.ATION AND EVALUATION VALSARTAN
..IGIOTENSIN RECEPTOR BLOCKER

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IN

PHARMACEUTICS

Submitted
By

SAHAYA AROCKIADASS A S

(Reg No:261211161)

Under the guidance of

Prof.Dr.M.Murugan, M.Pharm., Ph.D.,

DEPARTMENT OF PHARMACEUTICS

EDAYATHANGUDY.G.S PILLAY COLLEGE OF PHARMACY

NAGAPATTINAM-611002

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Prof.Dr.M.Murugan, M.Pharm., Ph.D.,

Professor cum Director,

Department of Pharmaceutics,

Edayathangudy.G.S.Pillay College of Pharmacy,

Nagapattinam – 611 002.

CERTIFICATE

This is to certify that the dissertation entitled **"FORMULATION AND EVALUATION VALSARTAN ANGIOTENSIN RECEPTOR BLOCKER "** submitted by **SAHAYA AROCKIADASS A S**(Reg No:261211161) in partial fulfillment for the award of degree of Master of Pharmacy to the Tamilnadu Dr. M.G.R Medical University, Chennai is an independent bonafide work of the candidate carried out under my guidance in the Department of Pharmaceutics, Edayathangudy.G.S.Pillay College of Pharmacy during the academic year 2013-2014.

Place: Nagapattinam **Prof.Dr.M.Murugan, M.Pharm., Ph.D.,**

Date:

Prof.Dr.D.Babu Ananth,M.Pharm., Ph.D.,

Principal,

Edayathangudy.G.S.Pillay College of Pharmacy,

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Place: Nagapattinam **Prof.Dr.D.BabuAnanth,M.Pharm., Ph.D.,**

Date:

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CONTENTS

INTRODUCTION

The colloidal drug carriers have the potential to afford site specific as well as targeted drug delivery. The idea of using submicron drug delivery systems for drug targeting was conceived and developed after Paul Ehrlich originally proposed the idea of tiny drug-loaded magic bullets over a hundred year ago. Among these carriers, liposomes and micro/nanoparticles have been the most extensively investigated. Liposomes present some technological limitations including poor reproducibility and stability, and low drug entrapment efficiency. Nevertheless, several low molecular weight drugs are now commercially available which employ this technology.

Nanotechnology

The word “Nano” is derived from Greek word Dwarf , means “a billionth ” .A Nanometer is billionth of a meter, which is 250 millionth of an inch , about 1/80,000 of the diameter of a human hair or 10 times of the diameter of hydrogen atom. The term ‘Nanotechnology’ was coined by Prof. Norio Taniguchi, Tokyo Science University in 1974 to describe the precision manufacture of materials with nanometers tolerances and was unknowingly appropriated by Drexler in his 1986 book ‘Engines of creation: The Coming Era of Nanotechnology.

Nanoparticles

Nanoparticles are sub-nano sized colloidal structure of synthetic or semi synthetic polymer .The first reported nanoparticles were based on non biodegradable polymeric system (polyacrylamide, polymethyl-methaacrylate, polystyrene). The polymeric nanoparticles can carry drug(s) or proteineous substances, i.e. antigen(s). These bio-active agents are entrapped in polymer matrix as particulates or solid solution or may bound to particle surface by physical adsorption or chemically. The drug(s) may be added during preparation of nanoparticle or to the previously prepared nanoparticles. The structure of nanoparticles was shown in Figure. 1.

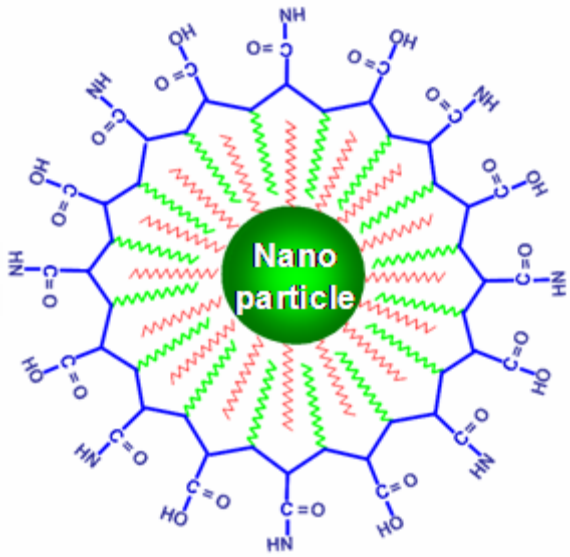

Figure: 1, Structure of nanoparticle

The term particulates are suggestively general and doesn't account for morphological and structural organization of system. Nanomedicine is an emerging field of medicine with novel applications.

Nanomedicine is a subset of nanotechnology, which uses tiny particles that are more than 10 million times smaller than the human body. In nanomedicine, these particles are much smaller than the living cell. Because of this, nanomedicine presents many revolutionary opportunities in the fight against all types of cancer, neurodegenerative disorders and other diseases.[1]

Polymeric nanoparticles

Polymeric nanoparticles, which possess a better reproducibility and stability profiles than liposomes, have been proposed as alternative drug carriers that overcome many of these problems. Nanoparticles are solid colloidal particles with diameters ranging from 1-1000 nm. They consist of macromolecular materials and can be used therapeutically as adjuvant in vaccines or drug carriers, in which the active ingredient is dissolved, entrapped, encapsulated, adsorbed or chemically attached. Polymers used to form nanoparticles can be both synthetic and natural polymers.

The polymeric nanoparticles are prepared from biocompatible and biodegradable polymers in size between 10-1000 nm where the drug is dissolved, entrapped, encapsulated or attached to a nanoparticle matrix. Depending upon 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. The field of polymer nanoparticles is quickly expanding and playing an important role in a wide spectrum of areas ranging from electronics, photonics, conducting materials, sensors, medicine, biotechnology, pollution control and environmental technology.

Polymeric nanoparticles are promising vehicles for drug delivery by easy manipulation to prepare carriers with the objective of delivering the drugs to specific target; such an advantage improves the drug safety. Polymer-based nanoparticles effectively carry drugs, proteins, and DNA to target cells and organs. Their nanometer-size promotes effective permeation through cell membranes and stability in the blood stream. Polymers are very convenient materials for the manufacture of countless and varied molecular designs that can be integrated into unique nanoparticle constructs with many potential medical applications. Several methods have been developed during the last two decades for preparation of PNPs, these techniques are classified according to whether the particle formation involves a polymerization reaction or nanoparticles form directly from a macromolecule or preformed polymer or ionic gelation method.[2,3]

Advantages of polymeric nanoparticles

- Increases the stability of any volatile pharmaceutical agents, easily and cheaply fabricated in large quantities by a multitude of methods.
- They offer a significant improvement over traditional oral and intravenous methods of administration in terms of efficiency and effectiveness.
- Delivers a higher concentration of pharmaceutical agent to a desired location. The choice of polymer and the ability to modify drug release from polymeric nanoparticles have made them ideal candidates for cancer therapy, delivery of vaccines, contraceptives and delivery of targeted antibiotics.
- Polymeric nanoparticles can be easily incorporated into other activities related to drug delivery, such as tissue engineering.

Mechanisms of drug release

The polymeric drug carriers deliver the drug at the tissue site by any one of the three general physico-chemical mechanisms.

- By the swelling of the polymer nanoparticles by hydration followed by release through diffusion.

- By an enzymatic reaction resulting in rupture or cleavage or degradation of the polymer at site of delivery, there by releasing the drug from the entrapped inner core.
- Dissociation of the drug from the polymer and its de-adsorption/release from the swelled nanoparticles.[4,5]

Polymers used in preparation of nanoparticles

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

- Chitosan
- Gelatin
- Sodium alginate
- Albumin

Synthetic polymers

- Polylactides(PLA)
- Polyglycolides(PGA)
- Poly(lactide co-glycolides) (PLGA)
- 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)[2]

There are two types of nanoparticles depending on the preparation process: nanospheres and nanocapsules. Nanospheres have a monolithic-type structure (matrix) in which drugs are dispersed or adsorbed onto their surfaces. Nanocapsules exhibit a membrane-wall structure and drugs are entrapped in the core or adsorbed onto their exterior. The term “nanoparticles” is adopted because it is often very difficult to unambiguously establish whether these particles are of a matrix or a membrane type.[6,7,8]

Nanoparticles not only have potential as drug delivery carriers as they offer non-invasive routes of administration such as oral, nasal and ocular routes, but also show to be good adjuvant for vaccines. Despite these advantages, there is no ideal nanoparticle system available. Most of nanoparticles prepared from water-insoluble polymers are involved heat, organic solvent or high shear force that can be harmful to the drug stability.

Moreover, some preparation methods such as emulsion polymerization and solvent evaporation are complex and require a number of preparation steps that are more time and energy consuming. In contrast, water-soluble polymers offer mild and simple preparation methods without the use of organic solvent and high shear force.

Criteria for ideal polymeric carriers for nanoparticles & nanoparticle delivery systems

- Polymeric carriers
- Easy to synthesize and characterize
- Inexpensive
- Biocompatible
- Biodegradable
- Non-immunogenic
- Non-toxic
- Water soluble

Nanoparticle delivery systems

- Simple and inexpensive to manufacture and scale-up
- No heat, high shear forces or organic solvents involved in their preparation process
- Reproducible and stable
- Applicable to a broad category of drugs; small molecules, proteins and polynucleotides
- Ability to lyophilize
- Stable after administration
- Non-toxic[9,10]

LITERATURE REVIEW

Among water-soluble polymers available, chitosan is one of the most extensively studied. This is because chitosan possesses some ideal properties of polymeric carriers for nanoparticles such as biocompatible, biodegradable, nontoxic, and inexpensive. Furthermore, it possesses positively charge and exhibits absorption enhancing effect. These properties render chitosan a very attractive material as a drug delivery carrier. In the last two decades, chitosan nanoparticles have been extensively developed and explored for pharmaceutical applications. [11]

Properties of chitosan making it suitable for oral delivery

• Biocompatibility and biodegradability

• Permeation enhancing effect

• Mucoadhesiveness

• pH sensitiveness

• Mild gelation conditions

CHITOSAN NANOPARTICLE

Chitosan based Nanoparticles have advantages particularly for the design and engineering of novel Nanoparticulate drug delivery systems, due to their desirable properties such as:

- Biocompatibility,
- Biodegradability,
- Bio– and mucoadhesivity, and
- Hydrophilic character that facilitate the administration of poorly absorbable drugs across various epithelial barriers, such as corneal, nasal and intestinal mucosa.

Chitosan Nanoparticles have been shown to provide sustained release of both hydrophilic and hydrophobic drugs and are prepared by three distinct methods including ionic gelation, precipitation using tripolyphosphate and crosslinking methods using gluteraldehyde. The method used for preparation determines the entrapment efficiency, loading efficiency, and particlesize. Particle size of the Chitosan Nanoparticles generally depends on molecular weight of chitosan used, concentration of chitosan solution and amount of cross linker. Increasing the concentration of chitosan increases the viscosity of chitosan solution thus making smaller sized particle formation difficult.

An additional advantage of this type of system is that they can be produced under aqueous and fairly mild conditions, thus effectively, being especially suitable to preserve the bioactive conformation of delicate macromolecules (e.g. hormones, antigens, DNA, RNA, growth factors), that otherwise would be prone to enzymatic degradation and hydrolysis. Most frequently Chitosan Nanoparticles are formed according to a bottom–up approach as a result of a self–assembling or crosslinking processes in which the molecules arrange themselves into ordered

nanoscale structures either by physical or covalent inter– or intramolecular interactions. In these nanostructures, the drug can be entrapped or attached to the Nanoparticles matrix. Chitosan Nanoparticles have been prepared by several methodologies, including physical crosslinking by ionic gelation by specific ions such as pentasodium tripolyphosphate (TPP) or EDTA. In particular, chitosan – TPP Nanoparticles have been utilized as a drug delivery platform for a wide range of active molecules. [12, 13]

Chitosan

Chitosan is a biopolymer that has received much attention and has been extensively studied for micro- and nonoparticles preparation. Properties like biodegradability, low toxicity and good biocompatibility make it suitable for use in biomedical and pharmaceutical formulations as antidiabetic agents, anti-inflammatory drugs, immobilization of enzymes and protein, ophthalmology. Chitosan, a linear polyaminosaccharide is obtained by alkaline deacetylation of chitin, which is the second abundant polysaccharide next to cellulose. Chitin is the main component of protective cuticles of crustaceans such as crabs, shrimps, prawns, lobsters and cell walls of some fungi such as *Aspergillus* and mucor. The structure of chitin and chitosan is shown in Figure. 2.

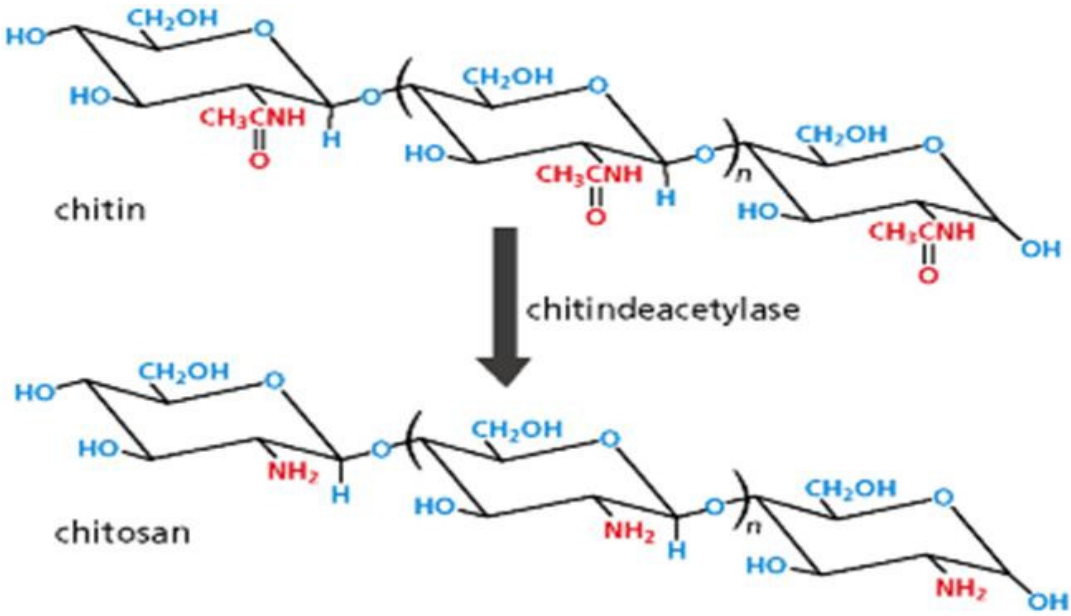

Figure: 2, Structure of chitin and chitosan

Chitin is a homopolymer composed of β-(1,4)-linked Nacetyl- glucosamine units while chitosan comprises copolymers of glucosamine and N-acetylglucosamine. Although chitin is insoluble in most solvents, chitosan is soluble in most organic acidic solutions at pH less than 6.5 including formic, acetic, tartaric,and citric acid. It is insoluble in phosphoric and sulfuric acid. Chitosan is available in a wide range of molecular weight and degree of deacetylation. Molecular weight and degree of deacetylation are the main factors affecting the particle size, particles formation and aggregation.

Chitosan has one primary amino and two free hydroxyl groups for each C6 building unit. Due to the different ways of applications, chitosan has been formulated as powder, gels and films, sponges, intragastric floating tablets and especially spherical particles (micro- and nanoparticles). [14,15]

Specifications & characteristics of pharmaceuticalgrade chitosan

The pharmaceutical requirements for chitosan include: a white or yellow appearance (powder or flake), particle size < 30 m, density between 1.35 and 1.40 g/cm3, a pH of 6.5 to 7.5, moisture content < 10%, residue on ignition <0.2%, protein content <0.3%, degree of deacetylation 70% to 100%, viscosity <5 cps, insoluble matter <1%, heavy metals (As) <10 ppm, heavy metals (Pb) <10 ppm**,** and no taste and smell.

Different techniques used for the preparation of nanoparticles using natural polymers

Common methods to prepare chitosan nanoparticles are ionic gelation, coacervation or precipitation, emulsion-droplet coalescence, reverse micellar, and self-assembly chemical modification. The ionic gelation process is commonly used to prepare chitosan nanoparticles because it is a very simple and mild method. This process can be performed either by chemical or physical crosslinking.

1. Emulsion Cross linking

In this process, chitosan solution is emulsified in oil (w/o emulsion). The aqueous droplets are stabilized using a suitable surfactant. The emulsion is then reacted with an appropriate crosslinking agent such as glutaraldehyde, to stabilize

the polysaccharide droplets. The Nanoparticles are then washed and dried. Ohya *et al* reported for the first time the preparation of Chitosan Nanoparticles containing 5-fluorouracil using w/o emulsion method followed by glutaraldehyde cross linking. These pioneering studies demonstrated the feasibility of preparing Chitosan Nanoparticles that could bind and delivery drugs. Major drawbacks of this method are associated with the use of organic solvents and cross linking agents that may adversely affect the stability of proteins. Moreover, glutaraldehydes cross linked Nanoparticles present negative effects on cell viability. [16,17,18]

2. Spray-drying

Spray-drying is a well-known technique to produce powders, granules or agglomerates from the mixture of drug and excipient solutions as well as suspensions. The method is based on drying of atomized droplets in a stream of hot air. In this method, chitosan is first dissolved in aqueous acetic acid solution, drug is then dissolved or dispersed in the solution and then, a suitable cross-linking agent is added. This solution or dispersion is then atomized in a stream of hot air. Atomization leads to the formation of small droplets, from which solvent evaporates instantaneously leading to the formation of free flowing particles. Various process parameters are to be controlled to get the desired size of particles. Particle size depends upon the size of nozzle, spray flow rate, atomization pressure, and inlet. Huang et al. prepared chitosan–iron oxide Nanoparticles with various chitosan: iron oxide ratios by spray-drying. [19, 20]

3. Reverse Micellar Method

In this method surfactant is first dissolved in an organic solvent to produce reverse micelles. To this, an aqueous solution of chitosan and drug are added with constant vortexing to avoid any turbidity. The aqueous solution is kept in such a way as to keep the entire mixture in an optically transparent microemulsion phase. Additional amount of water may be added to obtain Nanoparticles of larger size. To this solution, a crosslinking agent is added and the mixture kept overnight under constant stirring. The organic solvent is then evaporated to obtain the transparent dry mass. The material is dispersed in water, followed by the addition of a suitable salt, which helps to precipitates the surfactant out. It is then centrifuged and the supernatant decanted, which contains the drug-loaded Nanoparticles. The aqueous dispersion is immediately dialysed through dialysis membrane for about 1 hr. and the liquid is lyophilized to dry powder. [21]

4. Template Polymerization

In this technique, chitosan is firstly dissolved in an acrylic monomer solution under magnetic stirring. Due to the electrostatic interaction, the negatively charged acrylic monomers align along the chitosan molecules. After complete dissolution of chitosan, the polymerization is started by adding the initiator (K2S2O8) under stirring at 70ºC. The complete polymerization leads to the appearance of an opalescent solution, indicating the Nanoparticles formation. The Nanoparticles solution are then filtered and dialysed to remove the residual

monomers and initiator. The obtained Nanoparticles are positively charged and present a size in the range of 50 to 400 nm. [22]

5. Precipitation

There are two kinds of approaches for Nanoparticle precipitation. One is desolvation, in which flocculant (commonly sodium sulfate) is added to a water solution of chitosan and solubility of chitosan is decreased by the combination of water and sodium sulfate, leading to the precipitation of Nanoparticles due to hydrogen bonding between molecules. This method was first applied by Berthold et al to prepare chitosan microspheres. Technical improvements then enabled Tian and Groves prepare 600- to 800-nm chitosan Nanoparticles. The other type is based on diffusion of emulsified solvent. Under the action of emulsified solvent, the water phase containing chitosan is dispersed in the organic phase encapsulating the drug, where turbulence appears between the interfaces of the two phases and chitosan is precipitated, resulting in the generation of Nanoparticles. In this method, organic solvent is used and the large Nanoparticles obtained restricting their application. [23]

6. Ionotropic gelation or Ionic gelation

Chitosan NP prepared by ionotropic gelation technique was first reported by Calvo, and has been widely examined and developed. The mechanism of chitosan NP formation 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. [24]

7. Microemulsion method

Chitosan NP prepared by microemulsion technique was first developed by Maitra. 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, chitoan 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 lyophilyzation. 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 alter 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.

8. Emulsification solvent diffusion method

El-Shabouri reported chitosan NP prepared by emulsion solvent diffusion method, which originally developed by Niwa employing PLGA. 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 (e.g., the use of organic solvents) and the high shear forces used during nanoparticle preparation. [25, 26,27,28,29]

9. 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. The complexes size can be varied from 50 nm to 700 nm. [30]

Among all the mentioned methods, ionic gelation technique is selected to prepare the chitosan nanoparticles of valsartan. Here the ionic gelation process is dicussed briefly.

Ionic gelation

Ionic gelation (IG), earlier known as 'ion-induced gelation', results in nanoparticles and microparticles. The mucoadhesive properties of chitosan, due to molecular attractive forces formed by electrostatic interaction between positively charged chitosan and negatively charged mucosal surfaces is well documented. These properties may be attributed to

(a) Strong hydrogen bonding groups like -OH, -COOH

(b) Strong charges

(c) High molecular weight

(d) Sufficient chain flexibility

(e) Surface energy properties favoring spreading into the mucus

Cross linking agents

It is worth noting that chitosan has a high density of amine groups in its backbone and the amine groups are protonized to form -NH3 + in acidic solution. These positively charged groups in chitosan can be chemically cross-linked with dialdehydes such as glutaraldehyde and ethylene glycol diglycidyl ether, or physically cross-linked with multivalent anions derived from sodium tripolyphosphate (TPP), citrate and sulphate.

Advantages of tripolyphosphate

Glutaraldehyde and ethylene glycol diglycidyl ether are toxic and can cause irritation to mucosal membranes. Physically cross-linked chitosan gels have been used in drug delivery systems due to their enhanced biocompatibility over chemically cross-linked chitosan. Non-toxicity and quick gelling ability of TPP are the important properties that make it a favourable cross-linker for ionic gelation of

chitosan. In addition, TPP has been also recognized as an acceptable food additive by the US Food and Drug Administration.

Moreover, the process of ionic gelation of chitosan with TPP as a cross-linker is feasible for the scale-up of entrapment in a particle processing operation. Chitosan nanoparticles prepared by TPP as an anionic cross-linker are homogeneous, and possess positive surface charges that make them suitable for mucosal adhesion applications. The properties of ionically cross-linked chitosan are influenced by electrostatic interactions between the anionic cross-linker and chitosan. [31, 32]

Nature of interaction

This interaction depends on the variables such as: anionic molecular structure, its charge density and molecular concentration, pH of chitosan solution, and physical properties of chitosan, i.e., molecular weight and degree of deacetylation (DDA). Several studies have investigated the effect of these variables on the properties of ionically produced chitosan, its drug entrapment efficiency and drug release behavior. [33]

In the ionic gelation method, the positive or negative charge of the hydrophilic polymer is complexed with a multivalent cationic (e.g. calcium chloride) or polyanionic (e.g. sodium tripolyphosphate) to form highly viscous gel particles with a size in the range of a nanometer. Ionic gelation method was

developed by Calvo and Coworkers for the preparation of chitosan nanoparticles. In this method polymer solutions and polyanion solutions are mixed to form nanoparticles.

Counterions

The counterions used for ionotropic gelation can be divided into two major categories:

(i) Low molecular weight counterions

(e.g. CaCl2, BaCl2, MgCl2, CuCl2, ZnCl2, CoCl2, pyrophosphate, tripolyphosphate, tetrapolyphosphate, octapolyphosphate, hexametaphosphate and [Fe(CN)6]-4 / [Fe(CN)6]-3;

(ii) High molecular weight ions

(e.g. octyl sulphate, lauryl sulphate, hexadecyl sulphate, cetylstearyl sulphate). The ionotropic gelation method is very simple and mild. In addition, reversible physical crosslinking by electrostatic interaction instead of chemical crosslinking avoids the possible toxicity of reagents and other undesirable effects. [34]

Mechanism of cross linking

The basic mechanism involved in the formation of nanoparticles is the electrostatic interactions between positively charged amino groups present in

polymer and negatively charged anion. The mechanism of cross linking was shown in Figure. 3.

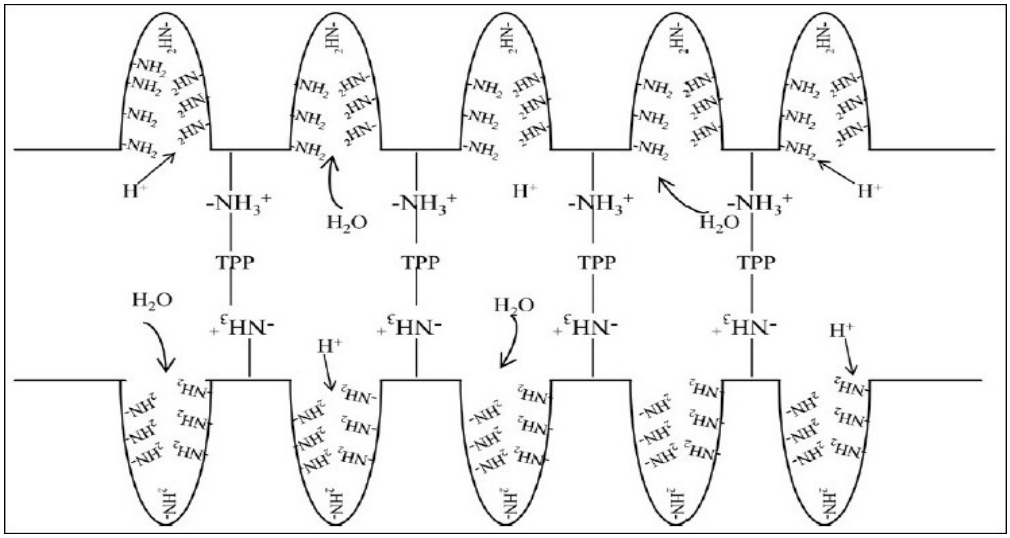

Figure: 3. Mechanism of cross linking

In other words it can be seen that in the ionic gelation method, due to interaction the material undergoes transition from liquid to gel phase. The obtained chitosan nanoparticles generally are of small size in the range of 200-500nm.

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.

The basic concept is that a polycationic polymer in aqueous solution passes, in appropriate conditions, from sol to dispersed gel following electrostatic crosslinking with an adequate anionic substance. This technique has been used with several quaternized chitosans carrying fixed, pH-independent positive charges, the most known of which is *N*-trimethyl chitosan (TMC).

Sodium tripolyphosphate (TPP) has widely been employed as the ionotropic crosslinker. The nanoparticles prepared by ionotropic gelation of quaternized chitosans with TPP were generally 200-300 nm in size, i.e., smaller than those obtained by the same method starting from plain chitosan which, by the way, showed lesser stability and tended to re-dissolve after some time from formation. The zeta potential was always positive, in the 10-20 mV range.

The solution of the chitosan derivative into which the TPP solution was dripped would often contain a surfactant, usually Tween 80, to hinder nanoparticle aggregation and facilitate their re-dispersion after centrifugation. In fact, centrifugation was necessary to clear the particles of non-encapsulated drug. [35]

The thiolated nanoparticles formed by ionotropic gelation with TPP were stabilized via oxidation of thiols with H2O2 which formed interchain disulfide

bonds. These would bestow gastroresistance on the particles, which would be particularly appropriate in case of oral administration of the nanoparticle formulation. However the presence of some non-oxidized thiols on the nanoparticle surface was needed to confer enhanced mucoadhesivity on such a surface.

Applications of chitosan nanoparticles

Carrier for various drugs

1. Carrier of gene drugs

As a gene carrier, conventional virus has the disadvantages of low transfection rate and cell toxicity, and even causes serious immune response. As a nonvirus carrier, chitosan has excellent biocompatibility and biodegradation, which has led to increasing application of chitosan nanoparticles in gene drug delivery. Gene silencing mediated by double-stranded small interfering RNA (siRNA) has been widely investigated as a potential therapeutic approach for diseases caused by genetic defects. However, its application is restricted by rapid degradation and poor cell absorption. Drug loading of chitosan nanoparticles prepared by ionic gelation by Katas and Alpar reached 100%, protecting well siRNA from nuclease degradation.

With natural positive ion chitosan as a carrier material and using electrostatic interaction of polyelectrolyte, siRNA of silencing green fluorescent protein was compounded directly by Liu55 to prepare stable siRNA nanoparticles with a complex rate of 83% to 94%.

2. Carrier of protein drugs

Protein drugs can be degraded easily by enzymes in vivo and have poor permeability and stability as well as a short half-life. However, chitosan can protect protein well and promote the contact between drug and biomembrane, thereby improving bioavailability. Gan and Wang showed that changing the size and surface charge of chitosan–bovine serum albumin nanoparticles could regulate the encapsulation efficiency and release kinetics of bovine serum albumin, but it was difficult to control the burst release of protein of high molecular weight.

Zhang used insulin and cationic β-cyclodextrin to form a complex encapsulated into alginate–chitosan nanoparticles. Binding rate and drug-loading amount were 87% and 9.5%, respectively, and cumulative release of insulin in simulated intestinal fluid reached 40%. Insulin was protected well in the nanoparticles core, avoiding the degradation in simulated gastric fluid, as well as the structure of insulin during release. Glycol chitosan nanoparticles modified by 5β-cholanic acid (HGC) and RGD (Arg-Gly-Asp) polypeptide were easily encapsulated into nanoparticles with a drug-loading amount greater than 85%.

3. Carrier of anticancer chemical drugs

Chitosan itself has a certain antitumor activity and its positive charge can neutralize the negative charge on the tumor cell surface, resulting in selective absorption. Thus, chitosan nanoparticles can increase drug concentration in the tumor site and improve therapeutic effects. Doxorubicin/methoxy PEG grafted

carboxymethyl chitosan nanoparticles with higher cell toxicity could enter cell and inhibit tumor-cell proliferation effectively. Paclitaxel chitosan nanoparticles had a high encapsulation rate of 94.0% ± 16.73% with sustained-release effect. Cell toxicity testing showed that paclitaxel–chitosan nanoparticles had a higher toxicity than that of paclitaxel alone, and with a higher cell uptake rate.

4. Carrier of other drugs

Chitosan nanoparticles also can load other drugs including antivirus drugs, antiallergic drugs, and hormone drug. Hao and Deng prepared acyclovir-loaded chitosan nanoparticles with a drug loading of 17.8% and an encapsulation rate of 87.5% by an ionic cross-linking method. Li and Luan prepared tranilast-loaded chitosan nanoparticles for allergic diseases with a particle size of 285.5 nm and an encapsulation rate of 82.4%. [36]

5. Parenteral administration

Nano-sized particles can be administered intravenously because the diameter of the smallest blood capillary is approximately 4 μm. The bio-distribution of nanoparticles can vary depending on the size, surface charge and hydrophobicity of the administered particles. Particles greater than 100 nm in diameter are rapidly taken up by the reticuloendothelial system (RES) in the liver, spleen, lung and bone marrow, while smaller-sized particles tend to have a

prolonged circulation time. Negatively-charged particles are eliminated faster than positively-charged or neutral particles.

The most promising drugs that have been extensively studied for delivery by this route are anticancer agents. Following intravenous injection, many nanoparticle systems including chitosan NP exhibited a marked tendency to accumulate in a number of tumors. One possible reason for the phenomenon may involve the leakiness of tumor vasculature. Doxorubicin loaded chitosan NP showed regression in tumor growth and enhance survival rate of tumor-implanted rats after IV administration.

In addition, chitosan NP less 100 nm in size have been developed which showed to be RES evading and circulate in the blood for considerable amount of time. Delivery of antiinfectives such as antibacterial, antiviral, antifungal and antiparasitic drugs is another common use of nanoparticles. The low therapeutic index of antifungal drugs, short half-life of antivirals and the limited ability of antibiotics to penetrate infected cells in intracellular compartments make them ideal candidates for nanoparticle delivery.

Peroral administration

The idea that nanoparticles might protect labile drugs from enzymatic degradation in the gastrointestinal tract (GIT) leads to the development of nanoparticles as oral delivery systems for macromolecules, proteins and poly-nucleotides. This approach was extensively studied after a report that blood glucose levels were reduced in diabetic rats following the oral administration of

insulin nanoparticles. Limiting nano-sized particles to less than 500 nm in diameter seems to be a key factor in permitting their transport through the intestinal mucosa most probably through an endocytotic mechanism.

However, besides the enzymes, mucus layer, which hamper diffusion of drug molecules and nanoparticles, and the epithelial absorption barriers are main hurdles against gastrointestinal protein drug absorption. Therefore, drug bioavailability can be improved by controlling the particle size along with prolonging the residence time of drug carrier systems in GIT. Among polymeric nanoparticles, chitosan NP showed to be attractive carriers for oral delivery vehicle as they promote absorption of drug.

The absorption promoting effect of chitosan has been extensively studied by several research groups and found to be due to a combination of mucoadhesion and transient opening of tight junctions in the mucosal cell membrane which have been verified both *in vitro* and *in vivo*. The mucoadhesive properties of chitosan are due to an interaction between positively charged chitosan and negatively charge of mucin which provide a prolonged contact time between the drug and the absorptive surface, and thereby promoting the absorption.

Chitosan muco-adhesion is also supported by the evidence that chitosan increases significantly the half time of its clearance. Furthermore, *in vitro* studies in Caco-2 cells have shown that chitosan is able to induce a transient opening of tight junctions thus increasing membrane permeability particularly to polar drugs, including peptides and proteins. Recent studies have shown that only protonated

soluble chitosan, in its uncoiled configuration, can trigger the opening of the tight junctions, thereby, facilitating the paracellular transport of hydrophilic compounds.

This property implies that chitosan would be effective as an absorption enhancer only in a limited area of the intestinal lumen where the pH values are below or close to its pKa. Although chitosan was able to open up the tight junctions, the uptake of particle > 50 nm could not be explained by a widening of the intercellular spaces. Mechanism of chitosan NP transport across GIT is most probably through adsorptive endocytosis. Electrostatic interaction between positively charged chitosan and negatively charged sialic acid of mucin causes association of chitosan NP to the mucus layer and subsequently internalization via endocytosis.

Recently, chitosan was demonstrated to promote the nasal absorption of insulin in rats and sheep. However, the insulin-chitosan powder, chitosan blended with insulin using pestle and mortar, showed to have bioavailability greater than chitosan NP containing insulin.

Non-viral gene delivery vectors

Although viruses can efficiently transfer genes into cells, concerns such as host immune response, residual pathogenicity, and potential induction of neoplastic growth following insertional mutagenesis have led to the exploration of non-viral gene transfer systems. These latter delivery systems are generally considered to be safer since they are typically less immunogenic and lack mutational potential. There are usually considered to be five primary barriers that

must be overcome for successful gene delivery: *in vivo* stability, cell entry, endosome escape, intracellular trafficking and nuclear entry. Cationic polymers and lipids have both shown promise as gene delivery agents since their polycationic nature produces particles that reduce one or more of these barriers. For example, by collapsing DNA into particles of reduced negative or increased positive charge, binding to the cell surface and enhanced endocytosis may be promoted.

In many cases, cationic polymers seem to produce more stable complexes thus offering more protection during cellular trafficking than cationic lipids. Among cationic polymers, PEI is particularly promising as a vector given its relatively high level of transfection in a number of target organs by various delivery routes. The high charge density of PEI is thought to be a key factor that contributes to its high transfection efficiency. Unfortunately, the polycationic nature of PEI also appears to be the main origin of its marked toxicity, a property it shares with many other polycations (e.g. polylysine).

This toxicity has severely limited its use as a gene delivery vector *in vivo*. On the contrary, chitosan is a cationic polymer with extremely low toxicity. It showed significantly lower toxicity than poly-L-lysine and PEI. Additionally, it enhances the transport of drug across cell membrane as discussed earlier. Chitosan as a promising gene delivery vector was first proposed by Mumper. Chitosan mediates efficient *in vitro* gene transfer at nitrogen to phosphate (N/P) ratio of 3 and 5. At these ratios, small chitosan-DNA complexes can be prepared in the range

of 50-100 nm with a positively surface charge of approximately +30 mV. Sato *et al.* found that *in vitro* chitosan-mediated transfection depends on the cell type, serum concentration, pH and molecular weight of chitosan.

Delivery of vaccines

Nanoparticles often exhibit significant adjuvant effects in parenteral vaccine delivery since they may be readily taken up by antigent presenting cells. Moreover, oral and nasal delivery of nanoparticles are thought to have the potential to provide mucosal protective immune responses, one of the most desired goals of modern vaccinology. The submicron size of nanoparticles allows them to be taken up by M-cells, in mucosa associated lymphoid tissue (MALT) i.e. gut-associated, nasal-associated and bronchus-associated lymphoid tissue, initiating sites of vigorous immunological responses. Immunoglobulin A (IgA), a major immunoglobulin at mucosal surface, and the generation of B-cell expressing IgA occur primarily in MALT. The B-cell then leaves the MALT and reaches systemic circulation where they clonally expand and mature into IgA plasma cells. Therefore, providing not only protective IgA at the pathogen entered sites, but also systemic immunity. There are two main administration routes for mucosal vaccine delivery, oral and nasal. The main targeted for oral delivery vaccine are Peyer's patches. By incorporating vaccine into nanoparticles systems, the vaccine is protected against enzymatic degradation on its way to the mucosal tissue and efficiently taken up by M-cells.

In contrast to oral administration, nasal administered vaccines have to be transported over a very small distance, remain only about 15 minutes in the nasal cavity, and are not exposed to low pH values and degradative enzymes. Thus, nasally delivery vaccines may not necessary formulated as nanoparticles as discussed earlier. It may be administered as solution or powder with absorption enhancing agent to slow down mucociliary clearance process and thereby prolong the contact time between the formulation and nasal tissue. Among the polymers used to form vaccine nanoparticles, chitosan is one of the most recently explored and extensively studied as prospective vaccine carriers. Its absorption promoting effect is believed to improve mucosal immune response.

The mechanism of action of chitosan in improving transport of drug across mucosal membrane can be explained by the same theory as discuss earlier in peroral administration section. Illum *et al.* successfully developed chitosan vaccines containing influenza, pertussis and diphtheria antigens for nasal delivery. They demonstrated that these vaccines produced a significant antibody level in mice, both serum and secretory IgA. Despite the potential carrier for mucosal delivery vaccine, chitosan has also been reported to act as an adjuvant for systemic vaccine delivery such as increasing the accumulation and activation of macropharges and polymorphonuclear cells. Activation of macropharges is initiated after uptake of chitosan. Furthermore, chitosan has also been widely explored as the application for DNA mucosal vaccines. [37, 38]

Ocular administration

Nanoparticles have been found to be potential carriers for ocular delivery following the observation that various types of nanoparticles tend to adhere to the ocular epithelial surface. The resulting prolonged residence time of nanoparticles leads to a much slower elimination rate compared to conventional ophthalmologic formulations, thereby improving drug bioavailability. As a consequence, nanoparticles have been developed for targeted ophthalmic delivery of anti-inflammatory, antiallergic and beta-blocker drugs.

Among mucoadhesive polymers explored now, chitosan has attracted a great deal of attention as an ophthalmic drug delivery carrier because of its absorption promoting effect. Chitosan not only enhance cornea contact time through its mucoadhesion mediated by electrostatic interaction between its positively charged and mucin negatively charged, its ability to transient opening tight junction is believed to improve drug bioavailability. Felt *et al.* found that chitosan solutions prolonged the cornea resident time of antibiotic in rabbits.

Chitosan also shown to be a low toxic material, ophthalmic formulation based on chitosan exhibited an excellent tolerance after applied chitosan onto the rabbit's corneal surface. Besides employing chitosan NP to improve drug transport via ocular, chitosan-coated nanoparticles can also be utilized as it exhibited ability to enhance the corneal penetration. In addition, De Campos *et al.* found that after ocular administration of chitosan NP in rabbits, most of drugs were found in extra-ocular tissue, cornea and conjunctiva, while negligible drug were found in

intraocular tissues, iris/ciliary body and aqueous humor. Together, these results suggested that chitosan NP showed to be attractive material for ocular drug delivery vehicle with potential application at extra-ocular level.

Valsartan delivery systems based on chitosan-TPP nanoparticles are reported in research works performed previously. A shortcoming of chitosan-based nanoparticles as drug release system is that such particles release 30-70% of the drug within 3-6 h placed in release environment. This is due to the mechanism of burst release that has been considered as a slow release in many studies. [39]

Burst release management is a major challenge in the development of drug delivery systems because it may lead to inefficient delivery and significant toxicity hazards. The degree of burst release generally depends upon the nature of the polymer, drug molecular structure and its molecular weight, polymer/ drug ratio, relative affinities of the drug and polymer and the aqueous phase.

Polymer coating is another burst release control method. Tavakol et al have studied this method for *N,O*-carboxymethyl chitosan (NOCC) beads coated with chitosan. They produced beads of NOCC and alginate with ionotropic gelation method and then, coated the beads with chitosan. The effect of coating and drying methods on the swelling and release behaviour of the prepared beads has been

investigated. The results of this work indicate that burst release has not been observed in chitosan coated beads. Using additional barrier layers is naturally an efficient method to manipulate drug release profiles and reduce the burst release, though it is inherently an expensive method due to the additional materials employed and difficulties associated with controlling the barriers quality.

In a study, Sarmento and co-workers prepared insulin-loaded alginate– CS-NPs by ionotropic pre-gelation of dilute alginate solution with calcium chloride followed PEC formation with CS. Particle size, association efficiency of insulin into alginate NPs and loading capacity were influenced by various process and formulation variables such as time and speed of stirring, alginate guluronic acid content, CS molecular weight and initial alginate/CS mass ratio. Upon optimisation, high association efficiency (92%) and loading capacities (14.3%) of insulin were achieved, and the NPs displayed controlled release in gastric pH (50%) for up to 24 h while extensive insulin release was noticed in intestinal pH (75%)

Chitosan, a cationic polysaccharide, is one of such biodegradable polymers, which has been extensively exploited for the preparation of nanoparticles for oral controlled delivery of several therapeutic agents. In recent years, the area of focus has shifted from chitosan to chitosan derivatized polymers for the preparation of oral nanoparticles due to its vastly improved properties, such as better drug

retention capability, improved permeation, enhanced mucoadhesion and sustained release of therapeutic agents.

The main objective of this work done by Morteza et al., was to reduce the burst release of BSA-loaded chitosan nanoparticles prepared by a one-step simple method. Very few studies have focused on high loadings (10-15%) of proteins in micro/nanospheres with low burst release. Due to the nature of BSA, an optimum BSA delivery system must undergo burst release as low as possible while keeping drug loading as high as possible. The design of an optimum system is the main objective of the study.

Intrinsic characteristics of chitosan enhance the transport of bioactives across the cell membrane. For example, calcium, a poor absorbable nutrient, can be entrapped into chitosan in order to improve the bioavailability. Chitosan has many advantages with respect to nanoparticles preparation since it not only contains a number of free amine groups available for cross linking, but also for controlling the release of bioactives. [40]

AIM AND PLAN OF WORK

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Burst release management is a major challenge in the development of drug delivery systems because it may lead to inefficient delivery and significant toxicity hazards. The degree of burst release generally depends upon the nature of the polymer, drug molecular structure and its molecular weight, polymer/ drug ratio, relative affinities of the drug and polymer and the aqueous phase. [10]

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methods on the swelling and release behaviour of the prepared beads has been investigated. The results of this work indicate that burst release has not been observed in chitosan coated beads. [11]

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The main objective of the work done by Morteza et al., was to reduce the burst release of BSA-loaded chitosan nanoparticles prepared by a one-step simple method.

Chitosan nanoparticles were fabricated by ionotropic gelation technique which gives many advantages including a simple, mild preparation method without

the use of organic solvents and high shear force. Precise control of particle size is central in chitosan nanoparticles preparation. The ionotropic gelation of chitosan forms the inter and intramolecular linkages between positively charged amine group of chitosan and negatively charged polyanion such as tripolyphosphate.

Valsartan is a selective angiotensin II receptor antagonist used treatment of hypertension. It is poorly soluble in water. It is reported that bioavailability of valsartan is 23-25% after oral dose. Peak plasma concentration of valsartan achieves at 2-4 hours after a oral dose. Due to the reasons mentioned the drug valsartan selected and an attempt was made to formulate chitosan nanoparticles in two ways that is to formulate plain chitosan nanoparticles and alginate chitosan nanoparticles with calcium chloride to compare the burst control and to improve the bioavailability.[25]

The formulated chitosan nanoparticles will be an alternate for the formulations with chitosan with more burst release and can improve the bioavailability of the drug valsartan with less burst release.

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PLAN OF WORK

1. Determination of max of valsartan
2. Calibration curve for the drug in phosphate buffer pH 6.8
3. Drug polymer interaction study by using FTIR
4. Formulation of chitosan nanoparticles of valsartan by ionic gelation technique by using different concentrations of chitosan alone and chitosan with sodium alginate and calcium chloride to compare the burst release of drug in both systems.
5. Evaluation of particle size
6. Calculation of percentage yield.
7. Determination of percentage drug entrapment efficiency
8. Determination of zeta potential.
9. Measurement of mean particle size.
10. Evaluation of *in vitro* drug release.
11. Comparisons of *In –vitro* release pattern of optimized chitosan nanoparticles of valsartan with marketed formulations.
12. Accelerated stability studies of optimized chitosan nanoparticles of valsartan.

MATERIALS AND EQUIPMENTS

MATERIALS USED

1. Drug- Valsartan - Shasun laboratories
2. Chitosan - Shasun laboratories
3. Sodium alginate - Shasun laboratories
4. Calcium chloride - Shasun laboratories
5. Tripolyphosphate - Shasun laboratories
6. Acetic acid - S.D.Fine chem Ltd
7. Di-sodium hydrogen phosphate - Vin biotech systems
8. Calcium chloride - Nice chemicals
9. Sodium hydroxide - Nice chemicals

EQUIPMENTS USED

1. Mechanical stirrer - Bombay India ltd.
2. Malvern zeta sizer - SM, UK.
3. Electronic Balance - A&D Company, Japan.
4. Magnetic Stirrer - MC Dalal & co.
5. UV Visible Spectrophotometer - UV Pharma spec 1700, Shimadzu.
6. FTIR Spectrophotometer - Perkin Elmer.
7. Environmental chamber - Inlab equipments (Madras pvt ltd).
8. Centrifuge - Inlab equipments.
9. Freeze drier - Rocktech.

DRUG PROFILE

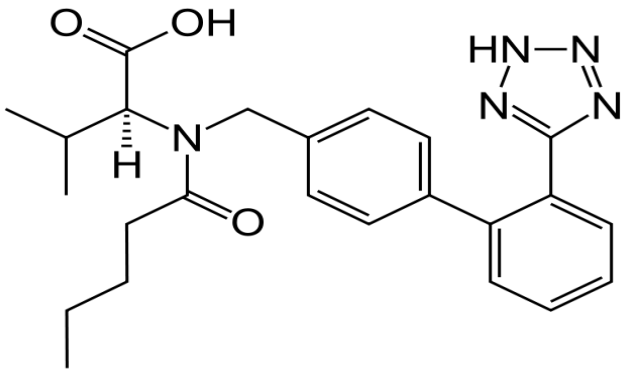
Valsartan:

Valsartan is an angiotensin-receptor blocker (ARB) that may be used to treat a variety of cardiac conditions including hypertension, diabetic nephropathy and heart failure. Valsartan lowers blood pressure by antagonizing the renin-angiotensin-aldosterone system (RAAS); it competes with angiotensin II for binding to the type-1 angiotensin II receptor (AT1) subtype and prevents the blood pressure increasing effects of angiotensin II. Unlike angiotensin-converting enzyme (ACE) inhibitors, ARBs do not have the adverse effect of dry cough. Valsartan may be used to treat hypertension, isolated systolic hypertension, left ventricular hypertrophy and diabetic nephropathy. It may also be used as an alternative agent for the treatment of heart failure, systolic dysfunction, myocardial infarction and coronary artery disease. [41]

Categories:

Antihypertensive Agents, Angiotensin II Receptor Antagonists

Structural formula:



Systematic name:

Valsartan is chemically described as N-(1-oxopentyl)-N-[[2'-(1H-tetrazol-5-yl) [1, 1'-biphenyl]-4-yl] methyl]-L-valine.

Chemical Formula: $C_{24}H_{29}N_5O_3$

Average mass: 435.518799 Da

Monoisotopic mass: 435.227051 Da

Appearance: white powder

Melting Point: 117 °C

Solubility: Slightly soluble in water, soluble in alcohol, Soluble to 100 mM in DMSO

Stability: Stable. Incompatible with strong oxidizing agents

Classes: Biphenyltetrazoles and Derivatives [42, 43,44]

Pharmacodynamics:

Valsartan belongs to a class of antihypertensive agents called angiotensin II receptor blockers (ARBs). Valsartan is a specific and selective type-1 angiotensin II receptor (AT1) antagonist which blocks the blood pressure increasing effects angiotensin II via the renin-angiotensin-aldosterone system (RAAS). RAAS is a homeostatic mechanism for regulating hemodynamics, water and electrolyte balance. During sympathetic stimulation or when renal blood pressure or blood flow is reduced, renin is released from granular cells of the juxtaglomerular apparatus in the kidneys. Renin cleaves circulating angiotensinogen to angiotensin I, which is cleaved by angiotensin converting enzyme (ACE) to angiotensin II.

Angiotensin II increases blood pressure by increasing total peripheral resistance, increasing sodium and water reabsorption in the kidneys via aldosterone secretion, and altering cardiovascular structure. Angiotensin II binds to two receptors: AT1 and type-2 angiotensin II receptor (AT2). AT1 is a G-protein coupled receptor (GPCR) that mediates the vasoconstrictive and aldosterone-secreting effects of angiotensin II.

Studies performed in recent years suggest that AT2 antagonizes AT1-mediated effects and directly affects long-term blood pressure control by inducing vasorelaxation and increasing urinary sodium excretion. Angiotensin receptor blockers (ARBs) are non-peptide competitive inhibitors of AT1. ARBs block the

ability of angiotensin II to stimulate pressor and cell proliferative effects. Unlike ACE inhibitors, ARBs do not affect bradykinin-induced vasodilation. The overall effect of ARBs is a decrease in blood pressure.

Mechanism of action:

Valsartan is an ARB that selectively inhibits the binding of angiotensin II to AT1, which is found in many tissues such as vascular smooth muscle and the adrenal glands. This effectively inhibits the AT1-mediated vasoconstrictive and aldosterone-secreting effects of angiotensin II and results in a decrease in vascular resistance and blood pressure. Valsartan is selective for AT1 and has virtually no affinity for AT2. Inhibition of aldosterone secretion may inhibit sodium and water reabsorption in the kidneys while decreasing potassium excretion. The primary metabolite of valsartan, valeryl 4-hydroxy valsartan, has no pharmacological activity.

Volume of distribution:

17 L (low tissue distribution)

Protein binding:

94 - 97% bound to serum proteins, primarily serum albumin.

Half life:

The initial phase $t_{1/2\,\alpha}$ is < 1 hour while the terminal phase $t_{1/2\,\beta}$ is 5-9 hours.

Metabolism:

Valsartan is excreted largely as unchanged drug (80%) and is minimally metabolized in humans. The primary circulating metabolite, 4-OH-valsartan, is pharmacologically inactive and produced CYP2C9. 4-OH-valsartan accounts for approximately 9% of the circulating dose of valsartan. Although valsartan is metabolized by CYP2C9, CYP- mediated drug-drug interactions between valsartan and other drugs is unlikely. [45,46,47]

Route of elimination:

83% of absorbed valsartan is excreted in feces and 13% is excreted in urine, primarily as unchanged drug.

Clearance:

2 L/h [IV administration], 4.5 L/h [heart Failure patients receiving oral administration 40 to 160 mg twice a day]

Indication:

May be used as a first line agent to treat uncomplicated hypertension, isolated systolic hypertension and left ventricular hypertrophy. Possibly will be used as a first line agent to delay progression of diabetic nephropathy. Losartan may be also used as a second line agent in the treatment of congestive heart failure, systolic dysfunction, myocardial infarction and coronary artery disease in those intolerant of ACE inhibitors.

Side effects:

Valsartan may cause side effects like dizziness, headache, excessive tiredness, nausea, diarrhea, stomach pain, back pain, joint pain, blurry vision, cough, and rash.

Adult Hypertension:

The recommended starting dose of Diovan (valsartan) is 80 mg or 160 mg once daily when used as monotherapy in patients who are not volume-depleted. Patients requiring greater reductions may be started at the higher dose. Diovan may be used over a dose range of 80 mg to 320 mg daily, administered once a day. The antihypertensive effect is substantially present within 2 weeks and maximal reduction is generally attained after 4 weeks. If additional antihypertensive effect

is required over the starting dose range, the dose may be increased to a maximum of 320 mg or a diuretic may be added. Addition of a diuretic has a greater effect than dose increases beyond 80 mg.

Storage:

The drug should be stored at 25°C (77°F).[48,49]

Applications in Pharmaceutical Formulations or Technology

Glacial and diluted acetic acid solutions are widely used as acidifying agents in a variety of pharmaceutical formulations and food preparations. Acetic acid is used in pharmaceutical products as a buffer system when combined with an acetate salt such as sodium acetate. Acetic acid is also claimed to have some antibacterial and antifungal properties.

Description

Glacial acetic acid occurs as a crystalline mass or a clear, colorless volatile solution with a pungent odor.

Pharmacopeial Specifications

Test	JP XV	PhEur 6.0	USP 32

Identification Characters	+	+	+
Freezing point	≥514.58C	≥514.88C	≥ 515.68C
Nonvolatile matter	≤ 41.0mg	≤40.01%	≤41.0mg
Sulfate	+	+	+
Chloride	+	+	+
Heavy metals	≤410 ppm	≤45 ppm	≤45 ppm
Iron	—	≤ 45 ppm	—
Readily oxidizable impurities	+	+	+
Assay	≥599.0%	99.5–100.5%	99.5–100.5%

Typical Properties

Acidity/alkalinity

pH = 2.4 (1M aqueous solution);

pH = 2.9 (0.1M aqueous solution);

pH = 3.4 (0.01M aqueous solution).

Boiling point - 1188C

Dissociation constant **-** pKa = 4.76

Flash point - 398C (closed cup); 578C (open cup).

Melting point - 17 □C

Refractive index - n D^{20} = 1.3718

Solubility

Miscible with ethanol, Ether, Glycerin, Water, and other fixed and volatile oils

Specific gravity

1.045

Stability and Storage Conditions

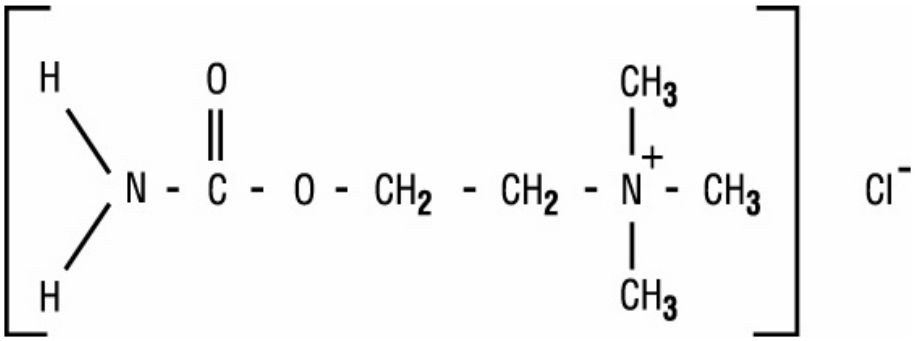
Acetic acid should be stored in an airtight container in a cool, dry place.

Incompatibilities

Acetic acid reacts with alkaline substances. [50,51,52]

1. Calcium Chloride

Structural Formula



Nonproprietary Names

Calcium Chloride Dihydrate

Calcium Chloride Hexahydrate

Synonyms

Calcii chloridum dihydricum; calcii chloridum hexahydricum.

Chemical Name and CAS Registry Number

Calcium chloride anhydrous [10043-52-4]

Calcium chloride dihydrate [10035-04-8]

Calcium chloride hexahydrate [7774-34-7]

Empirical Formula and Molecular Weight

CaCl2 110.98 (for anhydrous)

CaCl2_2H2O 147.0 (for dihydrate)

CaCl2_6H2O 219.1 (for hexahydrate)

Functional Category

Antimicrobial preservative, therapeutic agent, water-absorbing agent.

Applications in Pharmaceutical Formulation or Technology

The main applications of calcium chloride as an excipient relate to its dehydrating properties and, therefore, it has been used as an antimicrobial preservative, as a desiccant, and as an astringent in eye lotions. Therapeutically, calcium chloride injection 10% (as the dehydrate form) is used to treat hypocalcemia.

Description

Calcium chloride occurs as a white or colorless crystalline powder, granules, or crystalline mass, and is hygroscopic (deliquescent).

Typical Properties

Acidity/alkalinity - pH= 4.5–9.2 (5% w/v aqueous solution)

Boiling point - >1600˚ C (anhydrous)

Density (bulk) - 0.835 g/cm3 (dihydrate)

Melting point - 772 ˚ C (anhydrous), 176 ˚ C (dihydrate), 30 ˚ C (hexahydrate).

Solidification temperature 28.5–30˚ C (hexahydrate)

Solubility - Freely soluble in water and ethanol (95%), insoluble in diethyl ether.

Stability and Storage Conditions

Calcium chloride is chemically stable; however, it should be protected from moisture. Store in air tight containers, in a cool and dry place.

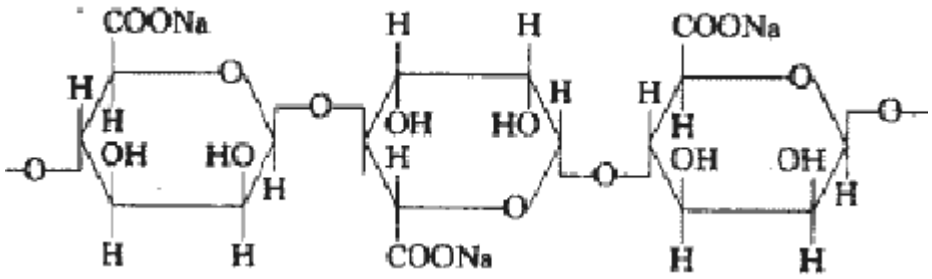
Incompatibilities

Calcium chloride is incompatible with soluble carbonates, phosphates, sulfates, and tartrates. It reacts violently with bromine trifluoride, and a reaction

with zinc releases explosive hydrogen gas. It has an exothermic reaction with water, and when heated to decomposition it emits toxic fumes of chlorine. [53,54,55]

2. Sodium Alginate

Structural Formula



Nonproprietary Names

Sodium Alginate

Synonyms

Alginato sodico, algin, alginic acid, sodium salt, E401, Kelcosol, Keltone, natrii alginas, Protanal, sodium polymannuronate.

Chemical Name and CAS Registry Number

Sodium alginate [9005-38-3]

Empirical Formula and Molecular Weight

Sodium alginate consists chiefly of the sodium salt of alginic acid, which is a mixture of polyuronic acids composed of residues of Dmannuronic acid and L-guluronic acid. The block structure and molecular weight of sodium alginate samples have been investigated.

Functional Category

Stabilizing agent, suspending agent, tablet and capsule disintegrant, tablet binder, viscosity increasing agent.

Applications in Pharmaceutical Formulation or Technology

Sodium alginate is used in a variety of oral and topical pharmaceutical formulations. In tablet formulations, sodium alginate may be used as both a binder and disintegrant; it has been used as a diluent in capsule formulations.

Sodium alginate has also been used in the preparation of sustained-release oral formulations since it can delay the dissolution of a drug from tablets, capsules, and aqueous suspensions. The effects of particle size, viscosity and chemical composition of sodium alginate on drug release from matrix tablets have been described.

In topical formulations, sodium alginate is widely used as a thickening and suspending agent in a variety of pastes, creams, and gels, and as a stabilizing agent for oil-in-water emulsions. Recently, sodium alginate has been used for the

aqueous microencapsulation of drugs, in contrast with the more conventional microencapsulation techniques which use organicsolvent systems.

It has also been used in the formation of nanoparticles. The adhesiveness of hydrogels prepared from sodium alginate has been investigated, and drug release from oral mucosal adhesive tablets, buccal gels, and vaginal tablets based on sodium alginate have been reported. The esophageal bioadhesion of sodium alginate suspensions may provide a barrier against gastric reflux or site-specific delivery of therapeutic agents. Other novel delivery systems containing sodium alginate include ophthalmic solutions that form a gel in situ when administered to the eye; an in situ forming gel containing paracetamol for oral administration; nasal delivery systems based on mucoadhesive microspheres; and a freeze-dried device intended for the delivery of bone-growth factors. Hydrogel systems containing alginates have also been investigated for delivery of proteins and peptides.

In addition, sodium alginate microspheres have been used in the preparation of a footmouth disease DNA vaccine, and in an oral vaccine for Helicobacter pylori; chitosan nanoparticles coated with sodium alginate may have applications in mucosal vaccine delivery systems.

Therapeutically, sodium alginate has been used in combination with an H2-receptor antagonist in the management of gastroesophageal reflux, and as a hemostatic agent in surgical dressings.

Alginate dressings, used to treat exuding wounds, often contain significant amounts of sodium alginate as this improves the gelling properties. Sponges composed of sodium alginate and chitosan produce a sustained drug release and may be useful as wound dressings or as tissue engineering matrices. Lyophilized wound healing wafers composed of sodium alginate have been found to exhibit large reductions in viscosity following gamma irradiation. Sodium alginate is also used in cosmetics and food products;

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Description

Sodium alginate occurs as an odorless and tasteless, white to pale yellowish-brown colored powder.

Typical Properties

Acidity/alkalinity pH - 7.2 (1% w/v aqueous solution)

Solubility - Practically insoluble in ethanol (95%), ether, chloroform, and ethanol/water mixtures in which the ethanol content is greater than 30%. Also, practically insoluble in other organic solvents and aqueous acidic solutions in which the pH is less than 3.

Slowly soluble in water, forming a viscous colloidal solution. Viscosity (dynamic) various grades of sodium alginate are commercially available that yield aqueous solutions of varying viscosity. Typically, a 1% w/v aqueous solution, at 20 ˚ C, will have a viscosity of 20–400 mPa s (20–400 cP). Viscosity may vary depending upon concentration, pH, temperature, or the presence of metal ions. Above pH 10, viscosity decreases 11.

Stability and Storage Conditions

Sodium alginate is a hygroscopic material, although it is stable if stored at low relative humidities and a cool temperature. Aqueous solutions of sodium alginate are most stable at pH 4–10. Below pH 3, alginic acid is precipitated. A 1% w/v aqueous solution of sodium alginate exposed to differing temperatures had a viscosity 60–80% of its original value after storage for 2 years. Solutions should not be stored in metal containers. Sodium alginate solutions are susceptible on storage to microbial spoilage, which may affect solution viscosity. Solutions are ideally sterilized using ethylene oxide, although filtration using a 0.45 mm filter also has only a slight adverse effect on solution viscosity. Heating sodium alginate solutions to temperatures above 708C causes depolymerization with a subsequent loss of viscosity. Autoclaving of solutions can cause a decrease in viscosity, which may vary depending upon the nature of any other substances present. Gamma irradiation should not be used to sterilize sodium alginate solutions since this process severely reduces solution viscosity. Preparations for external use may be

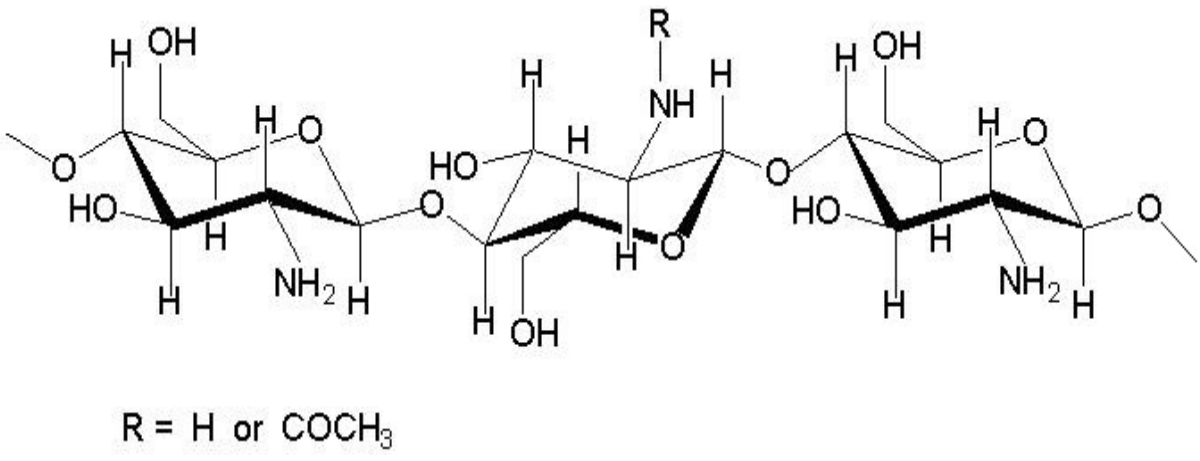
preserved by the addition of 0.1% chlorocresol, 0.1% chloroxylenol, or parabens. If the medium is acidic, benzoic acid may also be used. The bulk material should be stored in an airtight container in a cool, dry place.

Incompatibilities

Sodium alginate is incompatible with acridine derivatives, crystal violet, phenylmercuric acetate and nitrate, calcium salts, heavy metals, and ethanol in concentrations greater than 5%. Low concentrations of electrolytes cause an increase in viscosity but high electrolyte concentrations cause salting-out of sodium alginate; salting-out occurs if more than 4% of sodium chloride is present. [56,57]

3. Chitosan

Structural formula



Nonproprietary Names

Chitosan Hydrochloride

Synonyms

2-Amino-2-deoxy-(1,4)-b-D-glucopyranan, chitosani hydrochloridum, deacetylated chitin, deacetylchitin, b-1,4-poly-D-glucosamine, poly-D-glucosamine, poly-(1,4-b-D-glucopyranosamine).

Chemical 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-acetylglucosamine. 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. A 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 10 000–1 000 000, and vary in degree of deacetylation and viscosity.

Functional Category

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

Applications in Pharmaceutical Formulation or Technology

Chitosan is used in 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, use as a component of mucoadhesive dosage forms.

Description

Chitosan occurs as odorless, white or creamy-white powder or flakes. Fiber formation is quite common during precipitation and the chitosan may look ‘cottonlike’.

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 physicochemical 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-acylation 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.

Acidity/alkalinity pH = 4.0–6.0 (1% w/v aqueous solution)

Density 1.35–1.40 g/cm3

Glass transition temperature 203˚ C

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.

Particle size distribution

<30 mm

Solubility

Sparingly soluble in water, practically insoluble in ethanol (95%), In other organic solvents and neutral or alkali solutions at pH above approximately 6.5. Chitosan dissolves readily in dilute and concentrated solutions of most organic

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. The PhEur 6.5 specifies that chitosan should be stored at a temperature of 2–8˚ C.

Incompatibilities

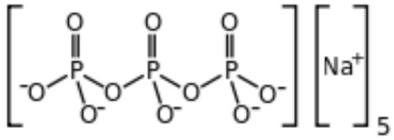
Chitosan is incompatible with strong oxidizing agents. [58,59]

4. **Sodium triphosphate**

Sodium triphosphate

(STP, sometimes STPP or sodium tripolyphosphate or TPP) is an inorganic compound with formula $Na_5P_3O_{10}$. It is thesodium salt of the polyphosphate penta-anion, which is the conjugate base of triphosphoric acid. It is produced on a large scale as a component of many domestic and industrial products, especially detergents. Environmental problems associated with eutrophication are attributed to its widespread use.

Structural formula:



IUPAC name

Pentasodium triphosphate

Other names

Sodium tripolyphosphate, polygon, STPP

Molecular formula

$Na_5P_3O_{10}$

Molar mass

367.864 g/mol

Appearance

White powder

Density

2.52 g/cm^3

Melting point

622 °C

Solubility in water

14.5 g/100 mL (25 °C)

Properties

STPP is a colourless salt, which exists both in anhydrous form and as the hexahydrate. The anion can be described as the pentanionic chain $[O_3POP(O)_2OPO_3]$. Many related di-, tri-, and polyphosphates are known including the cyclic triphosphate $P_3O_9^{3-}$. It binds strongly to metal cations as both a bidentate and tridentate chelating agent.

Food applications

STPP is a preservative for seafood, meats, poultry, and animal feeds. It is common in food production as E number E451. In foods, STPP is used as

an emulsifier and to retain moisture. Many governments regulate the quantities allowed in foods, as it can substantially increase the sale weight of seafood in particular. The United States Food and Drug Administration lists STPP as "generally recognized as safe." [60,61]

Other uses

Other uses (hundreds of thousands of tons/year) include "ceramics, leather tanning (as masking agent and synthetic tanning agent - SYNTAN), anticaking, setting retarders, flame retardants,paper, anticorrosion pigments, textiles, rubber manufacture, fermentation, antifreeze." TPP is used as a polyanion crosslinker in polysaccharide based drug delivery.

EXPERIMENTAL METHODS

CALIBRATION CURVE FOR VALSARTAN

PREPARATION OF CALIBRATION MEDIUM

27.218 gm of potassium-di-hydrogen phosphate was dissolved in sufficient amount of distilled water to make 1000ml of 0.2M solution. 8 gm of sodium hydroxide was dissolved in sufficient amount of distilled water to make one 1000ml of 0.2M solution. From the above solutions 50ml of potassium-di-hydrogen phosphate solution and 22.4ml of sodium hydroxide solutions were mixed and made up to 200ml with distilled water to get phosphate buffer solution of pH 6.8.

Preparation of standard curve for valsartan

The standard stock solution of valsartan was prepared by dissolving a known amount of drug in phosphate buffer pH 6.8. From the above stock solution, different concentrations of 10, 20.....50λg /ml was prepared in same solution of phosphate buffer pH 6.8. The resulting solution was scanned in UV Spectrophotometer to find mmax and the absorbance was measured at λmax (250nm). The standard curve was plotted by taking concentration in X-axis and absorbance in Y-axis. The standard curve was used to estimate drug content and percentage drug release.

FORMULATION OF VALSARTAN CONTAINING PLAIN CHITOSAN NANOPARTICLES AND ALGINATE CHITOSAN NANOPARTICLES BY IONIC GELATION METHOD

The formulations of valsartan containing chitosan nanoparticles were prepared by ionic gelation technique and there were nearly six formulations formulated with different concentrations of chitosan and Tripolyphosphate. Among the six formulations three were prepared with chitosan alone and three were prepared with sodium alginate and calcium chloride to get optimum size of nanoparticles and to study the burst release pattern with chitosan nanoparticle alone and chitosan with sodium alginate and sodium chloride. The formulations were made by mixing (1:1, 1:2. 1:3) different ratios of drug and polymer in both plain chitosan nanoparticles and with sodium alginate chitosan nanoparticles.

The concentration of sodium alginate was kept 0.1 %, 0.2% and 0.3% w/v in three formulations of sodium alginate chitosan nanoparticles.

Preparation of plain chitosan nanoparticles

Chitosan nanoparticles were synthesized via the ionic gelation of chitosan with TPP anions. Chitosan was dissolved in acetic aqueous solution at various concentrations. The concentration of acetic acid in aqueous solution was 1.5 time higher than that of chitosan. The pH of chitosan solution was adjusted to 5.5 by 2 N NaOH solution after adding specified quantity of valsartan into chitosan solution. The TPP solution (1 mg/mL) was prepared by double-distilled water. Chitosan nanoparticles were spontaneously fabricated with the dropwise addition of 5 mL of the chitosan solution to 2 mL of TPP solution under magnetic stirring (1000 rpm, 1 hour) at room temperature. The opalescent suspension was formed

under the same above mentioned conditions. The nanoparticles were separated by centrifugation at 10,000 rpm and 14°C for 30 minutes, freeze-dried and stored at 5 ± 3°C. The weights of freeze-dried nanoparticles were also measured. The formulation of chitosan nanoparticles is shown in Table.1.

Preparation of alginate-chitosan nanoparticles

The chitosan nanoparticles were prepared by the same procedure, except that various amounts of sodium alginate were dissolved in the TPP solution before adding the chitosan-valsartan solution. Chitosan nanoparticles were formed upon adding the TPP solution to chitosan- valsartan solution and mixing. After ten minutes of mixing these two solutions, 0.9 g calcium chloride was added to the suspension which was stirred for 20 min. Finally, the nanoparticles were isolated by centrifugation at 6000 rpm for 40 min. The formulation of alginate-chitosan nanoparticles is shown in Table. 2. [7,16,19]

EVALUATION OF THE VALSARTAN CONTAINING CHITOSAN NANOPARTICLES

Drug-polymer compatibility study

Drug-polymer compatibility study was carried out by FTIR (ATR). Spectra were recorded for pure drug, polymer and for drug and polymer physical mixture. The FTI-IR spectrum pure drug sample and pure polymer sample with formulation were recorded to find out whether any interaction of drug with polymer was present in formulations. [18,62]

Practical yield

Freeze dried nanoparticles were collected and weighed to determine practical yield (PY) from the following equation, [17,63,64]

$$PY\ (\%) = \frac{\text{Nanoparticles weight}}{\text{Theoretical mass of drug + polymer + TPP}} \times 100\%$$

The individual values for three replicates were determined, and their mean values are reported.

Characterization of nanoparticles for particle size analysis

The particle size of the Nanoparticles was evaluated by Scanning Electron Microscope (SEM) to find out the size of the nanoparticles and to study the surface

morphology of the nanoparticles. Dried particles were taken in a piece of black tape and attached to the sample holder. Particle morphology was determined under low vacuum. The SEM method provides a finest approach to find out particles size and surface morphology when compared to other methods. [20,65]

Measurement of mean particle size

The mean size of the nanoparticles was determined by photocorrelation spectroscopy (PCS) on a submicron particle size analyzer (Malvern Instruments) at a scattering angle of 90°. A sample of 0.5mg of the nanoparticles suspended in 5 ml of distilled water was used for the measurement. [21 23, 66, 67]

Determination of percentage of drug entrapment efficiency

The Nanoparticle suspension was centrifuged at 12000 rpm in cooling centrifuge at 15oC for 10 min. The supernatant fluid was analysed spectrophotometrically.

Amount of Drug in the Nanoparticles

$$\text{Drug Entrapment (\%)} = \frac{\text{Amount of Drug fed in to system}}{\text{Amount of Drug fed in to system}} \times 100$$

The above method can be useful to find out the percentage of drug entrapped into the nanoparticles. [20,68]

Determination of zeta potential

The zeta potential of the drug-loaded chitosan nanoparticles was measured on a zetasizer (Malvern Instruments) by determining the electrophoretic mobility in a micro electrophoresis flow cell. Zeta potential is highly useful for physical stability of colloidal dispersions and it can be measured by determination of the movement velocity of the particles in an electric field. It limits ranged from – 200mV to + 200mV. All the samples were measured in water at 25 °C in triplicate and the samples were diluted 10 times before measurement of zeta potential. The zeta potential was measured by Malvern zetasizer UK. [21 26,69]

Evaluation of *in vitro* drug release

The chitosan nanoparticles, after separation by ultracentrifugation, were re-dispersed in 5ml buffer solution PBS solution of pH 6.8, placed in a dialysis membrane bag, tied and immersed in 150ml buffer in 250ml beaker. The entire

system was stirred continuously at 37 °C by using a magnetic stirrer. At pre-determined time intervals, 5ml of the release medium was removed and replaced with 5ml of fresh buffer solution. The amount of drug in the release medium was evaluated by UV spectrophotometry at 250nm. [25, 70]

Stability studies

Valsartan nanoparticles were stored at 2-8°C in refrigerator and 30º C ± 2º C/65% ± 5% RH in humidity chamber for 60 days to find out the stability of the formulations. The optimized formulation stored in the sealed aluminium foil. The optimized formulation was analyzed after 30 and 60 days for appearance, percentage drug entrapment efficiency and *in vitro* drug release. [20, 71]

RESULTS AND DISCUSSION

CALIBRATION CURVE OF VALSARTAN

Calibration curve of valsartan was done in phosphate buffer pH-6.8. Valsartan shows λmax of 250 nm in phosphate buffer pH-6.8. The correlation coefficient was 0.999. Hence, Valsartan obeys the beer's law within the concentration range of 10 to 50µg/ml. The calibration plot of valsartan in phosphate buffer pH-6.8 was showed in fig.5.

FORMULATION OF VALSARTAN CONTAINING PLAIN CHITOSAN NANOPARTICLES AND ALGINATE CHITOSAN NANOPARTICLES BY IONIC GELATION METHOD

Six formulations of valsartan as plain and alginate chitosan nanoparticles were prepared by using various concentrations of (1:1, 1:2, 1:3) chitosan and valsartan were formulated by using ionic gelation technique. The drug concentration kept as constant for each formulation (40mg).

The two main reasons for selection of the method was to compare the release pattern of plain chitosan nanoparticles with alginate nanoparticles to show the burst release of drug in plain chitosan and less burst release in alginate chitosan nanoparticles.

EVALUATION OF FORMULATION

Drug-polymer compatibility study

FT-IR spectra of pure drug valsartan, chitosan and combination of drug and polymer were obtained. The figures are shown in Fig 6, Fig 7 and in Fig 8. FTIR Spectra showed peaks around 1655, 1534 and 3309 cm-1, reflecting the acetylamino I, acetylamino II and (NH2) groups, respectively. The characteristic peaks of sodium alginate included O-H at 3397 cm-1, COO- (asymmetric) at 1617, COO- (symmetric) at 1429 and 1028 cm-1 for C-O-C stretching. The spectrum was indication of intramolecular and intermolecular hydrogen bonds which were formed and enhanced between chitosan and alginate molecules. From the obtained spectra it was observed that all the characteristics peaks of valsartan were present in the combination spectra thus indicating the compatibility of the drug with the polymer used. It shows that there was no significant change in the chemical integrity of the drug.

Practical yield

The practical yields of the formulations were calculated and it was nearly 90 – 95 % for all the formulations.

Characterization of nanoparticles for particle size analysis

The particle size analysis of the nanoparticles reveals that the particle sizes were ranges from 100 – 200 nm and the particles were in nanometer size range. The SEM photography of the formulations was shown in Figure.9.

Measurement of mean particle size and PDI

The mean particle size and PDI was found in range of 105 – 198 nm and 0.075 - 0.129 respectively for the plain chitosan nanoparticles and the mean particle size and PDI was found to be 115 – 220 nm and 0.251 – 0.392 respectively. The both the plain chitosan nanoparticles and alginate chitosan nanoparticles were found be within nanometer size range. The Values were shown in Table.3 and in figure 10 and 11.

Determination of percentage of drug entrapment efficiency

The drug entrapment efficiency was ranges from 74.26 % - 78.56 % for the plain chitosan nanoparticles and it shows the increase in concentration of chitosan leads to increase in entrapment efficiency. The drug entrapment efficiency of alginate chitosan nanoparticles ranges from 80.23 – 85.12 % and the concentration of chitosan and alginate both increases the drug entrapment efficiency when the concentration increases. The Values were shown in Table.3 and in Figure 12.

Determination of zeta potential

Zeta potential of the plain chitosan nanoparticles was found in the range of -12.05±46 to -16.04±04 mV and the chitosan alginate nanoparticles range from -12.23±05 to -15.06±57 mV. The results of Zeta potential revealed that all formulations were found stable. The Values were shown in Table.3.

Evaluation of *In vitro* drug release

The *In-vitro* drug release studies were carried out by using dialysis bag. The data of cumulative percentage drug release of the formulations were shown in Table.4, and in Figure 13. The cumulative percentage drug release after 60 h was studied.

The percentage drug release of plain chitosan nanoparticles ranges from 91.45 – 99.13% at the end of 60 hrs. The percentage drug release of alginate chitosan nanoparticles ranges from 60.12 – 85 23 % at the end of 60 hrs. In alginate chitosan nanoparticles the interaction between chitosan matrix and alginate and ionotropic gelation of sodium alginate with CaCl2 in alginate chitosan nanoparticles may also contribute to enhanced cross-linking density of the matrix. Increased cross-linking density may lead to lower diffusion of the drug from the matrix leads to decreased burst release and rate of release. Among all the formulation the F6 which contains high concentration of alginate and chitosan shows less burst release and sustained release of 60.12% which was chosen as optimized formulation.

Stability studies

Stability studies were carried out to find out the stability and changes in appearance and entrapment efficiency for optimized formulation F6. The formulation was stored at 30±2°C/65±5% RH for two months. The results were shown Table 5 and figure 14. The results showed that there was no significant difference in appearance and entrapment efficiency.

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