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Chitosan Nanoparticles - An Emerging Trend In Nanotechnology

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Abstract

The application of nanotechnology for the treatment, diagnosis, monitoring, and control of biological systems has recently been determined by the National of Health (NIH) as nanomedicine. The strategy of Nanoparticle delivery plays a significant impact on global Pharmaceutical planning and marketing. Polymeric nanoparticles are used to control the drug release, to improve the dissolution of poorly soluble drugs in addition to improve the bioavailability of degradable substances such as protein. They also enhance the uptake of hydrophilic substances across the epithelial layers and have the potential for intracellular drug delivery. The submicron size range of nanoparticles is 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. Thus nanoparticle formulations are more advantageous over traditional dosage forms. The main aim of the present review deals with the nanoparticles of chitosan, which is a natural and bio-degradable polymer. The review focuses on the isolation, purification, characteristic features, derivatives of chitosan, preparation techniques, evaluation methods and applications of chitosan nanoparticles.

Keywords: chitosan, nanoparticles, bioavailability, biodegradable polymer.

Introduction

In the present scenario, nanotechnologies have gained significance and revolutionized the application of medicines. Before 1970 there was a false notion that pharmaceutical suspensions cannot be administered through I.V route but a research paper of "Peter Speiser" in 1976 showed that nanoparticles of antigen can be prepared for the purpose of vaccination and the activity can be sustained for a better immune response [1]. "Christian de Duve" was awarded nobel prize for the discovery of lysomotropic effect of the nanoparticles and also showed that nanocapsules can incorporate compounds into biological cells which cannot permeate through the cellular barriers[2][3].

Nanoparticles (NPs) are familiar for transporting drugs, release the drugs in the target site which mostly undergo degradation in the biological fluids and for drugs that cannot readily diffuse across the barriers [4]. They are used to control and manipulate drugs which are essential for betterment of human health, and biomolecules which are essential for life and to improve the quality of life [5]. Nanoparticles that are formulated with water insoluble polymers use organic solvents, heat or high shear force which may affect the stability of the drugs. Moreover, the preparation methods are complex and time consuming. In contrast, water-soluble polymers offer mild and simple preparation methods without the use of organic solvent and high shear force. Among water-soluble polymers available, chitosan is one of the most extensively studied. Chitosan is a natural polymer with biocompatibility, biodegradability, hydrophilicity and protective properties, broadly used in the delivery of peptides, proteins and polynucleotides, due to maintaining the structure, activity and protect from degradation. As it is hydrogel in nature it prolongs the in-vivo circulation time of the components.[6] Each and every chemical entity contains its own inherent physicochemical properties like charge, lipophilicity, solubility, particle diameter, geometry, and aggregation tendencies which can be modified to enable different pharmacological activities [7, 8]. So physicochemical properties affects the biocompatibility and bioavailability which inturn influence the administration of the drugs, so such a modification of physicochemical properties of chemical entity for pharmacological activity and biocompatibility through nanoparticle approach is a novel and prominent area of research in the drug delivery[9]. Surface modification of these nanoparticulate systems with hydrophilic polymers is the most common way to control the opsonization process and to improve the surface properties of the system.

Chitosan nanoparticles have the potential to chelate metal ions such as palladium, copper, silver, iodine catalyst which can be used in health products, and insecticides. The derivatives of chitosan such as cyanoethyl chitosan are widely used in dialysis and insulating papers, hydroxy ethyl glycol chitosan in enzymological studies and dialysis and glutaraldehyde chitosan used in enzyme immobilization [10].

Low molecular weight chitosan nanoparticles are having good solubility, biocompatibility, active in biological systems, and hence used to deliver the non-viral vector gene [11, 12, 13]. The reactions between "thiol" and primary amino groups of chitosan produce

thiomers. Thiolation of chitosan forms a disulfide bond with glycoproteins of mucus thereby enhancing the mucoadhesive nature, increases permeation, and also observed the antiprotease activity [14]. Several thiolated derivatives of chitosan have been synthesized like chitosan cysteine [15], chitosan-thiobutylamidine, chitosan-thioglycolic acid [16] and chitosan-glutathione conjugates [17].

Recently tri-methyl chitosan nanoparticles are developed to deliver the vaccines and showed the maximum immune responses in mice, indicating that trimethyl coated chitosan nanoparticles are potential carriers of vaccines [18].

This review is an overview of chitosan and chitosan nanoparticles and focuses on isolation, purification, characteristic features, derivatives of chitosan, stability, preparation techniques of chitosan nanoparticles, evaluation methods and its applications.

Chitosan

Chitosan is a heteropolymer and it consists of β (1-4) 2-acetamido-2-deoxy-β-D-glucopyranose (N-acetyl glucosamine) and 2-amino-2-deoxy-β-D-glucopyranose (D-glucosamine) units, randomly or distributed as blocks throughout the biopolymer. It is the N-deacetylated derivative of chitin, but the N-deacetylation is almost never complete (19). Chitosan has one primary amine group and two free hydroxyl groups for each monomer with a unit formula of $C_6H_{11}O_4N$ with the molecular weight of 3.8 – 2000KD.[20] Chitosan exists as off white to yellow colored course ground flakes, and powder with glass transition temperature 203°C and degree of deacetylation is 50-95%. [21-23]

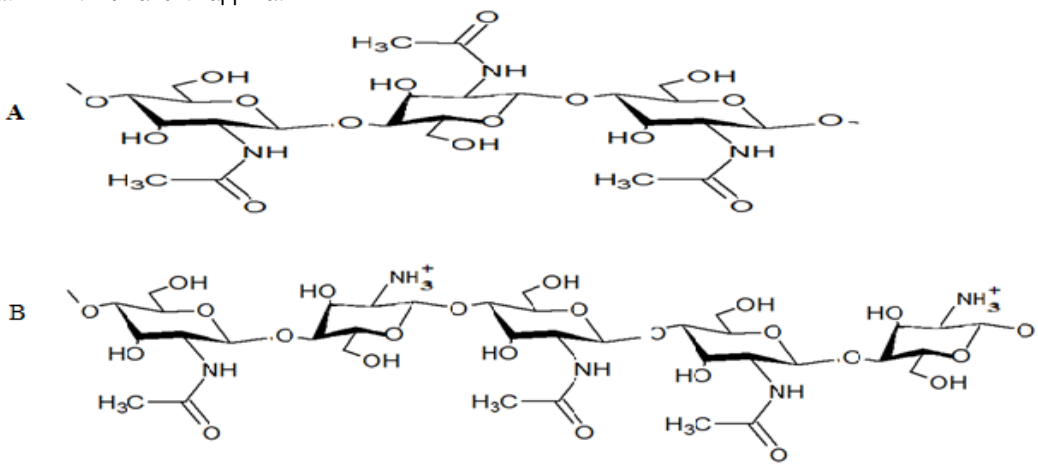


Figure 2 Structure of (A) Chitin and (B) Protonated Chitosan

Solubility of Chitosan

Solubility of chitosan in organic acids was limited, but it is soluble in acids like acetic acid, formic acid, and lactic acid. The effect of pH was more predominant in dissolving chitosan. While preparing the solution the pH must be adjusted to below 6, at this pH the protonation of amino groups will takes place producing NH_3^+ ion which is an important prerequisite for dissolving chitosan. 1% acetic acid concentration is the most commonly used for dissolving, also it is also soluble in 1% hydrochloric acid. But it is insoluble in inorganic acids like "sulphuric acid, and phosphoric acids".

As previously mentioned solubility of chitosan solution is mostly affected by pH, and it's solution stability also affected by pH, above pH 7 the stability of chitosan solution was poor. At pH below 4 it forms a stable solution. It forms a precipitation or gelation and poly ion complex which results in formation of gel at higher pH(>7).[24]

Production of Chitosan

Production of chitosan takes place in four steps. They are

De-acetylation

De-proteinization

Demineralization

De-colorization

Depending on the source and method of preparation and also on physiological conditions, chitosan is not a single chemical entity, but varies in composition. Chitosan is a deacetylated form of chitin to form a soluble amine salts. The acetyl content must be less than 4- 4.5% to form the soluble product and the degree of deacetylation must be 80 to 85% or higher. Commercially, it is manufactured by a chemical method. First, wash the sources such as crab or shrimp shells and grinded into powdered form and then it is deproteinized by treatment with an aqueous 3-5% solution of sodium hydroxide. Then it is neutralized and demineralized at a room temperature by treating it with aqueous 3-5% of hydrochloric solution to form a white or slightly pink precipitate of chitin. Then chitin is deacetylated by treatment with an aqueous 40-45% of sodium hydroxide solution and the precipitate is then washed with water. The insoluble part is removed using an aqueous 2% acetic acids solution. The supernatant solution is then neutralized with an aqueous sodium hydroxide solution to obtain a purified chitosan. [25] The extent of deacetylation mainly depends upon alkali concentration, time and temperature employed throughout the

process. For example, increasing temperature or strength of sodium ydroxide solution can remove acetyl groups, resulting in chitosan molecules of varied range of molecular weight with different physicochemical properties and applications.

Degree of deacetylation

Chitosan is a semi-crystalline polymer and the degree of crystallinity is a function of the degree of deacetylation. The nature of crystallinity is maximum for both chitin (0% deacetylated) and chitosan (100% deacetylated). Chitin is N-deacetylated to such an extent that the formed chitosan becomes soluble in dilute aqueous acids. The process of deacetylation involves the removal of acetyl groups from the molecular chain of chitin, leaving behind a free amino group (-NH2). Chitosan versatility depends mainly on the degree of chemically reactive amino groups. To increase the amino group content of chitosan and higher deacetylation, chitosan is subjected to repeated alkaline treatment. Increasing either the temperature or strength of the alkaline solution can also enhance the removal of acetyl groups from chitin. The degree of deacetylation in chitosan is the ratio of 2-acetamido-2-deoxy-D-glucopyranose to 2-amino-2-deoxy-D-glucopyranose structural units. The degree of deacetylation of chitosan, which determines the content of free amino groups, can be employed to differentiate between chitin and chitosan. Commercially available chitosan has degree of deacetylation ranging from 50 to 90%. Conversion of chitin into chitosan increases degree of deacetylation, and thereby alters the charge distribution of chitosan molecules. It is known that the charge density along the chain increases with an increase in the degree of deacetylation, and the chain flexibility of chitosan molecules can be manipulated by changing the degree of deacetylation.[26]

Determination of deacetylation of chitosan

Degree of deacetylation of chitosan was determined by titrimetric method. In this method, 1% chitosan solution prepared using acetic acid was added to phosphoric acid in the ratio of 1:1 (v/v) and the mixture was titrated against 0.1M NaOH using phenolphthalein as an indicator. The degree of deacetylation of the chitosan can be obtained using the following formula

$$\text{Degree of Deacetylation} = 100 - 2.303 \frac{V_{ab} - V_o}{m}$$

Where *m* = amount of chitosan (mg) used and *V* = difference of the 0.1 M NaOH used between the chitosan solution and standard.

Purification of Chitosan

Various grades of chitosan were purified by the following methods:

Cleaning the samples by the removal of insoluble particles

Disperse the chitosan in distilled water and then solubilized using acetic acid, followed by filtration using whatmann® filter paper grade 541.

Through washing by dialysis

In addition to first method, Chitosan was precipitated from filtered chitosan solution and titrated with 1 N NaOH until pH value of 8.5 was attained followed by centrifugation and resuspending of chitosan in distilled water. Then the chitosan suspensions were dialyzed against distilled water for 2 days using dialysis tubing. Finally, chitosan suspensions retrieved from the dialysis tubes were frozen at -80°C and lyophilized in a freeze dryer.

Extensive purification

In this method, 1 ml of 10% w/v aqueous solution of sodium dodecyl sulfate (SDS) was added to the resuspended solution of chiosan and stirred for 30 min. Dithiothreitol (DTT) was also added directly to solution of chitosan and the mixture was heated at 90-95°C with continuous stirring for 5 min in the hood. After leaving the solution under stirring at room temperature overnight, 3.3 ml of 5% w/v ethylene diamine tetra acetic acid (EDTA) was added and stirred at room temperature for 2 additional hours. The water insoluble chitosan precipitate was collected by centrifugation at 4,300 rpm for 30 min and washed several times with distilled water by resuspending and re-centrifugation for 30 min. Chitosan was resuspended in distilled water and dialyzed for 2 days using dialysis tubing, changing the water triplicate. Finally, chitosan suspensions were retrieved from the dialysis tubes, frozen at -80°C and lyophilized.

Derivatives of Chitosan

Chitosan has large number of applications in pharmaceutical dosage forms and its further application can be exploited by modification of basic structure to obtain derivatives with wide range of properties.

N-Trimethylene chloride chitosan

N-Trimethylene chloride is a quaternary derivative of chitosan after methylation. It has superior aqueous solubility, intestinal permeability as well as higher absorption over a wide pH range. The high degree of substitution was observed with decrease in solubility. Quaternization of N-Trimethyl chloride chitosan decreases the transepithelial electrical resistance and thereby enhances drug absorption properties.[27]

Trimethyl chitosan (TMC)

The TMC can be synthesized by quaternization of the amino groups of the parent polymer. The quaternization methods include use of methylating agent as methyl iodide and dimethylsulfate or the sequence of reductive alkylation via Schiff base formation. Trimethylation of chitosan generates the N, N, N-trimethyl derivative with permanent positive charges and water solubility.

TMC proved to be a derivative of chitosan with superior solubility and basicity, even at low degrees of quaternization, compared to chitosan and salts. Trimethyl chitosan nanoparticles are a superior vehicle for increasing the solubility of *candesartan-cilexetil* compared to trimethyl chitosan, gum arabic or commercial water soluble Chitosan.[28] This derivative has shown promising results as drug delivery agent as well as a DNA delivery agent.[29] Thymopentin-loaded TMC nanoparticles on oral administration show higher efficiency for ratio of lymphocyte CD4+/CD8 than thymopentin alone.[30] Self-assembled nanoparticles prepared by TMC and poly (gamma-glutamic acid) for oral delivery of insulin showed superior stability of nanoparticles in a broader pH range and sustained release profile of insulin with permeation enhancement observed.[31] Compared with non-modified TMC nanoparticles, alginate modified TMC nanoparticles had a stronger effect on enhancing drug transport through transcellular pathway but an equal effect on enhancing drug transport through paracellular pathway. TMC nanoparticles are potential carriers for oral protein/vaccine delivery.[32] TMC nanoparticles containing ovalbumin as a model antigen (TMC/OVA nanoparticles) and an immunopotentiator (TMC/OVA/immunopotentiator nanoparticles) was formulated.[33] TMC nanoparticles have been shown to increase the immunogenicity of subunit antigens after nasal and intradermal administration.

Thiolated derivatives of chitosan

Thiolated derivatives of chitosan known as thiomers have been produced via immobilization of thiol groups on the primary amino groups of chitosan backbone. Thiolation of chitosan has also demonstrated to improve the mucoadhesive properties of chitosan through disulfide bonds with cysteine-rich domains of mucus glycoproteins. Permeation enhancement and antiprotease activity have also been observed with thiolated chitosan. Synthesis of different thiolated derivatives of chitosan including chitosan cysteine, chitosan-thiobutylamidine, chitosan-thioglycolic acid and chitosan-glutathione conjugates. Chitosan-glutathione conjugates were prepared from unmodified and thiolated polymers, characterized, and compared. Thiolation improves the solubility of Chitosan without any significant alteration in size and charge of nanoparticles. Nanoparticulate system with mucoadhesion properties was composed of a core of polymethyl methacrylate surrounded by a shell of thiolated chitosan (Ch-GSH-pMMA) for enhancing oral bioavailability of docetaxel (DTX).The pharmacokinetic study was carried out in vivo using wistar rats. The half-life of DTX-loaded nanoparticles was about 9 times longer than oral DTX used as positive control. The oral bioavailability of DTX was increased to 68.9% for DTX-loaded nanoparticles compared to 6.5% for positive control. [34] Docetaxel nanoparticles showed a high cytotoxicity effect in the Caco-2 and MCF-7 cell lines after 72 hours. [35] Nanoparticles of Letrozole prepared from thiolated chitosan (TCS)-dextran sulfate (DS) may represent a useful approach for targeting its release at its site of absorption, sustaining its release and improving its mucosal availability in the treatment of breast cancer.[36]

Chitosan Esters

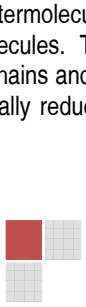
Chitosan esteric forms (glutamate, succinate, phthalate) have a different solubility profile. These esteric forms were insoluble in acidic condition and provide sustained release in basic condition. Ordinary chitosan can be dissolved in acidic water but not in alkaline. Whereas Succinyl chitosan exihibits opposite behavior. It can function as a drug carrier for longer retention, low toxicity and accumulation at tumour. Chitosan (CS) esters based matrix has been used successfully in many formulations such as in colon-specific oral delivery of sodium diclofenac.[37] N succinyl chitosan (NSC) nanoparticles had potential as a local sustained delivery system for hydrophobic antitumor drug, Hydroxycamptothecin. In vivo studies, the NSC nanoparticles showed tumor targeting and significant suppression of tumor growth after S.C. injection (close to the tumor) to mice bearing S180 sarcoma tumor. A histopathological analysis of the tumor tissues indicated that NSC nanoparticles had a lethal effect on the sarcoma cell.[38] The 5-fluorouracil-loaded NSC nanoparticles showed good anti-tumour activity against Sarcoma 180 solid tumour and mild toxicity.[39]

Chitosan conjugates

Chitosan can be conjugated with bio-active excipients for delivery of active ingredients. Chitosan conjugates such as chitosan-4-thiobutylamidine, 5-methylpyrrolidinone chitosan, showed enhanced absorption as well as mucoadhesive properties. Chitosan is attached with the enzyme inhibitor. The polymer formed after attaching the enzyme retained the muco-adhesive properties and prevents the degradation of drugs by inhibiting enzymes, such as trypsin and chymotrypsin. This conjugated Chitosan demonstrated promise delivery of sensitive peptide drugs such as calcitonin.[40] The polymer–drug conjugates are composed of a water-soluble polymer that is chemically conjugated to a drug via a biodegradable spacer. The spacer is usually stable in the bloodstream but cleaved at the target site by hydrolysis or enzymatic degradation. Such drug conjugates can be selectively accumulated at the tumor site by the EPR effects, followed by release of the drug by cleavage of the spacer. Eg: Doxorubicin-conjugated glycol chitosan (DOX–GC) with a cis-aconityl spacer, Low molecular weight chitosan conjugated with paclitaxel (LMWC-PTX) was also synthesized by chemical conjugation of LMWC and PTX through a succinate linker, which can be cleaved at physiological conditions.

Cross-linking agents used in preparation of nanoparticles

Cross-linking occurs when a reagent introduces intermolecular bridges or cross-links between polysaccharide molecules. The cross-linking agent reacts with macromolecule linear chains and/or itself in an alkaline medium. The cross-linking drastically reduces



segment mobility in the polymer and a number of chains are interconnected by the formation of new inter chain linkages. A three dimensional network is then formed. If the degree of crosslinking is high, the matrix of the polymers becomes insoluble in water and organic solvents.[41]

Classification of Cross-Linking Agents

Cross-linking agents are broadly classified on the basis of interaction of cross-linkers with chitosan during cross-linking.
Physical cross-linking
Chemical cross-linking

Physical cross-linking

Polysaccharides form cross-linking network with counter ions at the surface. High counter ion concentration would require longer times to achieve complete cross-linking of the polysaccharide. This method gives nanoparticles with reversibility and is considered biocompatible due to the lack of harsh preparation condition or toxic crosslinkers. Ionically-crosslinked nanoparticles are generally pH sensitive, which is a suitable feature for stimuli-sensitive controlled release. Ionic cross linking occurs by using Tripolyphosphate (TPP), sulphuric acid, inorganic ions such as $Fe(CN)_6^4$, $Fe(CN)_6^3$ citrate and calcium ions as cross linkers.[42]

Chemical cross-linking

Cross-linking agents reacts with polysaccharides forming either intermolecular or intramolecular covalent bonds. Covalently crosslinked polysaccharide nanoparticles enable the network structure to be permanent since irreversible chemical links are formed unless biodegradable or stimuli-responsive crosslinkers are employed. Though there is drastic change in pH, the rigid network allows absorption of water and bioactive compounds without dissolution of the nanoparticles. The concentration of cross linking agent and cross-linking time affects the degree of chemical cross-linking.[41] Chemical cross-linking is carried out by using glutaraldehyde formaldehyde, cinnamaldehyde, genipin, low toxic di and tricarboxylic acids (cuccinic acid, malic acid, tartaric acid and citric acid) vanillin, epichlorhydrin etc., [43-50]

Stability of Nanoparticles

Maintaining the stability of nanoparticles is a very important aspect in the field of nanotechnologies with respect to pharmaceuticals, the nanoparticle formulations should have stability until use, or else the therapeutic efficacy will be lost and which shows impact on the human health.
The stability of nanoparticles in suspension depends on the two forces like “Brownian motion and gravitational forces”. These two forces play a major role in the stability. As nanoparticles are tiny and submicron sized particles they are in a continuous random motion which is called as Brownian motion, while in a random motion the interparticular collision may lead to settling of particles thereby affecting stability. The colloidal nanoparticle suspension may be de-stabilized by the addition of other components to the formulation.[51]
The physical instability of nanoparticles is due to “agglomeration of particles, bridging flocculation, coagulation, hetero coagulation”.[52]

Chemical stability of nanoparticles

It depends on the storage conditions like temperature, P^H of the medium, composition of the formulation, type of polymer used, and molecular weight of polymer used in formulation. Hydrolytic degradable polymers will degrade at low rate if temperature and P^H are controlled.
E.g. Lemoine et. al,(1996) studied the effect of type of polymer in stability of nanoparticles and showed that PLGA 50:50 is less stable than PLGA 75:25.
For poly-caprolactone nanoparticles the initial molecular weight donot affect the degradation.[53]
P^H of the media also influences the stability of nanoparticles, the ph effect was more prominent in polylactides. The polylactide nanoparticles are more stable in the ph of biological fluids, at a temperature of 4^0 C. The instability of polymeric nanospheres is due to hydrolysis in the ph environment. In the acidic $P^{H,}$ it causes non-random cleavage of ester bonds of polymer chain by producing soluble derivatives including lactic-acid.
The chemical integrity of drug in nanoparticles is another fundamental aspect of stability.[54]
Most of the drugs sensitive to ph, hence ph should be optimized to control the leakage of drug. During the encapsulation of strong hydrophilic drugs precipitation in the aqueous phase causes nano-crystals, that may be misinterpreted as nanoparticles.[55]
In some instances the addition of anionic-surfactants in the dispersion cause a more rapid degradation of polymer[PLGA], when strong nucleophilic groups were added in the formulation.[54]
The storage temperature has a crucial effect on the long-term stability of nanoparticles.
E.g. Polylactide and polycaprolactone nanoparticles were stored for 350 days at 5^0C, it caused only slight changes in the molecular weight. At 37^0 C there was a rapid degradation of both polymers. However when PLA nanoparticles were stored at 25^0 C, changes in molecular weight of polymer were observed after 4 months of storage.

Stabilization of nanoparticles by lyoprotectants

Water replacement hypothesis was considered to be the suggested mechanism of stabilization of nanoparticles by lyoprotectants. In this mechanism hydrogen bonds will be formed between the nanoparticles and lyoprotectants. They protect the structure of nanoparticles by acting as a water substituents. Among several lyoprotectants disaccharides were found to be most effective lyoprotectants(e.g. disaccharides most effectively protect “griseofulvin-lipids” nanoparticles.[56]
Freeze drying improves the long term stability of nanoparticles, by converting colloidal suspension in to solid state which will prevent

the aggregation of particles thereby increasing the stability of particles. It also prevents the breakage of polymer, which decreases the diffusion of drug from nanoparticles.

E.g. The nanocapsules of polycaprolactone were freeze dried by using PVP as a lyoprotectant under extreme conditions of temperature and humidity and found that the nanocapsules were stable for 6 months.[57]

The size of freeze-dried solid lipid nanocapsules (SLN) remained stable after three months of storage under two storage temperatures: 5 C and 40 C at 75% relative humidity. The stored nanocapsules did not exhibit any oil leakage after 3 months storage.[58]

Dehydroemetine nanoparticles for treating visceral leishmaniasis have been freeze-dried using glucose 5% as cryoprotectant. These freeze-dried nanoparticles could be stored at 20 C for 24 months without any modification of their size or the level of drug binding.[59]

Freeze dried of solid lipid nanoparticles of Azidothymine with trehalose was found to be more stable, when compared to other stabilizers.[60]

The stability studies of poly (methylidene malonate 2.1.2) (PMM 212) nanoparticles which were freeze dried was conducted. The tests were performed for a period of 12 months under various storage conditions of temperature and light. By conducting the test it was observed that PMM 212 nanoparticles showed significant changes at 40^0 c, the alterations are decrease in P^H of suspension, modification of HPLC chromatogram of compounds, and the reduction of in-vitro cytotoxicity. PMM 212 is lyophilized and it retained it's properties in darkness, at room temperature, and in light.[61].

Some of cryoprotectants used in literature for the freeze-drying of nanoparticles

Glucose [62]
Sucrose [63]
Trehalose [64]
Lactose [65]
Mannitol [66]
Sorbitol [67]
Aerosil (colloidal silicon dioxide) [68]
Maltose [64]
Poly(vinyl pyrrolidone) [69]
Fructose [70]
Dextran [71]
Glycerol [66]
Poly(vinyl alcohol [71]
Glycine, Gelatine [72]
Hydroxypropyl-β-cyclodextrin [73]

Mechanisms Of Drug Release From The Polymer [74]

The delivery of drug form the polymeric carrier involves any one of the following steps.

After ingestion of the formulation,

The polymeric nanoparticles absorb water and starts swelling followed by diffusion of drug from the swollen matrix of the polymer.

The cleavage or degradation of the polymer by the enzymatic reaction at the site of drug delivery, there by the drug is released from the inner core.

Dissociation of the drug from the polymer and released from the swelled nanoparticles.

Preparation Methods

Chitosan nanoparticles are prepared by various methods like

Ionotropic Gelation

It is the simple and easy method for the preparation of nanoparticles. Electrostatic interactions between cationic amino group of chitosan and negatively charged anions of cross-linking agent like TPP are responsible for the formation of nanoparticles. Chitosan is dissolved in acetic acid to form polymer solution. Crosslinking agent is added to water to produce solution. In the presence or absence of stabilizing agents like Tween 80, Polyethylene glycol, crosslinking agent is added to the chitosan solution to form the nanoparticles instantaneously under stirring at room temperature. The size and surface charge of particles can be modified by varying the ratio of chitosan and stabilizer.[75]

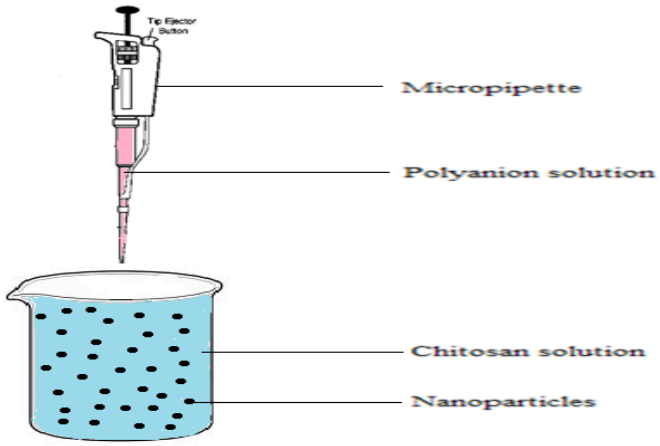


Figure 3 Iontotropic gelation technique

Premix Membrane Emulsification Technique

The dispersed phase is prepared by dissolving the chitosan in water. The continuous phase is prepared from the mixture of liquid paraffin, petroleum ether and emulsifier. Emulsion is prepared by dispersing the chitosan solution in continuous phase by low-seed stator homogenization and then transferred into the premix reservoir. The coarse emulsion is extruded through the porous glass membrane under high pressure. The formed nanodroplets

were further cross-linked to obtain chitosan nanoparticles. The chitosan nanoparticles size from 300 nm to 1.85μm could be obtained by premix membrane emulsification method by changing the pore size of the membrane. The polydispersity index could be as low as 0.027 under optimized conditions. It is rapid and potential technique to prepare nanoparticles of uniform size.[76, 77] [Figure 4].

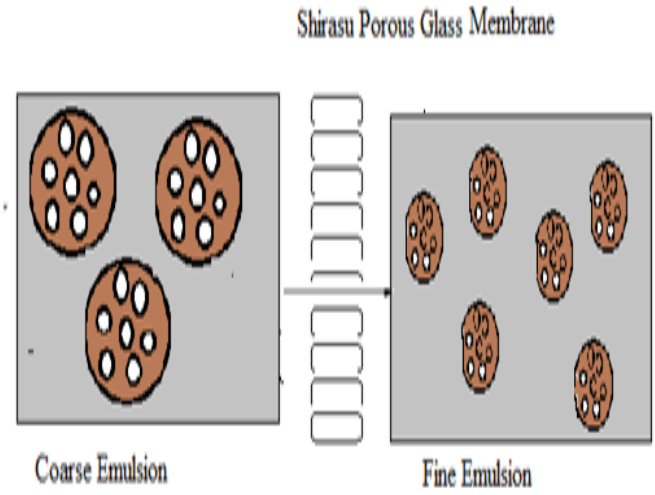


Figure 4 Premix membrane emulsification technique

Micro emulsion Method

In this technique, chitosan nanoparticles form in the aqueous core of reverse micellar droplets and subsequently cross-linked through glutaraldehyde. It involves addition of surfactant to n-Hexane. Then, chitosan in acetic solution and the cross linking agent like glutaraldehyde were added to surfactant/hexane mixture under continuous stirring at room temperature. The total setup is kept overnight for the formation of nanoparticles in the presence of surfactant. A low pressure is used to remove the organic solvent. The process results in the cross-linked chitosan nanoparticles. The excess surfactant was then removed by precipitating with $CaCl_2$ and the precipitant was removed by centrifugation. The final nanosphere 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 concentration of cross linking agent which alters the degree of cross linking. Nevertheless, some disadvantages associated with this method are use of organic solvent, time-consuming preparation process, and complexity in the washing step.[78, 79]

Emulsification Solvent Diffusion Method

This technique is based on the partial miscibility of an organic solvent with water. An O/W emulsion is obtained by injection of an organic phase into aqueous phase containing chitosan solution and stabilizing agent (i.e. poloxamer) under mechanical stirring, followed by high pressure homogenization. The emulsion is diluted by addition of large amount of water to overcome organic solvent miscibility in water. As a result of the diffusion of organic solvent into water, polymer precipitation occurs, leading to the formation of nanoparticles. This method is applicable for hydrophobic drug and showed high percentage of drug entrapment. The limitations of this method include harsh processing conditions (e.g., the use of organic solvents) and the high shear forces applied during nanoparticles preparation [Figure 5].

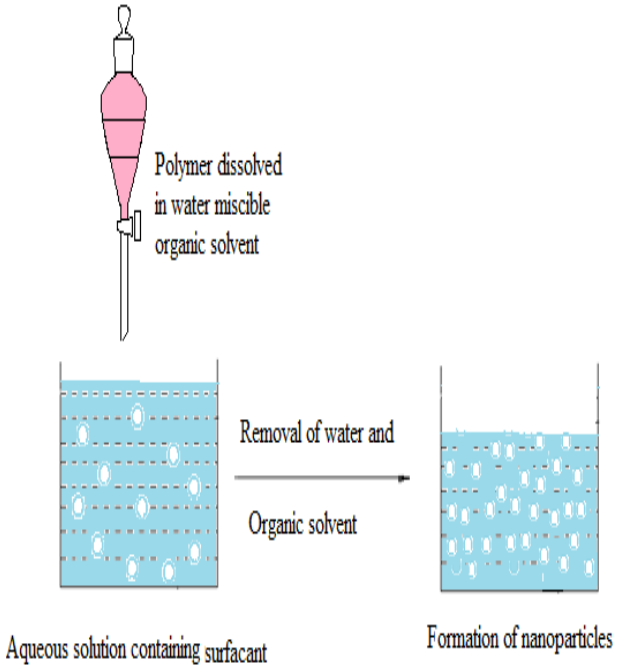


Figure 5 Emulsification solvent diffusion method

Polyelectrolyte Complex (PEC)

This is the simple and mild technique for the preparation of nanoparticles since no harsh conditions are involved. Polyelectrolyte complexes are formed by self-assembly of the cationic charged polymer and alginate. The mechanism involved in the formation of complex involves the charge neutralization between cationic polymer and alginate due to the polyelectrolyte component self assembly leading to a fall in hydrophobicity. Several cationic polymers (i.e. gelatin, polyethyleneimine) also possess this property. The nanoparticles were spontaneously formed after addition of alginate solution into chitosan which was priorly dissolved in acetic acid solution, under mechanical stirring at room temperature. The complexes size range from 50 nm to 700 nm.[80] [Figure 6].

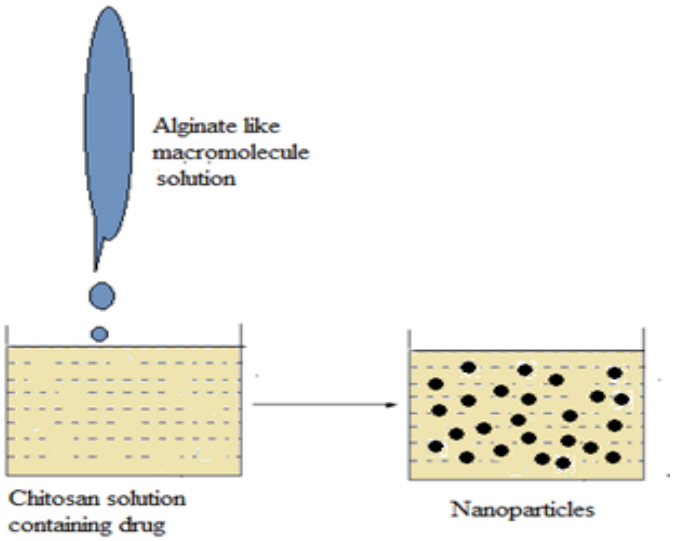


Figure 6 Polyelectrolyte complex technique

Reverse Micellar Technique

Preparation of ultrafine polymeric nanoparticles with narrow size distribution can be obtained using reverse micellar medium.[81] Aqueous core of the reverse micellar droplets can be used as a nanoreactor to prepare such particles, since the size of the reverse micellar droplets usually lies between 1 and 10 nm and are highly monodispersed.[82] In this method, the surfactant is dissolved in an organic solvent in order to prepare reverse micelles. To this, aqueous solutions of Chitosan and drug are added with constant vortexing to avoid any turbidity. Additional amount of water may be added to obtain nanoparticles of larger size. To this transparent solution, a cross-linking agent is added and cross-linking is achieved after stirring overnight. The material is dispersed in water and then adding a suitable salt, precipitates the surfactant out and the mixture is subjected to centrifugation. The supernatant solution is decanted which contains the drug-loaded nanoparticles. The aqueous dispersion is immediately dialyzed using dialysis membrane for about 1 h and the obtained liquid is lyophilized to get dry powder. [Figure 7]

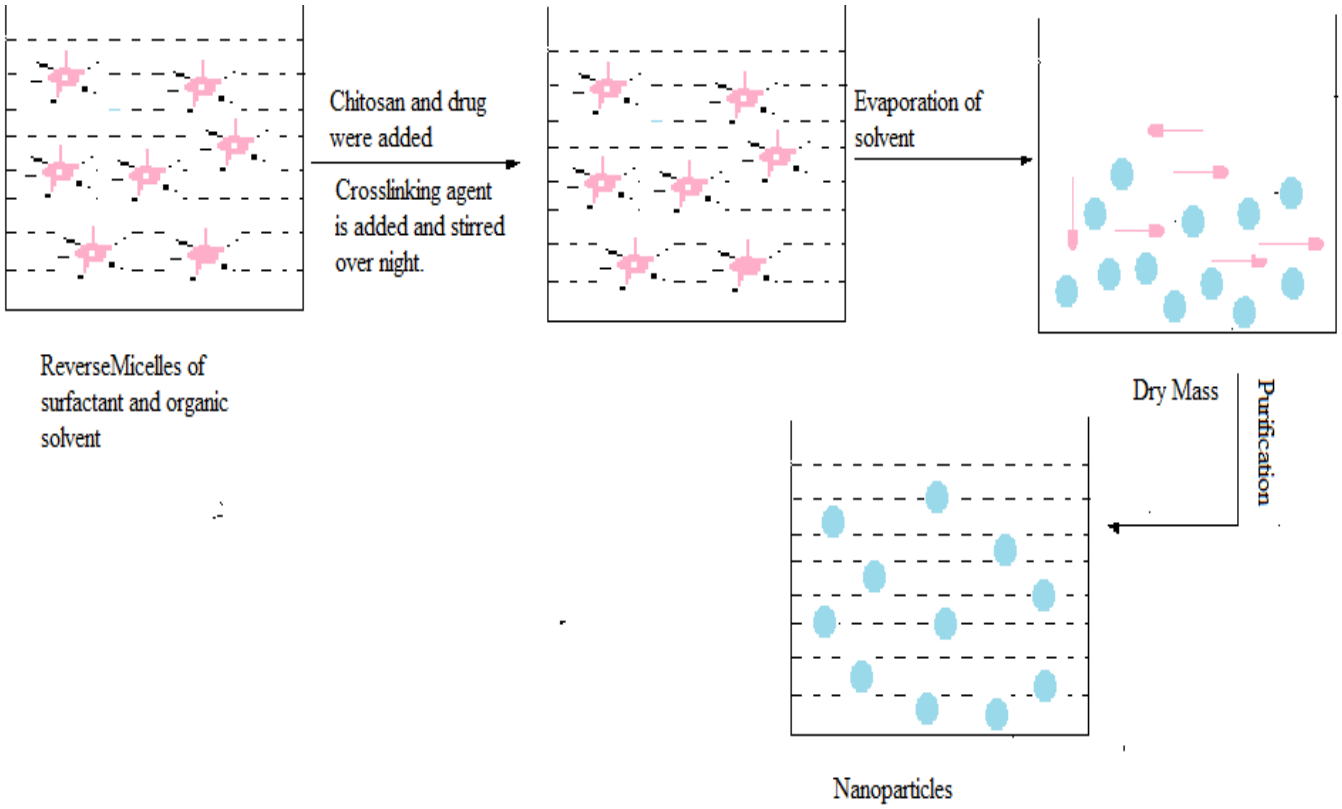


Figure 7 Reverse micellar technique

Emulsion-Droplet Coalescence Method

A nanoparticle formed under this technique involves/utilizes the mechanism of both emulsion cross-linking and precipitation methods. However, in this method, instead of crosslinking the stable droplets, precipitation is induced by allowing coalescence of Chitosan droplets with sodium hydroxide droplets. First, a stable emulsion containing Chitosan solution along with drug is produced in liquid paraffin oil and then, another stable emulsion containing Chitosan aqueous solution made of sodium hydroxide is produced in the same manner. When both emulsions are mixed under high-speed stirring, droplets of each emulsion would collide at random and coalesce, thereby precipitating Chitosan droplets to give small size particles of chitosan nanoparticles.[83] [Figure 8

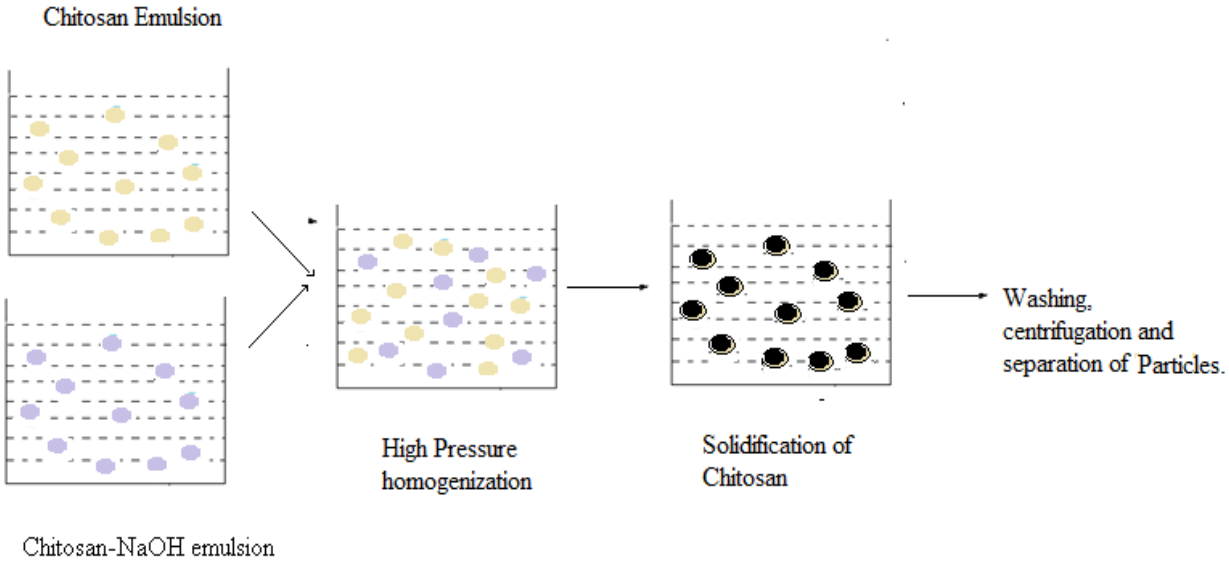


Figure 8 Emulsion- droplet coalescence technique

Spray-drying

Spray-drying method can be used as a one-step preparation of nanoparticle powder. Mannitol microspheres containing chisosan nanoparticles-loaded protein was prepared by this technique.[84] Chitosan–iron oxide nanoparticles with various chitosan: Iron oxide ratios by spray-drying.[85] Atomic absorption spectrometry results implied that chitosan had strong chelation with iron. Meanwhile, Fe_3O_4 was crystallized and distributed in the chitosan matrix. These chitosan–iron oxide nanoparticles were stable in water with strong super paramagnetism.

Emulsification and cross-linking

In this method, chitosan nanoparticles were prepared by addition of cross linking agents to the w/o emulsion to hardened the particles. The reactive amino groups of chitosan undergo a covalent cross-linking with the aldehyde groups of glutaraldehyde, which is added after the emulsion formation and, consequently, after nanoparticle production. The final particle size was demonstrated to be highly dependent on stirring speed, as well as on the extent of cross-linking. It has many disadvantages like tedious procedures and the application of harsh cross-linking agents.[86, 87] [Figure 9].

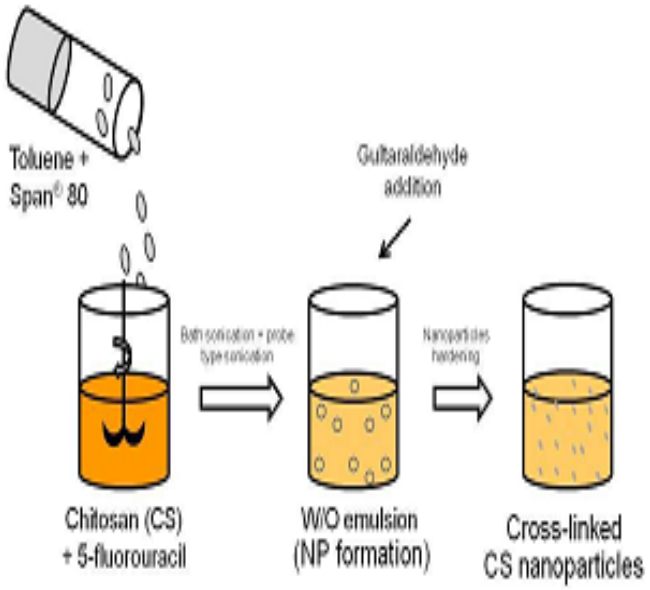


Figure 9 Emulsification and cross-linking technique

Modified ionic gelation with radical polymerization

This method is derived from ionic gelation, but introduces a modification, because chitosan gelation occurs concomitantly with the polymerisation of acrylic acid monomers. The first step occurs at room temperature and consists in stirring an aqueous monomer solution of acrylic or methacrylic acid with an aqueous solution of oppositely charged chitosan. In some cases, polyethylene glycol (PEG) or polyether (polethylene glycol-polypropylene glycol-polyethylene glycol) are also added to the reaction medium, either separately into the monomer solution or following mixing with chitosan.

The opposite charges of chitosan and acrylic or methacrylic acid lead to an ionic interaction, while radical polymerisation of the latter is initiated by the addition of potassium persulfate. This reaction takes place under a nitrogen stream and the temperature is usually raised to 60 - 70 ºC. The polymerisation reaction lasts for approximately 6 h, after which the formed suspension of nanoparticles is allowed to settle overnight. Finally, unreacted monomers are removed by dialysis or subsequent washes of the formed particles with distilled water. Parameters such as chitosan/acrylic monomers ratio and polymers concentration have been found to strongly affect the physicochemical characteristics of the nanoparticles properties.[88] [Figure 10].

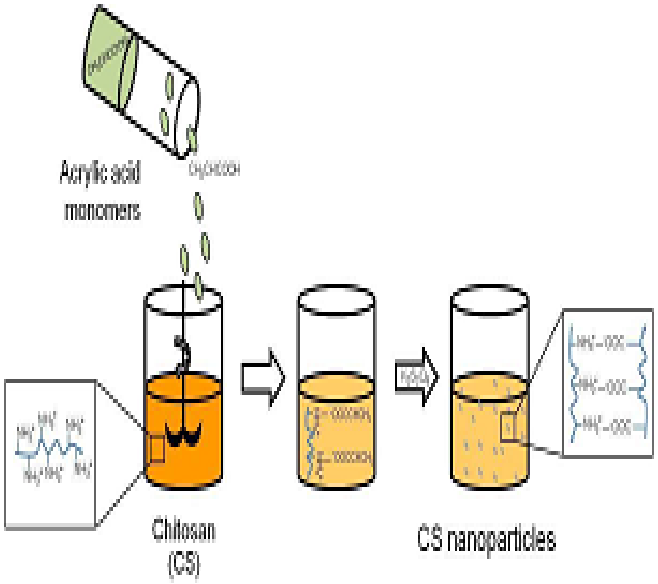


Figure 10 Modified ionic gelation with radical polymerization

Desolvation

The method of desolvation is also frequently referred to as simple coacervation or phase separation and involves a macromolecular aggregation brought about by partial desolvation of fully solvated molecules. The use of desolvating agents to produce chitosan particles was reported for the first time for the preparation of micron-sized carriers. Substances such as sodium sulfate acetone used as precipitating agents. The preparation of chitosan nanoparticles by this method is very simple and mild as it involves the dropwise addition of the solvent competing agent of greater hydrophilicity (e.g. sodium sulfate) into a previously formed chitosan solution. As the salt enters in contact with the aqueous environment of chitosan solution, a progressive elimination of salvation water surrounding chitosan occurs as a consequence of the higher affinity of water for the salt. Factors such as chitosan molecular weight, chitosan concentration, amount of desolvating agent and stirring rate have been found to strongly affect the final characteristics of nanoparticles.[89] [Figure 11]

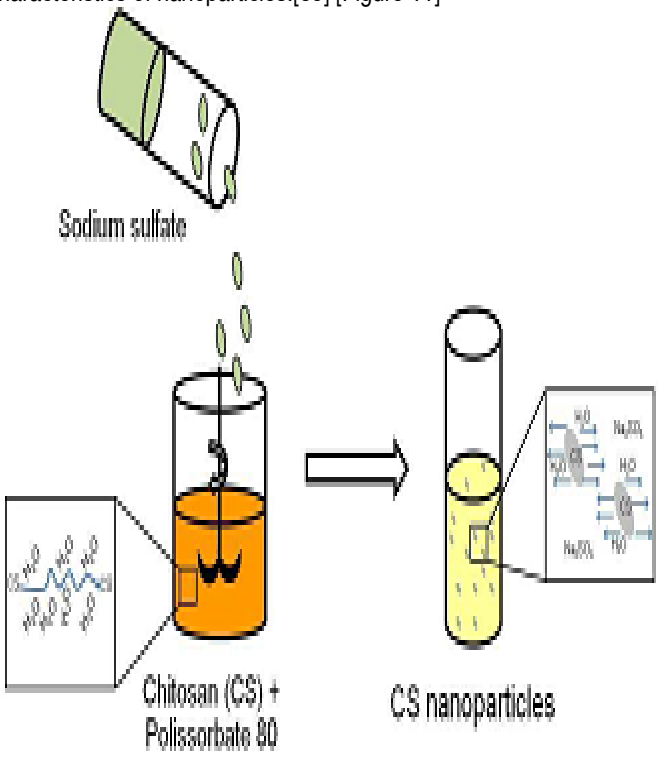


Figure 11 Desolvation Technique.

Table 1:Characterization Of Chitosan Nanoparticles [90-92]

Parameter	Instrument	Method/Specifications
Compatibility studies	Fourier Transform Infrared (FTIR) Spectra Studies	Specified quantity of potassium bromide and samples was blended, compressed to the pellet and analyzed from 400 to 4000 cm^{-1}
Thermal analysis	Differential scanning calorimetry (DSC)	Temperature of glass transition may be determined. Freeze-dried nanoparticles along with lyoprotectant (to prevent shrinkage) were analyzed.
Determination of particle size	Scanning Electron Microscopy (SEM) (Ex: JEOL JSM T330A)	Dried film of nanoparticles coated on 25nm thick Gold coated slab
	Transmission electron microscopy (TEM) (Ex: JEM-100 CX, Japan)	A drop of nanoparticle solution was placed on a copper grid with polyvinyl formal film
	Atomic force microscopy (Ex: Multimoda III, Veeco, USA)	Samples were prepared by adsorbing the polysaccharide nanoparticles on a silicon substrate modified with a PEI and PEI/PSS sublayer and mean hydrodynamic diameter of swollen polysaccharide nanoparticles was estimated by light scattering
	Quasi-elastic light scattering (QELS)	Air-cooled argon ion laser operated at 488 nm as Light source using 128-channel digital correlator. The time dependence of the intensity autocorrelation function of the scattered intensity was derived which gave the self-diffusion of the particles.
Zeta potential	Zeta sizer (Ex: Brookhaven's Zeta Plus apparatu (Brookhaven Instruments Corporation)	Based on electrophoretic mobility of drug loaded nanoparticles
Surface analysis	X-ray diffraction (XRD)	Diffraction patterns of samples were obtained at room temperature with powder diffractometer using a Cu- K radiation, a current of 30 mA and voltage of 45KV
	X-ray diffraction Spectroscopy (XPS)and Time of flight-Secondary Ion Mass Spectroscopy (TOF - SIMS)[68]	To determine their chemical composition by placing the sample on polished monocrystalline silicon wafer (Sample holder) XPS→Al-K radiation was passed, photoelectrons are collected at an angle of 90^0 relative to sample surface (as monolithic spots of 400 μm diameter, 0.1256 mm^2 area) and analyzed by constant analyzer energy (CAE). TOF-SIMS→ Samples were bombarded with a pulsed bismuth ion beam (Bi^{3+}) generated with a liquid metal ion gun (25 keV) and at 45º incidence. Secondary ions are generated are extracted with 10kV and TOF was measured in reflectron mass spectrometer.
Investigation of pH Sensitivity of Chitosan Nanoparticles	pH meter, Zeta Sizer	Nanoparticles were incubated for 1 and 3 h in phosphate buffer solutions with different pH values of 3, 4, 5, 6, and 7.4 (maintained by HCl (37%) and NaOH (50 (w/v)%) and particle size distribution was measured

Evaluation Of Chitosan Nanoparticles

Percentage yield of Nanoparticles

Thoroughly dried nanoparticles were collected and weighed accurately. The percentage yield was then calculated using formulae given below:[93]

$$Percentage\ yield = \frac{Mass\ of\ nanoparticles\ obtained}{Total\ weight\ of\ drug\ and\ polymer} X\ 100$$

Estimation of Amount of Drug Incorporated to Chitosan Nanoparticles

Accurately weighed known quantity of drug loaded nanoparticles from each batch is taken and dispersed in 100 ml of normal saline and sonicated for half an hour. The solution is centrifuged at 10000 rpm for 15 min, and the absorbance is determined using UV spectrophotometer with plain chitosan nanoparticles as reagent blank. [94]

Study on *In-vitro* Drug Release

The known quantity of nanoparticles is taken in a 250 ml conical flask and to it 100 ml of suitable buffer is added. Then the flask is kept in a shaker cum incubator and shaker is adjusted to 40-50 horizontal stokes / min at 37ºC. From this, 2 ml is withdrawn at various time intervals while replacing it with fresh normal saline of 2 ml. The samples are centrifuged and filtered. From the filtrate 1 ml of the sample is withdrawn and diluted to 10 ml with normal saline and the drug content is analyzed by UV Spectrophotometer.

Determination of Kinetics of Drug Release

In-vitro release data obtained is fit into a suitable model in order to predict and correlate the release behaviour of the drug from the polymer matrix. Hence the dissolution data is fitted according to the well-known exponential equation, which is often used to describe the drug release behaviour from polymeric system. The equation, which is used to describe drug release mechanism is:

$$\frac{M_t}{M_\infty} = kt^n$$

Where m_t/m is the fraction release of the drug at time't', 'k' is the constant which indicates the properties of the macromolecular polymer system, and 'n' is the release exponent which indicates of the mechanism of release. The 'n' value is used for the analysis of drug release mechanism from the drug-loaded nanoparticles.[95]

In-vivo biodistribution Studies

In-vivo bio distribution studies are carried out using suitable animal models and divided into groups. On the first day, group I is treated with free drug with appropriate dose intravenous route. The similar concentrations of drug loaded nanoparticles are administered to group II and suitable buffer as solvent control for group III. After 18 h of injection, animals are sacrificed, then blood was taken and plasma is separated out, and also different organs like liver, lung, kidney and spleen are extracted out and homogenized in suitable buffer saline followed by centrifugation. Supernatant of the homogenized tissue are analyzed by using HPLC to estimate the bio distribution of the drug administered.[91]

Stability study of nanoparticles

The formulation of nanoparticles were separated into three portions one portion was kept at room temperature, second at 45°C, and third at 4°C for one month. At weakly intervals samples were determined spectro-photometrically using phosphate buffer saline pH 7.4 and drug content were estimated.

Blood compatibility

Whole blood was collected from healthy men of age 22–31 years old, added with EDTA, and mixed thoroughly. On centrifuging whole blood at 1000 rpm for 20 min, red blood cells (RBCs) were collected. The RBCs were washed thrice with a saline solution before being diluted with a buffer to prepare erythrocyte stock solutions with fixed concentrations of hemoglobin (3:1 centrifuged erythrocytes: buffer saline solution). Prior to sonication, freeze dried nanoparticles were redispersed in a saline solution to give 0.2 % suspensions. 100 µL of suspension with different concentrations were added to 1 ml of an erythrocyte stock solution. The mixtures were incubated for 1 h at 37ºC in water bath. After the centrifugation at 4000 rpm for 10 min, an aliquot of the supernatant was analyzed spectrophotometrically, by dissolving in 2 ml of saline. The saline solution alone was used as a negative control (0 % lysis) and the distilled water as a positive control (100 % lysis). The hemoglobin released was determined spectrophotometrically (UV 1650 PC Shimadzu) at 540 nm. Percent hemolysis was calculated using the formula

$$\%\ Hemolysis = \frac{Absorbance\ of\ sample}{Absorbance\ of\ the\ positive\ control}\ X\ 100$$

Applications

Parenteral administration

Particles greater than 100 nm in diameter are rapidly taken up by the reticulo endothelial system (RES) in the liver, spleen, lung and bone marrow, while smaller-sized particles tend to have a prolonged circulation time. Nanosized particles can be administered intravenously because of small diameter blood capillary of approximately 4 µm. The biodistribution of nanoparticles can vary depending on the surface charge, size, and hydrophobicity of the administered particles.[96] Negatively-charged particles are eliminated faster than positively-charged or neutral particles.[97] The creation of a hydrophilic coating such as polyethylene glycol (PEG) or a nonionic surfactant on hydrophobic carriers significantly prevent their uptake by opsonins and also improves their circulation time. Hydrophilic coating along with neutral surface charge is a viable approach to decrease macrophage phagocytosis and thereby improve the therapeutic efficacy of formulation. The most promising drugs that have been extensively used for delivery through this route are anticancer agents such as 5-Flurouracil, cytarabine, doxorubicin, paclitaxel etc. Following intravenous administration, many nanoparticles including chitosan nanoparticles (NP) accumulated in a number of

tumors. The main possible reason for the phenomenon may involve the leakiness of tumor vasculature.[75] Nanoparticles for targeting an anti-Alzheimer's drug tacrine to the brain. A higher concentration of drug tacrine was observed in liver, spleen and lungs with the nanoparticles after 1 h of post injection the rats (decapitation method).[98]

Peroral administration

This route is suitable for the administration of molecules which are acid labile and sensitive to enzyme degradation such as macromolecules, proteins, polynucleotide's. This approach was extensively studied after a report that blood glucose levels were reduced in diabetic rats following the oral administration of insulin nanoparticles.[99] Limiting nanosized 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 endocytosis mechanism.[100] 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.[101] Therefore, drug bioavailability can be improved by controlling the particle size along with prolonging the residence time of drug carrier systems in GIT.[102] Among polymeric nanoparticles, chitosan NP showed to be attractive carriers for oral delivery vehicle as they promote absorption of drug.

Non-viral gene delivery vectors

In order to overcome the disadvantages associated with viral vectors like pathogenicity, host immune responses, induction of neoplastic growth following insertional mutagenesis, non-viral gene transfer systems were explored.[103] There are usually considered to be primary barriers for successful gene delivery because of cell entry, endosome escape, intracellular trafficking, and nuclear entry *in vivo* stability. Cationic polymers like chitosan and lipids both have shown promise as gene delivery agents since they produce particles of polyatomic nature that reduce one or more of these barriers for absorption. 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 through barriers.[104] In many cases, cationic polymers offer more stable complexes and favoring more protection during cellular trafficking than cationic lipids.[105] Nanoparticles for gene delivery efficacy of DNA polyplexes composed of chitosan and Factor VIII DNA. Transgene DNA was detected in both local and systemic tissues following oral administration of the chitosan nanoparticles to hemophilia A mice. [106]

Delivery of vaccines

Nanoparticles may be readily taken up by antigen presenting cells and exhibit significant adjuvant effects in parenteral vaccine delivery.[107] Chitosan nanoparticles were widely apt for the modern vaccinology facilitated by the oral and nasal delivery of nanoparticles with mucosal protective immune responses. The submicron size of nanoparticles promotes their uptake up by M-cells, in mucosa associated lymphoid tissue (MALT) i.e. gut-associated, nasal-associated and bronchus-associated lymphoid tissue and other targeting sites of vigorous immunological responses.[108]. Chitosan-based DNA flu vaccine for intranasal administration was formulated.[109]

Ocular administration

The administration of nanoparticles through this route provides prolonged residence time by adhering to ocular epithelial tissue compared to conventional ophthalmologic formulations, thereby improving drug bioavailability. This suggests that the nanoparticles are potential carriers for ocular delivery.[110] As a consequence, nanoparticles have been developed for targeted ophthalmic delivery of antiallergic, anti-inflammatory and beta-blocker drugs.[86] Mucoadhesive chitosan sodium alginate nanoparticles as submicroscopic reservoirs for prolong ophthalmic delivery of gatifloxacin.[111]

Dental administration

Chitosan was widely used as an effective medicament in various fields of medicine and dentistry. Chitosan could be used as an anti-inflammatory root canal dressing material for periapical lesions in endodontics. Chitosan stimulates the fibroblastic cells to release chemotactic inflammatory cytokines, especially interleukin 8 (IL-8).[112] Histological findings indicate that chitosan induces the migration of polymorphonuclear leukocytes and macrophages in the applied tissue at the early stage of dental disease.[113] The chitosan nanoparticles administered orally had the ability to hinder the acid tolerance response (ATR) of adhered Streptococcus mutans.[114] Fluoride chitosan nanoparticles can be used for remineralization and prevention of dental caries.[115]

Colon drug delivery

Chitosan shows degradation in the colon and hence it was used in colon drug delivery. Hyaluronic acid-coupled chitosan nanoparticles bearing 5-fluorouracil (5-FU) were prepared by an ionotropic gelation method for the effective delivery of the drug to the colon tumors.[116] These nanoparticles showed enhanced cellular uptake by HT-29 colon cancer cells compared to the uncoupled nanoparticles.[117] Nanoparticles formulated with pH-sensitive properties and specific biodegradability for colon-targeted delivery of satranidazole.[118]

Liver delivery

Chitosan nanoparticles were used for liver delivery by employing passive trapping of nanoparticles by reticuloendothelium or active targeting based on recognition between hepatic receptor and ligand bearing particulates.[119] Lactosaminated N-succinyl-chitosan (Lac-Suc), synthesized by reductive amination of N-succinyl-chitosan and lactose using sodium cyanoborohydride, as

a liver specific drug carrier.[120, 121] In the other study, glycyrrhizin (GL) was conjugated to the surface of chitosan nanoparticles by ionic gelation process. These nanoparticles were developed for a drug delivery system targeting the liver through a specific interaction between GL and hepatocytes.

Kidney delivery

Chitosan drug conjugates have been used for drug targeting to the kidney in the form of drug-carrier conjugates. However, drug conjugates often suffer from renal toxicity, cardiovascular side effects, and poor biocompatibility.[122, 123] Therefore, researchers have devoted their efforts in developing highly safe carrier systems for the drugs. Randomly 50% N-acetylated low molecular weight chitosan (LMWC) selectively accumulated in the kidneys, especially in the renal tubes after intravenous injection into mice.[98] In an attempt to develop drug delivery system for renal targeting, the authors conjugated prednisolone to LMWC (19 kDa) through a succinic acid spacer. The distribution of the conjugates in the kidney was found to be 13 fold higher than that of prednisolone alone.

Lung delivery

Chitosan-modified poly (lactic-coglycolic acid) nanoparticles containing paclitaxel (C-NPs paclitaxel) was prepared by a solvent evaporation method. The study demonstrated that the in vitro uptake of the nanoparticles by a lung cancer cell line (A549) was significantly increased by chitosan modification. Under acidic tumor conditions, C-NPs became more positive and interacted strongly with the negatively charged tumor cells. The enhanced interaction between C-NPs and tumor cells at the acidic microenvironment might be the underlying mechanism of lung tumor-specific accumulation of paclitaxel from C-NPs-paclitaxel. Formulation of chitosan and glycol chitosan nanoparticles containing the surfactant Lipoid S100 for the systemic delivery of low molecular weight heparin for pulmonary administration.[124]

Cancer-targeted drug delivery

The conventional cancer chemotherapeutics includes high toxicity of most anticancer drugs, following systemic administration due to indiscriminate distribution of drugs towards disease and healthy cells. In addition, anticancer drugs often suffer from poor solubility in water and thus need to use organic solvents or detergents for clinical applications, resulting in undesirable side effects such as venous irritation and respiratory distress. Therefore, designing a distinct carrier system that encapsulates a large quantity of drugs and specifically targets tumor cells is indispensable for successful cancer therapy.[125] Physical targeting using Chitosan-based stimuli-sensitive formulations and chitosan based magnetic nanoparticles are also developed. Formulation of Doxorubicin–dextran conjugate into long circulating CS nanoparticles to minimize cardiotomacromolecular drugs in tumor tissues. The specific passive accumulation of macromolecules occurs near the defective tumor vasculature with disorganized endothelium at the tumor site and a poor lymphatic drainage system. Since then, researchers have capitalized this concept for the delivery of various drugs by conjugating them with polymers or encapsulating within nanoparticles. Nowadays, it is evident that long circulating macromolecules (polymer–drug conjugates) and nanosized particulates (such as micelles and liposomes) accumulate passively at the tumors due to the EPR effect.[127]

Active targeting — Receptor-mediated endocytosis (RME)

The accumulation of drugs in tumor tissue does not always lead to the successful therapy if the drug does not reach the target site of the tumor cell such as the cell membrane, cytosol, or nucleus. Therefore, a more effective method should be discovered such that the therapeutic agents are able to reach their molecular targets. Cancer cells often over-express some specific antigens or receptors on their surfaces, which can be utilized as targets in modern nanomedicine. Active targeting can be achieved by chemical modification of nanosized drug carriers with targeting components that precisely recognize and specifically interact with receptors on the targeted tissue. Direct conjugation of the targeting moiety to drugs failed to demonstrate their improved activity on cancer treatment. This was due to decrease in the biological activity of the drugs, compromised by conjugation of the targeting moiety. In addition, conjugation badly affected the targeting molecule by disrupting receptor/ligand recognition. To avoid this disadvantage, researchers developed an efficient drug delivery system comprised of (a) active chemotherapeutic drug, (b) targeting moiety, and (c) a nanosized carrier made up of polymers or lipids. In this system, the therapeutic agents are physically entrapped in the carrier.[128]

Table 2: List of some reported Chitosan nanoparticles

S.N	NAME OF DRUG	METHOD	REFERENCES
1	Atorvastatin calcium	Solvent evaporation method	[129]
2	Amphoteracin B	Ionic gelation method	[130]
	Amoxycillin		[131]
	5-Fluorouracil		[132]
	Isoniazid		[92]
	Dexamethasone Sodium Phosphat		[133]
	Arbutin		[134]
	Ampicillin trihydrate		[135]
	Acyclovir		[136]
	Gatifloxacin		[111]
	Cytarabine		[97]
	Mercaptopurine		[137]
	Docetaxel		[138]
	Carboplatin		[139]
	Tamoxifen		[140]
	Tenofovir		[141]
	Thymoquinone		[142]
3	Carboplatin-Fe@C	Reverse micro emulsion method	[143]
4	Paclitaxel	Emulsification solvent Evaporation	[144]
	Bovine serum albumin		[145]
	Letrozole		[36]
5	5-fluorouracil	Emulsion Droplet Coalescence Method	[146]
	Stavudine		[147]
	5-fluorouracil		[148]
6	Salmon calcitonin	Spray drying	[149]
	Cetirizine dihydrochloride		[150]
	Lomustine		[151]
	Tramadol hcl		[152]

Table 3: List of various patents of chitosan nanoparticles

S.no	Inventor name	Publication number and date	Brief result	Applications	Reference
1	Marta Domingo et al.	EP1243688 A1 Sep 25, 2002	Preparation of Nanochitosan of various forms like fibers, yarns, knitted fabrics and fabrics.	Possess the soothing and anti-inflammatory action	[153]
2	Allan Emerson David et al.	WO2005117844 A2 Dec 15, 2005	Method development for encapsulation of Chitosan polymer in a surface modified network of colloid formi materials like silica to form mucoadhesive nano composites.	For the treatment of stomach ulcers and also serves as antibacterial.	[154]
3	Julio Fernandes et al.	US20060105049 A1 May 18, 2006	Preparation of folic acid and chitosan Chitosan nanoparticles to deliver therapeutic agent of Interest	For the treatment of non-viral novel drug delivery with improved transfection efficiency	[155]
4	Fernandez M. et al.	CA2602031 A1 Sep 21, 2006	Preparation of chemically modified Chitosan nanoparticles with polyethyleneglycol and loaded with bioactive agents.	For the treatment of diseases and also serves for cosmetic purposes.	[156]
5	Makiko Aimi et al.	WO2007086613 A1 Aug 2, 2007	Development of safe nanoparticles made from highly biocompatible materials without the use of a surfactant or synthetic polymer and followed by cross linking.	Imparts safety	[157]
6	Sun Heang Heo et al.	WO2008060096 A1 May 22, 2008	Preparation of folic acid conjugated water soluble Chitosan nanoparticles	Served as Gene carriers and shows high gene expression.	[158]
7	Peter Kauper and Carsten Laue	US20080254078 A1 Oct 16, 2008	Development of new binary system of hydrophilic nanoparticles and microparticles, using chitosan and polyanionic polysaccharides	Used as carrier systems for drugs, pro drugs, proteins and peptides, enzymes, vitamir and fragrances.	[159]
8	Alekha K. Dash, William J. Trickler	WO2008105852 A3 Nov 6, 2008	Development of Chitosan nanoparticles composed of glyceryl monooleate loaded with bioactive agents.	For the treatment of breast, pancreatic, colon, prostate, and other cancers	[160]
9	Hsing-Wen Sung and Hosheng Tu.	WO2006073950 B1 Dec 11, 2008	Introduced the method of preparation of insulin loaded chitosan- poly-□-glutamic acid nanoparticles.	To enhance the permeability for paracellular drug delivery of bioactive agents.	[161]
10	Morten andreasen et al.	WO2009010071 A Jan 22, 2009	Prepatation of bio-active Osteopontin/siRNA-chitosan nanoparticles	To inhibit bacterial growth and for treatment of bacterial infections.	[162]
11	Jackie Y. Ying et al.	WO2008136773 A8 Jan 29, 2009	Development of derivatives of chitosan oligomeric or polymeric glucosamines with surfactants loaded with bio-active agents	For the treatment of molecular imaging agents, biosensing agents or drug delivery agents	[163]

12	Fernandez M. et al.	EP2026772 A1 Feb 25, 2009	Development of micro- and nanoparticulated systems composed of chitosan (or other cationic polymers) and hyaluronic acid	To improve the interaction and absorption of nanoparticles with epithelial barriers and also used for Cosmetic purposes and gene therapy.	[164]
13	Emir Baki Denkbas et al.	WO2009048958 A2 Apr 16, 2009	Formulation of siRNA incorporated chitosan particles and Methods introduced for delivering a therapeutic agent or diagnostic agent	Helps in predicting prognosis of ovarian cancer ovarian cancer	[165]
14	Peter Kauper, and Carst Laue	US20090117195 A1 May 7, 2009	Preparation of the nanoparticles constituted by two hydrophilic polyme like alginate and chitosan derivative	Improves the physicochemical properties of the micro- or nanoparticles.	[166]
15	Flemming Besenbacher et al.	WO2009012786 A3 Jun 18, 2009	Development of chitosan-siRNA nanoparticles targeting a mRNA encoding a pro-inflammatory cytokines.	For the treatment of inflammatory diseases	[167]
16	Emir Baki Denkbas et al.	WO2009048958 A3 Jul 16, 2009	Development of Chitosan nanoparticles loaded with Zeste homologue (EZH2) for ovarian cancer.	For Predicting the prognosis of diseases like ovari cancer	[168]
17	Youngnam Cho et al.	WO2009091992 A1 Jul 23, 2009	Development of non toxic chitosan or silica polymer surfaced microcolloid nanoparticles	To target the damaged nerve tissues	[169]
18	Yue Chen et al.	US20090252803 A1 Oct 8, 2009	Development of lung targeted drug delivery system where carrier is modified with glycyrrhetinic acid for loading bio-active agents	For the treatment of abdominal pain over the liver area, jaundice and itch.	[170]
19	Arun Kumar et al.	WO2009105278 A3 Oct 15, 2009	Pre-loading of Sertoli cells with chitosan nanoparticles coupled with or without the drug curcumin and pre-labele with a fluorescent cell marker	For the treatment of lung diseases.	[171]
20	Silke Krol and Gallardo Julian Lopez-Viota	EP2123262 A1 Nov 25, 2009	Development of gold-creatine Chitosan Nanoparticles	For the treatment of stroke and serves as neuro protective	[172]
21	Julio C. Fernandes and Françoise Winnik.	US20090324726 A1 Dec 31, 200	Development of nanoparticles comprising of Folate-PEG-chitosan conjugate and plasmid	For the treatment of arthritis using gene therapy	[173]
22	Cheng-Hsien Chen et al.	US20100086613 A1 Apr 8, 2010	Preparation of Chitosan-DNA or Chitosan-protein complexes	Used for the diagnostic, therapeutic and biological industrial purposes.	[174]
23	Peter Kaeuper, Carstnen Laue	US20100092572 A1 Apr 15, 2010	Preparation of chitosan nanoparticles comprising of ribonucleic acid	For the treatment of genetic diseases.	[175]
24	Santosh Kumar et al.	WO2010013224 A4 May 14, 201	Introduced the methods of producing Chitosan nanoparticles loaded w pure Curcumin to increase the bioavailability of the same.	For the treatment of cancers, inflammatory disease alzeihmer's disease, cholesterol gall stone, and diabetes	[176]

25	Magdolna Bodnar and Janos Borbely	US7740883 B2 Jun 22, 2010	Methods introduced for preparing Chemically modified chitosan derivatives with one or more mono-, di-, tri- and polycarboxylic acids t get nano-sized derivatives	Chitosan derivatives used as detergents, additives for pharmaceutical composition and for drug delivery, and DNA carrie system	177]
26	Moon-Hee Sung et al.	WO2010151076 A3 May 5, 2011	Development of modified chitosan nanoparticles using poly gamma glutamate helped in making of vaccine and Adjuvar composition	Increases antibody production, and made resistant virus and bacterial infections	[178]
27	Swadeshmukul Santra	US20110158901 A1, Jun 30, 201	Development of Chitosan based nanoparticles incorporating diagnostic agents and target-specific ligand.	Used for biological imaging.	[179]
28	Sukhdeep Dhadwar et al.	WO2012040832 A1 Apr 5, 2012	Development of Chitosan nanoparticles comprising a nucleic acid that encodes a therapeutic protein	For the treatment of genetic diseases.	[180]
29	Kiran Sonaje et al.	US8153153 B1 Apr 10, 2012	Development of Chitosan- poly-glutamic acid nanoparticles with at leas one bioactive agent	To enhance absorption and permeability.	[181]
30	Moon-Hee Sung	US20120164174 A1 Jun 28, 2012	Development of poly-gamma-glutamic acid chitosan nanoparticles comprising of vaccine composition	Used in the treatment of viral and bacterial infections and in cancers to increase the productio of antibodies.	[182]
31	Ke-Ming Liang et al.	US8211475 B2 Jul 3, 2012	Preparation of chitosan nanoparticles in water Phase	To lower the dosage of drugs thereby reduces the side effects caused by high dosage can be reduced	[183]
32	Noah Ben- Shalom et al.	US20120189704 A1 Jul 26, 2012	Preparation of Chitosan gels in different concentration s of acids at physiological pH and temperature.	It Provides the slow-release of drugs and regenerative medicine.	[184]
33	Hsing-Wen Sung et al.	US8287905 B1 Oct 16, 2012	Introduced the method of preparation of insulin loaded nanoparticles c chitosan and γ-PGA.	For the treatment of hypoglycemia.	[185]
34	Swadeshmukul Santra et al.	US20120269729 A1 Oct 25, 2012	Development of chitosan-based nanoparticles stabilized with a hydrophilic dispersing agent, such as polyglutamic acid (PGA).	Used for imaging and therapeutic drug/gene delive	[186]
35	Rebecca A. Bader and Nan Zhang.	US20120294904 A1 Nov 22, 201	Development of poly(sialic acid) (PSA)-based N,N,N-trimethylchitosan (TMC) nanoparticles loaded with Methotrexate	For treating of systemic diseases and neurologica disorders	[187]
36	Nora Dr. Laryea et al.	EP1992230 B1 Jul 3, 2013	Silylation of the natural substance like Chitosan with the organosilanes to form nanoparticles	Used for coating purpose, aircraft or watercraft, carpets, textile under shields and boot liners.	[188]
37	Cliff Wong et al.	US20130224282 A1 Aug 29, 2013	Development of Multistage nanoparticles using various polymers varyi their size based on the physiological barriers.	For the treatment and/or diagnosis of disease, e.g. cell proliferative diseases such as cancer.	[189]

Conclusion

The review has concluded that the chitosan is the versatile biopolymer and has great utility in controlled release and targeting of the drug molecules. Chitosan nanoparticles serve as the promising drug delivery system because it is a natural polymer, with biocompatible, biodegradable and bioadhesive properties. Existence of Chitosan in various molecular weights and its chemical derivatives promotes the flexibility in the formulation development. Chitosan nanoparticles prepared by simple methods as chitosan are soluble in water. Nanosize range improves absorption thereby bioavailability. These may avoid degradation of drugs and related side effects. Chitosan nanoparticles offer versatile routes of administration, especially non-invasive routes i.e. per oral, nasal, and ocular mucosa, which are preferable routes of administration. These nanoparticles serves as a promising drug delivery and are suitable for the wide range of drugs like macromolecules, bio-molecules like proteins, thermolabile and hydrophobic drugs

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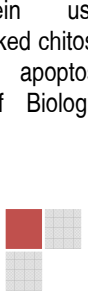

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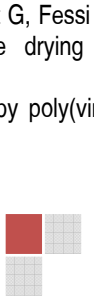

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