that proposed variables affected particle size. The admixing of protein to polysaccharide solution prior to nanoparticle formation lead to increased entrapment efficiency therefore polysaccharide based nanoparticles are promising carrier for biologically active compound.

Moradhaseli S. *et al*., 2013 investigated the effectiveness of ICD-85 (venom derived peptides) loaded sodium alginate nanoparticles (NPs) against human carcinoma. Nanoparticles were fabricated by ionic gelation pre gelation technique and evaluated for physicochemical

properties, *in-vitro* release profile, *in-vitro* cytotoxicity assay. The results revealed that high loading capacity and sustained release behavior of ICD-85 from loaded NPs can effectively inhibit proliferation of HEp-2 (cancer cell) cell line compared to free ICH -85 as evaluated by MTT test.

Sankar G.P.et *al*., 2013 suggested that ethambutol alone created roughness and significantly cleaved the surface of cell wall. Rifampacin alone created pores on the cell wall of *M smegmatis*. These finding gave better understanding of activity of the drug molecules and use of this cocept may help to improve the existing drug molecules for the treatment of Tuberculosis.

Sarie F.N. *et al*., 2013 investigated and reported the efficacy of alginate nanoparticles as a carrier with adjuvant and enhancement in immunogenicity due to prolonged release behavior. The study involved the preparation of diphtheria toxoid loaded nanoparticles by ionic gelation technique. The study revealed the encapsulation process did not affect the antigenic integrity and activity. Guinea pigs immunized with the diphtheria toxoid-loaded alginate nanoparticles showed highest humoral immune response than conventional vaccine.

Ramesh C.N. *et al*., 2012 fabricated 5 fluorouracil loaded nanoparticles by ionic gelation method using sodium alginate and chitosan which were then suspended in chitosan solution i.e. chitosan coated sodium alginate- chitosan nanoparticles(CH-SA-CH).The results revealed that size, encapsulation efficiency were dependent on molar ratio of chitosan and alginate. The chitosan coating over alginate chitosan nanoparticle changed morphology of surface of nano-carrier but enhanced muco-adhesion due to chitosan coating, increased bioavailability compared to uncoated nanoparticles.

Chopra M. *et al*., 2012 formulated Streptomycin loaded chitosan-alginate nanoparticle using ionotropic pregelation method. Concentration of 0.75mg/ml of chitosan, 1% (w/v) of calcium chloride and stirring time 90 min constituted the optimum conditions in formulation development. The optimized batch had particle size and percentage encapsulation efficiency of 328.4nm and 93.32% respectively. The result concluded that increased polymer and cross linker concentration lead to increase in particle size. Encapsulation efficiency first showed an

increase followed by a decrease on increasing the polymer concentration, whereas it increased with an increase in cross linker concentration.

Daniel L. C. *et al*., 2012 fabricated mesoporous silica nanoparticle (MSNP) of rifampicin and isoniazid and coated with cyclodextrin (pH-operated valves that open only at acidic pH). The Study revealed that cyclodextrin fuctionalized Isoniazid MSNP kills *M. tuberculosis* within macrophages significantly more effectively than a free drug. These studies showed that MSNP provide a versatile platform that can be functionalized to optimize the loading and intracellular release of specific drugs for the treatment of tuberculosis.

Debjit B. *et al*., 2012 reviewed and summarized the ability of controlled release formulation to deliver the encapsulated drug at predictable and reproducible rate for predetermined period in particular site. The result concluded that such systems offer more advantages over traditional methods, including tailoring of drug release rate, protection of sensitive drugs, increased patient compliance and comfort.

Drew R.E. *et al.*, 2012 investigated the effect of ligand density, receptor density and nanoparticle size on cell targeting. The results indicated that intermediate ligand density provided statistically significant improvement in cell binding compared to higher or lower ligand density. The study was justified by use of folic acid ligand.

Gupta V.K. *et al*., 2012 formulated and investigated the effect of drug concentration, polymer concentration, crosslinking agent and stirring speed on 5-Flurouracil loaded nanoparticles. Nanoparticles were prepared by Ionotropic pregelation method. The results showed optimum particle size (246 nm) and maximum drug entrapment (89.90%) was obtained in drug polymer ratio 05:75, cross-linking agents 2 ml, stirring rate 800 rpm and stirring time 90 min.

Jawahar N. *et al*., 2012 reviewed the importance of nano particulate systems and methods for delivering anti-tubercular drugs directly to the lungs via the respiratory route. Also suggested various drug delivery systems using polymers, lipids, and proteins to serve as inhalable anti-tubercular drug carriers. Encapsulation of old drugs in new weapons i.e. nano systems has

emerged as an attractive and promising substitute that enhances therapeutic effectiveness and minimizes undesirable side effects of the drugs.

Karakousis P. *et al.,* 2012 reviewed and described that all health policy makers, clinicians, research community and patients, must work together to support the development of new, highly active, universally accessible short course regimen for the treatment of drug susceptible and drug resistant TB. They also discussed that we all must ensure a high level of implementation of 'DOTS' specially in high burden, resource limited areas in order to achieve goal of TB free world.

Nagavarma B V N *et al.,* 2012 reviewed and described the different methods available for production of polymeric nanoparticles. The result showed that despite certain technological challenges, nanoparticles have been showed great promise for the development of drug delivery system.

Nagavarma B.V.N. *et al.,* 2012 reviewed and described various preparation techniques for production of polymeric nanoparticles. The drug loaded nanospheres and nanocapsules can be produced by simple, safe and reproducible techniques. The result revealed that the nanoparticle preparation method is marked best due to no need of toxic reagents, allows economic scaling up, can be easily optimized to improve yield and entrapment efficiency.

Nesamony J. *et al.,* 2012 synthesized calcium alginate nanoparticles from pharmaceutical micro-emulsion. The sonication of micro emulsions for 1 h approximately resulted in 350nm sized calcium alginate nanoparticles. Further BSA was incorporated in calcium alginate nanoparticles and release profile studied. The study indicated initial burst release followed by a sustained-release.

Pratap Y.P. *et al.,* 2012 Investigated and developed the simple, rapid, accurate, sensitive and specific method for estimation of drug in same dosage form. The opportunity was taken handle the challenges during treatment which led to emergence of drug resistant TB. The main cause of resistance is non-adherence behavior towards multi dosage regimen. The recovery study confirmed the validity of proposed method as 99.56 % Isoniazid and 100.14% Pyridoxine was recovered and determined by simultaneous equation.

Raj V *et al.*, 2012 fabricated and evaluated rifampicin loaded chitosan and Polyethylene glycol 600 (PEG) nanoparticles by ionic gelation method. Nanoparticles were characterized for various parameters such as loading capacity, encapsulation efficiency, SEM, FTIR and *in-vitro drug release* profile. The result concluded PEG binding with CS-RIF changed surface properties of nanoparticles and significantly achieved prolonged retention of drug compared to non-coated CS-RIF.

Sanjay B. *et al.*, 2012 reported stability studies are prerequisite for the approval and acceptance of pharmaceutical product as it ensures the quality, safety, efficacy of product throughout the shelf life. Stability studies are carried out as per guidelines issued by ICH, WHO or other agencies. The result concluded that the stability study is the key procedural component in the pharmaceutical development programme for a new drug as well as new formulation. It should be carried out following proper scientific principles and after understanding of the current regulatory requirements and as per the climatic zone.

Vatsal R. *et al.*, 2012 investigated the effect of PEG binding on rifampicin loaded chitosan nanoparticles (CS-RIF). The carrier system (chitosan nanoparticle) was prepared by ionic gelation method. The research revealed that functionalization with PEG resulted in increased particle size, drug encapsulation also with significantly prolonged retention compared to non-coated CS-RIF.

Abhishek G. *et al.*, 2011 reviewed and described various methods of preparation, characterization, release and applications of polymeric nanoparticles. Also described its utility in various areas such as drug delivery, tissue targeting, cancer treatment, diagnostic agent and imaging pupose. The result concluded that these targeted delivery system changes the pharmacokinetic and pharmacodynamics properties of drug moiety.

Gupta J. *et al.*, 2011 formulated the chloramphenicol loaded alginate chitosan nanoparticles by counter ion induced gelification technique. The formulated batches were evaluated for *in-vitro* drug release and stability studies. Drug loaded nanoparticles were found to be physically and chemically stable at storage temp 2-8°C ± 1°C and 25°C ± 1°C. Dissolution study revealed that the drug release was better in phosphate buffer i.e. slow and continuous for 24

hrs. The result concluded nanoparticles of natural polymer could be a promising approach in solubility enhancement of hydrophobic drugs.

Gupta M. *et al.*, 2011 highlighted challenges for a drug molecule to reach its destination i.e. in complex cellular network of an organism. Targeted drug delivery approach improves the efficacy and reduces side effects of drug. Result concluded target based drug delivery has significant advantages over conventional therapy.

Holloway K.L. *et al.*, 2011 described tuberculosis as a re-emerging disease so the major problem in developing and developed countries today. The bone lesions occur in 3-5% of active tuberculosis cases and can be used to diagnose the disease in ancient skeletal remains. The results disclosed that the frequency of bone lesion due to tuberculosis decrease significantly through time. Leison distribution changed from spinal lesion to extra- spinal lesion in later time period.

Kaur S.P. *et al.*, 2011 Formulated and evaluated rivastigmine loaded chitosan tripolyphosphate nanoparticles by ionic gelation method. The study involved preparation of five batches with variable polymer concentration. Optimized batch 1:3 ratio showed entrapment efficiency, particle size and polydispersity index as 83.74%, 258 nm and 0.261 respectively. The results concluded the suitability of chitosan nanoparticles as a potential carrier for sustained delivery of drugs.

Krishna S.A. *et al.*, 2011 reviewed and discussed considerable research interest in nanoparticle based drug delivery. Various polymers have been used in formulation development of nanoparticles but natural polymers have gained special attention such as chitosan and alginate. Result concluded natural polymer based nano-carriers are biologically safe, non-toxic, biocompatible and biodegradable polysaccharide. Chitosan and alginate have gained more attention as drug delivery carriers.

Nitish K. *et al.*, 2011 reviewed and discussed the major challenges like development of multidrug resistant TB, requirement of high dose, subsequent intolerable toxicity associated with conventional TB treatment. The result concluded that novel anti-TB remains a priority so the development of the nanoparticle based drug delivery systems for currently used agents

represents cost effective and promising alternative in treatment and prevention of TB.

Sagar R Mudshinge *et al.*; 2011 investigated that nanotechnology provide wide range of synthetic nanostructures. These nanoscale tools can control and manipulate bio molecular and supramolecular assemblies in order to improve the human quality. The result showed that the human illness (cancer, cardiovascular disease, genetic disorders) and longevity could be better understood by these nano therapeutics and approaches

Shailja A. K. *et al.*, 2011 Reviewed and described the benefits of natural polymers in targeted drug delivery system via nanoparticles. Natural polymer have gained special attention as drug delivery carrier because of their better stability, low toxicity, simple and mild preparation method and providing versatile routes of administration. The article involved various techniques for the preparation of nanoparticles using natural polymers chitosan, alginate and proteins.

Shegokar R. *et al.*, 2011 described the importance of nanotechnology in field of therapeutics. This innovative approach suggested that infection could be controlled at molecular level. Nanotechnology is promising strategy in treatment of TB with improved drug bioavailability and reduction of the dosing frequency. The results showed nanotechnology based rational targeting may improve therapeutic success by limiting adverse drug effects and requiring less frequent administration regimes, ultimately resulting in more patient's compliance and thus attain higher adherence levels.

Shegokar R. *et al.*, 2011 reviewed nanoparticle based drug delivery systems represent cost effective, practical and alternative potential TB chemotherapy. Improved drug bioavailability, reduction of dosing frequency, feasibility of the versatile routes of drug administration, long term stability serve as better management of the disease. The result concluded natural polymers (e.g., alginate and chitosan) represent alternative perspective in drug delivery field.

Soval L.P. *et al.*, 2011 reviewed and focused on classification, preparation methods and advantages of nanoparticles over present pharmaceutics, which often characterized by poor bioavailability, higher patient cost and inefficient treatments, increased risk of toxicity or even

death. The result showed that nanotechnology enabled drug delivery is opening prospective future in pharmaceutics and have significant impact on drug delivery sector.

Yueling Z. *et al.*, 2011 formulated insulin loaded alginate chitosan microspheres by membrane emulsification technique in combination with ion (Ca^{2+}) and polymer (chitosan) solidification. The study investigated the effect of loading ways on loading efficiency and immunological activity. Results suggested that chitosan solidification process had higher loading efficiency (56.7%) and remarkable activity maintenance (99.4%). The blood glucose level of diabetic rats could be effectively reduced and stably kept for a long time (60 h) after oral administration of the insulin-loaded alginate–chitosan microspheres.

Angshuman B. *et al.*, 2010 fabricated lopinavir loaded alginate nanoparticles by insitu nanoemulsion polymer cross linking method. The study included the effect of different encapsulating solvents on nanoparticles characterstics. Among different batches the 1:6 drug polymer ratio showed best release pattern and controlled drug release over a period of 24 hour. The release followed Higuchi kinetics rather than first order kinetics, indicating diffusion controlled drug release.

Muhammed R. P.E. *et al*., 2010 developed chitosan nanoparticles for first line antitubercular drug isoniazid, to enhance bioavailability and to reduce dose frequency. Chitosan nanoparticles of various concentrations were prepared by ionic gelation nanoparticles which showed good encapsulation efficiency, good release profile with first order release kinetics.

Partha S. *et al.*, 2010 fabricated ampicillin trihydrate loaded chitosan nanoparticles by ionic gelation method and evaluated for physicochemical properties and release profile. Concentration of 0.35% w/v of chitosan, 0.40% w/v of tripolyphosphate and sonication time 20 min constituted the optimum conditions in formulation development. *In vitro* release data showed an initial burst followed by slow sustained drug release. The result concluded that polymer; cross linking agent concentration and sonication time are rate limiting factors in development of optimized formulation.

Rohitas M. *et al*., 2010 developed a simple, sensitive, rapid, accurate, precise and economical procedurefor the simultaneous estimation of Mesalazine and Prednisolone in combined dosage

.The method involved the use of absorbance maxima of Mesalazine and Prednisolone in simultaneous equation (Vierodt's method). The recoveries of Mesalazine and Prednisolone from the standard mixture solution were found to be 99.04% and 99.92% respectively. The results concluded that combined drugs could be quantified easily without interference of excipients.

Takka S. *et al.*, 2010 fabricated Bovine serum- albumin loaded beads by ionotropic gelation of alginate with calcium and chitosan. The proposed work investigated the effect of the sodium alginate and chitosan concentration on the particle size and loading efficacy of beads. The results concluded that chitosan concentration significantly influenced particle size and encapsulation efficiency of chitosan–alginate beads ($p < 0.05$). Decreasing the alginate concentration resulted in an increased release of albumin in acidic media. The rapid dissolution of chitosan–alginate matrices in the higher pH resulted in burst release of protein drug.

Vedha H.B.N. *et al.*, 2010 explained despite availability of potential curative pharmacotherapeutics for over 50 years, the length of treatment and pill burden hampered the patient life style and lead to low compliance with less adherence to the administration schedules. These are the main cause of therapeutic failure and development of multidrug resistant strains. The result concluded that nanoparticulate system are capable to target the site of TB hence reduces dosing frequency and improves healthcare system.

Wilson B. *et al.*, 2010 fabricated tacrine loaded chitosan nanoparticles by spontaneous emulsification method. Drug loaded formulated batches showed initial burst followed by continuous slow release. Results suggested coating of nanoparticles with Polysorbate 80 slightly reduced the drug release from the nanoparticles. The biodistribution of these particles after intravenous injection in rats showed that nanoparticles coated with 1% Polysorbate 80 altered the biodistribution pattern of nanoparticles.

Zhong M. *et al.*, 2010 Reviewed and discussed, despite established therapeutic efficacy of existing drugs the potency towards disease causing microorganism is decreased. The reason might be due to lack of accessibility to target site. The results revealed that nanostructured

biomaterials have unique properties such as small size, large surface area, functionalizable structure. These properties facilitate the administration of anti-microbial drug and overcoming limitations in traditional antimicrobial therapeutics.

Adlin J.J. *et al*., 2009 attempted to prepare flutamide loaded chitosan nanoparticles by ionic gelation method. The study aimed to formulate sustained release dosage form for a drug having half-life of 5-6 hrs. Also effect of core: coat ratio on physicochemical properties, drug content was studied. The results concluded that 1:4 gave better sustained release for about 12 hrs as compared to other formulations.

Gozare T. *et al.*, 2009 investigated the effect of various parameters such as polymer ratio $Cacl_2$/Alginate ratio and N/P ratio on the particle size distribution and loading efficacy of nanoparticles. Alginate chitosan nanoparticles were developed by ionic gelation technique. The optimized batch showed loading efficacy of 95.6%, average particle size 194 nm, zeta potential was about + 30 mV indicating good stability during storage.

Rajesh S. *et al*., 2009 reviewed and discussed about nano delivery systems which hold the great potential to overcome obstacles to the efficient target a number of diverse cell types. The result showed that small size, customized surface, improved solubility and multifuctionality of nanoparticles selectively affects the targeted organs.

Li P. *et al*., 2008 investigated the efficacy of CS nanoparticles for simultaneous delivery of 5-fluorouracil (5-FU) and leucovorin (LV). Combined drug encapsulated nanoparticles were fabricated by ionic gelation technology. The results concluded simultaneous release of drug 5-FU and LV had initial burst release followed by a constant and continuous release. The release of drugs was influenced by their initial drug concentration, showing that the release of drugs could be controlled by varying the initial drug concentration.

Motwani S.K. *et al*., 2008 investigated and reported mucoadhesive chitosan (CS)-sodium alginate (ALG) nanoparticles provide long term extraocular drug delivery. The result concluded that designed batches by design of experiments 3-factor, 3-level Box-Behnken statistical design helped in optimization of batches. The designed nanoparticles had average particle size from 205 to 572 nm (polydispersity from 0.325 to 0.489) and zetapotential from

17.6 to 47.8 mV. Nanoparticles revealed a fast release during the first hour followed by a more gradual drug release during a 24h period following a non-Fickian diffusion process.

Padayatchi N. *et al.*, 2008 reviewed and reported the growing number of cases of extensively drug resistant tuberculosis (XRD-TB) and challenges associated with existing therapeutic system. The result concluded that the current failing TB programmes could be improved by borrowing strategies such as decentralization of care, treatment expertise, high levels of treatment adherence. The longer we support a failed system or wait for a perfect solution, the more the devastation continue to grow.

Ping L. *et al.*, 2008 developed and characterized the nifedipine loaded chitosan alginate nanoparticles (CS/ALG) by ionotropic method. The study included the effect of different pH media (pH 1.5, 7.4, 6.8) on release of drug from nanoparticles. The release profile concluded the fast release of drug at simulated intestinal fluid (pH 6.8 and pH 7.4) while slow release in simulated gastric fluid (pH-1.5). The release profile also reflected the initial burst release in all three media, followed by continuous, controlled drug release. The release was best explained by fickian diffusion.

Shirwaikar A. *et al*; 2008 explained herbal excipients are promising biodegradable materials and are compatible with the excipients in drug delivery systems. Herbal excipients are non-toxic, freely available, less expensive compared to their synthetic counterpart. The result concluded that in coming years, there is going to be continued interest in natural excipients to have better materials for drug delivery systems.

Rolee S. *et al.*, 2007 formulated and investigated two drug loaded (isoniazid and rifampicin) microparticles for macrophage targeting. The micro-particles were prepared by spray drying method using poly (lactic acid) and exposed to cultured J774 mouse macrophage. The results revealed that micro-particle phagocytosis induced response in infected murine macrophage which indicated the activation of innate bacterial mechanism.

Boonsongrit Y *et al.*, 2006 investigated the effect of pH on entrapment efficiency, physicochemical properties and drug release profile of nano/ microparticles prepared by ionic interaction. Study included three model drugs (insulin, diclofenac sodium, and salicylic acid)

with different pI or pKa. The result concluded that the entrapment efficiency is affected by formulation pH. The ionic interaction between drug and chitosan was low and too weak to control the drug release so high burst release of drugs from chitosan micro/nanoparticles was observed regardless of the pH of dissolution media.

Zahoor A. *et al.*, 2006 fabricated anti-tubercular drug loaded alginate nanoparticles by controlled cation induced gelification method. The nanoparticles were administered orally to mice and therapeutic efficacy was evaluated in *M. tuberculosis* H37Rv infected mice. The results revealed that TB infected mice in three oral dose spaced 15 days apart resulted in complete bacterial clearance from organs compared to 45 conventional doses of orally administered free drugs.

Rama P. T. *et al;* 2005 reviewed and described the causes of high mortality in case tuberculosis disease. A number of anti-TB drugs are ineffective against disease so internationally efforts are being made to develop new anti-tubercular drugs. The result suggested that a large of drug targets from cell wall biosynthesis, nucleic acid biosynthesis and many other biosynthetic pathways are being unraveled throughout the world and are being utilized for drug development.

Shanmugam S. *et al.*, 2005 described that products of natural sources have become an integral part of human health care system because of some side effects and toxicity of synthetic drugs. The result suggested that natural polymers have wide scope in food, cosmetic and medical field as compared to synthetic polymers. Natural polymers have achieved great success in development of therapeutic system.

Barry C. E. *et al.*, 2004 described traditional drug delivery efforts have focused on killing of actively growing *Mycobacterium tuberculosis* and treating resistant strains but they are unlikely to significantly reduce duration of treatment and disease mortality. The result concluded recent technology-genome scale biology, leading to a far better understanding of the genes and enzymes that *Mycobacterium* required for its long term survival. It is a potential drug target in treatment of TB.

Khuller G. K. *et al*., 2004 investigated the emerging problems in treatment of tuberculosis specially Latent T.B. Current chemotherapy of mycobacterial are inadequate in achieving optimal drug concentrations inside the cells so Liposomal based drug delivery system have sparked a renewed interest in treatment of mycobacterial infection. The result concluded that the versatility of liposomes in incorporation of hydrophilic / hydrophobic components, biodegradability, biocompatibility, non-toxic nature, and sustained release behavior makes them innovative candidates for the delivery of anti-tubercular drugs.

Panchgnula R. *et al*., 2004 reviewed and explored that the formulation of rifampicin alone showed variability in bioavailability. The bioequivalence trials reported the problems and clear "myth and assumptions" regarding rifampicin bioavailability from fixed dose combination formulations. Hence different approaches are required to solve issue of rifampicin bioavailability on basis of BCS and ADME.

Pandey R. *et al*., 2004 developed and characterized anti-tubercular drug loaded alginate chitosan microspheres. The encapsulation efficiency ranged 65-85%, drug loading was 200-280 mg of drug per gram microspheres, mean half-life and residence time increased by 13 -15 fold. The results revealed that the administration of therapeutic dose of microspheres cleared bacilli in 10 days from *Mycobacterium tuberculosis* H37Rv-infected guinea pigs. This clearance was equivalent to conventional treatment of 6 weeks.

Kuo M.R. *et al*., 2003 reported that tuberculosis and malaria together result in a 5 million death annually. The spread of multi drug resistance in pathogenic causative agents underscore the need of active compounds with novel inhibitory properties. The result showed that two novel class of compounds were identified which do not require any activation and are effective against wild type and drug resistant strains of tuberculosis and malaria.

Smith L. *et al*., 2003 reviewed that tuberculosis is major leading cause of death worldwide from single infectious organism. Now a days multidrug resistant tuberculosis is a biggest challenge even after most advancement in treatment. The better diagnostic techniques, control measures and treatment options are desperately needed along with worldwide commitment to battle this age-old disease.

Shiratsuchi H. *et al.,* 2000 investigated the effects of T lymphocytes on intracellular *Mycobacterium avium* replication. In study the separated adherent monocytes were infected with *Mycobacterium avium* and cultured with lymphocyte. The results revealed that CD4 lymphocyte diminished the anti-mycobacterial property while CD81 (T cells) increased intracellular *M. avium* growth. Hence T cells play important role in intracellular growth of *M. avium* in monocyte.

Ramachandran R. *et al., 1999* aimed to quantify the socioeconomic impact of tuberculosis on patients and their family in urban and rural areas. The interview was scheduled on 17 focus groups and data collected regarding socioeconomic demographic characteristics, employment, income particulars, expenditure on illness and effect on children from newly detected sputum positive tuberculosis patients. The result concluded that the total cost and particularly indirect costs due to TB were very high care giving activities of female patients decreased significantly.

Paranjape R. S. *et al.,* 1997 screened total 4618 tuberculosis patients of Pune between 1991and 1996 for anti HIV antibodies. The method of assay was enzyme immune assay (EIA) and rapid EIA or western blot test. The result concluded that sera prevalence of HIV among newly diagnosed tuberculosis patients rose from 3.2 percent in1991 to 20.1 percent in 1996.

Rajanarivony M. *et al.,* 1993 presented new approach for preparation of nanoparticles. The method involved the controlled gelification phenomenon of alginate by calcium ion followed by Poly L lysine. The results revealed that size of the particles are greatly dependent on the order of addition of calcium and poly-L-lysine to the sodium alginate solution and concentration of polymers. For evaluation of drug loading capacity the doxorubicin was used as model drug. The results indicated that alginate nanoparticles are interesting carriers because the drug-loading capacity could be > 50 mg of doxorubicin per 100 mg of alginate.

Dannenberg A.M *et al., 1993* reviewed the susceptibility of bacilli towards antimicrobial therapy and fight against drug resistant TB. The result suggested that the knowledge of the two main type of immune response against tuberculosis and use of it to manipulate those mechanisms leading to precisely designed recombinant BCG vaccines as effective attack tool

on current epidemic.

1.8 RESEARCH ENVISAGED

Even though the *mycobacterium tuberculosis* was identified about 130 years ago but exact understanding of pathogenesis of tuberculosis is still deficient. The most serious and challenging problem in society is the treatment of asymptomatic TB (Latent TB) The persistence of latent tuberculosis is due to ability of *Mycobacterium tuberculosis to* subvert host immune responses to survive and grow in the infected macrophages. Whenever immune system weakens the dormant bacilli transform to active bacilli and carried to distant organs and tissues and now such person can infect another. World Health Organization has listed 30 countries with high TB burden rate among them three countries contributed almost half of the world's cases of MDR-TB i.e. India (24%), China (13%), Russian Federation (10%). About 1.7 billion i.e. 23% of world's population are estimated to have latent TB infection and so are at the risk of developing TB disease during their life time (WHO report., 2018).

The delivery of anti-tubercular drugs by nanoparticulate delivery system offers the potential advantage over free drug to target infected macrophages. Hence macrophage targeted, natural polymer nanoparticulate system offers opportunity to deliver drug intracellularly for eradication of *Mycobacterium* present in the macrophage cells as dormant bacilli.

1.9 OBJECTIVE OF STUDY

1. To deliver the drug to intracellular infected cells i.e. macrophages.
2. To achieve target specificity towards macrophages by use of ligand molecule.
3. To improve the bioavailability of drug.
4. To shield the drug from degradation by use of nanoparticulate approach.
5. To reduce drug toxicity.

1.10 Plan of Work

1. Procurement of drugs and Polymers.

2. Preformulation studies of drug / Identification of drug.

2.1 a) Physical appearances.

b) Solubility parameter.

c) λ max determination of drugs.

d) Functional group analysis.

2.2. Drug excipient interaction study.

3. Formulation development.

a) Selection of Polymer.

b) Selection of suitable, feasible formulation method.

a) Optimization of formulation parameters.

4. Evaluation of formulation.

a) Particle size and shape evaluation.

b) Particle size distribution.

c) Entrapment efficiency.

d) Loading efficiency.

e) *In vitro* drug release study.

f) Stability studies.

5. *In-vitro* anti-tubercular screening test of optimized batch.

6. Macrophage cell (J774) line study of best batch.

7. Cytotoxicity study best batch.

8. Statistical analysis

9. Computation and compilation.