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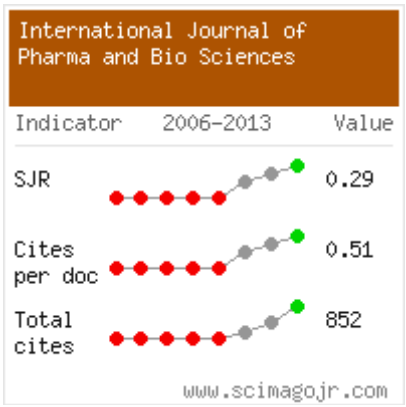

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Review Article **Nanotechonology**

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CHITOSAN NANOPARTICULATE AND THEIR APPLICATIONS: A REVIEW

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ABSTRACT

Chitosan has reached a prominent position as the carrier-forming material, as diverse methods can be applied to produce nanoparticles using that excipient. Chitosan polymers are extensively used for the delivery of an active pharmaceutical ingredient. They can form a matrix or membrane that can control the release of a drug over a prolonged period of time or sustained release of drugs, thus avoiding repetitive dosing. Several methods have been developed to produce chitosan nanoparticles, since those have been shown to offer attractive advantages as drug delivery carriers. Chitosan is an effective material for biomedical applications because of their biocompatibility, biodegradability and non-toxicity, apart from their antimicrobial activity and low immunogenicity, which clearly points to an immense potential for future development. This review summarizes the biomedical applications of multifunctional chitosan based nanomaterials in drug delivery and as a support medium for enzyme immobilization.

KEYWORDS: ***chitosan, polymers, biocompatability, biodegradable, non-toxic, antimicrobial, enzyme immobilization.***

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INTRODUCTION

Nanotechnology is being increasingly explored in science and industry for widely different applications. Nanotechnology and polymers have captivated a tremendous interest in many areas such as the pharmaceutical industry and therapeutic innovation among others. Polymers are the macromolecules composed of repeating structural units of monomers connected by covalent chemical bonds and this process is known as polymerization. There are many types of polymers including natural and synthetic moiety. Natural polymers such as proteins (collagen, silk and keratin), carbohydrates (starch, glycogen,chitosan) are widely used materials for conventional and novel dosage forms. These materials are chemically inert, nontoxic, less expensive, biodegradable, eco-friendly and widely available (1, 2). Chitosan, a natural polymer is extensively used for the delivery of an active pharmaceutical ingredient. They can form a matrix or membrane that can control the release of a drug over a prolonged period, thus avoiding repetitive dosing.

Chitosan Polymer

Chitosan (CS) is a polysaccharide, similar in structure to cellulose. Both are made of linear β(1-4)-linked Monosaccharides. The primary amine groups of chitosan render special properties that make CS very useful in pharmaceutical applications. Compared to many other natural polymers, chitosan has a positive charge and is mucoadhesive.(3) Chitosan is obtained from the deacetylation of chitin, a naturally occurring and abundantly available (in marine crustaceans) biocompatible polysaccharide. However, applications of chitin are limited as compared to CS because chitin is structurally similar to cellulose, but chemically inert. Acetamide group of chitin can be converted into an amino group to give CS, which is carried out by treating chitin with concentrated alkali solution. Chitosan is relatively reactive and can be produced in various forms such as powder, paste, film, fiber, etc.(4,5).

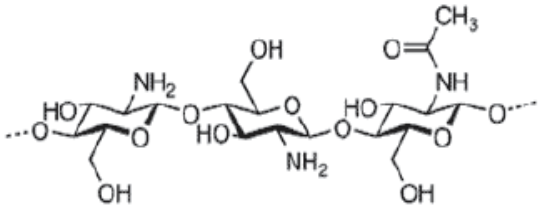

Fig. 1. Chitosan's structure

Commercially available CS has an average molecular weight ranging between 3800 and 20,000 Daltons and is 66% to 95% deacetylated. Chitosan, being a cationic polysaccharide in neutral or basic pH conditions, contains free amino groups and hence, are insoluble in water. In acidic pH, amino groups can undergo protonation thus, making it soluble in water. Solubility of Chitosan depends upon the distribution of free amino and N-acetyl groups as shown in the above structure of chitosan(6). Usually 1–3% aqueous acetic acid solutions are used to solubilize Chitosan. Chitosan is biocompatible with living tissues since it does not cause allergic reactions and rejection. It breaks down slowly to harmless products (amino sugars), which are completely absorbed by the human body If the degree of deacetylation and molecular weight of CS can be controlled, then it would be a material of choice for developing micro/nanoparticles. Chitosan has many advantages, particularly for developing micro/nanoparticles.

Chitosan nanoparticle

Chitosan polymers are extensively used for the delivery of an active pharmaceutical ingredient.

They can form a matrix or membrane that can control the release of a drug over a prolonged period, thus avoiding repetitive dosing. They can be used to form nano carriers to deliver drugs, in particular poorly soluble drugs or biotechnology-based drugs. Both systems can protect the drug from degradation. Moreover, when the carrier is functionalized by a targeting agent, the encapsulated drug may be selectively released inside or near a specific organ. Polymeric delivery systems can modify the pharmacokinetics of a drug, leading to a higher therapeutic index by decreasing the side effects and increasing efficacy.(7) Chitosan nanoparticles due to their small size and large surface area to volume ratio have special characteristics, which make them favourable carriers for different drugs specially hydrophobic drugs in cancer drug delivery application.(8)

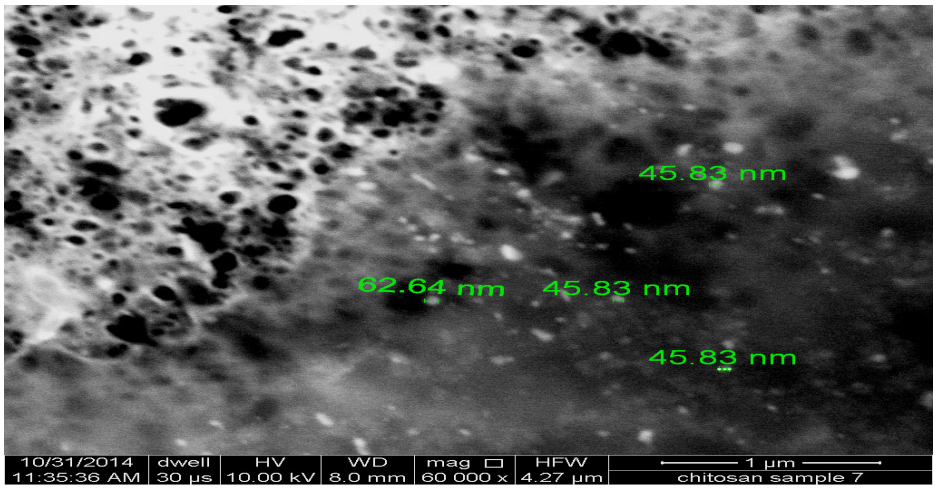

Figure 2
SEM images of Chitosan nanoparticles formed by ionotropic gelation method.(47)

Properties of chitosan making it suitable for oral delivery

- Biocompatibility and biodegradability
- Permeation enhancing effect
- Mucoadhesiveness
- pH sensitiveness
- Mild gelation conditions

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

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

Chitosan Nanoparticles have been shown to provide sustained release of both hydrophilic and hydrophobic drugs and are prepared by three distinct methods including ionotropic gelation, precipitation using tripolyphosphate and crosslinking methods using gluteraldehyde. They self aeembles to form a micelle like structure where hybrophobic head is protected and hydrophilic portion extrudes outside through which they can accomodate or encapsulate hydrophobic drugs as shown in fig.3. (Sunita et al,2013). The method used for preparation determines the entrapment efficiency, loading efficiency, and particle size.

Particle size of the Chitosan Nanoparticles generally depends on molecular weight of chitosan used, concentration of chitosan solution and amount of cross linking agent. Increasing the concentration of chitosan increases the viscosity of chitosan solution thus making smaller sized particle formation difficult.

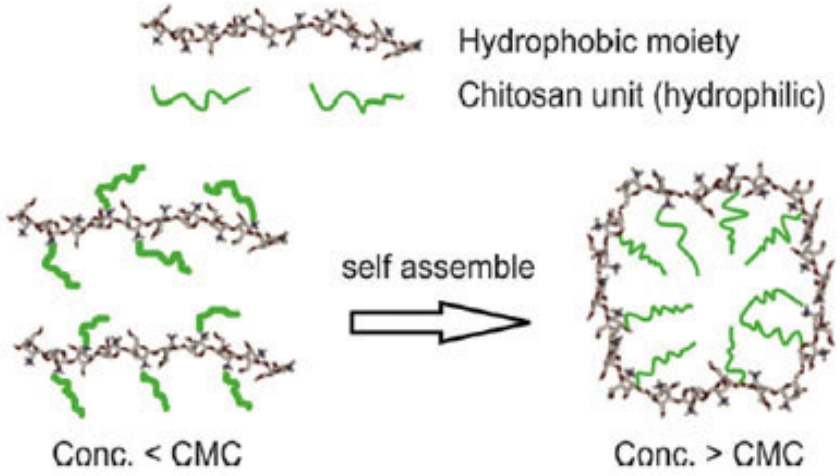

Figure 3
Self assembly of chitosan nanoparticle above Critical micellization concentration.

Chitosan Nanoparticles are formed according to a bottom up approach as a result of a self–assembly or crosslinking processes in which the molecules arrange themselves into ordered nanoscale structures either by physical or covalent inter or intramolecular interactions. In these nanostructures, the drug can be entrapped or attached to the Nanoparticles matrix. Chitosan Nanoparticles have been prepared by several methodologies, including physical crosslinking by ionic gelation by specific ions such as pentasodium tripolyphosphate (TPP) or EDTA. In particular, chitosan TPP Nanoparticles have been utilized as a drug delivery platform for a wide range of active molecules.

1. Ionic gelation method

Ionic gelation method is most commonly used for preparation of chitosan nanoparticles. This method was first reported in 1997(9). In this method appropriate concentration of chitosan is dissolved in acetic acid. Sodium tripolyphosphate is most commonly used crosslinking agent. Both of these phases are dissolved in separate glass bottles and mixed under stirring leads to formation of chitosan nanoparticles due to inter and intra molecular interaction between chitosan and sodium tripolyphosphate. Anticancer drug can be loaded in these chitosan nanoparticles during mixing between chitosan and sodium tripolyphosphate. Size of nanoparticles can be varied by changing degree of deacetylation of chitosan.

2 Desolvation method

In this method, desolvating agents are used to produce chitosan particles. It was reported for the first time for the preparation of micron-sized carriers (10). But now a day, this method is used most frequently for production of chitosan nanoparticles. Sodium sulfate and acetone are the most commonly used precipitating agents. Chitosan nanoparticles are formed by dropwise addition of sodium sulfate into chitosan solution. Due to greater affinity of salt to water, water surrounding chitosan get eliminated, resulting in precipitation, inducing desolvation of chitosan. So chitosan nanoparticles are produced.

3.Spray-drying

Spray-drying becomes a good technique to improve the stability of colloidal nanoparticles. Optimization and evaluation of spray dried

chitosan nanoparticles containing doxorubicin have been reported (11). Preparation of lomustine loaded chitosan nanoparticles by spray drying and *in vitro* cytostatic activity on human lung cancer cell line L132 have been reported(12). Effect of crosslinking agents (sodium tripolyphosphate (TPP) and sodium hexametaphosphate (HMP) were studied on the drug leaching, water uptake of hydrogels, drug release from matrix and its mechanism.

4. Covalent cross-linking

Chitosan nanoparticles can be prepared by covalent crosslinking method. In this method, covalent bond form between chitosan chain and a functional cross-linking agent. The covalent cross linking occurs between reactive amino groups of chitosan with the aldehyde groups of glutaraldehyde, which is added in solution after the emulsion formation and leads to nanoparticle production(13). Anticancer drug 5-fluorouracil has been encapsulated by cross-linking glutaraldehyde with amino groups in the molecular chain of chitosan(14).

The Biomedical Applications of Chitosan

Chitosan has been extensively used in tissue engineering including bone, cartilage, liver, and nerve tissue engineering (15). Tissue engineering is aimed to develop biocompatible substitutes to restore, maintain, or improve biofunction of dysfunctional human tissues or organs. Since chitosan is biodegradable and nontoxic, it has been formulated into a varieity of forms such as film, powder, gels. In addition, various modification of chitosan could be made to improve the cell seeding. In bone tissue engineering, chitosan has been shown to promote cell growth and mineral rich matrix deposition(16). In cartilage tissue engineering, glycosamine glycans (GAGs) plays a pivotal role in modulating chondrocytes morphology, differentiation and function(17). While, chitosan is chosen as a scaffold material in cartilage tissue engineering due to its structural similarity to GAGs (18). In live tissue engineering, the bioartificial liver needs to provide an extracellular cell matrix (ECM) like surrounding for the seeded primary hepatocytes or liver stem cells. Also for the similarity with GAGs which are components of ECM, chitosan frequently serves as the scaffold material for hepatocytes culture (19, 20). In nerve engineering, artificial tubes are the effective means to repair injured nerves by bridging of nerve stumps. Chitosan is suitable for nerve regeneration due to its biocompatible and biodegradable properties. In addition, bioadhesive chitosan could favour the adhesion of nerve cells to the tube lumen(21). Chtiosan has been widely applied in drug delivery in a verity of forms such as film or membrane (22), hydrogel system(23) and particulate system(24). A big number of drugs and biomolecules could be delivered by chitosan-based drug delivery systems, i.e., proteins/peptides, genes, antibiotics and chemotherapeutics. With the favorable biodegradable and nontoxic properties, chitosan could be fabricated to hydrogel through gel gelation without the use of cross-linking moieties through electrostatic, hydrophobic, and hydrogen bonding forces between polymer chains or covalently cross-linking available –NH and –OH groups on chitosan by various cross-linkers such as small molecule cross-linkers, polymers or photosensitive agents. For example, genipin, a naturally derived cross-linking agent is effective to crosslink chitosan which contains amino groups(25). The chitosan-based particulate systems will be discussed in the following section. Besides tissue engineering and drug delivery applications, chitosan also has been used in wound-healing formulations. For a wound-healing formulation, it should protect the wound from bacterial infection as well as promote healing process. Chitosan was found to induce wound-healing on its wound and produce less scarring due to its antibiotic property(26). In addition, endothelial cell proliferation enhanced growth factors such as fibroblast growth factor-2 (FGF-2) could be enclosed in the formulation to accelerate this wound-healing process(27). Because of the excellent non-toxic, hydrophilic and cationic properties, chitosan has also been frequently used in bioimaging. Some metal or magnetic based imaging agents are commonly in a colloid form to avoid agglutination. Chitosan are always used as coating or conjugation material to stabilize these imaging agents and decrease

their toxicity(28). Besides, as coating material, the imaging agents such as super paramagnetic iron oxide(29), gadolinium (30) etc. could be encapsulated or entrapped in chitosan nanoparticles as well.

Other applications of Chitosan Nanoparticle

As discussed in the section of chitosan microparticles, the oral drug delivery has the advantages of convenience and good patient compliance. However, the enzymatic digestion and low mucosal absorption are the main barriers for some drugs which are sensitive to these harsh conditions. Based on the favorable bioadhensive and enhanced permeability properties chitosan, it has been formulated to nanoparticles to deliver these drugs. Beside above mentioned advantages of chitosan microparticles, chitosan nanoparticles also have its size advantages which can allow them more easily absorbed by mucosal epithelium cells. Sarmento et al. reported that (31) alginate/chitosan nanoparticles administered orally to diabetic rats were found effective for oral insulin delivery [32] reported that water-soluble chitosan nanoparticles enhance and prolong the intestinal absorption of bovine serum albumin Pulmonary and nasal drug administrations are also frequently used means due to their large absorbance surface area. Al-Qadi et al. [33] reported a new dry powder system consisting of microencapsulated insulin-loaded chitosan nanoparticles. The mild preparation conditions of chitosan nanoparticles protected the insulin bioactivity well, whereas, the final spray dried powder provided an adequate aerodynamic property for deposition in deep lungs. The assessment of the plasmatic glucose levels following intratracheal administration to rats revealed that the microencapsulated insulin-loaded chitosan nanoparticles induced a more pronounced and prolonged hypoglycemic effect compared to the controls. Shahnaz et al. [34] et al. reported a thiolated nanoparticle to enhance the bioavailability for the nasal application of leuprolide. The inter and/or intramolecular disulfide formation within the NPs network stabilized obtained nanoparticles and achieved a sustained leuprolide release over 6 hours. Ciliary beat frequency study demonstrated that thiolated chitosan nanoparticles could be considered as suitable additives for nasal drug delivery systems.

Ocular Drug Delivery

The short residence time, drug drainage and frequent instillation are the major drawbacks of the conventional drugs for ophthalmic diseases. Chitosan nanoparticles have been used to deliver ocular drugs to solve above limitations. De la Fuente et al. [35]. reported a hyaluronic acid-chitosan nanoparticles to deliver genes to the cornea and conjunctiva. Both of chitosan and hyaluuronic are the polysaccharide with good bioadhisive and permeability enhancement properties, besides, hyaluronic acid is known for its implication in several processes, such as the regeneration of corneal and conjunctival epithelial cells, through an interaction with the CD44 receptor. Their results indicated that hyaluronic acid-chitosan nanoparticles were able to target and further transfer genes to the ocular surface.

Vaccine Delivery

Nanoparticles often present significant adjuvant effects in parenteral vaccine delivery due to their effective uptake by antigen presenting cells. The nanoscaled size allows nanoparticles to be taken up by M-cells in mucosa-associated lymphoid tissue (MALT), i.e., gut-associated, nasal-associated and bronchus-associated lymphoid tissues, to initiate vigorous immunological responses. Chitosan nanoparticles have been used in vaccine delivery due to their bioadhesive, biocompatible, biodegradable and permeation-enhancement properties. They can be effectively uptaken by phagocytotic cells inducing strong systemic and mucosal immune responses against antigens. Zheng et al. [36] investigated the immune stimulation mechanisms of ovalbumine (a frequently used antigen model) loaded chitosan nanoparticles. They suggested that chitosan nanoparticles had a strong potential to increase both cellular and humoral immune responses and elicited a balanced Th1/Th2 response, and that chitosan nanoparticles may be a safe and efficacious adjuvant candidate suitable for a wide spectrum of prophylactic and therapeutic vaccines.

Gene Delivery

As a nonviral gene carrier, chitosan nanoparticles are able to reduce the risk of cell toxicity and even induce strong immune responses. To date, chitosan nanoparticles have been applied to deliver DNAs, and RNAs mainly through the complex coacervation between polycationic chitosan and polyanionic nucleic acid molecules. Among them, one hot scope in life biology since the last decade of century is the gene silencing technique which is induced by long double-stranded RNA dsRNA), small interfering RNAs (siRNA) or microRNAs (miRNA). One limitation of the delivery of siRNAs into cells is rapid degradation and poor cell absorption of these small molecules. Katas et al. [37] reported a 100% protecting of siRNAs from nuclease degradation by chitosan nanoparticles. However, generally gene transfection efficiency of chitosan nanoparticles is lower than that of viral gene carriers. Improving transfection efficiency is a challenge for using chitosan nanoparticles as a gene carrier. Mansouri et al. [38] reported a folic acid modified chitosan nanoparticles to improve gene tranfection efficiency. Their results revealed that the obtained folic acid-modified chitosan nanoparticles showed a low cell toxicity and were able to condense DNA effectively with ideal size and zeta potential.

Chitosan Nanoparticles as Enzyme Immobilization Support Medium

Chitosan is known as an ideal support material for enzyme immobilization because of its many characteristics like improved resistance to chemical degradation and avoiding disturbance of metal ions to enzyme (39). Enzymes have been immobilized onto chitosan supports with different particle size. For practical purposes, carrier beads with size falling into millimeter range are mainly used (40, 41). Under the scale of nano, nanomaterials have characteristics, such as magnetism and large surface area to volume ratio. These characteristics are in favour of immobilization of the enzymes (42,43). Recent studies indicated that the performance of enzyme immobilization onto chitosan nanoparticles is higher than that of the biocatalyst immobilized onto chitosan microparticles (44). The result can be related to better distribution of the enzyme onto the support due to high specific surface area and numerous active functional groups available for fixing the enzyme molecules. Immobolization of enzymes onto biopolymers nanoparticles has shown some benefits like improving their stability to pH and temperature, resistance to proteases and other denaturing compounds, as well as an adequate environment for their repeated use or controlled release (45). In the last decades, immobilized enzymes onto nanoparticles have been also considered for biosensors and food packaging applications (46).

CONCLUSION

This review emphasizes recent research on different aspects of chitosan based nanomaterials,including the preparation methods, its biomedical and other applications of chitosan based nano material and also as a support material for enzyme immobilization. Chitosan nanoparticles due to their highest specific surface area are much proper for immobilization of higher amount of enzymes. As compared to chitosan macro and microparticles, higher activity values of immobilized enzyme onto chitosan nanoparticles is explained again by better distribution of the enzyme onto the larger surface area of nanoparticles. Many drugs have problems of poor stability, water insolubility, low selectivity, high toxicity, side effects and so on. Clinically useful drug delivery systems need also to deliver a certain amount of a drug that can be therapeutically effective over an extended period of time. Such requirements can be met through the nano scale drug delivery systems. Chitosan NPs are good drug carriers because of their good biocompatibility, biodegradability and non toxicity and can also be readily modified by attaching functional groups. As a new drug delivery system, they have attracted increasing attention for their wide applications. Chitosan nanoparticles are now being modified for sustained/ controlled release and targeting. Although Chitosan NPs can be easily modified to carrier, coat and encapsulate hydrophobic drugs, further investigation is required on the biocompatibility of modified Chitosan NPs and its derivatives.

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