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CHAPTER 1 INTRODUCTION Page 1 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 26 th 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

CHAPTER 1 INTRODUCTION Page 2 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 Tripathi 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 DS 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 19 th century, the TB cases were very rare but since then TB incidences increased progressively due to increased growing population density caused by industrialization (Nilesh 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 Murray 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 Ranjita 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

CHAPTER 1 INTRODUCTION Page 3 bacilli may enter in the body or may invade through epithelial surface (Dannenberg AM 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 TM 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

CHAPTER 1 INTRODUCTION Page 4 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.

CHAPTER 1 INTRODUCTION Page 5 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 KL 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

CHAPTER 1 INTRODUCTION Page 6 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

CHAPTER 1 INTRODUCTION Page 7 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 Y by T-cells on exposure to TB antigen B Elisa test / Quantiferon test(Enzyme linked immune assay) Measures INF Y by T-cells on exposure to PPD (purified protein derivatives) antibody mixture. C Quantiferon Gold /Elisa test Measures INF Y 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

CHAPTER 1 INTRODUCTION Page 8 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 MR 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 HM 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.,).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

CHAPTER 1 INTRODUCTION Page 9 as XDR TB as these strains are virtually untreatable which cause of high lethality in infected individual (Alladi Mohan et al). Table 1.2 Types of TB (Iseman Md 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 Hari BN et al; 2010) Drug Dose (mg) BCS Class Isoniazid 300 III Rifampacin 300 II Pyrazinamide 500 III Ethambutol HCl 400 III Rifabutin 150 II

CHAPTER 1 INTRODUCTION Page 10 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 lal pal 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,

CHAPTER 1 INTRODUCTION Page 11 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 Tran et al., 2017). The advantage of using nanoparticles as a drug delivery system includes the following (Rajesh Singh 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

CHAPTER 1 INTRODUCTION Page 12 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 et al., 2016). Thus before commercialization and clinical use of these nanosystems the associated practical problems should be overcome (Brajesh et al., 2016). 1.2.1 Ideal properties desired for nanoparticulate drug delivery system (Siddhi Pandhare 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

CHAPTER 1 INTRODUCTION Page 13 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 lal pal 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 (Macheal Kohler 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

CHAPTER 1 INTRODUCTION Page 14 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

CHAPTER 1 INTRODUCTION Page 15 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

CHAPTER 1 INTRODUCTION Page 16 1.2.3

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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. 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

CHAPTER 1 INTRODUCTION Page 17 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

CHAPTER 1 INTRODUCTION Page 18 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 Rangari et al., 2015)

CHAPTER 1 INTRODUCTION Page 19 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 (Nagaverma 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 Rangari., 2015).

CHAPTER 1 INTRODUCTION Page 20 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

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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

CHAPTER 1 INTRODUCTION Page 21 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. NON -BIODEGRADABLE POLYMERIC NANOPARTICLES SYNTHETIC POLYMERIC NANOPARTICLES POLYMERIC NANOPARTICLES NATURAL POLYMERIC NANOPARTICLES BIODEGRADABLE POLYMERIC NANOPARTICLES

CHAPTER 1 INTRODUCTION Page 22 1.2.5 Importance of natural polymers over synthetic polymers (Kusum kaushik 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 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.

CHAPTER 1 INTRODUCTION Page 23 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.

0: https://www.researchgate.net/publication/315012217_Recent_Applications_of_Natural_Polymers_in_Nanodrug_Delivery 52%

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 A Sailaja P 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

0: https://www.researchgate.net/publication/315012217_Recent_Applications_of_Natural_Polymers_in_Nanodrug_Delivery 80%

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

0: https://www.researchgate.net/publication/315012217_Recent_Applications_of_Natural_Polymers_in_Nanodrug_Delivery 83%

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

CHAPTER 1 INTRODUCTION Page 24 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.

CHAPTER 1 INTRODUCTION Page 25 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

CHAPTER 1 INTRODUCTION Page 26 conventional excipient and specifically as a tool in polymeric controlled drug delivery (Tonnesen HH 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 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 Sachan et al; 2009).

CHAPTER 1 INTRODUCTION Page 27 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 (Kuen yong et al, 2012). After extraction, the alginate can be further purified and then converted to either salt or acid. Fig 1.9 Sodium alginate production

CHAPTER 1 INTRODUCTION Page 28 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 yong Lee 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

CHAPTER 1 INTRODUCTION Page 29 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 (Shamkhani AL 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,

CHAPTER 1 INTRODUCTION Page 30 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

CHAPTER 1 INTRODUCTION Page 31 concentration are used to localize the drug carrier to predetermined site (Jose M Morachis 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

CHAPTER 1 INTRODUCTION Page 32 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.

CHAPTER 1 INTRODUCTION Page 33 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

CHAPTER 1 INTRODUCTION Page 34 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

CHAPTER 1 INTRODUCTION Page 35 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

CHAPTER 1 INTRODUCTION Page 36 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 5 H 5 N 3 O Drug Category Anti-tubercular drug Physical Properties White, crystalline powder, odourless and slightly bitter taste. Melting Point 192 o C 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

CHAPTER 1 INTRODUCTION Page 37 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 6 H 7 N 3 O

CHAPTER 1 INTRODUCTION Page 38 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 and must be activate 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.

CHAPTER 1 INTRODUCTION Page 39 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 6 H 7 O 6 Composition linear copolymer with homopolymeric 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

CHAPTER 1 INTRODUCTION Page 40 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 6 H 11 NO 4) n Composition linear copolymer with homopolymeric 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

CHAPTER 1 INTRODUCTION Page 41 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 5 P 3 O 10 Molecular Weight linear copolymer with homopolymeric 376.86 g/mol 3 pH 9.8 Solubility NaTPP is easily soluble in water about 20g/100ml (200 o C). It may

CHAPTER 1 INTRODUCTION Page 42 be clear to slightly hazy Toxicity The products are not considered to be toxic during the natural course of handling. The food grade STPP 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

CHAPTER 1 INTRODUCTION Page 43 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 Melting Point 772 o C (anhydrous); 176 o C (dehydrate); 30 o C (Hexahydrate).

CHAPTER 1 INTRODUCTION Page 44 Empirical Formula CaCl 2 , CaCl 2 .2H 2 O, CaCl 2 .6H 2 O. Molecular Weight 110.98 g/mol Boiling point < 1600 o C 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

CHAPTER 1 INTRODUCTION Page 45 Molecular Structure Empirical Formula C 19 H 19 N 7 O 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

CHAPTER 1 INTRODUCTION Page 46 Molecular Structure Empirical Formula C 6 H 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.

CHAPTER 1 INTRODUCTION Page 47 1.7 REVIEW OF LITERATURE Sujit kumar Debhnath 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 Mayur kumar

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. 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. Susan Swindells 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.

CHAPTER 1 INTRODUCTION Page 48 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. 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. Zhaohui Ge 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 Choi 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,

CHAPTER 1 INTRODUCTION Page 49 liver, kidney, spleen until 24 h post nebulization. The drug loaded CHNPs have excellent chemotherapeutic efficacy on mycobacterium than free drug. Khan MA 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. 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. Kusum Kaushik 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.

CHAPTER 1 INTRODUCTION Page 50 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. 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. Hou DZ 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. Balaji RA et al., 2015 revealed that lipophilic drug levofloxacin could be entrapped within chitosan/ alginate (

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>
> 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.

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>
> The release profile was characterized by an initial burst effect in phosphate buffer solution, followed by a continuous and controlled release.

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-

CHAPTER 1 INTRODUCTION Page 51 dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay in A549 cell lines along with hemocompatibility. Emilia Szymanska 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. Yolandy lemmer 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. Siddhi pandhare 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. Patricia Severino 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. Dheda 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

CHAPTER 1 INTRODUCTION Page 52 new drugs and rapid diagnostics for tuberculosis. Government Funding had strengthened global control efforts, research and TB eradication programmes. Kidenya 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. 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. Mojtaba Salouti 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. 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. Daisy S Chella Kumari et al., 2013 prepared methotrexate (BCS Class –III) loaded alginate nanoparticles by ionic gelation method using Box Behnken method. The objective of research

CHAPTER 1 INTRODUCTION Page 53 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 SS 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. Attia Shafie et al., 2013 formulated Betamethasone sodium phosphate loaded mucoadhesive chitosan-sodium alginate nanoresevoir by ionotropic gelation method and investigated

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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

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an initial burst release of the drug followed by slow sustained release over 24, 48 or 72 hours depending on the formulation parameters.

Sarie F 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. S Kumaran 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

CHAPTER 1 INTRODUCTION Page 54 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. Masalova 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. Ramesh C Nagarwal 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. Daniel L. Clemens 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

CHAPTER 1 INTRODUCTION Page 55 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. 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. Raj V 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. 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. Gupta VK 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. 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

CHAPTER 1 INTRODUCTION Page 56 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. 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. Debjit Bhownik 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. Pratap Y Pawar 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. Sanjay Bajaj 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. Drew 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

CHAPTER 1 INTRODUCTION Page 57 statistically significant improvement in cell binding compared to higher or lower ligand density. The study was justified by use of folic acid ligand. 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 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. V Raj 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. Shegokar Ranjita S 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. Kaur SP et al., 2011 Formulated and evaluated rivastigmine loaded chitosan tripolyphosphate nanoparticles by ionic gelation method. The study involved preparation of five batches with

CHAPTER 1 INTRODUCTION Page 58 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. Yueling zhang 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. Gupta Manish 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. Shailja A K et al., 2011 Reviewed and described the benefits of natural polymers in targeted drug delivery system via nanoparticles. Natural polymer

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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

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for the preparation of nanoparticles using natural polymers chitosan, alginate and proteins.

Gupta Jitendra 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 .

CHAPTER 1 INTRODUCTION Page 59 Shegokar 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. Nitish kumar 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. Holloway KL 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. Soval lal pal 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. A. Krishna Sailaja P 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.

CHAPTER 1 INTRODUCTION Page 60 Abhishek Garg 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. 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. 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. 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

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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

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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.

Partha saha 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.

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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. Sagar R Mudshinge et al.; 2010 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. Vedha Hari BN 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. Muhammed Rafeeq PE 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.

CHAPTER 1 INTRODUCTION Page 62 Zhang 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. 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. Adlin JJ 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. Rajesh singh 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. Ping Li 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.

CHAPTER 1 INTRODUCTION Page 63 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 SK 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 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. 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 Sharma 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.

CHAPTER 1 INTRODUCTION Page 64 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

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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.

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The ionic interaction between drug and chitosan was low and too weak to control the drug release

so

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high burst release of drugs from chitosan micro/nanoparticles was observed regardless of the pH of dissolution media.

Zahoor Ahmed et al., 2006 fabricated anti-tubercular drug loaded alginate nanoparticles by controlled cation induced gelification method. The nanoparticles were administered orally to mice and

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therapeutic efficacy was evaluated in M. tuberculosis H37Rv infected mice.

The results revealed that TB infected mice in three oral dose

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spaced 15 days apart resulted in complete bacterial clearance from organs compared to 45 conventional doses of orally administered free drugs.

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. Rama P Tripathi 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. 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

CHAPTER 1 INTRODUCTION Page 65 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. 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. 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.

CHAPTER 1 INTRODUCTION Page 66 Kuo MR 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. H Shiratsuchi 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

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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

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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

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evaluation of drug loading capacity the doxorubicin was used

CHAPTER 1 INTRODUCTION Page 67 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.

CHAPTER 1 INTRODUCTION Page 68 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.

CHAPTER 1 INTRODUCTION Page 69 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

CHAPTER 1 INTRODUCTION Page 70 9. Computation and compilation. CHAPTER 2

CHAPTER 1 INTRODUCTION Page 71 MATERIALS AND METHODOLOGY 2.1 MATERIALS 2.1.1 Materials used in present research work Table 2.1 List of Ingredients Used S. No. Ingredient Company Name 1. Isoniazid (API) Micro Lab, Mumbai 2. Pyrazinamide (API) Micro Lab, Mumbai 3. Sodium Alginate Sigma Aldrich Chemical, Bangalore 4. Calcium Chloride Hi Media, Mumbai 5. Chitosan Sigma Aldrich Chemical, Bangalore 6. Sodium Tripolyphosphate Yarrow Chem Chemical, Mumbai 7. Glacial Acetic acid CDH Lab, New Delhi, India 8. Tween 80 CDH Lab, New Delhi, India 9. D-mannose Yarrow Chem Chemical, Mumbai. 10. Folic Acid SD Fine Chemical, Mumbai. 11. MB7H9 media Sigma Aldrich Chemical, Bangalore 12. ADC growth supplement Sigma Aldrich Chemical, Bangalore

CHAPTER 1 INTRODUCTION Page 72 13. Resazurin Dye Sigma Aldrich Chemical, Bangalore 14. Rhodamine B Sigma Aldrich Chemical, Bangalore 15. Antibiotic-Antimycotic Gibco BRL, USA 16. RPMI 1640 culture medium (DMEM) Sigma Aldrich Chemical, Bangalore 17. Tetrazolium salt Sigma Aldrich Chemical, Bangalore 18. Fetal bovine serum RMBIO,Biotechno labs,New Delhi. 19 Phosphate buffered saline Sigma Aldrich Chemical, Bangalore 2.1.2 Instruments used in present research work.

CHAPTER 1 INTRODUCTION Page 73 Table 2.2 List of Instruments Used. Sr. No. Instrument Manufacturer Name 1. UV Spectrophotometer UV 1700 Shimadzu 2. Magnetic Stirrer Tarson ceramic Spinot, chennai, India 3. Electronic Weighing Balance Afcoset, Mumbai, India 4. Sonicator PCI Analytics Pvt. Ltd , India 5. Centrifuge Remi Motors, Mumbai, India. 6. Pipettes Riviera glass pvt limited, Mumbai. 7. Double Distillation Apparatus Riviera glass pvt limited, Mumbai. 8. Zeta Sizer Malvern zetasizer Instrument (UK) 9. Nano Plus HD Nano Plus-3, Particulate systems :a division of micromeritics (US) 10. Vortex Shaker Popular India 11. FTIR Shimadzu, Perkin Elmer (US) 12. Dialysis Membrane Hi Media, India 13. FE SEM Tescan Mira 3, Australia 14. Burette (50 ml) Riviera glass Pvt limited, Mumbai. 15. Lyophilizer LABCONCO-Freeze zone 2.5 plus, India 16. pH Meter Elico Li 127, Hyderabad(India) 17 Beaker Riviera glass Pvt limited, Mumbai

CHAPTER 1 INTRODUCTION Page 74 18 Centrifuge (High RPM with cooling) Eppendorf, Model 5804 R Chennai 19 Microplate reader Biotek, Synergy Neo-2, India 20 Stability chamber REMI Laboratory instruments, Mumbai, India. 2.2 METHODOLOGY

CHAPTER 1 INTRODUCTION Page 75 2.2.1 PREFORMULATION STUDIES OF ISONIAZID: Drug was received as a gift sample from Micro Labs, Mumbai. 2.2.1.1 Identification of drug 2.2.1.1.1 Physical Appearance Isoniazid was physically examined for identification of drugs. The drug was examined for organoleptic properties. The drug was found to be colorless, odourless, crystalline powder. 2.2.1.1.2 Melting Point Melting point of drug was determined by using thiels tube apparatus. First capillary was taken and sealed at one end; sealed capillary was filled with drug and attached to thermometer using a thread. Thiels apparatus was filled with liquid paraffin and thermometer along with tied capillary was placed high enough so that liquid paraffin doesn‟t enter into capillary. The side arm was heated using burner to heat the sample until melting point was observed. The temperature at which sample begins to melt and the temperature at which it melts completely was noted. The procedure was repeated three times and mean melting point was calculated. Table 2.3 Melting point of Isoniazid S. No. Experimental melting point(° C) Average melting point(° C) Mean melting point(° C) 1 168-172 170 171.5±1.5 2 170-174 172 3 170-175 172.5 2.2.1.1.3 Solubility analysis of Isoniazid in various solvents

CHAPTER 1 INTRODUCTION Page 76 The solubility study of Isoniazid was performed in various aqueous, non-aqueous solvents. Excess of drug (500mg) was added in fixed solvent quantity (1ml) of each solvent in a screw cap container and kept on rotary shaker for 24 h. After 24 hour solution was centrifuged at 15000 rpm, supernatant was taken for determination of absorbance and matched to I.P solubility chart. Table 2.4 Solubility Chart as per I.P (1996) Description Approx. Volume(g/ml) Very soluble Less than 1 Freely Soluble 1to 10 Soluble 10 to 30 Sparingly Soluble 30 to 100 Slightly Soluble 100 to 1000 Very Slightly Soluble 1000 to 10000 Practically Insoluble Greater than 10000 Table 2.5 Solubility of Isoniazid in various solvent S. No Solvents Approximate volume of solvent (ml) /gram of solute Category as per IP 1 Water 28ml Soluble 2 Methanol 239ml Slightly soluble 3 Ethanol 385ml Slightly soluble 4 Chloroform 496ml Slightly soluble 5 Ether 1094ml very slightly soluble 2.2.1.2 ANALYTICAL METHOD DEVELOPMENT FOR ISONIAZID BY UV- VISIBLE SPECTROPHOTOMETER.

CHAPTER 1 INTRODUCTION Page 77 UV spectroscopy method was developed for the analysis of drug using double beam Shimadzu 1700 UV spectrophotometer. 2.2.1.2.1 Determination of λ max of Isoniazid Isoniazid solution of 100µg/ml was prepared in phosphate buffer (7.4 pH). The spectrum of the solution was obtained by using spectrophotometer (UV-1700 Pharma spec. Shimadzu). The sample was scanned at a range of 400-200 nm. The UV spectrum of isoniazid was obtained by scanning the above solution. A working λ max of isoniazid was obtained at 262nm. Fig 2.1 UV Spectrum of Isoniazid 2.2.1.2.2 Preparation of calibration curve of Isoniazid A standard stock solution of the pure drug (Isoniazid) was prepared by taking 10mg of drug, dissolving in 100ml of Phosphate buffer (7.4 pH) in 100ml volumetric flask to get 0.1mg/ml (100µg/ml) of solution. Using stock solution, various dilutions in the range 2, 4, 6, 8, 10 µg/ml conc. were prepared. The absorbance of all the dilutions were determined using

UV spectroscopy at 262nm and calibration (standard curve) was plotted. Table 2.6 Calibration curve of Isoniazid

CHAPTER 1 INTRODUCTION Page 78 2.2.1.3 SPECTRAL ANALYSIS (FTIR) OF ISONIAZID The functional group analysis (IR) was done at IIT, Kanpur. Infrared spectrum of any compound gives information about the groups present in particular compound. An infrared spectrophotometer for recording the spectra in the infrared region consists of an optical system capable of providing the monochromatic light in the region of 4000 to 400cm -1 and means of measuring the intensity of transmitted light and the incident light. In FTIR study sufficient quantity of isoniazid was placed in to sample holder and scanned from 4000cm -1 to 650cm -1 using ATR FTIR. Spectra of Isoniazid obtained was examined for peaks. Fig. 2.2 IR Spectra of Isoniazid Concentration (µg/ml) Absorbance (262nm) 0 0.000 2 0.062 4 0.122 6 0.178 8 0.239 10 0.296

CHAPTER 1 INTRODUCTION Page 79 2.3 PREFORMULATION STUDIES OF PYRAZINAMIDE 2.3.1 Identification of drug 2.3.1.1 Physical Appearance Drug was received as a gift sample from Micro Labs, Mumbai. Drug sample was physically examined for identification of drugs. The drug was white to off-white in color, odourless and crystalline powder. 2.3.1.2 Melting Point Melting point of Pyrazinamide was determined by using thiels tube apparatus. First capillary was taken and sealed at one end; sealed capillary was filled with drug and attached to thermometer using a thread. Thiels apparatus was filled with liquid paraffin and thermometer along with capillary tube was tied and placed high enough so that liquid paraffin doesn‟t enter in to capillary. The side arm was heated using burner to heat the sample until melting point was observed. The temperature at which sample begins to melt and the temperature at which it melts completely was noted. The procedure was repeated three times and mean melting point was taken. Table 2.7 Melting point of Pyrazinamide S. No. Experimental melting point(° C) Average melting point(° C) Mean melting point(° C) 1 189-192 190.5 190 o ±1 2 188-191 189.5 3 189-193 191 2.3.1.3 Solubility analysis of Pyrazinamide in various solvents

CHAPTER 1 INTRODUCTION Page 80 The solubility study of Pyrazinamide was performed in various aqueous, non-aqueous solvents. Excess of drug (500mg) was added in fixed solvent quantity (1ml) of each solvent in a scew cap container and kept on rotary shaker for 24 h. After 24h solution was centrifuged at 15000 rpm, supernatant was taken for determination of absorbance and matched to IP solubility chart. Table 2.8 Solubility of Pyrazinamide in various solvent S. No Solvents Approximate volume of solvent (ml) /gram of solute Category as per I.P (1996) 1 Chloroform 46.4 Soluble 2 Water 58.9 Soluble 3 Ethanol 345 Slightly soluble 4 Methanol 267.8 Slightly soluble 2.3.2 ANALYTICAL METHOD DEVELOPMENT FOR PYRAZINAMIDE BY UV- VISIBLE SPECTROPHOTOMETER. UV spectroscopy method was developed for the analysis of drug using double beam Shimadzu 1700 UV spectrophotometer. 2.3.2.1 Determination of λ max of Pyrazinamide Pyrazinamide solution of 100µg/ml was prepared in phosphate buffer (7.4 pH). The spectrum of the pure drug sample solution was obtained by using spectrophotometer (UV- 1700 Pharma spec. Shimadzu). The sample was scanned at a range of 400-200 nm. The UV spectrum of Pyrazinamide was obtained by scanning the above solution. A working λ max of Pyrazinamide was obtained at 268.5nm.

CHAPTER 1 INTRODUCTION Page 81 Fig. 2.3 UV Spectrum of Pyrazinamide 2.3.2.2 Preparation of

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calibration curve of Pyrazinamide A standard stock solution of the pure drug (Pyrazinamide) was prepared by dissolving 10mg of drug in 100ml

phosphate buffer (7.4 pH) in 100ml volumetric flask to get 0.1mg/ml (100µg/ml) of solution. Using stock solution, various dilutions in the range 2, 4, 6, 8, 10 µg/ml concentration were prepared. The absorbance of all the dilutions were determined using UV spectroscopy at 268.5 nm and calibration (standard curve) was plotted. Table 2.9 Calibration curve of Pyrazinamide Concentration (µg/ml) Absorbance (268.5) 0 0.000 2 0.140

CHAPTER 1 INTRODUCTION Page 82 2.3.3 SPECTRAL ANALYSIS OF PYRAZINAMIDE The functional group analysis (IR) was done at IIT, Kanpur. Infrared spectrum of any compound gives information about the groups present in particular compound. An infrared spectrophotometer for recording the spectra in the infrared region consists of an optical system capable of providing the monochromatic light in the region of 4000 to 650cm -1 and means of measuring the quotient of the intensity of transmitted light and the incident light. In FTIR study sufficient quantity of Pyrazinamide was placed in to sample holder and scanned from 4000cm -1 to 650 cm -1 using ATR FTIR. Spectra of Pyrazinamide obtained and examined for peaks. Fig. 2.4 IR Spectra of Pyrazinamide 4 0.278 6 0.420 8 0.572 10 0.712

CHAPTER 1 INTRODUCTION Page 83 2.4 DRUG EXCIPIENT INTERACTION STUDY The drug excipient interaction study was done at IIT, Kanpur. 2.4.1 I.R spectrum of drugs and polymers (Formulation 1). Drugs (Isoniazid, Pyrazinamide) and polymers (chitosan, sodium tripolyphosphate) were taken in equal amount to form a physical mixture. The physical mixture was placed in mortar and triturated. Finally for FTIR study sufficient quantity of mixture was placed in to sample holder and scanned from 4000cm -1 to 650cm -1 using ATR FTIR. Spectra of physical mixture was obtained and examined for peaks. Fig 2.5 IR spectrum of drug polymer interaction (Formulation I) 2.4.1.1 I.R spectrum of drugs, polymers and ligand (D-mannose). Drugs (Isoniazid, Pyrazinamide) and polymers (chitosan, sodium tripolyphosphate, D mannose) were taken in equal amount to form a physical mixture. The physical mixture was placed in mortar and triturated. Finally for FTIR study sufficient quantity of mixture was placed in to sample holder and scanned from 4000cm -1 to 650cm -1 using ATR FTIR. Spectra of physical mixture was obtained and examined for peaks.

CHAPTER 1 INTRODUCTION Page 84 Fig 2.6 I.R spectrum of drugs, polymers and ligand (D-mannose) 2.4.1.2 I.R spectrum of drugs, polymers and ligand (Folic acid) Drugs (Isoniazid, Pyrazinamide), polymers (chitosan, sodium tripolyphosphate, folic acid) were taken in equal amount to form a physical mixture. The physical mixture was placed in mortar and triturated. Finally for FTIR study sufficient quantity of mixture was placed in to sample holder and scanned from 4000cm -1 to 650cm -1 using ATR FTIR. Spectra of physical mixture was obtained and examined for peaks.

CHAPTER 1 INTRODUCTION Page 85 Fig 2.7 I.R spectrum of drugs, polymers and ligand (Folic acid) 2.4.2 IR spectrum of drugs and polymers (Formulation 2) Drugs (Isoniazid, Pyrazinamide) and polymers (chitosan, calcium chloride, sodium alginate) were taken in equal ratio to form a physical mixture. The physical mixture was placed in mortar and triturated. Finally for FTIR study sufficient quantity of mixture was placed in to sample holder and scanned from 4000cm -1 to 400cm -1 using ATR FTIR. Spectra of physical mixture was obtained and examined for peaks.

CHAPTER 1 INTRODUCTION Page 86 Fig 2.8 IR spectrum of drug polymer interaction (Formulation II) 2.4.2.1 I.R spectrum of drugs, polymers and ligand (D-mannose) Drugs (Isoniazid, Pyrazinamide), polymers (chitosan, calcium chloride, sodium alginate, D mannose) were taken in equal ratio to form a physical mixture. The physical mixture was placed in mortar and triturated. Finally for FTIR study sufficient quantity of mixture was placed in to sample holder and scanned from 4000cm -1 to 650cm -1 using ATR FTIR. Spectra of physical mixture was obtained and examined for peaks. Fig 2.9 I.R spectrum of drugs, polymers and ligand (D-mannose)

CHAPTER 1 INTRODUCTION Page 87 I.R spectrum of drugs, polymers and ligand (Folic acid) Drugs (Isoniazid, Pyrazinamide), polymers (chitosan, calcium chloride, sodium alginate, folic acid) were taken in equal ratio to form a physical mixture. The physical mixture was placed in mortar and triturated. Finally for FTIR study sufficient quantity of mixture was placed in to sample holder and scanned from 4000cm -1 to 650cm -1 using ATR FTIR. Spectra of physical mixture was obtained and examined for peaks. Fig 2.10 I.R spectrum of drugs, polymers and ligand (Folic acid) 2.5 SELECTION OF ANALYTICAL WAVELENGTH FOR SIMULTANEOUS ESTIMATION OF DRUGS A stock solution (100µg/ml) of pure drug Pyrazinamide and Isoniazid were prepared in phosphate buffer (7.4 pH). Both stock solutions were further diluted separately with phosphate buffer to get 2, 4, 6, 8, 10 µg/ml. The spectrum of both solutions was obtained by using UV Visible spectrophotometer (UV Visible 1700 Pharma Spec, Shimadzu) at 268.5 and 262 nm respectively. Finally from overlay spectra isobestic λ max (isoabsorptive point) was determined as 252nm.

CHAPTER 1 INTRODUCTION Page 88 Fig 2.11 Overlay Spectra of drug mixture (Isoniazid and Pyrazinamide) 2.5.1 Simultaneous estimation method for combined drugs 2.5.1.1 Simultaneous equation method The simultaneous equation method for analysis of drugs is based on the absorption of two drugs at their wavelength maxima. The two maxim wavelength selected in the method are 262nm and 268.5 nm for isoniazid (INH) and pyrazinamide (PYZ) respectively. The stock solutions of both drugs were further diluted separately with 7.4 pH buffer to get a series of standard solution 2, 4, 6, 8, 10µg/ml of INH and PYZ. The absorbance was measured at the selected wavelengths and absorptivities for both the drugs at respective wavelengths were determined. Absorptivity value of drugs was calculated by using following formula. ---------- eqn No. 2.1 Concentrations of drugs in sample were determined by using following formula. Cx = (A2aY1-A1aY2) / (aX2aY1-aX1aY2) ------- eqn No. 2.2

CHAPTER 1 INTRODUCTION Page 89 Cy = (A1aX2-A2aX1) / (aX2aY1-aX1aY2) ------- eqn No 2.3 Cx and Cy are concentration of INH and PYZ respectively, aX1 and aX2 are the absorptivity value of INH at 262nm and 268.5 nm respectively. aY1 and aY2 are absorptivity of PYZ at 262 and 268.5nm respectively. A1 and A2 are the absorbances of the diluted sample (mixture) at 262nm and 268.5nm respectively. 2.6 FORMULATION DEVELOPMENT OF CHITOSAN NANOPARTICLES The nanoparticles were formulated by ionic gelation method using natural polymer chitosan and sodium tripolyphosphate. 2.6.1 Ionotropic gelation method Chitosan –sodium tripolyphosphate nanoparticles were prepared by ionotropic gelation method. In a 500 ml beaker 1% acetic acid solution was prepared and from the same 100 ml was taken in to three beakers. To these different beakers 0.1g, 0.2g, 0.3g of chitosan was added to get different concentration of chitosan 0.1%, 0.2%, 0.3%. After overnight they were mixed thoroughly and pH was raised to 4.6-4.8 with 10N NaOH. Further in these different concentrations of chitosan solutions 0.1g each of both drugs (INH and PYZ) were added, 0.5ml of tween 80 (in 30 ml chitosan solution) and stirred for 10 minutes at 1000 rpm at room temperature. Sodium tripolyphosphate (NaTPP) solutions of different concentrations 0.25%, 0.5%, 0.75% were also prepared and added to chitosan solution to get nanoparticles. The TPP solution was added drop-wise to a chitosan solution under magnetic stirring at room temperature till opalescent mixture is obtained. Opalescence in solution showed the formation of nanoparticles in solution. Chitosan nanoparticles were formed on addition of 9.5 ml of sodium tripolyphosphate solution to 30 ml chitosan solution under magnetic stirring, followed by 1 hr sonication at 25 ° C (Bath sonicator).In order to prepare nanoparticles at different TPP:Chitosan ratio, the concentration of TPP and chitosan was varied as per coded batches.

CHAPTER 1 INTRODUCTION Page 90 Fig 2.12 Formulation development of chitosan nanoparticles 2.6.1.1 3 2 Factorial design as an optimization tool for chitosan nanoparticle formulation Optimization of experiments can lead to useful savings of scientific resources. Factorial experimental designs are commonly used to optimize experiments and discover which factor influence the outcome of experiments and what levels of these factors lead to a test with a better response. In present study optimization tool was factorial design matrix. A two factor, three level factorial design was implemented to study the individual effects of each factor and their interaction on the characteristics of nanoparticles (Parhi et al., 2015; Mehta et al., 2007). The independent variables, X1 chitosan and X2 sodium TPP concentrations were set at three levels to obtain nine possible batches. The critical qualities expected for nanoparticles were particle size and entrapment efficiency. A 3 2 factorial design matrix is summarized in Table 2.10 and Table 2.11. Table 2.10 Values of independent variables for chitosan nanoparticles Independent variable Low level(-1) Medium level (0) High level (+1) Conc. of chitosan-X1(%w/v) 0.1% 0.2% 0.3% Conc. of Na TPP-X2 (%w/v) 0.25% 0.5% 0.75%

CHAPTER 1 INTRODUCTION Page 91 Table 2.11 Design codes for batches of chitosan nanoparticles Batch No. X1 (Conc. of Chitosan %w/v) X2 (Conc. of Na TPP %w/v) 1N +1 +1 2N 0 0 3N -1 -1 4N +1 -1 5N 0 +1 6N -1 0 7N +1 0 8N 0 -1 9N -1 +1 2.7 FORMULATION DEVELOPMENT FOR ALGINATE-CHITOSAN NANOPARTICLES The nanoparticles were formulated by ionic gelation method using natural polymers Sodium alginate and Chitosan, Calcium chloride as cross linking agent. 2.7.1 Ionotropic pregelation method

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Alginate/chitosan nanoparticles containing antitubercular drugs

were prepared by

a two-step procedure based on the ionotropic

pre-gelation of

poly-anion with calcium chloride followed by

poly-cationic crosslinking

through a protocol earlier described (Rajaonarivony et al., 1993).

The different concentration of sodium alginate 0.05%, 0.075%, 0.1% were prepared in double distilled water and kept for overnight. Then mixed thoroughly and pH was

CHAPTER 1 INTRODUCTION Page 92 maintained to 4.9 with 1 N HCl. Further in these, different concentrations of sodium alginate solutions 0.1g each of both drugs were added and stirred for 10 minutes using magnetic stirrer (Tarson Ceramic Spinot , Chennai). The different concentration of chitosan 0.05%, 0.07%, 0.09% were prepared in 1% acetic acid and kept for overnight. Next day contents were mixed thoroughly and pH was raised to 4.6 with 10N NaOH. A 18mM CaCl 2 solution was prepared in distilled water. Sodium alginate nanoparticles were formed by adding 1.2 ml of 18 mM calcium chloride solution dropwise into a beaker containing 20 ml of different concentration of alginate solution which was under magnetic stirring.

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Then, 2.2 ml of different concentration (0.05–0.09%, w/w) chitosan solution was added drop wise into the pregel over 60 min,

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to provide a colloidal dispersion with final pH of 4.7. Nanoparticles were held with an additional stirring for 60 min to improve curing (

Ping Li et al, 2008). Subsequently, nanoparticles were centrifuged at 12000 rpm (Eppendorf, Model 5804 R Chennai) at 5°C and pellet was collected. The obtained pellets were washed thrice with distilled water and centrifuged to get final pellet. The pellet was re- dispersed in distilled water and freeze dried using lyophilizer (LABCONCO, Freeze Zone 2.5 Plus, India). Fig 2.13 Formulation development of alginate-chitosan nanoparticles

CHAPTER 1 INTRODUCTION Page 93 2.7.2 3 2 Factorial design as optimization tool for alginate chitosan nanoparticles development The study involved 3 2 factorial design matrix as a optimization tool. A two factor, three level factorial design was implemented to study the individual effects of each factor and their interaction on the characteristics of nanoparticles (Parhi et al., 2015; Mehta et al., 2007). The independent variable, X1 sodium alginate concentration and X2 chitosan concentration were set at three levels to obtain nine possible batches. Factor (X3) was kept constant in all formulated batches. The critical qualities expected in nanoparticles were particle size and entrapment efficiency. A 3 2 factorial design matrix is summarized in Table 2.12 and 2.13. Table 2.12 Values of independent variables Independent variable Low level (-1) Medium level (0) High level (+1) Conc. of alginate -X1(%w/v) 0.05% 0.075% 0.1% Conc. of chitosan-X2(%w/v) 0.05% 0.07 % 0.09% Conc. of CaCl 2 -X3 (%w/v) 18mM 18mM 18mM Table 2.13 Design codes for batches of Alginate- Chitosan nanoparticles Batches X1 (Conc. of alginate %w/v) X2(Conc. of Chitosan %w/v) X3(Conc. of CaCl 2 %w/v) 1S 0 0 18mM 2S -1 +1 18mM 3S +1 -1 18mM 4S +1 0 18mM 5S -1 -1 18mM

CHAPTER 1 INTRODUCTION Page 94 2.8 EVALUATION PARAMETERS FOR NANOPARTICLES 2.8.1

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Particle size, zeta potential and Poly-dispersity index The particle size and zeta potential of nanoparticles were

determined by dynamic light scattering using Malvern zetasizer Nano ZS and Nanoplus (Malvern instrument limited, Malvern, UK). Prior to measurement, 1mg/ml sample of centrifuged pellet was prepared in water and sonicated for 3 minutes. The diluted 1ml sample was taken in a disposable cuvette and analyzed at 25 o C with angle of detection 90 o . The particle size distributions of nanoparticles were reported as a polydispersity index (PDI), the values near to 1 shows homogeneity. Zeta potential measures the surface charge over the particles which is crucible parameter for stability of nanoparticulate system. Sample volume used for analysis was kept constant. All measurements were performed and recorded. 2.8.2 Particle surface morphology Nanoparticles

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morphology such as shape, surface and occurrence of aggregation phenomenon was studied by

FE SEM (TESCAN MIRA3 ,Australia) at IIT Kanpur. For analysis the freeze dried nanoparticles were mounted on metal studs and coated with gold sputter in a high vaccum evaporator. The Gold coated nanoparticles were examined at accelerating voltage 5kV and photographs were taken. 2.8.3 Drug entrapment and loading efficiency The entrapment and loading efficiency of nanoparticles were determined by separating nanoparticles by ultracentrifugation at 12000

rpm at 5 ° C for 30 minutes. The obtained pellet 6S 0 +1 18mM 7S 0 -1 18mM 8S -1 -0 18mM 9S +1 +1 18mM

CHAPTER 1 INTRODUCTION Page 95 was weighed and amount of free drug in the supernatant was measured by using UV Spectrophotometer against appropriate blank at wavelength of 262 (maxima of INH), 252 (isobestic point) and 268.5 (maxima of PYZ) nm. Finally the amount of drug entrapped and loaded in nanoparticles was calculated by following formula. ---------- eqn No. 2.4 ---------- eqn No. 2.5 2.8.4 In-vitro drug release study The in-vitro drug diffusion from the formulation was studied by using dialysis membrane-150 (HI MEDIA, molecular cut off between 12000-14000 Dalton) and modified diffusion apparatus. The freshly prepared dissolution media of 1.2 pH (simulated gastric media) and 7.4 pH (phosphate buffer) was used in study. Dialysis membrane-150 was previously soaked in dissolution medium and tied to one end of the specially designed glass cylinder (open at both ends).To the centrifuged pellet, 5 ml buffer was added and formulation was accurately placed into this assembly. The cylinder was attached to a stand and suspended in 100 ml of dissolution medium maintained at 37± 0.5 o C so that the membrane just touched the receptor medium surface. The dissolution medium was stirred at low speed using magnetic stirrer. Aliquots, each of 5 ml were withdrawn at regular intervals (0.25, 0.5, 1, 2, 3, 4, 5, 6, ---------- 14h) and replaced by an equivalent volume of buffer in receptor compartment to maintain the sink conditions. The aliquots were suitably diluted with phosphate buffer 7.4 pH and analyzed for drug content by UV visible spectrophotometer at 252 nm (isoabsorptive point), 262 (λ max of INH) and 268.5 (λ max of PYZ). The quantity of drug equivalent to 5 mg of drug was taken for diffusion study.

CHAPTER 1 INTRODUCTION Page 96 2.8.4.1 Kinetic modeling

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In order to understand the kinetic and mechanism of drug release, the results of in-vitro drug release study of nanoparticles were fitted with various kinetic equations like zero order (Cumulative % release

vs. time), first order (log % drug remaining vs. time), Higuchi‟s model (cumulative % drug release vs. square root of time),

Peppas plot (log of cumulative % drug release vs. log time). R 2 (Coefficient of correlation) and k (release rate constant) values were calculated for the linear curve obtained by regression analysis of above plots. 2.8.4.1.1 Drug release kinetic

0: https://www.researchgate.net/publication/51051231_Paraquat-loaded_alginatechitosan_nanoparticles_Preparation_characterization_and_soil_sorption_studies 88%

Whenever a new dosage form is developed, it is necessary to ensure that

dosage form shows dissolution in proper manner. The focus on dissolution/release studies is continuously increasing. For the quantitative analysis of drug release from any dosage form it is easier to obtain the correct values by using suitable mathematical formulas as a function of characteristics of some dosage form. Kinetic models describes the amount of drug dissolved from solid dosage form as

a function of test time „t "or C=f(t). Some analytical definitions of the C(t) are commonly used function like zero order, first order, Hixon Crowell, Higuchi,

Korsemeyer Peppas . 2.8.4.1.1.1 Objectives of mathematical Model 1. Designing of new drug delivery system based upon the general release expression. 2. Prediction of the drug release rate form dosage form and drug diffusion behavior through polymers, thus avoid excessive experiment. 3. Optimization of the release kinetic. 4. Physical mechanism of drug transport is determined by comparing of release data with mathematical models. 5. Prediction of effects of design parameters like shape, size and compositon on the overall drug release rate.

CHAPTER 1 INTRODUCTION Page 97 6. Accurate prediction of drug release profile and improve overall therapeutic efficacy and safety of these drugs. 2.8.4.1.1.2 Application of mathematical Model The Mathematical model equation can be used to design new systems by selecting the optimal geometry, method of formulation and size. Mathematical modeling aids in predicting the drug release rate and diffusion behavior from these systems by an appropriate model, thereby reducing the number of experiments needed. Mathematical modeling of controlled drug delivery can help provide a scientific knowledge base concerning the mass transport mechanisms which are involved in the control of the drug release. Thus mathematical modeling can significantly facilitate the optimization of existing and the development of new pharmaceutical products. The systemic use of model saves money and time. The mathematical approaches may help researchers to develop high effective drug formulations and more accurate dosing regimen. To describe the drug release rate from different drug delivery systems a large number of models have been developed. Some of important models are: ? Zero order kinetic models. ? First order kinetic model. ? Higuchi model. ? Korsemeyer-peppas model. ? Hixon- crowell model. 2.8.4.1.2 Zero order To study release kinetics, data obtained from in vitro drug release were plotted as cumulative amount of drug released versus time. Drug dissolution from dosage form that do not aggregate and release the drug slowly and zero order kinetics defines the process of constant drug release from drug delivery system and where release rate is independent of its concentration.

CHAPTER 1 INTRODUCTION Page 98 2.8.4.1.3 First order The model is used to describe the absorption and elimination of some drugs, although it is difficult to understand the mechanism on the theoretical basis. The data obtained was plotted as log cumulative percentage drug remaining versus time. 2.8.4.1.4 Higuchi model Higuchi model is used to describe drug release from matrix system. The model is applicable to the different geometrics

and porous system. Data obtained was plotted as cumulative percentage of drug release versus square root of time. 2.8.4.1.5 Hixon – crowell model The model describes the release of dose from system, where, there is change in surface area and diameter of particle or tablet. This model considers the release rate is limited by drug particle dissolution rate and not by diffusion that might occur through the polymeric matrix. This model is used to describe the release profile keeping in mind the surface of drug particle diminishes during dissolution. Plot is made between cube root of drug percentage remaining versus time. 2.8.4.1.6 Korsemeyer-peppas model Korsemeyer- Peppas model describes the release of drug from a polymeric system. To illustrate the mechanism of drug release, first 60% of drug release data was fitted in Korsemeyer-Peppas model.

drug release studies were plotted as log cumulative percentage drug release versus log time. 2.9 IN-VITRO ANTITUBERCULAR SCREENING OF DRUG LOADED NANOPARTICLES BY MICROPLATE ALAMAR BLUE ASSAY (MABA) The in vitro screening of anti-mycobaterial activity of combined drug loaded nanoparticles was done at CDRI, Lucknow. The nanoparticles were dissolved in DMSO to make 10mg/ml and seeded in MB7H9 media (100µg/ml) enriched with ADC growth supplement (10%V/V) with decreasing double dilutions starting with 50 µg/ml(for 250 µg/ml)

CHAPTER 1 INTRODUCTION Page 99 in 96 wellplate. 150 µg/ml of culture (Mycobacterium tuberculosis H37Ra,10 6 CFU/ml) was added in each well except blank (negative control). Culture control, Blanks (media alone), Rifampicin and streptomycin were taken as test control. Plate was incubated for 5 days at 37 o C in an incubator. On 6 th day, 25 µl Resazurin (0.01%w/v, stock conc.) was added and plate was incubated further till pink color in control wells were observed. The colour change from purple to pink was assessed visually and fluorescence was measured at 530 ± 25nm and 590 ± 25nm for excitation and emission respectively by synergy biotech plate reader. The 90% inhibitory concentration was calculated by plotting fluorescence values. Table 2.14 Coded formulated nanoparticles for MABA test S. No. Formulation Sample code 1 Formulated sodium alginate nanoparticles (3S) K1 2 Formulated chitosan nanoparticles (8N) K2 2.10 PREPARATION OF LIGAND ATTACHED ALGINATE-CHITOSAN NANOPARTICLES OF OPTIMIZED BATCH 2.10.1 Purpose of ligand in nanocarriers The use of ligands in nanocarriers causes surface modification and promotes macrophage phagocytosis. Ligands were added in two steps a) during nanoparticle formulation and b) after the formation of nanoparticles to promote surface adsorption too. Fig 2.14 Varied surface behaviour between plain and ligand attached nanoparticles

CHAPTER 1 INTRODUCTION Page 100 2 .10.2 Ligand attached alginate-chitosan nanoparticle formulation development. Sodium alginate nanoparticles were prepared by two step procedure. 1) Added drop wise 6 ml of 18 mM calcium chloride solution into a beaker containing 0.1% alginate concentrate. The alginate concentrate (alginate-0.1g, anti-tubercular drugs-0.1 g each and ligand-5mg) was maintained under magnetic stirring. This causes

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the

pre-gel formation. 2) Further 12 ml of 0.05% chitosan solution was added drop wise into the pre-gel

over 60 min,

to get colloidal dispersion with final pH of 4.7. After getting colloidal dispersion, 5 mg of ligand mannose or folic acid was added to promote surface adsorption of ligand over nanoparticles.

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Nanoparticles were held with an additional stirring for 60 min. to improve curing.

Further, nanoparticles were centrifuged at 12000 rpm and pellet was collected. The obtained pellets were lyophilized to get freeze dried ligand attached nanoparticles. The obtained freeze dried nanoparticles were coded for macrophage cell line study and macrophage toxicity test. Table 2.15 Coded ligand bound formulation S. No. Formulation Sample code 1 Alginate nanoparticles S1 2 Alginate nanoparticles with Folic acid as ligand S2 3 Alginate nanoparticles with D-mannose as ligand S3 2.11 MACROPHAGE CELL (J774) LINE STUDY 2.11.1 Preparation of Fluorescent nanoparticles Freeze dried nanoparticles (5mg) were redispersed in 5 ml DMSO solution followed by the addition of 0.5 ml NaOH (0.1M) and rhodamine B was dissolved in methanol (10 mg/ml). The reaction was allowed to proceed for 4 hours in the dark room temperature. The prepared dye labeled nanoparticles were centrifuged and washed with sterile 7.4 pH required. Finally, particles were suspended in 1 ml RPMI with 10% FBS.

CHAPTER 1 INTRODUCTION Page 101 2.11.2 Phagocytosis Assay Cells (J774) were plated at density of 5000cells/well in 100µl into each well of the 96 well culture plates. Culture plates were placed in to a 37 o C CO 2 incubator overnight before performing assay. For phagocytosis assay, medium of the cells were replaced with or without nanoparticles. Cells were further incubated for 30 minutes to allow phagocytosis. After 30 minutes, medium was removed and cells were washed with PBS once and finally 100 µl of PBS was added to each well. Readings were recorded at 530 nm with sensitivity of 50. 2.12 MACROPHAGE CYTOTOXICITY TEST (MTT assay) 2.12.1 Isolation and culture of primary cultures of mice peritoneal macrophages (MPMF) Male Balb/c mice (5-6 weeks old, 20+2g) obtained from the animal breeding colony of CSIR-Indian Institute of Toxicology Research (CSIR-IITR), Lucknow (U.P; India) were acclimatized and cared as per CPCSEA norms. Bacillus Chalmette Guerin (BCG) stocks were thawed and resuspended at a density of 10 -7 CFUs in 0.5 ml of sterile PBS without Ca +2 /Mg +2 . The peritoneal macrophage activation was done by challenging Balb/c mice with BCG (10 -7 CFUs in 0.5 ml) intraperitoneally and left for five days. Six animals were sacrificed on day 6 and abdomen of each mouse was soaked with 70% ethanol. Then a small incision was made along the midline with sterile scissor. Abdominal skin was retracted

manually to expose the intact peritoneal wall and PBS (10 ml) was injected in the peritoneal cavity essentially spleen side. All the peritoneal fluid after aspiration was centrifuged at 400xg for 10 minutes at 4°C and the supernatant was discarded. The pelleted cells were re-suspended in RPMI medium containing 10% fetal bovine serum (FBS), 0.2% w/v sodium bicarbonate (NaHCO 3), 1% antibiotic-antimycotic (Gibco BRL, USA) with gently tapping the bottom of the tube and pipetting up and down several times. Cells were counted on a hematocytometer and approximate 1x10 6 cells were seeded in 6-well tissue culture plates. The cells were allowed to adhere to substrate by culturing them for 1-2h in humidified 5% CO 2 environment at 37°C. Non-adherent cells were removed by gently washing the cells with PBS (3x1 ml) and the remaining adhered macro-phage cells were maintained as such. The adherent cells comprised of more than 95% of macrophages.

CHAPTER 1 INTRODUCTION Page 102 2.12.2 Culture of cell lines The cell lines such as HaCaT, A549 and HepG2 used in this study, were obtained from National Centre for Cell Sciences, Pune, India, and maintained as per the standard protocols at CSIR-Indian Institute of Toxicology Research, Lucknow. The cells were cultivated in RPMI 1640 culture medium (DMEM) from Sigma Aldrich (St. Louis, MO), supplemented with 10% FBS, 0.2% NaHCO 3 , 1% antibiotic-antimycotic. Cultures were maintained at 37°C in a humidified 5% CO 2 environment. 2.12.3 MTT assay Non-cytotoxic dose of coded test samples S1, S2 and S3 was identified in HaCaT, A549, HepG2 human cell lines and primary cultures of macrophages isolated from peritoneal cavity. Cytotoxicity assessment was done using standard endpoint i.e., tetrazolium bromide MTT (3- (4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide) assay fallowing the protocol of Kashyap et al 2011. In brief, cells (1x10 4 cells/well) were seeded in 96-well tissue culture plates and incubated in the CO 2 incubator for 24 h at 37°C. Then the medium was aspirated and cells were exposed to medium containing either of sample number 1, 2 or 3 (0.001-100 µg/ml) for 24-96 h at 37°C in 5% CO 2 -95% atmosphere under high humid conditions. Tetrazolium salt (10 µl/well; 5 mg/ml of stock in PBS) was added 4 h prior to completion of respective incubation periods. At the completion of incubation period, the reaction mixture was carefully taken out and 200 µl of culture grade DMSO was added to each well. The content was mixed well by pipetting up and down several times until dissolved completely. Plates were then incubated for 10 minutes at room temperature and color was read at 550 nm using Multiwell Microplate Reader (Synergy HT, Bio-Tek, USA). The unexposed sets were also run parallel under identical conditions that served as a basal control. 2.12.4 Neutral Red Uptake assay The assay was carried out following the protocol described earlier (Siddiqui et al., 2008). Cells were exposed to compound in identical experimental setup as to MTT assay. Upon the completion of incubation period, medium was aspirated and NRU salt (50µM/mL in medium) was added 100 µl per well plate and incubated for 3 h. Then, the reaction mixture was carefully taken out and plates were washed with washing solution (100 µl/ well)

CHAPTER 1 INTRODUCTION Page 103 containing 1% CaCl 2 (w/v) and 0.5% HCHO (v/v) to remove unincorporated dye. Washing solution was removed and mixture of 200?l; 1% acetic acid and 50% ethanol was added. The plates were kept on rotor shaker for 10 min at room temperature and then analyzed at 540 nm using multi-well microplate reader (Synergy HT, Bio-Tek, USA). Unexposed sets were also run under identical condition and served as control.

2.12.5 LDH release assay Lactate dehydrogenase (LDH) release assay is a method to measure the membrane integrity as a function of the amount of cytoplasmic LDH released into the medium. The assay was carried out using readymade commercially available LDH assay kit for in vitro cytotoxicity evaluation (TOX-7, Sigma St. Louis, MO., USA). The assay was based on the reduction of NAD by the action of LDH. The resulting reduced NAD (NADH +) was utilized in the stoichiometric conversion of a tetrazolium dye. The resulting colored compound was measured using multiwell plate reader at wavelengths 490 and 690 nm. In brief, the cells were exposed with (0.001-100 µg/ml) for different time periods after the completion of respective time periods the cells were processed for LDH release assay similar to MTT assay. Culture plates were removed from CO 2 incubator as per the experimental schedule and centrifuged at 250 x g for 4 min. Then supernatant of each well was transferred to a fresh flat bottom 96 well culture plate and processed further for enzymatic analysis as per the manufacturer‟s instructions. 2.13 STABILITY STUDIES It is one of the major criteria for any rational design of a dosage form. The stability studies were carried out on best formulation according to International Conference on Harmonization (ICH) guide lines. The study was carried out to assess the stability of ligand (folic acid) attached alginate chitosan nanoparticles containing anti-tubercular drugs. The samples were taken in sealed screw capped glass containers. Glass containers were then stored at 5 o C ± 3 o C in refrigerator, 25 o C ± 2 o C/60 ± 5 % RH at room temperature, at 30 o C ± 2 o C/ 65 ± 5 % RH and at 40 o C ± 2 o C/75± 5% RH in humidity controlled stability chamber(Tsai M et al., 2011). The samples were withdrawn at 1, 3, 6 months to analyse the drug content and any change in physical appearance (Jonassen. H et al., 2012). The drug content was analysed by ultracentrifugation of test samples at 12000 rpm for 30min. Further amount of drug in

CHAPTER 1 INTRODUCTION Page 104 supernatant was assessed by UV visible spectrophotometer at 252nm, 262nm and 268.5 nm. The drug content was calculated as per formula (Masilamani et al; 2012). ----------eqn No. 2.11 Table 2.16 Stability test storage conditions for drug products (As per ICH Guidelines) (Arunachalam A et al., 2013) Intended storage condition Stability test method ICH Test temperature and humidity (period in months) Room temperature Long term 25 ° C ± 2 ° C/ 60 ±5% RH (6 month) Intermediate 30 ° C ± 2 ° C/ 65 ±5% RH (6 month) Accelerated 40 ± 2 o C/ 75 ±5% RH (6 month) Refrigerated Long term 5 o C ± 3 o C (6month) 2.14 STATISTICAL ANALYSIS All the results were expressed as Mean ± Standard deviation.

CHAPTER 1 INTRODUCTION Page 105 CHAPTER 3 RESULTS & DISCUSSION 3.1 PREFORMULATION STUDIES OF ISONIAZID 3.1.1 Identification of drug 3.1.1.1 Physical appearance Isoniazid was physically examined for organoleptic properties. It was found to be white, odourless, crystalline powder. Table 3.1 Physical appearance of Isoniazid S. No. Characteristics Observation 1 State Solid, Crystalline nature 2 Colour White 3 Odour Odourless 4 Taste Bitter 3.1.1.2 Melting Point The melting point of Isoniazid was found to be 171.5 ±1.5 by thiels tube method. Table 3.2 Melting Point of Isoniazid

CHAPTER 1 INTRODUCTION Page 106 S. No. Drug Melting Point 1 Isoniazid 171.5 ±1.5 Data shown are the mean ± SD (n=3). 3.1.1.3 Solubility studies The solubility study of Isoniazid was determined in various aqueous and non-aqueous solvents. Isoniazid was soluble in water,

slightly soluble in methanol, ethanol and chloroform and very slightly soluble in ether. 3.1.2 ANALYTICAL METHOD DEVELOPMENT FOR ISONIAZID BY UV-VISIBLE SPECTROPHOTOMETER. UV Spectroscopy method was developed for the analysis of Isoniazid, using double beam Shimadzu 1700 UV spectroscopy. 3.1.2.1 Detertmination of λ max of Isoniazid Fig 3.1 λ max of Isoniazid The optimized λ max of Isoniazid was found to be 262nm. 3.1.2.2 Calibration curve of Isoniazid

CHAPTER 1 INTRODUCTION Page 107 The absorbance of prepared dilutions (2, 4, 6, 8, 10 µg/ml) were measured by UV- Visible spectrophotometer at its own λ max 262 nm and thereafter at λ max 268.5 nm belonging to PYZ against 7.4 pH buffer . The absorbance so obtained is tabulated in table and calibration curve was plotted. The regressed data is shown as straight line. The regression coefficient was found to be 0.9995 and 0.9983 indicating good linearity as shown in Fig 3.2 and Fig 3.3. The calibration curve obeyed Beers Lamberts law in the concentration range 2-10 µl. Fig 3.2 Standard curve of Isoniazid at 262nm Fig 3.3 Standard curve of Isoniazid at 268.5nm 3.1.3 SPECTRAL ANALYSIS (FTIR) OF ISONIAZID On comparing the IR spectrum of sample (Isoniazid) and Reference spectrum, it was observed that all characteristic peaks of drugs were found as shown in Fig 3.4

CHAPTER 1 INTRODUCTION Page 108 Fig 3.4 IR Spectra of Isoniazid Table 3.3 IR characterization of Isoniazid S. No. Wave no.(cm -1) Vibration mode May be due to 1. 3000.1 C-H str Aromatic ring 2. 3107.6 N-H str Primary amine 3. 1322.8 C=C str Aromatic ring 4. 1407 C=N str pyridine ring 5. 1663.9 C=O str Amide-I 6. 1542.4 N-H bending Amide-II 7. 841.56 C-H def (oop) Aromatic ring 3.2 PREFORMULATION STUDIES OF PYRAZINAMIDE 3.2.1 Identification of drug 3.2.1.1 Physical appearance

CHAPTER 1 INTRODUCTION Page 109 The Drug sample was studied for its organoleptic properties. The drug was white in colour, odourless, crystalline powder. Table 3.4 Physical properties of Pyrazinamide S. No. Characteristics Observation 1 State Solid, Crystalline nature 2 Colour White 3 Odour Odourless 4 Taste Bitter 3.2.1.2 Melting Point Melting point of Pyrazinamide was found to be 190 ±1 o C by thiels tube method. 3.2.1.3 Solubility study The solubility study of Pyrazinamide was determined in various aqueous and non- aqueous solvents. Pyrazinamide was soluble in water, chloroform, slightly soluble in ethanol, methanol. 3.2.2 ANALYTICAL METHOD DEVELOPMENT FOR PYRAZINAMIDE BY UV – VISIBLE SPECTROPHOTOMETER. 3.2.2.1 Detertmination of λ max of Pyrazinamide

CHAPTER 1 INTRODUCTION Page 110 Fig 3.5 λ max of Pyrazinamide The optimized λ max of Pyrazinamide was found to be 268.5 nm. 3.2.2.2 Calibration curve of Pyrazinamide The absorbance of prepared dilutions (2, 4, 6, 8, 10 µg/ml) were measured by UV- Visible spectrophotometer at λ max of PYZ i.e. 268.5 nm and thereafter at λ max 262nm belonging to INH against 7.4 pH buffer. The absorbance so obtained is tabulated in table and calibration curve was plotted. The regressed data showed straight line. The regression coefficient was found to be 0.9995 and 0.9974 respectively, indicating good linearity as shown in Fig 3.6 and Fig 3.7. The calibration curve obeyed Beers Lamberts law in the concentration range 2-10 µl.

CHAPTER 1 INTRODUCTION Page 111 Fig 3.6 Standard curve of Pyrazinamide at 268.5 nm Fig 3.7 Standard curve of Pyrazinamide at 262 nm 3.2.3 SPECTRAL (FTIR) ANALYSIS OF

PYRAZINAMIDE On comparing the IR spectrum of sample (Pyrazinamide) and Reference spectrum, it was observed that all characteristic peaks of drugs were found as shown in Fig 3.8

CHAPTER 1 INTRODUCTION Page 112 11 Fig 3.8 IR Spectrum of Pyrazinamide Table 3.5 IR characterization of Pyrazinamide S. No. Wave no.(cm -1) Vibration mode Due to 1. 3149.7 C-H str Aromatic ring 2. 3406.6 N-H str Primary amine 3. 1519, 1430.3 C=C str Aromatic ring 4. 1575.1 C=N str Pyrazine ring 5. 1701.3 C=O str Amide-I 6. 1607.8 N-H bending Amide-II 7. 780.82 C-H def (oop) Aromatic ring 3.3 DRUG EXCIPIENT INTERACTION STUDY 3.3.1 IR spectrum of drugs and polymers (Formulation 1) The characteristic absorption peaks of Isoniazid, Pyrazinamide were observed in recorded IR spectra of physical mixture containing drugs (INH and PYZ) and excipients (chitosan, sodium tripolyphosphate) used in formulation 1. The FTIR results revealed that there was no

CHAPTER 1 INTRODUCTION Page 113 interaction between drugs (INH and PYZ) and excipients (chitosan, sodium tripolyphosphate) used in formulation. IR Spectra is shown in Fig 3.9. Fig 3.9 IR spectrum of drug polymer interaction (Formulation I) Table 3.6 IR Characteristics of Drugs and polymer interaction (Formulation 1) S. No. Wave no.(cm -1) Vibration mode Due to 1. 3384.33 N-H str Primary amine 2. 2916 C-H str Aromatic ring 3. 2850.6 C-H str Methyl group 4. 1666.50 C=O str Amide I 5. 1551.7 N-H ben Amide-II 6. 1411.6 C=N str Pyrazine/Pyridine 7. 1019.1 C-O-C ring stre Pyranose 8. 888.28 C-H def (oop) Aromatic ring 3.3.1.1 I.R spectrum of drugs, polymers and ligand (D-mannose)

CHAPTER 1 INTRODUCTION Page 114 The characteristic absorption peaks of Isoniazid, Pyrazinamide were observed in recorded IR spectra of physical mixture containing drugs (INH and PYZ) and excipients (chitosan, sodium tripolyphosphate, D-mannose) used in formulation 1. The FTIR results revealed that there was no interaction between drugs (INH and PYZ) and excipients (chitosan, sodium tripolyphosphate, D mannose) used in formulation. IR Spectra is shown in Fig 3.10. Fig 3.10 I.R spectrum of drugs, polymers and ligand (D-mannose) (Formulation I) Table 3.7 IR Characteristics of Drugs, polymer and ligand (D-mannose) interaction S. No. Wave no.(cm -1) Vibration mode May be due to 1 3410.33 N-H str Primary amine 2 2920.7 C-H str Aromatic ring 3 2850.6 C-H str Methyl group 4 1665.98 C=O str Amide I 5 1411.6 N-H bending Amide II 6 1271.4 C=N str Pyrazine/Pyridine

CHAPTER 1 INTRODUCTION Page 115 7 1023.8 C-O-C ring str Pyranose 3.3.1.2 I.R spectrum of drugs, polymers and ligand (Folic acid) The characteristic absorption peaks of Isoniazid, Pyrazinamide were observed in recorded IR spectra of physical mixture containing drugs (INH and PYZ) and excipients (chitosan, sodium tripolyphosphate, Folic acid) used in formulation 1. The FTIR results revealed that there was no interaction between drugs (INH and PYZ) and excipients (chitosan, sodium tripolyphosphate and folic acid) used in formulation. IR Spectra is shown in Fig 3.11. Fig 3.11 I.R spectrum of drugs, polymers and ligand (Folic acid) Table 3.8 IR Characteristics of Drugs, polymer and ligand (Folic acid) interaction S.No. Wave no.(cm -1) Vibration mode May be due to 1 3362.57 N-H stre Primary amine

CHAPTER 1 INTRODUCTION Page 116 2 2925.4 C-H stre Aromatic ring 3 2855.3 C-H stre Methyl group 4 1667.45 C=O str Amide I 5 1589.1 N-H bending Amide II 6 1266.7 C=N str Pyrazine/ Pyridine 7 1023.8 C-O-C ring str Pyranose 3.3.2 IR spectrum of drugs and polymers

(Formulation 2) The characteristic absorption peaks of Isoniazid and Pyrazinamide were observed in recorded IR spectra of physical mixture containing drugs (INH and PYZ) and excipients (chitosan, sodium alginate and calcium chloride)used in formulation 2. The FTIR results revealed that there was no interaction between drugs (INH and PYZ) and excipients (sodium alginate, chitosan, calcium chloride) used in formulation. IR Spectra is shown in Fig 3.12. Fig 3.12 IR spectrum of drug polymer interaction (Formulation 2) Table 3.9 IR Characteristics of Drugs and polymer interaction (Formulation 2)

CHAPTER 1 INTRODUCTION Page 117 S. No. Wave no. (cm -1) Vibration mode Due to 1. 3414.34 N-H str (asym) Primary amine 2. 3285.23 N-H str (sym) Primary amine 3. 3174.51 C-H str Aromatic ring 4. 1714.20 C=O str Amide I 5. 1666.84 N-H bend Amide-II 6. 1613.70 C=N str Pyrazine /Pyridine 7. 1479.41,1378.83 C=C ring str Aromatic ring 8. 786.5 C-H bend (oop) Aromatic ring 3.3.2.1 I.R spectrum of drugs, polymers and ligand (D-mannose) The characteristic absorption peaks of Isoniazid and Pyrazinamide were observed in recorded IR spectra of physical mixture containing drugs (INH and PYZ) and excipients (sodium alginate, chitosan, calcium chloride, D-mannose) used in formulation 2. The FTIR results revealed that there was no interaction between drugs (INH and PYZ) and excipients (sodium alginate, chitosan, calcium chloride, D-mannose) used in formulation2. IR Spectra is shown in Fig 3.13 Fig 3.13 I.R spectrum of drugs, polymers and ligand (D-mannose)

CHAPTER 1 INTRODUCTION Page 118 Table 3.10 IR Characteristics of drugs, polymers and ligand (D-mannose) interaction S.No. Wave no.(cm -1) Vibration mode May be due to 1 3388.01 N-H str Primary amine 2 2930 C-H str Aromatic ring 3 2850.6 C-H str Methyl group 4 1666.26 C=O str Amide I 5 1407 N-H bending Amide II 6 1266.7 C=N str Pyrazine/Pyridine 7 1019.1 C-O-C ring str Pyranose 3.3.2.2 I.R spectrum of drugs, polymers and ligand (Folic acid) The characteristic absorption peaks of Isoniazid and Pyrazinamide were observed in recorded IR spectra (sodium alginate, chitosan, calcium chloride, folic acid) used in formulation 2. The FTIR results revealed that there was no interaction between drugs (INH and PYZ) and excipients (sodium alginate, chitosan, calcium chloride, folic acid) used in formulation 2. IR Spectra is shown in Fig 3.14.

CHAPTER 1 INTRODUCTION Page 119 Fig 3.14 I.R spectrum of drugs, polymers and ligand (Folic acid) Table 3.11 IR Characteristics of drugs, polymers and ligand (Folic acid) interaction S.No. Wave no.(cm -1) Vibration mode Due to 1 3325.23 N-H str Primary amine 2 3001.3 C-H str Aromatic ring 3 2859.6 C-H str Methyl group 4 1667 C=O str Amide I 5 1556.4 N-H bending Amide II 6 1411.6 C=N str Pyrazine/Pyridine 7 1023.13 C-O-C ring str Pyranose 3.4 SELECTION OF ANALYTICAL WAVELENGTH FOR SIMULTANEOUS ESTIMATION OF DRUGS 3.4.1 Overlay spectrum of Isoniazid and Pyrazinamide for simultaneous estimation of drugs. In overlay spectrum, individual spectra of isoniazid and pyrazinamide were overlapped to find all points of intersection. The observed intersection points in overlay spectrum were at 225nm, 252 nm, 283.5 nm with absorbance 0.160A, 0.165 A and 0.078 A as shown in table 3.12. The highest intersection point was considered as isobestic point for analysis of drugs in combined formulation.

CHAPTER 1 INTRODUCTION Page 120 Fig 3.15 Overlay spectra of INH and PYZ with intersection point 225 nm Fig 3.16 Overlay spectra of INH and PYZ with intersection point 252nm

CHAPTER 1 INTRODUCTION Page 121 Fig 3.17 Overlay spectra of INH and PYZ with intersection point 283.5nm Table 3.12 Isobestic Point Determination Intersecting Points I st II nd III rd Isobestic Point λ max (nm) 225 252 283.5 252 Absorbance 0.160 0.165 0.078 0.165 λ max (Isobestic Point) for simultaneous estimation of Pyrazinamide and Isoniazid was found to be 252nm. 3.5 CHARACTERIZATION AND EVALUATION OF CHITOSAN NANOPARTICLES Isoniazid and Pyrazinamide loaded chitosan nanoparticles were prepared by ionotropic gelation method using chitosan and sodium tripolyphosphate. Total nine batches were formulated as per 3 2 factorial design. Various factors affecting characteristic of nanoparticles were studied. Table 3.13 Different variables used in chitosan nanoparticle preparation

CHAPTER 1 INTRODUCTION Page 122 Independent Variable Low level (-1) Medium level (0) High level (+1) Conc. of chitosan (X1) 0.1% (w/v) 0.2%(w/v) 0.3% (w/v) Conc. of NaTPP (X2) 0.25 % (w/v) 0.5% (w/v) 0.75% (w/v) Table 3.14 Experimental result data with various factors and their responses on formulated batches of chitosan nanoparticles Batches Conc. of Chitosan % (w/v) Conc. of Na TPP % w/v Particle size (nm) Zeta Potential (mV) PDI 1N 0.3 0.75 1044.4± 20.23 +18.89±0.21 0.443 2N 0.2 0.5 716.7± 19.54 +20.19±0.23 0.360 3N 0.1 0.25 381.3±23.56 +22.18±1.02 0.278 4N 0.3 0.25 658.4± 26.77 +28.16±0.87 0.286 5N 0.2 0.75 908.0±15.76 +16.14 ±0.72 0.364 6N 0.1 0.5 712.9±24.54 +16.56±0.55 0.286 7N 0.3 0.5 740.0±25.87 +22.17±0.32 0.294 8N 0.2 0.25 414.3±27.13 +26.52±0.67 0.296 9N* 0.1 0.75 00 00 00 All the data shown are the mean ± SD (n=3). * Batch 9N shown no particles during measurement. It may be due to the minimum zeta potential (very close to zero) that caused settling of particles and failure of formulated batch. 3.5.1 Particle size analysis of formulated chitosan nanoparticles The ionic interaction between chitosan (positively charged) and TPP (negatively charged) resulted in formation of positively charged chitosan nanoparticles at room temperature. The mean particle size and size distribution of formulated batch were analyzed by Nano plus HD (DLS Analyzer). Particle size and PDI of formulated batches ranged from 414.3 ± 2.71 to

CHAPTER 1 INTRODUCTION Page 123 1044 ± 20.23 nm and 0.278 to 0.443 as shown in Table 3.14 and 3.15. The PDI (Polydispersity index) value < 0.5 indicates narrow size distribution of particles. The Particle size of best batch (8N) was found to be 414.3±27.13nm. Table 3.15 Different Particle size of formulated batches of chitosan nanoparticles Batches Particle size (nm) 1N 1044.4 ± 20.23 2N 716.7 ± 19.54 3N 381.3 ± 23.56 4N 658.3 ± 26.77 5N 908.0 ± 15.76 6N 712.9 ± 24.54 7N 740.0 ± 25.87 8N 414.3 ± 27.13 9N 00 All the data shown are the mean ± SD (n=3). 3.5.2 Zeta potential of formulated chitosan nanoparticles The particle size and surface charge are critical determinants for fate of delivered nanoparticles. Zeta potential is surface charge that greatly affects the particle stability in colloid system through electrostatic repulsion. The particle aggregation is less likely to occur in case of highly charged particles i.e. above ±30mV. Zeta potential values in all formulated batches ranged between +14.19 ± 0.21to +28.16 ± 0.87 mV. This indicates moderate stability of formulations as shown in Table 3.16. The Positive zeta potential value is due to availability of free NH 3 + . The zeta potential of best

batch (8N) was found to be +26.52 ± 0.67 mV. Table 3.16 Different Zeta Potential of formulated batches of chitosan nanoparticles Batches Zeta Potential (mV)

CHAPTER 1 INTRODUCTION Page 124 1N +18.89±0.21 2N +20.19±0.23 3N +22.18±1.02 4N +28.16±0.87 5N +16.14±0.72 6N +16.56±0.55 7N +22.17±0.32 8N +26.52±0.67 9N 00 All the data shown are the mean ± SD (n=3). 3.5.3 SEM (Scanning Electron Microscopy) study of chitosan nanoparticles The shape and surface morphology of optimized batch (8N) was studied by FE-SEM (scanning electron microscopy). SEM analysis revealed that the use of factorial design resulted particles with target size and narrower size. The particles were found to be spherical in shape with a smooth surface as shown in fig 3.18 (a) & 318 (b) (a) (b) Fig 3.18 (a) & (b) SEM images of chitosan nanoparticles

CHAPTER 1 INTRODUCTION Page 125 3.5.4 Entrapment efficiency and drug loading capacity of chitosan nanoparticles The entrapment efficiency (%) and drug loading capacity (%) of various batches (1N - 9N) were determined. The results revealed that increase in polymer concentration lead to increase in particle size, entrapment efficiency and drug loading capacity to certain concentration but after that there was no significant increase in entrapment efficiency and drug loading capacity as shown in Table 3.17. The expected reason for this might be that increased concentration of polymer lead to increased viscosity which avoided aggregation of drug in nanoparticles, hence reducing the availability of drug for entrapment. The entrapment efficiency increased up to 56.96± 0.31% and 65.45 ± 0.023% while drug loading capacity increased up to 16.23 ± 0.21% and 16.78 ± 0.76% for INH and PYZ respectively. Table 3.17 Drug entrapment efficiency and loading capacity of formulated batches of chitosan nanoparticles Batches Particle size (nm) Entrapment efficiency (%) Drug loading efficiency (%) INH PYZ INH PYZ 3N 381.3 ± 23.56 55.32 ± 0.34 62.15 ± 0.12 11.34 ±0.23 14.23 ± 0.32 8N 414.3 ± 27.13 55.29 ± 0.06 63.14 ±0.29 11.99 ±0.78 14.65 ± 0.24 4N 658.3 ± 26.77 56.13 ± 0.08 64.35 ± 0.35 12.76 ± 0.12 15.32±0.28 6N 712.9 ± 24.54 56.93 ±0.23 65.13 ± 0.16 14.87 ± 0.54 15.64 ±0.06 2N 716.7 ± 19.54 56.96 ± 0.31 65.45 ± 0.023 16.23 ± 0.21 16.78 ±0.67 7N 740.0 ± 25.87 56.0 ± 0.05 64.98 ± 0.15 15.23 ±0.18 15.89±0.24 5N 908.0 ± 15.76 32.63 ±0.24 31.13 ±0.32 6.54 ±0.054 7.34 ±0.18 1N 1044.4 ± 20.23 32.04 ± 0.32 29.34 ±0.01 5.34 ± 0.32 6.21 ± 0.13 9N 00 00 00 00 00 All the data shown are the mean ± SD (n=3). * Batch 9N formulation failed.

CHAPTER 1 INTRODUCTION Page 126 3.5.5 EFFECT OF VARIOUS VARIABLES ON PARTICLE SIZE OF CHITOSAN NANOPARTICLES (CS NPs) 3.5.5.1 Effect of Chitosan concentration on particle size of CS NPs To study the effect of chitosan concentration on particle size, three set of experiments (3N, 8N, 4N), (6N, 2N, 7N) and (9N, 5N, 1N) were designed. In each set TPP concentration was kept constant but chitosan concentration was varied. Different concentrations of chitosan 1.0, 2.0, 3.0 (mg/ml) in each set, with ratio of 3:1:: CS:TPP (v/v) resulted in formation of nanoparticles. The results revealed that increase in concentration of chitosan lead to increased particle size. It may be due to increased viscosity of inner phases which lead to increased cross linking and increase in particle size. Table 3.18 Effect of chitosan on particle size of various formulated batches of CS NPs SET-I Batches Chitosan Conc.(mg/ml) Na TPP Conc. (mg/ml) Particle size (nm) 3N 1.0 2.5 381.3±23.56 8N 2.0 2.5 414.3±27.13 4N 3.0 2.5 658.3± 26.77 SET-II Batches Chitosan Conc.(mg/ml) Na TPP Conc. (mg/ml) Particle size(nm)

6N 1.0 5.0 712.9±24.54 2N 2.0 5.0 716.7± 19.54 7N 3.0 5.0 740.0±25.87 SET-III Batches Chitosan Conc.(mg/ml Na TPP Conc. (mg/ml) Particle size(nm) 9N 1.0 7.5 00 5N 2.0 7.5 908.0±15.76 1N 3.0 7.5 1044.4± 20.23 All the data shown are the mean ± SD (n=3).

CHAPTER 1 INTRODUCTION Page 127 Fig 3.19 Effect of Chitosan concentration on particle size of CS NPs 3.5.5.2 Effect of Tripolyphosphate concentration on particle size of CS NPs Three sets of experiments (3N, 6N, 9N), (8N, 2N, 5N) and (4N, 7N, 1N) were designed using 3 2 factorial design. The chitosan concentration was kept constant in all batches of each set but varied concentration of TPP 2.5,5.0,7.5 mg/ml was used in each set. Ratio of 3:1:: CS:TPP (v/v) resulted in formation of chitosan nanoparticles. The results reflect that TPP concentration affects particle size of chitosan nanoparticles. Increase in conc. of TPP resulted into increased particle size as can be depicted from Fig 3.20. Table 3.19 Effect of TPP on Particle size of various formulated batches of CS NPs SET-I Batches Chitosan Conc.(mg/ml) Na TPP Conc. (mg/ml) Particle size (nm) 3N 1.0 2.5 381.3 ± 23.56 6N 1.0 5.0 712.9 ± 24.54 9N 1.0 7.5 00 SET-II Batches Chitosan Na TPP Conc. Particle size(nm)

CHAPTER 1 INTRODUCTION Page 128 Conc.(mg/ml) (mg/ml) 8N 2.0 2.5 414.3 ± 27.13 2N 2.0 5.0 716.7 ± 19.54 5N 2.0 7.5 908.0 ± 15.76 SET-III Batches Chitosan Conc.(mg/ml) Na TPP Conc. (mg/ml) Particle size(nm) 4N 3.0 2.5 658.3 ± 26.77 7N 3.0 5.0 740.0 ± 25.87 1N 3.0 7.5 1044.4 ± 20.23 All the data shown are the mean ± SD (n=3). Fig 3.20 Effect of TPP on Particle size of CS NPs 3.5.6 EFFECT OF VARIOUS VARIABLES ON ZETA POTENTIAL OF CHITOSAN NANOPARTICLES (CS NPs)

CHAPTER 1 INTRODUCTION Page 129 3.5.6.1 Effect of Chitosan concentration on Zeta Potential of CS NPs The effect of chitosan concentration on zeta potential was studied through three set of experiments (3N, 8N, 4N), (6N, 2N, 7N) and (9N, 5N, 1 N) designed by 3 2 factorial design. The chitosan concentration was varied in each set from 1-3(mg/ml) while TPP concentration was kept constant in all batches of each set as shown in Table 3.20. It is evident from the findings that the zeta potential increased in each set on increasing chitosan concentration from 1-3 mg/ml at fixed TPP. It may be due to increased attribution of NH 3 + by increased chitosan concentration. The more availability of NH 3 + by increased chitosan concentration will increase positive value of zeta potential as shown in Fig 3.21. Table 3.20 Effect of Chitosan on Zeta Potential of various Formulated batches of CS NPs SET-I Batches Chitosan Conc.(mg/ml) Na TPP Conc. (mg/ml) Zeta Potential (mV) 3N 1.0 2.5 +22.18±1.02 8N 2.0 2.5 +26.52±0.67 4N 3.0 2.5 +28.16±0.87 SET-II Batches Chitosan Conc.(mg/ml) Na TPP Conc. (mg/ml) Zeta Potential (mV) 6N 1.0 5.0 +16.56±0.55 2N 2.0 5.0 +20.19±0.23 7N 3.0 5.0 +22.17 ±0.32 SET-III Batches Chitosan Conc.(mg/ml Na TPP Conc. (mg/ml) Zeta Potential (mV) 9N 1.0 7.5 00 5N 2.0 7.5 +16.14±0.72 1N 3.0 7.5 +18.89±0.21 All the data shown are the mean ± SD (n=3).

CHAPTER 1 INTRODUCTION Page 130 3.21 Effect of Chitosan on zeta potential of CS NPs 3.5.6.2 Effect of Tripolyphosphate concentration on Zeta Potential of CS NPs The comparative results of zeta potential are shown in fig 3.22. It was performed through three set of experiments (3N, 6N, 9N), (8N, 2N, 5N) and (4N, 7N, 1N). The batches in each set had varied TPP concentration (2.5-7.5mg/ml) while chitosan concentration was kept constant as shown in

Table 3.21. It can be depicted from Fig 3.22 that zeta potential of nanoparticles decreased in each set on increasing TPP concentration. The decrease in zeta potential may be due to de-protonation of amine by TPP as a result decrease in positive value of zeta potential. Table 3.21 Effect of TPP on Zeta Potential of various Formulated batches of Chitosan Nanoparticles. SET-I

CHAPTER 1 INTRODUCTION Page 131 Batches Chitosan Conc.(mg/ml) Na TPP Conc. (mg/ml) Zeta Potential(mV) 3N 1.0 2.5 +22.18±1.02 6N 1.0 5.0 +16.14±0.72 9N 1.0 7.5 00 SET-II Batches Chitosan Conc.(mg/ml) Na TPP Conc. (mg/ml) Zeta Potential(mV) 8N 2.0 2.5 +26.52±0.67 2N 2.0 5.0 +20.19±0.23 5N 2.0 7.5 +16.14±0.72 SET-III Batches Chitosan Conc.(mg/ml Na TPP Conc. (mg/ml) Zeta Potential(mV) 4N 3.0 2.5 +28.16±0.87 7N 3.0 5.0 +22.17±0.32 1N 3.0 7.5 +18.89 ±0.21 All the data shown are the mean ± SD (n=3). Fig 3.22 Effect of TPP on zeta potential of CS NPs 3.6 IN-VITRO DRUG RELEASE STUDY OF CS NPs The in vitro drug release studies of selective batches were carried out in 0.1N HCl (pH 1.2) and phosphate buffer (pH 7.4) using modified diffusion apparatus at 37 o C for 2 h and 14 hr respectively. The batches for drug release study were selected on the basis of particle size

CHAPTER 1 INTRODUCTION Page 132 and zeta potential. In vitro release of various batches ranged from 27.66 ±0.88% to 43.97 ± 1.56% and 42.66 ±0.74% to 57.80 ± 1.40 % for INH and PYZ respectively at pH 1.2 (0.1N HCl) for 2h while at pH 7.4 (phosphate buffer) the drug release ranged from 54.39 ± 1.43% to 84.82 ± 2.54% and 61.48 ± 1.52% to 71.24 ± 1.23% for INH and PYZ in 14 h. The optimized batch had showed initial burst release of 20.28% ± 1.43 and 23.62% ± 1.07 followed by controlled release of drug 84.82 ± 2.54 % and 61.48 ± 1.52 % for INH and PYZ respectively at 14 hr. Initial burst release indicates that the drug was localized outwards the nanoparticles. Different release models were applied to determine the best fit model. The release was best explained by zero order as it showed highest regression value (R 2 = 0.983 for INH and R 2 =0.976 for PYZ). Korsemeyer peppas equation was applied to understand the mechanism of drug release. The release exponent „n" was 0.374 and 0.281 for INH and PYZ respectively, which indicates zero order release with fickian diffusion. The in vitro release of drug also depends upon particle size. The small size indicates more surface area i.e. more surface area come in contact with medium resulting in faster release. Batch 8N with particle size approx. 414.3 ± 27.13nm showed drug release up to 84.82 ± 2.54% and 61.48 ± 1.52% for INH and PYZ respectively at 14 hr. Fig 3.23 Cumulative % drug release of INH and PYZ from various batches of CS NPs at pH 7.4 3.7 CUMULATIVE % DRUG RELEASE AND RELEASE KINETIC STUDY OF OPTIMIZED BATCH (8N) of CS NPs

CHAPTER 1 INTRODUCTION Page 133 Cumulative drug release study of optimized batch 8N was done using modified diffusion apparatus in phosphate buffer 7.4pH for 14 hours. Table 3.23 Cumulative drug release of optimized batch (8N) of CS NPs Time (Hr) Cumulative Drug Release (%) 0 0 0 0.25 20.28 ± 1.43 23.62 ± 1.23 0.5 20.60 ± 1.33 23.94 ±1.67 1.0 21.13 ± 1.54 24.47 ±1.21 1.5 21.88 ± 1.07 25.21 ± 0.94 2.0 22.02 ± 1.75 27.02 ± 0.96 2.5 23.83 ± 1.62 28.83 ± 1.43 3.0 29.33 ± 1.11 27.66 ± 1.12 3.5 34.47 ± 1.21 29.47 ±1.74 4.0 37.41 ± 1.13 30.74 ±1.98 4.5 37.84 ± 1.32 31.17 ± 1.77 5.0 44.43 ± 1.76 32.77 ± 1.98 5.5 45.43 ± 1.34 35.43 ± 1.57 6.0 51.06 ±1.29 36.06 ± 1.83 6.5 52.02 ± 1.11 37.02 ± 2.01 7.0 52.55 ± 1.45 37.55 ±2.16 7.5 54.75 ± 1.87 38.09 ± 1.56 8.0 55.11 ± 1.36 45.11 ± 1.07 8.5 55.46 ± 1.76 47.13 ± 1.04

CHAPTER 1 INTRODUCTION Page 134 9.0 58.30 ± 2.34 48.30 ± 1.25 9.5 59.18 ± 1.65 50.85 ± 1.23 10.0 59.82 ± 1.22 51.49 ± 1.62 10.5 64.26 ± 2.34 54.26 ± 1.33 11.0 70.50 ± 2.87 53.83 ± 1.98 11.5 73.01 ± 1.43 54.68 ± 1.43 12.0 76.67 ± 1.32 55.00 ± 1.23 12.5 78.05 ± 1.09 56.38 ± 1.21 13.0 80.99 ± 1.12 57.66 ± 1.9 13.5 84.65 ± 2.54 57.98 ± 1.54 14.0 84.82 ± 1.43 61.49 ± 1.43 All the data shown are the mean ± SD (n=3). Fig 3.24 Cumulative % drug release of optimized batch (8N) of CS NPs 3.8 RELEASE KINETIC STUDY 3.8.1 Zero Order Plot for the optimized batch (8N) of CS NPs

CHAPTER 1 INTRODUCTION Page 135 Fig 3.25 Plot of Zero order release kinetics of the optimized batch (8N) of CS NPs 3.8.2 First order Plot for the optimized batch (8N) of CS NPs Fig 3.26 Plot of First order release kinetics of optimized batch (8N) of CS NPs 3.8.3 Higuchi Plot for the optimized batch (8N) of CS NPs

CHAPTER 1 INTRODUCTION Page 136 Fig 3.27 Plot of Higuchi release kinetic of the optimized batch (8N) of CS NPs 3.8.4 Hixon-Cowell Plot for the optimized batch (8N) of CS NPs Fig 3.28 Plot of Hixon crowell release kinetic of optimized batch (8N) of CS NPs 3.8.5 Korsemeyer-Peppas Plot for the optimized batch (8N) of CS NPs 3.8.5.1 Korsemeyer- Peppas Plot for INH

CHAPTER 1 INTRODUCTION Page 137 Fig 3.29 Plot of Korsemeyer-Peppas release kinetic of optimized batch (8N) for INH 3.8.5.2 Korsemeyer- Peppas Plot for PYZ Fig 3.30 Plot of Korsemeyer-Peppas release kinetic of optimized batch (8N) for PYZ 3.9 CHARACTERIZATION AND EVALUATION OF ALGINATE-CHITOSAN (ALG- CS) NANOPARTICLES (NPs). Isoniazid and Pyrazinamide loaded alginate nanoparticles were prepared by ionotropic gelation method using chitosan, calcium chloride and sodium alginate. Total nine batches were

CHAPTER 1 INTRODUCTION Page 138 fabricated as per 3 2 factorial design. Various parameters affecting characteristics of nanoparticles were studied. Table 3.24 Values of independent variables in ALG-CS nanoparticle preparation Independent variable Low level (-1) Medium level (0) High level (+1) Conc. of alginate (X1) 0.05% 0.075% 0.1% Conc. of chitosan (X2) 0.05% 0.07 % 0.09% Conc. of CaCl 2 (X3) 18mM 18mM 18mM Table 3.25 Experimental result data with various factors and their responses on formulated batches of ALG –CS nanoparticles. All the data shown are the mean ± SD (n=3). 3.9.1 Particle size analysis of ALG –CS NPs Batches Conc. of sodium alginate (%w/v) Conc. of CaCl 2 (mM) Conc. of Chitosan (%w/v) Particle Size (nm) Zeta Potential PDI 1S 0.075 18mM 0.07 474.1±15.28 0.030 ±0.59 0.427 2S 0.050 18mM 0.09 649.5±26.65 -13.7 ±0.34 0.340 3S 0.100 18mM 0.05 539.7 ±23.3 -26.4 ±0.55 0.482 4S 0.100 18mM 0.07 551.8±39.02 -7.47 ±0.53 0.561 5S 0.050 18mM 0.05 269 ± 21.43 -19.5 ± 0.77 0.203 6S 0.075 18mM 0.09 654.9±15.67 0.011 ±0.45 1.00 7S 0.075 18mM 0.05 289.5±21.23 0.003 ± 0.38 0.970 8S 0.050 18mM 0.07 504.4±19.54 -4.99 ±0.64 0.530 9S 0.100 18mM 0.09 1090 ± 23.12 -17.97 ±0.64 0.546

CHAPTER 1 INTRODUCTION Page 139 The ionic interaction between chitosan, calcium chloride (positively charged) and sodium alginate (negatively charged) resulted in formation of negatively charged alginate- chitosan nanoparticles at room temperature. The desirable particle size was achieved at pH 4.9 and pH 4.6 for Sodium alginate and chitosan solution. As at this pH, the carboxyl groups of alginate and amine groups of chitosan are mostly ionized, therefore allowing stronger interaction between CS and ALG, leading to the formation of more

compact and smaller nanoparticles. The mean particle size and size distribution of fabricated batches ranged from 269 ± 21.43 to 1090 ± 23.12nm and 0.203 to 1. The PDI (Polydispersity index) value < 0.5 indicates narrow particle size distribution. The particle size of optimized batch (3S) was found to be 539.7 ± 23.3nm. Table 3.26 Different Particle size of formulated batches of ALG –CS NPs Batches Particle size (nm) 1S 474.1±15.28 2S 649.5± 26.65 3S 539.7 ± 23.3 4S 551.8±39.02 5S 269 ± 21.43 6S 654.9±15.67 7S 289.5±21.23 8S 504.4±19.54 9S 1090 ± 23.12 All the data shown are the mean ± SD (n=3). 3.9.2 Zeta Potential of formulated ALG –CS NPs The zeta potential of formulated batches of ALG –CS NPs were determined by Malvern zeta sizer and it ranged from -26.4 ± 0.26 mV to +0.030 ± 0.59 mV. This indicates the moderate stability of some formulations as it has been reported earlier that the value of

CHAPTER 1 INTRODUCTION Page 140 zeta potential less than -30 mV and higher than + 30 mV assures the stability of nanoparticle in colloidal system. The zeta potential of optimized batch 3S was found to be -26.4± 0.26 mV. The negative value obtained for zeta potential indicated that the nanoparticles are negatively charged due to availability of free carboxylate group (COO -) of ALG polymers over nanoparticles. Table 3.27 Different Zeta Potential of formulated batches of ALG-CS NPs Batches Zeta Potential (mV) 1S 0.030 ±0.59 2S -13.7 ±0.34 3S -26.4 ±0.55 4S -7.47 ±0.53 5S -19.5 ± 0.77 6S 0.011 ±0.45 7S 0.003 ± 0.38 8S -4.99 ±0.64 9S -17.97 ±0.64 All the data shown are the mean ± SD (n=3). 3.9.3 SEM Study of ALG-CS NPs The morphological characteristic of formulated optimized batch (3S) was examined by FE-

SEM. The images revealed that particles were having smooth surface and were spherical in shape.

CHAPTER 1 INTRODUCTION Page 141 (a) (b) Fig 3.31 (a) and (b) SEM image of alginate nanoparticles 3.9.4 Entrapment efficiency and drug loading of formulated batches of ALG-CS NPs Entrapment efficiency (%) and drug loading (%) of formulated ALG-CS NPs (batches 1S-9S) were determined by given formula below. The results indicated that increase in polymer lead to increase in entrapment efficiency (%) as well as loading capacity (%) to certain concentration but after that there was no significant increase in entrapment and loading efficiency. Entrapment efficiency increased up to 72.31 ± 0.06 % and 73.45 ± 0.21 % for INH and PYZ while loading efficiency increased upto 23.14 ± 1.23% and 19.87 ± 0.98% for INH and PYZ respectively. Table 3.28 Drug entrapment efficiency and loading capacity of formulated batches of ALG-CS NPs Batches Particle size Entrapment efficiency (%) Loading Capacity (%)

CHAPTER 1 INTRODUCTION Page 142 (nm) INH PYZ INH PYZ 5S 269 ± 21.43 65.42 ± 0.54 64.15 ± 0.34 16.34 ± 0.34 17.45 ± 0.22 7S 289.5 ± 21.23 67.26 ± 0.34 63.14 ± 0.57 17.54 ± 0.44 17.98 ± 0.65 1S 474.1 ± 15.28 67.35 ± 0.19 68.35 ± 0.65 18.44 ± 0.87 19.87 ± 0.98 8S 504.4 ±19.54 68.73 ± 0.63 72.13 ±0.85 20.65 ± 0.34 20.86 ± 0.12 3S 504 ± 19.54 70.2 ± 0.24 73.4 ± 0.21 23.14 ± 1.23 19.54 ± 0.67 4S 539.7 ± 23.3 72.31 ± 0.06 65.98 ± 0.52 22.45 ± 0.45 19.02 ±0.43 2S 551.8 ±39.02 24.34 ± 0.76 23.34 ± 0.45 08.67 ± 0.99 07.89 ± 0.23 6S 654.9 ±15.67 22.63 ± 0.45 20.13 ± 0.87 10.23 ± 0.23 08.45 ± 0.43 9S 1090 ± 23.12 15.0 ± 0.65 20.0 ± 0.45 07.76 ± 0.43 06.88 ± 0.34 All

the data shown are the mean ± SD (n=3). 3.9.5 EFFECT OF VARIOUS VARIABLES ON PARTICLE SIZE OF ALGINATE- CHITOSAN NANOPARTICLES (ALG-CS NPs). 3.9.5.1 Effect of Chitosan concentration on particle size of of ALG-CS NPs As per 3 2 factorial design, total 3 set of experiments were designed (5S, 8S, 2S), (7S, 1S, 6S), (3S, 4S, 9S) with varying concentration of chitosan concentration (0.05, 0.07, 0.09 %) in each set at constant alginate concentration as shown in Table 3.29. Particle size of formulated alginate-chitosan (AL-CS) nanoparticles was found to be affected by chitosan concentration. The results revealed that increasing the concentration of chitosan lead to increase in particle size. The reason behind this may be attributed to increase in viscosity of inner phase and increased cross linking with alginate. Table 3.29 Effect of chitosan on particle size of various formulated batches of ALG-CS NPs

CHAPTER 1 INTRODUCTION Page 143 SET-I Batches Chitosan Conc.(%w/v) Sodium alginate Conc. (% w/v)) Particle size (nm) 5S 0.05 0.050 269 ± 21.43 8S 0.07 0.050 504.4±19.54 2S 0.09 0.050 649.5±26.65 SET-II Batches Chitosan Conc.(%w/v) Sodium alginate Conc. (% w/v) Particle size(nm) 7S 0.05 0.075 289.5±21.23 1S 0.07 0.075 474.1±15.28 6S 0.09 0.075 654.9±15.67 SET-III Batches Chitosan Conc.(%w/v) Sodium alginate Conc. (% w/v) Particle size(nm) 3S 0.05 0.100 539.7 ±23.3 4S 0.07 0.100 551.8 ±39.02 9S 0.09 0.100 1090 ± 23.12 All the data shown are the mean ± SD (n=3). Fig 3.32 Effect of chitosan concentration on Particle Size of ALG-CS NPs

CHAPTER 1 INTRODUCTION Page 144 3.9.5.2 Effect of alginate concentration on particle size of ALG-CS NPs Total three sets (5S, 7S, 3S), (8S, 1S, 4S), (2S, 6S, 9S) of experiment were designed to study the effect of alginate concentration on nanoparticles. In three sets the alginate concentration (0.050, 0.075, and 0.100 %) was varied but chitosan concentration was kept constant as shown in Table 3.30. The results revealed that particle size of formulated batches were affected by alginate concentration. Increasing concentration of alginate resulted in increased particle size. The possible reason could be due to more availability of functional group (COO -) with increase in alginate concentration, that interacts with calcium and chitosan cations.It is interpreted that with the increase in interactions, the formation of number of chains increases and hence the particle size increased. Table 3.30 Effect of alginate on particle size of various formulated batches of ALG-CS NPs SET-I Batches Chitosan Conc.(% w/v) Sodium alginate Conc. (% w/v) Particle size (nm) 5S 0.05 0.050 269 ± 21.43 7S 0.05 0.075 289.5±21.23 3S 0.05 0.100 539.7 ±23.3 SET-II Batches Chitosan Conc.(%w/v) Sodium alginate Conc. (% w/v) Particle size(nm) 8S 0.07 0.050 504.4±19.54 1S 0.07 0.075 474.1±15.28 4S 0.07 0.100 551.8±39.02 SET-III Batches Chitosan Conc.(%w/v) Sodium alginate Conc. (% w/v) Particle size(nm)

CHAPTER 1 INTRODUCTION Page 145 2S 0.09 0.050 649.5±26.65 6S 0.09 0.075 654.9±15.67 9S 0.09 0.100 1090 ± 23.12 All the data shown are the mean ± SD (n=3). Fig 3.33 Effect of alginate on particle size o of ALG-CS NPs 3.9.6 EFFECT OF VARIOUS VARIABLES ON ZETA POTENTIAL OF ALGINATE- CHITOSAN NANOPARTICLES (ALG-CS NPs). 3.9.6.1 Effect of Chitosan concentration on Zeta potential of ALG-CS nanoparticles The effect of chitosan concentration on zeta potential was studied through three sets (5S, 2S), (7S, 6S), (3S, 9S) of experiments. In different sets, the chitosan concentration was varied but alginate concentration was kept constant as shown in Table 3.31. The zeta potential value of nanoparticles were increased in each set with increase in the concentration of CS from lower level 0.05 to higher 0.09 % as shown in Fig 3.34.

The increased concentration of chitosan bring about more availability of protonated amine (NH 3+) even after neutralization of carboxylate group of alginate, hence the addition of chitosan decreases the negative potential of NPs .

CHAPTER 1 INTRODUCTION Page 146 Table 3.31 Effect of Chitosan concentration on Zeta potential on various formulated batches of ALG-CS NPs SET-I Batches Chitosan Conc.(%w/v) Sodium alginate Conc. (% w/v) Zeta Potential (mV) 5S 0.05 0.050 -19.5 ± 0.77 2S 0.09 0.050 -13.7 ±0.34 SET-II Batches Chitosan Conc.(%w/v) Sodium alginate Conc. (% w/v) Zeta Potential (mV) 7S 0.05 0.075 0.003 ± 0.38 6S 0.09 0.075 0.011 ±0.45 SET-III Batches Chitosan Conc.(%w/v) Sodium alginate Conc. (% w/v) Zeta Potential (mV) 3S 0.05 0.100 -26.4 ±0.55 9S 0.09 0.100 -17.97 ±0.64 All the data shown are the mean ± SD (n=3). Fig 3.34 Effect of chitosan concentration on zeta potential of ALG-CS nanoparticles

CHAPTER 1 INTRODUCTION Page 147 3.9.6.2 Effect of alginate concentration on Zeta potential of ALG-CS nanoparticles Three set (5S, 3S), (8S, 4S), (2S, 9S) of experiments were designed to study the effect of alginate concentration on zeta potential. Among all three sets, the alginate concentration was varied while chitosan concentration was kept constant as shown in Table 3.32. The zeta potential value of nanoparticles were decreased (negative potential increased) in each set with increase in alginate concentration from lower level 0.050 to higher 0.100 % at fixed chitosan concentration as shown in Fig 3.35. The increased concentration of alginate bring about more interaction between negatively charged COO - of alginate with positively charged NH 3+ of chitosan. Therefore it can be interpreted that the addition of alginate contributed more negative potential in NPs. Table 3.32 Effect of alginate concentration on Zeta potential of various formulated batches of ALG-CS nanoparticles SET-I Batches Chitosan Conc.(%w/v) Sodium alginate Conc. (% w/v)) Zeta Potential (mV) 5S 0.05 0.050 -19.5 ± 0.77 3S 0.05 0.100 -26.4 ±0.55 SET-II Batches Chitosan Conc.(%w/v) Sodium alginate Conc. (% w/v) Zeta Potential (mV) 8S 0.07 0.050 -4.99 ±0.64 4S 0.07 0.100 -7.47 ±0.53 SET-III Batches Chitosan Conc.(%w/v) Sodium alginate Conc. (% w/v) Zeta Potential (mV) 2S 0.09 0.050 -13.7 ±0.34 9S 0.09 0.100 -17.97 ±0.64 All the data shown are the mean ± SD (n=3).

CHAPTER 1 INTRODUCTION Page 148 Fig 3.35 Effect of alginate concentration on zeta potential of ALG –CS nanoparticles 3.10 IN-VITRO DRUG RELEASE STUDY OF VARIOUS FORMULATED BATCHES OF ALG-CS NANOPARTICLES The in vitro drug release studies of selected batches (2S, 3S, 5S) were carried out on the basis of particle size, zeta potential, entrapment efficiency. The study was carried out in simulated gastric fluid (pH 1.2) and phosphate buffer (pH 7.4) using modified diffusion apparatus at 37 ° ± 0.5 ° C for 10 hr. In vitro drug release of various batches ranged from 48.53±2.45 to 74.53 ± 2.53% for INH and from 37.55 ± 1.87 to 57.87 ± 2.04 % for PYZ at 7.4 pH. The optimized batch (3S) had shown the initial burst release of 5.04 ± 0.45% and 19.68 ± 0.87% followed by slow sustained release of drug 74.53 ± 2.53 % and 57.87 ± 2.04 % for INH and PYZ respectively at 10 hr. Initial burst release indicates that the drug was localized outward the nanoparticles. Different release models were applied to determine the best fit model. The release was best explained by first order as it showed highest regression value (R 2 = 0.9703 for INH and R 2 =0.9401 for PYZ). Korsemeyer peppas equation was applied to understand the mechanism of drug release. The release exponent „n" was 0.4501 and 0.6986 for INH and PYZ respectively, indicating that

matrix nanosphere had non fickian diffusion. Table 3.33 In-vitro drug release profile of alginate chitosan nanoparticles at pH 1.2

CHAPTER 1 INTRODUCTION Page 149 Time (Hr) %Release INH (B-2S) %Release PYZ (B-2S) % Release INH (B-5S) %Release PYZ (B-5S) %Release INH (B-3S) %Release PYZ (B3S) 0.25 4.32± 0.08 3.09±.058 7.23± 0.07 9.17±0.32 04.32± 0.08 7.18±0.27 1.0 5.39±0.65 5.33±0.08 6.23±0.05 7.40±0.56 07.19±0.98 7.23±0.93 2.0 7.88±0.59 5.46±0.23 8.16±0.11 8.83±0.87 9.38±0.92 8.48 ±0.34 All the data shown are the mean ± SD (n=3). Table 3.34 In-vitro drug release profile of alginate chitosan nanoparticles at pH 7.4 Time (Hr) %Release INH (B-2S) %Release PYZ (B-2S) % Release INH (B-5S) %Release PYZ (B-5S) %Release INH (B-3S) %Release PYZ (B-3S) 0.25 20.28± 1.36 23.61±1.76 24.53± 1.23 32.87±0.45 5.03 ± 0.45 19.68±0.87 1.0 20.60±1.98 23.93±1.98 26.73 ±1.12 33.40±1.56 23.65± 1.02 20.31±2.65 2.0 21.13±1.99 24.46±2.65 27.16±1.11 33.83±1.87 28.61± 2.14 23.61±1.88 2.5 21.87 ±2.06 25.21±2.12 33.08±1.56 38.08± 2.56 34.46 ± 2.09 24.46 ±2.45 3.0 22.02± 2.45 27.02±1.86 33.19±1.56 38.19± 1.34 41.41 ± 2.56 28.08±2.09 3.5 23.83 ±2.34 28.83±2.54 36.34± 1.76 39.68± 1.45 43.36± 1.68 31.70±2.76 4.0 29.32± 1.56 28.16± 1.45 37.51±2.87 40.85±1.34 53.40±2.56 33.40±1.65 4.5 34.46± 1.45 29.46± 1.55 38.47±2.65 41.80 ±1.22 56.45±0.64 34.78±2.53 5.0 37.41±1.67 30.74± 1.87 39.53±2.33 42.87± 1.45 58.19± 2.61 41.06±2.43 5.5 37.83±1.45 31.17±2.56 44.71±1.67 46.38±1.05 58.29±2.18 48.29±2.67 6.0 44.43 ±2.56 32.76± 2.89 44.78±1.56 49.78± 1.33 60.74±2.56 50.74±2.87 7.0 45.42±2.65 35.42±2.67 45.95±1.54 50.95± 1.78 64.46±2.45 54.46±2.33 8.0 51.06±3.65 36.06± 2.55 46.09±1.76 52.76 ±2.65 69.57±2.01 54.57±2.90 9.0 52.02±2.13 37.02±1.89 46.21±1.87 54.57±2.66 71.59±2.32 56.59±2.54 10.0 52.55±1.98 37.55±1.87 48.53±2.45 55.872±1.76 74.53± 2.53 57.87±2.04 All the data shown are the mean ± SD (n=3).

CHAPTER 1 INTRODUCTION Page 150 Fig 3.36 Cumulative % drug release of INH and PYZ from various batches of ALG-CS NPs at pH 7.4 3.11 CUMULATIVE % DRUG RELEASE AND RELEASE KINETIC STUDY OF OPTIMIZED BATCH (3S) OF ALG-CS NANOPARTICLES Cumulative drug release study of optimized batch 3S was done by using modified diffusion apparatus in phosphate buffer 7.4 pH for 10 hours. Table 3.35 Cumulative drug release from optimized batch (3S) of ALG-CS NPs Time (Hr) Cumulative Drug release (%) 0 0.000 ± 00 0.00 ± 00 0.5 5.03 ± 0.045 19.68 ± 0.87 1.0 23.65 ± 1.02 20.31 ± 2.65 2.0 28.61 ± 2.14 23.61 ± 1.88 2.5 34.46 ± 2.09 24.46 ± 2.45

CHAPTER 1 INTRODUCTION Page 151 3.0 41.41 ± 2.56 28.08 ± 2.09 3.5 43.36 ± 1.68 32.70 ± 2.76 4.0 53.40 ± 2.56 33.90 ± 1.65 4.5 56.45 ± 0.64 34.78 ± 2.53 5.0 58.19 ± 2.61 46.76 ± 2.43 5.5 58.29 ± 2.18 48.99 ± 2.67 6.0 60.74 ± 2.56 50.94 ± 2.87 7.0 64.46 ± 2.45 54.46 ± 2.33 8.0 69.57 ± 2.01 56.57 ± 2.90 9.0 71.59 ± 2.32 56.99 ± 2.54 10.0 74.53 ± 2.53 57.87 ± 2.04 All the data shown are the mean ± SD (n=3).

CHAPTER 1 INTRODUCTION Page 152 Fig 3.37 Cumulative % drug release from optimized batch (3S) of ALG-CS NPs 3.12 RELEASE KINETIC STUDY 3.12.1 Zero Order Plot of the optimized batch (3S) of ALG CS NPs Fig 3.38 Plot of Zero order release kinetics for the optimized batch (3S) of ALG CS NPs 3.12.2 First order Plot of the optimized batch (3S) of ALG CS NPs

CHAPTER 1 INTRODUCTION Page 153 Fig 3.39 Plot of First order release kinetics for the optimized batch (3S) of ALG CS NPs 3.12.3 Higuchi Plot the optimized batch (3S) of ALG CS NPs

Fig 3.40 Plot of Higuchi release kinetic for the optimized batch (3s) of ALG CS NPs 3.12.4 Hixon-Cowell Plot the optimized batch (3s) of ALG CS NPs

CHAPTER 1 INTRODUCTION Page 154 Fig 3.41 Plot of Hixon crowell release kinetic for the optimized batch (3s) of ALG CS NPs 3.12.5 Korsemeyer- Peppas Plot for the optimized batch (3s) of ALG CS NPs 3.12.5.1 Korsemeyer- Peppas Plot for INH Fig 3.42 Plot of Korsemeyer-Peppas release kinetic of INH for optimized batch-3S 3.12.5.2 Korsemeyer- Peppas Plot for PYZ

CHAPTER 1 INTRODUCTION Page 155 Fig 3.43 Plot of Korsemeyer-Peppas release kinetic of PYZ for optimized batch-3S 3.13 IN-VITRO ANTITUBERCULAR SCREENING OF DRUG LOADED NANOPARTICLES BY MICROPLATE ALAMAR BLUE ASSAY (MABA) The optimized batches (3S and 8N) of nanoparticles were evaluated for in vitro anti- tubercular screening by MABA at CDRI, Lucknow. The study confirmed that the formulated nanoparticles (8N and 3S) of different natural polymer possess antimycobacterial activity. The MIC 90 for chitosan and alginate-chitosan nanoparticles was found to be 50 (µg/ml) and 25 (µg/ml) respectively. 3.14 CELL (J774) UPTAKE STUDY OF LIGAND ATTACHED ALGINATE- CHITOSAN NANOPARTICLES OF OPTIMIZED BATCH The ligand (folic acid and D mannose) decorated alginate-chitosan nanoparticles were prepared due to its more stability in stomach as well as in intestinal pH. These nanoparticles were studied for phagocytosis by J774 cell line study. The study revealed alginate nanoparticles with folic acid (S2) as ligand exhibited best phagocytosis compared to S1 and S3.

CHAPTER 1 INTRODUCTION Page 156 Fig 3.44 Image of Cell line J-774 Fig 3.45 Image of internalization of rhodamine dye treated drug loaded nanoparticles by macrophage cell (J774) 3.15 CYTOTOXICITY STUDY The data of cytotoxicity activity of S1, S2, S3 nanoparticles upon independent exposure are summarized in Fig 3.46-3.48. A time and dose dependent decrease in percent cell viability was observed in all the cells used in the study. However, none of the concentration could induce

CHAPTER 1 INTRODUCTION Page 157 loss of cell viability upto statistically significant level in any cell type. HaCat, a normal human cell line was used as positive control against the human cancer cell lines of different origin viz., HepG2 (Hepatoma cells), A549 (Lung epithelium carcinoma cells) and primary cultures of peritoneal macrophages. But, no cell specific changes could be recorded and there was a non-significant loss of cell viability with more or less equal magnitude in all the cell types. Hence, the effect of chemicals could be speculated due to cytostatic responses, not due to cytotoxicity or any anti-cancer activity. Hence all three test compounds (S1, S2, S3) were found to be non-cytotoxic/ Non-anti-carcinogenic upto 100 µg/ml concentrations for a long term exposure i.e., till 96 h in human cancer cells of different origin as well as in primary cultures of peritoneal macrophages. Table 3.36 MTT assay for coded sample S1

CHAPTER 1 INTRODUCTION Page 158 Table 3.37 MTT assay for coded sample S2 S1 Conc(

µg/ml) 0.001 (µg/ml) 0.01 (µg/ml) 0.1 (µg/ml) 1 (µg/ml) 10 (µg/ml) 100 (µg/ml)

Cells HaCat 24h 115 ± 7 107 ± 9 103 ±5 101±3 97±5 94±6 HepG2 109 ±7 105±9 99 ±4 94 ±6 91±3 87±5 A549 106± 5 103 ±7 98 ±4 95 ±8 92±4 89±5 Macrophages 103±9 99±5 94±7 91±5 88±3 83 ±4 HaCat 48h 112±10 105±5 101± 7 98±4 95±4 91±3 HepG2 103±9 97±5 93±3 89±6 83±4 76±4 A549 99±7 94±4 89±6 84±4 79±3 73±5 Macrophages 98±6 93±4 87±7 82±5 77±4 71±5 HaCat 72h 109±5 102 ±7 97±6 94±3 91±7 89±4 HepG2 96±4 91±6 84±5 77±7 71±5 67±3 A549 93±4 88 ±5 83±3 79±5 74±6 69±3 Macrophages 91±4 85±6 79±7 74±4 68±4 63±6 HaCat 96h 101±10 96 ±6 91 ±6 86±5 81±4 78±5 HepG2 91±6 85±4 79±7 72±3 66±5 59±4 A549 88±6 83±4 77±7 71±4 63±5 56±3 Macrophages 84±7 79±4 72±3 65±5 58±4 71±6

CHAPTER 1 INTRODUCTION Page 159 Table 3.38 MTT assay for coded sample S3 S2 Conc(

0: 8b7b436f-85c8-4cb0-b31d-27103b0e0c61 100%

µg/ml) 0.001 (µg/ml) 0.01 (µg/ml) 0.1 (µg/ml) 1 (µg/ml) 10 (µg/ml) 100 (µg/ml)

Cells HaCat 24h 109±10 106±7 102±5 98±4 95±6 91±4 HepG2 107±5 103±7 97±4 91±6 87±4 83 ±5 A549 102±6 99±4 94±7 88±5 83±2 78±4 Macrophages 101± 8 96±5 91±7 87±4 85±5 81±3 HaCat 48h 107±6 102±4 97±7 93±3 90±6 88±4 HepG2 101±8 95±5 90±3 86±6 79±5 72±4 A549 95±5 91±7 87±3 81±4 77±3 71±5 Macrophages 95±4 91±6 85±4 79±7 74±4 68±5 HaCat 72h 104 ±7 98±4 93±6 89±6 85±4 82±5 HepG2 92±5 86±3 81±6 74±4 69±6 63±4 A549 91±8 86±5 81±7 75 ±4 69±6 63±4 Macrophages 88±8 83±6 76±4 71±5 65±2 61±3 HaCat 96h 99±7 95±3 91±6 87±4 83±3 76±5 HepG2 88 ±4 83±6 76±7 68±3 62±5 57±4 A549 86±6 81±3 75±5 68±7 61±4 54±5 Macrophages 81±7 74±4 68±6 61±2 54±5 48±3

CHAPTER 1 INTRODUCTION Page 160 S3 Conc.

0: 8b7b436f-85c8-4cb0-b31d-27103b0e0c61 100%

µg/ml 0.001 (µg/ml) 0.01 (µg/ml) 0.1 (µg/ml) 1 (µg/ml) 10 (µg/ml) 100 (µg/ml)

Cells HaCat 24h 118±13 113±7 107±9 103±5 101±6 97±4 HepG2 114 ±9 109±7 105±5 99±6 94±4 90±5 A549 111±11 107±9 102±7 98±4 94±6 91±5 Macrophages 107±7 103±5 97±8 93±6 90±5 86±4 HaCat 48h 115±9 108±6 104±5 101±7 97±5 93±4 HepG2 108±8 102±5 97±7 92±5 87±3 81 ±4 A549 106±7 101±5 94±4 89±6 82±4 78±5 Macrophages 102±6 97±4 91±6 86±3 82±4 76±5 HaCat 72h 111±6 106±4 102±8 98±5 95±3 91±5 HepG2 103±6 96±4 89±7 82±5 76±4 71±3 A549 102±8 94±4 87±7 81±5 77±6 71±4 Macrophages 97±5 91±4 86±6 79±5 72±6 67±4 HaCat 96h 105±5 101±7 96±4 91±3 86 ±5 81±3 HepG2 98±4 91±6 86±5 79±7 71±3 66±5 A549 96±4 88±6 81 ±5 77±7 69±5 61±4 Macrophages 92±4 86±6 78±3 71±6 66±4 58±5

CHAPTER 1 INTRODUCTION Page 161 Fig 3.46 Cytotoxicity study for sample S1 Fig 3.47 Cytotoxicity study for sample S2

CHAPTER 1 INTRODUCTION Page 162 Fig 3.48 Cytotoxicity study for sample S3 3.16 STABILITY STUDIES Pyrazinamide and Isoniazid loaded (optimized batch) alginate-chitosan nanoparticles were stored in screw capped glass containers and estimated for drug content at pre-determined intervals. The results concluded that formulations were stable for 6 months at 25

o C±2 o C / 60± 5% RH, 30 o C±2 o C / 65 ± 5% RH, 40 o C±2 o C / 75± 5% RH, 5 o C±3 o C. The formulation was found to be more stable at 5 o C±3 o C and least at higher temperature. Table 3.39 Stability data for optimized batch (3S) of sodium alginate nanoparticle Storage Condition 25 o C±2 o C / 60 ± 5% RH 30 o C±2 o C / 65 ± 5% RH 40 o C±2 o C / 75± 5% RH 5 o C ± 3 o C Time 1 month INH 98.23 ± 0.16 97.34 ± 0.23 96.56 ± 0.25 98.89 ± 0.12 PYZ 98.45 ± 0.23 98.01 ± 0.32 96.89 ± 0.67 98.65 ± 0.30 2 month INH 97.97 ± 0.96 97.02 ± 0.58 95.15 ± 0.86 98.72 ± 0.45 PYZ 98.03 ± 1.42 97.45 ± 0.79 95.94 ± 0.54 98.33 ± 0.18

CHAPTER 1 INTRODUCTION Page 163 3 month INH 97.34 ± 0.70 96.45 ± 0.69 94.43 ± 0.61 97.46 ±1.23 PYZ 97.67 ± 1.03 97.23 ± 1.16 95.29 ± 0.63 97.78 ± 0.67 4 month INH 96.98 ± 0.28 96.20 ± 0.79 94.21 ± 0.38 97.12 ± 0.54 PYZ 97.12 ± 0.55 96.75 ± 0.92 95.02 ± 0.56 97.63 ± 0.87 5 month INH 96.45 ± 1.01 95.98 ± 0.37 93.89 ± 0.64 97.09 ± 0.43 PYZ 96.78 ± 0.56 96.06 ±1.13 94.74 ± 0.87 97.13 ± 0.37 6 month INH 96.08 ± 0.76 95.16 ±1.08 93.50 ± 0.55 96.92 ± 0.56 PYZ 96.28 ± 0.80 95.60 ± 0.19 94.12 ± 0.39 96.87 ± 0.54 All the data shown are the mean ± SD (n=3). Fig 3.49 Effect of degradation on drug content of alginate nanoparticle of optimized batch-3S at 5 o C±3 o C

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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		CS/ALG nanoparticles using a very simple ionotropic pre-gelation technique, strong electrostatic interactions exist in the nanoparticles. The nanoparticles with a diameter of 20-50 nm	

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The release profile was characterized by an initial burst effect in phosphate buffer solution, followed by a continuous and controlled release.		The release profile was characterized by an initial burst effect in three media, followed by a continuous and controlled release	

18	52%	18: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3614711/ 52%

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

simulated intestinal fluid (SIF, pH6.8) and phosphate buffer solution (pH7.4), while the release was slow in simulated gastric fluid (SGF, pH1.5).

The release profile was characterized by an initial burst effect in three media, followed by a continuous and controlled release

phase, the drug release

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have gained more attention as drug delivery carriers because of their better stability,low toxicity,simple and mild preparation method and providing versatile routes of administration.

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spaced 15 days apart resulted in complete bacterial clearance from organs compared to 45 conventional doses of orally administered free drugs.

spaced 15 days apart resulted in complete bacterial clearance from the organs, compared to 45 conventional doses of orally administered free drugs. 13

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were prepared by

a two-step procedure based on the ionotropic

pre-gelation of

poly-anion with calcium chloride followed by

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Alginate–chitosan nanoparticles were prepared in a two-step procedure based on the ionotropic gelation of polyanion with calcium chloride followed by

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39 86%

SEM. The images revealed that particles were having smooth surface and were spherical in shape.

39: https://docplayer.net/59659226-Preparation-of-sodium-alginate-nanoparticles-by-desolvation-technique-using-iso-propyl-alcohol-as-desolvating-agent.html 86%

SEM. The images revealed that the particles were having smooth texture and were spherical in shape.

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6 87%

linked D glucosamine (deacetylated unit) and N-acetyl-D-glucosamine (acetylated unit).

6: https://www.researchgate.net/publication/262643753_Alginatechitosan_nanoparticles_for_encapsulation_and_controlled_release_of_vitamin_B2 87%

linked D-glucosamine (deacetylated unit) and N-acetyl-D-glucosamine (acetylate unit)

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34 88%

Whenever a new dosage form is developed, it is necessary to ensure that

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Whenever a new solid dosage form is developed or produced, it is necessary to ensure that

35 69%

a function of test time „t "or C=f(t). Some analytical definitions of the C(t) are commonly used function like zero order, first order, Hixon Crowell, Higuchi,

35: https://www.researchgate.net/publication/51051231_Paraquat-loaded_alginatechitosan_nanoparticles_Preparation_characterization_and_soil_sorption_studies 69%

a function of the test time, t or Q=f(t). Some analytical definitions of the Q(t) function are commonly used, such as zero order, first order, Hixson-Crowell, Weibull, Higuchi,

Instances from: https://www.researchgate.net/publication/281181047_Preparation_and_Characterization_of_Novel_Drug-Inserted-Montmorillonite_Chitosan_Carriers_for_Ocular_Drug_Delivery

7 68%

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)

7: https://www.researchgate.net/publication/281181047_Preparation_and_Characterization_of_Novel_Drug-Inserted-Montmorillonite_Chitosan_Carriers_for_Ocular_Drug_Delivery 68%

the potential of montmorillonite as a sustained car- rier in the preparation of drug-loaded nanoparticles for prolonged ocular application. Nanopar- ticles were prepared by ionic gelation of chitosan with sodium tripolyphosphate (TPP).

Instances from: https://www.omicsonline.org/peer-reviewed/formulation-and-evaluation-of-betamethasone-sodium-phosphate-loaded-nanoparticles-for-ophthalmic-deliveryp-12576.html

10 58%

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

10: https://www.omicsonline.org/peer-reviewed/formulation-and-evaluation-of-betamethasone-sodium-phosphate-loaded-nanoparticles-for-ophthalmic-deliveryp-12576.html 58%

The effect of changing different formulation parameters (pH of chitosan solution, sodium alginate concentration, calcium chloride concentration, chitosan concentration, drug concentration and the addition of tween 80) on the physicochemical properties and in vitro release of the drug loaded nanoparticles was studied. The mean particle size ranged from 16.8 to 692 nm and

11 100%

an initial burst release of the drug followed by slow sustained release over 24, 48 or 72 hours depending on the formulation parameters.

11: https://www.omicsonline.org/peer-reviewed/formulation-and-evaluation-of-betamethasone-sodium-phosphate-loaded-nanoparticles-for-ophthalmic-deliveryp-12576.html 100%

an initial burst release of the drug followed by slow sustained release over 24, 48 or 72 hours depending on the formulation parameters.

Instances from: https://estudogeral.sib.uc.pt/bitstream/10316/5866/1/file71d15070522e4521a63d26e06428e79f.pdf

29 72%

Then, 2.2 ml of different concentration (0.05–0.09%, w/w) chitosan solution was added drop wise into the pregel over 60 min,

29: https://estudogeral.sib.uc.pt/bitstream/10316/5866/1/file71d15070522e4521a63d26e06428e79f.pdf 72%

Then, 25 ml of different concentration (0.05–0.09%, w/w) chitosan solution was added dropwise into the pre-gel over 120 min.

30 66%

to provide a colloidal dispersion with final pH of 4.7. Nanoparticles were held with an additional stirring for 60 min to improve curing (

30: https://estudogeral.sib.uc.pt/bitstream/10316/5866/1/file71d15070522e4521a63d26e06428e79f.pdf 66%

to provide a colloidal dispersion with final pH of 4.7, but further modified to provide a final pH of 4.2 and 5.2.

Nanoparticles were held with an addi- tional stirring for 30 min to improve curing,

32 76%

morphology such as shape, surface and occurrence of aggregation phenomenon was studied by

32: https://estudogeral.sib.uc.pt/bitstream/10316/5866/1/file71d15070522e4521a63d26e06428e79f.pdf 76%

morphology such as shape and occurrence of aggregation phenomena, was studied by

38 83%

38: https://estudogeral.sib.uc.pt/bitstream/10316/5866/1/file71d15070522e4521a63d26e06428e79f.pdf 83%

Nanoparticles were held with an additional stirring for 60 min. to improve curing.	Nanoparticles were held with an addi- tional stirring for 30 min to improve curing,

Instances from: https://www.researchgate.net/publication/236909856_Chitosan-Alginate_Nanoparticles_as_a_Novel_Drug_Delivery_System_for_Nifedipine

24 61%

new approach for preparation of nanoparticles. The method involved the controlled gelification phenomenon of alginate by calcium

24: https://www.researchgate.net/publication/236909856_Chitosan-Alginate_Nanoparticles_as_a_Novel_Drug_Delivery_System_for_Nifedipine 61%

new approach for the preparation of nanoparticles is presented. The method is based on control of the gelification phenomenon of alginate by calcium

25 91%

size of the particles are greatly dependent on the order of addition of calcium and poly-L-lysine to the sodium alginate solution

25: https://www.researchgate.net/publication/236909856_Chitosan-Alginate_Nanoparticles_as_a_Novel_Drug_Delivery_System_for_Nifedipine 91%

size of the particles formed is greatly dependent on the order of addition of calcium and poly-L-lysine to the sodium alginate solution.

26 63%

evaluation of drug loading capacity the doxorubicin was used

CHAPTER 1 INTRODUCTION Page 67 as model drug. The results indicated that alginate nanoparticles are interesting carriers

26: https://www.researchgate.net/publication/236909856_Chitosan-Alginate_Nanoparticles_as_a_Novel_Drug_Delivery_System_for_Nifedipine 63%

because the drug-loading capacity could be < 50 mg of doxorubicin per 100 mg of alginate.

Evaluation of the drug-loading capacity was done with doxorubicin as a drug model. The results indicate that alginate nanoparticles are interesting carriers because the drug-loading capacity could be < 50 mg of doxorubicin per 100 mg of alginate.

Instances from: https://www.researchgate.net/publication/315012217_Recent_Applications_of_Natural_Polymers_in_Nanodrug_Delivery

3 52%

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

3: https://www.researchgate.net/publication/315012217_Recent_Applications_of_Natural_Polymers_in_Nanodrug_Delivery 52%

Although the synthetic polymers display chemical

stability, their unsatisfactory biocompatibility still limits their potential

clinical applications [7,15,40]. Because the natural polymers always

show low/non toxicity, low immunogenicity and therea?er good

biocompatibility, they have been the preferred polymers in drug delivery

4 80%

is obtained by partial N- deacetylation of chitin found in shells of crustacean

4: https://www.researchgate.net/publication/315012217_Recent_Applications_of_Natural_Polymers_in_Nanodrug_Delivery 80%

is obtained from the partial N-deacetylation of chitin

found in the shells of crustacean.

5 83%

of glucosamine and N acetyl glucosamine linked by 1-4 glucosidic bonds. Chitosan is

5: https://www.researchgate.net/publication/315012217_Recent_Applications_of_Natural_Polymers_in_Nanodrug_Delivery 83%

of glucosamine and

N-acetyl glucosamine linked by β 1-4 glucosidic bonds and is

Instances from: https://www.researchgate.net/publication/329268575_Formulation_and_Evaluation_of_Betamethasone_Sodium_Phosphate_Loaded_Nanoparticles_for_Ophthalmic_Delivery

14 55%

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

14: https://www.researchgate.net/publication/329268575_Formulation_and_Evaluation_of_Betamethasone_Sodium_Phosphate_Loaded_Nanoparticles_for_Ophthalmic_Delivery 55%

Bovine serum albumin-loaded beads were prepared by ionotropic gelation of alginate with calcium chloride and chitosan. The effect of sodium alginate concentration and chitosan concentration on the particle size and loading efficacy

15 100%

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.

15: https://www.researchgate.net/publication/329268575_Formulation_and_Evaluation_of_Betamethasone_Sodium_Phosphate_Loaded_Nanoparticles_for_Ophthalmic_Delivery 100%

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.

17 81%

17: https://www.researchgate.net/publication/329268575_Formulation_and_Evaluation_of_Betamet

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

hasone_Sodium_Phosphate_Loaded_Nanoparticles_for_Ophthalmic_Delivery 81%

The effect of such parameters as polymer ratio, CaCl2/Alginate ratio and N/P ratio on ... [Show full abstract] the particle size distribution and loading efficacy

33 79%

In order to understand the kinetic and mechanism of drug release, the results of in-vitro drug release study of nanoparticles were fitted with various kinetic equations like zero order (Cumulative % release

vs. time), first order (log % drug remaining vs. time), Higuchi‟s model (cumulative % drug release vs. square root of time),

33: https://www.researchgate.net/publication/329268575_Formulation_and_Evaluation_of_Betamethasone_Sodium_Phosphate_Loaded_Nanoparticles_for_Ophthalmic_Delivery 79%

In order to understand the kinetics of drug release, the results of

in vitro drug release study of nanoparticles were ?tted with various

kinetic equations like zero (cumulative % drug release vs time), ?rst

order (log cumulative % drug remaining vs time), and Higuchi’s model

(cumulative % drug release vs. square root of time).

?

Instances from: https://www.ncbi.nlm.nih.gov/pubmed/16275050

19 100%

three model drugs (

insulin, diclofenac sodium, and salicylic acid) with different pI or pKa.

19: https://www.ncbi.nlm.nih.gov/pubmed/16275050 100%

Three model drugs (insulin, diclofenac sodium, and salicylic acid) with different pI or pKa

20 91%

The ionic interaction between drug and chitosan was low and too weak to control the drug release

20: https://www.ncbi.nlm.nih.gov/pubmed/16275050 91%

the ionic interaction between drug and chitosan is low and too weak to control the drug release.

21 100%

high burst release of drugs from chitosan micro/nanoparticles was observed regardless of the pH of dissolution media.

21: https://www.ncbi.nlm.nih.gov/pubmed/16275050 100%

high burst release of drugs from chitosan micro/nanoparticles was observed regardless of the pH of dissolution media.

Instances from: cc93617b-3aa3-48e7-ac25-e1826baa6caf

37 64%

the

pre-gel formation. 2) Further 12 ml of 0.05% chitosan solution was added drop wise into the pre-gel

37: cc93617b-3aa3-48e7-ac25-e1826baa6caf 64%

the alginate pre gel. Then different concentrations of 25 mL of chitosan solution was added drop wise to the pre gel

Instances from: cae93491-f71a-494c-9148-3756d71079f3

31	58%

Particle size, zeta potential and Poly-dispersity index The particle size and zeta potential of nanoparticles were

31: cae93491-f71a-494c-9148-3756d71079f3	58%

Particle Size, Zeta Potential and Morphology The particle size, size distribution and zeta potential of the fabricated chitosan nanoparticles were

Instances from: 63da9acc-e2d7-472b-b0bc-c9c142a65146

36	100%
To study the release kinetics, data obtained from in-vitro	

36: 63da9acc-e2d7-472b-b0bc-c9c142a65146	100%
To study the release kinetics, data obtained from in vitro	

Instances from: 8b7b436f-85c8-4cb0-b31d-27103b0e0c61

1 75%	1: 8b7b436f-85c8-4cb0-b31d-27103b0e0c61 75%
Preparation of nanoparticles The choice of appropriate method for manufacturing of nanoparticles depends on	preparation of nanoparticles. The selection of appropriate method for the preparation of nanoparticles depends on
2 95%	2: 8b7b436f-85c8-4cb0-b31d-27103b0e0c61 95%
are matrix system in which drug is physically and uniformly dispersed (	are matrix system in which the drug is physically and uniformly dispersed.
40 100%	40: 8b7b436f-85c8-4cb0-b31d-27103b0e0c61 100%
µg/ml) 0.001 (µg/ml) 0.01 (µg/ml) 0.1 (µg/ml) 1 (µg/ml) 10 (µg/ml) 100 (µg/ml)	µg/ml 30 µg/ml 20 µg/ml 10 µg/ml 5 µg/ml 40 µg/ml 30 µg/ml 20
41 100%	41: 8b7b436f-85c8-4cb0-b31d-27103b0e0c61 100%
µg/ml) 0.001 (µg/ml) 0.01 (µg/ml) 0.1 (µg/ml) 1 (µg/ml) 10 (µg/ml) 100 (µg/ml)	µg/ml 30 µg/ml 20 µg/ml 10 µg/ml 5 µg/ml 40 µg/ml 30 µg/ml 20
42 100%	42: 8b7b436f-85c8-4cb0-b31d-27103b0e0c61 100%
µg/ml 0.001 (µg/ml) 0.01 (µg/ml) 0.1 (µg/ml) 1 (µg/ml) 10 (µg/ml) 100 (µg/ml)	µg/ml 30 µg/ml 20 µg/ml 10 µg/ml 5 µg/ml 40 µg/ml 30 µg/ml 20

Instances from: 2a865b68-839c-4b51-bf2a-b0f8fb6dcb97

27	50%
calibration curve of Pyrazinamide A standard stock solution of the pure drug (Pyrazinamide) was prepared by dissolving 10mg of drug in 100ml	

27: 2a865b68-839c-4b51-bf2a-b0f8fb6dcb97	50%
Calibration Curve of Cimetidine A stock solution of 100 mcg/ml of Cimetidine was prepared in phosphate buffer pH 7.4 by dissolving 10 mg of drug in 100ml	

Instances from: 6a904f44-8825-4be8-b745-0d7d6c8e7a2c

16	89%	16: 6a904f44-8825-4be8-b745-0d7d6c8e7a2c	89%
In vitro release data showed an initial burst followed by slow sustained drug release.		In vitro release data of optimized formulation showed an initial burst followed by slow sustained drug release.	