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A REVIEW ON ROLE OF CHITOSAN NANOPARTICLES IN ANTIBACTERIAL DRUG DELIVERY

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ABSTRACT

Nanoparticles provide a broad range of applications due to their site-specific action and sustained release of drugs. Several drugs loaded nanoparticles proved the local and systemic action by site-specific delivery of drugs. Chitosan nanoparticles act as potential drug carriers due to their biocompatible, biodegradable and non-toxic properties. Different techniques like ionic gelation prepare chitosan nanoparticles, coacervation, spray drying, emulsion crosslinking, reverse micellar, sieving methods. Sodium tripolyphosphate is used as a chemical crosslinking agent in the preparation. Drug loading in chitosan nanoparticles is carried out by incorporation or incubation. Release of drug from chitosan nanoparticles is governed by diffusion through the swollen matrix, release from the surface of particles, and release due to polymer erosion. Particle size, morphology, and surface properties are evaluated by transmission electron microscopic and scanning electron microscopic studies. Chitosan nanoparticles has a potential application in different drug delivery systems owing to its sustained release properties making them essential in recent nanoparticle studies. Chitosan nanoparticles are used as a delivery system for most of the antibacterial drugs such as Vancomycin, Cephalexin, Levofloxacin, Ampicillin, Ciprofloxacin and so on for their high efficacy and sustained release. The literature study has confirmed the increased efficacy of antibacterial drugs in the combination of chitosan nanoparticles.

Keywords: Polymer Nanoparticles; Chitosan; Sodium Tripolyphosphate; Ionic gelation Method; Crosslinking; Antibacterial

INTRODUCTION

Nanotechnology is a well-known field having numerous applications in medical therapeutics, agriculture, forensic science, and textiles **[1]**. Nanotechnology witnesses the bridge between the physical and biological sciences by implementing nanostructures at varied branch of sciences, notably in drug delivery systems based on nanoparticles **[2]**. Nanoparticles are solid colloidal particles showing different shapes, sizes, and structures. The shape of nanoparticles can be spherical, conical, spiral cylindrical, hollow, flat, tubular, or irregular and range from 1 – 1000 nanometers. Nanoparticles exhibit distinctive biological, physical, and chemical properties at their nanoscales compared to their higher scales **[3]**. Nanoparticles are designed to control surface properties, particle size, and pharmacologically active agents release to achieve site-specific action of drug at therapeutically active range. Nanoparticles have an essential role in oral, systemic, transdermal, pulmonary, and other routes for their site-specific action, enhanced bioavailability, and drug stability by altering the molecular weight, polymer ratio, particle size, and fabricating conditions a lot of drug-releasing profiles can be attained. Factors like the type of polymer, molecular weight, composition, organic solvent property, concentration and nature of emulsifier, mechanical strength of stirring, temperature, and pH determine the properties of the resulting pharmaceutical nanoparticle **[4]**.

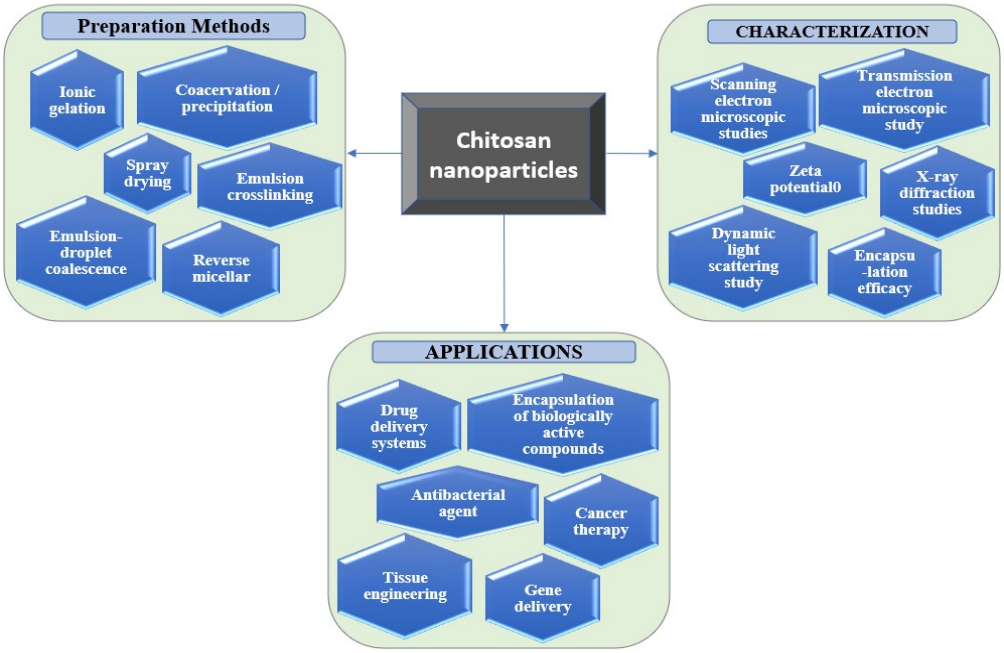
Nanoparticles are classified as organic and inorganic nanoparticles. In other means, they are classified as ceramic, metal, semiconductor, carbon-based, and polymeric nanoparticles. Standard organic nanoparticles are liposomes, dendrimers, ferritin, and micelles, and they are non-toxic and biodegradable. Micelles, liposomes have a hollow-core known as nano-capsules and are susceptible to heat and light. The organic nanoparticles are employed for targeted drug delivery in the biomedical field. Inorganic nanoparticles are categorized as metal and metal oxide nanoparticles **[5]**. Fullerenes and carbon nanotubes are carbon-based nanoparticles. Fullerenes are prepared by the globular hollow-cage like carbon allotropic forms. Carbon nanotubes are elongated, tubular structures. The chemical vapor deposition technique synthesizes them. Metal nanoparticles are prepared by metal precursors and possess unique optoelectrical properties. Ceramic nanoparticles are inorganic, nonmetallic solids and produced by means of heat and successive cooling. They are found in

dense, amorphous, porous, polycrystalline, or hollow forms. Semiconductor nanoparticles possess properties between metals and nanometals. Polymeric nanoparticles are polymer-based nanoparticles that are commonly nanospheres or nano capsular shaped **[6]**.

Nanoparticles are suitable for a wide selection of route of administration of drugs. They have advantages like increased shelf-stability, sustain and control drug release patterns, suitable for combinational therapy, increased bioavailability, targeted drug delivery, and the development of safer medicines. Disadvantages are that high manufacturing cost leads to increased product cost, solvents used in preparation are toxic in nature. Applications in the pharmaceutical field are nanoparticles as a proteins and peptides delivery system, as tumor-targeting delivery systems, in gene therapy for delivery of genes, as dermal and transdermal delivery system, in ocular delivery systems for increased drug release, as pulmonary drug delivery for the administration of solid-lipid nanoparticles, as carriers for nasal vaccine delivery to induce both mucosal and systemic immune response, as a drug discovery in identifying and validating a target, in molecular diagnostics, as biosensors and bio-labels employed for determination of pathological proteins and a biochemical indicator associated with disease, and in cosmetics like sunscreens, hair care, moisturizers, anti-wrinkle cream, toothpaste **[7–9]**.

Various synthetic and natural origin polymeric materials are considered for preparing nanoparticles because of their studied biodegradability and biocompatibility. Chitosan is a natural biopolymer used for the formulation of nanoparticles **[10]**. Chitosan is a natural poly-cationic biopolymer and is the composition of 1 → 4-linked 2-amino-2-deoxy-β-D-glucose. Chitosan is a deacetylated chitin derivative that is obtained from the shells of marine crustaceans and the fungi cell-wall **[11]**. Chitosan is produced by processing chitin using a strong alkali solution, that converts the acetamide group of chitin into an amino group. Chitosan is available in different forms such as powder, film, paste, etc., **[12]**. Chitosan, which contains free amino acids, is insoluble in neutral or basic pH conditions and is soluble in acidic pH conditions. Chitosan is most commonly solubilized in 1-3 % acetic acid solutions. Chitosan as it is biocompatible with living tissues, it does not cause any allergic reactions because it breaks down into amino sugars and is fully absorbed by the human body **[13]**. Primary amine groups of chitosan exhibit specific properties that produce useful pharmaceutical applications.

Chitosan consists of a positive charge responsible for mucoadhesive, when compared to several other natural polymers **[14]**. Chitosan opens the tight junctions of the epithelium and acts as a penetration enhancer, and promotes paracellular and transcellular passage of drugs. Chitosan is obtainable in varied molecular weights varying from 3,800 to 20,000 Da and with various grades of the degree of deacetylation. Chitosan features mucoadhesive properties due to their electrostatic interaction, molecular weight, ability to hydrogen bond formation, chitosan chain flexibility, and ease of spreading into the mucus. Ease of chemical modification of structure advantage chitosan as superior polysaccharide polymer **[15]**. Chitosan and chitosan oligomers have significant interest in pharmacological activities like antitumor, antimicrobial, and wound-healing. Chitosan shows inhibition of gram-negative and gram-positive bacteria **[16]**.



Figures 1: Preparation methods, characterization techniques and applications of Chitosan nanoparticles

Chitosan nanoparticles that are formulated by using sodium tripolyphosphate (TPP) as a chemical cross-linking agent were used as potential nanocarriers in the pharmaceutical field. The amino groups of chitosan are crosslinked with the negatively-charged phosphate groups of TPP to form positively charged chitosan nanoparticles **[17]**. The distribution of particle size of chitosan nanoparticles depends on the stoichiometry, concentration, and mixing procedure of chitosan-TPP, degree of deacetylation, and molecular weight. Ionic strength and p^H are the other parameters that define the particle size of the chitosan nanoparticles **(Figure 1) [18]**.

The advantages of chitosan nanoparticles are less toxic, site-specific targeting, possess mucoadhesive character, enhanced biocompatibility, stability, and increased therapeutic index. The limitations over the advantages of chitosan nanoparticles are less mechanical resistance, difficulty in controlling pore size, intrinsic properties of chitosan can be affected due to preparation by crosslinking, low solubility in neutral and alkaline pH. Method of preparation has to be changed based on the delivery of drug **[19]**. Various methods used for the formulation of chitosan nanoparticles are ionic gelation method, covalent cross-linking method, precipitation method, reverse micellar method, emulsion-droplet coalescence method, and microemulsion method. Chitosan nanoparticles have numerous possible applications in oral drug delivery, parenteral drug delivery, ocular drug delivery, mucosal drug delivery, gene delivery, vaccine delivery, etc. The present review on chitosan nanoparticles addresses the method of preparation, characterization, and recent trends in antibacterial activity of chitosan-based nanoparticle drug delivery system.

PREPARATION METHODS OF CHITOSAN NANOPARTICLES

Various techniques were used for the preparation of chitosan nanoparticles. The selection of preparation methods depends on the factors such as stability of the active agent, particle size of nanoparticles, reproducibility of release kinetic profile, and stability of final product. Some methods used in the preparation of chitosan nanoparticles were detailed below,

Ionic gelation

This is the preferable method for the preparation of chitosan nanoparticles because of its simple and mild process. This process undergoes intra and intermolecular cross-linkages mediated by anionic molecules, and ionic gelation is carried out by the electrostatic interaction of an amino group of chitosan with a negatively charged group of polyanion of tripolyphosphate. By altering the ratio within the chitosan and stabilizer, the nanoparticles size and surface charge can be maintained. At first, Bodmeier *et al*. reported the formation of chitosan nanoparticles by slowly dropping the chitosan solution dropwise into a tripolyphosphate solution. Later, many researchers investigated its applications in pharmaceutical usage **[20]**.

The principle in this method is, the cation of chitosan is produced by dissolving chitosan in an aqueous acetic acid solution. This cationic solution is added dropwise to the polyanionic solution of tripolyphosphate under constant stirring. On continuous stirring, complexation takes

place between oppositely charged ions, and precipitation is produced due to ionic gelation, and spherical particles are formed. Insulin loaded chitosan nanoparticles are produced

by ionic gelation method by dissolving insulin in tripolyphosphate solution and by adding chitosan solution to this under continuous stirring **[21, 22]**.

Coacervation/precipitation

Coacervation is liquid-liquid phase separation; when two solutions carrying oppositely charged ions are combined, it results in ionic complex formation. As chitosan is an acidic soluble cationic polymer, by adding to an aqueous alkali solution, results in precipitation formation. In this method, chitosan nanoparticles are produced by blowing or dropping chitosan solution through a compressed air nozzle into an alkali solution like ethanamide or sodium hydroxide to form coacervate droplets. Filtration or centrifugation techniques are used for the separation of particles and purified by washing them with hot and cold water respectively. The particle size can be controlled by varying compressed air pressure and the spray nozzle diameter. Release of drug from particles can be controlled by using a crosslinking agent. Chitosan-DNA nanoparticles are produced by the coacervation method **[23, 24]**.

Spray drying

Spray drying is described as a prominent method that produces granules, powders, agglomerates from the drug-excipient solution or suspension. This technique provides an easy and efficient process for the preparation of protein-loaded chitosan nanoparticles. The principle involved in this method is drying atomized droplets using steam of hot air. In this technique, chitosan is dissolved in acetic acid solution, then the drug is dissolved in this solution, and a crosslinking agent is added to this solution. The resulting solution is then atomized into a stream of hot air, which results in formation of small particles converting as free-flowing particles on solvent evaporation. The particle size of nanoparticles depends upon the nozzle size, atomization pressure, spray flow rate, and extent of crosslinking. Using this technique, He *et al*. formulated chitosan nanoparticles to deliver famotidine and cimetidine **[25, 26]**.

Emulsion crosslinking

This method involves the preparation of a water-in-oil (W/O) emulsion and the addition of a crosslinking agent dropwise to this emulsion that helps in the hardening of formed droplets. The functional amino group of chitosan encounter crosslinking with the aldehyde group of glutaraldehyde. This forms the nanoparticles out of the

emulsion. The nanoparticles formed are separated, washed frequently with n-hexane followed by alcohol, and dried.

The nanoparticles size is managed by maintaining the speed of the aqueous droplets into the emulsion, controlling the stirring speed and extent of the crosslinking agent. The limitations to this method include the application of harsh crosslinking agents and a tedious preparation procedure. Glutaraldehyde used as a crosslinking agent may cause over toxicity. Jameela *et al*. prepared chitosan microspheres to deliver desirable concentrations of progesterone over prolonged periods **[27–29]**.

Emulsion-droplet coalescence method

Tokumitsu *et al*. invented the novel emulsion-droplet coalescence method, that involves both the principles of emulsion cross-linking and precipitation. The coalescence of chitosan droplets with sodium hydroxide solution induces precipitation. This method involves the preparation of two emulsions; first, chitosan aqueous solution containing drug is added to the liquid paraffin oil at constant stirring to form a stable W/O emulsion. Later, an aqueous sodium hydroxide containing chitosan is added to liquid paraffin oil to give another W/O emulsion. By mixing these two emulsions under high-speed stirring precipitates chitosan droplets of small size. The resulting product is centrifuged and filtered to obtain chitosan nanoparticles. Senthilnathan *et al*. reported that dexibuprofene nanoparticles are formulated by this method to study the treatment of pain due to arthritis by allowing the drug to release for 24 h **[30–32]**.

Reverse micellar method

Mitra *et al*. first introduced the preparation of chitosan nanoparticles by the reverse micellar method as a strategy for tumor-targeted delivery. Reverse micelles are thermodynamically stable nanometre-sized water droplets dispersed in the organic solvent because of the effect of surfactant. A reverse micellar medium is utilized for the preparation of the ultrafine polymeric nanoparticles. These nanosized particles consists of aqueous core that is used as the reactors to prepare such nanoparticles. In this method, the surfactant is added to the organic solvent to form reverse micelles to this a solution of the chitosan-containing drug is added on continuous stirring by avoiding the formation of turbidity. To this solution crosslinking agent is added on continuous stirring and left overnight on stirring for crosslinking. A transparent dry mass is obtained in evaporation of the organic solvent. The obtained material is washed with water by adding suitable salt; thus, the excess amount of surfactant

precipitates out. The drug-loaded nanoparticles are collected after centrifugation and filtration. Doxorubicin dextran conjugate encapsulated chitosan nanoparticles are produced by this method to improve therapeutic efficacy in treatment and tumors, which also reduces side effects **[33, 34]**.

Sieving method

The sieving method is a simple, novel technique for the formulation of chitosan microparticles and was found by Agnihotri *et al*. in sieving method, chitosan is dissolved in 4% acetic acid solution to produce a thick hydrogel, to this glutaraldehyde is added to form crosslinked chitosan hydrogel. This hydrogel is passed through a suitable mesh size to get microparticles. The excess amount of glutaraldehyde is removed from the resulting microparticles by washing them off with the 0.1N sodium hydroxide. The resulting nanoparticles are kept for drying in an oven at 40^{o}C. Clozapine-loaded chitosan microparticles prepared by this method have shown effective particle size and in-vitro drug release has been shown for 12 h **[35]**.

CHARACTERIZATION OF CHITOSAN NANOPARTICLES

Chitosan nanoparticles prepared are analysed for several parameters like morphological and surface characteristics, zeta potential, particle size analysis, specific surface properties, loading and encapsulation efficiency, drug content, in-vitro drug release, and characterization of the site-specific action. The techniques used for the characterization are discussed below,

Fourier transform infrared spectroscopic studies.

FT-IR studies are performed to study the drug-excipient compatibility. This technique gives information about the interaction between the chitosan chains and STPP at the molecular level.

Scanning electron microscopic (SEM) study

SEM is employed to analyse the size and surface properties of the nanoparticles. SEM employs an electron beam of high energy to scan the surface of the sample. It reports the shape and size of the nanoparticles. Jingle *et al*. stated that SEM results for the chitosan nanoparticles was found to be spherical shaped and size about 200 nm **[36]**.

Transmission electron microscopic (TEM) study

Transmission electron microscopy is implemented to study the size and surface morphology of the nanoparticles. The principle involved in TEM is that a high-energy beam of electrons is passed through the sample that irradiates the sample, and

the image is reported on a fluorescent screen and charged-coupled camera. The sample for the TEM should be a simple deposition of dilute suspension on carbon-coated copper grids. When investigated using TEM, Chitosan nanoparticles crosslinked with glutaraldehyde resulted in particle size of 90 nm with narrow size distribution **[37]**.

Dynamic light scattering (DLS) study

Dynamic light scattering technique is employed to study the particle size and size distribution of particles in the sample. The principle involved in the DLS technique is by measuring the temporal fluctuations of the scattered light due to the Brownian movement of the particles, thus particle size is calculated by Stokes-Einstein equation. The size of particles is measured by measuring the change in wavelength, polarization change, change in average intensity of the particles. DLS measures the properties of the particles in biological fluids or physiological buffers, making it a more suitable method **[38]**.

X-ray diffraction studies

X-ray diffraction study is carried out to analyse the degree of crystallinity of the nanoparticles in the sample. The physical state of the drug incorporated in the nanoparticles is also studied. It also gives information about the structure and different structural parameters such as crystallinity, crystal cracks, strain **[39]**.

Zeta potential

Zeta potential indicates the surface electric charges of the particles and are measured by Zetameter based on electrophoresis. The particles are dispersed in a medium, and an electric field is applied; thus, particles migrate towards the oppositely charged electrodes with a velocity that is equal to the zeta potential. Lasser Doppler Anemometer is used to measure the velocity of migration.

The other parameters are drug content, loading efficiency, encapsulation efficiency, and nanoparticle yield **[40]**.

1) $$\text{Encapsulation efficiency (\%)} = \frac{\text{amount of drug in a definite mass of the prepared particle (mg)}}{\text{Theoretical Amount of drug in the same mass (mg)}} \times 100$$

2) $$\text{Loading efficiency (\%)} = \frac{\text{amount of drug in a definite mass of the prepared particle (mg)}}{\text{Total mass of the particle (mg)}} \times 100$$

3) $$\text{Yield (\%)} = \frac{\text{Total weight of prepared particles (mg)}}{\text{Total weight of drugs and polymer used (mg)}} \times 100$$

DRUG LOADING INTO CHITOSAN NANOPARTICLES

There are two methods for the loading of drugs in the nanoparticles: incorporation (during nanoparticle formulation) and incubation (after particle formation). In these methods, the drug is either physically loaded into the matrix or adsorbed onto the surface. Water soluble and water-insoluble drugs can be loaded in the chitosan nanoparticles. Drugs that are water-soluble are loaded during the formation of the chitosan nanoparticles, while drugs that are water-insoluble drugs or that are soluble in acidic pH are loaded after the chitosan nanoparticles formation by soaking preformed nanoparticles in drug solution. Cisplatin-loaded chitosan nanoparticles are formulated by incorporation method for high encapsulation efficiency. Diclofenac sodium gets precipitated in acidic pH conditions, is loaded in chitosan nanoparticles by incubation method **[41]**.

DRUG RELEASE MECHANISM OF CHITOSAN NANOPARTICLES

Drug release from the polymeric nanoparticles like chitosan nanoparticles is controlled by several mechanisms like diffusion through swollen matrix, release from surface of particles, and release due to polymer erosion. The factors that govern the drug release from the chitosan nanoparticles are particle size and density, the extent of crosslinking, physiochemical properties of the drug. At specific conditions, drug release may follow beyond one type of drug release mechanism. In conditions like surface release, when the drug comes in contact with release medium, the adsorbed drug gets dissolved immediately. The entrapped drug also follows the exact mechanism results in burst release. Burst release of drugs can be prevented by increasing the density of crosslinking. Washing off nanoparticles with proper solvent can also control the burst effect, but it causes low encapsulation efficiency **[42]**.

The diffusion mechanism of drug release undergoes three steps. At first, swelling of the matrix takes place by penetration of water into particles, followed by conversion of the polymeric layer into the rubbery matrix; finally, the drug is diffused from the swollen matrix. Because of this, drug release from the particles is slow at the initial stage, and later, it becomes rapid **[43]**.

TOXICITY OF CHITOSAN NANOPARTICLES

US Food and Drug Administration has approved chitosan as a biocompatible and non-dangerous polymer for wound dressing and dietary. The degradation process of chitosan is catalysed either chemically or enzymatically. Chitosan degradation is

influenced by means of amino groups availability and degree of deacetylation. Increased charge density and degree of deacetylation increases the toxicity of chitosan **[44]**. The chitosan toxicity may increase or decrease by modification, and residual reactants should be removed carefully. Chitosan's having different molecular weights are little cytotoxic on human lymphoblastic leukaemia and human embryonic lungs cells.

This is vital to consider that drug-chitosan formulation may change the pharmacokinetics and biodistribution properties. In formulation like chitosan-plasmid DNA nanoparticles, distribution and in-vivo kinetics are determined by nanoparticle characteristics. Charge interaction may alter the cellular uptake kinetics. By balancing and reducing the positive charges on chitosan, molecules influence its interaction with cells and decrease chitosan toxicity.

APPLICATIONS OF CHITOSAN NANOPARTICLES

Chitosan nanoparticles are natural polymeric nanoparticles that possess great physiochemical, biological, and antimicrobial properties that make them friendly environmental materials and do not harm humans **[45]**. Unique properties possessed by chitosan nanoparticles find a various extent of applications.

Chitosan nanoparticles in oral drug delivery system

Because of the ease of administration, oral drug delivery is considered the appropriate route for drug administration. Several advantages like small large surface area, particle size, and potential modifiable surface area of nanoparticles make it more advantageous in oral drug delivery. When compared to other drug delivery systems like liposomes, nanoparticles increase GIT stability of acid-labile drugs. Formulation of chitosan as polymeric nanoparticles has numerous applications in oral drug delivery **[46]**. Chitosan nanoparticles are used to treat both systemic and local GIT diseases. Chitosan nanoparticles loaded with antibiotics are used to treat the local GIT diseases like ulcerative colitis, intestinal infection, and suppression of *Helicobacter pylori*. Chitosan nanoparticles loaded with amoxicillin and clarithromycin have shown a local GIT effect in eradicati*ng H. pylori* **[47]**. 5-aminosalicylic acid-loaded chitosan microspheres are developed for the treatment of ulcerative colitis. These chitosan microspheres are coated with cellulose acetate butyrate to prevent the burst of the drug in the stomach due to the presence of acidic pH **[48]**.

Chitosan nanoparticles in nasal drug delivery system

The nasal drug delivery system is a non-invasive method used to deliver drugs to the respiratory system, systemic circulation, and brain. Drug delivery is quickly possible into the systemic circulation due to the nasal cavity's well vascularized and thin mucosa. Hepatic first-pass metabolism is another advantage of this route for the systemic and local effects of the drug **[49]**. The challenging aspects in the nasal delivery system are the high secretion rate, short residence time, and the low permeability of the nasal membrane. Mucoadhesive properties and small nanoparticle size provide the retention and penetration of drug-loaded nanoparticles **[50]**. Chitosan microparticles loaded with insulin when delivered through the nasal route have shown a significant reduction in blood glucose levels in rats and rabbits **[51]**. Antigen-loaded chitosan nanoparticles, when administered intranasally, resulted in the induction of antibodies at several sites of the respiratory tract and intestine **[52]**.

Chitosan nanoparticles in pulmonary drug delivery system

As the pulmonary route provides huge absorptive surface area and better blood supply, both systemic and local treatment of drugs can be achieved. It provides rapid and sustained drug release, high efficacy, and no hepatic first-pass metabolism. Proteolytic degradation, alveolar lining fluid, bronchial mucus layer act as barriers for the drug delivery by pulmonary route **[53]**. Drug delivery through this route may be oral inhalation or intranasal. The lumen airway of this route allows the administration of particles of very small size at low concentrations. This route affects drug diffusion by the shape, particle size, and surface electric charge of administered particles **[54]**. Recent studies have shown that chitosan nanoparticle drug delivery to the lungs is possible as chitosan provides mucoadhesive properties due to a positive charge on surface. It also provides the increased drug uptake due to tight intercellular junctions of the lung epithelium can be opened by the positive charge of chitosan **[55]**. An in-vivo study of inhalation of rifampicin-loaded chitosan nanoparticle dry powder has resulted in sustained drug release for 24 hrs. with increased plasma-drug concentration and extended mean residence time of drug **[56]**.

Chitosan nanoparticles in transdermal and topical drug delivery system

The largest organ in the body, the skin offers a large surface area which can be used to administer drugs through topical and transdermal routes. It consists of different layers that act as a natural barrier for the penetration of several therapeutic agents into the systemic circulation.

Macromolecules and drugs with low molecular weight are formulated for transdermal and topical delivery systems **[57]**. Chitosan nanoparticles have been formulated for their extensive use in topical and transdermal routes. Chitosan nanoparticles loaded with retinol vitamin A derivative have formulated for their use in wrinkles and acne treatment **[58]**. Fabrication of acyclovir-loaded chitosan nanoparticles results in reduced drug photodegradation, results in improved penetration and stability of the drug **[59]**.

Chitosan nanoparticles in parenteral drug delivery system

The parenteral route involves the incision or puncture in the body for administration of drug, thus known as an invasive route of drug administration. Intravenous parenteral administration and biodistribution of prepared drug nanoparticles are based on the particle's size, surface charge, and hydrophilic/hydrophobic nature **[60]**. Nanosized particles can be administrated intravenously due to the smallest diameter of the blood capillary. Hydrophobic nanoparticles can easily penetrate and distributes into the tissues when compared to that of hydrophilic particles. Hydrophilic nanoparticles that are smaller than 100nm coated with PEG or other non-ionic type surfactants will bypass the phagocytosis. PEGlyation of nanoparticles could extend the circulation time of particles and avoid entrapment in the reticuloendothelial system **[61, 62]**. Administration of several drugs loaded in chitosan particles through the parenteral route resulted in enhanced pharmacological effects and decreased systemic toxicity. Many anticancer drugs loaded chitosan nanoparticles formulations are studied for the parenteral route of studies. Doxorubicin, when administered intravenously, resulted in suppression in tumor growth and increased survival rate in experimental animals **[63]**. Chitosan nanoparticles are commonly used to deliver antiparasitic, antiviral, antibacterial, and antifungal drugs **[64, 65]**. Paclitaxel-loaded chitosan nanoparticles, when administrated through this route, reported reduced toxicity and an increase in tolerated dose when compared to that of the commercial dosage form **[66]**.

Other applications of chitosan nanoparticles

Chitosan nanoparticles have many other applications other than those stated above. Chitosan nanoparticles have shown a better therapeutic action in the ocular delivery system because of low toxicity. Due to mucoadhesive and absorbing promoting properties of chitosan nanoparticles cause prolonged ocular residence time. Targeted delivery of antiallergic, anti-inflammatory,

beta-blocker drugs at the ocular region is achieved by chitosan nanoparticles **[67]**.

Mumper *et al*. have successfully proposed chitosan as a potential gene delivery vector because of its less toxicity when compared to that of other vectors **[68]**. Glycosylated-chitosan-graft-dextran DNA complexes were developed for the targeted liver delivery system. Chitosan is a vaccine carrier that has been studied extensively because its absorption-promoting effect can increase the mucosal immune response. Because macrophage activation has been shown after chitosan uptake, chitosan works as an adjuvant for vaccine delivery **[69]**. Vaccines containing diphtheria, influenza, and pertussis antigens were developed successfully in chitosan nanoparticle formulation for nasal delivery **[70]**.

Chitosan nanoparticles loaded drugs after coating with polysorbate 80 are used for the brain-targeted delivery **[71]**. Chitosan, because of its mucoadhesive effect, has been studied extensively in the mucoadhesive drug delivery system. Chitosan nanoparticles show increased intestinal absorption of the drug through the mucoadhesive drug delivery system **[72]**. Chitosan nanoparticles are used for controlled drug release systems because of their high affinity towards cell membrane **[73]**. Chitosan nanoparticles have been considerably used for the insulin delivery carriers. Nasal administration of insulin-loaded chitosan nanoparticles has shown increased systemic absorption **[74]**.

RECENT TRENDS OF CHITOSAN NANOPARTICLES IN ANTIBACTERIAL ACTIVITY

Chitosan exhibits a potential antibacterial activity against bacteria like *Salmonella choleraesuis, Staphylococcus aureus, Pseudomonas aeruginosa*. Chitosan nanoparticles also showed antifungal activity against *Aspergillus niger, Candida albicans* **[75]**. Various studies reported that chitosan had shown an increased antibacterial effect on gram-positive bacteria compared to that of gram-negative bacteria. The mechanism of action is by electrostatic interaction of positively charged amino groups of chitosan with the negatively charged bacterial cell wall; resulting in cell disruption, thus altering membrane permeability and causing cell death **[76]**. Several drugs loaded chitosan nanoparticles are examined for their antibacterial activity has been discussed below.

Hafizi *et al*. 2021, have prepared vancomycin-loaded chitosan nanoparticles by ionotropic-gelation method using TPP as a cross-linking agent. The study reported sustained drug release of vancomycin when loaded in chitosan nanoparticles and no

interaction between the drug and chitosan. 40% of the drug is released in 9 hours.

Elassal *et al*. 2019, have prepared cephalexin-loaded chitosan nanoparticles by ionic gelation method to study the improvement of antibacterial activity of cephalexin. The antibacterial effect was studied on seven types of bacteria. The study reported the better antibacterial property of cephalexin-loaded nanoparticles with good stability, sustained release, and reduced activity **[77]**.

López-López *et al*. 2019, have prepared levofloxacin-loaded chitosan nanoparticles using TPP as a chemical cross-linking agent by ionic-gelation method for the parenteral route of administration. The study reported that nanocarriers help in the sustained release of drugs and reduce side effects **[78]**.

Porras-Gómez *et al*. 2018, used a modified ionic gelation method to formulate ampicillin-loaded chitosan nanoparticles using sodium tripolyphosphate as a chemical cross-linker to study their antibacterial activity on *Escherichia coli*. In-vitro studies of this formulation shown an initial burst followed by sustained release of drug and increased surface charge, results in increased growth inhibition rate of *E. coli* **[79]**.

Shabana *et al*. 2017, have formulated ciprofloxacin-loaded chitosan nanoparticles by ionotropic gelation method using tripolyphosphate as a chemical cross-linking agent against *E. coli* and *S. aureus*. The results show that these formulations inhibit the growth of two strains of gram-negative and gram-positive microorganisms. The minimum inhibitory concentration of chitosan nanoparticle formulation is 50% less than that of the drug itself **[80]**.

CONCLUSION

Nanoparticles in drug delivery enhance site-specific action with fewer side side-effects when compared to that of other delivery systems. Chitosan, a natural polymer, has properties like biocompatible, biodegradation, and non-toxic and is used in nanoparticle formulations. Chitosan shows inhibition of both gram-negative and gram-positive bacteria. Chitosan nanoparticles possess improved bioavailability, mucoadhesive property, and increased therapeutic action. Because of these special properties, chitosan nanoparticles have a varied range of applications in oral drug delivery, parenteral, ocular, mucosal, gene, and vaccine delivery. Chitosan nanoparticles have become a prominent delivery system for the development of several antibacterial drugs loaded formulations.

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