Chitosan Nanoparticles Synthesis From Shrimp Shell Waste For Application in The Pharmaceutical Industry

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ABSTRACT
Chitosan is an amino polysaccharide. This is a versatile, second most abundant natural polymer. Due to its biocompatible, biodegradable and non-toxic nature, it is also known as a biomaterial. These properties clearly indicate that chitosan has great potential in the fields like drug delivery, gene delivery, cell imaging, sensors, food, agriculture and treatment as well as diagnosis of some diseases like cancer. Chitosan based nanomaterial have superior physical and chemical properties such as high surface area, porosity, tensile strength, conductivity, photo-luminescent as well as increased mechanical properties. Chitosan can be prepared by processing shrimp waste (shell) which involves partial deacetylation of chitin. Biowastes are eco-friendly to produce typical nanoparticles with well-defined chemical composition, size, and morphology. This review paper highlights the recent developments in the area of producing nanoparticles from biowastes e.g. eggs and shrimp peels etc. The paper also discusses about preparation of chitosan-based nanoparticles from Shrimp shell waste and its application in pharmaceutical industry.

Keywords: Chitosan, Shrimps, Nanoparticles, Pharmaceutical Industry.

INTRODUCTION
Seafood processing industry does packaging and processing after culture or capture of shellfishes. Peeling of shrimps/ prawns gives required product meat and generates waste. By this way, the shellfish industry that is prominent in all costal countries generates about 60,000 to 80,000 tons of waste [1]. Even though the wastes are biodegradable, the dumping off large quantities makes degradation process slow resulting in accumulation of waste overtime, which is a major environmental concern. A quick and effective solution to this is recycling of shell wastes and extraction of commercially viable substances like chitin from them. Chitin on its own has
various applications, but due to insolubility in water and organic solvent, it is not widely used. This can further be deacetylated to form chitosan that has many uses [2].

Chitosan is the second most abundant polysaccharide next to cellulose on the earth. It is a linear polymer composed of β-(1–4)-2-amino-2-deoxy-D-glucopyranose units. It is white, hard, inelastic and nitrogenous polysaccharide [3] with great potential for wide range of uses due to its biodegradability, biocompatibility, antibacterial activity, non-toxicity and versatile chemical and physical properties [4]. Pharmaceuticals industry, paper production, textiles, wastewater treatment, biotechnology, cosmetics, food processing and agriculture are few of the many areas where chitosan is useful [5-9]. The presence of primary amine groups makes chitosan an excellent cell transfectant. Like polyethylene amine, chitosan exhibits a “proton sponge” effect, which refers to the swelling behavior of the polymer on encountering an acidic pH inside the cell’s endosome, making it an efficient carrier for therapeutic molecules [10].

However, chitosan is macromolecule, which significantly marks its application. To overcome this drawback, the use of chitosan fabricated nano/submicron chitosan is effective. In recent years, nanotechnology has shown significant inclination for the preparation of drug carriers. Under the scale of nano, nanomaterials have characteristics such as magnetism and large surface area, which are favorable for drug immobilization. Many studies have mainly reported the preparation of chitosan nanoparticles and their applications in the carrier of drugs and other pharmaceutical application. Since last two decades, science is trying to prepare CPNPs using different methods. The present review paper tries to provide an overview about synthesis of chitosan nanoparticles using simple yet effective methods and its application in pharmaceutical industry.

MATERIALS AND METHODS

(a) Sample Preparation

Collect shrimps like *Penaeus scardinus* and *Penaeus monodon* from seacoast. Remove their shells and operculum. Place all these waste in ziploc bags and refrigerate overnight. Measure the weight of crushed shrimp’s exoskeletons wet samples placing them on foil paper. Dry the samples in oven at 65°C until one gets constant weight.

(b) Extraction of Chitosan

A combination of three procedures - Deproteinization, Demineralization and Deacetylated will provide chitosan [11-13]. Treat five gms of shrimp shell waste with 4% NaOH at room temperature for 24hours. Drain the alkali from the shells and wash with distilled water repeatedly until pH drop to neutral. This process causes deproteinization of shells. For demineralization to yield chitin, treat the deproteinized shells with 4% HCl at room temperature for 12hours. Drain off the acid from chitin, wash with distilled water and finally dry at room temperature. Repeat the process with 2% NaOH and 1% HCl. The chitin obtained still has a slight pink hue.
Further decolourisation can be achieved by soaking chitin in 1% potassium permanganate for 30 mins followed by 1% oxalic acid for 30 mins to 2 hours. The decolourised chitin can be deacetylated to form chitosan by treating with 65% NaOH for 3 days at room temperature. Alkali should be drained off and washed repeatedly with distilled water until pH lowers. Chitosan can be further dried at room temperature and stored. (Figure.1)

![Preparation of Chitin & Chitosan](image)

**Figure.1:** Preparation of Chitin & Chitosan [34]

(C) Preparation of Nanoparticals

Over the past 30 years, chitosan NP preparation technique has been developed based on chitosan microparticles technology. There are at least five methods available: ionotropic gelation, microemulsion, emulsification solvent diffusion an, polyelectrolyte complex and Green Synthesis of Ag/Cts/PEG Nanocomposites. The most widely developed methods are ionotropic gelation, self assemble polyelectrolyte and These methods offer many advantages such as simple and mild preparation method without the use of organic solvent or high shear
force. Thus, they would be applicable to a broad categories of drugs including macromolecules which notorious as labile drugs. In general, the factors found to affect nanoparticles formation including particle size and surface charge are molecular weight and degree of deacetylation of chitosan. The entrapment efficiency is found to be dependent on the pKa and solubility of entrapped drugs. The drug is mostly found to be associated with chitosan via electrostatic interaction, hydrogen bonding, and hydrophobic interaction [14].

**Coacervation or Ionotropic Gelation**

Chitosan NP prepared by ionotropic gelation technique was first reported by Calvo and has been widely examined and developed by Janes. The mechanism of chitosan NP formation is based on electrostatic interaction between amine group of chitosan and negatively charge group of polyanion such as tripolyphosphate. This technique offers a simple and mild preparation method in the aqueous environment. First, chitosan can be dissolved in acetic acid in the absence or presence of stabilizing agent, such as poloxamer, which can be added in the chitosan solution before or after the addition of polyanion. Polyanion or anionic polymers was then added and nanoparticles were spontaneously formed under mechanical stirring at room temperature. The size and surface charge of particles can be modified by varying the ratio of chitosan and stabilizer.

![Figure 2](image)

**Figure 2** Schematic representation of ionic gelation method[35]

**Microemulsion Method**

Chitosan NP prepared by microemulsion technique was first developed by Maitra. This technique is based on formation of chitosan NP in the aqueous core of reverse micellar droplets and subsequently cross-linked.
through glutaraldehyde. In this method, a surfactant was dissolved in N-hexane. Then, chitosan in acetic solution and glutaraldehyde were added to surfactant/hexane mixture under continuous stirring at room temperature. Nanoparticles were formed in the presence of surfactant. The system was stirred overnight to complete the cross-linking process, which the free amine group of chitosan conjugates with glutaraldehyde. The organic solvent is then removed by evaporation under low pressure. The yields obtained were the cross-linked chitosan NP and excess surfactant. The excess surfactant was then removed by precipitate with CaCl₂ and then the precipitant was removed by centrifugation. The final nanoparticles suspension was dialyzed before lyophilization. This technique offers a narrow size distribution of less than 100 nm and the particle size can be controlled by varying the amount of glutaraldehyde that alters the degree of cross-linking. Nevertheless, some disadvantages exist such as the use of organic solvent, time-consuming preparation process, and complexity in the washing step.

**Emulsification Solvent Diffusion Method**

*El-Shabouri* reported chitosan NP prepared by emulsion solvent diffusion method, which originally developed by *Niwa et al.* employing PLGA. This method is based on the partial miscibility of an organic solvent with water. An o/w emulsion is obtained upon injection an organic phase into chitosan solution containing a stabilizing agent (i.e. poloxamer) under mechanical stirring, followed by high pressure homogenization. The emulsion is then diluted with a large amount of water to overcome organic solvent miscibility in water. Polymer precipitation occurs as a result of the diffusion of organic solvent into water, leading to the formation of nanoparticles. This method is suitable for hydrophobic drug and showed high percentage of drug entrapment. This technique presents several advantages, such as high encapsulation efficiencies (generally >70%), no need for homogenization, high batch-to-batch reproducibility, ease of scale-up, simplicity, and narrow size distribution. The major drawbacks of this method include harsh processing conditions (e.g., the use of organic solvents) and the high shear forces used during nanoparticles preparation. As with some of the other techniques, this one is efficient in encapsulating lipophilic drugs [15].

![Figure 3](image-url) **Figure.3** Schematic representation of the emulsification/solvent diffusion technique[35]
Polyelectrolyte Complex (PEC)

Polyelectrolyte complex or self-assemble polyelectrolyte is a term to describe complexes formed by self-assembly of the cationic charged polymer and plasmid DNA. Mechanism of PEC formation involves charge neutralization between cationic polymer and DNA leading to a fall in hydrophilicity. Several cationic polymers (i.e. gelatin, polyethylenimine) also possess this property. Generally, this technique offers simple and mild preparation method without harsh conditions involved. The nanoparticles spontaneously formed after addition of DNA solution into chitosan dissolved in acetic acid solution, under mechanical stirring at or under room temperature. The complexes size can be varied from 50 nm to 700 nm.

Green Synthesis of Silver/Chitosan/Polyethylene Glycol Nanocomposites

Silver nanoparticles (Ag NPs) have emerged as one of the most intensively studied areas in the field of nanotechnology. Huang et al. [16] reported the synthesis of different metal-chitosan nanocomposites (NCs) in aqueous solution by the reduction of corresponding salts with NaBH₄. The green synthesis is a concept that is introduced to define the method used in synthesis, which is favored over solvent medium. This is because it is environmentally friendly and contains a reducing agent that is benign to the environment. Besides, it also utilizes a non-toxic stabilizer in forming Ag NPs [17,18]. In addition, Wei et al. [19] also carried out research on Cts-based silver NCs by reducing silver nitrate (AgNO₃) salts with non-toxic and biodegradable Cts. It appeared that the exclusion of NaBH₄ in the synthesis made it “greener” as compared to the method reported by Huang et al. [20]. Polyethylene Glycol (PEG) is a water-soluble polymer with a general formula H(OCH₂CH₂)nOH. PEG is also a good stabilizer for Ag NPs based on the conclusions made by several research studies as mentioned in this paper [21–23]. In one of these research works, Luo et al. reduced AgNO₃ in the presence of PEG. The researchers suggested that stabilization can be obtained due to the free polymer chains in solution, where formation of aggregates is denied because of steric hindrance. From their observation, they also proposed that increasing the molecular weight of the polymer would help in forming stable Ag NPs [24]. The chemical reduction method is commonly used to prepare Ag NPs in industrial applications because of its great advantages in generating high yields and readiness to perform the method [25]. Hence, based on the principle of green synthesis [26], Cts and PEG were used as the stabilizer and solid support to prepare the silver nanoparticles. However, also use Cooper and ferrous for synthesis of nanoparticles of Chitosan polymers.
PHARMACEUTICAL APPLICATIONS OF CHITOSAN NANOPARTICLES

Ocular Administration

Among mucoadhesive polymers explored now, chitosan has attracted a great deal of attention as an ophthalmic drug delivery carrier because of its absorption promoting effect. Chitosan not only enhance cornea contact time through its mucoadhesion mediated by electrostatic interaction between its positively charged and mucin negatively charged, its ability to transient opening tight junction is believed to improve drug bioavailability [27]. Chitosan Nanoparticles solutions prolonged the cornea resident time of antibiotic in rabbits as demonstrated by De Campos et al. and Chitosan Nanoparticles remained attached to the rabbits’ cornea and conjunctiva for at least 24 hr. In addition, De Campos et al. found that after ocular administration of Chitosan Nanoparticles in rabbits, most of drug was found in extraocular tissue, cornea and conjunctiva, while negligible drug were found in intraocular tissues, iris/ciliary body and aqueous humor. Together, these results suggested that Chitosan Nanoparticles showed to be attractive material [28].

Nasal Delivery

The nasal mucosa is an attractive route for the delivery of vaccines because it has a relatively large absorptive surface and low proteolytic activity. Importantly, nasally administered vaccines can induce both local and systemic immune responses. Mucoadhesive, hydrophilic Chitosan Nanoparticles have received much attention to overcome problem of absorption of nasally administered proteins obstacles and deliver protein antigens via the nasal route, because they strongly attach the mucosa increasing mucin viscosity. Amidi prepared and characterized protein loaded Trimethyl Chitosan Nanoparticles as a nasal delivery system. It was observed that trimethyl Chitosan Nanoparticles have a high loading efficiency and capacity up to 50%. The release studies showed that more than 70% of the protein remained associated with the Nanoparticles for at least 3 hr of incubation in PBS (pH 7.4), at 37°C. In vivo uptake studies indicated the transport of the protein across the nasal mucosa.

Delivery of Vaccines

Nanoparticles often exhibit significant adjuvant effects in parenteral vaccine delivery since they may be readily taken up by antigen presenting cells. Moreover, oral and nasal delivery of Nanoparticles are thought to have the potential to provide mucosal protective immune responses, one of the most desired goals of modern vaccinology [29]. The submicron size of Nanoparticles allows them to be taken up by M-cells, in mucosa associated lymphoid tissue(MALT) i.e. gut-associated, nasal-associated and bronchus-associated lymphoid tissue, Illum et al initiating sites of vigorous immunological responses. Immunoglobulin A (IgA), a major immunoglobulin at mucosal surface, and the generation of B-cell expressing IgA occur primarily in MALT. The
B-cell then leave the MALT and reach systemic circulation where they clonally expand and mature into IgA plasma cells. Therefore, providing not only protective IgA at the pathogen entered sites, but also systemic immunity. Despite the potential carrier for mucosal delivery vaccine, chitosan has also been reported to act as an adjuvant for systemic vaccine delivery such as increasing the accumulation and activation of macrophages and polymorphonuclear cells. Activation of macrophages is initiated after uptake of chitosan. Furthermore, chitosan has also been widely explored as the application for DNA mucosal vaccines. For instance, a chitosan-based DNA flu vaccine has been developed. This system showed high antibody level in mice after intranasal administration [30-32]

**Parenteral Administration**

Following intravenous injection, many nanoparticle systems including chitosan NP exhibited a marked tendency to accumulate in a number of tumors. One possible reason for the phenomenon may involve the leakiness of tumor vasculature. Doxorubicin loaded chitosan NP showed regression in tumor growth and enhance survival rate of tumor-implanted rats after IV administration. In addition, chitosan NP less than 100 nm in size have been developed which showed to be RES evading and circulate in the blood for considerable amount of time. Delivery of antiinfectives such as antibacterial, antiviral, antifungal and antiparasitic drugs is another common use of nanoparticles. The low therapeutic index of antifungal drugs, short half-life of antivirals and the limited ability of antibiotics to penetrate infected cells in intracellular compartments make them ideal candidates for nanoparticle delivery. Thus, it has been suggested that nanoparticles should improve the therapeutic efficacy while decreasing the toxic side effects of these drugs. In theory, chitosan NP are very attractive carrier system for these drugs as they offer many advantages such as hydrophilic surface particles, nano-size of less than 100 nm.

However, to the best of my knowledge, chitosan NP as a tool to deliver these drugs have not yet been examined [33].

**Nonviral Gene Delivery Vectors**

Chitosan is a cationic polymer with extremely low toxicity. It showed significantly lower toxicity than poly-L-lysine and PEI. Additionally, it enhances the transport of drug across cell membrane as discussed earlier. Chitosan as a promising gene delivery vector was first proposed by Mumper. Chitosan mediates efficient *in vitro* gene transfer at nitrogen to phosphate (N/P) ratio of 3 and 5. At these ratios, small chitosan-DNA complexes can be prepared in the range of 50-100 nm with a positively surface charge of approximately +30 mV. Sato *et al.* found that *in vitro* chitosan-mediated transfection depends on the cell type, serum concentration, pH and molecular weight of chitosan. Hela cells were efficiently transfected by this system even
in the presence of 10% serum. In contrast, chitosan have not been able to transfect HepG2 human hepatoma
cells and BNL CL2 murine hepatocytes. The transfection efficiency was found to be higher at pH 6.9 than that
at pH 7.6. [34]

CONCLUSION

The main goal of this review was to give an overview of different preparation techniques available for
production of Chitosan nanoparticles. It is worth mentioning that preparation of CPNPs is a state-of-art
technology. The drug-loaded nanospheres or nanocapsules can be produced by simple, safe, and reproducible
techniques available. Depending on the physic-chemical characteristics of a drug, it is possible to choose the
best method of preparation and the polymer to produce nanoparticles with desired size range with good
entrainment efficiency of the drug. Nanoparticle preparation methods have been marked by three aspects: 1)
need for less toxic reagents 2) simplification of the procedure to allow economic scale-up and 3) optimization to
improve yield and entrainment efficiency. The limitations like one particular process or technique is not suitable
to all drugs, post preparative steps, such as purification and preservation, incomplete or discontinuous film,
inadequate stability of certain active components remain unsolved. Instead of that, we mostly use Ionic gelation
and Green synthesis route for synthesis of CPNPs, as these methods are most efficient, effective and simple
compared to other methods. Despite these technological challenges, nanoparticles have shown great promise for
the development of drug delivery system.

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