Review Article

Chitosan – A Novel Biopolymer as A Potential Drug Delivery Vehicle

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ABSTRACT

Chitosan is a natural linear amino polysaccharide produced from the deacetylation of chitin obtained from crustaceans and insects. Chitosan structure consists of 2-acetamido-d-glucose and 2-amino-d-glucose units linked with glycosidic linkages. It is a versatile compound due to presence of reactive amino and hydroxyl groups making it easily available for chemical reactions. Various functional chitosan derivatives have been prepared using ionic interactions and other chemical modifications. Chitosan is known to exhibit excellent properties such as biodegradability, biocompatibility, non-toxicity and easy absorption which led to significant research towards industrial, pharmaceutical and biomedical applications. This review discusses the importance and characteristics of chitosan and its derivatives by describing various aspects including biological properties, chemical properties, techniques of preparation and its applications.

Keywords: Chitosan, biocompatible, biodegradable, pharmaceutical applications, biomedical applications.

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INTRODUCTION

With the ever-increasing demands of a rising human population, the competition for global resources is becoming even direr. On the one hand, high-performance materials are required for advancements in defence, space exploration and biomedical research. On the other hand, environmental issues related to toxicity, sustainability and cost-effectiveness need to be addressed. To overcome these increasing challenges, researchers around the world strive to produce technologies and materials that have positive impacts on the living conditions within society, which minimize environmental impacts and production costs. Polymers obtained from natural resources is one of the promising strategies employed and in recent years, the biopolymers have been considered as potential eco-friendly substitute for the use of non-biodegradable and renewable materials.

The field of nanopolymers is greatly increasing and playing an important role in a broad spectrum of areas ranging from biotechnology, electronics, conducting materials, sensors, photonics, medicine, pollution control and environmental technology.[1] They are designed from biodegradable and biocompatible polymers in size range of 10-1000nm.[2] An advantage of polymeric nanoparticles includes - Increased stability of any volatile pharmaceutical agents fabricated easily in large quantities by a multitude of methods. In terms of efficiency and effectiveness, it offers a significant improvement over traditional methods of oral and intravenous administration.[3] Also, helps in delivering a higher concentration of pharmaceutical agent to a desired location. It further reveals the choice of polymer and the ability to modify drug release from polymeric nanoparticles which have made them ideal candidates for cancer therapy, delivery of vaccines, contraceptives and delivery of targeted antibiotics. Polymeric nanoparticles can be incorporated in various activities related to drug delivery (tissue engineering).[4]

Many authors have reported about the significance and efficacy of chitosan nanoparticles. It has been intensively developed, not only for its scientific interest but also for many technological area applications: gene delivery in shrimp,[5] factorial designs,[6] adsorption of heavy metals and also adsorption of dyes etc. Chitosan is the most commonly used natural polymer in the preparation of polymeric nanoparticles.[7-9] It is a cationic polysaccharide, obtained by partial deacetylation of chitin. The amine and –OH groups endow chitosan with many special properties, making it applicable in many areas and easily available for chemical reactions.[10] Natural chitosan has captivated greater attention in pharmaceutical and biomedical fields due to its beneficial functional properties. Recently, chitosan has paid a great attention in pharmaceutical and biomedical research due to its several advantages: a) biocompatible, biodegradable with non-toxicity polysaccharide, b) easy movement across cell membrane, c) efficient drug protection till it reaches the target, d) releasing of drug in a regulated manner and e) carrier molecule degradation upon drug delivery and other numerous features are rapidly applied to delivery drugs, wound healing, antibacterial[11]and antioxidant properties, anti-inflammatory, antiulcerogenic[12] and anticancer activity.[13] The positive charge of chitosan is responsible for enhanced drug absorption by cells.[14] In addition, chitosan acts as excellent moisturizing agent due to its water retention capacity.[15] It also provides absorption promoting effect that prolongs the contact time between substrate and cell membrane.[16]

The solubility of chitosan decreases with the increase in the molecular weight. The degree of deacetylation is one of the fundamental parameters that can affect the properties and functionality of chitosan. Chitosan nanoparticles have complete power over its own physical, chemical and morphological characteristics that actually determine their applications.

TECHNIQUES OF PREPARATION

The property of nanopolymers has to be optimized based on the specific applications. In order to achieve the properties of interest, the mode of preparation plays a vital role. Thus, it is highly beneficial to have preparation techniques at hand to obtain PNPs with the desired properties for a particular application.

1. Ionic Gelation Technique

Chitosan are hydrophilic natural polymers and have been used to synthesize biocompatible NPs by the ionic gelation method. This technique involves an interaction of a cation (or an anion) with an ionic polymer to generate a highly cross linked structure.[17] By ionic gelation, Calvo and co-workers developed a method for preparing hydrophilic chitosan nanoparticles. It involves the material undergoing transition from liquid to gel due to ionic interaction conditions at room temperature.[18]

Amir Dustgani et al. in 2008 prepared Dexamethasone Sodium Phosphate loaded chitosan nanoparticles by ionic gelation method. The method involves a mixture of two aqueous phases- one is the polymer chitosan, a di-block co-polymer ethylene oxide or propylene oxide (PEO-PPO) and the other is a poly anion sodium tripolyphosphate. A positively charged amino group of chitosan interacts with negative charged tripolyphosphate to form coacervates with a size in the range of nanometer. Coacervates are formed as a result of electrostatic interaction between two aqueous phases.[19]

Sujima Anbu in 2016 used 1% (w/v) of the plant extract mixed with 0.5% (w/v) of TPP. The solution is added drop wise into the chitosan solution containing 0.5% (w/v) chitosan and 1% (v/v) acetic acid under gentle magnetic stirring. The solution has been incubated for 20 mins and used for characterization. The mechanism behind is the physical cross linking of chitosan with the multivalent anions derived from sodium tripolyphosphate.[20] The properties such as the quick gelling ability and non-toxic nature make the TPP favourable cross-linker for ionic gelation. The pH value of the reaction medium has a significant role for the formation of nanoparticles. It influences the positive charge to neutralize with the gradual de-protonation of ammonium groups aiding in the formation of smaller nanoparticles. Thus, makes chitosan water-soluble and increases its nature of bioadhesivity.[21] The other previous research studies have concluded that the properties of chitosan nanoparticles obtained through interaction between chitosan and TPP are dependent on many parameters that are inherent to the preparation method.

2. Micro Emulsion Method.

Chitosan nanoparticles prepared by micro emulsion technique were first developed by Maitra. Basically it is based on involvement of chitosan in the aqueous core of reverse micellar droplets and followed by cross-linked through glutaraldehyde. In this technique, involvement of surfactant is must to n-hexane. Then prepared chitosan/acetic solution and glutaraldehyde is mixed with the surfactant/hexane and continuously stirred at room temperature. One author describes the use of surfactant; these molecules are amphiphilic in nature that are in presence of water or any organic solvent form a spherical aggregate.[22] The final solution is kept overnight, for removal of organic solvent used a low pressure.

Many authors described in their papers, the excess surfactant which is used particularly in experiment was removed by precipitating with $CaCl_2$ and found precipitate has been removed by centrifugation. The final nano sphere suspension is dialyzed before lyophilisation and by this technique, a particle of narrow size distribution of less than 100nm is found.[23]

3. Polyelectrolyte Complex (PEC)

It is a very simple technique for preparations of nanoparticles since no tough condition are involved. It formed by interaction between anionic and cationic charged polymer, followed by charge neutralization.

A previous study explains this method with the chitosan. 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 50nm-700nm.[24] These are used for delivery of proteins, peptides, drugs and plasmid DNA.[25] Effects of pH, MW and concentration play a main role in this method to determine the size and yield of nanoparticles.

4. Emulsification/solvent Diffusion (ESD)

This method is originally developed by Niwa et al. employing PLGA. It is a modified solvent diffusion method that requires no homogenisation. This is the specific method for preparation of CSNPs and involves the addition of water-miscible solvents (e.g. methylene chloride and acetone) along with organic solvents (e.g. dichloromethane or chloroform). Solvent is eliminated by evaporation or filtration, according to its boiling point. There have been very limited studies on this method, but it has proven to be suitable for encapsulating hydrophobic drugs.[26] This method offers great reproducibility, simplicity, narrow size and easy scale up. But, it eliminates high volumes of water from suspension and during emulsification; there is leakage of drug from water soluble to saturated aqueous external phase.

APPLICATIONS OF CHITOSAN NANOPARTICLES (CSNPs)

Over the last few years CSNPs have gained considerable attention due to their inherent biological properties. They are being used in a variety of different products and applications ranging from pharmaceuticals, food packaging, bio-sensing, fuel cell manufacturing, larvicidal and mosquitocidal activity, and waste-water treatment.

1. Synthesizing and stabilizing the optically active materials

The cationic polyelectrolyte nature of CS was found to be advantageous for stabilizing fascinating photonic materials including plasmonic nanoparticles, semiconductor nanoparticles, fluoroscent organic dyes, and luminescent transitional and lanthanide complexes. Compared to reviews published on the usage of CS for various other applications, the optically active materials are very limited. Therefore, this review highlighted the different works involving some of the molecular-nano systems that are prepared or stabilized using the CS polymer.[27]

2. Pre-oral administration

As nanoparticles can protect labile drugs from enzymatic degradation in gastrointestinal tract, they serve as potent oral delivery systems for proteins, macromolecules and polynucleotides.[28]

Chitosan nanoparticles are attractive carriers for oral delivery vehicle as they promote better absorption of drug. Several research groups have studied the absorption promoting effect of chitosan and founded, in mucosal cell membrane, a combination of mucoadhesion and transient opening of tight junctions.[29]

Using Chitosan, Captopril mucoadhesive microspheres were prepared successfully in combination with copolymers (HPMC K4M, eudragit RS-100 and polycarbophil) in different proportions. This research study concluded that F9 showed better drug entrapment, mucoadhesive property and controlled drug release. Thus, the formulated chitosan based mucoadhesive microspheres seem to be a potential candidate as an oral gastro retentive controlled drug delivery system in prolonging the drug retention in stomach and increasing the bioavailability of drug.[30]

3. Waste water treatment

Chitosan nanoparticles are used in removal/recovery of heavy metal ions from wastewaters, removal and binding of dye, in biological denitrification, as a dehydration agent and in sludge treatment.[31]

4. Antibacterial

With rising population, the use of antibiotics has significantly increased to treat the diseases. Thus, the overuse of these antibiotics has resulted in the rise of antibiotic-resistant bacteria. Therefore, materials with non-conventional antibacterial systems that avoid the development of antibiotic-resistant "superbug" species like methicillin-resistant *Staphylococcus aureus* (MRSA) are in high demand. Chitosan is highly attractive compared to other antibacterial agents as it inhibits the growth of a wide variety of bacteria by exhibiting many advantages such as killing a wider range of bacteria, higher killing rates and lower toxicity toward mammalian cells.[32]

The CS-stabilized metal and metal-oxide hybrid nanoparticles are expected to be more stable, less toxic and are expected to exhibit higher antibacterial efficiency due to the presence of the CS stabilizer with the metal or metal-oxide nanoparticles.[33] A recent research study demonstrated that the antimicrobial effect of AgNPs strongly depended on their size- because the smaller the size of nanoparticles, the larger is the surface area in contact with the bacterial cells and hence, greater is the interaction with cells. The diameter of zone of inhibition also increased as the size of the nanoparticles decreased.[34]

The chitosan fits within the principles of "Green Synthesis", which is favoured over other chemical reduction methods because it avoids the use of harsh chemicals, reducing and stabilizing agents.[35]

In these works, CS has shown to exhibit a triple role as a solvent medium, stabilizing medium and reducing medium. Some researchers have extensively demonstrated the ability of a CS medium to act as both reducing and stabilizing agent to form different sized AgNPs and ZnONPs in the absence of any other reducing or stabilizing agents.[36] In summary, the eco-friendly nature of CS and its biocompatibility have attracted much attention in the area of nanomedicine as an effective antibacterial agent.

5. Antioxidant

Oxidative stress is considered a critical factor in various degenerative diseases, as well as in the normal process of aging. Antioxidant mechanisms include scavenging ROS, activating detoxifying proteins, or preventing the generation of ROS. Finding natural antioxidants is important, because they can protect the human body from free radicals and slow the progress of many chronic diseases.[37] Therefore, using functionalized CS to obtain polysaccharide-based compounds with antioxidant properties is of growing importance.[38] The oxidant scavenging activity of CS is due to the strong hydrogen-donating ability of CS, as well as its ability to chelate metal ions. CS polymer has been shown to form very stable macromolecular radicals when reacted with certain oxygen species. Overall, a higher concentration of low molecular weight CS has a positive influence on antioxidant activity. Also, CS has been used as an effective antioxidant for human serum albumin (HSA), which is a major target of oxidative stress in uraemia and other vascular disorders.[39] Unfortunately, many methods of obtaining functionalized CS involve using toxic chemicals such as carbodiimide, ammonium persulfate, or formaldehyde. Therefore, newer approaches involve enzymatic cross-linking, which is attractive due to an enzyme's high specificity and environmental friendliness.[38]

6. Drug delivery

One of the most promising and useful forms of chitosan are CSNPs. Extensive reviews detailing the evaluation of the chemistry of CSNPs formation and their impact on drug delivery systems for the treatment of diseases like cancer, are well documented.[40,41] Among various drug delivery platforms, nanoparticles-based delivery systems are known to exhibit several advantages such as target specific drug delivery, sustained release, enhanced solubility of hydrophobic drugs, increased concentration of the drug at the tumor site, and reducing immunogenicity. CSNPs have also been investigated for the delivery of chemotherapeutic agents, cancer imaging agents and are used to deliver chemotherapeutic drugs to tumors via the enhanced permeability and retention effect.[42]

Min et al. demonstrated the use of hydrophobically-modified glycol CSNPs to deliver camptothecin (CPT). Insoluble CPT was encapsulated in the glycol CSNPs with a loading efficiency above 80%.[43]

Thus, engineering nanostructures from CS polymer under the most benign and facile conditions is highly desirable in order to take advantage of all the intrinsic biocompatible properties of CS polymer.

7. Bioimaging and Cancer Research

Though there are multiple applications of luminescent CS hybrids, we have selected the most prevalent works. Although great progress has been achieved relative to the diagnosis and therapy for various cancers in last decades, cancer remains one of the leading causes of death. Patients treated with conventional chemotherapy commonly suffer from severe side effects. One of the goals of chemotherapy is to develop a drug delivery system that can intelligently trigger the drug release targeted at the cancer cells, to reduce the drug's side effects on the patient and improve the overall therapeutic efficacy.[44] As ICG is photo-degraded in aqueous solution and is unstable at high temperature, resulting in loss of absorption and fluorescence. All of these disadvantages restrict its use in Photothermal therapy (PTT). Therefore, recently a CS-based ICG-containing nanostructure for effective molecular tumor imaging has been developed by Song et al. to overcome these disadvantages and an effort has been made to improve ICG's photo and thermal-stability, pharmacokinetics and biodistribution in tumor tissue.[45]

Carbon Dots (CDs) have also been studied for targeted release of cancer drugs. CDs with unique optical properties have been incorporated into different nanomaterials for applications as higher quality membranes, catalysts, drug carriers, MRI contrast agents and nanodevices. As per a recent study, when these optically active nanoparticles are embedded into materials such as CS, the resultant hybrid material can be applied in the sensing field[44] and can inhibit tumor growth.

8. Gene therapy

Gene therapy can be broken into two main approaches. In the first approach, there is gene augmentation to upregulate tumor suppressor genes. The second approach involves gene knockdown using short, interfering RNA (siRNA). For successful nanoparticle gene therapy, the therapeutic genes have to be protected from gene cleavage enzymes and transported into the targeted intracellular compartments.[46] Oligonucleotide-coated NPs conjugates are useful for drug delivery, gene therapy and diagnostics.[47] Kenneth et al. reported that the CS/siRNA nanoparticles enhance the green fluorescent protein gene knockdown in both human lung carcinoma cells and murine peritoneal macrophages.[48] These NP-oligonucleotide structures showed high levels of cellular uptake and transfection efficiency. Moreover, these structures showed resistance against degradation by nuclease with minimal immune response; low toxicity; and highly effective gene regulating capabilities.[47]

9. Sensing

Irrespective of various platforms used in sensing, a polymer support to load the indicator is an integral part of the sensor.[49]

Baranwal et al. have summarized many sensing applications using CS polymer. The group explained the significance and impact of CS polymer for biosensing, highlighting its potential versus other biological polymers like poly-lysine, poly-glutamate, and alginic acid. Many optically active materials including AuNPs and AgNPs have been fabricated with CS polymer for developing electrochemical immune-sensors and electrochemical enzyme biosensors.[50]

In the case of optical sensors, Bhatnagar et al. have constructed a nanobiosensor for diagnosis of invasive *Aspergillosis* using CS-stabilized AuNPs.[51] An excellent work by Wang et al. resulted in fabrication of biosensors based on a combination of CS and Prussian blue dye. The biosensors included a glucose sensor, glutamate sensor and a galactose sensor. The sensors' interference with ascorbic acid and uric acid were also selectively analyzed. The biosensors based on a CS platform were able to detect glucose, galactose and glutamine in human blood serum and in fermented solutions.[52]

Realizing the significance of heparin in the regulation of various physiological and pathological processes, many sensors have been fabricated based on different platforms. CS-stabilized AuNPs were prepared and based on the intensity of scattered light, the presence vs. absence of heparin was investigated.[53]

10. Cytotoxic activity

A very recent research investigation was carried out on Synthesis of bioactive chemicals cross-linked Sodium Tripolyphosphate (TPP)- Chitosan nanoparticles for enhanced cytotoxic activity against Human ovarian cancer cell line (PA-1). This study strongly suggested that the fabricated phytochemicals cross-linked CSNPs from *G.sylvestre* leaf extracts exhibited enhanced cytotoxic activity and it could be effectively used as a promising anticancer agent in the near future because of its stimulated cytotoxic activity and reduction in the nanodrug dosage.[54]

11. Larvicidal activity

Several advanced research studies have been performed over the years on mosquito breeding and larvicidal activity by using jasmine extracts. But, a recent study by Varun Tyagi in 2017 has depicted that benzyl alcohol in jasmine oil revealed moderate-high rate of larval mortality. Another recent research on CSNP synthesis by TPP has revealed its potential mosquito larvicidal activity against 3rd instar of *A.egypti* that has been contemplated with the varieties of chitosan.[55]

CONCLUSION

Chitosan is a natural second most abundant aminated polycationic polysaccharide. The solubility of chitosan decreases with the increase in molecular weight. The degree of deacetylation is a fundamental parameter that affects the functionality of chitosan. It offers excellent physiological properties such as biocompatibility, biodegradability, bio adhesiveness, nontoxicity and easy movement cross the membrane. Its unique chemical nature, positive charge, presence of reactive amino and hydroxyl groups provides wide applications.

Green synthesis of CSNP is a major focus on modern nanotechnology research. Various techniques of preparation of CSNP have been demonstrated such as ionic gelation, poly-electric complex, micro emulsion, emulsification and solvent diffusion.

Chitosan polymer can be used in waste water treatment by efficient removal of heavy metal ions, dyes and organic pollutants.

CSNP proved to exhibit antimicrobial activity by mechanism which includes ionic surface interaction resulting in cell wall leakage, inhibition of mRNA and protein synthesis by penetrating into nuclei of microbes and chelating metal ions provoking the suppression of essential nutrients to microbial growth.

The antioxidant activity of chitosan has been established by strong proton donating ability and high degree of quaternization. Phenolics and polyphenolic compounds condensed with chitosan to form natural prodrugs exhibits positive influence on antioxidant activities.

In addition to the aforementioned nature of chitosan- it's potentially used as a carrier for target drug delivery, controlled release and enhanced solubility of hydrophobic drugs.

Chitosan due to its cationic and low immunogenicity can be used as a non-viral vector material for efficient gene delivery. Positively charged Chitosan can be easily complexed with DNA and protected from nuclease degradation and hence used in transfection.

Chitosan-a multipurpose biomaterial, is known to exert effects against several types of cancer through induction of apoptosis and cell cycle arrest. Chitosan and its derivatives have been reported to selectively permeate through membrane of cancer cells and show anticancer activity through cellular enzymatic, antiangiogenic, immunoenhancing and antioxidant defense mechanism.

Medicinal oil encapsulated Chitosan capsule shows potential larvicidal activity. The mortality mechanism is owed to the interaction mediated by the electrostatic forces forming the protonated ammonium group and the antagonistic residues presumably competing with calcium for electro-negative sites on the membrane surface of the larvae.

Along with the biomedical and pharmaceutical application, Chitosan also has industrial and biotechnological applications such as a hydrocolloid in cosmetic products , immobilization of enzymes in food processing , water engineering, manufacture of paper and biodegradable packaging material for food wrap and other products, textile industry, agriculture, fixing agent for the acid dyes in developing photographs, presence of amino and hydroxyl groups in chitosan makes it an useful chromatographic support for separation of DNA and fabrication of solid state batteries.

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