CHITOSAN NANOCOMPOSITE OF METOPROLOL SUCCINATE: ASSESSMENT & ITS EVALUATION FOR THE PREVENTION OF HYPERTENSION IN THE MORNING

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I further certify that neither this dissertation nor any part of it has been submitted to any other University or Institute for award of any degree or diploma. I am pleased to forward this dissertation for evaluation.

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DECLARATION OF ORIGINALITY AND COMPLIANCE OF ACADEMIC ETHICS

I hereby declare that the dissertation entitled "***CHITOSAN NANOCOMPOSITE OF METOPROLOL SUCCINATE: ASSESSMENT & ITS EVALUATION FOR THE PREVENTION OF HYPERTENSION IN THE MORNING***" is a bonafide and genuine research work carried out by me under the supervision of Dr. Ketousetuo Kuotsu, Professor, Department of Pharmaceutical Technology, Jadavpur University. All information in this document have been obtained and presented in accordance with the academic rules & ethical conduct. I also declare that as required by these rules and conduct; I have fully cited and referenced all materials & results that are not original to this work.

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PREFACE

The dissertation is performed for the partial requirement of the degree of Master of Pharmacy. The present research work entitled "**CHITOSAN NANOCOMPOSITE OF METOPROLOL SUCCINATE: ASSESSMENT & ITS EVALUATION FOR THE PREVENTION OF HYPERTENSION IN THE MORNING**" was designed to improve the bioavailability of Metoprolol Succinate by employing Ionic Gelation method.

Metoprolol Succinate is a beta-blocker medication used primarily to treat high blood pressure, angina (chest pain), and heart failure. It works by blocking beta-adrenergic receptors in the heart, which reduces heart rate, decreases blood pressure, and lowers the heart's demand for oxygen. This helps prevent conditions like heart attacks and strokes. Metoprolol Succinate belongs to BCS Class I which means it has high solubility and high permeability. The objective of the study is to improve the bioavailability of Metoprolol Succinate.

The Ionic Gelation Method is a widely used technique for the preparation of polymeric nanoparticles. In this process, the polymer is dissolved in an aqueous solution and then crosslinked with counter-ion or polyanion to form nanoparticles. The method is favoured for its simplicity, mild conditions, and ability to produce stable nanoparticles without the need for harsh chemicals or high temperatures. Ionic Gelation allows for efficient encapsulation of active ingredients, enabling controlled release and improved bioavailability.

This thesis is divided into NINE chapters describing fundamentals, methodologies, results, discussion, conclusion & reference. Chapter 1 is the introductory chapter which deals with nanoparticle drug delivery system, their types, preparation and applications. Chapter 2 describes aims and objectives of the research work. Chapter 3 is about literature review on various other works that had been done on this regard. Chapter 4 describes the materials & methodologies used in this research work. Chapter 5 contains the tables and graphs whereas Chapter 6 discusses the result obtained through various evaluations. Chapter 7 contains the conclusion section which gives a brief outline of results that obtained from the entire work. Chapter 8 includes the future scope about this research work. Chapter 9 consists of list of references that are being used to successfully complete the thesis work.

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INTRODUCTION

1.1 NANOPARTCLES

In numerous fields of applications, nanoparticles (NPs) and nanostructured materials (NSMs) are a rapidly expanding techno-economic sector and an active research subject. Nanotechnology is the emerging science that deals with nanometers scale and nanoparticles are one of the building blocks in nanotechnology. Recently from last few years, nanotechnology and polymers together have captivated a tremendous interest in many areas including pharmaceutical industry and therapeutic innovation among others.[1]The term nanomaterial is described as "a manufactured or natural material that possesses unbound, aggregated or agglomerated particles where external dimensions are between 1–100 nm size range", according to the EU Commission.[2]The British Standards Institution proposed the following definitions for the scientific terms that have been used:

- Nanoscale: Approximately 1 to 1000 nm size range.
- Nanoscience: The science and study of matter at the nanoscale that deals with understanding their size and structure-dependent properties and compares the emergence of individual atoms or molecules or bulk material related differences.
- Nanotechnology: Manipulation and control of matter on the nanoscale dimension by using scientific knowledge of various industrial and biomedical applications.
- Nanomaterial: Material with any internal or external structures on the nanoscale dimension.
- Nano-object: Material that possesses one or more peripheral nanoscale dimensions.
- Nanoparticle: Nano-object with three external nanoscale dimensions. The terms nanorod or nanoplate are employed, instead of nanoparticle (NP) when the longest and the shortest axes lengths of a Nano-object are different.
- Nanofiber: When two similar exterior nanoscale dimensions and a third larger dimension are present in a nanomaterial, it is referred to as nanofiber.
- Nanocomposite: Multiphase structure with at least one phase on the nanoscale dimension.
- Nanostructure: Composition of interconnected constituent parts in the nanoscale region.
- Nanostructured materials: Materials containing internal or surface nanostructure.[1]

Nano particles size ranges from 1 to 100nm which are made up of metal, metal oxides, organic matter, carbon. Nanoparticles vary in size, shape, and composition depending on the material they are made of. Due to their small size and large surface area, they exhibit unique physical and chemical properties. Nanoparticles can be prepared both from natural polymers such as

protein, polysaccharide or synthetic polymer such polystyrene. Heat, an organic solvent, or strong shear force are used in the creation of synthetic polymer nanoparticles, which may compromise the stability of the medicine. On the other hand, natural polymer-based nanoparticles provide gentle and straightforward preparation techniques without requiring strong shear forces or organic solvents.[3]

1.2. HISTORY AND DEVELOPMENT OF NANOMATERIALS

More than 4,500 years ago, humans already took advantage of the reinforcement that natural asbestos nanofibers might provide for ceramic Matrixes.[4] More than 4,000 years ago, the Ancient Egyptians used NMs in hair colour, based on a synthetic chemical procedure to create PbS NPs with a diameter of about 5 nm.[5] Similarly, in the third century BC, Egyptians created and employed "Egyptian blue," the first synthetic pigment, by melting a mixture of quartz and glass that was as small as a nanometer.[6] Egyptian blue is a complex combination of SiO_2 and $CaCuSi_4O_{10}$ (both in glass and quartz). Archaeological research has revealed the widespread usage of Egyptian blue as a decorative material in several ancient Roman Empire countries, including Egypt, Mesopotamia, and Greece. The metallic nanoparticle era began when Egyptians and Mesopotamians began employing metals to make glass in the 14th and 13th century BC, which was when the first metallic nanoparticles were synthesized chemically.[7] These substances might be the first synthetic NMs used in a useful application. Red glass tinted by surface plasmon excitation of Cu NPs has been discovered at Frattesina di Rovigo (Italy) and dates back to the late Bronze Age (1200–1000 BC).[8] Similarly, it has been observed that Cu NPs and cuprous oxide (cuprite Cu_2O) are present in Celtic red enamels that date back to the 400–100 BC period.[9] Yet the most well-known instance of the use of metallic NPs in antiquity is found in a Roman glass workpiece. The Lycurgus Cups are a type of dichroic glass from the fourth century that changes colour depending on the direction of light. It shows green when light is coming from the front and red when light is coming from the back. According to recent research, the Lycurgus Cups also have roughly 10% Cu and Ag–Au alloy NPs at a ratio of 7:3.[10]

Later, colloidal Au and Ag NPs were added to create the red and yellow stained glass found in churches built during the Middle Ages. Mesopotamians began decorating with metallic shine on glazed ceramics in the ninth century. Because of the unique Ag and/or Cu NPs that were separated inside the outermost glaze layers, these decorations displayed incredible optical

qualities. These ornaments are an example of metal nanoparticles that, in specific reflection settings, exhibit iridescent vivid green and blue colours. A double layer of Ag NPs (5–10 nm) in the outer layer and larger ones (5–20 nm) in the inner layer was discovered by TEM investigation of these ceramics. Interference effects resulted from the continuous distance between the two layers, which was measured to be around 430 nm. Because of the first layer's light scattering, the phase shift is caused by the dispersed light from the second layer. When scattering, this incoming light wavelength-dependent phase shift results in a new wavelength. Furthermore, the best examples of natural NM utilization since antiquity are clay minerals with a thickness of a few nanometers. According to reports, wool and clothing in Cyprus were washed with clay as early as 5000 BC.[11] The synthesis of a colloidal Au NP solution was described by Michael Faraday in 1857, marking the beginning of the history of NMs in science and the first scientific account of NP manufacture. Additionally, he disclosed that Au colloids' optical properties differ from those of their corresponding bulk counterparts. This was most likely one of the first reports to describe and observe quantum scale effects.[12] Subsequently, Mie (1908) provided an explanation for why metal colloids have certain colours. SiO_2 nanoparticles were produced in the 1940s to replace carbon black in rubber reinforcing.

Even with all of the additional potential advantages, one of the main uses for NPs is still to enhance materials' appearance by utilizing their advantageous size and shape effects. Furthermore, the bulk application of passive NMs embedded in an inert (polymer or cement) matrix to form a nanocomposite is frequently the only way that NMs are used commercially. Samsung launched Silver NanoTM, an antibacterial technology that uses ionic Ag NPs, in 2003 for their vacuum cleaners, air conditioners, refrigerators, washing machines, and air purifiers. In the construction of automobiles, NPs and NSMs are widely utilized as fillers in tires to increase traction on the road, as fillers in the car body to increase rigidity, and as clear layers for heated, mist- and ice-free window panes. Mercedes-Benz introduced an NP-based clear coat for metallic and non-metallic paint finishes into series production by the end of 2003. The coating improves shine and boosts resistance to scratches. Ferrofluids, often known as liquid magnets, are ultra-stable suspensions of tiny magnetic nanoparticles that possess superparamagnetic characteristics. The liquid will macroscopically magnetize when a magnetic field is applied, which causes the NPs to align along the direction of the magnetic field.[13] Enhancing Earth-based astronomical telescopes using adaptive optics and magnetic mirrors composed of ferrofluids that may change form have been the subject of recent research. Commercially available TiO_2 NPs have the capacity to sensitize dyes in solar cells.[14] When

Logitech released an external iPad keyboard that runs on light in the summer of 2012, it was the first significant commercial application of dye-sensitized solar cells. The human serum albumin NP substance AbraxaneTM containing paclitaxel was produced, marketed, and introduced to the pharmaceutical industry in 2005. Approximately 1814 consumer goods based on nanotechnology were offered for sale in more than 20 countries in 2014.[15]

Nanoparticles are commonly employed as transport agents for *in vivo* medication and gene delivery as well as contrast agents for medical imaging applications. Nanoparticles can penetrate the body and reach a particular tissue more directly and efficiently because of their small size. Moreover, nanoparticles can transfer molecules—like those found in medications—faster and with less discomfort in order to identify and treat illnesses. Numerous parameters, including size, hydrophilicity, conjugation power with different medications, biodegradability, biocompatibility, and hydrophilicity, must be taken into account before using nanomaterials in the treatment of disease. Over the last few years chitosan nanoparticles, have gained considerable attention in present scenario due to their inherent biological properties.

1.3 MERITS & LIMITATIONS OF NANOPARTICLES

1.3.1. Merits of Nanoparticles

(a) Ease of modifying nanoparticle surface properties and particle size to target drugs both passively and actively after parenteral administration.[16]

(b) Using nanosized quantum dots based on immunofluorescence to label particular bacteria, which makes it easier to identify and get rid of them.

(c) Nanotechnology is a growing field in many industries, including aquaculture, and it has numerous applications in areas like nutrition.

(d) Reproduction, water purification, fishing, and disease control as well as the reduction of toxicity and negative effects.

(e) The preparation of nanoparticles using biodegradable materials enables sustained drug release at the target site over the course of days or even weeks.[3]

(f) Nanoparticles are so small, they easily pass through tiny capillaries and are absorbed by cells, enabling effective drug accumulation at the body's target sites.[17]

(g) Nanotechnology can make fabrics more durable because NPs have a high surface energy and a large surface area to volume ratio.

(h) Nano supplements can be easily added using the encapsulation technique for effective drug and nutritional delivery.

(i) Nano barcodes are used to label food products for safety and to track their distribution.[18]

1.3.2. Limitations of Nanoparticles

(a) The cellular environment is extremely reactive to nanoparticles due to their small size and large surface area.

(b) Non-biodegradable particles may build up at the medication delivery site when they are utilized, which could result in a persistent inflammatory reaction.

(c) The therapy cannot be stopped because of the restricted targeting capabilities of nanoparticles.

(d) The development of nanotechnology can incur much higher costs.[16]

1.4. SELECTION OF POLYMER:

Polymers, building blocks of nanoparticles belong to natural and synthetic origins. The selection of matrix materials depends on many factors including:

(a) Size of nanoparticles required.

(b) Inherent properties of the drug, e.g., aqueous solubility and stability.

(c) Surface characteristics such as charge and permeability.

(d) Degree of biodegradability, biocompatibility and toxicity.

(e) Drug release profile desired.

(f) Antigenicity of the final product.

1.5. NEED FOR NANOPARTICLES BASED DRUG FORMULATION

The use of nanoparticles as therapeutic and diagnostic agents, as well as to improve medication delivery, is crucial and urgent for a number of reasons. One of them is that conventional medications that are now marketed for injection or oral use aren't always produced in the best possible formulation for each product. More creative carrier systems are needed for products comprising proteins or nucleic acids in order to increase their effectiveness and shield them from unintended destruction.[19]Notably, particle size directly affects the majority of drug delivery modalities' efficiency (with the exception of intravenous and solution). Drug

nanoparticles exhibit greater bioavailability, increased solubility, and the capacity to penetrate the blood-brain barrier (BBB), enter the pulmonary system, and be absorbed through the tight connections of skin endothelial cells because of their tiny size and huge surface area.[20] Particularly, natural and synthetic polymer-based nanoparticles, both biodegradable and non-biodegradable, have drawn increased interest due to their ability to be tailored for drug delivery, enhance bioavailability, and offer a regulated release of medication from a single dose; by means of adaptation, the system can stop endogenous enzymes from breaking down the drug.[21]

Moreover, the creation of novel drug delivery methods is giving pharmaceutical companies still another reason to expand. Pharmaceutical companies are developing new formulations of their existing medications due to innovative drug delivery. Although the patients will benefit from these novel formulations, they will also generate a strong market force that will propel the creation of ever more efficient delivery systems.[22]

Application of Nanoparticle Technology:

(a) Drug Delivery Systems: **(i)** ***Targeted Delivery***: Ligands that bind only to receptors on diseased cells can be used to functionalize nanoparticles. This ensures that medicines are precisely delivered to malignant or infected tissues, reducing systemic toxicity.

(ii) ***Controlled Release*****:** Drugs encapsulated in nanoparticles allow for prolonged release profiles that sustain therapeutic levels for long stretches of time, lowering dosage frequency and enhancing patient adherence.

(iii) Breaking through Biological Barriers**:** Bypassing physiological barriers like the blood-brain barrier, nanoparticles can improve the way that problems of the central nervous system are treated.

(b) Cancer Therapy: (i) ***Enhancement of Chemotherapy*****:** with the direct delivery of chemotherapeutic medicines to tumour areas, nanocarriers can increase drug concentration in cancer cells while protecting healthy tissues.

(ii) Photothermal and Photodynamic Therapy**:** By converting light into heat or reactive oxygen species, gold nanoparticles and other photoreactive nanomaterials can specifically kill cancer cells.

(iii) Gene therapy**:** Using siRNA or CRISPR/Cas9 components, nanoparticles can alter the expression of certain genes in cancer cells, providing a means of oncogene silencing or gene repair.

(c) Medical Imaging: (i) *Contrast Agents*: Gold nanoparticles improve contrast in computed tomography (CT) images, while magnetic nanoparticles improve contrast in magnetic resonance imaging (MRI).

***(ii) Fluorescent Markers*:** Real-time, high-resolution imaging of biological tissues is made possible by quantum dots and other fluorescent nanoparticles, which help in the early detection and monitoring of disease.

(d) Antimicrobial Applications: (i) *Antibacterial Coatings*: To stop biofilm formation and hospital-acquired illnesses, silver nanoparticles are added to coatings for medical equipment. **(ii) *Wound Healing*:** Antimicrobial drugs are delivered to wound sites using nanoparticle formulations, which encourage healing and lower the risk of infection.[23]

1.6. POLYMER

Chitosan

Chitosan is a fibre or polysaccharide that is made when chitin is N-deacetylated. A biopolymer found naturally in the shells of crustaceans, including shrimp, lobsters, and crabs, is called chitin.[24] Moreover, microbes like yeast and fungi contain chitosan. Chitosan's molecular makeup is made up of N-acetyl-glucosamine and glucosamine units: The degree of deacetylation (DD) determines the units' repeatability. Chitosan is insoluble at neutral pH values due to its equilibrium acid association constant (pKa) of approximately 6.5 on the amine groups. It becomes soluble at acidic pH values below 6.5, when the chitosan molecule has a positive charge.[25] Biological targets of interest are molecules that, despite being less soluble in their unionized state, are more readily able to cross lipophilic barriers between them. The presence of amine groups in the protonatable molecular structure of chitosan is directly influenced by the degree of deacetylation. However, some inorganic acids, such as sulfuric and phosphoric acids, insoluble in chitosan. There is a wide range of molecular weights and deacetylation levels available for chitosan. When it comes to the synthesis of chitosan nanoparticles, the degree of deacetylation and molecular weight directly affect the size, form, and degree of aggregation of the particles in solution.[26]

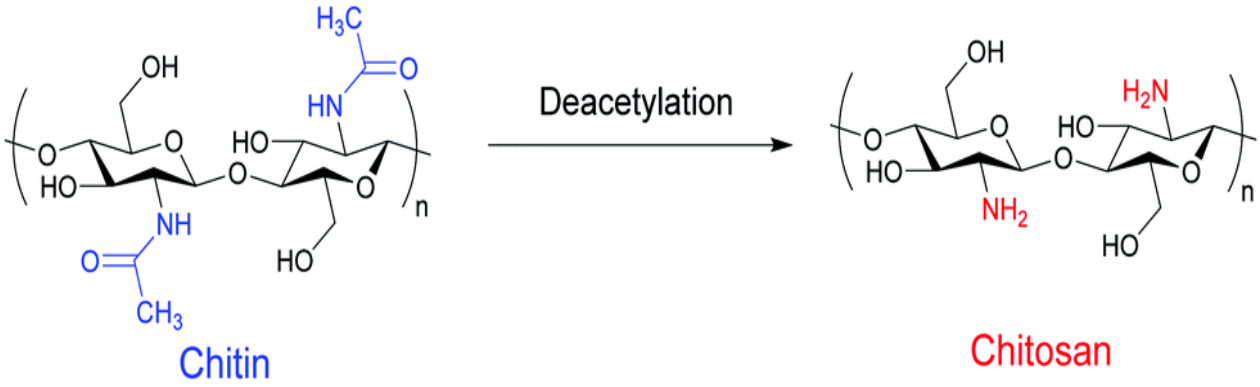


Fig.1.6.1 Conversion of Chitin into Chitosan

It is possible to create chitosan nanoparticles (CNPs) with the desired Nano-scale properties, like small size, specific surface and interface effects, and quantum size effects. Chitosan is inexpensive and widely accessible, making it useful in a variety of applications. In particular, chitosan offers a wide range of medical uses, such as efficient drug delivery systems and wound dressings that speed up wound healing. It is utilized in food processing as an ingredient and food-coating material and in agriculture for the manufacturing of fertilizer.

Physicochemical, Biological and Pharmacological Properties of Chitosan		
Physical Properties:	Particle Size	30mm
	Density	1.35-1.40 g/cc
	pH	6.5-7.5
	Solubility	Insoluble in water but soluble in acids
Chemical properties		
○ Cationic Polyamine		
○ High charge density at pH 6.6		
○ Adheres to negatively charge surfaces		
○ Forms gels with polyanions		
○ High molecular weight, linear polyelectrolyte		
○ Viscosity- high to low chelates certain transitional metals		
○ Reactive hydroxyl/ amino group		
Biological Properties		
○ Nontoxicity		
○ Biocompatibility		
○ Biodegradability		
Pharmacological Properties		
○ Hypocholesterolemic action		
○ Wound-healing properties		
○ Antacid and antiulcer activity		
○ Antifungal and antibacterial activity		

Table 1. Properties of chitosan

1.6.1. Chemistry of Chitosan

Three reactive or functional groups make up chitosan: two hydroxyl groups at C3-OH and C6-OH, and an amino group (-NH2) at C2-NH2. Compared to the hydroxyl group at C3-OH, the

one at C6-OH has greater chemical activity. Another way to think of the glycosidic bond is as a functional group that permits chemical alterations to create a polymer with unique characteristics.[27] Chemical alteration at the amino group yields N-modified chitosan derivatives with the use of appropriate chemicals, whereas chemical modification at the hydroxyl groups yields O-modified chitosan derivatives, which offer enhanced physicochemical qualities.[28] When N-cinnamyl replacing O-amine group is added to chitosan, it becomes significantly more hydrophobic and effective against Staphylococcus aureus, Bacillus cereus, Escherichia coli, and Pseudomonas aeruginosa.[29] A chitosan-based glycol polymer that modifies C6 N-quaternary ammonium-O-sulfobetaine is soluble in watery solutions and binds to lectins with good affinity.[30] Furthermore, it has been demonstrated that this modified chitosan possesses antibacterial action. It has been demonstrated that O-acylated chitosan nanofibers containing fatty acid and anhydride side groups exhibit variable levels of hydrophobic and hydrophilic values, which are closely correlated with the length of the substituted acyl group's chain. When used as a coagulant and flocculant agent, chitosan quaternary ammonium salt effectively combats Microcystis aeruginosa cyanobacteria. When coupled with Fe3O4 nanoparticles, the quaternary ammonium ions of chitosan can function as a bio adsorbent for methyl orange and chromium (VI). Similarly, organ transplantation and the restoration of organ function can benefit from a hydrogel made from chitosan cross-linked with glutaraldehyde, and glyoxal.[31] Targeted radiation Therapy has been applied to Ho-166, Sm-153, and Lu-166 radionuclides that have been cross-linked with chitosan.[32,33]

1.6.2. Properties of chitosan in a drug delivery system:

- Anionic drug delivery properties
- Mucoadhesive properties
- Permeation enhancing properties
- Bio adhesive

(a)Anionic drug delivery properties:

When a simple drug dissolution technique, like diffusion, membrane layer with hand grip erosion, or osmotic, is unable to accomplish a drug release procedure, ionic correlation-mediated retardation is frequently employed. The latter method can be applied to cationic medications by employing anionic polymeric ingredients, such as carboxyl methyl cellulose, alginate, or poly acrylates. Chitosan, on the other hand, is the exclusive choice for anionic

delivery systems. To determine the medication release mechanism for the anionic drug naproxen, chitosan was employed as a medication contributor matrix. It was discovered that there was a more noticeable interaction between the therapeutic agent and chitosan, and that stable complexes, which are used to manufacture the medication, could also form and last longer as measured by ionic cross-linking. For instance, the enoxaparin/chitosan Nano particle delivery method produced more stable complexes and greatly enhanced drug absorption. Chitosan can be used to homogenize a variety of anionic polymeric excipients, including carrageenan, pectin, and polyacrylates, leading to high-density, rather stable complexes. On the other hand, homogenizing chitosan with an alternative to inorganic polymer anions and multivalent anionic anions, like sulphate or tri polyphosphate, can yield a similar outcome.

(b)Mucoadhesive properties:

Its cationic nature may be the cause of the chitosan. Hydrophobic interactions with the mucoadhesive components may also provide support. When chitosan is combined with different anionic polymeric excipients, such as carbomer and hyaluronic acid, its mucoadhesive qualities become weaker. Since adhesive bonds within mucoadhesive polymers typically fail due to their incompatibility with integrating both the polymer and the mucous gel layer, a polymer with strong cohesive characteristics is required to achieve significant mucoadhesive qualities. These cohesive qualities swing to being rather weak in the case of chitosan. It can be improved through the creation of complexes containing multivalent inorganic anions, multivalent anionic polymeric components, and multivalent anionic medicinal therapies. This method is only partially effective because it blocks the cationic raft foundation of chitosan, which is what allows cohesion over ionic interactions when employing the mucous. shown that buserelin has a markedly improved oral bioavailability. However, when chitosan and polyanionic carbomer were combined in the same formulation, this particular effect could no longer be obtained. The trimethylation of chitosan's main amino group gives the polymer a more cationic temper.

(c)Permeation enhancing properties:

Based on chitosan's favourable effects, it was discovered that these effects are responsible for the permeation enhancer mechanism. This mechanism can interact with chitosan's cell membrane to produce a structural establishment of proteins linked to tight junctions. Thetrimethylation strategy did not lead to the further development of permeation-enhancing characteristics by a main amino group that conducts to a more pronounced cationic character.

It was shown that the toxicity and enhanced permeability were relevant to the structural characteristics of chitosan, taking into account the molecular mass and degree of DE acetylation. The increased epithelium penetrability of chitosan with high molecular mass and high degree of deacetylation may be attributed to the molecular mass and other permeation-enhancing polymers such as polyacrylates.

(d)Bio adhesiveness:

The chitosan molecules amino and carboxyl groups can combine with the glycoprotein in mucus to create a hydrogen bond, which is necessary for an adhesive action. Because mucoprotein in mucus has a positive charge, chitosan and mucus bind to one another to increase drug bioavailability, extend the duration of drug retention, and promote continuous drug release *in vivo.*[34]

1.7. CHITOSAN NANOPARTICLES

Polymer nanoparticles have received a lot of attention lately as a possible medication delivery vehicle. Because of their excellent biocompatibility, biodegradability, and unique drug release behaviour, biodegradable polymers like chitosan (CS) have been the subject of significant research. Chitosan nanoparticles have the potential to be used as gene, vaccine, and anticancer agent delivery vehicles. According to reports, chitosan nanoparticles have a small particle size and a high zeta potential. Chitosan is a polysaccharide that shares structural similarities with cellulose. Both are composed of linear monosaccharides connected to h-(1Y4). Chitosan differs significantly from cellulose in that it is made up of 2-amino-2-deoxy-h-d-glucan coupled with glycosidic bonds. Chitosan has unique qualities that make it a valuable ingredient in pharmaceutical applications because of the primary amine groups. Chitosan is more mucoadhesive and has a positive charge than a lot of other natural polymers. As a result, it finds widespread use in applications related to drug delivery. The process of deacetylating chitin, a biocompatible polymer that is abundantly accessible in marine crustaceans, yields chitosan. However, because chitin is chemically inert and physically comparable to cellulose, its applications are restricted when compared to chitosan. Chitin can be treated with a strong alkali solution to transform its acetamide group into an amino group, which will yield chitosan. According to Zhao *et al.*, nanoparticles are defined as solid particles or particulate dispersions having a size between one and one thousand nm. To put it simply, chitosan nanoparticles are nanoparticles made from chitosan or its derivatives.[35] Due to its distinct polymeric cationic

nature, good biocompatibility, non-toxicity, biodegradability, mucoadhesive, and absorption-enhancing properties, the N-deacetylated derivative of chitin is an intriguing biopolymer from which to produce nanoparticles (Kunjachan *et al.*).[36] The two main characteristics that make chitosan significant in the production of nanoparticles are its cationic nature, which permits ionic cross-linking with multivalent anions, and the fact that it is a linear polyamine with several free amine groups that are easily available for cross-linking (Agnihotri *et al.*).[37] According to Qi *et al*., the special properties of chitosan nanoparticles may offer a better affinity for negatively charged biological membranes and site-specific targeting in vivo.[38] Thus, medications, enzymes, and DNA can all be successfully encapsulated using it (Bowman and Leong 2006; Colonna *et al.*).[39] These nanoparticles have several uses across numerous industries, making them perfect materials for controlled release systems (Corradini *et al*.).[40] The physical, chemical, and morphological properties of chitosan nanoparticles are what give them their unique bioactivity; these properties are influenced by the system's circumstances as well as the manufacturing technique. It is evident that the particles' morphologies are dissimilar. Chitosan nanoparticles demonstrate consistent assemblage forms, including spheres, snowflakes, and rounds.[41]

The following are some advantages of using chitosan nanoparticles as a drug delivery system:

(a) Nanoparticles are little molecules that preserve their properties and function as stable molecules.
(b) The nanoparticle's particle size can be changed to deliver the medication we need.
(c) The release of the medication at the intended location and during transportation.
(d) Using magnetic guiding or attaching certain ligands to particle surfaces allows for site-specific targeting.
(e) The nanoparticle can be administered by a variety of methods, including parenteral, intraocular, nasal, and oral.
(f) Nanoparticles have a deep penetration into the body's confined spaces.
(g) The low toxicity, improved stability, and biodegradability of nanoparticles allow for easy administration by oral, nasal, parenteral, and other routes.

1.8. NANOMEDICINAL APPLICATION

Based on current trends, it is conceivable that nanomedicine will help to bring the next leap in developing advanced therapy, imaging, drug delivery, and the treatment of fatal diseases. Chitosan nanoparticles are natural materials that are widely used in medicinal applications due to their hydrophilic, nontoxic, biocompatible, and bio-degradable nature. Because CNPs have these properties, they are highly suitable for a wide range of drug delivery, gene therapy, and tissue engineering applications. Some of these applications are discussed below.

(a)Drug Delivery

The demonstrated potential use of CNPs as drug delivery systems has provided opportunities for the development of a largely expanded range of CNP-based delivery vehicles. Due to its biocompatibility, chitosan is classified by the United States Food and Drug Administration as GRAS (Generally Recognized as Safe). As noted above, the presence of the amino and hydroxyl functional groups, as well as the glycosidic bond, enables the loading of CNPs with drug molecules and DNA. Because CNPs are soluble in acidic aqueous solutions, sustainable chemistry may be employed in their synthesis without the use of harmful organic solvents. An additional advantage of using CNPs is that, through mucoadhesion, they enable the controlled release of drugs in vivo. CNPs have several important potential applications for the delivery of drugs, namely, parenteral, ocular, oral, pulmonary, nasal, buccal, and vaginal, in addition to applications in cancer therapy, tissue engineering, etc. A few of these applications of CNPs for drug delivery are discussed below.

(i) Ocular Drug Delivery

Because chitosan has mucoadhesive properties, the use of CNPs for controlled drug delivery is favourable via mucosal membranes.[42] CNPs undergo surface gel layer formation when in contact with near-neutral aqueous fluids, which may improve residence time on the mucosal surface and the efficacy of drug delivery to ocular tissue. CNPs cross-linked using sulfobutylether-β-cyclodextrin (SBE-β-CD) were utilized for investigations of their potential for ocular drug delivery by Mahmoud *et al*. These authors used econazole nitrate (ECO) to test for ocular antifungal efficacy in albino rabbits. Their results showed that the prepared CNPs were predominantly pseudo spherically shaped with average particle sizes ranging from 90 to 673 nm and Zeta potential values ranging from 22 to 33 mV. The ECO drug loading percent values ranged from 13 to 45%. The authors performed in vivo studies, which showed that the ECO-loaded CNPs had better antifungal ocular efficacy than an ECO solution, thus confirming

that chitosan nanoparticles are a promising ECO drug delivery vehicle for antifungal ocular treatment.[43] Santhi *et al.* used the emulsification preparation technique to synthesize fluconazole-loaded CNPs with an average particle size of 152.85 ± 13.7 nm. The authors used the cup-plate method to test the efficacy of antifungal treatment using fluconazole-loaded CNPs as compared to that of fluconazole eye drops. The fluconazole-loading capacity of their CNPs was found to be optimal at ≤ 50%. The authors concluded that the CNPs exhibited promising characteristics, including drug loading capacity, antifungal activity, and prolonged drug release, for fluconazole drug delivery for antifungal treatment.[44]

(ii) Oral Drug Delivery

Oral drug delivery is widely used because of several factors, including convenient drug administration, controlled delivery, low production cost, and patient compliance: However, challenges in conventional oral drug delivery include drug solubility issues in low-pH gastric fluids, the degradation and reduced activity of drugs due to the presence of enzymes, and the lack of adequate membrane permeability. Nanomedicine offers potential opportunities to overcome such challenges in oral drug delivery.[45] The various physicochemical properties of CNPs noted above, including mucoadhesion, biocompatibility, large surface-to-volume ratios, and drug conjugation versatility, make them suitable candidates for improving oral drug delivery. Pan *et al.* conjugated insulin to CNPs, which ranged 250–400 nm in size and were positively charged, and performed oral drug administration to diabetic rats. The authors reported that the CNP-assisted oral drug delivery resulted in the enhanced intestinal absorption of insulin in diabetic rats.[46] By modulating the dose of insulin loaded on the CNPs, the authors found that glucose levels in diabetic rats could be brought to normal levels for an extended period of time. Although the precise mechanism is not known, the authors conjectured that the CNPs improve the stability of insulin by providing protection in the gastrointestinal environment, thereby helping to increase the drug uptake. In order to study the potential improvement in the bioavailability of lipophilic drugs, such as cyclosporine, by nanoparticle encapsulation, El-Shabouriused cyclosporine-A-loaded CNPs for oral administration in dogs, whose blood samples were analysed at predetermined intervals after administration for drug uptake.[47] The mean size of the chitosan HCl nanoparticles was 148 nm with a Zeta potential of +31 mV. The results from this study showed that cyclosporine A bioavailability was increased by 73% when administered via CNP encapsulation compared to oral delivery using the commercial microemulsion Neoral®. El-Shabouri conjectured that the positively charged CNPs interact more strongly with negatively charged epithelial cells of the gastrointestinal tract than

neutral or negatively charged carriers, thereby resulting in greater permeability and bioavailability of the drug.

(iii) Pulmonary Drug Delivery

There are several physiological properties of the lungs that may enable enhanced drug delivery, including a relatively thin absorption barrier for drug uptake, a large surface area, and their extensive vascularity.[48]As with oral drug delivery, pulmonary drug delivery is conjectured to benefit from the physicochemical properties of CNPs, including a large loading capacity, mucoadhesion, a positive charge, an antibacterial property, and sustained drug delivery. Islam and Ferro have made an extensive review of CNP-based vehicles for pulmonary drug delivery. Several noteworthy studies have been made to investigate the efficacy of using CNPs for pulmonary drug delivery.[49] Yamamoto *et al.* used a poly (DL-lactide-co-glycolide) (PLGA) copolymer for the synthesis of CNPs that were loaded with elcatonin (used for lowering blood calcium) and aerolized for pulmonary drug delivery.[50] The results from their studies demonstrated the efficacy of the drug delivery, along with sustained drug release (up to 24 h), using CNP-PLGAs loaded with elcatonin when compared to the use of unmodified CNPs. The authors conjectured that the positively charged properties of the PLGA-modified CNPs enabled the opening of the tight junctions in the epithelial cells of the lungs, thus improving drug uptake.[51] Jafarinejad *et al*. prepared aerosolized CNPs loaded with itraconazole, which is an antifungal drug, with in vitro testing for pulmonary administration.[52] The study was made to test whether the low solubility of itraconazole in the gastrointestinal tract upon oral administration can be overcome by pulmonary drug delivery using CNPs. The authors reported an increased uptake of itraconazole using their aerosolized CNPs, particularly when leucine was added for aerosolization of the nanoparticles. Rawal *et al.* prepared CNPs loaded with rifampicin for pulmonary administration in rats in order to test for the efficacy of drug delivery in the treatment of tuberculosis.[53] The primary advantage in pulmonary drug delivery is the potential of eliminating the considerable adverse effects to the drug when administered orally. The in vitro study showed sustained release of the drug for up to 24 h and negligible toxicity.

(iv) Nasal Drug Delivery

The effective administration of peptides, nucleic acids, vaccines, and other drugs encapsulated in nanoparticles via nasal delivery is highly desirable because this route may induce a substantially more potent immune response; however, the nasal epithelium presents low permeability to hydrophilic molecules, whereas mucosal clearance and the mucus gel inhibit drug uptake in nasal passageways. Due to their mucoadhesion, biocompatibility, low toxicity,

and other properties, chitosan nanoparticles are postulated to be good candidates for an effective nasal delivery of drugs.[54] Shahnaz *et al.* synthesized thiolated CNPs (chitosan conjugated with thioglycolic acid) loaded with leuprolide, which is used to treat prostate cancer, the lining of the uterus, and uterine fibroids, to test whether this formulation could improve the bioavailability of the drug via nasal delivery.[55] Their results showed substantially improved bioavailability of leuprolide from nasal administration using thiolated CNPs in rats compared to the administration of leuprolide solution alone. One of the main complications in the conventional oral delivery of anti-epileptic drugs is the prevention of drug uptake in the brain due to the blood–brain barrier, resulting in drug resistance. Liu *et al.* used carboxymethyl-CNPs loaded with carbamazepine, which is an anti-epileptic drug, to study the bioavailability of the drug when administered intranasally.[56] The authors used CNPs that were found to have a particle size of ~219 nm with high entrapment efficiency (80%). From in vivo tests in mice, the authors concluded that carboxymethyl-CNPs carriers caused substantially improved bioavailability and enhanced brain-targeting of carbamazepine, when compared to the nasal administration of a carbamazepine solution. However, the authors report that the CNP-carrier administration is limited by the volume of the drug that can be delivered intranasally.

(v) Buccal Drug Delivery

Buccal drug delivery is a preferred route for the delivery of drugs, particularly ones with high molecular weights, that cannot be administrated by the oral route.[57] This is a transmucosal delivery mechanism that has advantages over the oral route, including overcoming drug degradation in the gastrointestinal tract and the first-pass metabolism effect. Thus, the buccal drug delivery method may lead to enhanced drug bioavailability and lower required doses of the drug.[58] Mazzarino *et al.* prepared films containing CNPs coated with polycaprolactone and loaded with curcumin, which has potential uses for the treatment of periodontal disease, for administration via the buccal mucosa route[59]. AFM and SEM characterization of the films showed the confirmed presence of CNPs in the films and that they were uniformly distributed throughout the films. *in vitro* studies conducted by the authors in simulated saliva solutions showed maximum swelling of the films due to a hydration of ~80% and the sustained delivery of curcumin, which are required for the successful treatment of periodontal disease.

(vi) Vaginal Drug Delivery

As with buccal drug delivery, the vaginal mucosa offers another transmucosal route for drug administration. Drugs are administered vaginally for two approaches: either for local treatment or for systemic effects whereby the drug passes through the vaginal mucosa and enters the

bloodstream.[60] Primarily due to their mucoadhesive and conjugation properties, chitosan nanoparticles may be ideally suited as drug carriers in vaginal drug administration for systemic effects. Nevertheless, there are several challenges for vaginal drug delivery including a low pH (3.8–4.5) vaginal environment, considerable fluid discharge, and extensive epithelial tissue folding.[61] Martínez-Pérez *et al.* prepared CNPs that were surface modified with PLGA and loaded with clotrimazole for vaginal drug administration.[62] In vitro studies revealed that delivery via CNP-PLGAs improved antifungal activity in relation to delivery without the use of CNPs, thus making CNP-PLGAs potentially useful for the treatment of fungal infections of the vagina. Similarly, in a separate study, Perineli *et al*. developed a hydrogel system containing hydroxypropyl methylcellulose (HPMC) and chitosan to treat fungal infection of the vagina caused by *Candida albicans* and non-albicans strains.[63] The HPMC-chitosan hydrogel was found to possess either CNPs or monomolecular chitosan and was loaded with metronidazole. In vitro studies and mucoadhesive tests revealed that both types of HPMC-chitosan hydrogel, whether containing CNPs or monomolecular chitosan, exhibited improved anti-*Candida* activity of all strains and enhanced mucoadhesive properties.

(b)Cancer Therapy

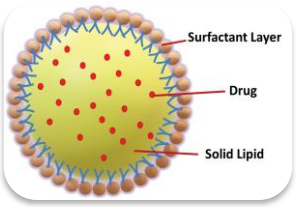
Chemotherapy remains an important therapy route for the treatment of cancers; however, because cytotoxic chemotherapeutic drugs cause chemical damage to both cancerous and noncancerous cells, this option produces considerable adverse effects in patients. Chitosan-based nanostructures (i.e., nanoparticles, nanocomposites, nanorods, etc.) are one class of polymer-based nanomaterials that are projected to play an important role in providing cancer-targeted therapies under controlled drug release that will minimize adverse effects. Mathew *et al.* prepared CNPs decorated with Mn-doped ZnS quantum dots as drug carriers and as a cancer cell imaging agent using fluorescent microscopy. In vitro studies using the CNPs loaded with 5-Fluorouracil on the MCF-7 breast cancer cells revealed that these carriers are useful for controlled and targeted drug delivery.[64] Sekar *et al*. synthesized CNPs loaded with ascorbic acid for tests of efficacy in potential targeted drug delivery in the treatment of cervical cancer.[65] The authors found from in vitro studies that the ascorbate-CNPs are effective in targeting HeLa cervical cells with no effect on human-diploid fibroblast (WI-38 strain) normal cells, thus demonstrating the potential use of these carriers as cancer drug delivery systems. Nascimento *et al.* developed chitosan-polyethylene glycol (PEG) nanoparticles loaded with silencing RNA (siRNA) that were specifically designed to target epidermal growth factor receptor (EGFR) proteins and silence the overexpressing Mad2 gene in tumour cells.[66] The authors report that

the use of CNP-PEG-siRNA nanoparticles loaded with cisplatin, in comparison to CNP-PEG-siRNA alone, had dramatically stronger inhibiting effects against cisplatin-resistant tumours in the lung. In addition, the CNP-PEG-siRNA-cisplatin nanoparticles enabled considerably reduced drug dosage with negligible adverse effects. Another approach to targeting tumour cells involves using glycol chitosan, which, due to its surfactant properties, can be used for the self-assembly of glycol-CNPs encapsulating tumour-targeting drugs.[67]

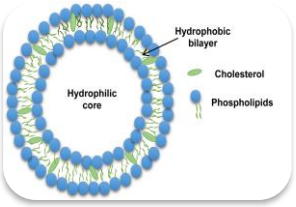
(c) Tissue Engineering

Due to their biocompatibility, biodegradability, non-toxicity, antibacterial activity, functionalizability, and other properties mentioned above, chitosan-based nanostructures are increasingly being used in and investigated for tissue engineering applications.[68] Chitosan is typically combined with other biopolymers or with bioactive nanoscale ceramic materials to formulate scaffolding for use in tissue engineering.[69] One of the more active areas in the application of chitosan-based scaffolds is for periodontal tissue engineering. Studies made on chitosan-based scaffolds generally show good efficacy in periodontal tissue engineering, but have poor mechanical strength. Investigations in utilizing bioactive bioceramics (e.g., hydroxyapatite, Bioglass, etc.) in composite form with chitosan in scaffolding may lead to improved mechanical strength properties.[70] Tayebi *et al.* recently developed a transparent composite scaffolding containing CNPs and polycaprolactone (PCL). Culture studies showed that human corneal endothelial cells attached appropriately to the CNP-PCL scaffolding in monolayer formation, indicating significant potential for corneal tissue engineering applications.[71]

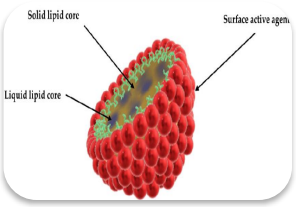
1.9. TYPES OF NANOPARTICLES



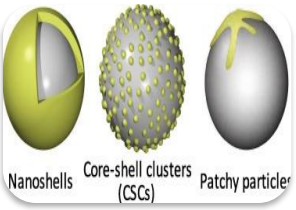
Solid Lipid Nanoparticle(SLNs)



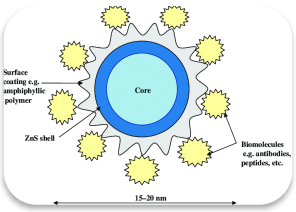
Liposomes



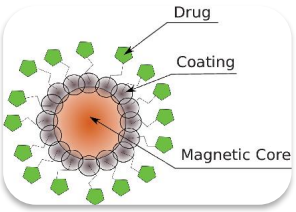
Nanostructured Lipid carriers(NLCs)



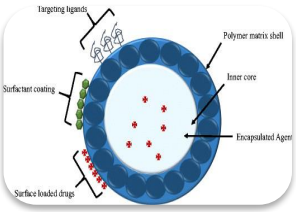
Nanoshells



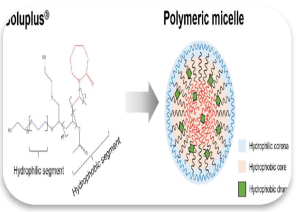
Quantum Dots



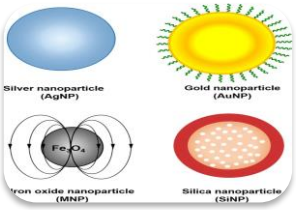
Super Paramagnetic Nanoparticles



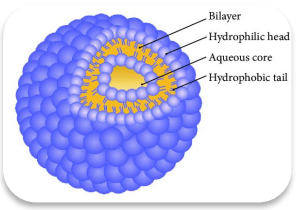
Polymeric Nanoparticles



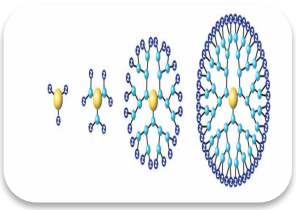
Polymeric Micelles



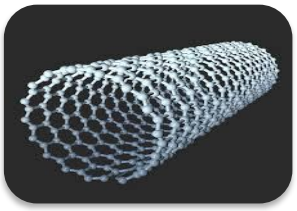
Inorganic Nanoparticles



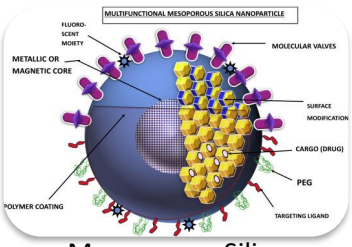
Niosomes



Dendrimers



Carbon Nanotubes



Mesoporous Silica Nanoparticles

Fig.1.9.1 Types of Nanoparticles

1.9.1. Solid Lipid Nanoparticles (SLNs)

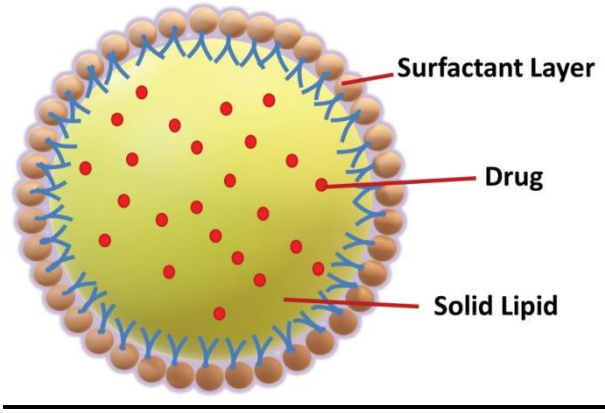


Fig.1.9.2 Structure of Solid Lipid Nanoparticle

Size of solid lipid nanoparticles (SLNs) ranges between 50–1000 nm, mainly comprise lipids that are in solid phase at the room temperature and surfactants for emulsification & offer unique properties such as small size, large surface area, high drug loading, the interaction of phases at the interfaces.[72]

Merits: -• The lipids used in SLNs are biocompatible and biodegradable and non- toxic.

- Organic solvents are not necessary for the production of SLNs.
- SLNs have a high degree of physical stability.
- Drug targeting and regulated drug release are both possible.
- Adding active substances to SLNs can increase their stability.
- SLNs can contain both hydrophilic and lipophilic pharmaceuticals.
- Large-scale SLN production is easy & can be sterilized.

Demerits: -•Lipid dispersions include a lot of water.

- The ability of hydrophilic drugs to load is limited.
- Polymorphic changes
- Particle size increases during storage.

1.9.2. Liposomes

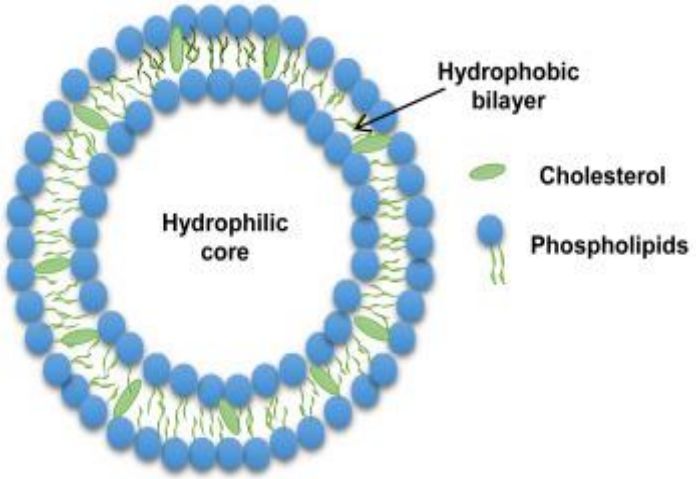


Fig.1.9.3 Structure of Liposome

Liposomes are spherical vesicle composed of lipid bilayer enclosing aqueous core which are capable of carrying bioactive agent such as drug.[73] Phospholipids have been accepted as safe substances, the likelihood of negative consequences is reduced. The hydrophobic bilayer prevents solutes, including medications, from passing through the core; yet, hydrophobic molecules can be absorbed into the bilayer, allowing the liposome to carry both hydrophilic and hydrophobic molecules. The lipid bilayer of liposomes can fuse with other bilayers such as the cell membrane, which promotes release of its contents, making them useful for drug delivery and cosmetic delivery applications. Liposomes that have vesicles in the range of nanometers are also called nanoliposomes. Liposomes can vary in size, from 15 nm up to several nm and can have either a single layer (unilamellar) or multiple phospholipid bilayer membranes (multilamellar) structure. Unilamellar vesicles (ULVs) can be further classified into small unilamellar vesicles (SUVs) and large unilamellar vesicles (LUVs).[72]

Merits: - Entrapment of both hydrophobic & hydrophilic drugs separated or simultaneously.

- Made up of natural ingredients
- Simple fabrication process
- Cost effectiveness
- The increase in the number of layers maybe beneficial to prevent or delays the release of active molecule
- Biodegradable & biocompatible

Demerits: -Reduction in entrapment efficiency due to size enlargement

- Higher physical instability during storage
- Drug leakage

1.9.3. Nanostructured Lipid Carriers (NLCs)

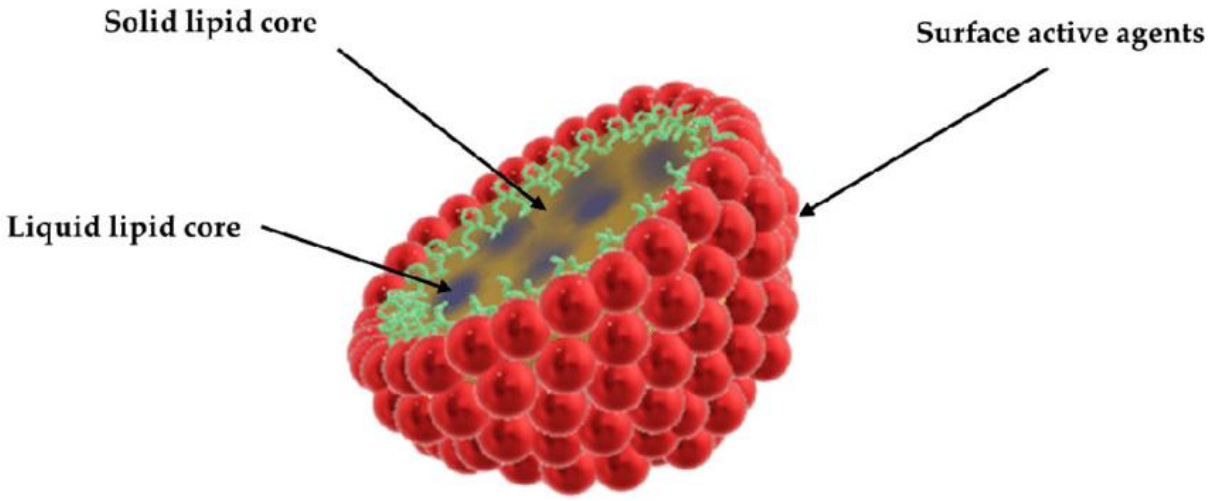


Fig.1.9.4 Structure of Nanostructured Lipid Carriers (NLCs)

Nanostructured Lipid Carriers are produced from blend of solid and liquid lipids, but particles are in solid state at body temperature. Lipids are versatile molecules that may form differently structured solid matrices, such as the nanostructured lipid carriers (NLC) and the lipid drug conjugate nanoparticles (LDC) that have been created to improve drug loading capacity. NLC can present an insufficient loading capacity due to drug expulsion after polymorphic transition during storage, particularly if the lipid matrix consists of similar molecules. Drug release from lipid particles occurs by diffusion and simultaneously by lipid particle degradation in the body. NLCs can generally be applied where solid nanoparticles possess advantages for the delivery of drugs.[72]

Merits: - Protecting drug from environmental conditions

- Low toxicity due to their biocompatible & biodegradable components and absence of organic solvents in their process
- Low cost
- Improved drug loading capacity

Demerits: - Difficulty in loading therapeutic protein

- Irritation and sensitizing action of surfactants

- Cytotoxic effects related to the nature of lipid matrix and concentration

1.9.4. Nanoshells

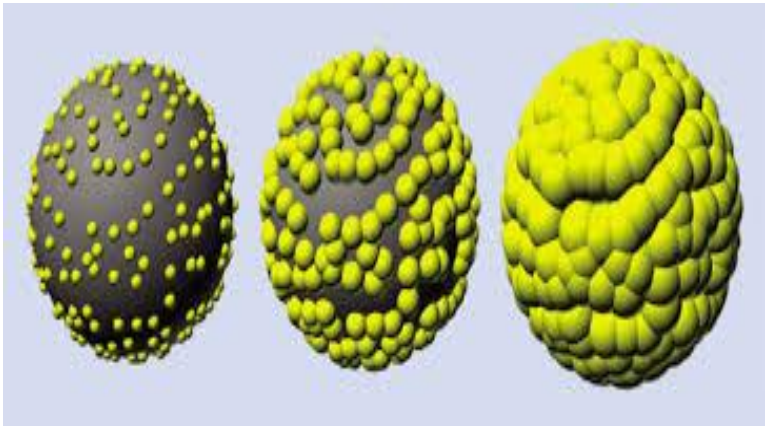


Fig.1.9.5 Structure of Nanoshells

Nanoshells are also known as core-shells, nanoshells are spherical cores of a particular compound (concentric particles) surrounded by a shell or outer coating of thin layer of another material, which is a few 1–20 nm nanometers thick Nanoshell particles are highly functional materials show modified and improved properties than their single component counterparts or nanoparticles of the same size.

Merits: -reduces the susceptibility of chemical and/or thermal denaturation.

Demerits: - Production of reactive oxygen species during photodynamic therapy

1.9.5. Quantum Dots

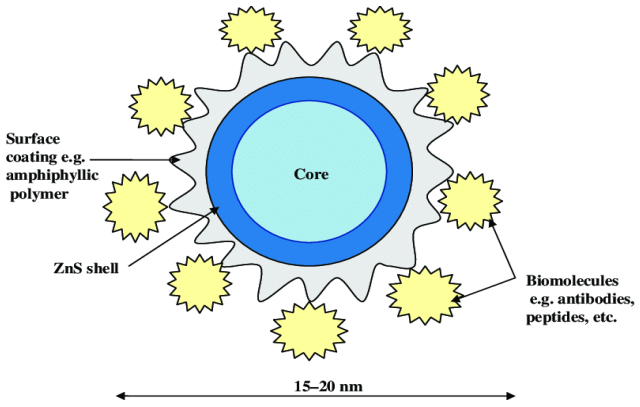


Fig.1.9.6 Structure of Quantum Dot

The quantum dots are semiconductor nanocrystals and core shell nanocrystals containing interface between different semiconductor materials. The size of quantum dots can be continuously tuned from 2 to 10 nm, which, after polymer encapsulation, generally increases to 5–20 nm in diameter.[74]

Merits: -Reduced Environmental impact

- Longer life span

Demerits: - CdSe based quantum dots are highly toxic and require stable polymer shell.

- The shells can alter the optical properties and it is also hard to control size of the particles.

1.9.6. Super Paramagnetic Nanoparticles

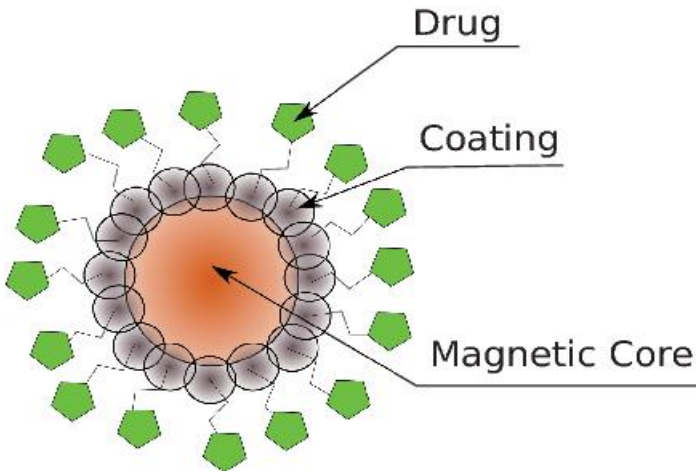


Fig.1.9.7 Structure of Super Paramagnetic Nanoparticles

Magnetic nanoparticles are small particles with a diameter of fewer than 100 nm that can be controlled by a magnetic field. Magnetic elements are used to make these particle materials. The magnetic sensitivity of these nanoparticles is used to classify them. Magnetic susceptibility of paramagnetic nanoparticles is higher than that of typical contrast forms. These nanoparticles are utilized for diagnostic and treatment strategies.[72]

Merits: -can be easily separated from water

- have high adsorption capacity
- can be reused.

Demerits: - Costly synthesis

- potential environmental impacts

1.9.7. Polymeric Nanoparticles

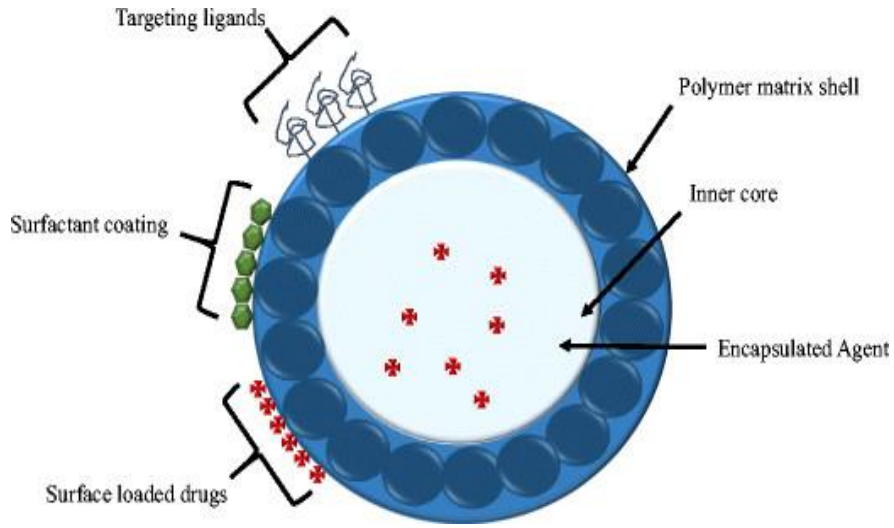


Fig.1.9.8 Structure of Polymeric Nanoparticle

Polymeric nanoparticles are colloidal particles composed of biodegradable polymer which can exist in two forms nanocapsule and nanosphere. Nanocapsule is prepared by dissolving or dispersing the drug in liquid core (oil or water) which is encapsulated with polymeric membrane. Nanosphere is fabricated by entrapping drug in polymeric matrix.[73]

Merits: - Drug release in controlled & sustained manner

- Incorporation of both hydrophilic & hydrophobic drugs
- Higher stability than lipid-based ones
- Tunable physical & chemical properties

Demerits: - Difficult for their scale up

1.9.8. Polymeric micelles

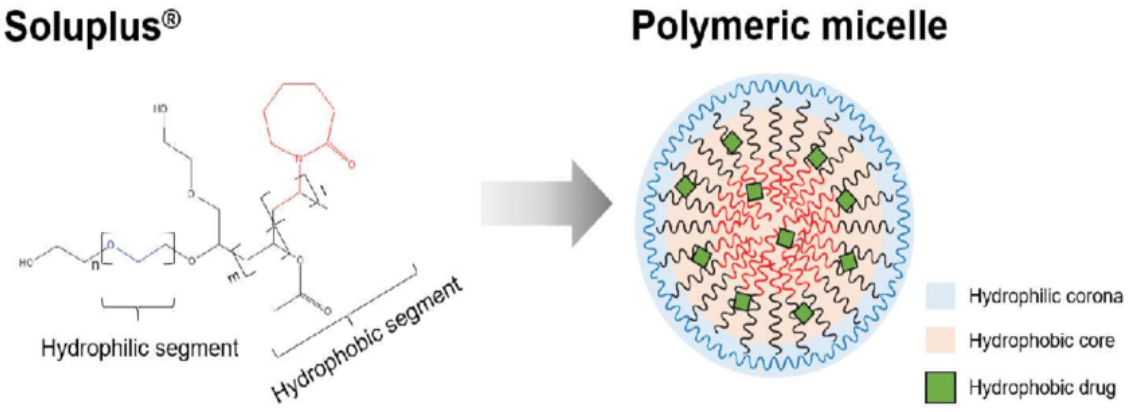


Fig.1.9.9 Structure of Polymeric Micelles

Polymeric micelles are spherical shell which self assembles using amphiphilic di- or tri-block copolymers in aqueous media.[73]

Merits: - Increasing solubility of highly lipophilic drugs

- Tunable physical & chemical properties
- Drug release in controlled manner
- Protecting drug from environmental conditions

Demerits: - Used only for lipophilic drugs

- Low drug loading capacity

1.9.9. Niosomes

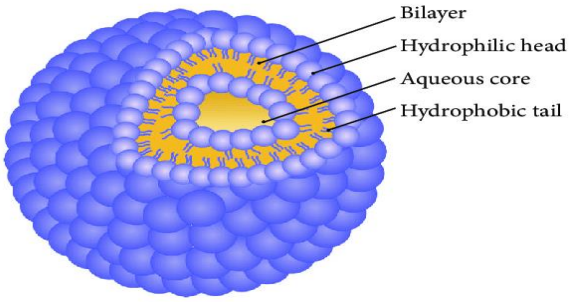


Fig.1.9.10 Structure of Niosome

Niosomes are a type of molecular cluster formed in an aqueous phase by the self-assembly of non-ionic surfactants. Niosomes have a unique architecture that allows them to function as a new delivery method that can accommodate both lipophobic and lipophilic agents. Niosomes consist of non-ionic surfactants, they are characterized by their non-toxicity, high stability and they are considered to be a replacement to liposomes.[75]

Merits: - Nonimmunogenic, non-toxic, biodegradable, biocompatible

- Improve therapeutic performance of drug
- Stable and osmotically active

Demerits: - leakage of entrapped drug

- Physically instable
- Aggregation
- Time consuming techniques

- May exhibit fusion, leaching or hydrolysis of entrapped drug which limits the shelf life

1.9.10. Dendrimers

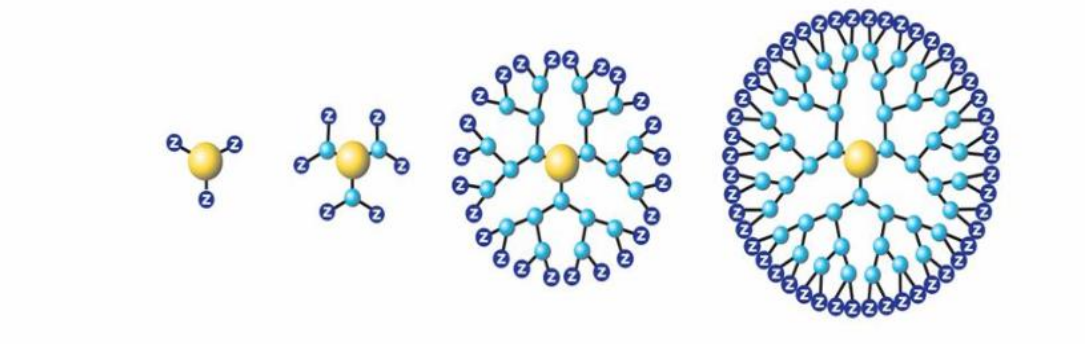


Fig.1.9.11 Structure of Dendrimer

Dendrimers which range from 1-100nm are nanosized, symmetrical molecules in which a small atom or group of atoms is surrounded by the symmetric branches known as dendrons. Dendrimer moiety consists of mainly a central core atom, secondly repetitive branching units, and terminal groups which affect the functionality of molecules. An increase in the generation of branching leads to the formation of different globular structures. They are highly customizable, with a range of sizes, surface functionalities, and drug-loading capacities.[76]

Merits: - Can be designed to have specific sizes and shapes

- high drug loading capacity can target specific tissues

Demerits: - Not a good candidate carrier for hydrophilic drugs

- High cost for their synthesis

1.9.11. Carbon Nanotubes

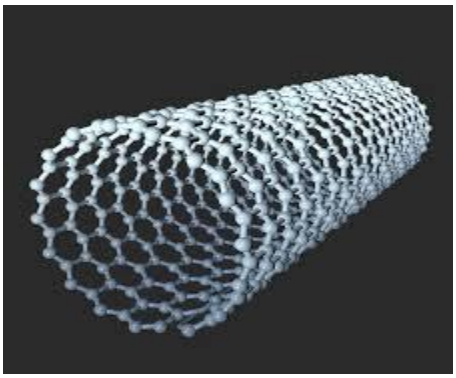


Fig.1.9.12 Structure of Carbon Nanotubes

Carbon nanotubes are tubular carbon-based structures. These tubes are made up of cylinders of graphite sheets that are sealed at one or both ends by Bucky balls and range in length from 1 to 100 nm. Single-walled nanotubes (SWNTs) and multiwalled nanotubes (MWNTs) are two designs that have recently gained popularity (MWNTs). C60-fullerenes are also found in typical configurations. They come in a variety of graphite cylinder configurations and are known for being cage-like and hollow (nanotubes and fullerenes).[77]

Merits: -Improved mechanical and electrical properties of materials

Demerits: -Poor solubility in water, low biodegradability, toxicity concerns

1.9.12. Mesoporous Silica Nanoparticles

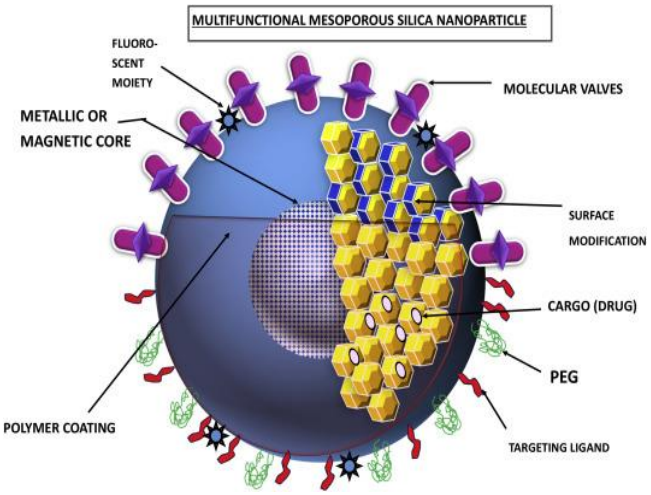


Fig.1.9.13 Structure of Mesoporous Silica Nanoparticles

Mesoporous silica nanoparticles (MSNPs) are inorganic nanoparticles or mesoporous form of silica having a particle size between 30 and 300 nm that can promote endocytosis by target cells with minimal toxicity. Mesoporous silica nanoparticles due to their low toxicity and high drug loading capacity, so they are used in controlled and target drug delivery system.[73]

Merits: - High pore volume & surface area

- Well defined surface properties
- Tunability of size and shape
- High drug loading capacity

Demerits: - Difficult in preparation of well ordered

- Scattered size distribution

1.10. Preparation of Nanoparticles

1. High Pressure Homogenization

(a)Hot Homogenization

(b)Cold Homogenization

2. Ultrasonication/High Speed Homogenization

3. Solvent Evaporation

4. Solvent emulsification-diffusion method

5. Supercritical fluid method

6. Microemulsion based method

7. Spray drying method

8. Double emulsion method

9. Precipitation technique

10. Salting out method

11. Phase Inversion Temperature (PIT) Method

12. Solvent Injection Method

1.10.1. High Pressure Homogenization

This technique is safe and potent for the production of SLNs, NLCs &Nano emulsions. High pressure homogenizers push a liquid with high pressure (between 100 and 2000 bar) to pass through a small opening (a few microns). The fluid accelerates to a very high velocity (over 1000 km/h) in a very short distance. Particle disruption occurs at submicron levels due to extremely high shear stress and cavitation pressures. The technique is based on the reduction of droplet and particle size under extreme pressure conditions. Two general approaches of HPH are hot homogenization and cold homogenization, work on the same concept of mixing the drug in bulk of lipid melt.[78]

(a) Hot Homogenization

In hot HPH, drugs are dissolved or uniformly distributed in molten lipids at temperatures that are normally 5–10 ◦C higher than the melting point of solid lipids. In general, the lipid content of the resulting SLNs and NLCs dispersion is about 5%–10% (w/v). To create a hot pre-emulsion, an aqueous phase containing surfactants is added separately and heated to the same temperature as the lipid melt while being constantly stirred. A piston-gap homogenizer is used to homogenize the resulting pre-emulsion at the same temperature. Generally, between 3 and 5 homogenization cycles at 500 to 1500 bars are sufficient to yield the necessary SLNs and NLCs. However, due to particle coalescence under extremely kinetic conditions, increasing cycle numbers and homogenization pressure may cause an increase in particle size. After homogenization, the Nano emulsions are cooled, which results in the formation of SLNs & NLCs.[68, 69]

(b) Cold Homogenization

In Cold Homogenization, when dissolving or dispersing medications in molten lipids, mixtures are rapidly cooled using liquid nitrogen or dry ice. Drug dispersions in lipid matrix are homogeneous due to this high cooling rate. Next, the lipid-drug combinations are reduced to a PS of 50–100 µm in a ball mill or mortar. After being suspended in cold aqueous solutions containing surfactants, the lipid microparticles are homogenized at low temperatures (e.g., 0–4 ◦C) typically over 5–10 cycles at 500 bars.[81]

1.10.2. Ultrasonication/ High Speed Homogenization

Ultrasonication and high-speed stirring (also known as high-shear homogenization) are common dispersing methods. One of the simplest and most economical methods for creating SLNs and NLCs is high-speed stirring. Using this process, drugs are uniformly dissolved or distributed after lipids are melted at temperatures 5–10 °C above the melting point of solid lipids among the liquid fats. The drug-lipid melt is then combined with an aqueous phase that contains surfactants (at the same temperature), and the mixture is homogeneously dispersed using a high-shear mixer. A hot oil/water (o/w) emulsion is formed due to the shear of intense turbulent eddies. SLNs and NLCs are formed by cooling these dispersions. This high-speed stirring is usually followed by ultra-sonication, which breaks droplets. High-speed stirring and ultra-sonication have been widely used in combination to achieve SLNs and NLCs dispersions with narrow particle distributions.[82]

1.10.3. Solvent Evaporation

One of the most common techniques for creating nanoparticles is the solvent evaporation method. This process consists of two steps: emulsifying the polymer solution into an aqueous phase in step one, and then evaporating the polymer solvent to cause nanosphere-sized polymer precipitation in step two. To create SLNs and NLCs, water-immiscible organic solvents such as chloroform, cyclohexane, dichloromethane, and toluene are used. To create Nano dispersions, drugs, and lipids are dissolved in a solvent or a solvent mixture and then emulsified in an aqueous phase. The organic solvent is then removed using a rotary evaporator or mechanical stirring. After solvent evaporation, lipid precipitation results in the formation of SLNs and NLCs.[83]

1.10.4. Solvent emulsification-diffusion method

The solvent emulsification-diffusion method is mainly used to produce polymeric Nano-carriers. This method is generally performed using organic solvents that are partially miscible with water (such as methyl acetate, ethyl acetate, isopropyl acetate, benzyl alcohol, and butyl lactate). To achieve the initial thermodynamic equilibrium of both phases, the organic solvent and water are first mutually saturated. An o/w emulsion is created by dissolving drugs and lipids in the water-saturated solvent, which is then emulsified under stirring in the aqueous phase (solvent-saturated water with stabilizer). Water is added to the emulsion in a volume ratio ranging from 1:5 to 1:10 to facilitate the solvent's diffusion into the continuous phase. Lipid precipitation causes SLNs and NLCs to develop spontaneously. The solvent is then removed by vacuum distillation or lyophilization.[84]

1.10.5. Supercritical fluid method

Several SLNs and NLCs production methods involved the use of supercritical fluids like supercritical CO2. For the supercritical fluid extraction of emulsions (SFEE) method, an o/w emulsion is prepared beforehand, followed by supercritical fluid extraction of the organic solvent. Typically, the emulsion is added to an extraction column from the top, and supercritical CO2 is introduced in a counter-current manner from the bottom. The solvent is quickly and completely removed, and this leads to lipid precipitation. Furthermore, the produced SLNs and NLCs have uniform particle size distribution.[85]

1.10.6. Microemulsion based method

The method involves diluting a microemulsion in a cold aqueous solution; the process creates a Nano emulsion, which in turn causes lipid precipitation to form SLNs and NLCs. In short, an aqueous phase comprising water and surfactant (pre-heated to the same temperature) is added under gentle stirring to generate a clear and thermodynamically stable microemulsion. The drug is dissolved in molten lipids at a temperature above the lipids melting point. Next, the microemulsion is gently mixed mechanically while being added to a cold aqueous solution (2–10 ˚C). The cold aqueous phase's volume is usually 25–50 times larger than the hot emulsions. Upon dilution, a Nano emulsion is formed and lipids immediately crystallize to form SLNs or NLCs.[86]

1.10.7. Spray drying method

Spray drying is a quick, easy, repeatable, and scalable method of drying that produces mild temperatures ideal for biopharmaceuticals that are heat-sensitive. Four phases are involved in spray drying: (1) heating the drying gas; (2) producing droplets; (3) drying the droplets; and (4) particle collecting. The process of Nanospray drying facilitates the production of smaller particle sizes compared to conventional spray dryers, hence enhancing the bioavailability and drug release of bioactive components. The benefits of drug-loaded nanoparticles are numerous and include higher stability, a better rate of cell penetration, a larger surface-to-volume ratio, and the ability to target release.[87]

1.10.8. Double emulsion method

The double emulsion method provides a means of producing SLNs and NLCs of hydrophilic drugs and biomolecules (e.g., peptides and proteins). According to this method, a drug and a stabilizer are dissolved in an aqueous solution and then emulsified in a water-immiscible organic phase containing lipids or in solvent-free molten lipids. These primary emulsions are dispersed in an aqueous phase containing a hydrophilic emulsifier to form water/oil/water (w/o/w) emulsion. After solvent evaporation, SLNs and NLCs dispersions are obtained due to lipid precipitation.[88]

1.10.9. Precipitation technique

The glycerides are dissolved in an organic solvent (e.g. chloroform) and the solution will be emulsified in an aqueous phase. After evaporation of the organic solvent the lipid will be precipitated forming nanoparticles.

1.10.10. Salting out method

Salting-out effect depends mainly on the separation of a water miscible solvent from aqueous solution. Both the drug and the polymer are dissolved in a vehicle in the first phase, which is then emulsified into an aqueous gel with the salting out agent and a colloidal stabilizer. Salting out agents (electrolytes, as well as non-electrolytes) and colloidal stabilizers have been used. This technique produces an oil/water emulsion, which is subsequently diluted with enough water to enhance solvent diffusion in the aqueous phase, allowing for the formation of nanospheres. Salting out technique is used for the synthesis of ethyl cellulose, PLA and Poly (methacrylic) acids nanospheres. This method has the advantage of minimizing the stress on the protein included in encapsulants formation and resulted in high efficiency and is easily scaled up.[89]

1.10.11. Phase Inversion Temperature (PIT) Method

In this method, drug, lipid, water, and surfactant are gently mixed, heated to a temperature above the phase inversion temperature of the surfactant. During the heating process (above the inversion temperature), surfactant is dehydrated, thus modifying its hydrophilic lipophilic balance, and subsequently its affinity for each phase, i.e., emulsion is inverted. Upon rapid cooling (e.g. using an ice bath), the surfactant becomes hydrophilic again, thus allowing formation of small particles of the NLCs. The advantages of this method are the low energy input and avoidance of organic solvents. Nevertheless, formed NLCs might possess poor stabilities.[90]

1.10.12. Solvent Injection Method

According to this method, lipids and drugs are dissolved in a water-miscible solvent (e.g., methanol, ethanol, isopropanol, or acetone) or a water-miscible solvent mixture. The aqueous phase is usually prepared by adding an emulsifier or an emulsifier mixture to water or a buffer solution. The organic phase is then quickly injected into the aqueous phase under continuous mechanical stirring using a needle.[91] Two principal mechanisms occur simultaneously and aid

each other to form SLNs and NLC. First, the solvent diffuses out of the droplets into the aqueous phase, which results in a droplet size reduction. As a consequence, lipid concentration within the droplets increases, which leads to the formation of local supersaturated regions stabilized by emulsifiers in the aqueous phase. Second, the emulsifiers reduce interfacial tension between water and solvent, and this leads to formations of small solvent-lipid droplets at the injection site. Due to the interfacial pulsation and turbulence during solvent diffusion, those droplets are broken into smaller droplets with essentially the same lipid concentrations. Therefore, in the solvent injection method, solvent diffusion results in the formation of tiny droplets and lipid precipitation. Emulsifiers play an important role in the determination of particle size and size distributions. When present, even at low concentrations (e.g., 0.1%), particle size reduced.[81, 82]

1.11. Methods of preparation of chitosan nanoparticles[94]

Different methods such as ionotropic gelation, emulsion cross-linking, nanoprecipitation, salting out etc have been used to prepare CS particulate systems. Selection of any of the methods depends upon factors such as particle size requirement, thermal and chemical stability of the active agent, reproducibility of the release kinetic profiles, stability of the final product and residual toxicity associated with the final product.

1.11.1. Ionotropic Gelation Method

The use of complexation between oppositely charged macromolecules to prepare CS nanoparticles has attracted much attention because the process is very simple and mild. In addition, reversible physical cross-linking by electrostatic interaction, instead of chemical cross-linking, has been applied to avoid the possible toxicity of reagents and other undesirable effects. Tripolyphosphate (TPP) is a polyanion, which can interact with the cationic CS by electrostatic forces. After Bodmeier *et al*, reported the preparation of TPP–CS complex by dropping CS droplets into a TPP solution, many researchers have explored its potential pharmaceutical usage. In the Ionic Gelation method, CS is dissolved in aqueous acidic solution to obtain the cation of CS. This solution is then added dropwise under constant stirring to polyanionic TPP solution. The chitosan molecules have abundant NH3 group which can react with negatively charged phosphoric ions of TPP to form cross-linked chitosan nanoparticles. During the process of cross-linking and hardening process water was extruded from the particles, which may help in sustaining the release of drug. Three kinds of phenomena were observed: solution, aggregation and opalescent suspension while preparing the nanoparticles. The last stage indicates the completion of the process. Insulin-loaded CS nanoparticles have been prepared by mixing insulin with TPP solution and then adding this to CS solution under constant stirring.[95]Lifeng Qi *et al.* have prepared the chitosan nanoparticles by ionotropic gelation method and also evaluated the antibacterial activity of chitosan nanoparticles. They have also characterized the particles by SEM, AFM and their MIC value were less than 0.25 g/ml, and the MBC values of nanoparticles reached 1 g/ml .AFM revealed that the exposure of S. choleraesuis to the chitosan nanoparticles lead to the disruption to the cell membranes and the leakage of cytoplasm. Zengshuan Ma *et al.* have prepared chitosan insulin nanoparticles by ionotropic gelation of chitosan with TPP anions. The ability of chitosan nanoparticles to enhance the intestinal absorption. The relative pharmacological activity and bioavailability of

insulin were investigated by monitoring the plasma glucose level of administration of various dose of insulin loaded chitosan nanoparticles.[96]

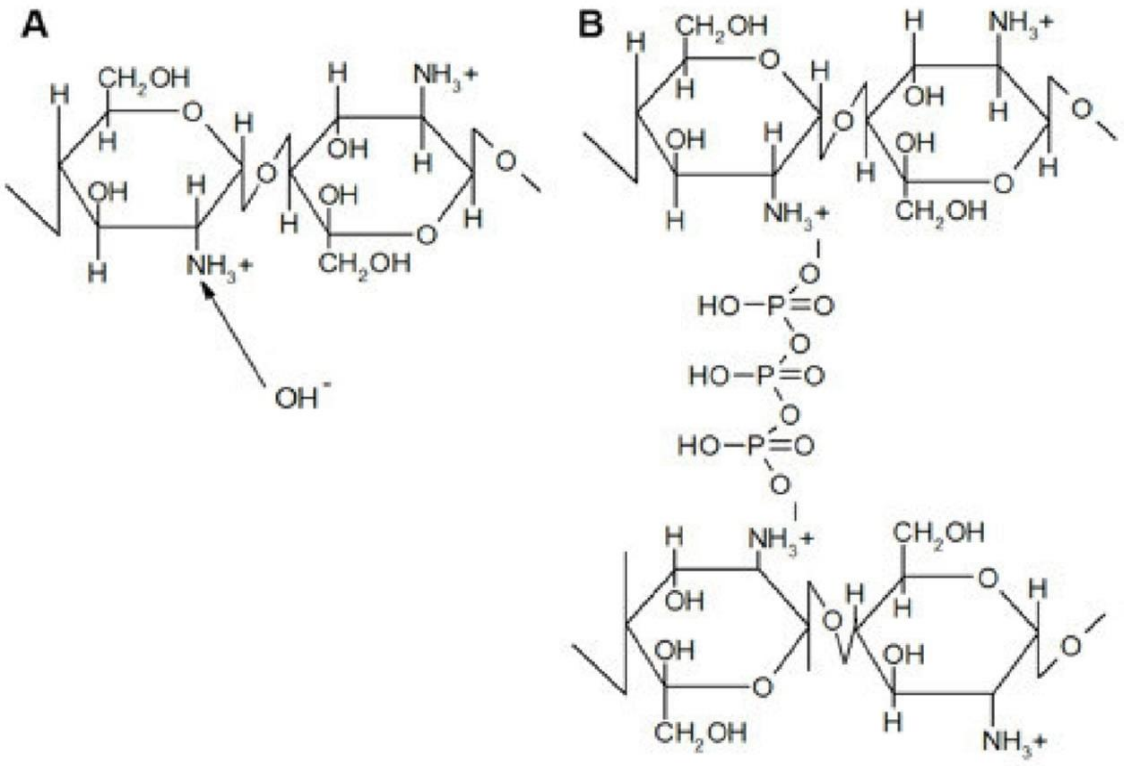


Fig. Interaction of chitosan with TPP (A) Deprotonation (B) Ionic Cross Linking

Advantages of ionotropic gelation method

• The method is very economic and simple

• The method requires less equipment and time

•Reversible physical cross-linking by electrostatic interaction, instead of chemical crosslinking, has been applied to avoid the possible toxicity of reagents and other effects.

• No use of organic solvent.

Disadvantages of ionotropic gelation method

The only disadvantage of TPP/CS nanoparticles is their poor mechanical strength

1.11.2. Cross-linking Emulsion

This method is commonly used to produce both chitosan nanoparticles and chitosan microparticles. The emulsion crosslinking method utilizes the reactive functional groups of chitosan to cross-link with a cross-linking agent. This method employs the development of a

water-in-oil emulsion by emulsifying chitosan solution in an oil phase. The aqueous droplets are stabilized using a suitable surfactant. The emulsion is then reacted with an appropriate cross-linking agent, such as glutaraldehyde, to stabilize the polysaccharide droplets. Finally, the nanoparticles are washed and dried. In this method, the size of the particles can be controlled by varying the size of the aqueous droplets. However, the amount of cross-linking agent used for hardening and the speed of stirring during the formation of the emulsion determine the particle size of the final product. However, this method has some drawbacks as well. The major ones are the use of organic solvents and harsh cross-linking agents, which ultimately have adverse effects on the stability of proteins and cell viability.

1.11.3. Droplet Coalescence Emulsion

This method's fundamentals include precipitation as well as emulsion cross-linking. Tokumitsu *et al.* first devised the technique to create chitosan nanoparticles laden with gadopentetic acid (Gd-DTPA) for gadolinium neutron-capture therapy (Gd-NCT). Two emulsions are made using a similar process in this procedure. First, a stable emulsion containing the medication in liquid paraffin oil and an aqueous chitosan solution is made. Next, an aqueous solution of NaOH containing chitosan is made into the second stable emulsion. After that, the two emulsions are combined while being agitated quickly. Random droplets from each emulsion then collide, combine, and eventually precipitate as tiny particles. The emulsion droplets contain the nanoparticles.

1.11.4. Coacervation/Precipitation

Using compressed air to create coacervated droplets, the chitosan solution was sprayed into sodium hydroxide, NaOH methanol, or ethane diamine alkaline solutions to generate the nanoparticles.

After that, the particles are separated and purified using centrifugation or filtration, and they are then repeatedly washed in hot and cold water. Furthermore, Berthold and Kreuter presented a very distinct technique for obtaining nanoparticles. Their method involves stirring and continuous sonication for 30 minutes while adding sodium sulphate solution dropwise to an acidic chitosan-containing surfactant solution.

1.11.5. Reverse Micelles

To create ultrafine polymeric nanoparticles with a limited size distribution, reverse micelle medium is employed. Thermodynamically stable liquid combinations of water, oil, and surfactant are known as reverse micelles. Reverse micelles hosted systems are unique in that they exhibit dynamic activity in contrast to emulsion polymerization using other current procedures produces ultrafine particles with a limited size range. In order to create reverse micelles, the surfactant was dissolved in an organic solvent. Brownian motion is applied to the randomly displaced micelle droplets. They reorganize into two different micelles after exchanging water content. Subsequently, the emulsion is continuously vortex with the addition of the aqueous phase containing the medicines and chitosan, causing the nanoparticles to develop in the reverse micelle core. The entire mixture is maintained in an optically clear micro emulsion phase by controlling the aqueous phase. Since, the size of the water-in-oil droplets increases linearly—that is, the micelle concentration falls with increasing water content—more water can be added to create larger-sized droplets. A cross-linking agent is added to this transparent solution while it is continuously stirred, and cross linking is accomplished by swirling for an entire night. Drugs differ in how much can be dissolved in reverse micelles; thus, the maximum amount must be found by progressively adding more medication until the clear micro emulsion turns into a translucent solution. To obtain the nanoparticles, the solvent must eventually evaporate and the dry material must then be filtered.

1.11.6. Molecular Self-Assembly

The molecular self-assembly is thought to be a potent method for producing nanostructured materials, it has attracted a lot of attention in a number of studies conducted in the fields of food, medicine, and agriculture. This approach creates opportunities for a wide range of applications because it is easy to use, adaptable, and economical. The process of self-assembly is described as the process of autonomous component organization into structurally well-defined aggregates is typified by the dissemination of specific molecular connections formed by non-covalent contacts, such as hydrophobic and/or electrostatic interactions. There are three ways to achieve the hydrophobic association with hydrophilic polymer to produce chitosan nanoparticles: first, hydrophobic chains can be grafted to a hydrophilic backbone; second, hydrophilic chains can be grafted to a hydrophobic backbone (grafted polymers); and third, alternating hydrophilic and hydrophobic segments can be achieved (block polymers).

Table2: The general overview of chitosan NPs preparation methods:

Method	**Main Principle(s)**	**Advantage(s)**	**Drawback(s)**
Emulsification and crosslinking	Covalent cross linking	• Simple process steps	•Use of harmful chemicals
Reversed micelles	Covalent crosslinking	•Ultrafine NPs below 100 nm	•Time-consuming process •Complex application
Phase inversion precipitation	Precipitation	•High encapsulation capacity for specific compounds	•Requires high shear force •Use of harmful chemicals
Emulsion-droplet coalescence	Precipitation		• Requires high shear force •Use of harmful chemicals
Ionic gelation	Ionic crosslinking	•Use of mild chemicals • Simple process • Ease of adjusting NP size	
Ionic gelation with radical polymerization	Polymerization and crosslinking		•Time-consuming process • Complex application
Self-assembly	Electrostatic and/or hydrophobic interaction	• Highly stable NPs •Use of mild chemicals • Adjustable	• Hard to control when carried out a large scale

AIMS &

OBJECTIVES

2. AIMS & OBJECTIVES

The present research work deals with the design and development of Metoprolol Succinate loaded chitosan nanoparticles using Ionic Gelation method and the in-vitro release of drug from the nanoparticles for improved drug delivery in cardiovascular treatment.

The present investigative work is carried out with the following objectives:

1. Determination of absorption maxima and preparation of the calibration curve of Metoprolol Succinate by UV-Visible Spectroscopy.

2. Preparation of chitosan nanoparticles by Ionic Gelation method.

3. Preparation of Metoprolol Succinate loaded chitosan Nanoparticle by Ionic Gelation method.

4. Optimization of formulation

5. *In-vitro* drug release of the prepared Nanoparticle using Franz Diffusion cell.

6. Characterization of the Nanoparticle:

   a) Fourier Transform Infrared Spectroscopy (FTIR).

   b) Determination of particle size, Polydispersity Index and zeta potential by Dynamic Light Scattering (DLS).

   c) Drug Loading Efficiency.

   d) Drug Entrapment Efficiency.

   e) X-Ray Diffraction (XRD).

   f) *In-vitro* drug release using Franz Diffusion cell

LITERATURE REVIEW

3. LITERATURE REVIEW

1. Afshar *et al.* developed a drug delivery system (DDS) using sodium alginate (SA)/polyvinyl alcohol (PVA) hydrogel containing Rosuvastatin-loaded chitosan (CS) nanoparticles. The researchers optimized the SA:PVA ratio and nanoparticle concentration, finding that a 7:3 ratio with 3 wt.% drug-loaded CS nanoparticles yielded optimal mechanical properties. The CS nanoparticles, sized 100-150 nm, were synthesized using ionic gelation. The DDS demonstrated controlled release of Rosuvastatin over 24 hours, with CS nanoparticles significantly influencing release behaviour. Cytotoxicity tests on human fibroblast cells showed high cell viability after 72 hours of incubation, indicating good biocompatibility. The authors concluded that this SA/PVA hydrogel containing drug-loaded CS nanoparticles shows great potential as a controlled release system for Rosuvastatin, offering a promising approach for drug delivery applications.[97]

(2) Agarwal *et al.* successfully prepared CSNPs using the ionic gelation method and characterized them through various techniques. UV-Vis spectroscopy, FTIR, DLS, and SEM were employed to analyse the nanoparticles' properties. The results revealed that the CSNPs were stable and spherical in shape, with an average size of 216 nm at the selected concentration. Zeta potential measurements using a Malvern Zeta Sizer Nano S showed a value of 50 mV, indicating good stability. These findings demonstrate the potential of chitosan nanoparticles for use as antimicrobial agents or drug delivery vehicles in pharmaceutical industries. The successful preparation and characterization of CSNPs through ionic gelation provide a foundation for further research and development in this field.[98]

(3) Koukaras *et al.* investigates the formation of chitosan nanoparticles through ionic gelation with tripolyphosphate (TPP) using density functional theory calculations. The researchers identified three primary ionic cross-linking configurations: H-link, T-link, and M-link, with H-links being the most probable due to their high interaction energies and spatial accessibility. Proton transfer was observed at close range, with maximum interaction energies varying from 12.3 to 68.3 kcal/mol depending on TPP protonation and chitosan-TPP coordination. Based on their findings, the authors proposed a mechanism for the impact of these linking types on nanoparticle formation. They also introduced the β ratio, an extension of the commonly used α ratio, which incorporates structural details of the oligomers. This research provides valuable insights into the molecular interactions involved in chitosan nanoparticle formation, which could inform future developments in this field.[99]

(4) Guo *et al*. developed chitosan-based nanoparticles to enhance the delivery and efficacy of 10-Hydroxycamptothecin (HCPT) for melanoma treatment. The nanoparticles, termed NPs/HCPT, were characterized by dynamic light scattering and zeta potential measurements, revealing an average diameter of 114.6 ± 4.1 nm. The researchers investigated the nanoparticles' cell uptake, cytotoxicity, apoptosis induction, and in vivo antitumor activity. Results showed that NPs/HCPT significantly decreased the viability of murine melanoma cell lines B16F10 and B16F1 in vitro and inhibited tumour progression in vivo. The positively charged nanoparticles were designed to improve tumour penetration and overcome the poor solubility and permeability issues associated with many chemotherapeutic drugs. The authors conclude that the cationic NPs/HCPT system shows promise as a drug delivery Nanosystem for melanoma chemotherapy.[100]

(5) Guibal *et al*. presents a simple and efficient method for synthesizing chitosan nanoparticles (CNPs) with sizes between 68-77 nm, which have potential applications in various fields including pharmaceuticals, agriculture, and water treatment. The researchers used ionic gelation with low molecular weight chitosan and tripolyphosphate as a crosslinker, combined with rigorous homogenization to produce high-yield, monodisperse CNPs. The nanoparticles were characterized using multiple techniques, including dynamic light scattering and scanning electron microscopy. The study explored the effects of pH, ionic strength, and different purification methods on CNP formation, with larger particles (95-219 nm) produced under certain conditions. The smaller CNPs (68-77 nm) obtained through homogenization and filtration demonstrated the ability to interact with negatively charged proteins and DNA, making them suitable precursors for developing virus surrogates in environmental water applications.[101]

(6) Perera *et al*. described about the preparation, characterization, and applications of chitosan nanoparticles. The authors discuss various methods for synthesizing these nanoparticles, including ionic gelation, microemulsion, and emulsification-solvent evaporation techniques. They highlight the importance of characterization techniques such as dynamic light scattering, zeta potential analysis, and electron microscopy in determining the physicochemical properties of chitosan nanoparticles. The paper also delves into the wide range of applications for these nanoparticles, including drug delivery, gene therapy, tissue engineering, and food preservation. The authors emphasize the biocompatibility, biodegradability, and non-toxicity of chitosan nanoparticles, making them attractive for biomedical and pharmaceutical applications. Overall,

this review provides a comprehensive overview of chitosan nanoparticles and their potential in various fields.[94]

(7) Du *et al.* investigated the antibacterial properties of chitosan nanoparticles loaded with various metal ions (Ag+, Cu2+, Zn2+, Mn2+, and Fe2+) against E. coli, S. choleraesuis, and S. Aureus. The nanoparticles were prepared using ionic gelation between chitosan and sodium tripolyphosphate. The researchers found that loading metal ions onto chitosan nanoparticles significantly enhanced their antibacterial activity, with the exception of Fe2+. Notably, chitosan nanoparticles loaded with Cu2+ demonstrated 21-42 times lower minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) compared to Cu2+ alone. The study also revealed a direct correlation between antibacterial activity and zeta potential of the nanoparticles. These findings suggest that metal ion-loaded chitosan nanoparticles, particularly those containing Cu2+, may offer promising antibacterial applications.[102]

(8) Nguyen Huy Hoang *et al* synthesized Chitosan nanoparticles through ionic gelation have emerged as a promising technology for sustainable agriculture and plant disease management. These nanoparticles can be loaded with various active ingredients such as copper, zinc, salicylic acid, and silicon to enhance their effectiveness CS-NPs have demonstrated efficacy in controlling plant diseases and promoting growth through both direct and indirect mechanisms. While many crops have been evaluated in controlled environments, field trials have been conducted on maize and soybean to manage specific diseases. The application of CS-NPs in agriculture has gained significant attention in recent years, with an increasing number of field studies being performed since 2019. As global challenges like population growth and climate change intensify the demand for food production, CS-NPs offer a potential solution for improving crop protection and enhancing agricultural sustainability.[103]

(9) Priyanka Rana *et al.* developed a novel mucoadhesive buccal film incorporating carvedilol nanosuspension to enhance bioavailability by avoiding first-pass metabolism. The tri-layered film consisted of a mucoadhesive layer, a drug-containing nanosuspension layer, and a backing membrane. The optimized nanosuspension had a particle size of 495 nm with a negative zeta potential. The drug gel layer contained 3% HPMC and 50 mg Carbopol 934P, while the mucoadhesive and backing layers were composed of 3% HPMC and 1% ethyl cellulose, respectively. In vitro drug release studies showed 69% release across synthetic membrane and 62.4% across porcine buccal mucosa over 9 hours. In vivo studies in rabbits demonstrated a

remarkable 916% increase in relative bioavailability compared to oral tablet formulation, with increased C_{max} and t_{max} due to enhanced surface area and bypassed hepatic metabolism.[104]

(10) Gutiérrez-Ruíz *et al* optimized the synthesis, purification, and freeze-drying of chitosan nanoparticles for biomedical applications. The researchers found that pH was the most influential factor in nanoparticle fabrication, significantly affecting particle size and polydispersity index. Optimal conditions yielded nanoparticles with an average size of 172.8 nm, PDI of 0.166, and zeta potential of 25.00 mV. The addition of surfactants (poloxamer 188 and polysorbate 80) improved stability during purification, with centrifugation increasing zeta potential and dialysis reducing particle size. Freeze-drying was successful using trehalose and sucrose as cryoprotectants, with the required sugar concentration depending on the purification method. The study concluded that surfactant selection should be based on formulation-specific benefits, and further research is needed regarding molecule loading.[105]

MATERIALS & METHODS

4. MATERIALS & METHODS

4.1 MATERIALS

4.1.1. CHEMICALS AND REAGENTS

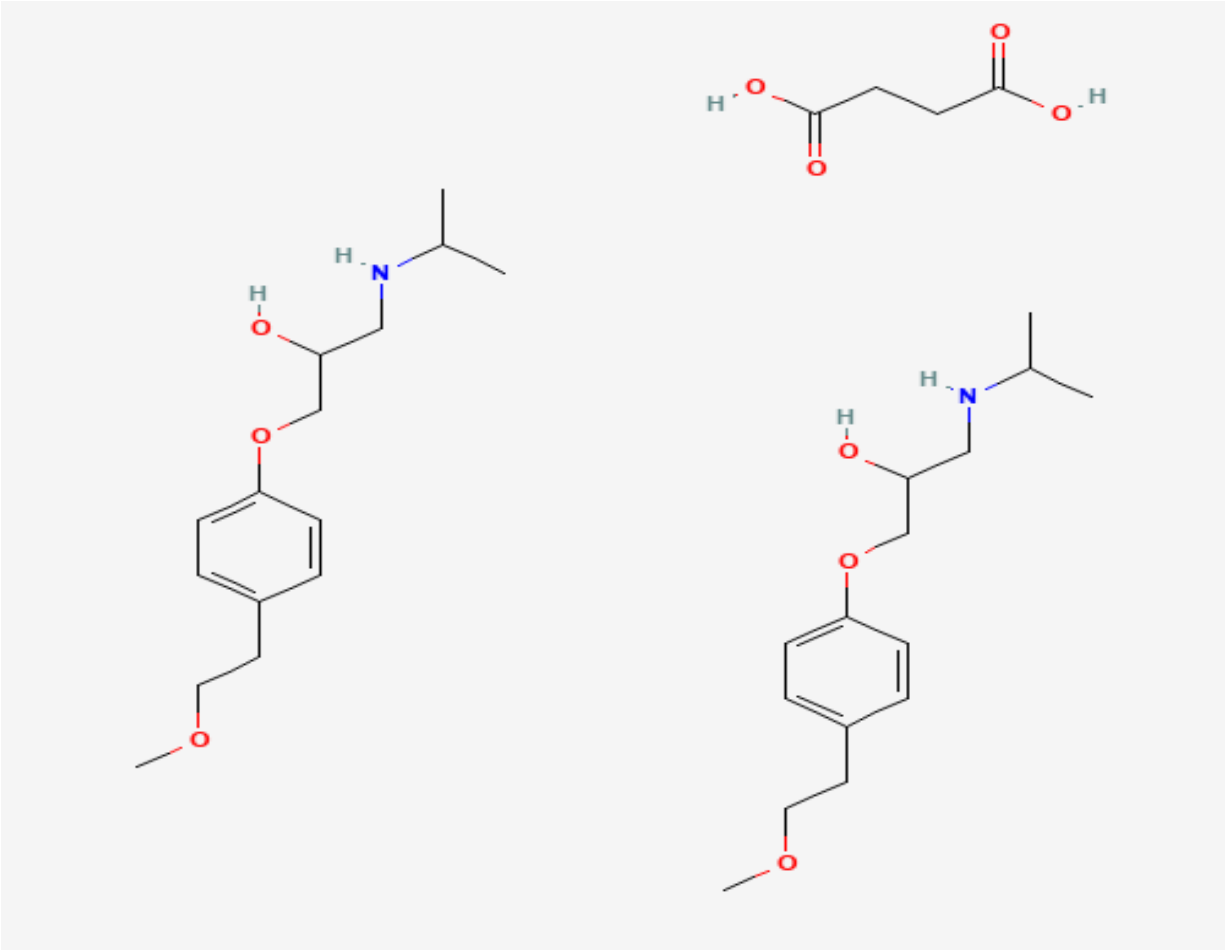
- Chitosan (Medium molecular weight) was purchased from Sisco Research Laboratories Pvt. Ltd., Maharashtra.
- Sodium Tripolyphosphate (Anhydrous) (STPP)was purchased from Loba Chemie Pvt. Ltd., Maharashtra.
- Metoprolol Succinate was purchased from Dr. Reddy Laboratories, Hyderabad
- Glacial acetic acid was purchased from Sisco Research Laboratories Pvt. Ltd., Mumbai.
- Sodium hydroxide pellets was purchased from Merck Pvt. Ltd., Mumbai.
- Potassium dihydrogen phosphate was purchased from Merck Life Science Pvt. Ltd., Mumbai.
- Disodium hydrogen phosphate was purchased from Merck Life Science Pvt. Ltd., Mumbai.
- Sodium chloride was purchased from Process Chemicals, Kolkata.
- Distilled water

4.1.2. DRUG PROFILE

Drug Name: Metoprolol Succinate

Metoprolol is a beta-blocker used in the treatment of hypertension and angina, and used to reduce mortality due to myocardial infarction.

Structure:



Chemical Formula: $C_{15}H_{25}NO_3$

Molecular weight: 652.8g/mol

IUPAC Name: butanedioic acid; 1-[4-(2-methoxyethyl) phenoxy]-3-(propan-2-ylamino) propan-2-ol

Half-life: The immediate release formulations of Metoprolol present a half-life of about 3-7 hours.

Clearance: The reported clearance rate on patients with normal kidney function is 0.8 L/min.

Route of elimination: Metoprolol is mainly excreted via the kidneys. From the eliminated dose, less than 5% is recovered unchanged

Mechanism of action: Metoprolol is a beta-1-adrenergic receptor inhibitor specific to cardiac cells with negligible effect on beta-2 receptors. This inhibition decreases cardiac output by producing negative chronotropic and ionotropic effects without presenting activity towards neither membrane stabilization nor intrinsic sympathomimetics.

Pharmacodynamics: Administration of Metoprolol in normal subjects is widely reported to produce a dose-dependent reduction on heart rate and cardiac output. This effect is generated due to a decreased cardiac excitability, cardiac output, and myocardial oxygen demand. In the case of arrhythmias, Metoprolol produces its effect by reducing the slope of the pacemaker potential as well as suppressing the rate of atrioventricular conduction.

Absorption: When Metoprolol is administered orally, it is almost completely absorbed in the gastrointestinal tract. The maximum serum concentration is achieved 20 min after intravenous administration and 1-2 hours after oral administration. The bioavailability of Metoprolol is of 100% when administered intravenously and when administered orally it presents about 50% for the tartrate derivative and 40% for the succinate derivative.

Volume of distribution: The reported volume of distribution of Metoprolol is 4.2 L/kg. Due to the characteristics of Metoprolol, this molecule is able to cross the blood-brain barrier and even 78% of the administered drug can be found in cerebrospinal fluid.

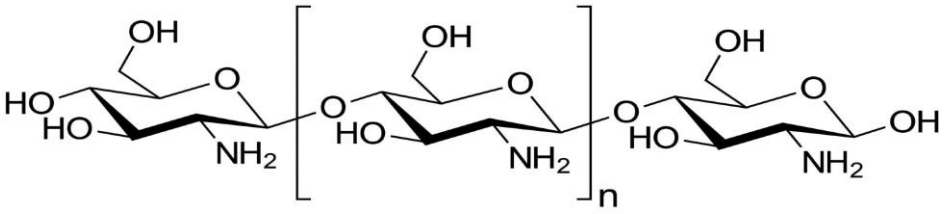
Protein binding: Metoprolol is not highly bound to plasma proteins and only about 11% of the administered dose is found bound. It is mainly bound to serum albumin.

Metabolism: Metoprolol goes through significant first-pass hepatic metabolism which covers around 50% of the administered dose.1 The metabolism of Metoprolol is mainly driven by the activity of CYP2D63 and to a lesser extent due to the activity of CYP3A4. The metabolism of Metoprolol is mainly represented by reactions of hydroxylation and O-demethylation.

Food Interaction: Interacts with alcohol, liquorice

4.1.3. EXCIPIENTS

(1) CHITOSAN



Specifications-

Appearance (Colour): White to pale yellow

Appearance (Form): Powder

Solubility (1% solution in 1% Acetic acid): Colourless to pale yellow

Loss on drying: max. 5%

Viscosity (20°C): 150 - 500 milli Pas

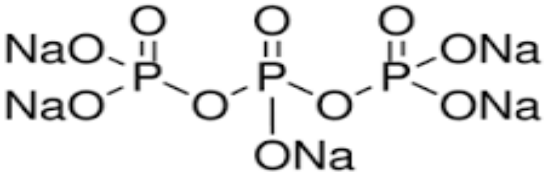
Deacetylated Degree: min. 90%

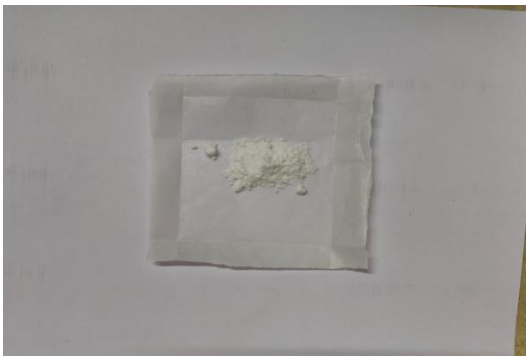
Storage: 8 to 25°C (Cool & Dry Area)

Shelf Life: maximum 60 Months

Application: Biocompatible, antibacterial and environmentally friendly polyelectrolyte with a variety of applications including water treatment, chromatography, additives for cosmetics, textile treatment for antimicrobial activity, novel fibres for textiles, photographic papers, biodegradable films, biomedical devices, and micro-capsule implants for controlled release in drug delivery.

(2) SODIUM TRIPOLYPHOSPHATE (ANHYDROUS)





Formula: $Na_5O_{10}P_3$

Synonyms: Penta sodium tripolyphosphate Anhydrous, STPP, Sodium triphosphate, Sodium triphosphate Penta basic, Sodium tripolyphosphate Penta basic.

Physical & Chemical Properties-

Appearance: White Powder

Tripoly w/w: 90%

pH (1% solution in water): 9-10

Physical state: Solid

Colour: White

Molecular mass: 367,86 g/mol

Odour: Odourless

Melting point: 622 °C

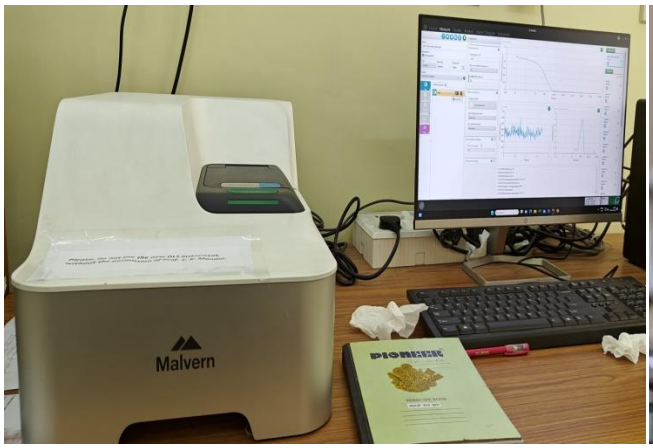
Solubility: Water: 145 g/l (20°C)

Density: > 1, 5 g/cm³

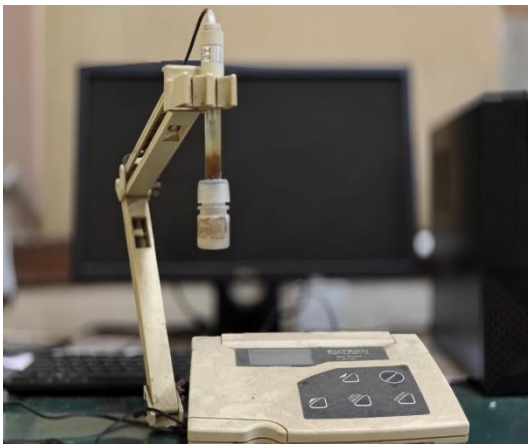
Uses: Cross-linker, pH regulator, Thickening agent, commonly used in the food industry as preservative, emulsifier and texturizer.

4.1.4. ANALYTICAL INSTRUMENT

a) Zeta Sizer



b) pH meter



c) UV- Visible Spectroscopy

d) Weighing Balance

e) FTIR

f)Lyophilizer

4.2. METHODOLOGY

4.2.1. Determination of Absorption Maximum of Drug

The UV spectrum's wavelength was chosen for the study of Metoprolol succinate. Metoprolol succinate was synthesized at a concentration of 100 µg/ml, and its UV spectra was scanned between 200 to 400 nm to identify the wavelength maximum.

4.2.2. Preparation of Calibration Curve of Metoprolol Succinate

Based on solubility property, Distilled water is selected for proper dissolving of Metoprolol succinate.

Preparation of Stock Solution

1 mg of metoprolol succinate that had been precisely measured was transferred to volumetric flask and then dissolved in 10ml of distilled water to achieve the desired concentration of 100 µg/ml. The standard stock solution was diluted- **2µg/ml, 4µg/ml, 6µg/ml, 8µg/ml, 10µg/ml**. The UV spectrum's wavelength was chosen for the study of Metoprolol succinate. Metoprolol succinate was dissolved in distilled water at a concentration of 100 µg/ml, and its UV spectra was scanned between 200 to 400 nm to identify the wavelength maximum.

4.2.3. Preparation of Drug Loaded Chitosan Nanoparticle

(a)Preparation of Chitosan solution

Chitosan Nanoparticles were synthesized by Ionic Gelation Method using Sod. TPP (Sodium Tripolyphosphate) as a crosslinking agent. First, Prepare the 1% (w/v) acetic acid solution. Then, weigh the desired amount of chitosan polymer and then add weighed amount of chitosan polymer in 1% (w/v) acetic acid solution with continuous stirring at room temperature until the achievement of transparent solution.

(b)Preparation of Metoprolol Succinate Loaded Chitosan-TPP nanoparticle:

Formulation 1: Prepare 1% (w/v) acetic acid solution by measuring 0.45ml acetic acid in50ml of distilled water. 25mg of chitosan, 10mg of sodium tri-polyphosphate and 25mg of Metoprolol succinate were accurately weighed. The weighed amount of chitosan was then dissolved in 1% (w/v) acetic acid solution under constant stirring at 500 rpm for about 2 hours

and the pH of the solution was adjusted to 5 by using 1M NaOH. Then the weighed amount of sodium tri-polyphosphate added to 20 ml of distilled water until the clear solution obtained. After achieving the clear solution of sodium tri-polyphosphate, weighed amount of metoprolol succinate is added to this sodium tri-polyphosphate solution. Then, this solution was added dropwise to the chitosan solution with syringe under continuous stirring at room temperature. After mixing the two solutions, a colloidal solution was formed. This colloidal solution was then centrifuged at 14,000rpm for 20 minutes. The nanoparticle pellet is collected and freeze dried using lyophilizer for further analysis. The supernatant solution is used for the determination of drug loading by UV- Visible Spectroscopy.

Formulation 2: Prepare 1% (w/v) acetic acid solution by measuring 0.45ml acetic acid in 50 ml of distilled water. 50mg of chitosan, 10mg of sodium tri-polyphosphate and 25mg of Metoprolol succinate were accurately weighed. The weighed amount of chitosan was then dissolved in 1% (w/v) acetic acid solution under constant stirring at 500 rpm for about 2 hours and the pH of the solution was adjusted to 5 by using 1M NaOH. Then the weighed amount of sodium tri-polyphosphate added to 20 ml of distilled water until the clear solution obtained. After achieving the clear solution of sodium tri-polyphosphate, weighed amount of Metoprolol succinate is added to this sodium tri-polyphosphate solution. Then, this solution was added dropwise to the chitosan solution with syringe under continuous stirring at room temperature. After mixing the two solutions, a colloidal solution was formed. This colloidal solution was then centrifuged at 14,000rpm for 20 minutes. The nanoparticle pellet is collected and freeze dried using Lyophilizer for further analysis. The supernatant solution is used for the determination of drug loading by UV- Visible Spectroscopy.

Formulation 3: Prepare 1% (w/v) acetic acid solution by measuring 0.45ml acetic acid in 50 ml of distilled water. 75mg of chitosan, 10mg of sodium tri-polyphosphate and 25mg of Metoprolol succinate were accurately weighed. The weighed amount of chitosan was then dissolved in 1% (w/v) acetic acid solution under constant stirring at 500 rpm for about 2 hours and the pH of the solution was adjusted to 5 by using 1M NaOH. Then the weighed amount of sodium tri-polyphosphate added to 20 ml of distilled water until the clear solution obtained. After achieving the clear solution of sodium tri-polyphosphate, weighed amount of Metoprolol succinate is added to this sodium tri-polyphosphate solution. Then, this solution was added dropwise to the chitosan solution with syringe under continuous stirring at room temperature. After mixing the two solutions, a colloidal solution was formed. This colloidal solution was then centrifuged at 14,000rpm for 20 minutes. The nanoparticle pellet is collected and freeze

dried using Lyophilizer for further analysis. The supernatant solution is used for the determination of drug loading by UV- Visible Spectroscopy.

Formulation 4: Prepare 1% (w/v) acetic acid solution by measuring 0.45ml acetic acid in 50 ml of distilled water. 100mg of chitosan, 10mg of sodium tri-polyphosphate and 25mg of Metoprolol succinate were accurately weighed. The weighed amount of chitosan was then dissolved in 1% (w/v) acetic acid solution under constant stirring at 500 rpm for about 2 hours and the pH of the solution was adjusted to 5 by using 1M NaOH. Then the weighed amount of sodium tri-polyphosphate added to 20 ml of distilled water until the clear solution obtained. After achieving the clear solution of sodium tri-polyphosphate, weighed amount of Metoprolol succinate is added to this sodium tri-polyphosphate solution. Then, this solution was added dropwise to the chitosan solution with syringe under continuous stirring at room temperature. After mixing the two solutions, a colloidal solution was formed. This colloidal solution was then centrifuged at 14,000rpm for 20 minutes. The Nanoparticle pellet is collected and freeze dried using Lyophilizer for further analysis. The supernatant solution is used for the determination of drug loading by UV- Visible Spectroscopy.

Formulation 5: Prepare 1% (w/v) acetic acid solution by measuring 0.45ml acetic acid in 50 ml of distilled water. 125mg of chitosan, 10mg of sodium tri-polyphosphate and 25mg of Metoprolol succinate were accurately weighed. The weighed amount of chitosan was then dissolved in 1% (w/v) acetic acid solution under constant stirring at 500 rpm for about 2 hours and the pH of the solution was adjusted to 5 by using 1M NaOH. Then the weighed amount of sodium tri-polyphosphate added to 20 ml of distilled water until the clear solution obtained. After achieving the clear solution of sodium tri-polyphosphate, weighed amount of Metoprolol succinate is added to this sodium tri-polyphosphate solution. Then, this solution was added dropwise to the chitosan solution with syringe under continuous stirring at room temperature. After mixing the two solutions, a colloidal solution was formed. This colloidal solution was then centrifuged at 14,000rpm for 20 minutes. The Nanoparticle pellet is collected and freeze dried using Lyophilizer for further analysis. The supernatant solution is used for the determination of drug loading by UV- Visible Spectroscopy.

Also, the drug encapsulation rate of Chitosan Nanoparticles was determined from a supernatant solution obtained after the centrifugation. The absorbance value of the unloaded drug in the supernatant was measured by a UV-Visible Spectrophotometer at λ=221nm using the calibration curve equation. Then, the drug encapsulation rate was calculated by considering the

initial weight of the drug added to the chitosan solution. In addition, samples of colloids containing chitosan nanoparticles with and without the drug were subjected to further analysis.

Prepare 1% (w/v) acetic acid solution

[1ml acetic acid in 100ml distilled water]

↓

Weigh required amount of Chitosan polymer

↓

Chitosan was dissolved in acetic acid to prepare chitosan polymeric solution

↓

Prepare aqueous solution of TPP crosslinking agent solution by adding desired amount of TPP in distilled water

↓

Add weighed amount of drug in TPP solution to produce Drug loaded -TPP solution

↓

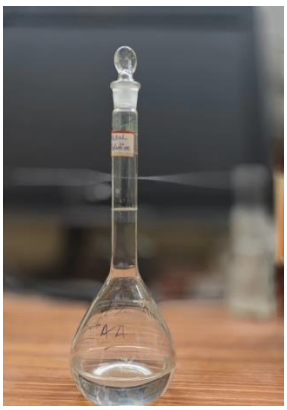
Add Drug loaded-TPP solution to Chitosan Polymeric solution dropwise with the help of syringe under continuous stirring at room temperature

↓

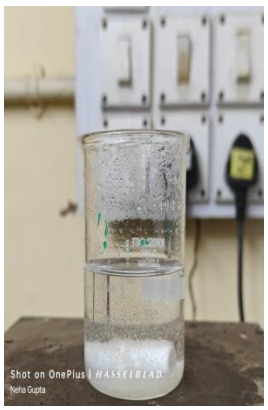
Colloid solution was obtained &then colloid was then centrifuged at 10,000rpm for 20minutes.

↓

Collect chitosan nanoparticles; was then dried using Lyophilizer & the dried chitosan nanoparticles were ready for further analysis.



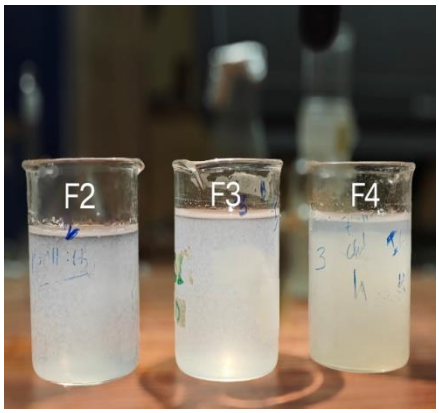
1% (w/v)
Acetic acid solution



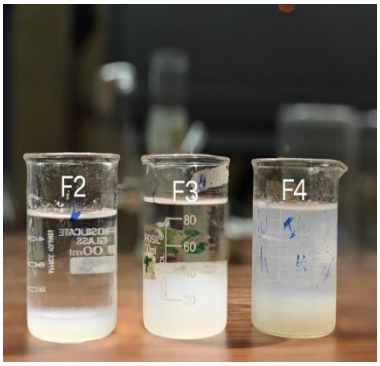
Chitosan solution

Drug Loaded sod. TPP solution

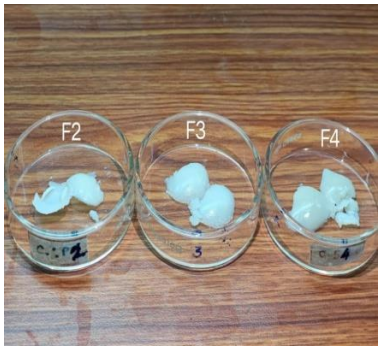
Slowly adding Drug Loaded Sod.TPP solution to Chitosan Solution with syringe



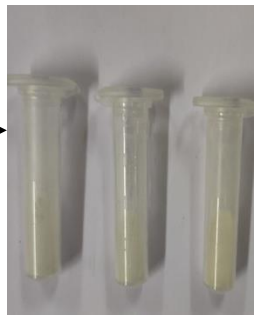
Colloidal solution formed after adding two solutions



After 30mins



Nanoparticle pellets collected after centrifugation Nanoparticles



Freeze Dried

Fig.4.2.1 Preparation of Drug Loaded Chitosan Nanoparticles

Table: Composition of Prepared Formulation

Formulation	Chitosan (50ml)	TPP	Drug	Supernatant Absorbance	Weight of Dried NPs
F-1	0.5mg/ml	0.5mg/ml	25mg	0.5243	30mg
F-2	1mg/ml	0.5mg/ml	25mg	0.5139	54.9mg
F-3	1.5mg/ml	0.5mg/ml	25mg	0.5478	116.2mg
F-4	2mg/ml	0.5mg/ml	25mg	0.4668	106.5mg

4.2.4. Characterization of Chitosan Nanoparticles

The nanoparticles formation is generally affected by the processing condition and time. They are characterized on the basis of their surface charge, morphology, particle size distribution, zeta potential, functional group analysis etc., using UV- Visible Spectrophotometer, electron microscope, dynamic light scattering, Fourier Transform Infrared spectroscopy (FTIR), X-Ray Diffraction (XRD).

(a)Ultraviolet–visible Spectroscopy (UV-Vis)-

To verify the formation of nanoparticles the solution was scanned in the range of 200–600 nm in a spectrophotometer using a quartz cuvette with 1% acetic acid solution as the reference.

(b) Fourier Transform Infrared Spectroscopy (FTIR)

FTIR study is conducted to confirm the synthesis of nanoparticles by determining their functional groups. Sample preparation for FTIR is done by gently triturating it with KBr which is then compressed into disks. The compressed disks are scanned against a blank KBr pellet background at 25°C to obtain the FTIR results. For every spectrum, a 32 scan interferogram was collected at transmittance/absorbance mode in the 4000–400 cm−1 region. The functional

groups of a chitosan nanoparticles consist of amide (·NH2) and hydroxyl (·OH) group, C·H, C·N, C·O, and P·O stretching. Generally, the FTIR peak at 3000–3500 cm−1 attributed to (·OH) and (·NH2) is the confirmatory peak for the formation of ChNPs.

(c)Particle Size Determination

The main principle of DLS is based on the Brownian movement of particles/molecules present in the solution that results from their collision with the randomly moving solvent particles. A laser beam is passed through the sample, and the fluctuation in scattered light due to the random motion of particles is detected by the photon detector. DLS is used for the measurement of average particle size, particle size distribution, polydispersity index (PDI), and zeta potential. PDI explains the polydispersity or monodispersity of particles in an aqueous medium. PDI value greater than 0.5 represents polydispersity and less than 0.5 normally shows the monodispersity of particles. Generally, monodisperse ChNPs exhibit the PDI value within the range of 0.2–0.4. Further, the surface charge of nanoparticles, also known as zeta potential, explains the stability of the nanoparticles, which is measured in the range of ±30 mV. The ChNPs show a positive zeta potential value that may vary from 11.2 ± 1.2 mV to 18.7 ± 0.4 mV. Further, the appropriate particle size determined by DLS showed that the ChNPs are nearly spherical in shape with size ranging from 50 to 200 nm.

(d)Loading &Entrapment Efficiency

Entrapment Efficiency& Loading Efficiency is a crucial parameter in the evaluation of nanoparticles-based drug delivery systems. It reflects the proportion of the drug successfully encapsulated within the nanoparticles compared to the total amount of drug used in the formulation process. High entrapment efficiency is desirable as it indicates effective drug loading and minimizes drug wastage. The drug encapsulation rate of Chitosan nanoparticles was determined from a supernatant solution obtained after the centrifugation. The absorbance value of the unloaded drug in the supernatant was measured by a UV–visible spectrophotometer at λ = 221 nm using the calibration curve equation. The % Entrapment Efficiency was calculated as follows:

$$\%EE = \frac{\text{Total amount of drug added to the formulation-Unbound Drug} \times 100}{\text{Total amount of drug added to the formulation}}$$

$$\%\text{Loading Efficiency} = \frac{\text{Total Given Drug} - \text{Supernatant Free Drug}}{\text{Weight of Nanoparticles}} \times 100$$

(e) X-Ray Diffraction

The nature and degree of crystallinity of CNPs can be studied using X-ray diffraction (XRD). XRD is the foremost analytical tool used to identify the crystalline phase(s) of materials. In addition to determining structural properties, XRD can also be used to measure the mean diameter of the nanoparticles. When measured in powder X-ray diffraction, the Scherrer equation may be used to determine the mean crystallite size of the material after corrections in the broadening of the diffraction peaks due to instrumental, strain, and lattice imperfections. The Scherrer equation relates the full width with the half-maximum (FWHM) of a given XRD peak of a specific crystalline phase (after the aforementioned corrections) to the mean size of the nanoparticles, assuming the typical size of a nanoparticles is the same as the size of a crystallite.

(f) *in-vitro* Drug Release Study

in-vitro release study was carried out by using Franz Diffusion cell apparatus. In brief, required amount of nanoparticles (different formulations) was accurately weighed and placed in 40ml of phosphate buffer saline ph7.4 in Franz diffusion cell at a temperature of 37 °C± 0.5 °C with 50 rpm stirring speed. At a particular time, interval 2ml sample was withdrawn and replenish with fresh buffer solution. The samples were analysed using UV-Visible spectrophotometer at 221 nm and the cumulative %drug release was calculated.

TABLES & GRAPHS

5. TABLES & GRAPHS

5.1. Determination of Absorption maxima of Metoprolol Succinate

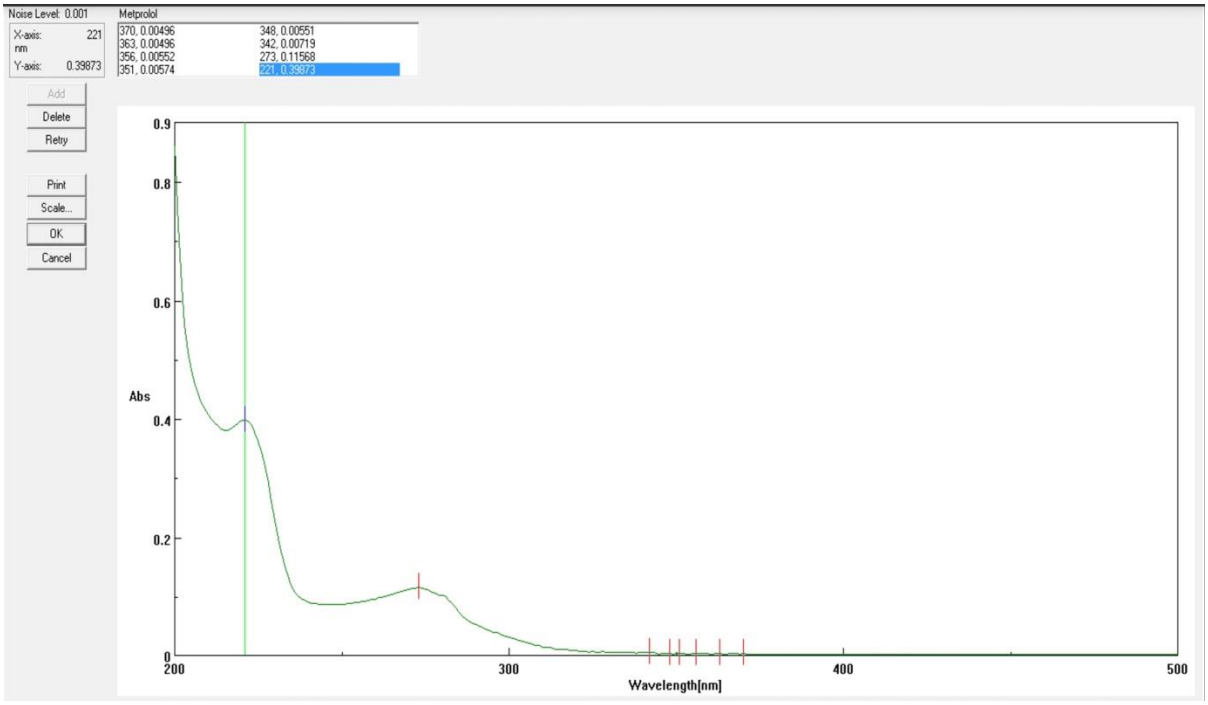


Fig.5.1.1 Determination Absorption maxima of Metoprolol Succinate

5.2. Calibration Curve of Metoprolol Succinate

Table:5.2.1. Drug concentration vs Absorbance of Metoprolol Succinate

Concentration(µg/ml)	Absorbance
2	0.1437
4	0.2305
6	0.3281
8	0.4463
10	0.5226

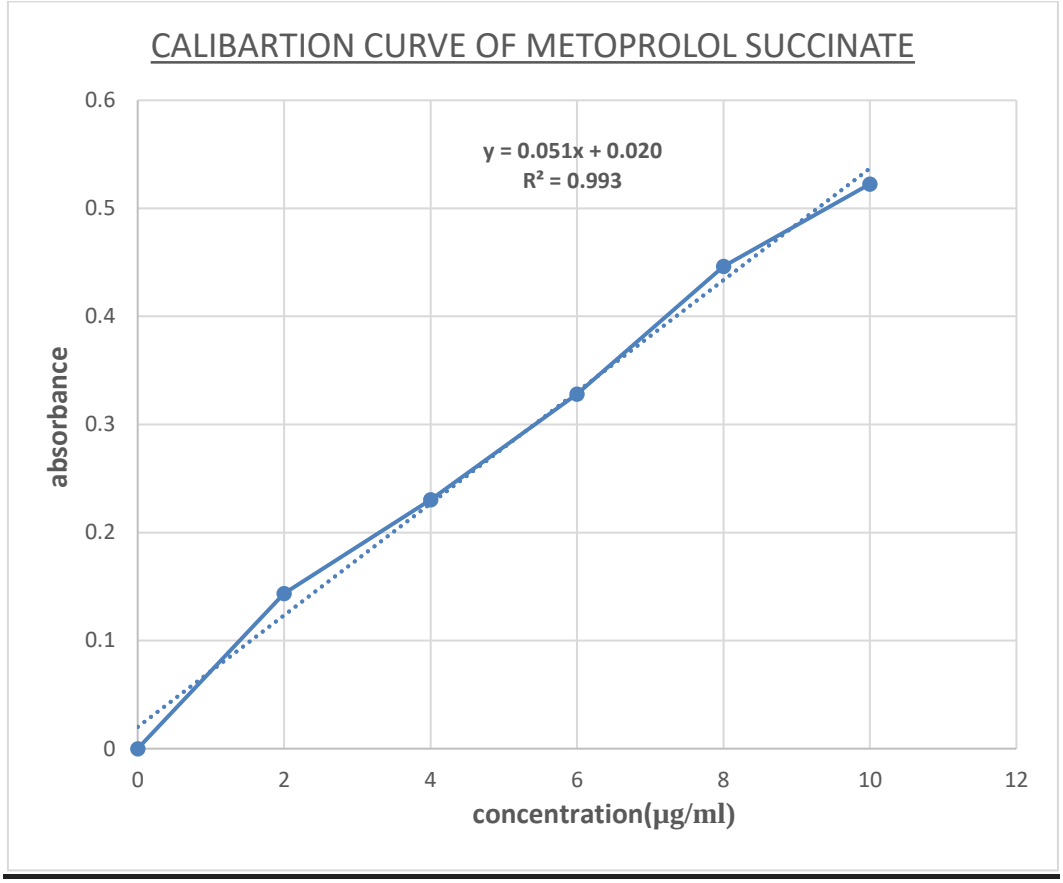


Fig.5.2.1 Calibration Curve of Metoprolol Succinate

5.3. Fourier Transform Infrared Spectroscopy

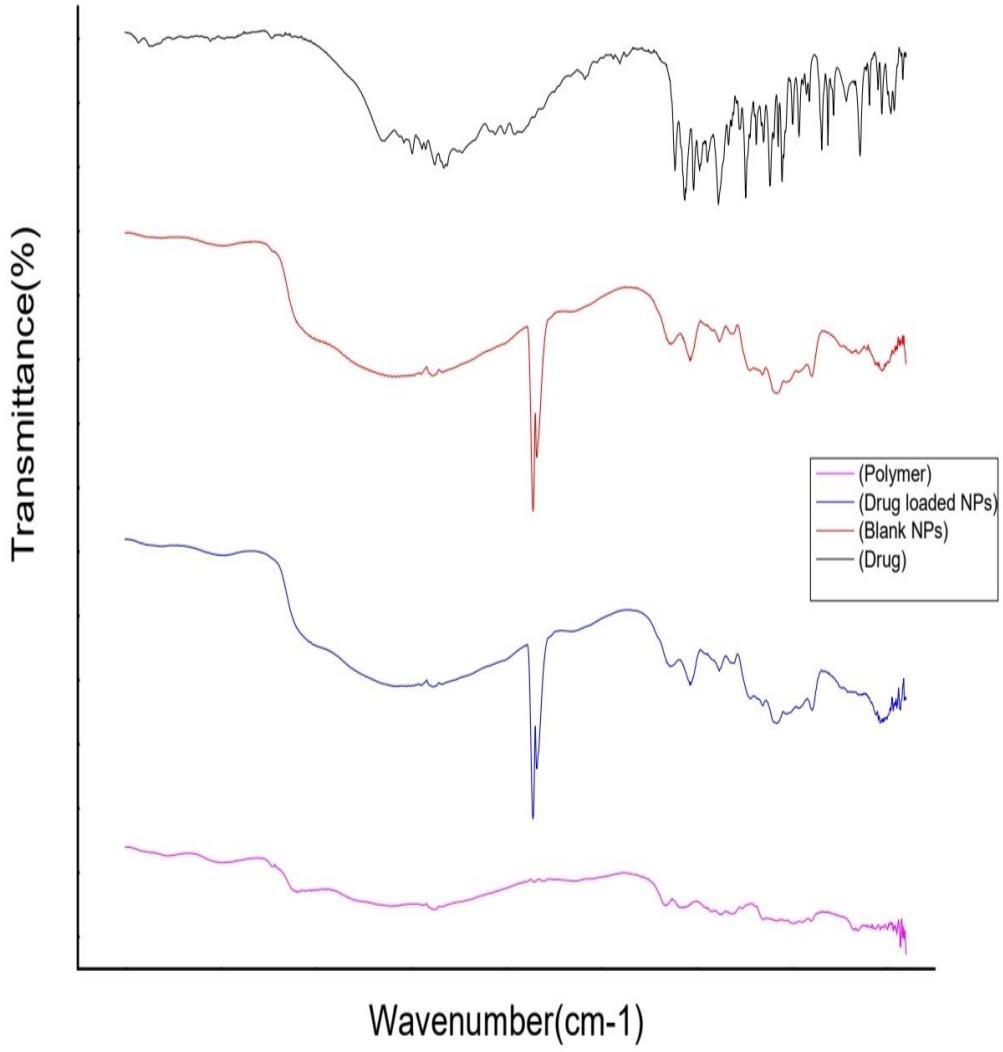


Fig.5.3.1 Comparative Fourier Transform Infrared Spectroscopy analysis of Polymer, Drug, Blank NPs, Drug Loaded NPs

5.4. Particle Size Determination

Table.5.4.1 Particle size, Zeta Potential and PDI of formulations

Formulation	Particle size (nm)	Zeta Potential(mV)	Polydispersity Index
F1	146.1	-22.42	1
F2	125.6	-17.75	0.439
F3	23.88	-10.14	0.3834
F4	108	-11.57	1

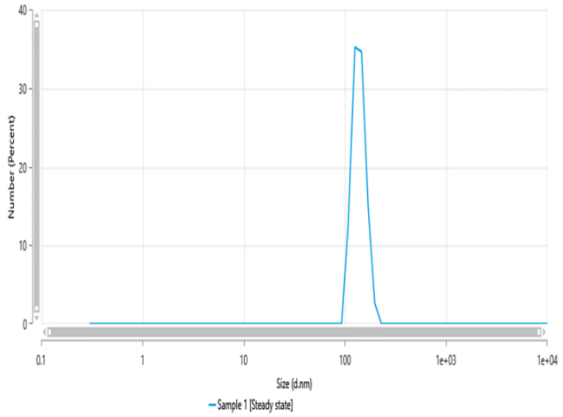


Fig.5.4.1 Particle Size Distribution of F1

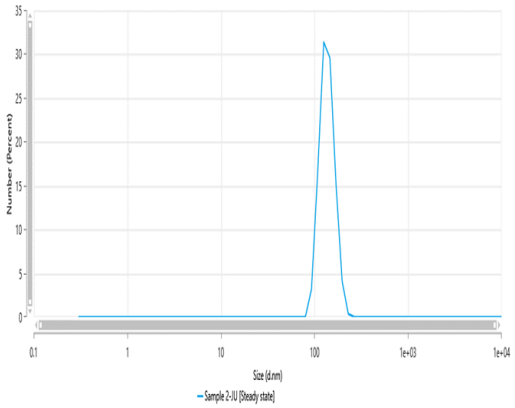


Fig.5.4.2 Particle Size Distribution of F2

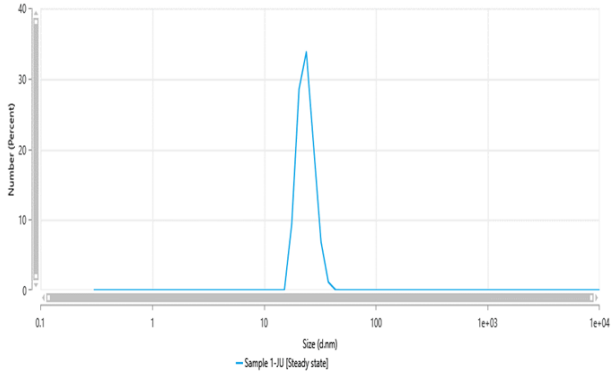


Fig.5.4.3 Particle Size Distribution of F3

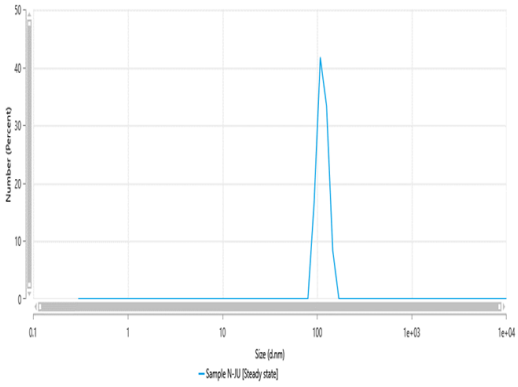


Fig.5.4.4 Particle Size Distribution of F4

5.5. Loading and Entrapment Efficiency

Table.5.5.1 Loading and Entrapment Efficiency of formulations

Formulation	Loading Efficiency (LE %)	Entrapment Efficiency (EE %)
F1	15.62%	96.75%
F2	**44.08%**	96.81%
F3	20.78%	96.61%
F4	22.79%	**97.11%**

5.6. X-Ray Diffraction

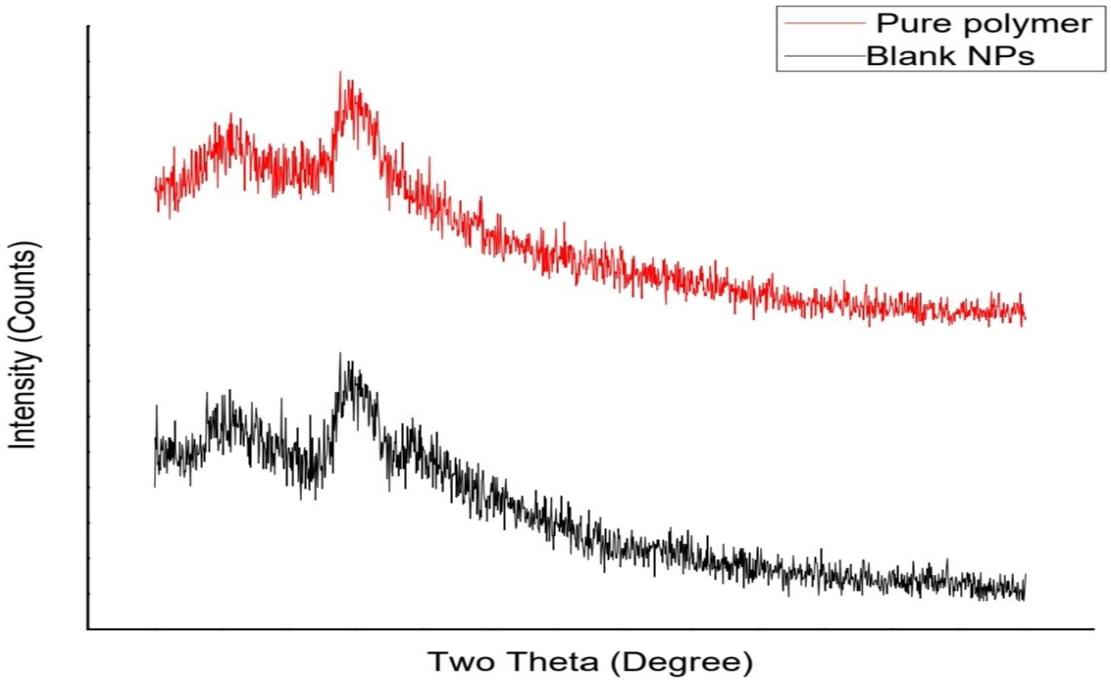


Fig.5.6.1 XRD graph of Polymer and Blank NPs

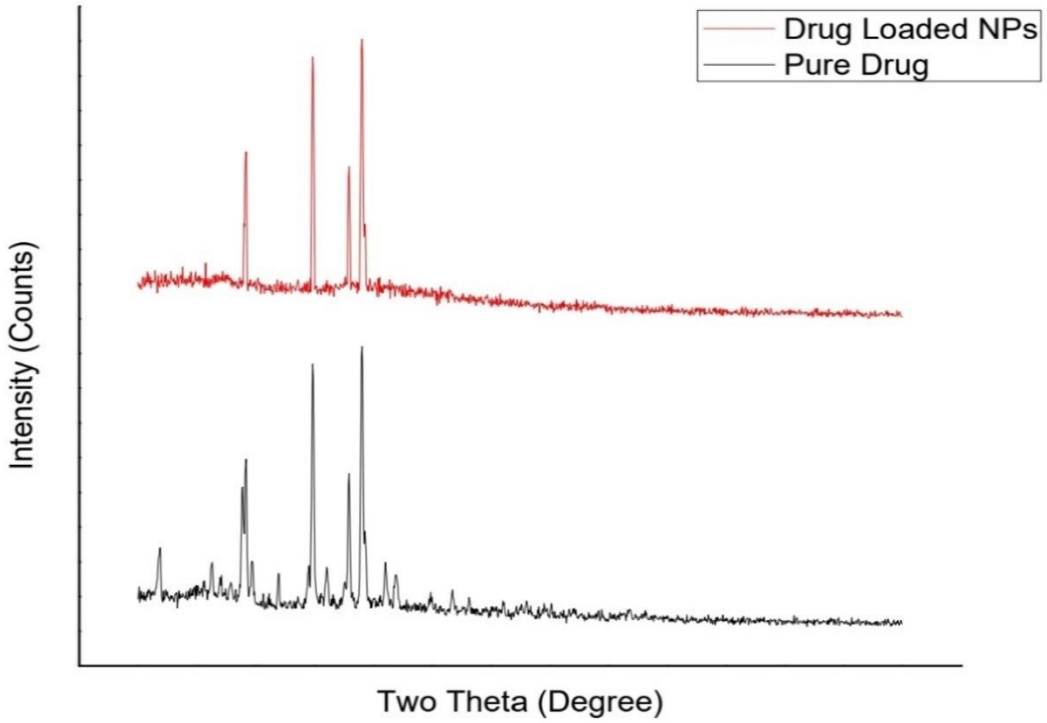


Fig.5.6.2 XRD graph of Drug Loaded NPs and Pure Drug

5.7. *In-vitro* drug release study using Franz Diffusion cell

Formulation2:

Table.5.7.1 *In-vitro* drug release study of F2

Time (mins.)	Concentration (per ml)	Concentration (in 40ml)	CAR (Cumulative amount of drug release) (µg)	CPR (Cumulative percentage of drug release) (%)
15	4.6847	196.7574	196.7574	3.93
30	6.3733	267.6786	277.048	5.54
45	8.7969	369.4698	391.5858	7.83
60	12.0754	507.1668	546.8766	10.93
90	18.1740	763.308	863.1802	17.26
120	18.1798	763.5516	899.7718	17.99
180	18.7001	785.4042	994.4056	19.88
240	17.8471	749.5782	1033.8018	20.67
300	18.166	762.972	1082.8898	21.65
360	18.4951	776.7942	1133.044	22.66
420	18.6344	782.6448	1175.8848	23.51
480	19.3210	811.482	1241.9408	24.83
540	19.6015	823.263	1292.4138	25.84
600	22.3056	936.8352	1445.189	28.90

Fig.5.7.1 *In-vitro* release of Metoprolol Succinate from NPs as Zero Order Model

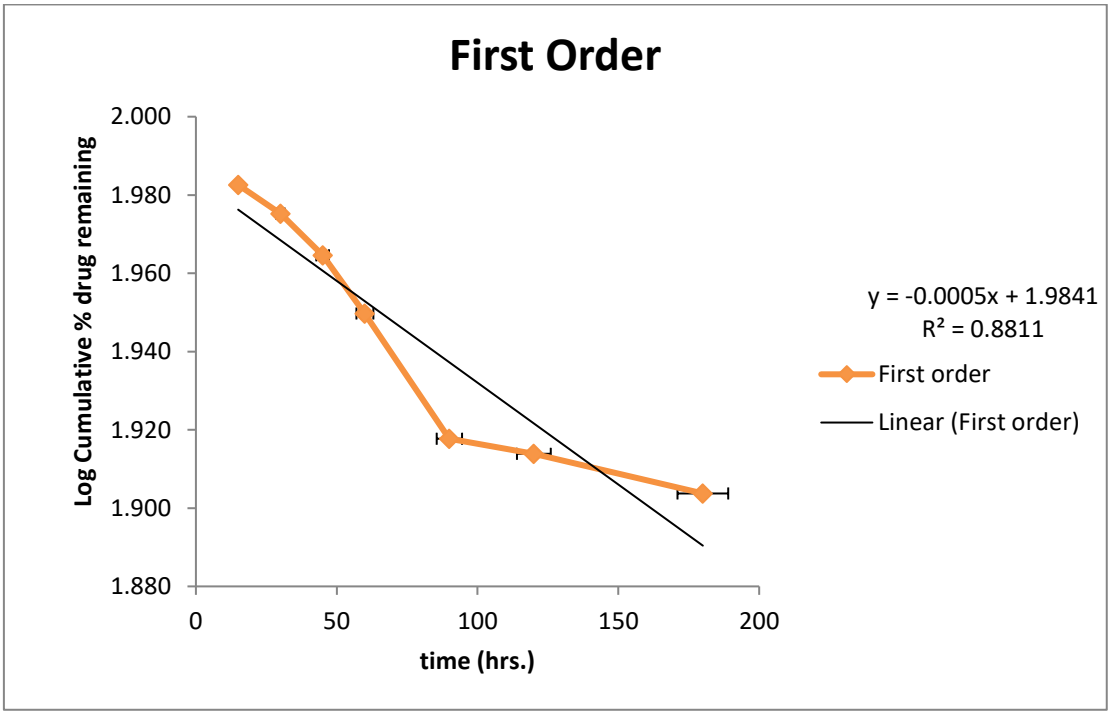


Fig.5.7.2 *In-vitro* release of Metoprolol Succinate from NPs as First Order Model

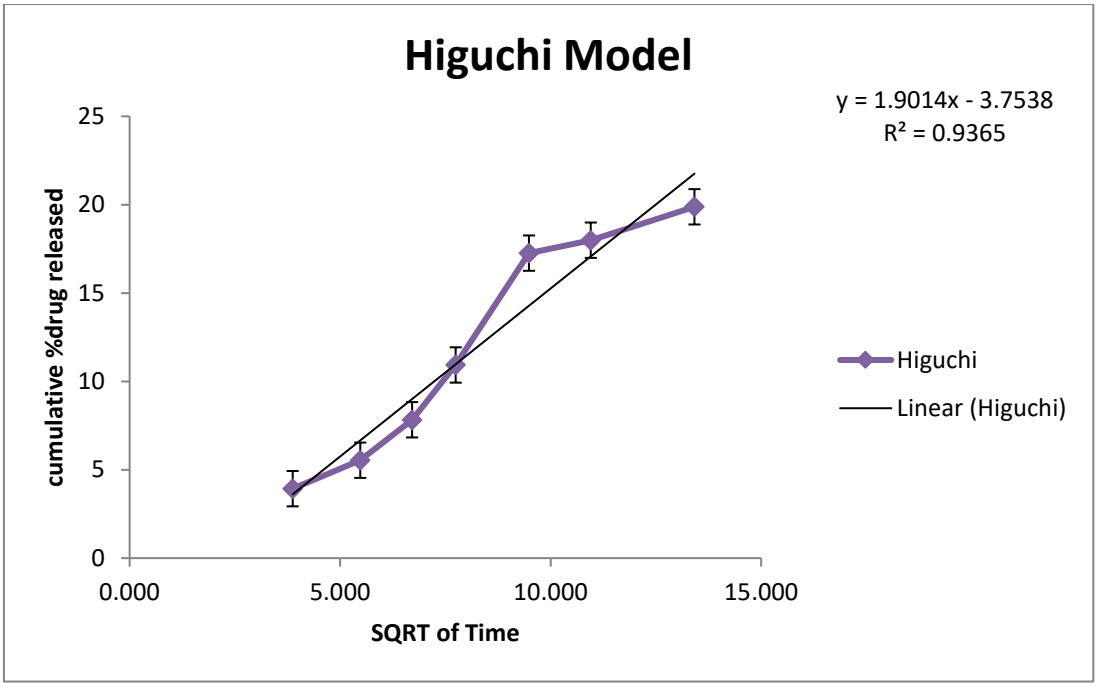


Fig.5.7.3 *In-vitro* release of Metoprolol Succinate from NPs as Higuchi Model

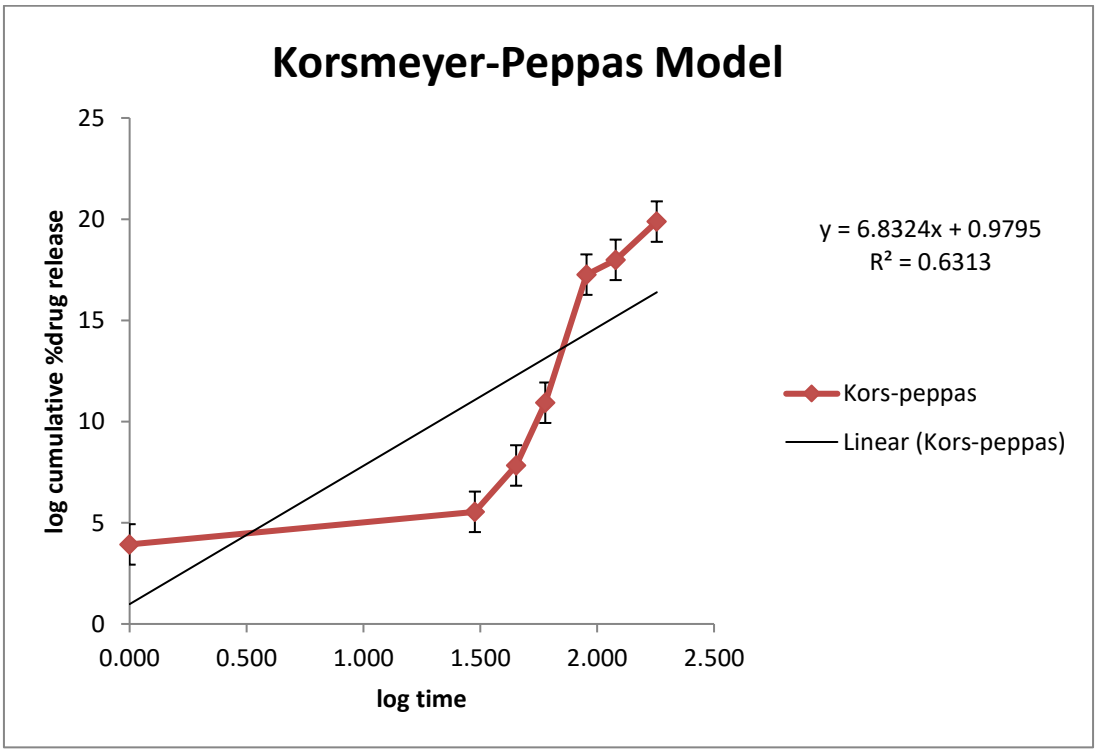


Fig.5.7.4 *In-vitro* release of Metoprolol Succinate from NPs as Korsmeyer- Peppas model

Formulation3:

Table.5.7.2 *In-vitro* drug release study of F3

Time	Concentration (per ml)	Concentration (in 40ml)	CAR (Cumulative amount of drug release) (µg)	CPR (Cumulative percentage of drug release) (%)
15	3.2630	137.0483	137.0483	2.74
30	3.4410	144.5222	151.0483	3.02
45	4.8568	203.9883	217.3964	4.34
60	6.4622	271.4158	294.5376	5.89
90	15.2940	642.3481	678.3944	13.56
120	18.5725	780.0464	846.6807	16.93
180	19.0348	799.4622	903.2415	18.06
240	19.0502	800.1121	941.961	18.83
300	18.9187	794.5880	974.5374	19.49
360	18.0657	758.7620	976.5489	19.53
420	19.0986	802.1431	1056.0615	21.12
480	19.1470	805.3114	1097.427	21.94
540	23.4816	986.2282	1316.6919	26.33
600	23.9071	1004.1005	1381.5274	27.63

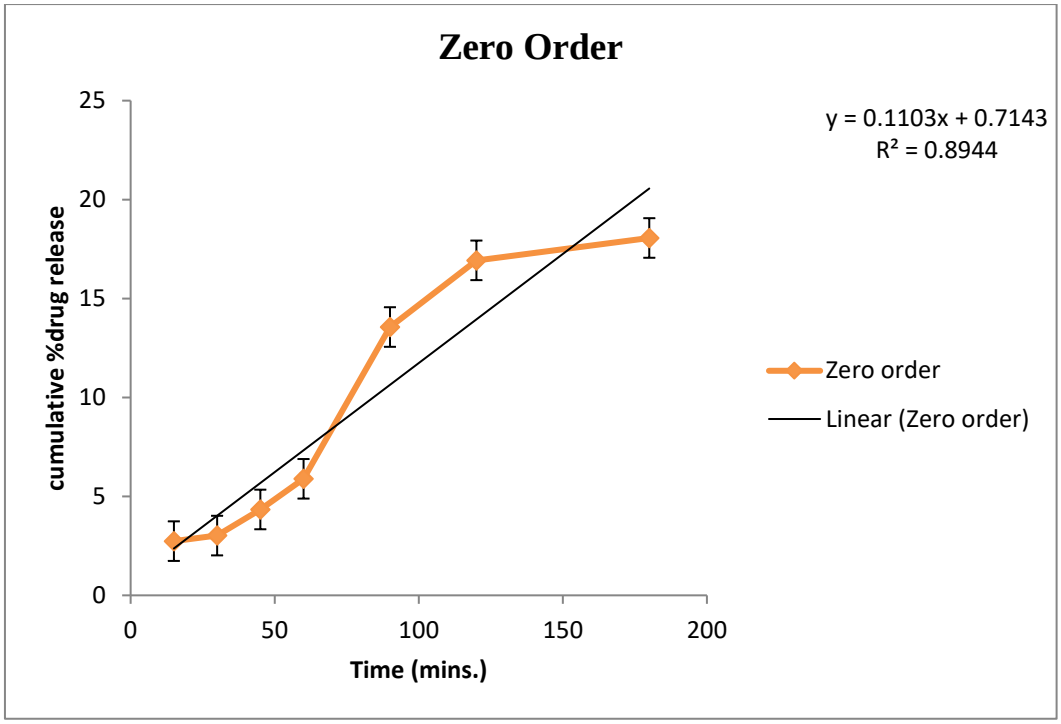


Fig.5.7.5 *In-vitro* release of Metoprolol Succinate from NPs as Zero Order Model

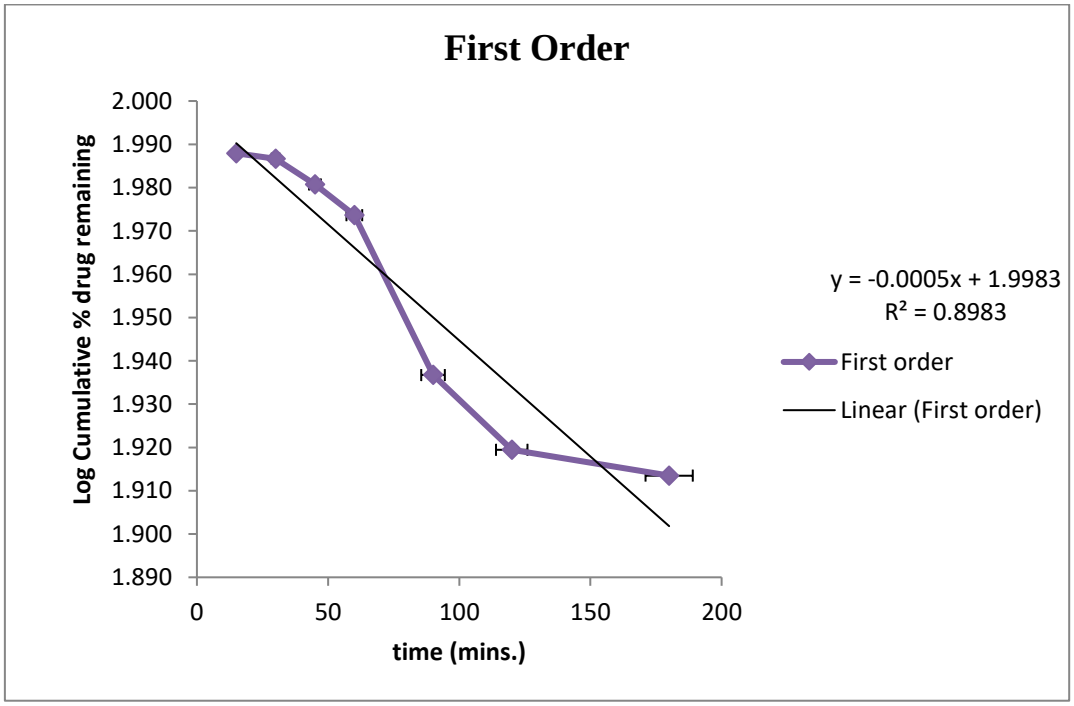


Fig.5.7.6 *In-vitro* release of Metoprolol Succinate from NPs as First Order Model

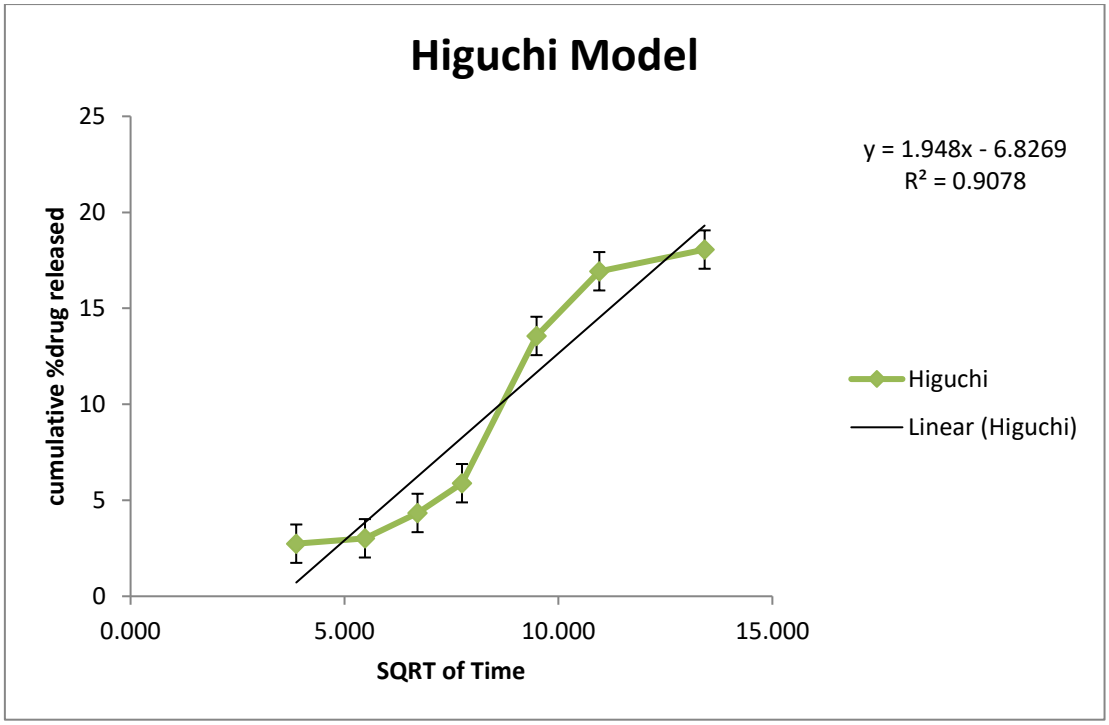


Fig.5.7.7 *In-vitro* release of Metoprolol Succinate from NPs as Higuchi Model

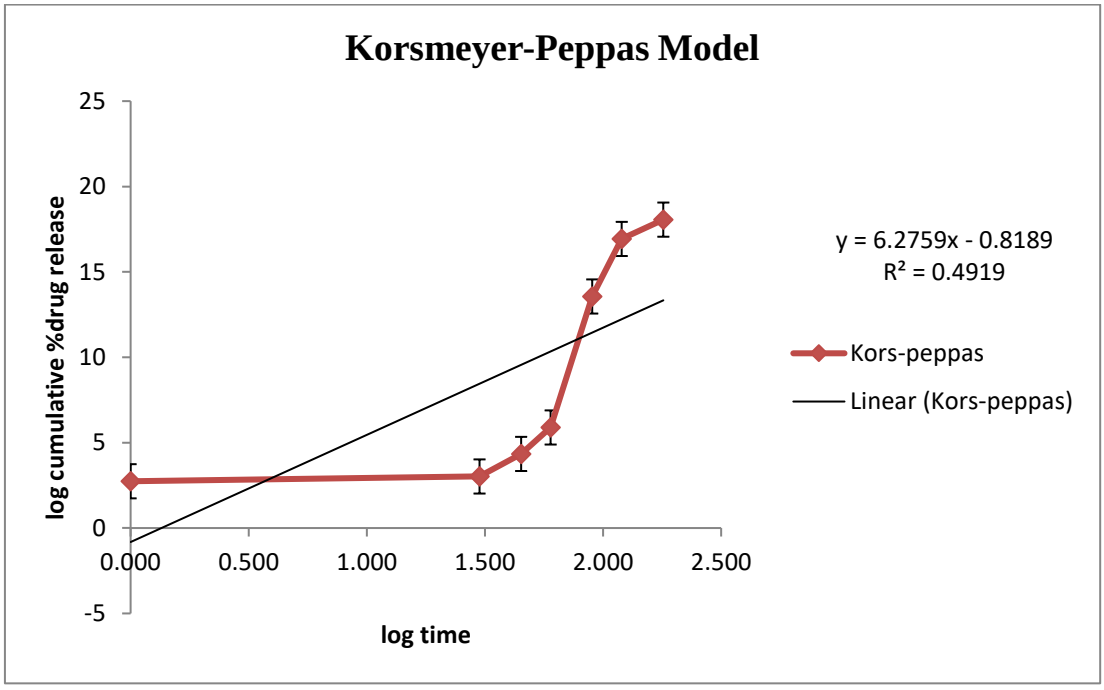


Fig.5.7.8 *In-vitro* release of Metoprolol Succinate from NPs as Korsmeyer- Peppas Model

Formulation4:

Table.5.7.3 *In-vitro* drug release study of F4

Time (mins)	Concentration (per ml)	Concentration (in 40ml)	CAR (Cumulative amount of drug release) (µg)	CPR (Cumulative percentage of drug release) (%)
15	16.1431	758.7272	758.7272	15.17
30	16.2727	764.8181	797.1043	15.94
45	16.3442	768.1818	833..0134	16.66
60	16.3887	770.2727	867.7928	17.35
90	17.1257	804.9090	935.2066	18.70
120	17.2011	808.4545	973.0035	19.46
180	18.0425	848	1046.9513	20.93
240	17.0928	803.3636	1038.4	20.76
300	17.1566	806.3636	1075.5856	21.51
360	17.8607	839.4545	1142.9898	22.85
420	18.4932	869.1818	1208.4385	24.16
480	17.2379	810.1818	1186.4249	23.72
540	17.6808	831	1241.7189	24.83
600	17.5371	824.2727	1270.3533	25.40

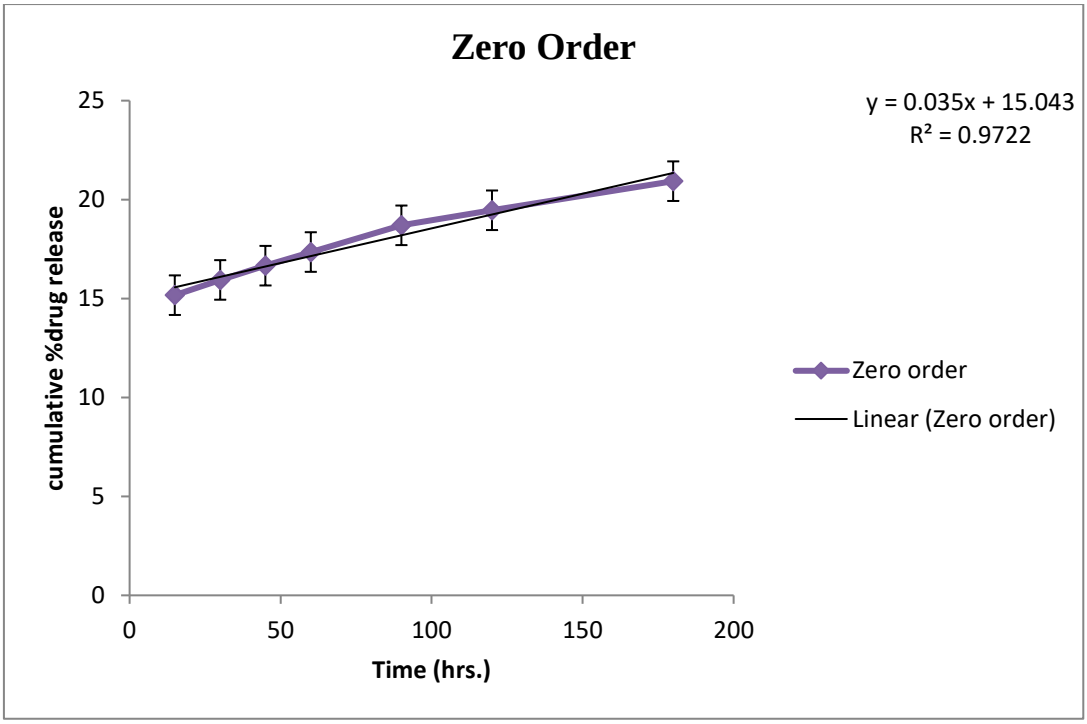


Fig.5.7.9 *In-vitro* release of Metoprolol Succinate from NPs as Zero Order Model

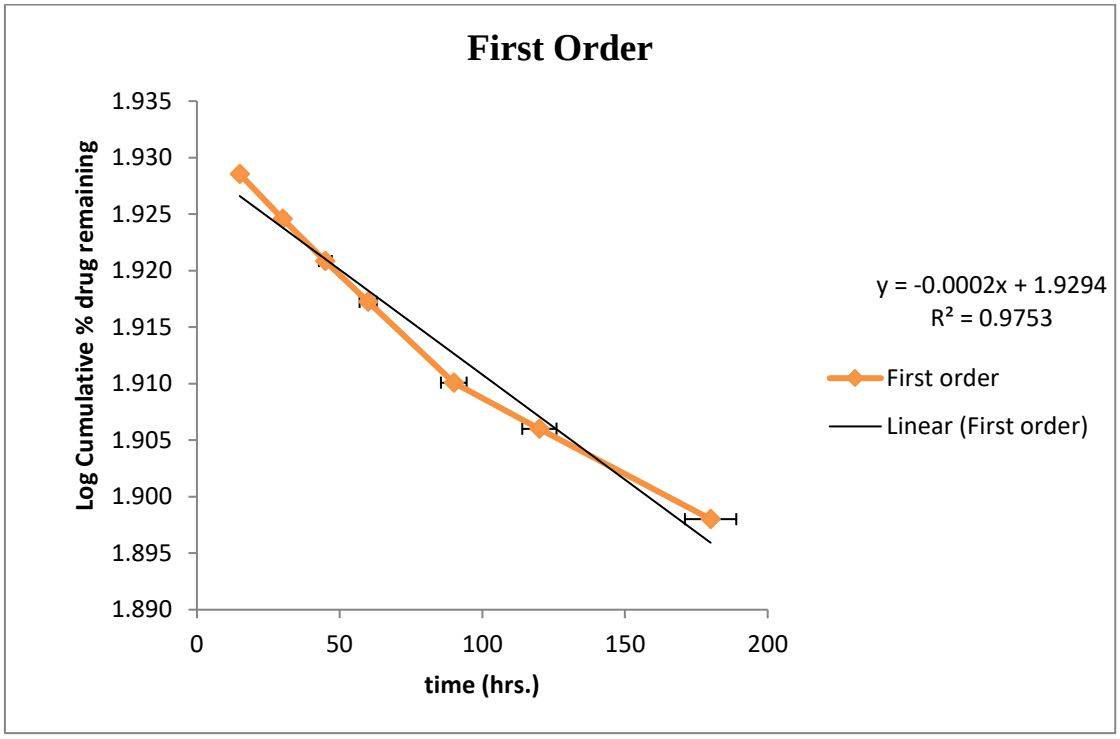


Fig.5.7.10 *In-vitro* release of Metoprolol Succinate from NPs as First Order Model

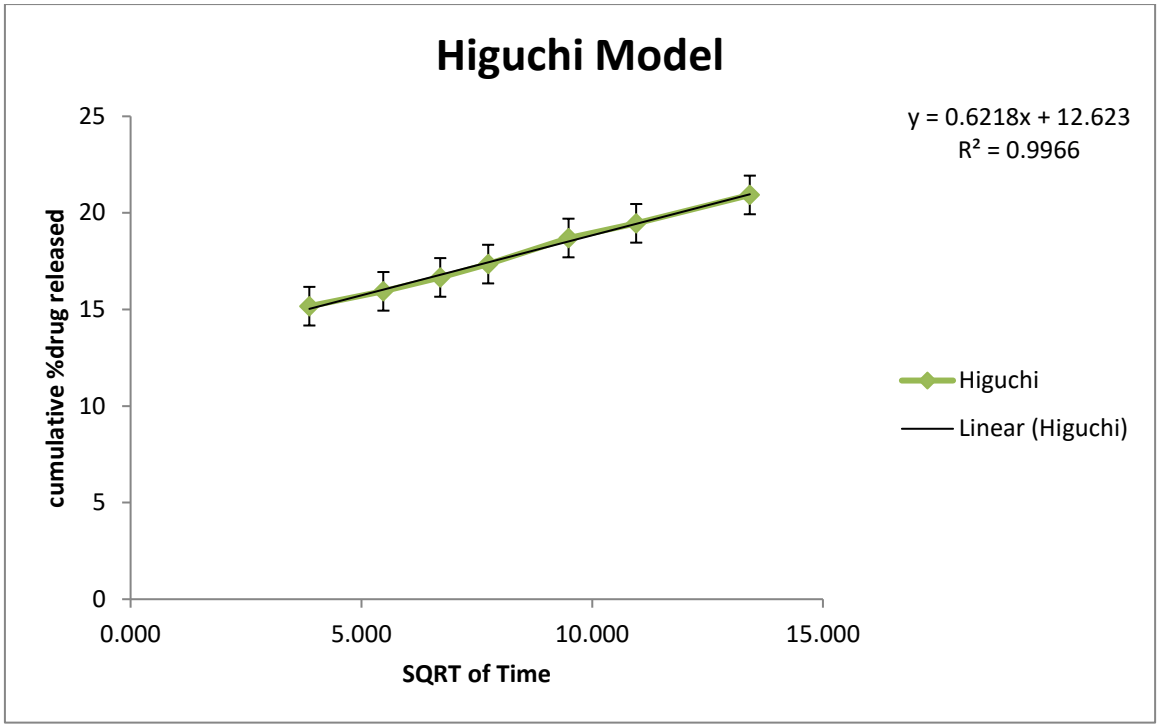


Fig.5.7.11 *In-vitro* release of Metoprolol Succinate from NPs as Higuchi Model

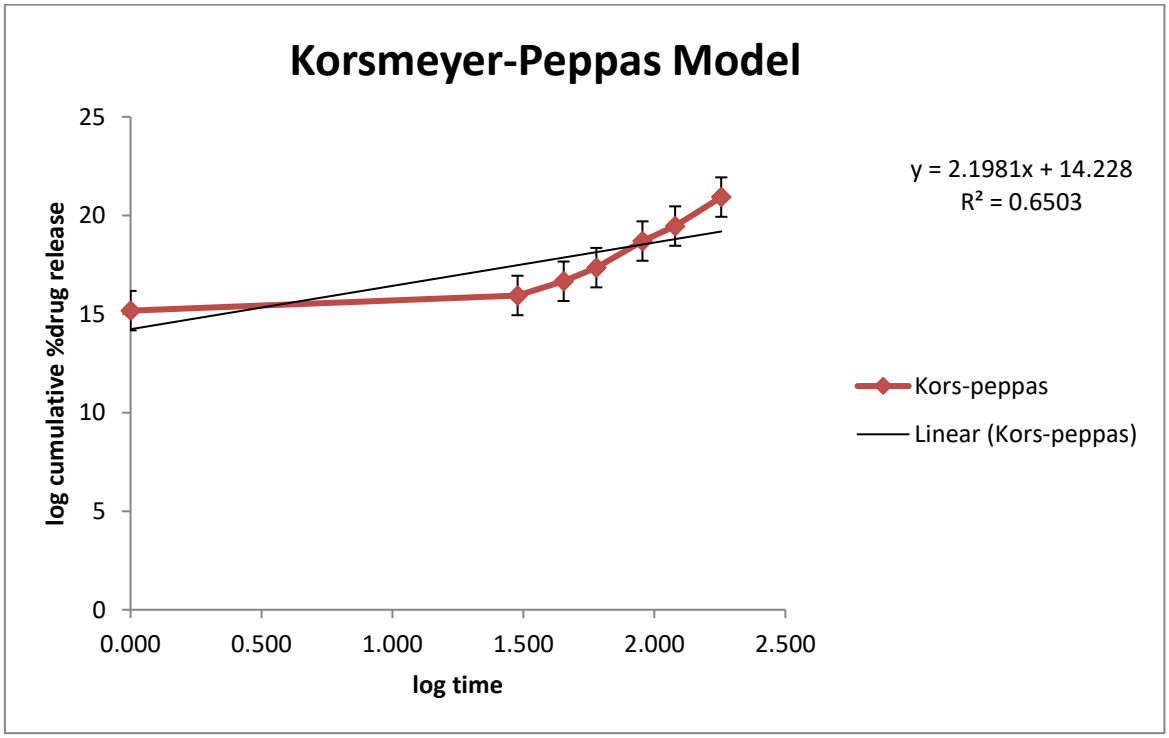


Fig.5.7.12 *In-vitro* release of Metoprolol Succinate from NPs as Korsmeyer- Peppas Model

Table.5.7.4 Comparative Release Profile

Model's	R^2 value
(Formulation2)	
(a)Zero Order	0.8733
(b)First Order	0.8811
(c)Higuchi Model	**0.9365**
(d)Korsmeyer- Peppas Model	0.6313
(Formulation3)	
(a)Zero Order	0.8944
(b)First Order	0.8983
(c)Higuchi Model	**0.9078**
(d)Korsmeyer- Peppas Model	0.4919
(Formulation4)	
(a)Zero Order	0.9722
(b)First Order	0.9753
(c)Higuchi Model	**0.9966**
(d)Korsmeyer- Peppas Model	0.6503

Table.5.7.5 Exponent n of the power law and drug release mechanism from polymeric controlled delivery systems

Exponent n slab	Sphere	Drug Release Mechanism
0.5	0.43	Fickian Diffusion
0.5<n<1.0	0.43<n<0.85	Anomalous Transport
1.0	0.85	Case II Transport

RESULTS & DISCUSSION

6. RESULTS & DISCUSSION

6.1. DETERMINATION OF ABSORPTION MAXIMA OF METOPROLOL SUCCINATE

The absorption maxima of the drug sample was determined by UV- Visible Spectroscopy. A 100µg/ml solution of Metoprolol Succinate was prepared using distilled water as solvent. This stock solution was scanned between 200 to 400 nm to identify the wavelength maximum and spectrum was obtained. The absorption maxima was found to be at 221 nm wavelength where absorbance was maximum at this wavelength as shown in Fig 5.1.1.[106]

6.2. CALIBRATION CURVE OF METOPROLOL SUCCINATE

The calibration curve of Metoprolol Succinate was obtained by plotting the different concentration in µg/ml of Metoprolol Succinate as shown in Table.5.2.1& Fig.5.2.1. The regression equation was calculated and was for the quantitative estimation of Metoprolol for drug loading. The correlation factor (R^2 value) was found to be 0.993. This shows the linearity of the calibration curve and suggests that the method is reliable for determining the concentration of Metoprolol Succinate in unknown samples. This calibration curve is critical for the quantification of Metoprolol Succinate in pharmaceutical products, ensuring accurate dosage determination and quality control in manufacturing processes.

6.3. FOURIER TRANSFORM INFRARED SPECTROSCOPY (FTIR)

FTIR spectral analysis was carried out in order to find out any possible chemical interactions between the drug and the polymer. The positions and the relative intensities of the absorption bands of Metoprolol Succinate loaded nanoparticles as well as that of the blank were compared with the Metoprolol Succinate and Polymer as shown in Fig.5.3.1. The polymer's FTIR spectrum shows C=O stretch around 1700 cm-1which is a strong absorption peak observed here corresponding to the carbonyl (C=O) stretching vibration; C-H stretch around 2800-3000cm-1, this region indicates the presence of aliphatic or aromatic hydrocarbons in the polymer. The Drug's FTIR spectrum shows N-H stretch around 3200-3500 cm-1 which is a broad peak in this region indicates the presence of amine or amide groups; O-H Stretch around 3200-3600 cm^{-1} shows the drug contains hydroxyl groups, this peak will appear broad and intense, possibly overlapping with the N-H stretch; C=O Stretch around 1650-1750 cm^{-1} shows a sharp peak here indicates the presence of carbonyl groups.[108] The Blank NPs spectrum shows

the peaks here should closely resemble those of the polymer since the blank nanoparticles are composed of the polymer without any drug. The C=O and C-H stretches should be prominent, with no additional peaks that would indicate the presence of the drug. The Drug loaded NPs FTIR spectrum shows shifted C=O Peak around 1700 cm^{-1} which may shift slightly compared to the pure drug or polymer. This shift can indicate an interaction between the drug and the polymer matrix, such as hydrogen bonding or ionic interactions. Peaks corresponding to the drug (e.g., N-H, O-H) should appear in the drug-loaded nanoparticles spectrum. These peaks might be slightly shifted or broadened compared to the pure drug, suggesting successful incorporation of the drug into the polymer matrix. The analysis of FTIR peaks reveals that the drug is successfully incorporated into the polymeric nanoparticles, as evidenced by the appearance and slight shifting of drug-related peaks in the drug-loaded nanoparticles spectrum. The observed shifts and broadening of peaks suggest interactions between the drug and the polymer, which are essential for stable drug encapsulation and controlled release properties.

6.4. PARTICLE SIZE DETERMINATION

The particle size distribution of Metoprolol Succinate was determined using dynamic light scattering (DLS) to assess the uniformity and suitability of the formulation of drug delivery. Different batches of Metoprolol Succinate loaded nanoparticles were prepared coded as F1, F2, F3, F4 by varying the concentrations of chitosan. The mean particle size of the formulations was between 23.88 to 146.1 nm as shown in Table.6.4.1.

The zeta potential of Metoprolol Succinate loaded chitosan nanoparticles were in the range of -22.42 mV to -10.14 mV as shown in Table.6.4.1. These values of zeta potential lie between +30mVto – 30mV which indicates that they are highly stable having strong electrostatic repulsion between particles and also the particles do not agglomerate.

6.5. LOADING EFFICIENCY (LD %)

F2 exhibited the highest loading efficiency at 44.08% suggesting that this formulation has a superior capacity to load the active ingredient effectively. F1 showed a lowest loading efficiency of 15.62%. F3 & F4 had significantly moderately loading efficiency of 20.78% and 22.79% respectively as shown in Table.5.5.1.

6.6. ENTRAPMENT EFFICIENCY (EE %)

F1 demonstrated an entrapment efficiency of 96.75% which suggests that the formulation is highly effective in encapsulating the active ingredient. F2 showed a slightly higher entrapment efficiency of 96.81%. F3 had an entrapment efficiency of 96.61% which is marginally lower than F1 and F2. Despite this, the formulation still exhibits high entrapment efficiency. F4 displayed the highest entrapment efficiency at 97.11%, suggesting that this formulation might have the most optimal conditions for encapsulation among the four tested formulations as shown in Table.5.5.1.[107]

6.7. X-RAY DIFFRACTION

Powder X-ray diffraction could provide further verification of drug crystal conversion. The PXRD pattern of pure drug Metoprolol, the polymer, the blank nanoparticles and the drug loaded nano particles were depicted in Figure 5.6.1 and 5.6.2. The diffraction pattern of pure drug showed characteristic high intensity peaks at 19.8, 19.85, 19.9, 19.95, 24, 24.05, 24.1 which indicates that the drug is present in the crystalline form, but in case of drug loaded nano particles, most of the peaks were absent indicating the transformation from crystalline to amorphous state.

6.8. *IN-VITRO* DRUG RELEASE STUDY USING FRANZ DIFFUSION CELL

The *in-vitro* release studies of formulations- F2, F3, F4; all the formulations prepared were evaluated by plotting amount of drug released vs. Time as shown in Fig.5.7.1- 5.7.12. The maximum Cumulative percentage of drug release of pure drug was found to be 76.95 % which was carried out up to 5 hrs and was discontinued because there was no increase in the release of drug after 5 hrs. The optimized formulation's CPR was found to be 28.90% within 10 hrs. F2 produces maximum release of 27.63 % which may be attributed due to lower concentration of polymer in initial nanoparticle preparation. F3 produces similar release profile as that of F2 however the CPR of F3 was found to be 28.90 % which shows that an incremental increase in concentration of polymer produces higher CPR. F4 produces release pattern which did not coincide with controlled release system thereby releasing the drug immediately after 1hr. This may be attributed to excessive polymeric concentration present leading to self-aggregation. The formulations were plotted against various kinetic models such as Zero Order, First Order,

Higuchi Model, Korsmeyer- Peppas Model. The drug release from all the formulations followed Higuchi model, as the plot observed between cumulative % of drug released vs. square root of time was found to be fit the model as compared to the other models. From the Higuchi model it can be said that the process of drug release is a diffusion process, as the correlation coefficient is 0.9078. The data was also analysed using Korsmeyer Peppas Model which is commonly used to describe drug release from polymeric system. The slope (m = 6.275) was calculated through linear regression of the plotted data (log cumulative % drug release vs. Log time). This slope corresponds to the release exponent (n) in the Korsmeyer-Peppas model. In this case n = 6.275, which is significantly higher than 1, indicating a super case-II transport mechanism, suggesting that the drug release is controlled by the relaxation or erosion from the nanocomposite.

CONCLUSION

7. CONCLUSION

This thesis successfully developed and evaluated Metoprolol Succinate loaded chitosan nanoparticles using the Ionic Gelation method, demonstrating their potential as an advanced drug delivery system for hypertension. The optimized nanoparticles exhibited desirable properties, including appropriate particle size, high encapsulation efficiency, and controlled drug release, which are essential for enhancing the therapeutic efficacy of Metoprolol Succinate.

In vitro studies revealed that the nanoparticles provided a sustained release of the drug up to 10hrs, potentially reducing the frequency of dosing and improving patient compliance. Stability testing confirmed that the formulation maintained its integrity under various storage conditions, indicating its suitability for long term use.

Overall, the findings of this research highlight the promise of chitosan-based nanoparticles as a novel and effective delivery system for Metoprolol Succinate. This work contributes to broader field of nanotechnology in drug delivery and set the stage for future studies aimed at further optimizing and validating this approach in vivo, with the ultimate goal of improving treatment outcomes for patients with hypertension. However, due to a lack of time, I was unable to complete all aspects of my studies as thoroughly as I would have liked. I wish I had more time to further refine and expand upon this research.

FUTURE SCOPE

8. FUTURE SCOPE

The research presented in this thesis on Metoprolol Succinate-loaded chitosan nanoparticles reveals several promising directions for future exploration. Initially, in vivo studies are essential to validate the findings from in vitro experiments. Assessing the pharmacokinetics, biodistribution, and therapeutic efficacy of these nanoparticles in animal models will provide crucial insights into their practical application and safety profile. These studies will help determine the real-world potential of the nanoparticles for improving drug delivery and therapeutic outcomes.

Moreover, the chitosan nanoparticle system holds promise for the encapsulation and delivery of other pharmacologically active compounds, potentially extending its application beyond Metoprolol Succinate. Future research should explore the system's versatility in delivering various drugs, particularly those with complex pharmacokinetic profiles, to enhance their bioavailability and therapeutic efficacy.

Further refinement of the nanoparticle formulation could involve experimenting with different crosslinking agents or incorporating additional polymers to improve characteristics such as drug loading capacity, release kinetics, and stability. Innovations in targeted delivery strategies, such as functionalizing nanoparticles with specific ligands or antibodies, could also enhance the precision of drug delivery, minimizing off-target effects and maximizing therapeutic outcomes.

Lastly, scaling up the ionic gelation method for industrial production is essential for the commercial feasibility of the nanoparticles system. Research should focus on optimizing the production process to ensure consistent quality and efficiency at a larger scale. Extended stability studies will also be necessary to determine the shelf life and storage conditions, ensuring the formulation's viability for long-term use.

Addressing these future directions will significantly advance the development of chitosan-based nanoparticles systems, potentially leading to more effective, targeted, and patient-friendly therapeutic solutions in hypertension and other areas of healthcare.

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