FORMULATION AND CHARACTERIZATION OF URAPIDIL LOADED CHITOSAN NANOPARTICLES FOR THE TREATMENT OF HYPERTENSION

A Dissertation submitted to

THE TAMILNADU DR.M.G.R. MEDICAL UNIVERSITY

CHENNAI-600 032.

In partial fulfillment of the requirements for the award of the degree of

MASTER OF PHARMACY
IN
PHARMACEUTICS

Submitted by

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COLLEGE OF PHARMACY
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TAMIL NADU

CERTIFICATE

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EXAMINERS

1.

Place: Chennai – 03

Date:

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Gratitude is the memory of the heart
-Jean Baptiste

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LIST OF ABBREVIATIONS

DDS	Drug Delivery System
NDDS	Novel Drug Delivery System
SDDS	Sustained Drug Delivery System
STPP	Sodium Tri PolyPhosphate
DSC	Different Scanning Colorimetry
FTIR	Fourier Transform Infra-Red spectroscopy
SEM	Scanning Electron Microscopy
TEM	Transmission electron microscopy
XRD	X-ray Diffraction Spectroscopy
UV	Ultra Violet Spectroscopy
PDI	Poly Dispersity Index
CS	Chitosan
NPs	Nanoparticles
BCS	Biopharmaceutical Classification System
RH	Relative Humidity
WHO	World Health Organization
C-NPs	Capsulated Nanoparticle

LIST OF SYMBOLS

cps	Centipoise
nm	Nanometer
cm	Centimeter
mV	Milli volt
kV	kilo volt
mA	Milliampere
µg	Microgram
ml	Millilitre
λmax	Maximum Wavelength of Absorption
mg	Milligram
g	Gram
sec	Seconds
min	Minute
h	Hours
%	Percentage
^{o}C	Degree Celsius
t	Time
k0	Zero order constant
h	Height
r	Radius
rpm	Revolution per Minute

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1. INTRODUCTION

1.1. DRUG DELIVERY SYSTEM

A drug delivery system (DDS) is defined as a formulation or a device that enables the introduction of a therapeutic substance in the body and improves its efficacy and safety by controlling the rate, time and place of release of drugs in the body. This process includes the administration of the therapeutic product, the release of the active ingredients by the product, and the subsequent transport of the active ingredients across biological membrane.[1]

The objective of any drug delivery system is to deliver a therapeutic amount of drug to the sites of action and to maintain the desired amount of drug level in the tissue or the body that can elicit a desired pharmacological effect without causing any serious adverse reactions.

A perfect drug delivery system possesses two elements: the capacity to target and to control the release of drug. Targeting will ensure high efficiency of the drug and reduce the side effects, especially when dealing with drugs that are presumed to kill cancer cells but can also kill healthy cells when delivered to them. The side effects can be reduced or prevented by the control of drug release.[2]

Drawbacks associated with conventional dosage forms[3]

An ideal dosage regimen in the drug therapy of any disease is one which immediately attains the desired therapeutic concentration of drug in plasma and maintains it constant for the entire duration of treatment. This is possible through the administration of drug delivery system in a particular dose and at particular frequency. The frequency of administration or dose interval of any drugs depends upon its life or mean residence time and its therapeutic index. In most cases, dosing interval is much shorter than the half-life of the drug, resulting in number of limitations associated with such a conventional dosage form which are,

- A drug with short biological half-life which needs a close succession

- The uncontrolled fluctuation of drug level may leads to either below effective range or over the effective range.
- Plasma concentration verses time profile of dosage form and it is difficult to achieve the steady state active drug level.
- The rise and fall of drug levels may give to accumulation of adverse effects, especially for a drug having less therapeutic index.

To overcome the above drawbacks, drug delivery system capable of controlling the rate of drug delivery, sustain the duration off therapeutics action or targeting the drug to a particular tissue was developed.

Reasons for development of drug delivery system[4]

Over the past three decades, a great deal of attention has been focused on the development of new drug delivery system. There are many reasons for the interest into this drug delivery system. They are

- As bringing new drug entities into the market is an expensive and time consuming process, development of new drug delivery is profitable.
- New system is needed to deliver novel, genetically engineered pharmaceuticals such as protein and peptides to their sites of action without biological inactivation.
- To improve therapeutically efficacy and safety of conventional drug by reducing size and number of doses.

1.2 NOVEL DRUG DELIVERY SYSTEM (NDDS)

Novel drug delivery (NDDS) is advanced drug delivery system which improves drug potency, control drug release to give a sustained therapeutic effect, provide greater safety; finally to target a drug specifically to a desired tissue. The basic goal of novel drug delivery system is to achieve a steady state blood or tissue level that is therapeutically effective and non-toxic for an extended period of time.

Conventional drug delivery involves the formulation of drug into suitable form, such as compared tablet for oral administration or a solution for IV administration. These

dosage forms have serious limitation in terms of higher doses required low effectiveness, toxicity and adverse effects.

NDDS are being developed rapidly, so as to overcome the limitation of conventional drug delivery[4]. Therapeutic benefits on novel drug delivery system over conventional dosage form.

1. Increased efficacy of drug
2. Site specific delivery
3. Decreased toxicity /side effects
4. Increased convenience
5. Shorter hospitalization
6. Viable treatment of previously incurable diseases
7. Potential for prophylactic application
8. Lower health care costs
9. Better patient compliance

The reasons for intense interest in NDDS is due to

- The recognition of the possibility of repeating successful drugs by applying concepts and techniques of controlled release drug delivery systems, coupled with the increasing expense in bringing new drug entities to market has encouraged the development of NDDS.
- NDDS are needed to deliver the novel, genetically engineered pharmaceuticals i.e. Proteins and peptides to their sites of action without incurring significant immunogenicity or biological inactivation.
- Treating enzymes deficient diseases and cancer therapies can be improved by better drug targeting.
- Therapeutics and safety drug administration by conventional route can be improved by more precise spatial and temporal placement within the body.[5]

Present scenario and future of NDDS[6]

In India major areas of research and development within the body for NDDS are

- Nanoparticles
- Liposomes
- Transdermal drug delivery
- Implants
- Oral system
- Micro encapsulation/ microcapsules
- Polymers in drug delivery.

Two prerequisites would be required to imagine the ideal drug delivery system. First it would be a single dose for the duration of treatment, whether it is for days or weeks as in the case of infection or for the life time of the patients, as in hypertension or diabetes. Second it should deliver the active entity directly to the site of action, thus minimizing the side effects. It is obvious that this imaginary delivery system will have changing requirements for different diseases states and different drugs. Hence, will able to deliver the therapeutic agent to a specific site for a specific period of time.

Non immediate release delivery systems may be divided into four categories

- Delayed release
- Sustained release, controlled release
- Prolonged release
- Targeted drug delivery
- Receptor release

Terminology

- **Sustained release**

  These drug delivery systems are designed to achieve a prolonged therapeutic effect by continuously releasing medication over an extended period of time after administration of single dose of drugs.

- **Controlled release**

  Controlled release dosage forms release the drug at constant rate which is predictable and also the release rate is reproducible from one unit to another. It provides plasma concentration that remains invariant with time.

- **Prolonged release**

  Prolonged release indicates that the drug is provided for absorption over a longer period of time than from a conventional dosage form. However there is an implication that onset is delayed because of an overall slower release rate from the dosage form.

- **Extended release**

  Extended release dosage forms release the drug slowly, so that plasma concentration is maintained at a therapeutic level for a prolonged period of time.

- **Delayed release**

  Delayed release indicates that the drug is not being released immediately following an administration but at latter time. E.g. Enteric coated tablets, Pulsatile release capsules.

- **Repeat action**

  These are the alternative system of sustained release which contains multiple doses of drug within the dosage form, and each dose is released at regular intervals.

- **Timed release**

  Timed release drug delivery system are used to obtain the drug release after a lag time of about 4-5 h. Enteric coated dosage forms of cellulose acetate phthalate are designed to provide protection in the stomach. Application of a thick coat causes a delay in the drug release in small intestine and delays the drug release. This time controlled drug release may be retarded up to 5h and targets the drug to the colon.

- **Site specific and receptor release**

  They are designed to target the drug directly to a certain biological location. In the case of site-specific release, the drug directly target to a certain organ or tissue, while in receptor release, the target on the particular receptor within the organ tissue.

1.3 SUSTAINED RELEASE PREPARATION[7]

Traditional drug delivery system has been characterized by immediate release and repeated dosing of the drug which might lead to the risk of dose fluctuation, this arises the need of a formulation with control release that maintain a near-constant or uniform blood level. Sustain release with the introduction of extended release matrix tablet have proved to be an effective tool to control the release of drug without involving the complex production procedures. By the sustained release method therapeutically effective concentration can be achieved in the systemic circulation over an extended period of time, thus achieving better compliance of patients.

Numerous sustained release oral dosage forms such as membrane controlled system, matrixes with water soluble/insoluble polymer or waxes and osmotic systems have been developed, intense research has recently focused on the designation of SR systems for poorly water soluble drugs.

However generating such a system requires certain consideration of which the half-life and the pharmacological action of the drug form an essential part. But making a consideration of the drawbacks seen with conventional drug delivery system (repeated dosing and dose fluctuation) the sustain release helps to achieve the following goals:

- Uniform release of drugs over prolong period of time
- Reduced dosing frequency
- Less fluctuating blood levels

In many instances, conventional method is more preferred to deliver the drug, but some drugs are unstable and toxic by frequently dosing. These kinds of drug have narrow therapeutic range and face solubility difficulties. In such cases, sustained drug delivery systems are used, which maintain the drug plasma level in the therapeutic index.

FACTORS AFFECTING THE DESIGN OF ORAL SUSTAINED RELEASE DOSAGE FORM

A) Pharmacokinetics and Pharmacodynamics factor:

1. **Biological half-life**

   Drug with biological half-life of 2-8 hrs are considered suitable candidate for sustain release dosage form, since this can reduce dosing frequency. However this is limited in that drugs with very short biological half-life may require excessive large amount of drug in each dosage unit to maintain sustained effects, forcing the dosage form itself to become limitingly large.

2. **Absorption**

   Rate of absorption of a sustained formulation depends upon release rate constant of the drug from the dosage form, and for the drugs that are absorbed by active transport the absorption is limited to intestine.

3. **Distribution**

   The distribution of drugs into tissues can be important factor in the overall drug elimination kinetics. Since it not only lowers the concentration of circulating drug but it also can be rate limiting in its equilibrium with blood and extra vascular tissue, consequently apparent volume of distribution assumes different values depending on the time course of drug disposition. Thus for design of sustain release products, one must have information of disposition of drug.

4. **Metabolism**

   The metabolic conversion of a drug is to be considered before converting into another form. Since as long as the location, rate, and extent of metabolism are known a successful sustain release product can be developed.

B) Drug properties relevant to sustain release formulation:

1. **Dose size**

   A dose size of 500-1000mg is considered maximal for a conventional dosage form. This also holds true for sustained release dosage forms. Since dose size consideration serves to be a parameter for the safety involved in administration of large amounts with narrow therapeutic range.

2. **Ionization, pka and aqueous solubility**

   Most drugs are weak acids or bases and in order for a drug to get absorbed, it must dissolve in the aqueous phase surrounding the site of administration and then partition into the absorbing membrane.

3. **Partition coefficient**

   Bioavailability of a drug is largely influenced by the partition coefficient, as the biological membrane is lipophilic in nature transport of drug across the membrane largely depends upon the partition coefficient of the drug. Drugs having low partition coefficient are considered as poor candidate for the sustained release formulation as it will be localized in the aqueous phase e.g.; Barbituric acid and vice versa

4. **Drug stability**

   When drugs are orally administered, they come across acid-base hydrolysis and enzymatic degradation. In this case, if the drug is unstable in stomach, drug release system which provides medication over extended period of time is preferred, whereas in contrast the drug unstable in intestine will face problem of less bioavailability.

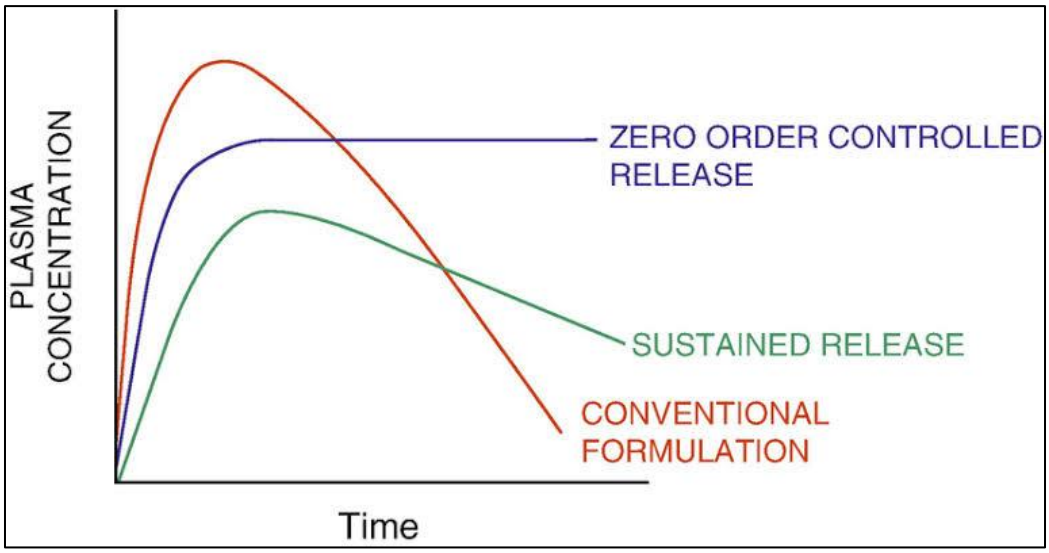


Figure 1.1: Comparison of conventional and controlled release profiles

APPROACHES IN SUSTAINED RELEASE DRUG DELIVERY SYSTEM

1. Dissolution controlled release system
2. Diffusion controlled release system
3. Dissolution and diffusion controlled release systems
4. Ion exchange resin-drug complexes
5. pH dependent formulation
6. Osmotic pressure controlled systems

MERITS OF SUSTAINED RELEASE DOSAGE FORMS

CLINICAL MERITS

1. Decreased local and systemic side effects
2. Reduced gastrointestinal irritation
3. Better drug utilization
4. Reduction in total amount of drug used
5. Minimum drug accumulation on chronic dosing
6. Improved efficiency in treatment
7. Optimized therapy
8. Less reduction in drug activity with chronic use
9. Improved patient compliance

COMMERCIAL MERTIS

1. Chances of illustration of innovative / technological leadership
2. Extension of product life cycle
3. Differentiation of product
4. Expansion of market
5. Extension of patent

DEMERITS OF CONVENTIONAL RELEASE DOSAGE FORM

1. If the drug has short half-life, it has to be administered frequently, so there are chances of missing the dose.
2. If the drug is not taken at periodic interval, peak valley plasma concentration time profile obtained is not steady.
3. The fluctuations of drug plasma level that occurs during conventional release may produce under medication or overmedication.
4. Poor patient compliance

1.4 NANOTECHNOLOGY

Nanotechnology is a rapid expanding field, encompassing the development of material with 5-200 nanometers in size. It has a wide range of applications in the fields of engineering, medicine and life sciences. Advances in nanotechnology were utilized in medicine for therapeutic drug delivery and treatment for a variety of diseases and disorders. The drug is dissolved and entrapped into biodegradable nanoparticles which are specially designed to absorb drug and protecting it against chemical and enzymatic degradation.[8]

History

The concept of using nanoparticles as vehicle for the drug delivery was first developed by speiser and khanna in the late 1960s and early 1970. Speiser first focused on the development of nanoparticles for vaccination purpose(tetanus, diphtheria) and other infections require multiple injections to build up antibody levels in the body that are sufficient for protection. It was hoped that due to the sustained release properties of nanocapsules or nanoparticles a constant immune stimulation would be achieved and only one injection would be required to achieve the necessary antibody response.

Gerd Birrenbach and jorgkreuter Helmut Kopf who student of speiser worked for further development works. Helmut Kopf was Peter Speiser's second graduate student working on the same process. However, his task was to obtain the first nanoparticles for sustained intravenous drug delivery. He bound nor ephedrine Hcl and 1 [4-(2-

biphenyloxy)-butyl] - 1 – ethypiperidinium bromide into the acrylamide nanoparticles and also performed a thorough chemical and physiochemical investigation of the nanoparticles and of the production process including a determination of residual monomers.

Another pioneer in nanoparticles, Patrick Couvreur joined Professor Speiser's group for a couple of month in 1976. In Zurich he worked on the process developed by Birrenbach and Speiser, and after his return to Brussels found the lysosomotropic effects of the nanoparticles. Shortly after this he produced the first rapidly biodegradable acrylic nanoparticles made of poly (methyl cyanoacrylate) and poly (ethyl cyanoacrylate). Most research with nanoparticles was carried out in Europe, the first product; Abraxane TM was put on the market by a US company, Abraxis Oncology. Still nanoparticles are under investigation for further development and application.[9]

Nanoparticulate Drug Delivery System[10]

Nanoparticles (NP) are colloidal drug delivery systems which are formulated by natural, synthetic and semi synthetic polymers. Particle size of NP ranges from 10nm to 1,000 nm in diameter. This colloidal drug delivery system shows different inner structure. Depending upon to the method of preparation, nanoparticles, nanospheres or nanocapsules can be obtained. Nanocapsules are systems in which the drug is confined to a cavity surrounded by a unique polymer membrane, while nanospheres are matrix systems in which the drug is physically and uniformly dispersed.

- Nanospheres in matrix type system
- Nanocapsules in reservoir type system

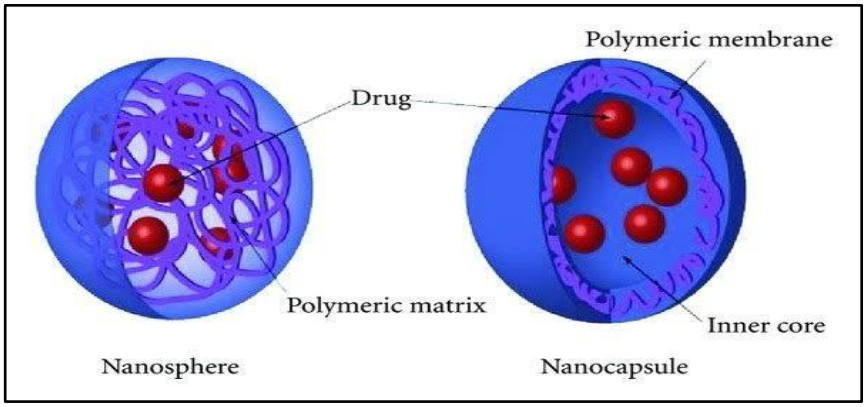


Figure 1.2: Types of Nanoparticles system

Nanoparticles can be used in targeted drug delivery at the site of disease to improve:

- The uptake of poorly soluble drugs
- The targeting if drugs to specific site
- Drug bioavailability.

Drug release from Nanoparticles

The nanoparticle is coated by polymer, which release the drug by controlled diffusion or erosion from the core across the polymeric membrane or matrix. The membrane coating acts as a barrier to release, therefore the solubility and diffusivity of drugs in polymer membrane becomes the determining factor in drug release. In general, drug release rate depends on

- ✓ Solubility of drugs
- ✓ Desorption of the surface bound/ adsorbed drug
- ✓ Drug diffusion through the nanoparticle matrix
- ✓ Nanoparticle matrix erosion/ degradation
- ✓ Combination of erosion / diffusion process.

Thus solubility, diffusion and biodegradation of the matrix materials govern the release process.

1.5 NANOPARTICLES

The solid colloids microscopic particles with size ranging between 10-1000 nm are known as nanoparticles. They can be defined as system which contains active ingredients dissolved encapsulation or adsorbed in matrix materials which are used as target delivery system. To see the effect of drug in target tissue, to increase stability against degradation through enzymes and for solubilization at intra vascular route they are formulated in the form of injection which consist spherical amorphous particle.

Formulations are less toxic in nature because in this co solvent is not used to solubilize the drug. Eukaryotic or prokaryotic cells had been founded to be larger in size than nanoparticles but for comparisons of their size they belong to size of virus or

antibody. During the designing of nanoparticle some control has to be taken such as their release pattern, their size and surface properties which determine site specific action at optimal rate with right dose regimen.

Advantages of nanoparticles

- The allowable size of nanoparticle to be administered via intravenously unlike colloidal system which would occlude in blood capillaries and needle.
- Due to its small size than microspheres and liposomes, they can easily pass through the sinusoidal spaces in the bone marrow and spleen as compared to other system with long circulation time.
- Due to their larger surface area, nanoparticles have higher loading capacity.
- It reduces the toxicity of liver.
- Nanoparticles increase stability of drug/protein against enzymatic degradation.
- Nanoparticles are safe and effective in site specific and target drug delivery system.
- To enhance the targeting moieties by adhering monoclonal antibodies with nanoparticles for specificity.
- It improves the solubility of poorly water soluble drugs.
- It improves bioavailability by reducing the fluctuations in the therapeutic ranges.
- They offer controlled rate of drug release and particle degradation characteristics that can be readily modulated by the choice of matrix constituents.[11]
- Suitable for combination therapy where two or more drug can be co- delivered.
- Both hydrophobic and hydrophilic drug can be incorporated.
- Development of new medicines which are safer.[12]

Disadvantage of nanoparticles[11]

1. The manufacturing costs of nanoparticle are high resulting in overall product cost.
2. Solvents are toxic in nature which is used in the preparation process.
3. Can start immune response and allergic reactions in body.
4. Extensive use of poly vinyl alcohol as stabilizer may have toxicity issues.
5. Nanoparticles are difficult to handle in physical form because of particle-particle aggregation occurs due their small size and large surface area.

Types of Nanoparticles[13]

Polymeric Nanoparticles: Biodegradable nanoparticles, as effective drug delivery system, are being applied extensively over the past few decades. Nanoparticles formulated from various natural and synthetic polymers have gained importance. This drug delivery system provides targeted drug delivery, increased bio-availability, and sustained release of drugs and protects from enzymatic degradation.

Fullerenes: A fullerenes is a molecule made of carbon in different shapes such as tubes, hollow-spheres and ellipsoid. Fullerene is similar to graphite in structure.

Nanotubes: Nanotubes (NTs) are cylindrical fullerenes. NTs have a closed end as well as open end. Fullerenes show various therapeutic properties such as targeting cancerous cells, binding specific antibiotic to the specific structure of bacteria etc.

Solid lipid Nanoparticles (SLNs): SLNs are lipids in nature which remains in solid phase at normal room temperature. SLNs are composed of solid hydrophobic core and a single coating layer of phospholipids. SLNs are stabilized by different surfactants for emulsification and also show many properties such as increased bio-degradability, increased bio-availability and drug targeting in the brain. SLNs have vast applications in cancer. SLNs have ability to accumulate tumor and also allow anticancer drugs delivery to the brain.

Super Paramagnetic Nanoparticle: Theses are attracted towards a specific magnetic field. When the magnetic field is removed, these cannot retain their residual

magnetism. Particle range in the size of 5nm to 100nm and used for selective magnetic bio-separation and can be visualized in magnetic resonance imaging (MRI). These work on the principle of magnetic field and heated to trigger the drug release. These have also shown major role in cancer therapy and diagnosis.

Nanostructure lipid carriers (NLC): NLC are prepared by using blend of solid lipids and liquid lipids. The particles remain in solid state at normal room temperature. Nanostructure lipid carriers (NLC) and the lipid drug conjugate (LDC) nanoparticles are prepared in the form of matrixes. These matrixes increase drug loading capacity and bio-availability. These also have applications in the fields of cosmetics, food, agricultural and used in the delivery of anti-inflammatory drugs.

Nanoshells: Nanoshells also known as core-shells are spherical cores of concentric particles which are surrounded by an outer coating of thin layer of another material. Nanoshells have biomedical imaging and therapeutic applications.

Gold Nanorods: gold nanorods were first prepared in mild-1990. This exhibit distinct optical and electronic properties and depends on shape, size and aspect ratios. These can be easily stabilized and conjugated to antibodies.

Quantum Dots (QD): The Quantum Dots are known as semiconductor Nano-crystals and core-shell. These are 2 nm to 10 nm in size. These are used as drug delivery system for various hydrophilic drugs such as small interfering RNA and anti-sense oligo-deoxy-nucleotide as well as targeting antibodies, peptides etc. QD have extensive applications in imaging contrast.

Ceramic nanoparticles: Ceramic nanoparticles are porous in nature and particle size is less than 50 nm. These possess distinct properties such as sol-gel process, work in ambient temperature condition and product produced of desired size, shape and porosity as well as effective in hiding the uptake by reticulo endothelial system.

Nanofibres: Nanofibres are produced by electro spinning technique in which fabrication of polymers in a fine and dense mesh works directly drop solution and requires an electric field. These have dimension less than 100 nm as mentioned. Polymeric

nanofibres are effective carriers for drug delivery and show advantages such as specific surface with small pore size, porosities, reduced toxicity and increased therapeutic level and bio-compatibility.

Nanoerythrosomes: Nanoerythrosomes are derived from a red blood cell membrane by the process of haemo-dialysis through filter. Nanovesicles are of defined pore size and composed of proteins, phospholipids and cholesterol. These can load a variety of biologically active agents such proteins. Nanoerthroysomes composed of a natural membrane allows the insertion of recombinant ligands along with better stability.

POLYMERIC NANOPARTICLES[14]

Polymeric nanoparticles (NPs) are particles within the size range from 1 to 1000nm and can be loaded with active compounds entrapped within or surface adsorbed onto the polymeric core. Polymeric NPs have shown great potential for targeted delivery of drugs for the treatment of several diseases. Advantages of polymeric NPs as drug carriers include their potential use for controlled release, the ability to protect drug and other molecules with biological activity against the environment, improve their bioavailability and therapeutic index.

Polymers used in preparation nanoparticles[15]

The polymers should be compatible with the body in the terms of adaptability (non-toxicity) and (non-antigenicity) and should be biodegradable and biocompatible.

Natural polymers: The most commonly used polymers in preparation of polymeric nanoparticles are

- Chitosan
- Gelatin
- Sodium alginate
- Albumin

Synthetic polymers: There are many synthetic polymers like

- Polylactides
- Polyglycolides
- Poly (lactide co- glycolides)
- Polyanhydrides
- Polyorthoesters
- Polycyanoacrylates
- Polycaprolactone
- Poly glutamic acid
- Poly malic acid
- Poly (N- vinyl pyrrolidone)
- Poly (methyl methacrylate)
- Poly (vinyl alcohol)
- Poly (acrylic acid)
- Poly acrylamide
- Poly (ethylene glycol)
- Poly (methacrylic acid)

Preparation of Nanoparticle[16]

The selection of the appropriate method for the preparation of nanoparticles depends on the physiochemical characteristics of the polymer and the drug to be loaded. On the contrary, the preparation techniques largely determine the inner structure, in vitro release profile and the biological fate of these polymeric delivery systems. Two types of systems with different inner structures are apparently possible including,

- A matrix type system consisting of the entanglement of oligomer or polymer units(Nano particles/Nanospheres)
- A reservoir type of system comprised of an oily core surrounded by an embryonic polymeric shell(Nano capsule)

The drug can either be entrapped within the reservoir or the matrix or otherwise be adsorbed on the surface of these particulate systems; the polymers are strictly structured to a nanomeric size particle using appropriate methodologies.

In the preparation of nanoparticles different types of matrix material are used such as polysaccharides, synthetic polymer and proteins. Various factors are involved in selection of matrix material to be used in preparations which are

1. Required nanoparticle size.
2. Permeability and surface charge of nanoparticle.
3. Level of biodegradability and biocompatibility must be optimum.
4. Material must not be toxic.
5. Solubility profile and stability of drugs should not be affected.
6. It should be show desired drug release profile.
7. Must not be immunogenic.

Methods of preparation[17]

- Amphiphilic macromolecules cross linking
    - Heat- cross linking
    - Chemical – cross linking
- Polymerization based methods
    - Polymerization of monomers in situ
    - Emulsion (micellar) polymerization
    - Dispersion polymerization
    - Interfacial condensation polymerization
    - Interfacial complexation
- Polymer precipitation / evaporation
    - Solvent extraction / evaporation
    - Solvent displacement / evaporation
    - Salting out

1.4 CHITOSAN NANOPARTICLES [18, 19]

Chitosan nanoparticles have gained more attention as drug delivery carriers because of their better stability, low toxicity, simple and mild preparation method, and providing versatile routes of administration. Their sub-micron size not only suitable for parenteral application, but also applicable for mucosal routes of administration, i.e., oral, nasal, and ocular mucosa, which are non-invasive route. The application for mucosal delivery is facilitated by Chitosan absorption enhancing the effect. Clinical investigations suggest that therapeutic nanoparticle can enhance efficacy and reduce side effects.

Preparation Methods for Chitosan Nanoparticles

- Ionotropic gelation
- Microemulsion method
- Emulsification solvent diffusion method
- Polyelelectrolyte complex (PEC)

1. **Ionotropic gelation method**

   Ionotropic gelation techniques of is based on electrostatic interaction between amine group of Chitosan and negatively charge group of polyanion such as tripolyphosphate. This technique offers a simple and mild preparation method in the aqueous environment. First, Chitosan can be dissolved in acetic acid in the absence or presence of stabilizing agent, such as poloxamer, which can be added in the Chitosan solution before or after the addition of polyanion. Polyanion or anionic polymers was then added and nanoparticles were spontaneously formed under mechanical stirring at room temperature. The size and surface charge of particles can be modified by varying the ratio of Chitosan and stabilizer.

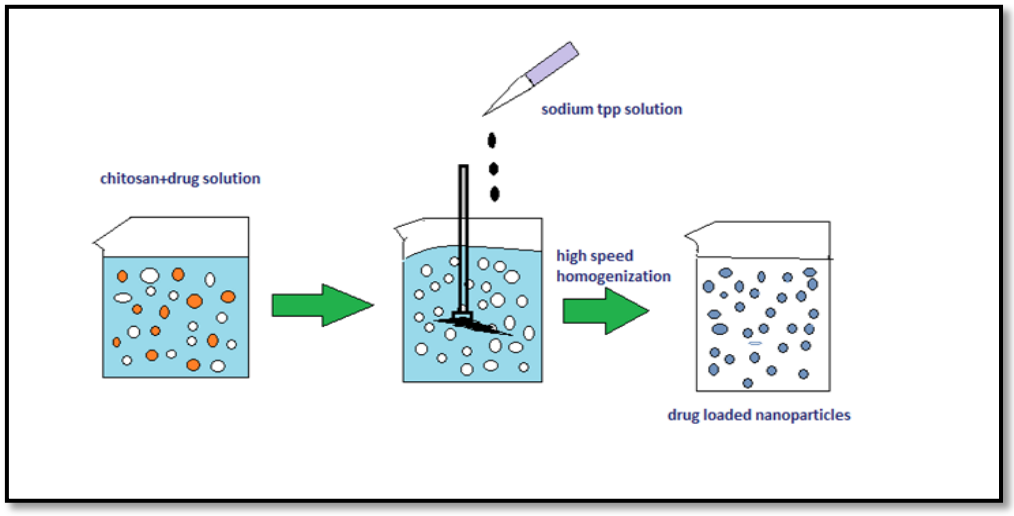


Figure 1.3: Schematic representation of Ionic gelation method

2. Microemulsion method

This technique is based on formation of Chitosan NP in the aqueous core of reverse micellar droplets and subsequently cross-linked through glutaraldehyde. In this method, a surfactant was dissolved in N-hexane. Then, Chitosan in acetic solution and glutaraldehyde were added to surfactant/hexane mixture under continuous stirring at room temperature. Nanoparticles were formed in the presence of surfactant. The system was stirred overnight to complete the cross-linking process, which the free amine group of Chitosan conjugates with glutaraldehyde. The organic solvent is then removed by evaporation under low pressure. The yields obtained were the cross-linked Chitosan NP and excess surfactant. The excess surfactant was then removed by precipitate with CaCl2 and then the precipitant was removed by centrifugation. The final nanoparticles suspension was dialyzed before lyophilization. This technique offers a narrow size distribution of less than 100 nm and the particle size can be controlled by varying the amount of glutaraldehyde that alters the degree of cross-linking. Nevertheless, some disadvantages exist such as the use of organic solvent, time-consuming preparation process, and complexity in the washing step.

3. Emulsification/ solvent diffusion method

This method is based on the partial miscibility of an organic solvent with water. An o/w emulsion is obtained upon injection an organic phase into Chitosan solution containing a stabilizing agent (i.e. poloxamer) under mechanical stirring, follow by high pressure homogenization. The emulsion is then diluted with a large amount of water to overcome organic solvent miscibility in water. Polymer precipitation occurs as a result of the diffusion of organic solvent into water, leading to the formation of nanoparticles. This method is suitable for hydrophobic drug and showed high percentage of drug entrapment. The major drawbacks of this method include harsh processing conditions (use of organic solvents) and the high shear forces used during nanoparticle preparation.

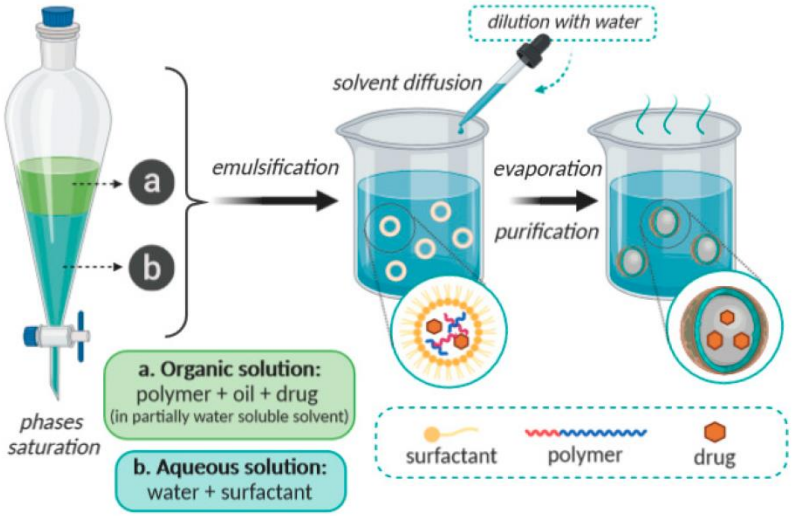


Figure 1.4: Schematic representation of the emulsification/ solvent diffusion method

4. Polyelectrolyte complex (PEC)

Polyelectrolyte complex or self-assemble polyelectrolyte is a term to describe complexes formed by self-assembly of the cationic charged polymer and plasmid DNA. Mechanism of PEC formation involves charge neutralization between cationic polymer and DNA leading to a fall in hydrophilicity as the polyelectrolyte component self-assembly. Several cationic polymers (i.e. gelatin, polyethylenimine) also possess this property. Generally, this technique offers

simple and mild preparation method without harsh conditions involved. The nanoparticles spontaneously formed after addition of DNA solution into Chitosan dissolved in acetic acid solution, under mechanical stirring at or under room temperature.

Route of Administration[20]

- Parenteral administration
- Ocular administration
- Oral administration
- Nasal administration
- Buccal administration
- Vaginal drug delivery
- Pulmonary drug delivery

Application in Drug Delivery[21, 22]

- Colon targeted drug delivery
- Mucosal delivery
- Cancer therapy
- Gene delivery
- Topical delivery
- Ocular delivery
- Vaccine delivery
- Tissue engineering
- Protein and peptide delivery

CHARACTERISATION OF NANOAPRTICLES[6, 23]

The nanoparticles are generally characterized for size, density, electrophoretic mobility, angle of contact and specific surface area.

Table 1.1: Characterization of Nanoparticles

PARAMETERS	CHARACTERIZATION METHODS
Particle size distribution	Photo correlation Spectroscopy (PCS), Laser defractometry, Transmission Electron Microscopy(TEM), Scanning Electron Microscopy(SEM)
Charge determination	Laser Doppler Anemometry, Zeta potentiometer
Carrier drug interaction	FTIR,XRD, Differential Scanning Calorimetry(DSC)
Nanoparticle Dispersion Stability	Critical Flocculation Temperature
In vitro drug release	Using Franz diffusion cell, Dialysis bag method, Open ended tube method

2. REVIEW OF LITERATURE

1. **Moeed et al., (2021)**[24]formulated the Chitosan-based nanoparticles for extended release of verapamil to reduce its dosing frequency and synthesized using the ionotropic gelation method. The formulations were optimized by cross linking of tripolyphosphate (TPP) with chitosan, and $CaCl_2$ sodium alginate at different concentrations. The best formulation for nanoparticles preparation involved using 0.06 % sodium alginate with 18 mM CaCl2 and of chitosan: TPP (5:1 ratio). The size of verapamil nanoparticles prepared with sodium alginate was 186 nm (p=0.02). Chitosan-based formulations had entrapment efficiency in the range of 38.63 – 50.58 %, while the entrapment efficiency of sodium alginate-based formulations was 11.70 – 40.57 %.
2. **Anand M et al., (2018)**[25]examined the larvicidal activities of extracted chitosan and synthesized Chitosan NPs from shrimp shells against third-instars larvae of mosquitoe Aedes aegypti (A. aegypti). The Chitosan Nanoparticles (CS NPs) exhibited higher larvicidal activity in comparison to extracted and commercial chitosan. The CS NPs were synthesized by a novel method of ionic gelation with sodium tripolyphosphate (STPP) as a reducing agent. The chitosan was morphologically characterized by Fourier transforms infrared (FTIR) spectra, X-ray diffraction (XRD) and scanning electron microscope (SEM), whereas the CS NPs were characterized using FTIR, XRD, atomic force microscopy (AFM) and high resolution transmission electron microscopy (HRTEM). The larvicidal activity of CS NPs was confirmed against third instars larvae of A. aegypti.
3. **Bangun H et al., (2011)**[26] formulated and evaluated the chitosan - tripolyphosphate (CS-TPP) nanoparticles suspension as an antibacterial agent by ionic gelation method with various concentration of CS and TPP. The formulation of 0.25% CS and 0.1% sodium TPP resulted in the smallest particle size (238.17nm) with spherical morphology, no sedimentation after centrifugation at 6000 rpm for 2 hours and gave the highest antibacterial activity against *Staphylococcus aureus* and *P.aeruginosa.*
4. **Harekrishna Roy et al., (2018)**[27]formulated the fast-dissolving tablets (FDT) of Urapidil by studying the effect of the variable for response with the help of Box-Behnken design (BBD), which successfully provided the significant value for the quadratic model. A total of 17 formulations were prepared by altering the proportion of cross carmellose sodium, spray dried lactose, and hydroxypropyl methylcellulose K4M by direct

compression technique. BBD Response surface and contour plots were plotted based on BBD and relationship between the variables and response (DT) were established. A perturbation graph was also plotted to identify the deviation of viable from the mean point. Thus, it can be concluded that the optimized formulation with desirable parameters can be obtained by BBD with the response and variable relation.

5. **Sukmavati A et al., (2018)**[28]investigated formulation aspects for chitosan- FA (folate) nanoparticle by addition of various concentration of tween 80 to achieve desired nano-size particles. The ionic gelation method was used for nanoparticle preparation using 0.05% w/v chitosan – FA with addition of 0.1 to 0.5% v/v of tween 80. The result showed that the high concentration of tween 80 during nanoparticle preparation lead to formation of smaller size particle. The 111.8 ±4.11 nm particle size was revealed by addition of 0.5% v/v tween 80 during chitosan –FA nanoparticle preparation loaded with active substances.
6. **Sharma R et al., (2017)**[29]developed and optimized carvedilol (CAR) loaded chitosan (CS) nanoparticles (NPs) prepared by ionic gelation method to enhance its oral bioavailability and comparing it with marketed tablets. The results indicated stable CAR-CS NPs with the value of ZP to be +32 mV ± 2 mV, the small particle size of 79.23 nm and high entrapment efficiency of 72.56%. The optimization of the formulation by Taguchi orthogonal array design exhibited C_3 T_5 $R_{11}S_{20}$ as the optimized batch with CS concentration of 0.3% w/v, tripolyphosphate (TPP) concentration of 0.5% w/v, CS: TPP volume ratio of 1:1 v/v and a stirring speed of 2000 rpm. The in vitro release study revealed sustained release of drug for 72 h with 74.84% cumulative drug release.
7. **Niaz T et al., (2016)**[30]fabricated Antihypertensive (AHT) nano-ceuticals in hydrophilic carriers of natural origin to improve drug solubility, protection and sustained release. AHT Nano-carrier systems encapsulating captopril, amlodipine and valsartan were fabricated using chitosan polymer by ionic gelation assisted ultra-sonication method. Drug encapsulation efficiencies of 92 ± 1.6%, 91 ± 0.9% and 87 ± 0.5% were observed for captopril, valsartan and amlodipine respectively. Zeta size results had confirmed that average size of chitosan nanoparticles was below 100 nm. Thus, here reported innovative AHT nano-ceuticals of polymeric origin can improve the oral administration of currently available hydrophobic drugs while providing the extended-release function.

8. **Roy H et al., (2016)**[31]formulated Fast dissolving tablet of Urapidil by direct compression technique using various concentration of Superdisintegrants like Crosscarmellose sodium (CCS), Cross povidone and Sodium starch glycolate (SSG). The formulated tablets were evaluated for Crushing strength, Friability, Thickness, Diameter, Weight variation, Drug content, Wetting time, Water absorption ratio, Disintegration time and Percentage of drug release.
9. **Sameer J Nadaf et al., (2016)**[32]developed an accurate, simple and precise UV Spectrophotometric method for estimation of Urapidil hydrochloride both in bulk and pharmaceutical formulation. The results showed that the concentration of Urapidil over a range of 2-14 µg/ml, obeyed Beers law. The correlation co-efficient for Urapidil was found to be 0.998 and the% relative standard deviation was within specification limits (< 2). Percent Recovery estimated was found to be 98.86±0.3725. This method showed satisfactory linearity and can be applied in regular laboratory analysis.
10. **Son S. Chaudhary et al., (2015)**[33]developed a Chronomodulated system comprised of dual approach; immediate release granules and pulsatile release mini-tablets filled in the hard gelatin capsule. Conventional therapies are incapable to target time points when actually the symptoms get worsened. The mini-tablets were coated using Eudragit S-100 which provided the lag time. To achieve the desired release, various parameters like coating duration and coat thickness were studied. The immediate release granules were evaluated for micrometrical properties and drug release, while mini-tablets were evaluated for various parameters such as hardness, thickness, friability, weight variation, drug content, and disintegration time and in-vitro drug release. Thus, the dual approach of developed chronomodulated formulation found to be satisfactory in the treatment of hypertension.
11. **Zeinab M.A Fathella et al., (2015)**[34] formulated chitosan-based nanoparticles loaded with ketorolac tromethamine (KT) intended for topical ocular delivery. NPs were prepared using ionic gelation method incorporating tri-polyphosphate (TPP) as cross-linker. The data suggested that the size of the NPs was affected by CS/TPP ratio where the diameter of the NPs ranged from 108.0 ± 2.4 nm to 257.2 ± 18.6 nm. The *in vitro* release profile of KT from CS NPs showed significant differences ($p < 0.05$) compared to KT solution. Furthermore, mucoadhesion studies revealed adhesive properties of the formulated NPs. The KT-loaded NPs were found to be stable when stored at different storage conditions for a period of 3 months.

12. **Omidreza Jafarieh et al., (2014)[35]** investigated anti-Parkinson's drug ropinirole (RH) to the brain using polymeric nanoparticles. Ropinirole hydrochloride - loaded chitosan nanoparticles were prepared by an ionic gelation method. The Ropinirole hydrochloride Chitosan Nanoparticles were characterized for particle size, polydispersity index, zeta potential, loading capacity, entrapment efficiency *in vitro* release study, and *in vivo* distribution after intranasal administration. The novel formulation showed the superiority of nose to brain delivery of RH using mucoadhesive nanoparticles compared with other delivery routes reported earlier.
13. **Priyanka Patil et al., (2014)[36]**optimized and evaluated doxorubicin loaded spray dried chitosan nanocarriers as a sustained release. Chitosan nanoparticles were prepared by using ionotropic gelation technique. Elucidated the influences of the 4 decision variables (i.e. chitosan concentration, cross-linking concentration, stirring speed, stirring time on the mean particle size, Entrapment efficiency, in-vitro release a four factorial / two level experimental designs was carried out by the design expert software. Among all 16 batches high drug loading 66%, particle size 126-1392 nm. Based on in-vitro release study formulations show biphasic pattern characterized by initial burst release followed by a slower and sustained release.
14. **Bhavin K. Patel et al., (2013)[37]** developed and optimized oral sustained release Chitosan nanoparticles of rifampicin by design of experiment. Chitosan Nanoparticles was prepared by modified emulsion ionic gelation technique. The 2^3 full-factorial design was employed by selecting the independent variables such as Chitosan concentration, concentration of tripolyphosphate, and homogenization speed in order to achieve desired particle size with maximum percent entrapment efficiency and drug loading. The design was validated by checkpoint analysis, and formulation was optimized using the desirability function. Particle size, drug entrapment efficiency, and drug loading for the optimized batch were found to be 221.9 nm, 44.17 ± 1.98% W/W, and 42.96 ± 2.91% W/W, respectively. In vitro release data of optimized formulation showed an initial burst followed by slow sustained drug release. In vitro study suggests that oral sustained release Chitosan Nanoparticles might be an effective drug delivery system for tuberculosis.
15. **Kirti Ranjan Parida et al., (2013)[38]** attempted to formulate the fast-dissolving tablets of Urapidil by direct compression technique using various concentration of Super disintegrants like Cross carmellose sodium (Ac-Di-Sol), Polyplasdone R-XL and Sodium

starch glycolate (SSG). The formulated tablets were evaluated for Crushing strength, Friability, Thickness, Diameter, Weight variation, Drug content, Wetting time, Water absorption ratio, Disintegration time and Percentage of drug release. Among them formulation AD3 containing 3% of Ac-Di-Sol exhibited complete release within 1 hour and disintegration time within 10 second. Accelerated stability study indicated no significant difference in assay and crushing strength. There was no chemical interaction between the drug and excipients during FT-IR study and DSC study considered in the present investigation.

16. **Mohammad Fazil et al., (2012)[39]**prepared the Rivastigmine (RHT) loaded chitosan nanoparticles (CS-RHT NPs) by ionic gelation method to improve the bioavailability and enhances the uptake of RHT to the brain via intranasal (i.n.) delivery. The brain concentration achieved from i.n. administration of CS-NPs (966± 20.66ng ml^{-1}; t_{max} 60 minutes) was higher significantly higher than those achieved after i.e. administration of RHT sol (387 ± 29.51 ng ml^{-1}; t_{max} 30 min), and i.n. administration of RHT solution (508.66 ± 22.50 ng ml^{-1}; t_{max} 60 min). These results suggest that CS-RHT NPs have better brain targeting efficiency and are a promising approach for i.n. delivery of RHT for the treatment and prevention of Alzheimer's disease (AD).
17. **Mohammadpour Dounighi et al., (2012)[40]**investigated Mesobuthus eupeus venom-loaded chitosan nanoparticles were prepared via ionic gelation of tripolyphosphate (TPP) and chitosan. The optimum encapsulation efficiency (91.1%) and loading capacity (76.3%) were obtained by a chitosan concentration of 2 mg/mL, chitosan-to-TPP mass ratio of 2 and M. eupeus venom concentration of 500 µg/ml. The nanoparticle size was about 370 nm (polydispersity index: 0.429) while the zeta potential was positive. The in-vitro release of nanoparticles showed an initial burst release of approximately 60% in the first ten hours, followed by a slow and much reduced additional release for about 60 hours.
18. **Amar Singh et al., (2011)[41]**prepared losartan potassium loaded chitosan nanoparticles by ionic gelation of chitosan with tripolyphosphate anions. Scanning Electron Microscopy indicated that the nanoparticles were found to be in nanometer range and showed ideal surface morphology. In vitro release behaviour from all the drug loaded batches were found to follow zero order and provided sustained release over a period of 24 hours. The developed formulation overcomes and could possibility be advantageous in terms of sustained release dosage forms of losartan potassium.

19. **ParthaSaha et al., (2010)[42]** developed ampicillin trihydrate-loaded chitosan nanoparticles by ionic gelation method and evaluated their antimicrobial activity. Parameters such as the zeta potential, polydispersity, particle size, entrapment efficiency and in vitro drug release of the nanoparticles were assessed for optimization. Concentrations of 0.35 %w/v of chitosan and 0.40 %w/v sodium tripolyphosphate and a sonication time of 20 min constituted the optimum conditions for the preparation of the nanoparticles. In vitro release data showed an initial burst followed by slow sustained drug release. Polymer and cross linking agent concentrations and sonication time are rate-limiting factors for the development of the optimized formulation. The chitosan nanoparticles developed would be capable of sustained delivery of ampicillin trihydrate.
20. **Adlin jino nesalin J et al., (2009)[43]** formulated and evaluated sustained release Nanoparticles of Flutamide. Flutamide, a substituted anilide, is a potent antiandrogenic that has been used in the treatment of prostate carcinoma having short biological half-life of 5-6 hrs.; Nanoparticles of Flutamide were formulated using chitosan polymer by ionic gelation technique. Nanoparticles of different core: coat ratio were formulated and analysed for total drug content, loading efficiency, particle size and in vitro drug release studies. From the drug release studies it was observed that nanoparticles prepared with chitosan in the core: coat ratio 1:4 gives better sustained release for about 12 hrs as compared to other formulations.
21. **Haibing He et al., (2009)[44]** developed and investigated the in vivo bioavailability of Modified-release pellets containing Urapidil. Lactose and MCC were used as the main fillers to prepare drug-layer pellets by the powder layering method using centrifugal granulation, and coated in a fluidized bed coater. Pellets with different drug release characteristics were prepared with a methacrylic acid copolymer aqueous dispersion. The bioavailability of the pellets (T_1, T_2, containing 30 mg Urapidil was determined in six healthy subjects after oral administration of a single dose, for a period of three weeks, in the form of a crossover design with a wash-out period of one week. The plasma concentrations of Urapidil were determined by HPLC with UV detection. The C_{max}, T_{max}, and MRT of T_1 were 311.7 ng/mL, 5.3 h and 8.3 h, respectively. T_1 was bioequivalent to R, with a relative bioavailability 110.9%. The C_{max} and T_{max}, of T_2 were 105.3 ng/mL and 13.3 h, respectively, and the relative bioavailability was 72.7%. This proves that the pH-dependent Urapidil pellets have a high bioavailability.

22. **Hui-Chia Yang et al., (2009)**[45]examined the effect that the molecular weight of chitosan had on the resulting nanoparticles' properties; the initial concentration of chitosan was held constant, but its molecular weight was decreased through the action of $NaNO_2$. The diameters of the nanoparticles-determined using dynamic light scattering and TEM techniques-decreased as the value of the viscosity of molecular weight of chitosan decreased. In addition, we prepared fluorouracil-loaded chitosan nanoparticles and characterized them using NMR spectroscopy. The encapsulation efficiency increased as the value of MW of chitosan decreased. The particles produced using 55-kDa chitosan had a mean diameter of 70.6 nm and a 66% drug loading.
23. **Zhuang X et al., (2009)**[46]investigated the efficacy and safety of Urapidil intravenous injected by micro-pump in the treatment of hypertensive emergency patients. There were 78 cases with hypertensive emergency that were treated with 50 mg Urapidil by intravenous micro-pump. The blood pressure, heart rate, adverse reactions were monitored and recorded before administration and 5 min, 10 min, 30 min, 60 min, 120 min after administration. Compared to that before administration, blood pressures were significantly decreased after administration, showing a significant difference. There was no significant difference in the heart rate. Urapidil intravenous injected by micro-pump was effective for the treatment of hypertensive emergency with fewer adverse reactions.

3. AIM AND PLAN OF WORK

3.1 AIM OF THE WORK

- ✓ To formulate Urapidil loaded Chitosan Nanoaparticles containing Chitosan as polymer by Ionic gelation method.
- ✓ Characterization and optimization of formulated Urapidil loaded Chitosan Nanoparticles.
- ✓ To enclose Urapidil loaded Chitosan Nanoparticles in Hard Gelatin Capsule and their character to be evaluated.

3.2 OBJECTIVE OF WORK.

- To improve solubility of Urapidil by Nanotechnology.
- The drug has low half-life and thus the development of Chitosan loaded Nanoparticle will provide a controlled release of the drug by retaining for a prolonged period of time.
- The frequency of drug administration is also decreased and thus improving patience compliance.

3.3 PLAN OF WORK

The present work is planned to carry out the formulation and evaluation Urapidil loaded Chitosan nanoparticles.

3.3.1Preformulationstudies

- Compatibility studies
  - Physical compatibility study
  - Chemical compatibility study by Fourier Transform Infra-Red spectroscopy
- Determination of melting point of pure drug
- Determination of λmax
- Determination of standard graph for Urapidil
- Solubility study of pure Urapidil

3.3.2 Formulation of Urapidil loaded Chitosan Nanoparticles

- Ionic gelation method
- Freeze drying process

3.3.3 Characterization of Urapidil loaded Chitosan Nanoparticles

- Percentage yield
- Drug content
- Entrapment efficiency
- *In vitro* release study of the formulation and pure drug

3.3.4 Characterization of optimized Urapidil loaded Chitosan Nanoparticles

- FTIR – optimized formulation
- Solubility studies of optimized nanoparticles formulations
- Scanning Electron Microscopy (SEM) analysis
- Particle size analysis
- Zeta potential

3.3.5 Preformulation studies of optimized Urapidil loaded Chitosan Nanoparticles

- Bulk density
- Tapped density
- Compressibility index
- Hausner's ratio
- Angle of repose

3.3.6 Optimized Urapidil loaded Chitosan Nanoparticles filled in Hard Gelatin Capsules

- **Post formulation studies**
  - Uniformity of weight
  - Disintegration test
  - Drug content
  - *In vitro* drug release

3.3.7 Release kinetics of optimized formulations

3.3.8 Stability studies

4. RATIONALE OF THE STUDY

4.1 Rationale of Disease[47]

Hypertension is designed as either a sustained systolic blood pressure of greater than 140 mm Hg or a sustained diastolic blood pressure of greater than 90 mm Hg. Hypertension results from the increased peripheral vascular arteriolar smooth muscle tone, which leads to increased arteriolar resistance and reduced capacitance of the venous system. Hypertension is also an important risk factor in the chronic kidney disease and heart failure.

4.2 Rationale of Drugs[38, 48]

- Urapidil is reported to block peripheral α_1- adrenoreceptor and have central action, which used in the treatment of Hypertension including Hypertension crises.
- It is rapidly absorbed from Gastro intestinal track and rapidly eliminated from the plasma with half life4.7 hours.
- Urapidil belongs to BCS class II drugs (low solubility and high permeability).
- To overcome the above problems (low solubility, shorter half life) of drug by formulating it as a polymeric nanoparticles using natural carrier.

4.3 Rationale of Chitosan based formulation[49, 50]

- Polymeric nanoparticles capable of loading hydrophilic and lipophilic drugs.
- Chitosan is used as a polymer because of its advantage like biodegradability, biocompatibility, non-toxicity, non-immunogenicity and low cast.
- Due to colon specific degradation by microbes in last part of GIT, Chitosan based nanocarrier system are extensively used in encapsulation of many drugs for site specific delivery. All these properties of Chitosan make this polymer a perfect drug delivery of systems for Anti-hypertensive(AHT) drugs as well as enhance their oral bioavailability, reduce the dosage, reduce dosage associated side effects and to control the release of AHT drugs *in vitro* and *in vivo* over longer period of time. It improves the patience compliance.
- It acts as an active drug delivery system in the field of oral, cancer therapies, vaccine and topical treatment.

5. DISEASE PROFILE[51-55]

Hypertension is a serious cardiovascular event which refers to rise in the arterial blood pressure. Due to raised blood pressure, heart has to work harder in order to pump adequate amount of blood to cope up with normal body functioning. If the same is not treated, it may lead to heart-related problems and may damage the organs like kidney, brain, and eyes. It is as such not a disease in itself but is a risk factor for major cardiovascular events like heart stroke, ischemic heart disease, myocardial infarction, and heart enlargement. According to WHO, Geneva, in 2008, hypertension resulted in 45% mortality rate because of ischemic heart disease and 51% mortality rate because of stroke. In 1980, 600 million people were suffering from hypertension while in 2008 this graph was raised to 1 billion raising a big concern for dealing with this condition effectively (WHO, 2013).

Blood pressure is measured in millimeters of mercury (mm Hg) and is recorded as two numbers usually written one above the other. The upper number is the systolic blood pressure - the highest pressure in blood vessels and happens when the heart contracts, or beats. The lower number is the diastolic blood pressure –the lowest pressure in blood vessels in between heart beats when the heart muscle relaxes. Normal adult blood pressure is defined as a systolic blood pressure of 120 mm Hg and a diastolic blood pressure of 80 mm Hg.

Table No. 5.1: Classification of Hypertension

Category	Systolic BP (mm Hg)	Diastolic BP (mm Hg)
Optimal	<120	<80
Normal	120–129	80–84
High normal	130–139	85–89
Grade 1 hypertension	140–159	90–99
Grade 2 hypertension	160–179	100–109
Grade 3 hypertension	≥180	≥110

Hypertension is sustained elevation of resting systolic BP (≥ 140 mm Hg), diastolic BP (≥90 mm Hg), or both. Hypertension with no known cause (primary: formerly, essential hypertension) is most common. Hypertension with an identified cause (secondary hypertension) is usually due to the chronic kidney disease or primary aldosteronism. Diagnosis is by Sphygmomanometry (using Sphygmomanometer). Tests may be done to determine cause, assess damage and identify other cardiovascular risk factors. Treatment involves lifestyle changes and drugs including Diuretics, Alpha blockers, Beta- blockers, ACE inhibitors, Angiotensin II receptors blockers and calcium channel blockers.

5.1 ETIOLOGY

Hypertension is either due to some cause or without identifiable cause. There are two types of hypertension.

1. Primary hypertension

2. Secondary hypertension

5.1.1 Primary hypertension

Primary (essential) hypertension is the most common form of hypertension, accounting for 90-95% of all cases of hypertension. Hemodynamic and physiologic components (plasma volume, activity of the Renin - Angiotensin system) vary that indicating the primary hypertension is unlikely to have a single cause. Even if one factor is initially responsible, multiple factors are probably involved in sustaining elevated blood pressure. In afferent systemic arterioles, malfunction of ion pumps on sarcolemmal membranes of smooth muscle cells may lead to chronically increased vascular tone. Heredity is a predisposing factor, but the exact mechanism is unclear. Environment factors (e.g. dietary sodium, obesity, stress) seem to affect only genetically susceptible people at younger ages: however, in patient >65, high sodium intake is more likely to precipitate hypertension. In almost all contemporary societies, blood pressure rises with aging and the risk of becoming hypertension in later life is considerable. Hypertension results from a complex interaction of genes and environmental factors.

Genetic factors

The role heredity in the etiology of essential hypertension has long suspected. E.g. occurrence of hypertension in twins.

Racial and environmental factors

During a survey in US, it was revealed that the incidence of hypertension in black is more than twins. Environmental factors like salt intake and obesity has influence on the hypertension.

Pathophysiology

Hypertension is a risk factor for atherosclerosis and cardiovascular complications like myocardial infarction, angina pectoris and congestive cardiac failure whether the cause of hypertension is known or unknown. In secondary hypertension, in less than 10% cases causes of hypertension are understood whereas in more than 90% cases, the mechanism of essential hypertension remains largely obscure. In general, two hemodynamic forces viz-cardiac output and peripheral resistance regulate the normal blood pressure. The role of kidney particularly in secondary hypertension by elaboration of rennin and subsequent formation of angiotensin II is well established. Chronic hypertension results in damage to end organs like eyes, brain, kidneys and heart. It is possible to correlate the duration of hypertension by considering the severity of end organ damage.

5.1.2 Secondary Hypertension

Causes include primary aldosteronism (thought to be most common), renal parenchymal disease (e.g. chronic glomerulonephritis or pyelonephritis, polycystic renal disease), endovascular disease, pheochromocytoma, Cushing's syndrome, congenital adrenal hyperplasia, hyperthyroidism and myxedema. Excessive alcohol intake and use of oral contraceptives are common causes of curable hypertension. It is secondary to some other underlying disease. When the actual cause is treated hypertension comes to normal. Underlying causes may be as follow

1. Renin – Angiotensin mediated hypertension
2. Endocrine disorders

5.2 TYPES OF HYPERTENSION

Renal hypertension

Renal diseases like renal vascular hypertension produce renal hypertension, which is associated with the renal artery. Renal hypertension may be produced by any one of three interrelated mechanisms

a) Activation of Renin- Angiotensin system.
b) Sodium and water retention.
c) Decreased release of vasodepressor materials.

Endocrine hypertension

A number of hormonal secretions may produce secondary hypertension like Adrenal glands, parathyroid glands and oral contraceptives. Usually, hyper secretion of tumor of endocrine glands. E.g. Cushing's syndrome, Primary Hyper Aldosteronism, Pheochromocytoma and oral contraceptives use may lead to release of Angiotensin I.

Neurogenic

Psychogenic polyneuritis, increased intracranial hypertension and secretion of spinal cord are the uncommon causes of secondary hypertension.

Contraction of Aorta

It causes secondary hypertension in the upper part of the body due to constriction. Diastolic hypertension results from changes in circulation.

5.3 RISK FACTORS:

5.3.1 Unmodified Risk factors

Age and gender

- The association between increasing age and increasing systolic blood pressure is thought to reflect the length of time people are exposed to modifiable risk factors. For any given age up to 65 years women tend to have a lower blood pressure than men after 65years, this trend is reversed. The cause is unknown. Prevalence also increases with age.

Ethnicity

- Hypertension is more prevalent in different ethnic groups, such as black Caribbean men and women and less prevalent in Bangladesh men and women, for example, some of the differences in prevalence are thought to be related to inherited to differences in the way the body reacts to salt and in blood pressure controlling hormones.

5.3.2 Modified risk factors

Excess salt

- Evidence has shown a strong association between salt intake and elevated blood pressure. In the UK, 65-70% of the salt we eat comes from processed foods such as bread, cereals, ready meals, etc. Average adult daily intake is 9g of salt per day. The daily recommended level is 6g for adult.

Overweight and obesity

- Obesity multiplies the risk of developing hypertension about four fold in men and three fold in women.

Physical inactivity

- People who don't take enough aerobic exercises (e.g.: running, walking and cycling) are more likely to have or to develop hypertension.

Excess alcohol

- Blood pressure rises when large amounts of alcohol are consumed, particularly when binge-drinking. A large study of almost 6000 men, aged 35-64 years, followed up 21 years, found that there was a strong correlation between alcohol consumption and mortality from stroke.

Diabetes

- Hypertension is more prevalent in people with Type I and Type II diabetes then in non diabetic population (whether they are overweight or not) as a consequence of kidney

damage and insulin resistance respectively. In England, as many as 70% of adult with Type II diabetes have hypertension, doubling the risk of cardiovascular event.

5.4 SYMPTOMS OF HIGH BLOOD PRESSURE

There is a common misconception that people with hypertension always experience symptoms, but the reality is that most hypertensive people have no symptoms at all. Sometimes hypertension causes symptoms such as,

- Headache
- Shortness of breath
- Dizziness
- Chest pain
- Palpitations of the heart
- Nose bleeds

It can be dangerous to ignore such symptoms, but neither can they be relied upon to signify hypertension. Hypertension is a serious warning sign that significant lifestyle changes are required. The condition can be a silent killer and it is important for everybody to know their blood pressure reading.

5.5 TREATMENT

- Diuretics
- Angiotensin Converting Enzymes (ACE) inhibitors
- Alpha adrenergic receptor blockers
- Beta adrenergic receptor blockers
- Calcium Channel Blockers
- Central adrenergic inhibitors
- Vasodilators

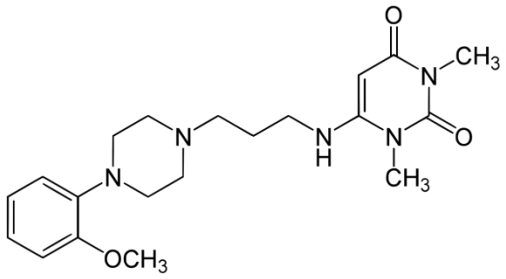
Lifestyle modification to reduce risk factors

6. DRUG PROFILE[32, 33, 56-60]

URAPIDIL

Urapidil is an antihypertensive agent derived from arylpiperazine. It is selective α_1-adrenoreceptor antagonist with central antihypertensive action which is increasingly used in the treatment of hypertension.

Chemical structure:



IUPAC name : 6-[3-(4-O-Methoxypheylpiperazin-l-yl) propylamino]-1, 3-dimethyluracil.

Molecular formula: $C_{20}H_{29}N_5O_3$

Molecular weight : 387.5

Description : White powder

Melting point : Between 157.0ºC and160.0ºC

Log p value : 1.6

Solubility : Insoluble in water, soluble in ethanol.

Indication : For the Management of hypertension including Hypertension Crisis.

BCS classification: BCS class II drug (low solubility, high permeability).

Pharmacokinetics:

Absorption: Orally administered Urapidil is rapidly absorbed.

Volume of Distribution: 0.77L/kg

Metabolism: Urapidil extensively metabolized in the liver, principally by hydroxylation. The aryl-p-hydroxylated urapidil (Metabolite 1) was predominant metabolite in humans.

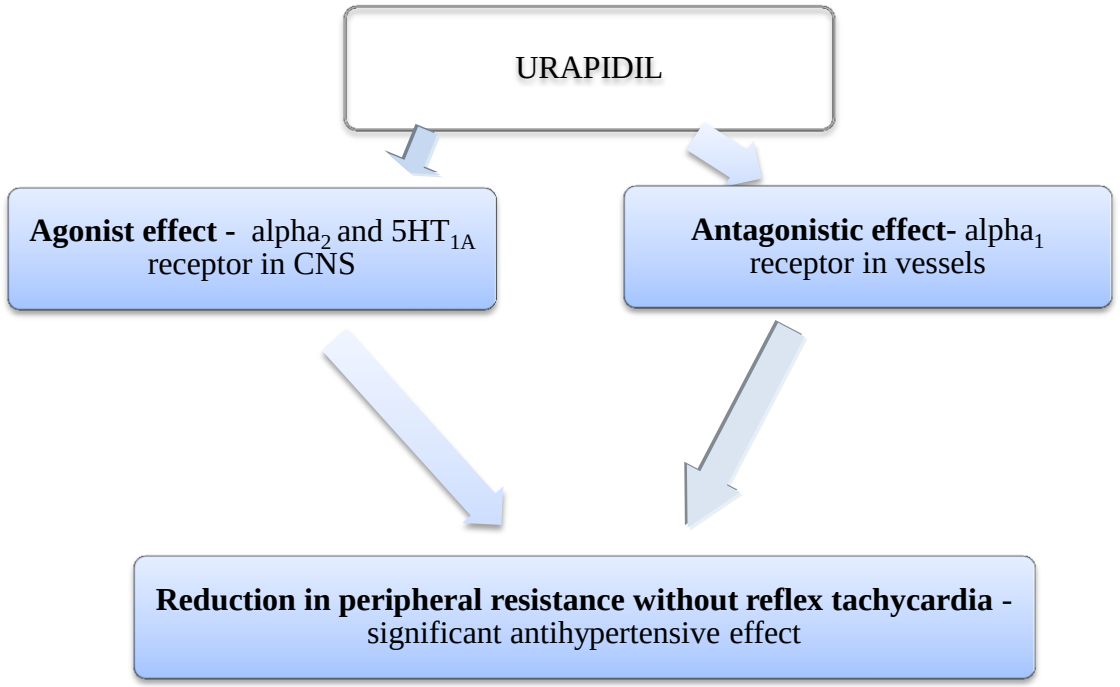
Protein binding **:** 80% bound to plasma protein.

Elimination Half life: 4.7 hours for Oral administration,

2.7 hours for Intravenous administration

Mechanism of action:

It is arylpiperazine derivative. It is postulated that the therapeutic effect is exhibited by blocking the peripheral α_1-adrenoreceptor and have central action. It produces a reduction in peripheral resistance and is fall in Systolic and Diastolic blood pressure without reflex tachycardia.



- Its action is mainly due to a postsynaptic α_{1-} adrenoreceptor antagonism that inhibits the vasocontriction action of catecholamines and reduces blood pressure by decreasing peripheral vascular resistance.
- It is improves myocardial oxygen consumption, systemic vascular resistance, left ventricular function and cardiac output.
- Two factors modify the pharmacokinetics of Urapidil in very old age and severe impairment of liver function.

Adverse effects:

Dizziness, Nausea, Headache, Fatigue, Hypotension, palpitation, Nervousness, pruritus and allergic reactions.

Dosage:

- In Hypertension 30-90 mg twice daily.
- In Hypertensive crisis Initial dose- 25mg given by slow intravenous injection over 20 seconds followed by infusion of 9- 30 mg /hour.

7. EXCIPIENT PROFILE

7.1 CHITOSAN[61, 62]

Nonproprietary names

- BP: Chitosan hydrochloride
- PhEur: Chitosani hydrochloridum

Synonyms

2-Amino-2-deoxy-(1,4)-b-D-glucopyranan; deacetylated chitin; deacetylchiyin; b-1,4-poly-D-glucosamine;poly-D-glucosamine;poly-(1,4-b-D-glucopyranosamine).

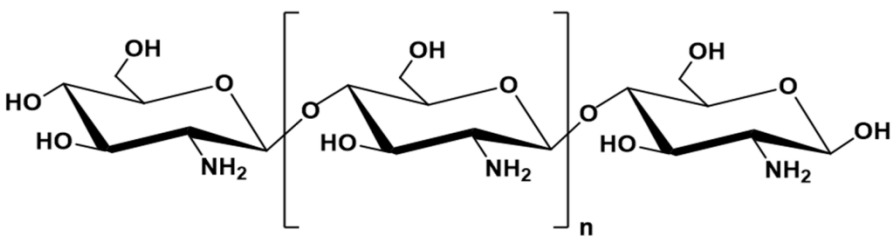
IUPAC name and CAS registry number

Poly-b-(1, 4)-2-Amino-2-deoxy-D-glucose (9012-76-4).

Empirical formula and molecular weight

Partial deacetylation of chitin results in the production of Chitosan, which is a polysaccharide comprising copolymers of glucosamine and N-acetyl glucosamine. Chitosan is the term applied to deacetylated chitins in various stages of deacetylation and depolymerization and it is therefore not easily defined in terms of its exact chemical composition. Clear nomenclature with respect to the different degrees of N-deacetylation between chitin and Chitosan has not been defined and as such Chitosan is not one chemical entity but varies in composition depending on the manufacturer. In essence, Chitosan is chitin sufficiently deacetylated to form soluble amine salts. The degree of deacetylation necessary to obtain a soluble product must be greater than 80-85%. Chitosan is commercially available in several types and grades that vary in molecular weight by 10000-1000000 and vary in degree of deacetylation and viscosity.

Chemical structure



Structure of Chitosan

Functional category

Coating agent; disintegrant; film forming agent; mucoadhesive; tablet binder; viscosity increasing agent.

Description

Chitosan occurs as odourless, white or creamy-white powder or flakes. Fiber formation is quite common during precipitation and the Chitosan may look 'cotton like'.

Typical properties

Chitosan is a cationic polyamine with a high charge density at pH < 6.5; and so adheres to negatively charged surfaces and chelates metal ions. It is a linear polyelectrolyte with reactive hydroxyl and amino groups (available for chemical reaction and salt formation). The properties of Chitosan relate to its polyelectrolyte and polymeric carbohydrate character. The presence of a number of amino groups allows Chitosan to react chemically with anionic systems, which results in alteration of physiochemical characteristics of such combinations. The nitrogen in Chitosan is mostly in the form of primary aliphatic amino groups. Chitosan therefore undergoes reactions typical of amines: for example, N-acetylation and Schiff reactions. Almost all functional properties of Chitosan depend on the chain length, charge density and charge distribution. Numerous studies have demonstrated that the salt form, molecular weight and degree of deacetylation as well as pH at which the Chitosan is used all influence how this polymer is utilized in pharmaceutical applications.

Description of Chitosan

PARAMETERS	**DESCRYPTION**
Appearance (powder or flake)	White or yellow
Particle size	Less than 30 µm
Viscosity(1% solution / 1%acid)	Less than 5 cps
Density	Between 1.35 to 1.40 g/cm3
Molecular weight	50000 to 200000 Da.
pH	4.0 to 6.0
Moisture content	More than 10%
Ash value	More than 2%
Mater insoluble in water	0.5%

Degree of acetylation	66% to 99.8%
Heavy metal (Pb)	Less than 10 ppm
Heavy metal (As)	Less than10 ppm
Protein content	Less than 0.3%
Loss on drying	Less than 10%
Glass transition temperature	203ºC

Moisture content

Chitosan adsorbs moisture from the atmosphere, the amount of water adsorbed depending upon the initial moisture content and the Temperature and Relative Humidity of the surrounding air.

Solubility

Sparingly soluble in water, Chitosan dissolves readily in dilute and concentrated solutions of most organic acids and to some extent in mineral inorganic acids (except phosphoric and sulfuric acids).

Viscosity (dynamic)

A wide range of viscosity types is commercially available. Owing to its high molecular weight and linear, unbranched structure, Chitosan is an excellent viscosity enhancing agent in an acidic environment. It acts as a pseudo plastic material, exhibiting a decrease in viscosity with increasing rates of shear. The viscosity of Chitosan solutions increases with increasing Chitosan concentration, decreasing temperature and increasing degree of deacetylation.

Stability and storage conditions

Chitosan powder is a stable material at room temperature, although it is hygroscopic after drying. Chitosan should be stored in a tightly closed container in a cool, dry place. Chitosan should be stored at a temperature of 2-8^0 C.

Preparation of Chitosan from raw materials

Chitosan is not a single chemical entity, but varies in composition depending on the source and method of preparation defined as sufficiently deacetylation of chitin to form a soluble amine salt. The degree of deacetylation must be 80 to 85% or higher or the acetyl content must be less than 4-4.5 % to form the soluble product. Chitosan is manufactured commercially by a chemical method. Firstly, the sources such as crab or shrimp shells are washed and grinded in to powdered form and then it is deproteinized by treatment with an aqueous 3-5 % solution of sodium hydroxide. The resulting product is neutralized and calcium is removed by treatment with an aqueous hydrochloric acid 3-5% solution at room temperature to precipitate chitin. N-Deacetylation of chitin is achieved by treatment with an aqueous 40-45 % solution at elevated temperature (110ºC), and the precipitate is washed with water. The insoluble part is removed by dissolving in an aqueous 2% acetic acid solution. The supernatant solution is then neutralized with an aqueous sodium hydroxide solution to obtain a purified Chitosan.

Safety

Chitosan is being investigated widely for use as an excipient in oral and other pharmaceutical formulations. It is also used in cosmetics. Chitosan is generally regarded as a nontoxic and nonirritant material. It is biocompatible with both healthy and infected skin. Chitosan has been shown to be biodegradable.

LD_{50} (mouse, oral) > 16g/kg

Applications in pharmaceutical formulation

Chitosan is used on cosmetics and is under investigation for use in a number of pharmaceutical formulations. The suitability and performance of Chitosan as a component of pharmaceutical formulations for drug delivery applications has been investigated in numerous studies. These include controlled drug delivery applications,

- Component of mucoadhesive dosage forms for rapid release
- Improved peptide delivery
- Colonic drug delivery systems
- Use for drug & gene delivery

Chitosan has been processed into several pharmaceutical forms including gels, films, beads, microspheres, tablets and coating for liposomes. Furthermore, Chitosan may be processed into drug delivery systems using several techniques including spray-drying, coacervation, direct compression and conventional granulation process.

7.2 SODIUM TRIPOLYPHOSPHATE (STPP) [63, 64]

Chemical structure:

IUPAC name : Pentasodium triphosphate

Synonyms : Sodium triphosphate, polygon

Molecular formula : $Na_5P_3O_{10}$

Molar weight : 367.864

Appearance : White power

Density : 2.52 g/cm^3

Melting point : 622^0 C

Solubility in water : 14.5 g/100ml (25^0C)

STTP is a colorless salt, which exists both in anhydrous form and as the hexahydrate. The anion can be described as the Penta-anionic chain [$O_3POP(O)_2OPO_3$]. Many related di, tri and polyphosphate including the acyclic triphosphate. It binds strongly to metal cations as both a bi-dentate and tri-dentate chelating agent.

Sodium tripolyphosphate (STPP), also known as pentasodium triphosphate, pentasodium tripolyphosphate or sodium triphosphate is used in a wide range of applications in the manufacture of cleaning products and food preservatives as well as in water treatment facilities. STPP is a sodium salt of triphosphoric acid that has the appearance of white crystal powder. It is odourless and is water soluble. In a highly controlled environment, it is made by combining monosodium phosphate and disodium phosphate.

Powerful cleaning agent: Sodium tripolyphosphate ($Na_5P_3O_{10}$) is a strong cleaning ingredient that typically can rid dishes and fabrics of soil and spots. Its key function is that it allows surfactants to work at their full potential. Also, it prevents deposition of soil and acts as a pH buffer. It softens detergent water and can be used as a water treatment as well. Many households cleaning products, including surface and toilet cleaners, contain STPP due to its cleaning components. Most chemical plants that make STPP list "detergent" as the primary area of use for this chemical.

Food additives:

The uses of STPP also include using it as a preservative. It can used to preserve foods such as red meats, poultry and seafood helping then to remain their tenderness and moisture. Pet food and animal feed have been known to be treated with STPP, serving the same general purpose as it does in human food. STPP also has been use in helping to preserve the quality of drinks such as milk and fruit juice.

Other Uses:

As a builder, sodium STPP mainly is used in detergents and soaps. This includes dishwasher detergents as well as laundry detergents. It is used in most gel, liquid, tablet and powder forms of each type of detergent. In the detergent, it helps strengthen the cleaning action.

It is used in paint as a pigment dispersant. Ceramics use it to help disperse clay and paper mills use it as an oil-resistant agent when coating paper. It additionally has been used as a tanning agent in making leather and the minerals in cement have been known to be treated with it. Sodium tripolyphosphate also has been used as an additive in toothpaste due to its cleaning action.

Cautions:

In generally is advised that STPP should be stored in a cool and well-ventilated area. Direct sunlight and damp areas usually should be avoided. If STPP comes into contact with skin, the area should be thoroughly rinsed. If eye contact is made, it is important to immediately flush the eyes.

8. MATERIALS AND METHODS

8.1 MATERIALS USED IN FORMULATION

For the formulation of Chitosan nanoparticles of Urapidil the following materials procured from the following manufacturer/ suppliers.

Table 8.1 List of material used in formulation

S.NO	NAME OF THE MATERIAL	PROCURED FROM	USE IN FORMULATION
1.	Urapidil	Adcock Ingram, Bangalore.	Active pharmaceutical ingredient
2.	Chitosan 50kD	Lab chemicals, Chennai.	Polymer
3.	Acetic acid	Lab chemicals, Chennai.	Solvent
4.	Sodium tripolyphosphate	Lab chemicals, Chennai.	Cross linking agent
5.	Tween 80	Sai Mirra Innopharm Pvt.Ltd, Chennai.	Surfactant
6.	Ethanol	Lab Chemicals, Chennai.	Solvent
7.	Potassium dihydrogen phosphate	Merck specialities Pvt.Ltd, Mumbai	Buffer Medium
8.	Sodium hydroxide	Lab Chemicals, Chennai.	Buffer medium
9.	Distilled water	Lab Chemicals, Chennai.	Solvent

8.2 EQUIPMENTS/ INSTRUMENTS USED IN FORMULATION

Table 8.2 List of Equipment / Instruments used

S.NO	EQUIPMENTS/ INSTRUMENTS	MANUFACTURERS / SUPPLIERS
1.	Electronic weighing balance	Mc Dalal, Chennai
2.	Magnetic stirrer	Remi Instruments, Mumbai
3.	High Speed Cooling centrifuge	Eppendorf Centrifuge 5810 R
4.	Ultra Sonicator	Lark, Chennai
5.	Freeze dryer	Mc Dalal, Chennai
6.	UV – Visible Spectrophotometer	Shimadzu, Japan
7.	Fourier Transform Infrared Spectroscopy	ABB MB3000
8.	pH meter	Labman scientific instrument (LMPH9), Chennai
9.	Melting point apparatus	Guna Enterprises, Chennai
10.	Particle size analyzer	Horiba Scientific Nano ZS100
11.	Zeta sizer	Horiba Scientific Nano ZS100
12.	Dissolution Apparatus	Labindia DS 8000, Chennai
13.	Disintegration Apparatus- Digital	Digital Programmable Disintegration apparatus, Rolex, India.
14.	Refrigerator	Samsung
15.	Stability chamber	Remi chem-6 plus

8.3 METHODOLGY

8.3.1 PREFORMULATION STUDIES

Preformulation study is defined as "Investigation of physical and chemical properties of the drug substance alone and combined with the excipients". Preformulation studies are the first step in the rational development of dosage form of drugs. It involves the application of biopharmaceutical principles to the physicochemical parameters of the drug with the goal of designing an optimum delivery system that is stable and bioavailable dosage form can be mass produced. The type of information needed will depend on the dosage form to be developed.

Thus the goals of the final study are,

1. To establish Physical characteristics
2. To establish its compatibility with the excipients
3. To determine kinetic rate profile [65,66]

8.3.1.1 Compatibility Studies[67]

The drug and excipients selected for the formulation were evaluated for physical and chemical compatibility studies.

Physical compatibility study

Each 100mg of powder drug, polymer and cross linking agent were weighed. Individual drug, polymer (Chitosan), sodium tripolyphosphate along with admixture of drug and excipients in airtight screw cap vials, kept at room temperature and at 40ºC and 75±2% RH. Any change in color of the physical mixture was observed visually.

Chemical compatibility study by FTIR

The chemical compatibility studies were conducted using Fourier Transform-Infrared (FTIR) spectroscopy, which was performed using a Shimadzu FTIR 8400 Spectrophotometer from 4000 to 400 cm^{-1} region. The method employed was Potassium bromide pellet method. In this method a small amount of finely ground solid samples (drug alone, Mixture of drug and excipients and the optimized formulation) intimately mixed with about 100 time its weight of powdered potassium bromide. The finely ground mixture was then passed under very high pressure in a press (at least 25,000 psig) to form a small pellets (1-2 mm thick and 1cm in diameter). The resulting pellet was transparent to IR radiation and was run as such.

Table No. 8.3 Composition of drug and excipients for FT-IR spectra

S.NO	INGREDIENTS
1	Drug
2	Drug + Chitosan
3	Drug + sodium tripolyphosphate
4	Drug + Chitosan + sodium tripolyphosphate

8.3.1.2 Determination of Melting point[68]

The melting point of Urapidil was determined by the capillary tube method as per USP. A Sufficient quantity of Urapidil powder was filled into the capillary tube to give a compact column of 4-6 mm in height. The tube was introduced in electrical melting point apparatus and the temperature was raised. The temperature at which the last solid particle of Urapidil in the tube passed into liquid phase was noted as melting point.

Preparation of 6.8 pH Phosphate buffer[69]

- 0.2M solution of potassium dihydrogen phosphate was prepared by dissolving 27.218gm of substance in 1000ml of distilled water.
- 0.2M solution of sodium hydroxide solution was prepared by dissolving 8gm of substance in 1000 ml of distilled water.
- 250 ml above prepared potassium dihydrogen phosphate solution & 112 ml of sodium hydroxide solution were mixed together and made up to 1000ml and pH was adjusted to 6.8.

8.3.1.3 Determination of lambda max (λ_{max}) [32]

100 mg of Urapidil was weighed and transferred to 100ml of volumetric flask. The drug was dissolved in 10 ml of ethanol and volume was made up to 100ml using phosphate buffer pH 6.8 to obtain a stock solution of 1000µg/ml (stock solution I). 10ml of this stock solution was again diluted with phosphate buffer pH 6.8 up to 100ml to obtain a solution of 100µg/ml (stock solution II). From the stock solution-II, 10 ml was pipette out in 100ml volumetric flask. The volume was made up to 100 ml using phosphate buffer pH 6.8 get a concentration of 10µg/ml. this solution was then scanned at 200-400nm in UV-Visible spectrophotometer to attain the absorption maxima (λ_{max}).

8.3.1.4 Standard curve for Urapidil [33]

100 mg of Urapidil was weighed and transferred to 100ml of volumetric flask. The drug was dissolved in 10 ml of ethanol and volume was made up to 100ml using phosphate buffer pH 6.8 to obtain a stock solution of 1000µg/ml (stock solution I). 10ml of this stock solution was again diluted with phosphate buffer pH 6.8 up to 100ml to obtain a solution of 100µg/ml (stock solution II). From the stock solution-II 2, 4,6,8,10,12 ml were transferred to series of 100 ml volumetric flasks. The volume was made up with phosphate buffer pH 6.8. The absorbance of these solutions was measured at 268nm against the blank.

8.3.1.5 Solubility studies of pure Urapidil[70]

Solubility of Urapidil pure drug was tested in distilled water and phosphate buffer pH 6.8. An excess amount of Urapidil pure drug was added in the pertinent media. The mixtures were stirred in a mechanical shaker at speed 50 rpm for 24 hours and the temperature was maintained at 37±0.5ºC. Visual inspection was carefully made to ensure there were excess Urapidil solids in the mixture, indicating saturation had been reached. Then the mixtures were filtered using 0.45µm filter and filtrates were suitably diluted with same media. The absorbance of the solution was measured at 268nm in UV-Visible spectrophotometer.

Table 8.4: Solubility characteristics[71]

Descriptive term	Parts of solvent required for part of solute
Very soluble	**Less than 1**
Freely soluble	**From 1 to 10**
Soluble	**From 10 to 30**
Sparingly soluble	**From 30 to 100**
Slightly soluble	**100 to 1000**
Very slightly soluble	**1000 to 10,000**
Practically insoluble or Insoluble	**10,000 or more**

8.3.2 FORMULATION DEVELOPMENT

8.3.2.1 Preparation of Chitosan Nanoparticles – Ionic gelation Method[72, 73, 74]

The preparation of Chitosan nanoparticles was based on ionic interaction between positively charged Chitosan solution and negatively charged STPP solution, with and without drug and it was prepared in the presence of Tween 80 as a re-suspending agent to prevent particle aggregation, at ambient temperature while stirring and Chitosan solution were raised to pH 4.6 to 4.7. Seven formulations (F1,F2,F3,F4,F5,F6,F7) of Urapidil loaded Chitosan nanoparticles were prepared by dissolving Urapidil in 30ml of Chitosan with varying concentrations (0.1,0.15,0.2,0.25,0.3,0.4,0.5%w/v) containing 0.5% w/v tween 80, TPP(0.1%w/v) was added drop wise under magnetic agitation(1000 rpm).The formed nanoparticle suspensions were lyophilized at -40^{o}C for 24hrs.

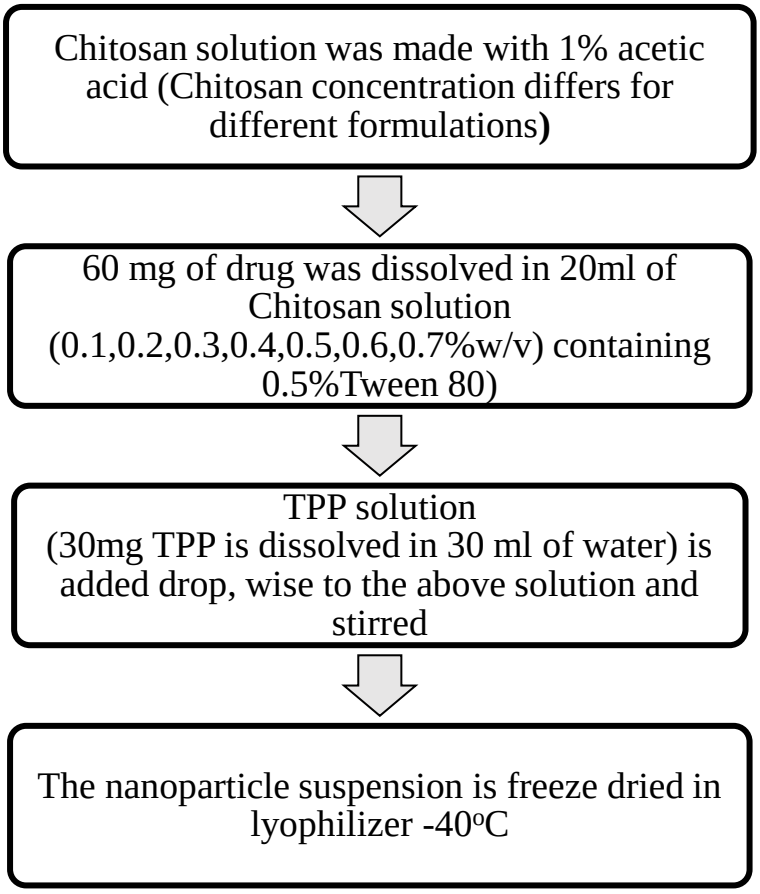


Table No.8.5: Composition of Nanoparticles

FORMULATION	POLYMER			DRUG (mg)	TWEEN 80 (%w/v)	SODIUM TRIPOLYPHOSPHATE		
	Chitosan (%w/v)	1% Aqueous acetic acid (ml)	Chitosan (mg)			TPP (%w/v)	Distilled water (ml)	TPP(mg)
F_1	0.1%	30ml	30	60	0.5	0.1	30	30
F_2	0.15%	30 ml	45	60	0.5	0.1	30	30
F_3	0.2%	30 ml	60	60	0.5	0.1	30	30
F_4	0.25%	30 ml	75	60	0.5	0.1	30	30
F_5	0.3%	30 ml	90	60	0.5	0.1	30	30
F_6	0.4%	30 ml	120	60	0.5	0.1	30	30
F_7	0.5%	30 ml	150	60	0.5	0.1	30	30

8.3.2.2 Freeze Drying [75]

Lyophilization is a promising way to increase the chemical and physical stability over extended period of time. Lyophilization had been required to achieve long term stability for a product containing hydrolysable drugs or a suitable product per oral administration.

The Urapidil loaded Chitosan suspension is kept in the freeze dryer at -20ºC in overnight and the flask were covered with parafilm sheets on the next day and perforated. After 24 hours the sample were kept inside the lyophilizer at temperature -40ºC and pressure below 15 Pascal (0.1pa) to remove the water from the samples. After lyophilization the dried powder is used for further studies.

8.4 CHARACTERIZATION OF URAPIDIL LOADED CHITOSAN NANOPARTICLE

All the formulated Urapidil loaded Chitosan Nanoparticles were evaluated for its percentage yield, drug content, entrapment efficiency, particle size, polydispersity index, zeta potential, *in vitro* drug release and kinetics of drug release.

8.4.1 Percentage yield[43]

The nanoaparticle yield was calculated according to the equation given below.

$$\text{Percentage yield (\%)} = \frac{\text{Practical yield}}{\text{Theoretical yield}} \times 100$$

8.4.2 Determination of Drug Content[76, 77, 89]

Equivalent to 60 mg of the prepared formulation were weighed and dissolved in minimum quantity of ethanol mixture and made up to 100ml with phosphate buffer pH 6.8. The solution kept for 24 hours and filtered to separate fragments. Drug content was analyzed after suitable dilution by UV- Visible spectrophotometer at a wave length 268nm against phosphate buffer pH 6.8 as blank. From the absorbance the drug content in the batches were calculated.

8.4.3 Drug entrapment efficiency[78]

For the determination of drug entrapment, the nanosuspension with known amount of drug was centrifuged at 4000 rpm for 15 minutes. The supernatant solution was separated. 5ml of supernatant was distributed with 100ml of phosphate buffer solution pH 6.8 and the absorbance was measured using UV-Visible spectrophotometer at 268nm using of phosphate buffer solution pH 6.8 as blank. The amount of drug unentrapped was calculated. The

percentage of entrapment efficiency was determined according to the following equation given below.

$$\% \text{ Drug Entrapment} = \frac{\text{Total amount of drug - Amount Of unbound drug}}{\text{Total amount of drug}} \times 100$$

8.4.4 Solubility Studies of Urapidil loaded Chitosan Nanoparticle[79]

The solubility of the Urapidil loaded Chitosan nanoparticles formulations were tested in various medium (distilled water and phosphate buffer pH 6.8) by adding an excess amount of formulations. The mixtures were stirred in a mechanical shaker at speed 50 rpm for 24 hours at room temperature. Visual inspection was carefully made to ensure there were excess urapidil solids in the mixture, indicating saturation had been reached. Then the mixtures were filtered using 0.45µm filter and filtrates were suitably diluted with same media. The absorbance of the solution was measured at 268nm in UV-Visible spectrophotometer.

8.4.5 *In vitro* drug release studies[80, 87]

The *in vitro* release rate studies of Urapidil loaded Nanoparticles formulations were carried out by dissolution test apparatus USP Type-I (Basket). Urapidil loaded Chitosan Nanoparticles were filled in capsule and placed in a dissolution medium and rotated at 100rpm. 10 ml of samples were withdrawn predetermined intervals up to 12 h and replaced with equal amount of phosphate buffer pH 6.8 for further dissolution testing the absorbance determined by spectrophotometrically at 268nm.

8.4.6 FTIR study of optimized formulations[67]

FT-IR spectra of optimized formulations of Chitosan Nanoparticle were recorded by grinding and dispersing the samples with micronized IR grade Potassium bromide powder and subjected to FT-IR measurement over the range of 4000- 400cm^{-1}.

8.4.7 Surface Morphology by SEM analysis[67, 76]

The Surface Morphology of the Chitosan nanoparticle can be measured by SEM at Anna University (Model Vega3 – Tescan, USA). Scanning Electron Microscopy was used to analyse particle size, shape and surface morphology of Nanoparticles. The sample was mounted directly onto the SEM sample holder using double sided sticking tape and image were recorded at different magnification at acceleration voltage of 10 kv using scanning electron microscope.

8.4.8 Particle size characterization[74]

The samples of the optimized formulations were analyzed for their particle size using Horiba Scientific SZ-100 particle size analyzer, Particle size (Z-average diameter), Polydispersity index (as a measure of the width of the particle size distribution) of Urapidil loaded Chitosan Nanoparticles dispersion is performed by dynamic light scattering also known as photon correlation spectroscopy (PCS) using a Horiba Scientific Nano SZ-100 at 25°C.

Prior to measurements all samples were diluted using ultra – purified water to yield a suitable scattering intensity. The diluted nanoparticles dispersion was poured into the disposable sizing cuvette which is then placed in the cuvette holder of the instrument and analyzed. Air bubbles were removed from the capillary before measurement.

8.4.9 Polydispersity Index (PDI) [78]

PDI indicates the width of the particle size distribution, which ranges from 0 to1. Monodisperse samples have a lower PDI value, whereas higher PDI value indicates a wider particle size distribution and the polydisperse nature of the samples can be calculated by following equation:

$$\mathbf{PDI = d/d\ avg}$$

Where,

d is the width of distribution donated by SD

d avg is the average particle size denoted by MV (nm) in particle size data sheet.

8.4.10 Zeta potential [81]

Zeta Potential is a crucial factor to evaluate the stability of colloidal dispersion surface charge on the Urapidil loaded chitosan NPs were determined using Horiba Scientific Nano ZS100. 1 ml of sample of Urapidil suspension was filled in clear disposable zeta cell ensured there was no air bubble within the sample and the system was set at 25°C temperature and the test can be carried.

8.5 PREFORMULATION STUDY OF OPTIMIZED NPs

8.5.1 Flow property measurements[82]

The flow properties are critical for an efficient tabletting and capsule filling operation. A good flow of the powder or granules is necessary to assure efficient mixing and acceptable weight uniformity for the compressed tablets and capsules. The flow property measurements include bulk density, tapped density, angle of repose, compressibility index and Hausner's ratio. The flow property measurements of Urapidil chitosan NPs are determined.

8.5.2 Bulk density (ρb) [83]

It is the ratio of total mass of powder to the bulk volume of powder. It was measured by pouring the weighed powder into a measuring cylinder and initial weight was noted. This initial volume was called the bulk volume. From this the bulk density was calculated according to the formula mentioned below. It is expressed in g/ml and is given by

$$\rho b = M/V_b$$

Where, M and V_b are mass of powder and bulk volume of the powder respectively.

8.5.3 Tapped density (ρ_t) [83]

It is the ratio of weight of the powder to the tapped volume of powder. The powder was introduced into a measuring cylinder with the aid of funnel and tapped for 300 times on a wooden surface at a 2 sec interval and the volume attained is the tapped volume.

$$\rho_t = m/V_t$$

8.5.4 Angle of repose (θ) [84]

The flow properties were characterized in terms of angle of repose, Carr's index and Hausners's ratio. For determination of angle of repose, the drug and the blend were poured through the walls of a funnel, which was fixed at a position such that its lower tip was at a height of exactly 2.0 cm above hard surface. The drug or the blends were poured till the time when upper tip of the pile surface touched the lower tip of the funnel. Angle of repose was calculated using following equation.

$$\theta = \tan^{-1} (h/r)$$

Where h= height of pile in cm; r = radius of pile in cm.

8.5.5 Carr's index or % compressibility[84]

It indicates powder flow properties. It is measured for determining the relative importance of interparticulate interactions. It is expressed in percentage and is given by

$$CI = \frac{\rho_t - \rho_b}{\rho_t}$$

Where ρ_t and ρ_b are tapped density and bulk density respectively.

8.5.6 Hausner's ratio[84]

Hausner's ratio is an indirect index of ease of powder flow. It is calculated by the following formula.

$$\mathbf{HR = \rho_t / \rho_b}$$

Where ρ_t and ρ_b are tapped density and bulk density respectively.

Table 8.6 Angle of Repose, Compressibility Index and Hausner's ratio

Flow property	Angle of repose (Ɵ)	Compressibility Index (%)	Hausner's ratio
Excellent	25-30	<10	1.00-1.11
Good	31-35	11-15	1.12-1.18
Fair	36–40	16-20	1.19-1.25
Passable	41-45	21-25	1.26-1.34
Poor	46-55	26-31	1.35-1.45
Very poor	56-65	32-37	1.46-1.59
Very very poor	>66	>38	>1.60

8.6 OPTIMIZED NPs FILLED IN HARD GELATIN CAPSULE[85]

The optimized Urapidil chitosan nanoparticles unstable in suspension form so it is lyophilized and converted into powder form. The dried powder is filled into size "0" hard gelatine capsule and each capsules containing 60mg equivalent of Urapidil.

8.7 EVALUATION OF OPTIMIZED CAPSULES[86]

8.7.1 Uniformity of weights

Intact capsule were weighed. The capsules were opened without losing any part of the shell and contents were removed as completely as possible. The shell was washed with ether and the shell allowed to stand until the odour of the solvent was no longer detectable. The empty shell was weighed. The procedure was repeated with a further 19 capsules. The average weight was determined. Not more than two of the individual weights deviate from the average weight by more than the percentage deviation and deviates by more than twice that percentage.

Table No 8.7 Uniformity of weights (I.P. Standard)

Average weight of capsule contents	Percentage deviation
Less than 300 mg	±10
300 mg or more	±7.5

8.7.2 Disintegration test

One capsules introduced in to each tube of the disintegration test apparatus. A disc may be added if necessary. The basket rack assembly is suspended in the beaker containing the liquid medium. The apparatus is operated and the time for disintegration is noted.

Table No.8.8 Disintegration time (I.P. Standard)

Type of capsules	Medium	Disintegration time
Hard capsules	Water	30 min
Soft capsules	Water	60 min

Enteric capsules	0.1 M Hydrochloric acid	Do not disintegrate for 2 Hour
	Mixed phosphate buffer pH 6.8	One hour

8.7.3 Drug content[87]

Five capsules were selected randomly and the average weight was calculated. The powder is removed completely. An amount of powder was equivalent to 60mg is dissolved with 10 ml of ethanol made up to 100 ml with Phosphate buffer pH 6.8. It was kept for overnight. The absorbance of solution was recorded at 268nm.

8.7.4 *In vitro* drug release study[44, 87]

The *in vitro* release of Urapidil Nanoparticle is evaluated by the using USP Type- I (Basket) dissolution test apparatus. Urapidil loaded Chitosan Nanoparticle filled equivalent amount in capsule and placed in a dissolution jar. Phosphate buffer pH 6.8 is used as dissolution medium and rotated at 100 rpm.10 ml of samples are withdrawn predetermined intervals of 1 hour and replaced with equal amount of phosphate buffer pH 6.8 for further dissolution testing the absorbance determined by spectrophotometrically at 268nm.

8.8 RELEASE KINETICS OF THE OPTIMIZED FORMULATIONS [88]

Different kinetic models such as zero order (cumulative amount of drug released vs. time), first order (log cumulative percentage of drug remaining vs. time), Higuchi model (cumulative percentage of drug released vs. square root of time), Korsmeyer-Peppas model and Hixson Crowell model were applied to interpret the drug release kinetics from the formulations. Based on the highest regression values for correlation coefficients for formulations, the best- fit model was decided.

To study the *in vitro* release kinetics of the optimized formulation, data obtained from dissolution study were plotted in various kinetics models.

- Zero-order
- First-order
- Higuchi
- Hixson-Crowell cube root law
- Korsmeyer-Peppas model

8.8.1 Zero order equation

The zero order release can be obtained by plotting cumulative % percentage drug released vs. time in h

$$C=K_0t$$

Where, K_0 = Zero order constant

T= time in h

8.8.2 First order

The graph was plotted as % cumulative drug release vs. time in h

$$Log\ C = Log\ C_0 – Kt / 2.303$$

Where, C_0 = initial concentration of drug,

K= First order

t= time in h

8.8.3 Higuchi kinetics

The graph was plotted with % cumulative drug release vs. square root of time.

$$Q = Kt^{1/2}$$

Where, K = constant reflecting design variable system (differential rate constant)

t = time in h

The drug release rate is inversely proportional to the square root of time.

8.8.4 Hixson and Crowell erosion equation:

To evaluate the drug release with changes in the surface area and the diameter of the particles, the data were plotted using the Hixson and Crowell rate equation. The graph was plotted by cube root of % drug remaining vs. time in h.

$$Q_0^{1/3} – Q_t^{1/3} = K_{HC}t$$

Where, Q_0 = Initial amount of drug

Qt = Amount of drug released in time t

K_{HC} = Rate constant for Hixson Crowell equation

8.8.5 Korsmeyer- Peppas equation

To evaluate the mechanism of drug release, t was further plotted in korsmeyer-Peppas equation as log cumulative % of drug released vs. log time

$$M_t / M_\alpha = Kt^n$$

Where, M_t / M_α = Fraction of drug released at time t

t = Release time

K=Kinetics constant (instructing structural and geometric characteristic of the formulation)

N= Diffusional exponent indicative of the mechanism of drug release

Table No 8.9 Diffusion exponent and solute release mechanism

Diffusion Coefficient	Overall solute diffusion mechanism
0.45	Fickian diffusion
0.45<n<0.89	Anomalous (non-fickian diffusion)
0.89	Case II transport
n>0.89	Super case II transport

8.9 STABILITY STUDIES[90, 91]

The optimized formulation (F5) is studied for stability for 90 days. The optimized formulation is divided into 3 groups. One group was kept at refrigeration (4±1ºC) temperature, second group at room temperature and the third group at 40±2ºC, 70±5% RH temperature. The formulation is withdrawn and analysed for change in physical appearance, drug content and entrapment efficiency at 30 days interval. The purpose of stability testing was to provide evidence on how the quality of a drug substance or nanoaprticles varies with time under influence of varies environmental factors such as temperature, humidity and light.

9. RESULTS AND DISCUSSION

9.1 PRE-FORMULATION STUDIES

- The optimization of a formulation can be done only after a thorough investigation of its physicochemical properties of the drug and excipients. The drug and the polymer must be compatible for a successful formulation.

9.1.1 COMPATIBILITY STUDIES

9.1.1.1 Physical Compatibility study

Table 9.1 Physical compatibility study of Drug and Excipients

S.No	Drug / Excipients	Description and Condition						
		Initial	At room temperature (in days)			At 40ºC ± 2ºC and 75% RH ± 2% (in days)		
			I	II	III	I	II	III
1	Urapidil	White coloured powder	NC	NC	NC	NC	NC	NC
2	Chitosan	Pale yellow coloured powder	NC	NC	NC	NC	NC	NC
3	Sodium tripolyphosphate	White coloured powder	NC	NC	NC	NC	NC	NC
4	Urapidil + Chitosan	Pale yellow coloured powder	NC	NC	NC	NC	NC	NC
5	Urapidil + Sodium tripolyphosphate	White coloured powder	NC	NC	NC	NC	NC	NC

*NC – No Change

Inference

The Physical compatibility study was performed for 3 months. There was no change of color therefore the drug and excipients are physically compatible with each other.

9.1.1.2 Chemical Compatibility Study

FT-IR spectroscopic study

FT-IR spectroscopy gives the possible information about the interaction between the drug and polymer. The results are follows

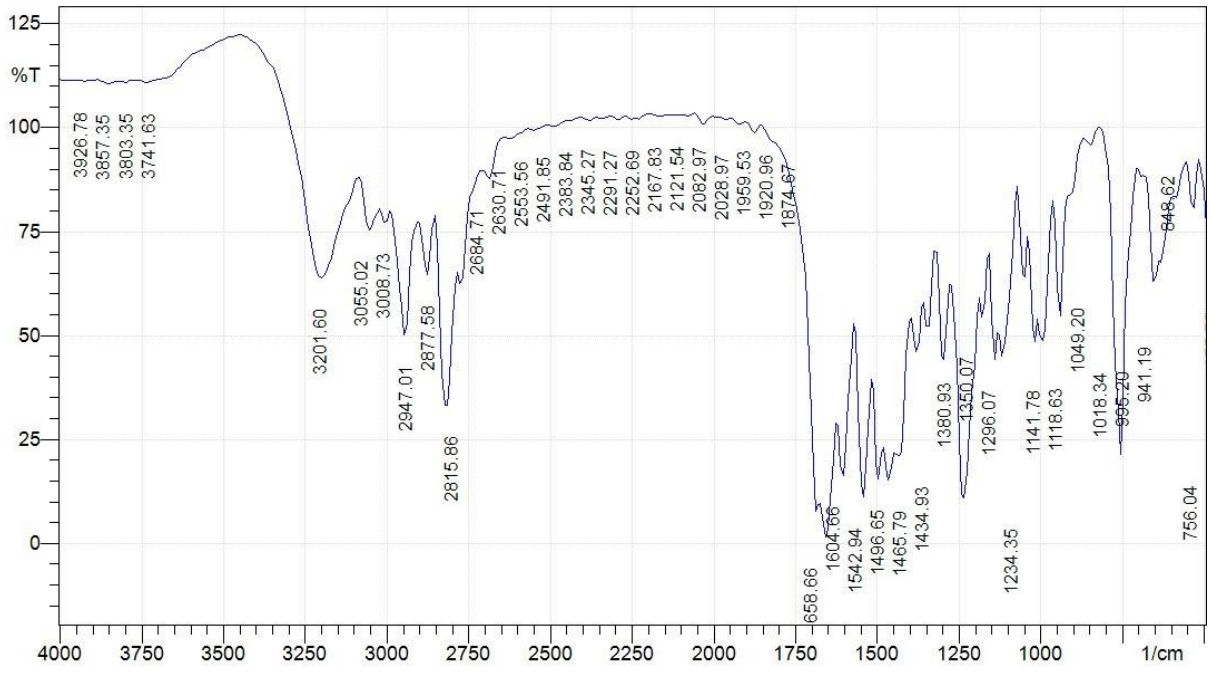


Figure 9.1: FT-IR spectrum of Urapidil

Table No 9.2: FT-IR spectral Interpretation of Urapidil

Type of vibration	Wave number (cm^{-1})
Aromatic C-H stretching	3055.02
C-N Stretching	1049.20
N-H Stretching	3201.60
C=O Stretching	1604.66
C-H Stretching	2947.01
N-C Stretching	1234.55
C-O-C Stretching	1380.93

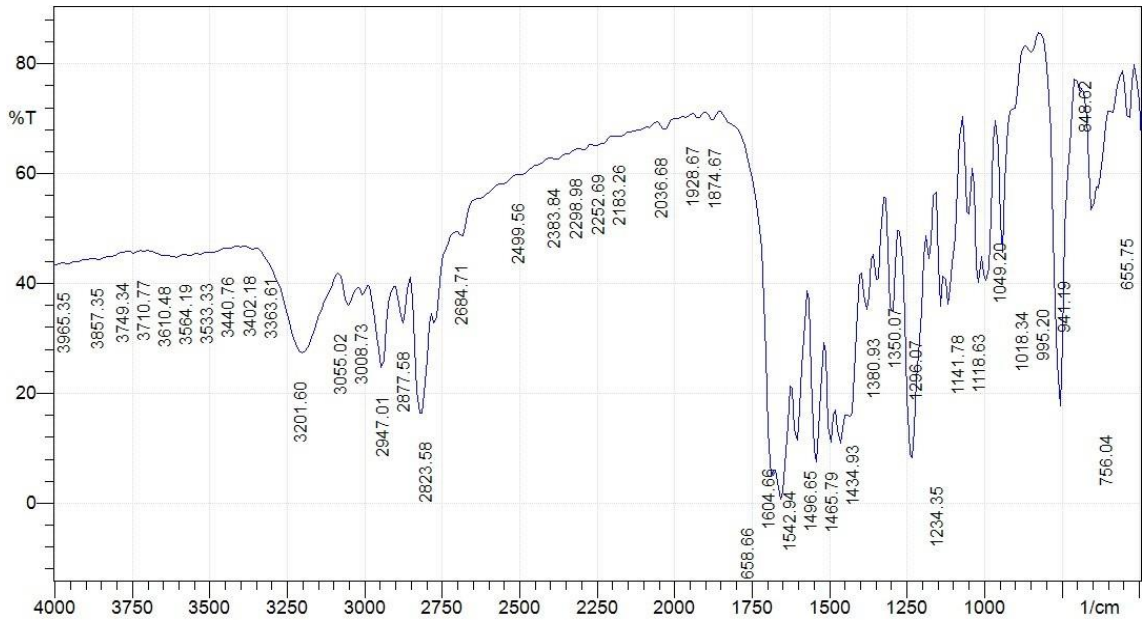


Figure 9.2: FT-IR Spectrum of Urapidil + Chitosan

Table No 9.3: FT-IR Spectral Interpretation of Urapidil + Chitosan

Type of vibration	Wave number (cm^{-1})
Aromatic C-H stretching	3055.02
C-N Stretching	1049.20
N-H Stretching	3201.60
C=O Stretching	1604.66
C-H Stretching	2947.01
N-C Stretching	1234.55
C-O-C Stretching	1380.93
NH_2	3402.18
C-O	1296.07
Phenolic- (O-H)	3201.60

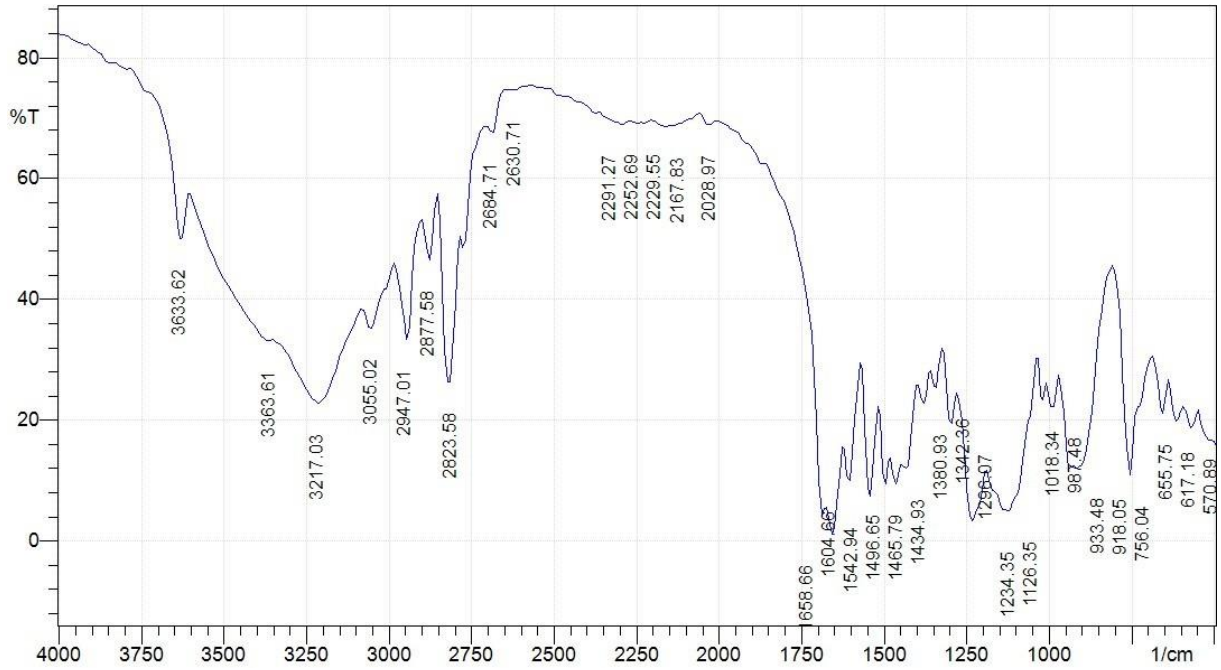


Figure 9.3: FT-IR spectrum of Urapidil + Sodium Tripolyphosphate

Table No 9.4: FT-IR Spectral Interpretation of Urapidil + Sodium tripolyphosphate

Type of vibration	Wave number (cm^{-1})
Aromatic C-H stretching	3055.02
C=O Stretching	1604.66
C-H Stretching	2947.01
N-C Stretching	1234.55
C-O-C Stretching	1380.93
Na	570.89
P-0	1126.35

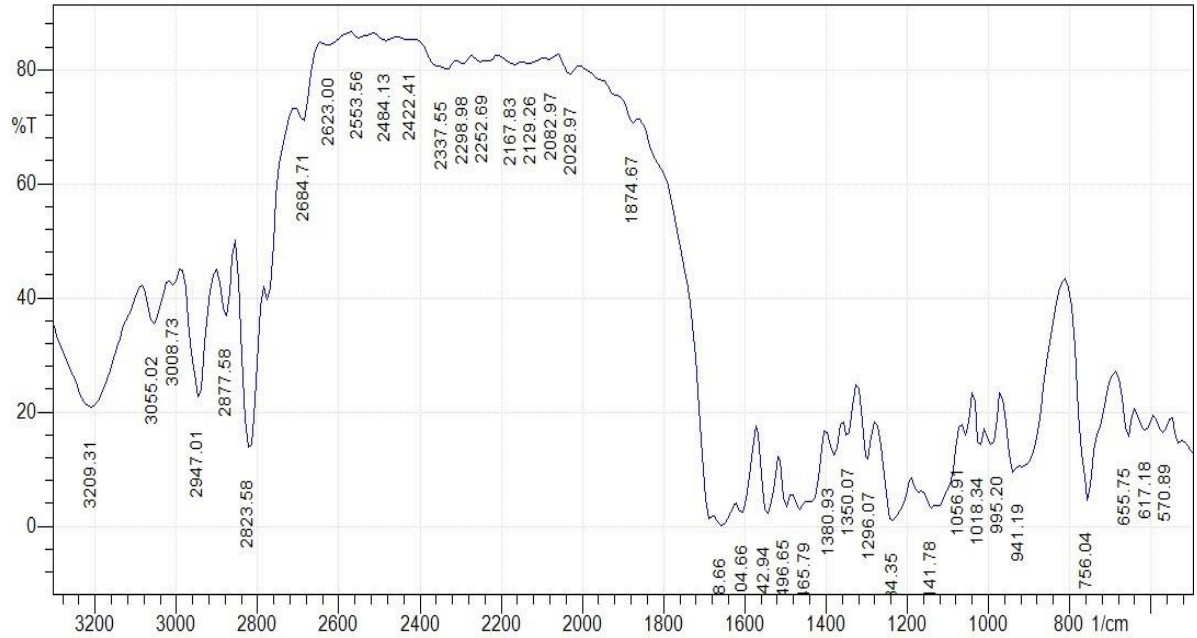


Figure 9.4 FT-IR spectrum of Urapidil + Chitosan + Sodium tripolyphosphate

Table 9.5 FT-IR spectral Interpretation of Urapidil + Chitosan + Sodium tripolyphosphate

Type of vibration	Wave number (cm^{-1})
Aromatic C-H	3055.02
C-N Stretching	1380.93
N-H Stretching	3209.31
C=0 stretching	1604.66
C-H Stretching	2947.01
N-C Stretching	1234.35, 1056.91
C-0-C Stretching	1350.07
C-O Stretching	1296.07
Na	570.89
P-O	1141.78

9.1.2. MELTING POINT

The melting point of Urapidil was measured using capillary tube method.

Table 9.6: Melting point of drug

S.No	Drug / Excipients	Specification	Observation
1	Urapidil	157ºC-160ºC	157ºC-158 ºC

Inference

The melting point of drug was studied and tabulated, which confirms the identification of Urapidil.

9.1.3 SOLUBILITY STUDY

The solubility study of Urapidil in different dissolution medium is performed by saturation solubility method.

Table 9.7: Saturation solubility of Urapidil in phosphate buffer pH 6.8 and Distilled water

Medium	pH	Solubility (mg/ml)
Phosphate buffer pH 6.8	6.8	0.004093
Distilled water	7.0	0.000216

Inference

The solubility of the drug at pH 6.8 was significantly higher than in that of distilled water. Pure drug of Urapidil in distilled water and phosphate buffer pH 6.8 was found be insoluble.

9.1.4 DETERMINATION OF LAMBDA MAX (λ_{max}) FOR URAPIDIL

The maximum absorbance of the Urapidil was studied. The maximum absorbance of the Urapidil was found to be 268nm. Hence the wavelength of 268nm was selected for analysis of drug in dissolution media.

9.1.5 CALIBRATION CURVE FOR URAPIDIL

Table 9.8: Data for standard curve of Urapidil in Phosphate buffer pH 6.8

S.No	Concentration (µg/ml)	Absorbance
1	0	0
2	2	0.1153±0.000374
3	4	0.2203±0.000287
4	6	0.3445±0.000432
5	8	0.4686±0.000455
6	10	0.6035±0.000424
7	12	0.7089±0.000497

Mean ± SD (n=3)

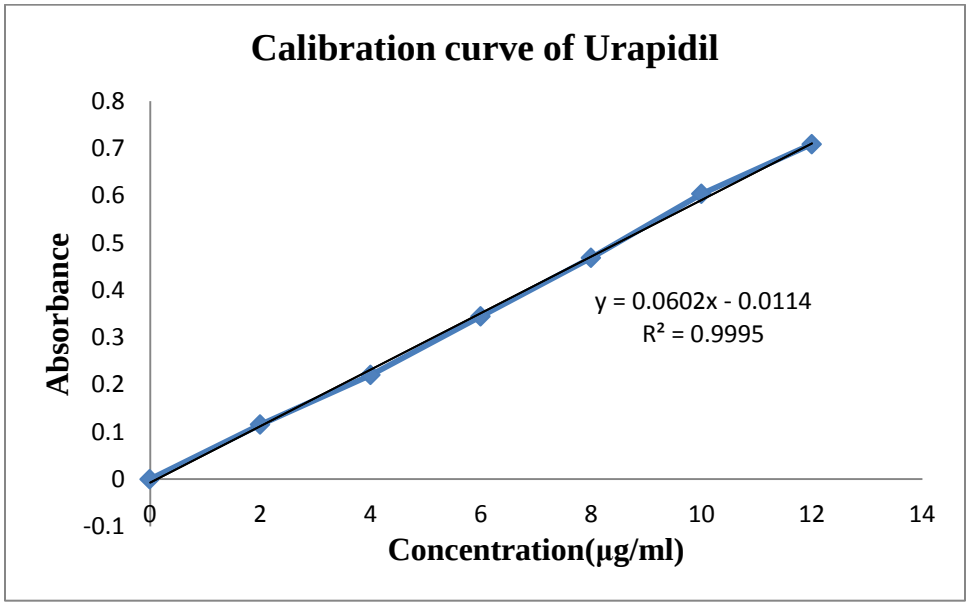


Figure 9.5: Standard curve of Urapidil in Phosphate buffer pH 6.8

Inference

The constructed calibration curve of Urapidil in Phosphate buffer pH 6.8 is shown figure 9.5. It was found that the solutions show linearity (R^2 = 0.9995) in absorbance at a concentration of 2-12 µg/ml and obeys Beer-Lambert's law.

9.2 FORMULATION OF URAPIDIL LOADED CHITOSAN NPs

Urapidil loaded Chitosan nanoparticles were prepared by Ionic-gelation method using Chitosan as polymer with different ratios and Sodium tripolyphosphate as cross linking agents. Formed nanoparticles were separated by centrifugation.

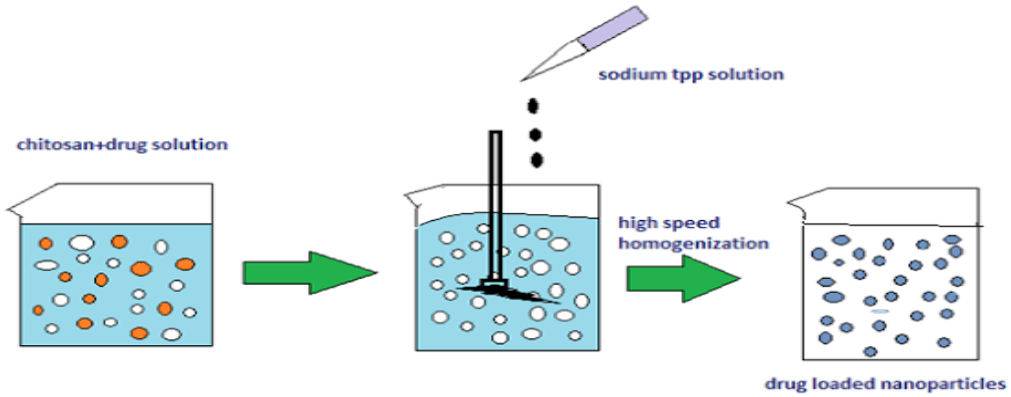


Figure 9.6: Ionic-gelation method

A) Before Lyophilization **B) After Lyophilization**

Figure 9.7: Urapidil loaded Nanoparticles before (A) and after (B) Lyophilization

9.3 CHARACTERIZATION OF URAPIDIL LOADED CHITOSAN NPs

The formulated Urapidil loaded Nanoparticles are characterized for Percentage yield, Drug content, Entrapment efficiency and the results were tabulated below.

Table 9.9 Characterization of Urapidil loaded Chitosan NPs

Formulations	Percentage yield (%)	Entrapment Efficiency (%)	Drug content
F1	79.09	83.40	77.16
F2	80.53	86.08	79.98
F3	81.90	88.65	82.52
F4	85.81	90.42	86.19
F5	**87.25**	**93.15**	**91.83**
F6	81.16	89.59	85.62
F7	78.84	85.61	82.10

9.3.1 PERCENTAGE YIELD

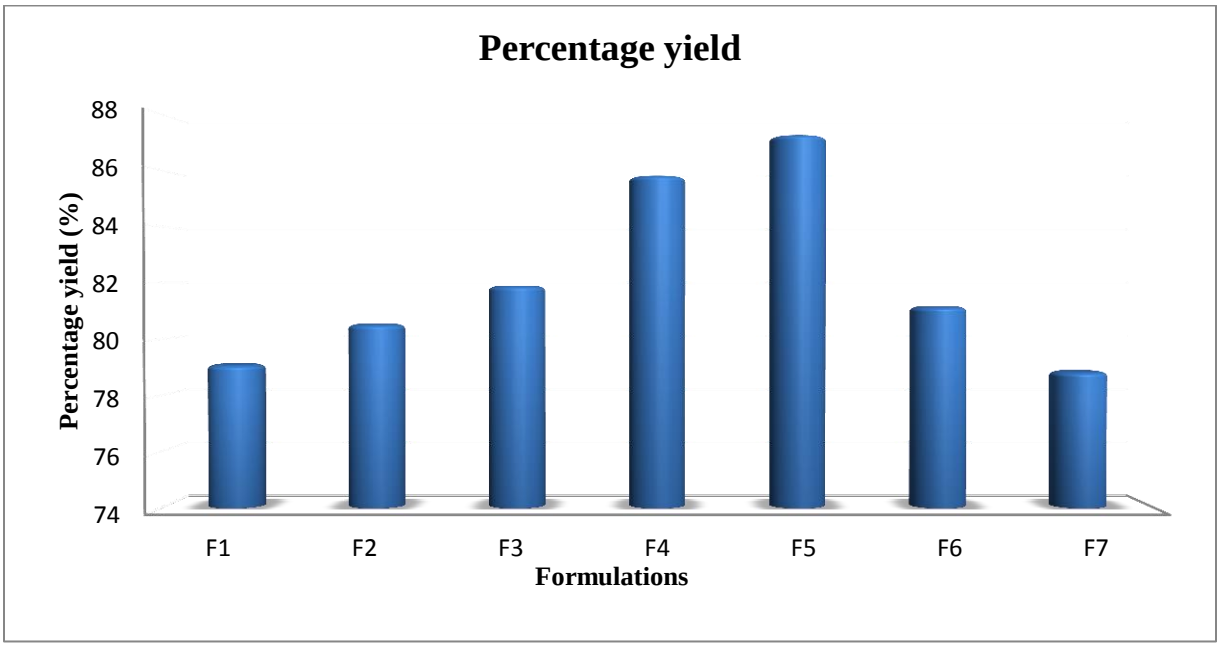


Figure 9.8: Percentage yield of Nanoparticle formulations

Inference

- Urapidil loaded Chitosan Nanoparticles were prepared and their percentage yield was calculated. The production yield of all batches was lies between 78.84 and 87.25%.
- The percentage yield of the formulation was not equal to 100% there was deviation with the percentage yield of the compounds. It may be due to the concentration of polymer in the formulation.
- F5 showed better yield shown in figure.9.8 compared to other six formulations. It was found that increases in the polymer concentration resulted in increased production yield but certain concentration it decreases due to its drug entrapment.

9.3.2 ENTRAPMENT EFFICIENCY

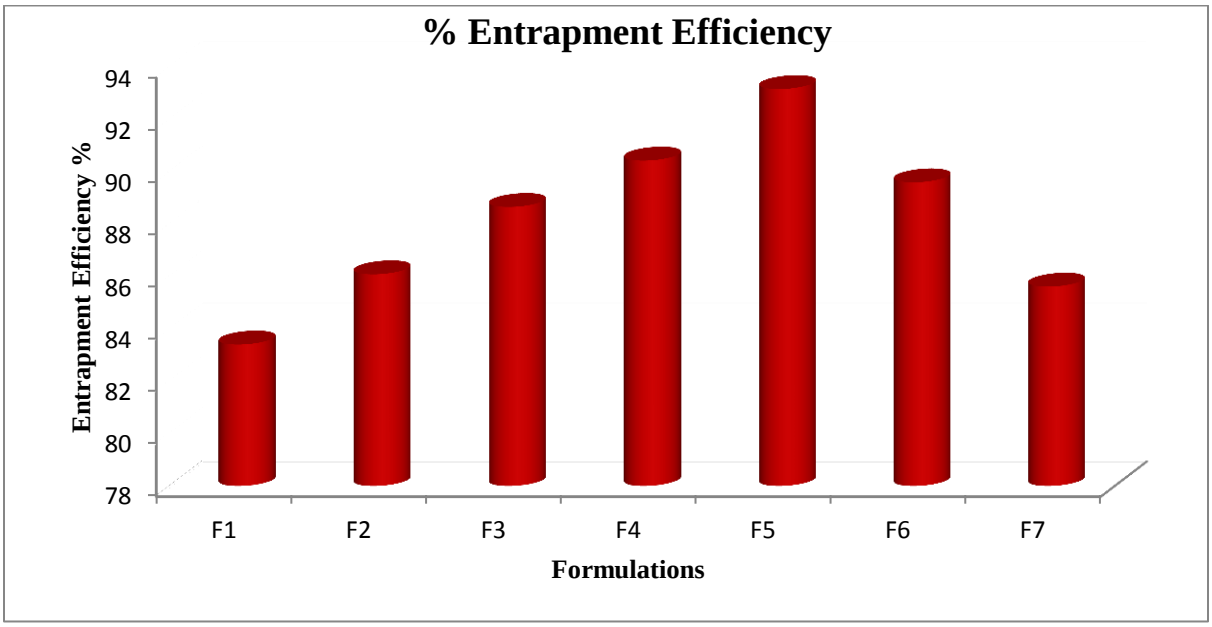


Figure 9.9: % Entrapment Efficiency of Nanoparticle formulations

Inference

- The entrapment of efficiency of the Chitosan Nanoparticles formulations was determined by centrifugation process. The entrapment efficiency of the formulations F1 to F7 was observed to lies between 83.40% and 93.15%.
- Low Chitosan concentration (1mg/ml) resulted aggregates. High Chitosan concentration (4mg/ml to 5mg/ml) resulted lesser encapsulation. Chitosan solution within the concentration of (3mg/ml) produced opalescent nanoparticle suspension. Further increases in concentration lead to decrease in encapsulation efficiency of Urapidil nanoparticles.
- Chitosan concentration (0.3% or 3mg/ml) promote the encapsulation of the drug (F5).

9.3.3 DRUG CONTENT

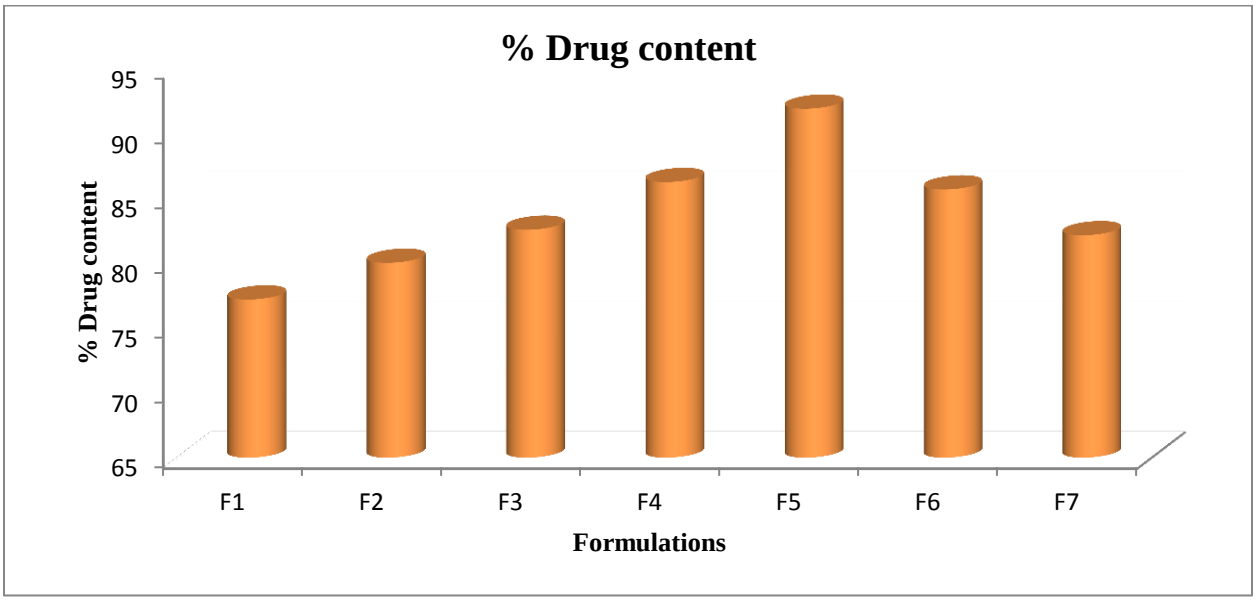


Figure 9.10: Drug content of Nanoparticle formulations

Inference

- The prepared formulations were analyzed for drug content and the data is reported in table 9.10. The particle size of the nanoparticle was increased by increasing polymer concentration.
- Due to increased particle size, the drug content also increased from 77.16% to 91.83% as on increasing polymer concentration 0.1% to 0.5%. There was no significant increase in drug content as polymer concentration increased. This probably caused by the increasing viscosity and hence poor dispersibility of Chitosan solution into aqueous phase.

9.3.4 SOLUBILITY STUDIES OF URAPIDIL LOADED NANOPARTICLES

The solubility study of Chitosan Nanoparticles formulations in distilled water and phosphate buffer pH 6.8 were studied by saturation solubility method. The Nanoparticles formulations compared with pure and tabulated below.

Table 9.10 Solubility of Nanoparticles and Urapidil in various medium

S.No	Formulation code	Solubility Medium	
		Distilled water pH 7 (mg/ml)	Phosphate buffer pH 6.8 (mg/ml)
1	Pure drug	0.000216	0.004093
2	F1	6.0998	6.9676
3	F2	6.7923	7.4355
4	F3	7.5888	8.7722
5	F4	7.9351	9.6748
6	F5	9.4933	13.251
7	F6	7.5190	8.1373
8	F7	6.4802	7.6360

Inference

- The solubility of Urapidil in distilled water and phosphate buffer pH 6.8 were found to be 0.000216 mg/ml and 0.004093 mg/ml respectively.
- The solubility of all formulations was improved (from insoluble to slightly soluble) compared to pure drug of Urapidil. Among all the formulations F5 show higher solubility in distilled water and phosphate buffer pH 6.8.

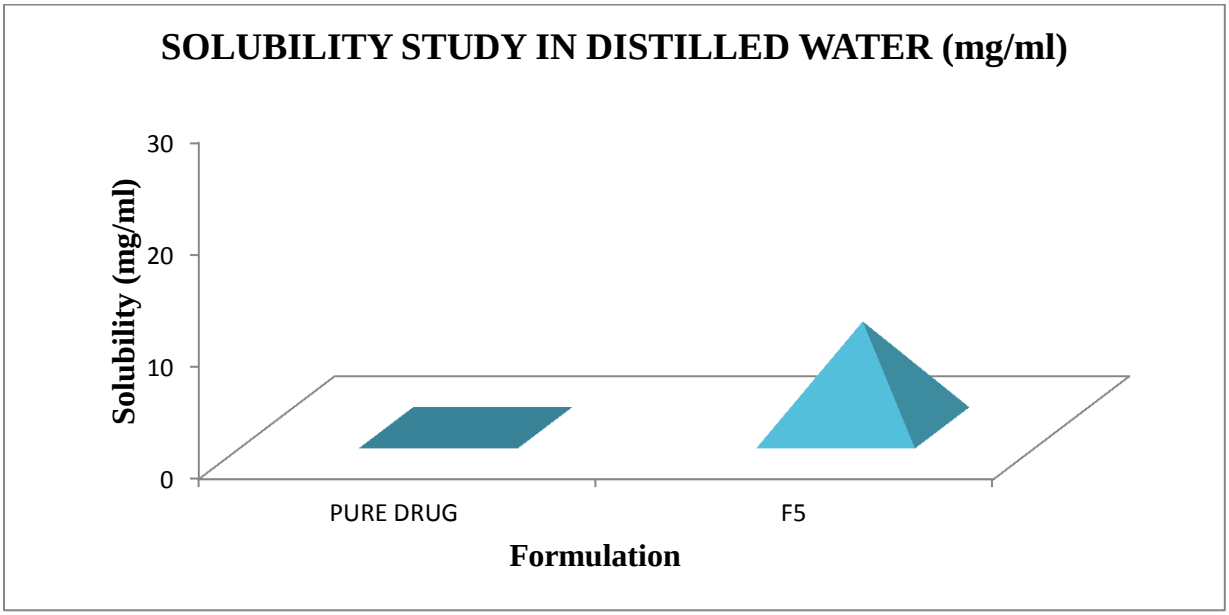


Figure 9.11: Solubility of optimized formulation F5 in Distilled water

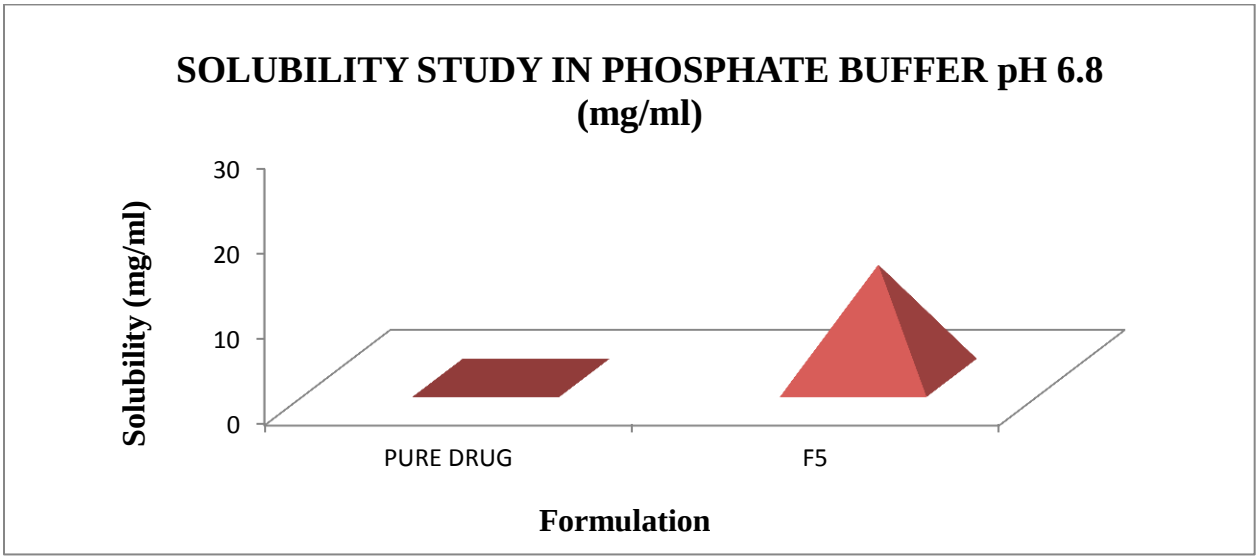


Figure 9.12 Solubility study of optimized formulation F5 in phosphate buffer pH 6.8

Inference

- Thus the solubility of formulation F5 in Distilled water and Phosphate buffer pH 6.8 were improved and compared with pure drug.

9.3.5 COMPARATIVE *IN VITRO* DRUG RELEASE FOR ALL FORMULATION

The formulated Nanoparticles preparation containing drug and polymer were evaluated for drug release and results were tabulated below

Table 9.11: *In vitro* drug release for all formulations

Time (h)	*In Vitro* Drug Release For Nanoparticles Formulations						
	F1	**F2**	**F3**	**F4**	**F5**	**F6**	**F7**
0.5	17.25	17.45	16.04	15.09	13.19	9.71	8.32
1	20.12	19.89	19.2	18.99	16.17	11.14	10.14
2	36.4	29.13	28.83	27.02	24.02	17.35	14.14
3	44.8	38.03	37.92	36.21	34.52	31.3	29.27
4	60.2	50.08	47.22	44.1	41.23	34.02	31.73
5	72.27	63.07	59.12	53.8	50.14	36.37	32.13
6	82.29	79.02	69.03	61.78	57.71	42.13	39.92
7	97.04	89.9	78.97	73.2	64.21	50.12	43.78
8	-	96.02	89.17	86.51	73.22	62.79	59.11
9	-	-	94.18	91.52	78.01	71.23	69.24
10	-	-	-	94.47	86.22	79.13	75.17
11	-	-	-	-	91.89	83.2	81.2
12	-	-	-	-	95.03	86.03	83.04

***In-vitro* Drug Release**

The formulations F1 TO F7 were prepared using Chitosan as a polymer (0.1%, 0.15%, 0.2%, 0.25%, 0.3%, 0.4% and 0.5%) and the Sodium tripolyphosphate as a cross linking agent.

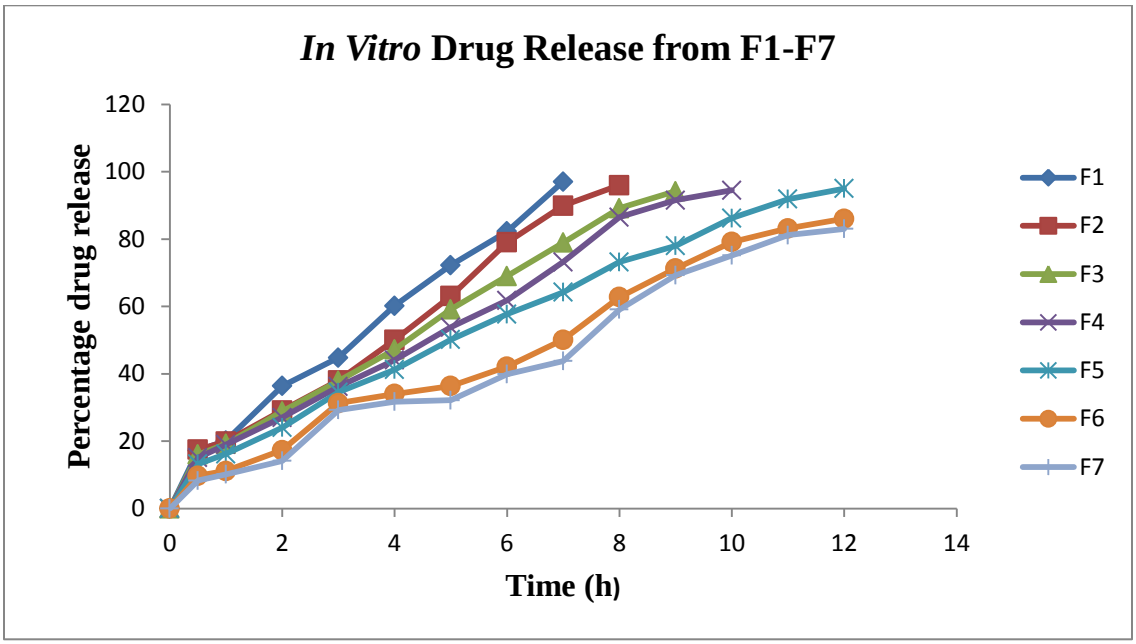


Figure 9.13: *In vitro* release study of Chitosan Nanoparticles (F1-F7)

Inference

- The *in vitro* drug release profile for formulated Urapidil loaded Chitosan Nanoparticles obtained from F1-F7 formulations were shown in figure 9.13.
- Among all the formulations F5 formulations shows 95.03% of drug release at the end of 12h in controlled manner. Thus F5 was selected as the optimized formulation.

9.4 CHARACTERIZATION OF OPTIMIZED URAPIDIL LOADED CHITOSAN NANOPARTICLES

9.4.1 FTIR Study of Optimized NPs

FTIR of optimized formulations of Urapidil loaded Chitosan Nanoparticles (F5)

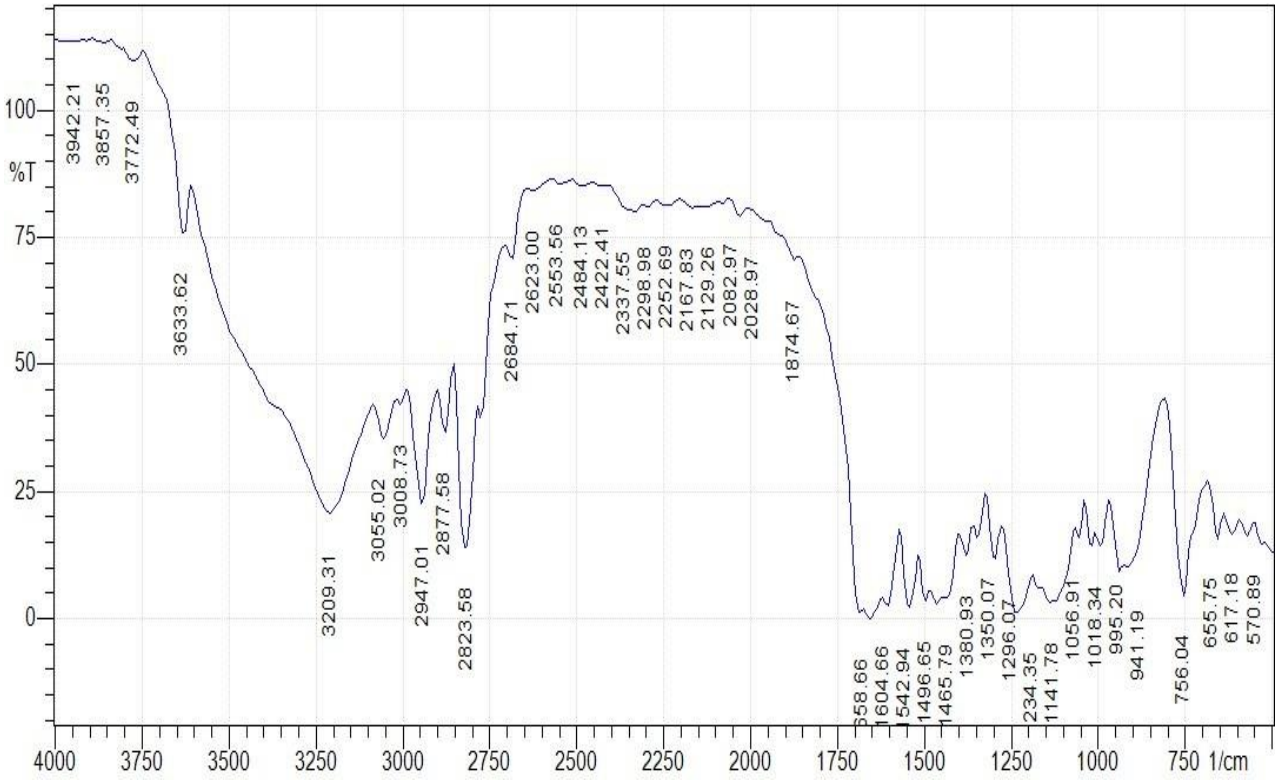


Figure 9.14 FT-IR spectral of Urapidil + Chitosan + Sodium tripolyphosphate

Table No: 9.12 FT-IR spectral Interpretation of Urapidil + Chitosan + Sodium tripolyphosphate

Type of vibration	Wave number (cm^{-1})
Aromatic C-H stretching	3055.02
C-N Stretching	1056.91
N-H Stretching	3209.31
C=O Stretching	1604.66
C-H Stretching	2947.01
C-N Stretching	1234.35
C-O-C Stretching	1380.93
Na	570.89
P-O	1141.78
Phenolic- (O-H)	3209.10
C-O	1296.07

Inference

- From the FTIR spectral studies, it is clearly evident that the optimized formulation F5 shows the presence of characteristic bonds for Urapidil, Chitosan. This indicates the absence of chemical interaction between the drug and excipients in this formulation.

9.4.2 SCANNING ELECTRON MICROSCOPE (SEM) ANALYSIS

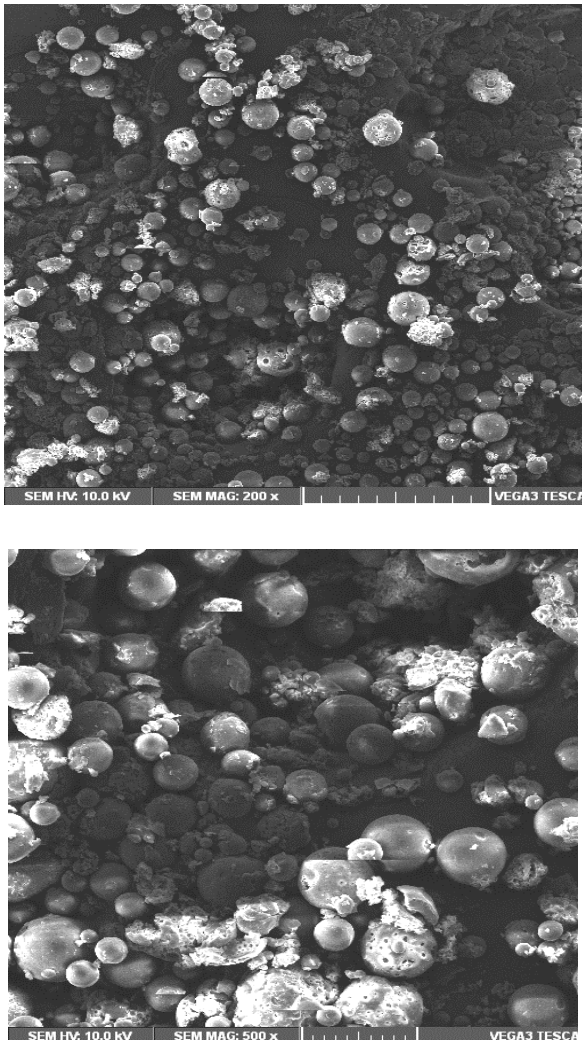


Figure 9.15 SEM Image of optimized formulation F5

Inference

- The shape and surface morphology of optimized formulations F5 was observed in scanning electron microscope.
- The SEM image of optimized formulation of F5 shows the prepared nanoparticles were spherical in shape with a smooth surface.

9.4.3 PARTICLE SIZE CHARACTERISATION

HORIBA Scientific

SZ-100

HORIBA SZ-100 for Windows [Z Type] Ver2.20

Measurement Results

Date	: 11 February 2022 14:32:08
Measurement Type	: Particle Size
Sample Name	: Chitosan F5
Scattering Angle	: 90
Temperature of the Holder	: 24.9 deg. C
Dispersion Medium Viscosity	: 0.897 mPa.s
Transmission Intensity before Meas.	: 25565
Distribution Form	: |Standard|
Distribution Form(Dispersity)	: Monodisperse
Representation of Result	: Scattering Light Intensity
Count Rate	: 386 kCPS

Calculation Results

Peak No.	S.P.Area Ratio	Mean	S. D.	Mode
1	1.00	188.3 nm	40.9 nm	160.3 nm
2	---	--- nm	--- nm	--- nm
3	---	--- nm	--- nm	--- nm
Total	1.00	188.3 nm	40.9 nm	160.3 nm

Cumulant Operations

Z-Average	: 229.0 nm
PI	: 0.372

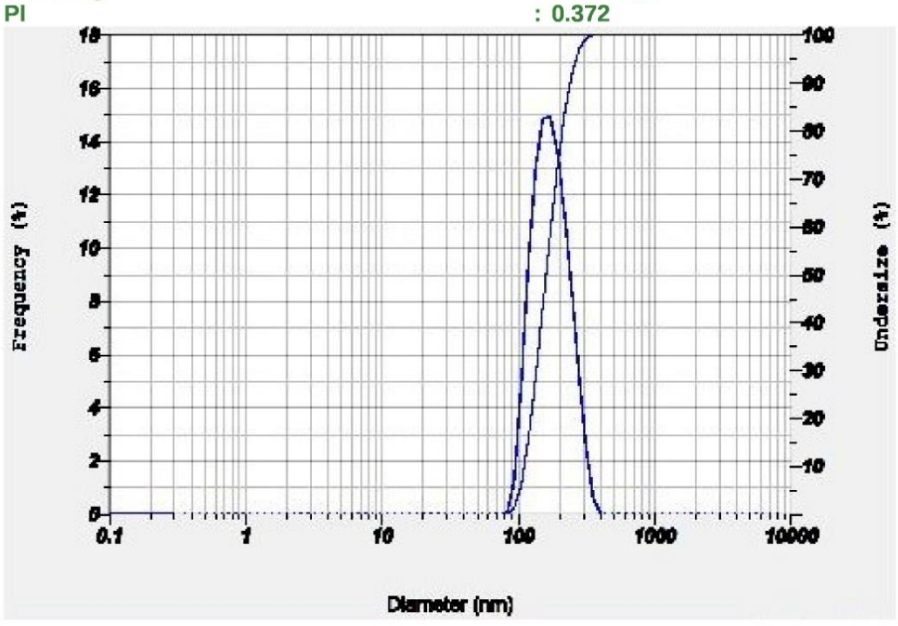


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Figure 9.16: Particle size distribution of F5

9.4.4 DETERMINATION OF ZETA POTENTIAL

HORIBA Scientific

HORIBA SZ-100 for Windows [Z Type] Ver2.20

SZ-100

Measurement Results

Measurement Results

Date	: 18 February 2022 11:38:42
Measurement Type	: Zeta Potential
Sample Name	: Chitosan F5
Temperature of the Holder	: 24.8 deg. C
Dispersion Medium Viscosity	: 0.887 mPa.s
Conductivity	: 0.509 mS/cm
Electrode Voltage	: 3.3 V

Calculation Results

Peak No.	Zeta Potential	Electrophoretic Mobility
1	25.8 mV	0.000199 cm2/Vs
2	--- mV	--- cm2/Vs
3	--- mV	--- cm2/Vs

Zeta Potential (Mean) : 25.8 mV

Electrophoretic Mobility Mean : 0.000199 cm^2/Vs

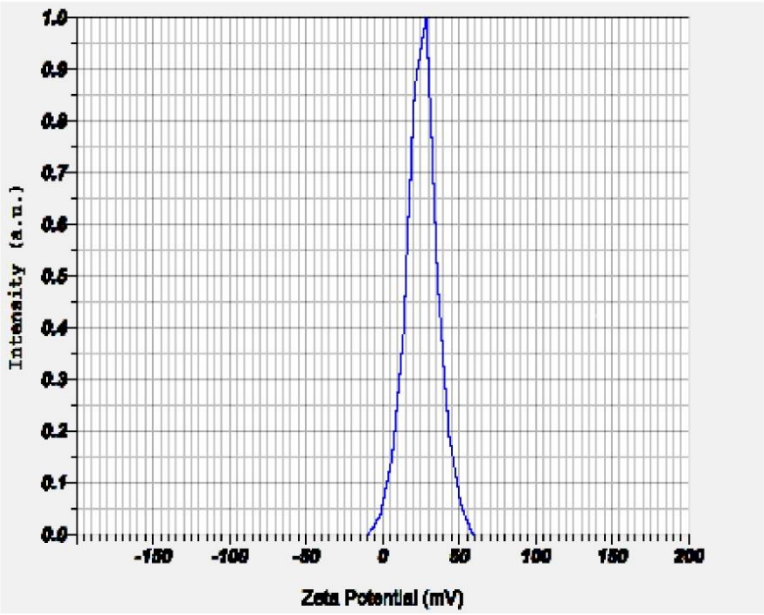

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Figure 9.17 Zeta potential of F5

Particle Size Characterization

Inference

Determination of particle size by Horiba scientific particle size analyzer

- The particle size analysis of optimized F5 formulation was observed 188.3 nm (Z-Average 229 nm) within nanometric range (Figure 9.16).

Determination of polydispersity by Horiba scientific particle size analyser

- A Polydispersity value of optimized F5 formulation was observed 0.372, which indicates uniformity of particle size in the formulation (Figure 9.16).

Determination of Zeta Potential by Zeta Sizer

- The zeta potential for the optimized formulations F5 was found to be 25.8mV and it shows that the formulation is stable. (Figure 9.17).

9.5 PREFORMULATION STUDIES OF OPTIMIZED NPs

After lyopilization the preformulation studies of optimized Chitosan Nanoparticles formulations were carried out to check the flow property. The optimized formulation F5 are evaluated for flow property and the results are shown in table 9.13

Table 9.13: Flow property measurements of optimized NPs

Formulation code	Bulk density (g/ml)	Tapped density (g/ml)	Carr's Index (%)	Hausner's ratio	Angle of repose (Ɵ)
Pure drug	0.2013±0.000531	0.29393±0.001247	32.75±0.3657	1.49±0.008165	51.14±1.3609
F5	0.3356±0.000245	0.3885±0.000327	13.62±0.00474	1.16±0.00471	33.20±0.1087

Mean ± SD (n=3)

Inference

The above results reveal that the optimized formulation F5 shows good flow property compared with Urapidil pure drug.

9.6 OPTIMIZED URAPIDIL LOADED CHITOSAN NPs FILLED IN HCG CAPSULES

- The optimized NPs were filled into "0" size hard gelatin capsules (HGC) without adding glidant or excipients because of good flow property can be observed.
- The filled each capsules containing 60 mg equivalent quantity of Urapidil.

9.7 POST FORMULATION STUDIES FOR URAPIDIL LOADED NANOPARTICULATE CAPSULES (C-NP)

9.7.1 Uniformity of weight

Table 9.14: Uniformity of weight in capsules

Formulation code	Average weight of capsule (g)
F5	0.1430±0.000479

Mean ± SD (n=20)

Inference

The Urapidil nanoparticulate capsules comply with the official test for Uniformity of weight.

9.7.2 Disintegration test

Table 9.15: Disintegration test for capsules

Formulation code	Disintegration Time
F5	8 mins 38 sec ±0.04966

Mean ± SD (n=3)

Inference

The Urapidil NP capsules comply with the official test for Disintegration test.

9.7.3 Drug content

Table 9.16: Drug content of optimized Formulations

Formulation code	Drug content
F5	99.01±0.0694

Mean ± SD (n=3)

Inference

The drug content of the optimized formulations (F5) was observed 99.01% for Nanoparticles prepared with Chitosan as a polymer.

9.7.4 *In vitro* release of optimized formulations and pure drug

The *in vitro* release date for optimized formulation F5 was compared with pure drug Urapidil and the results are tabulated below

Table 9.17: *In vitro* release of optimized and pure drug

Time (h)	Percentage drug release	
	F5	Pure drug
0.5	13.19	6.21
1	16.17	19.17
2	24.02	38.78
3	34.52	49.77
4	41.23	58.01
5	50.14	64.22
6	57.71	-
7	64.21	-
8	73.22	-
9	78.01	-
10	86.22	-
11	91.89	-
12	95.03	-

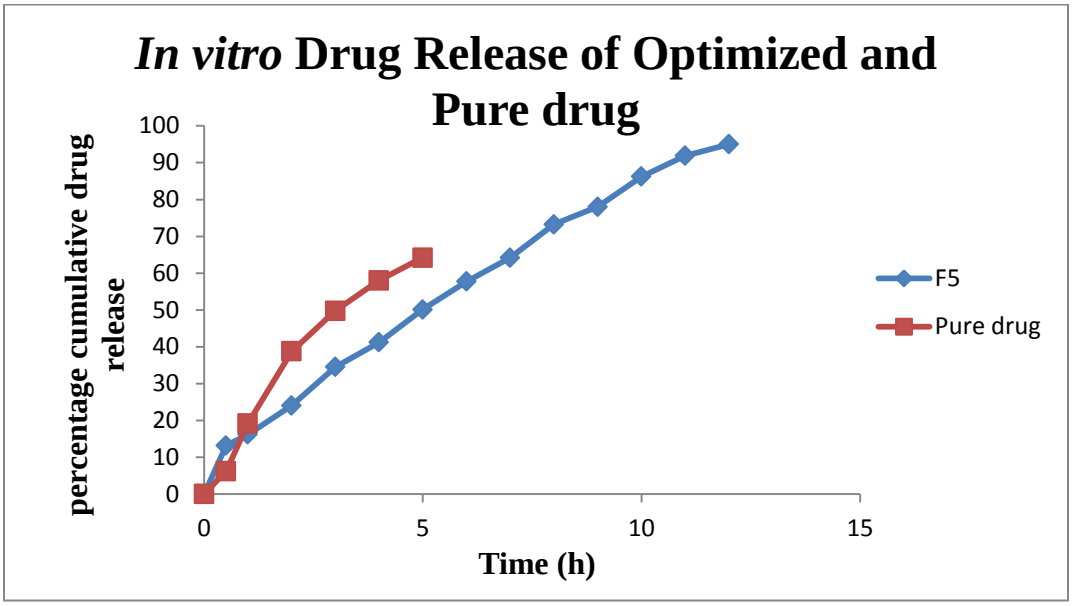

Figure 9.18: *In vitro* drug release of optimized formulation and pure drug

Inference

- The *in vitro* release profile for optimized Urapidil Chitosan Nanoparticles (F5) and pure drug of Urapidil were shown in figure 9.18. The result of optimized formulation show controlled release up to 12h, but pure drug shows 64.22% up to 5h.

9.8 RELEASE KINETIC OF OPTIMIZED FORMULATION

9.8.1 RELEASE KINETIC OF C-NPs. F5

Table No: 9.18 Release Kinetics of C-NPs.F5

Time (h)	% Cum. Drug Release	% Cum. Drug Remaining	Log % Cum. Drug Remaining	Square root of time	Log time	Log % Cum. Drug Release	cubic root of % drug Remaining
0	0	100	2	0	∞	∞	4.6416
0.5	13.19	86.81	1.9386	0.70711	-0.30103	1.1202	4.4278
1	16.17	83.83	1.9234	1	0	1.2087	4.3766
2	24.02	75.98	1.8807	1.4142	0.30103	1.3805	4.2355
3	34.52	65.48	1.8161	1.73205	0.4771	1.53807	4.0306
4	41.23	58.77	1.7692	2	0.60205	1.6152	3.8879
5	50.14	49.86	1.6978	2.23607	0.69897	1.70018	3.6806
6	57.71	42.29	1.6262	2.44949	0.7782	1.76125	3.4840
7	64.21	35.79	1.5538	2.64575	0.8451	1.8076	3.2955
8	73.22	26.78	1.4278	2.82843	0.9031	1.8646	2.9918
9	78.02	21.99	1.3422	3	0.9542	1.89215	2.8016
10	86.22	13.78	1.1393	3.162278	1	1.9356	2.3975
11	91.89	8.11	0.9090	3.316625	1.04139	1.96327	2.0091
12	95.03	4.97	0.6964	3.46410	1.07918	1.97786	1.7066

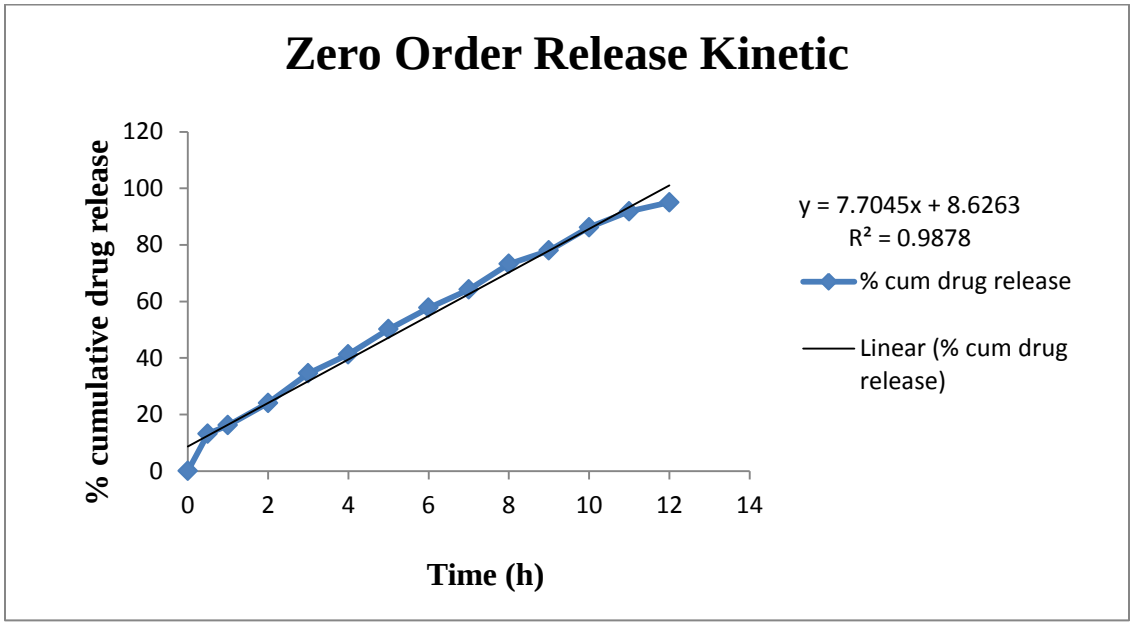

Figure 9.19: A plot of Zero Order Kinetics of optimized formulation (F5)

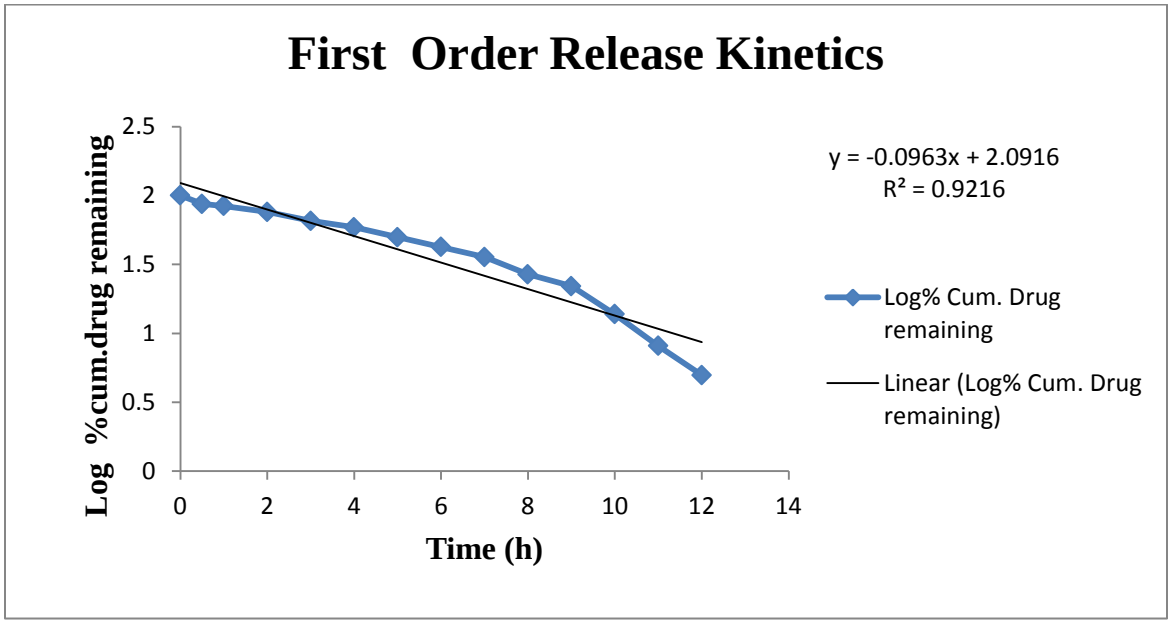

Figure 9.20: A plot of First Order Kinetics of optimized formulation (F5)

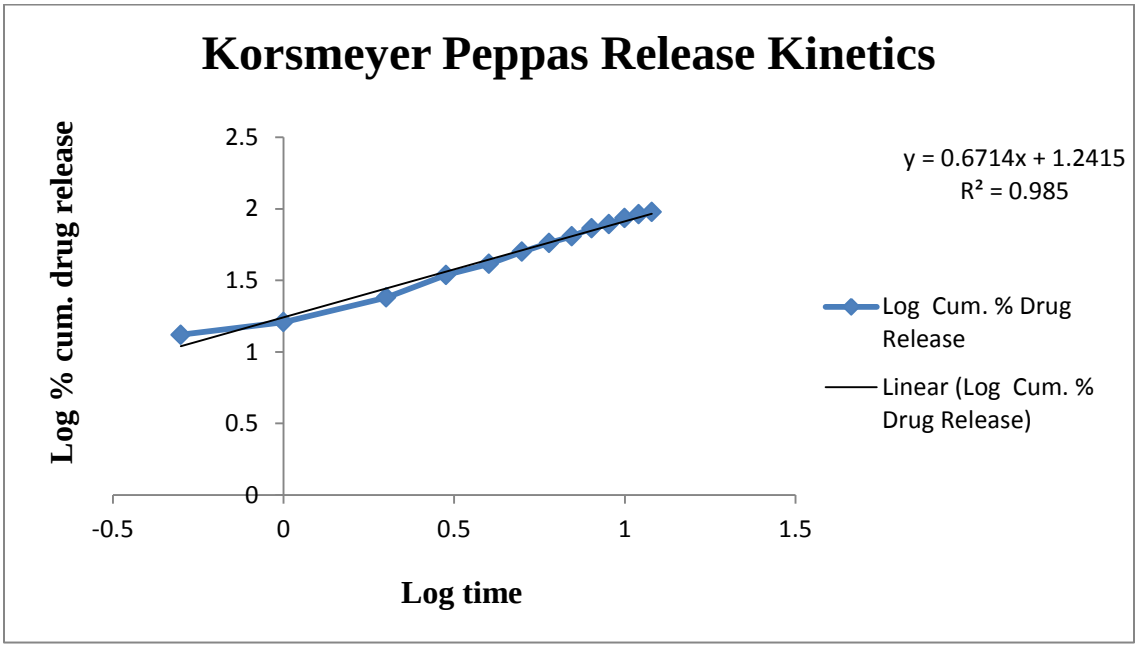

Figure 9.21: A plot of Korsmeyer Peppas Release Kinetics of formulation (F5)

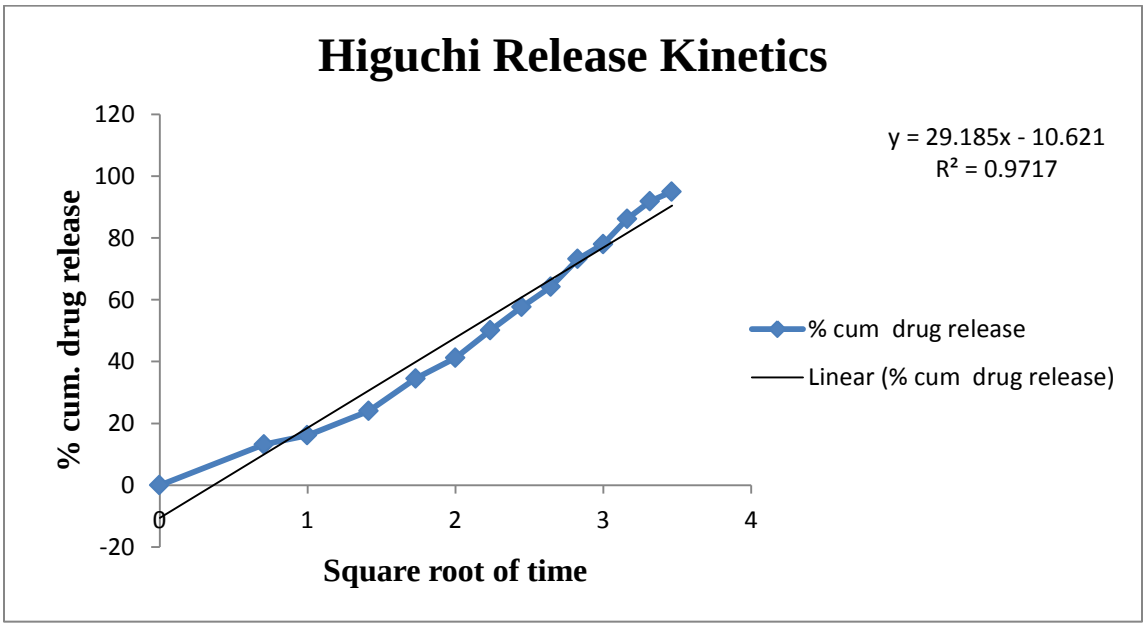

Figure 9.22: A plot of Higuchi Release Kinetics of optimized formulation (F5)

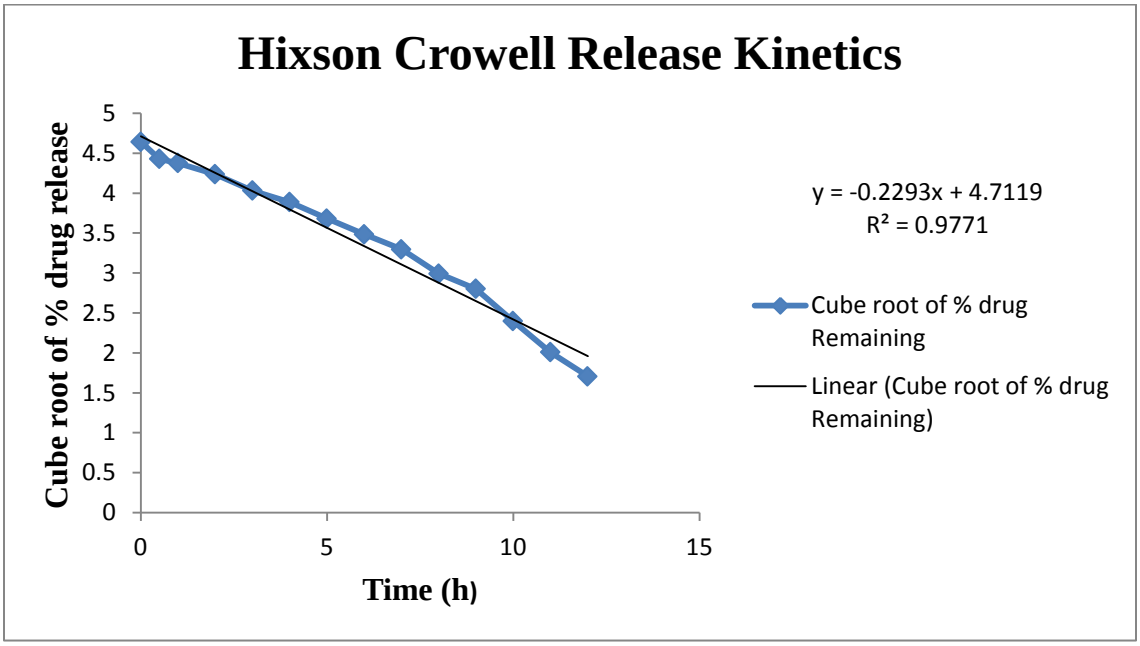

Figure 9.23: A plot of Hixson Crowell Release Kinetics of optimized formulation (F5)

The coefficient of determination (R^2) was taken as criteria for choosing the most appropriate model. The R^2 values of various models are given in table 9.19

Table No. 9.19: R^2 Values of F5 in various kinetic models

Kinetic Models	Coefficient of determination (R^2)
	F5
Zero order	0.9878
First order	0.9216
Korsmeyer and Peppas	0.985
Higuchi	0.9717
Hixson crowell	0.9771

Inference

- The *in vitro* release of optimized formulation F5 are fit into various kinetic models to find out the mechanism of drug release from Urapidil loaded Chitosan Nanoparticles.
- A good linearity was observed with the Zero order (R^2= 0.9878), the Zero order kinetics explains the controlled release of the prepared Nanoparticles over the period of 12 hours.
- Higuchi plot (R^2 = 0.9717) Show linearity, which indicates the rate of drug release through the mode of diffusion and to further confirm the diffusion mechanism, data was fitted into the Korsmeyer Peppas equation which showed linearity.
- The slope of the Korsmeyer Peppas plot (n = 0.671) was found to be more than 0.5 indicating the diffusion was anomalous diffusion (Non Fickian diffusion).
- Thus, the release kinetics of the optimized formulation was best fitted into Higuchi model that showed zero order drug release with anomalous diffusion (Non Fickian diffusion) mechanism.

9.9 STABILITY STUDIES

Table 9.20: Stability data for Optimized Formulation

Stability condition		Physical Appearance					Entrapment efficiency (%)					Drug content (%)				
		Initial	15	30	60	90	Initial	15	30	60	90	Initial	15	30	60	90
Refrigeration 4±2ºC	F5	NC	NC	NC	NC	NC	93.15	93.01	92.93	92.86	92.28	91.83	91.52	91.20	90.89	90.72
Room Temperature	F5	NC	NC	NC	NC	NC	92.85	92.79	92.55	92.23	91.98	91.72	91.26	90.78	90.54	90.21
At 40ºC±2ºC and 75% RH± 2%	F5	NC	NC	NC	NC	NC	92.90	92.27	91.88	91.39	90.89	91.42	91.02	90.44	89.91	89.43

*NC- No change

- The optimized formulation (F5) subjected to stability studies and the results are shown in table 9.20
- No significant change in, entrapment efficiency, drug content were observed at storage condition of 4±2ºC, room temperature, storage condition 40ºC±2ºC and analyzed after the end of 15,30,60 and 90 days.

10. SUMMARY AND CONCLUSION

- The purpose of this research was to prepare Urapidil loaded Chitosan Nanoparticles for controlled release of drug, to improve the solubility, reduce the dosing frequency, thereby increasing patient compliance to the therapy.
- Urapidil is formulated as Nanoparticles by ionic-gelation method using Chitosan as polymer, Sodium tripolyphosphate as a polyanionic agent (cross linking agent) and the lyophilized nanoparticles filled in hard gelatin capsules.
- The preformulation studies like melting point, determination of absorption maximum (268nm) were performed and the results evident that the drug and excipients are stable, safe and effective within the range.
- Physical compatibility study showed that the drug and excipients were physically compatible with each other.
- The chemical compatibility studies of Urapidil with excipients were physically analyzed by using FTIR Spectrometer. The results of the FTIR study proved that there was no interaction between the drug and polymer.
- Standard graph was drawn for Urapidil and it was found that the solutions show linearity (0.9995) and obeyed Beer – Lambert's law.
- Urapidil loaded Chitosan Nanopaticles prepared by ionic-gelation method using Chitosan as a polymer in different concentration (0.1%, 0.15%, 0.2%, 0.25%, 0.3%, 0.4%, and 0.5%). Sodium tripolyphosphate as a polyanionic agent (cross linking agent), Tween 80 as a deaggregating agent.
- All seven formulations characterized for percentage yield which found to be within the range of 78.84% to 87.25% and the entrapment efficiency of the formulations was observed between 83.40% to 93.15%. The results showed that the increase in polymer concentration, increase the entrapment efficiency. The entrapment efficiency was found to be higher in F5- 93.15% comparatively with other formulations.
- The Solubility analysis of Urapidil was carried out before and after formulation in distilled water and phosphate buffer pH 6.8. The results show that the solubility profile is improved after formulations (from insoluble to slightly soluble) compared with pure

drug. Thus the solubility of formulation F5 in distilled water and phosphate buffer pH 6.8 were improved (9.4933 mg/ml and 13.251 mg/ml) respectively.

- The *in vitro* release study was carried out for all seven formulations. The percentage of drug release in formulation F5 was found to be 95.03% at the end of 12h and the release profile was in controlled manner comparatively with other formulations.
- Based on the higher entrapment efficiency, drug content and prolonged *in vitro* drug release F5 was selected as optimized formulation.
- The FTIR studies of optimized formulation F5 shows there was no change in the individual peaks of the drug and the excipients. It concludes that there was no chemical interaction between the drug and excipients.
- The optimized formulations F5 are characterized for SEM analysis, particle size analysis and zeta potential.
- The SEM image showed that nanoparticles were spherical with smooth surface.
- The particle size analysis was done by Horriba scientific Nano SZ 100 particle size analyzer showed that mean particle size 188.3 nm and Z- Average 229.0 nm respectively.
- The Zeta potential study was done by Horriba scientific Nano SZ 100. The Zeta potential for the optimized formulations F5 was found to be 25.8mV and shows that the formulation is stable.
- Flow property measurements (Bulk density, Tapped density, Angle of repose, Carr's index and Hausner's ratio) were carried out for Urapidil pure drug and optimized Urapidil loaded Chitosan Nanoparticles. It revealed that the flow property of Chitosan Nanoparticles was good compared with pure drug.
- The optimized NPs are filled into "0" size hard gelatin capsules without adding glidant because of its good flow property.
- Post formulation parameters (uniformity of weight, disintegration test, drug content, and *in vitro* drug release) for nano particulate capsules were evaluated. The results were found to be complying with official specifications.
- The dissolution data of the optimized formulation was fitted to various kinetic models and the formulation F5 was best fitted to Zero order kinetics. The slope of the Korsmeyer Peppas plot indicating the diffusion was Anomalous diffusion (Non-Fickian diffusion).

- The optimized formulation F5 was subjected to accelerated stability study (Refrigeration and Room temperature) No significant change was found in appearance, drug content and entrapment efficiency at the end of 90 days in Refrigerator, Room temperature and at 40ºC±2ºC.
- From the overall results, it is clear that the formulations F5 containing 0.3% polymer concentration (Chitosan) is the optimal formulation, as it produces controlled drug release.

FUTURE SCOPE

- *In-vivo* study can be carried out.
- To study the pharmacokinetic and bio distribution of the drugs.
- To perform the Bioequivalence study.

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DEPARTMENT OF PHARMACEUTICAL CHEMISTRY

Porur, Chennai-116

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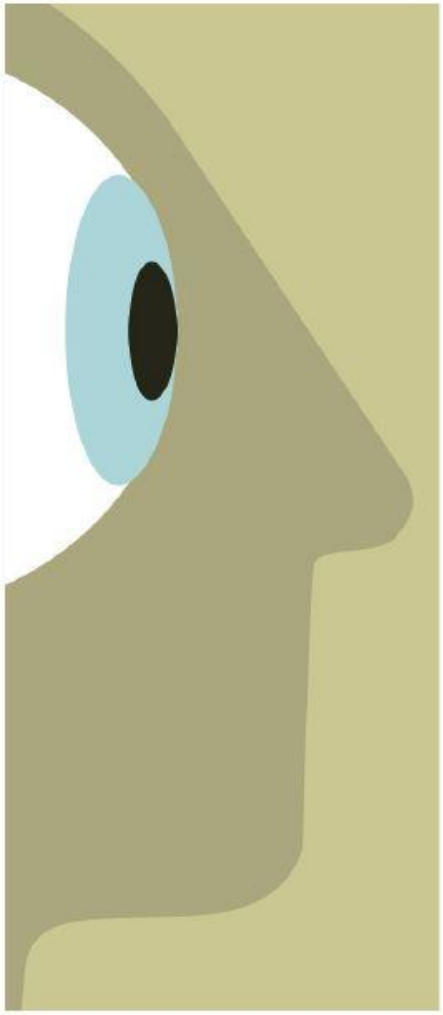

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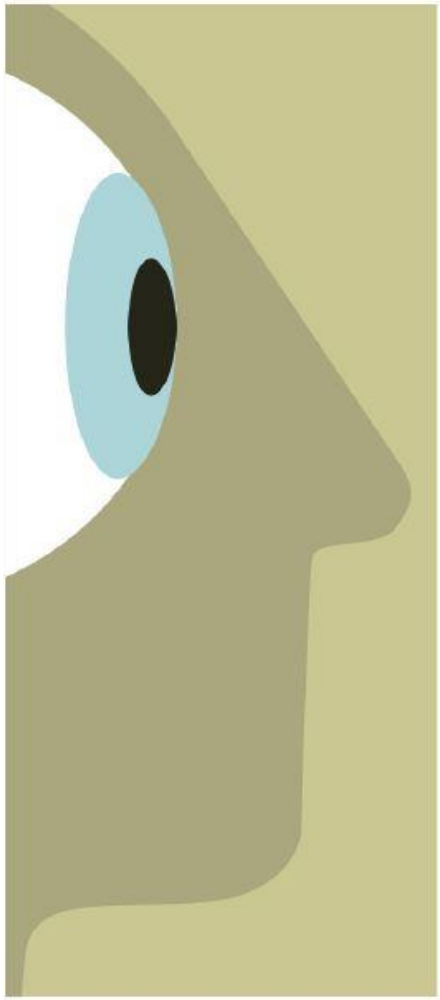

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INTRODUCTION

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