Chitosan Nanoparticles for Generating Novel Systems for Better Applications: A Review

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Abstract

The environmental sustainability and use of environmentally acceptable biomaterials are major areas of scientific and industrial research. In recent times, identification and application of suitable biopolymers instead of petroleum derived conventional polymers are gaining more attention. Chitosan and chitosan derived nanoparticles and nanocomposites are very promising due to their versatile properties and have found applications in various areas such as biomedical, foods, food packaging and personal care. Many studies suggest that chitosan nanoparticles and their derivatives are one of the best barrier and film coating materials for preserving the quality of foods and for delivering skin care products, mainly due to their biodegradability and anti-microbial properties. The present review gives insight on the progress made in the application of chitosan nanoparticles specifically in foods and food packaging, personal care products and in biomedical industry. Future studies shall address establishment of industrial scale up of chitosan nanoparticles, safety and quality aspects of these chitosan derived products for human use.

Keywords: Chitosan; Chitosan nanoparticles; Chitosan composites; Food packaging; Dietary fiber; Wound healing; Gene delivery

Introduction

Chitin is the second most abundant natural biopolyaminosaccharide polymer, next to cellulose. The exoskeleton of crustaceans, shell mollusks and to certain extent a few species of fungal cell wall contain chitin and chitosan. Chemically, chitosan is deacetylated derivative of chitin. The greatest advantage of chitin and chitosan are they are biodegradable, non-toxic and less antigenic nature [1]. Based on several studies chitin is converted into chitosan and the key four steps involved in the process are deproteinization, demineralization, decoloration and deacetylation [2]. Nanotechnology can produce variety of new materials which may have wide functional purposes. The major advantage of nanoparticles is that it provides more surface area and enhanced functional properties. Chitosan and chitosan particles are used to make a wide variety of compositions containing chitosan nanomaterials, nanofibers, films, nanoclay, colloids, composites, gels and other products either alone, in combination with other natural chemical or biological or biochemical compounds [3-4]. The physical properties of chitosan nanoparticles depend on concentration of chitosan used, amount of cross linking and molecular weight of the chitosan. Many methods have been used to prepare chitosan nanoparticles such as ionic gelation method using acetic acid and sodium tripolyphosphate [5]; desolvation method using sodium sulphate and acetone, spray drying and covalent cross linking [6-8].

Chitosan and chitosan nanomaterials have a wide array of applications in biomedical, pharmaceutical, nutraceutical, foods and personal care areas [9-11]. In health care industry, chitosan and chitosan materials are used as antimicrobial, antitumor, antiulcer, antidiabetic and cholesterol lowering agent. In the areas of food, food packaging and personal care, the antioxidative property, film forming capability, antimicrobial effect, clarifying effect, gelling nature, additive property, color stabilizing effect, anti-inflammatory and fiber like property of chitosan and chitosan derivatives make them an excellent choice [12-15]. In biomedical and pharmaceutical areas, chitosan nanomaterials play a pivotal role in delivery of genes and molecules, encapsulation, bioavailability enhancement, oral delivery of growth factors, insulin and optimal drug delivery [16-18]. Considering the importance of safety, quality, efficacy and environmental sustainability, the application of chitosan derived materials seem expanding as evidenced by rapid increase in publications, number of patents and products. Hence, in the present manuscript, we have attempted to review the current scientific developments in chitosan nanoparticles and their applications mainly in foods, personal care, and food packaging and also in other areas of applied biology and pharmaceutical sciences.

Chitosan in Foods

Application of chitosan in foods is gaining interest, specifically after the chitosan derived from shrimp has been recognized as Generally Recognized as Safe (GRAS) for common use in foods by the US Food and Drug Administration in 2011. The role of chitosan particles in foods could be viewed in broad categories based on their functions such as i) Antimicrobial property, ii) Additive property that includes functions such as color stabilization, emulsification, antioxidant activity and iii) Dietary fiber like property aiding water holding and fat entrapment, thereby imparting health benefit. Due to the multifunctional properties of chitosan, in the last decade the number of patents issued on the processing of chitosan and its derivatives related to the use in food industry has increased [15].
Antimicrobial property

Antimicrobial function of chitosan is known against wide variety of Gram-positive, Gram-negative bacteria, mould and yeast. The polycationic property of chitosan exhibits antimicrobial activity due to its ability to interact with negatively charged cell membranes of these microbes [19]. Both Gram positive and Gram negative bacteria have been found to be affected by chitosan having a minimum inhibitory concentration (MIC) in the range of 100 to 2000 ppm. The variations in the MIC values even for the same species across studies may be due to varying degree of acetylation of chitosan, since acetylation is associated with antimicrobial effect. Similarly, micro and nano chitosan particles have been found to show antifungal activities with MIC values ranging from 10-500 ppm against variety of species. Fungal species such as Botrytis cinerea and Drechslera sorokiana exhibited very low MIC of 10 ppm while several other fungi such as Candida albicans, Microsporum canis, Aspergillus fumigatus, Aspergillus parasiticus have shown higher MIC between 1000 and 2000 ppm, while for Trichophyton mentagrophytes and Byssoschlamys spp. the MIC values were found about 2000-5000 ppm [19]. In addition to the concentration of nanoparticle, size and zeta potential of both low and high molecular weight chitosan tend to influence the inhibitory effect against C. albicans and F. solani. However, only high molecular weight chitosan solution and nanoparticles were able to inhibit the growth of A. niger [20].

Three mechanisms have been put forward to explain the antimicrobial activity of chitosan: i) the positively charged chitosan and the negatively charged bacterial cell membrane, enhancing the membrane permeability [21]; ii) penetrating power of chitosan in to the nuclei of the microorganism leading to the inhibition of mRNA and protein synthesis; and iii) the chelating ability thereby creating an external barrier leading to essential nutrients suppression [19,22]. In general, it was observed that lower the molecular weight and the degree of acetylation, the higher would be the effectiveness of chitosan in reducing the growth and multiplication of microorganisms. Previous reports suggest that antimicrobial activity of chitosan is modulated by acidic conditions and this may be due to the loss of positive charges on the amino group at neutral pH and hence antimicrobial activity of chitosan may be decreased at neutral pH [23].

Several studies have focused on the antimicrobial action of the chitosan micro particles on pathogenic organisms, but little is known about the antimicrobial potential of chitosan and chitosan nanoparticles against food spoilage microorganisms. Yeast was completely eliminated following the addition of 0.3 g of chitosan per liter of pasteurized apple-elder flower juice stored at 7°C. The total count and more specifically the lactic acid bacterial counts increased at a slower rate. However, the increase of bacterial count was relatively lower as compared to the increase in the control [24]. Chitosan has been used as a clarifying agent in several juices including orange, pomegranate, apple and others. Earlier, use of chitosan in the treatment of fruit juices showed inhibition of yeast but failed to inhibit E. coli/O157:H7 suggesting that it may increase the food poisoning risk [25]. However, Abd and Niamah showed chitosan at a concentration of 0.2 to 1 g/L in apple juice could inhibit growth of some spoilage bacteria, mould and yeast [26]. Recent developments in the area of chitosan admixtures, especially arginine-functionalized chitosan showed antibacterial activity against E.coli/O157 in chicken juice [27].

Earlier we had indicated liposomes and chitosan derived materials could be better delivery agents for novel vaccines [28]. Furthermore, our collaborator’s work towards understanding the efficacy of Chitosan-dextran sulphate (CD) nanocapsules in delivering specific drugs against intracellular bacterial pathogens suggest that CD nanocapsules could effectively deliver specific antibiotics against intraphagosomal Salmonella leading to killing and clearing the pathogen [29]. These above findings along with other available evidences suggest that chitosan and chitosan derivatives seem to possess significant antimicrobial activity and potential drug delivering system.

Additive property

Food additives are integral component of any packaged food available today and are under regulatory guidelines. A plethora of additives are used primarily to preserve, blend, thicken and color food. Considering the wide variety of functional properties of chitosan and due to its natural source, chitosan in various forms and combination are one of the best known food additives. The importance of chitosan as food additive could be highlighted by the fact that about three decades back Japan and Korea have approved chitosan as a food additive [12]. Low molecular mass chitosan and chitosan oligosaccharides have been used generally as food additive and are suggested to have enhanced efficacy especially in acidic food products irrespective of the size and acetylation status of chitosan [30]. It is pertinent to note that chitosan acts as thickening agent and increases the viscosity of food without any allergic or toxic effect, due to its natural origin [12]. Chitin and chitosan have been shown to support stability on food emulsions. Also, microcrystalline chitin as additive distributes evenly and enhances flavour and taste of food by forming pyrazines during normal cooking temperatures with characteristic roasted taste and aroma [31].

Color stabilization

Chitosan binds to many classes of dyes such as disperse, acid, sulphur, reactive and others with high affinity due to the molecular structure and also the rate of diffusion of color appears similar in both cellulose and chitosan [12,32]. The influence of packaging made of chitosan on color stability in refrigerated ground beef has been studied previously [33]. Also, in fresh ground beef patties, chitosan in combination with rosemary extract showed synergistic effect resulting in the most intense red color stabilization and anti-oxidative protection [34]. Similarly, chitosan based edible films and packaging of fresh cut fruits and vegetables have been implicated strongly in color stabilization and retention, extending shelf life. However, one of the main limitations of their application is in oligosaccharide engineering due to difficulty to produce the specific glycosidic structures [30].

Antioxidant activity

Several studies have previously reported antioxidative properties of chitosan especially in food products that contain high amounts of unsaturated fatty acids which are sensitive to oxidation during storage [35,36]. Chitosan scavenges free radicals or chelate metal ions from the donation of a hydrogen or the lone pairs of electrons and this is considered responsible for the antioxidant ability and free radical scavenging activity of chitosan [37]. In addition, the low molecular weight of chitosan and the degree of quaternization appear to contribute to the antioxidant capacity of chitosan [38]. Addition of other naturally occurring phenolic antioxidant such as carvacrol or polyphenolic compounds with chitosan has been suggested to have synergistic effect as mutual prodrugs [38,39]. Low molecular weight chitosan oligomers and nanoparticles have been shown to have
significantly higher antioxidant activity when compared to higher molecular weight chitosan oligomers [40].

**Dietary fiber and health benefits**

Use of chitosan as a dietary fiber is one of the promising applications of chitroan in foods. Albeit chitosan does not exhibit classical properties of fiber, the high water binding nature, upper intestinal tract non digestibility and being a biopolymer supports chitosan with dietary fiber-like properties. However, the chemical form and the polymerization degree determine the efficacy of chitosan through oral administration. Solubility of chitosan is promoted in the acidic environment of the stomach and this allows chitosan to bind to fatty acids and lipids and it is proposed that greater portion of such lipids bound to chitosan is known to be excreted rather absorbed [41]. Several patented formulation of chitosan with other fibers in the form of tablets, capsules and drinkables are available as nutritional supplement [12]. The chitosan fibers have been implicated in management of body weight [42] and in lowering lipid levels [43].

Chitosan as a dietary fiber has been proposed to play important roles in fat entrapment, glucose dialysis and water holding capacity [12]. Further these authors concluded that chitosan acts similar to soluble fiber albeit the mechanism between vegetable soluble dietary fiber and chitosan might be different. Methods to determine the chitosan levels in foods seem to differ substantially and also the amount of detected chitosan depends on the presence of reducing sugars in the food, since Maillard reaction might happen between the chitosan and reducing sugar [12].

The beneficial effect of chitosan and chitosan derivatives in prevention and treatment of age-related dysfunctions especially in elderly such as diabetes mellitus, hypercholesterolemia, atherosclerosis, cardiovascular diseases, neurodegenerative diseases, and cancer has been reviewed earlier [44]. Liu et al. showed that in Streptozotocin treated rats which were fed with low and high molecular weight chitosan there was a significant decrease in liver gluconeogenesis and increase in skeletal muscle glucose uptake suggesting the potential effect of chitosan in alleviating diabetic hyperglycemia [45].

The role of chitosan in the areas of cardiovascular health and weight management is relatively less explored. Orally administered chitosan about 1.2 grams per day for 8 weeks mildly reduced hypercholesterolemia i.e. reduction of total and LDL cholesterol, one of the important risk factors in cardiovascular diseases [46]. The use of chitosan as nutraceutical product in maintaining cardiovascular health by regulating lipid profile and having benefit on anthropometric profile is reviewed recently [44,47]. Furthermore, studies carried out in rats fed with high cholesterol suggest that chitosan may exert effect on fecal bacterial enzyme functions, change the level of short-chain fatty acid concentrations and extend beneficial effect to the distal colon in rats [48]. Also, evidence suggests that the microbiota of the caecum in the rats supplemented with copper loaded chitosan nanoparticles changed beneficially [49]. Taken together, these findings on the dietary fiber like properties of chitosan and the ability of chitosan to bind to lipids provide compelling evidences to hypothesize that chitosan induced body weight reduction may be attributed to the alteration of the intestinal microbiota. However, detailed randomized clinical studies to delineate the effect of chitosan formulation in weight reduction need to be conducted.

**Chitosan in Gene Delivery**

Gene delivery has a great potential in treatment of many diseases by introducing the genetic materials into the living systems [50]. Modified forms of chitosans like O-alkylated chitosan, has been used in delivering genetic materials [51]. Chitosan has primary amine groups and these repeating groups become protonated in acidic conditions. Chitosan derivatives have better solubility and buffering capacities [52]. This property of chitosan derivatives has efficiently been used to transfact different types of cells lines for delivering genes. Chitosan alone may have lower transfection efficiency and to increase the efficiency, Oliveira and colleagues studied diethylamylene – chitosan derivatives which resulted in condensation of DNA [17]. Lu et al. observed that the transfection efficiency of chitosan-graft-polyethyleneimine DNA was similar to that of Lipofectamine 2000 in chondrocytes and synoviocytes without affecting cell viability [53]. Due to low cytotoxicity, the authors have suggested that chitosan might be a safe and efficient vector for gene delivery in chondrocytes and synoviocytes. It is thought that chitosan and polyethyleneimine complexes protect the DNA from serum degradation [54]. Previous study has demonstrated quaternized oligomers to be efficient than oligomeric chitosans and the size of the complexes ranged from 200-500 nm [55]. However, it was reported that release of DNA from chitosan could be changed by formulation conditions [18]. Li and Birchall tested cationic polymer chitosan for dispersability and reported higher deposition of chitosan on modified lactose formulation [56].

Various cells have been shown to have varying transfection efficiencies and studies have shown that HEK293 cells tend to take chitosan DNA micro particles more efficiently than HeLa and 3T3 cell lines [57]. In addition to increase in the levels of expression of reporter gene, DNA uptake was found different among various cell lines. It was also observed that degree of deacylation and molecular weight of chitosan play an important role in expression of genes [16]. The ratio of combinations modulated the expression levels and was attributed to electrostatic binding and particle stability [58].

In addition to the conventional methods of gene delivery, novel systems such as magnetic nanoparticles coated with chitosan and chitosan nanobubbles have also been studied. The spherical ferriferous oxide complexes were found to have significant effect on controlled release of DNA [18]. Others have shown that DNA loaded chitosan nanobubble shells with a diameter of about 300 nm with positive charge as a system for delivery of DNA and found efficiency in delivering genetic materials without affecting cell viability [59].

The storage stability of chitosan nanoparticles at various temperatures have also been examined by earlier workers [60]. In a series of studies, Mohammadi et al. showed Fibronectin Attachment Protein of Mycobacterium bovis (FAP-B) ligand was added to chitosan nanoparticles to form chitosan-DNA-FAP-B nanoparticles via electrostatic attraction [61]. Chitosan-DNA-FAP-B nanoparticles were stable for one month when stored at -20°C and retained their size and transfection efficiency when stored under these conditions and not at 5 and 24°C, suggesting higher temperatures not suitable for stability of the chitosan nanoparticles [60]. Taken together with the available laboratory evidences, gene delivery through chitosan particles seems to hold a great promise for the future.
Chitosan in Wound Healing

Chitosan is known to have potent intrinsic antimicrobial property and in addition to this, chitosan has also been found to exhibit efficiency in delivering antimicrobial agents having beneficial effect on wound healing [62]. Chitosan has been implicated to have effect on homeostasis, inflammation, migration, proliferation and maturation, the five different phases of wound-healing. Chitosan also acts as carrier for growth factors and promotes wound-healing accelerator not only by modulating the functions of inflammatory cells but also by increasing the tensile strength of wounds [62]. Chitosan has been used in treating burn injuries in rats and for skin grafting and bone wound repair in dogs [63]. Chitosan can be made into membranes, nanofibers, scaffolds and has excellent swelling, biodegradable and hydrophilic properties [64]. Various types of delivery formats have been made with chitosan and chitosan derivatives, such as chitosan mesh membrane, to study wound healing [65]. Histological and clinical studies have shown that with the application of chitosan mesh membrane, wound healing was faster with enhanced re-epithelialization and regeneration of granular layer compared to the wound treated without chitosan [65]. Chitosan powder has also been used for treating wounds and it was observed that it took only three weeks to heal the wounds applied with chitosan whereas in control groups without chitosan it took four weeks to heal the wounds [63]. Some workers have used a combination of chitosan, gelatin and curcumin for studying wound closure [66]. Workers have used a matrix composed of cotton fabric with chitosan that is embedded with silver nano particles and other researchers have attempted to combine chitosan and silver nanoparticles for improving wound healing properties chitosan [67]. In addition, chitosan complex with curcumin and tulsii has also been patented in a matrix to promote wound healing. The chitosan films were evaluated for water absorption capacity, tensile strength and anti-bacterial activity in albino rats and the wound healing was better in wounds treated with chitosan composite film along with gentamycin. A hydrogel film made of chitosan and sodium pyrrolidone nano silver oxide was found to be useful in enhancing wound healing due to its flexibility and other properties [68].

Researchers have also constructed nanofibers of chitosan as novel approach of using chitosan for wound healing. Imin-ochitosan was synthesized, its solution was electrospun and the authors obtained nanofiber web ranging from 70 – 400 nm and nanofiber webs showed an excellent antimicrobial property [69]. In another communication, electrospun nanofibers of chitosan and poly ethylene oxide were loaded with vascular endothelial growth factor [69] and platelet derived growth factor encapsulated nanoparticles for sustained release of growth factors. These encouraging results show that chitosan nanoparticles inhibited bacterial growth favoring accelerated wound healing and mediated quicker collagen deposition at the wound area [70].

The adhesion of chitosan to the wounded skin surface has also been studied and it was found that double the amount of chitosan formulation remained on the skin surface but the study could not find any difference in the detachment properties in comparison with a synthetic polymer adhesive [68]. More recently, chitosan and nanoparticle based films such as chitosan poly vinyl pyrrolidone nano silver oxide films and titanium dioxide were found more effective in wound healing compared to chitosan alone [71]. These above findings provide ample evidences to suggest that chitosan and chitosan nanoparticles may function as one of the best biopolymers available for wound healing.

Chitosan in Personal Care Applications

Various formulations of chitosan have been patented for multiple processes and products for functional applications [15]. Being a polymer, chitosan forms a film on hair or the skin, locks the moisture and thus provides sheen appearance to hair and skin. A combination of the salt of pyrithione with chitosan was shown to be a slow release. Chitosonic® acid (Carboxymethyl Captoprol Chitosan) can also be used in personal care products and Chitosonic® acid has recently been approved by the Personal Care Products Council as new cosmetic ingredient [72]. Interestingly, Chitosonic® acid is water soluble, can form a nano-network structure at concentration higher than 0.5% and can be made into nanosphere [72]. Chitosonic® acid has antimicrobial activities, anti-oxidant function, possesses good hydration activity and has shown less cytotoxicity as tested in L-929 cells [72]. Studies have shown that sulfated chitosan with varying molecular weights exhibited anti-coagulation activity similar to that of heparin [73]. Previous workers have used carboxymethyl chitosan and suggested importance of these chitosan variants in hydrogel and other formats for personal care products such as infant diapers, feminine hygiene and matrices for controlled release [74]. These workers have carried out study showing the structural properties of polyampholytecarboxymethyl chitosan-g-poly hydrogel and they observed the quantity of non-freezing water in the hydrogel appeared to increase gradually as the degree of cross-linking increased [74]. Hence, various studies support the importance and benefit of chitosan and chitosan derived variants for personal care products due to their different unique functional properties like film forming property, water resistance nature, anti-microbial activity, antioxidant activity and nontoxicity.

Chitosan Nanoparticles and Food Packaging

Over the years there is a tremendous increase in the use of non-biodegradable materials or plastics derived from petroleum products as food packaging materials. Such uncontrolled use of these materials resulted in the accumulation of plastic waste in the environment and pose serious concerns to the ecosystem [75]. Attempts have also been made from time to time from various parts of the world to address the demand for sustainability and environmental protection by exploring alternative and eco-friendly biomaterials for food packaging industries [76,77]. Hence, biopolymers are the best suitable alternatives to be exploited and developed into eco-friendly food packaging materials due to their biodegradability [78] and among these, chitosan is most favoured naturally available biopolymer. However, the major disadvantages of using chitosan are its poorer mechanical, thermal and barrier properties as compared to the commonly used non-biodegradable polymers from petroleum. Packing methods employed for such foods is mainly aimed to maintain quality, safety and value of foods [79]. The advancement of nanotechnology has been tremendously helping the progress of the chitosan based materials by the incorporation of newer nanoparticles thereby improving the functional properties of chitosan matrix having tremendous applications in food packaging industry [80]. Due to these properties chitosan films have been used as packing materials for maintaining the quality of preserved foods [81-84].

Many measures and parameters have been considered for “value” added or “active” packaging of foods which include antimicrobial and barrier permeability packing [85,86]. Such active packaging system ensures quality assurance, shelf life and safety concerns of the packed foods. Many materials have been used from time to time prepare edible films and coating to pack foods aiming at controlling
Table 1: Edible Chitosan coating/films improves shelf life of natural products.

| Product            | Function/Effect                                                                 | Reference |
|--------------------|--------------------------------------------------------------------------------|-----------|
| Mango              | Retarded weight loss and decline in sensory quality, inhibited growth of micro organisms | [114]     |
| Sponge Gourd       | Reduced weight loss and respiration rate, maintained firmness and visual appearance. Suppressed phenylalanine ammonia lyase activity, polyphenol oxidase and peroxidase activities | [115]     |
| Peeled  litchi fruit | Retarded weight loss and decline in sensory quality, reduced the increase in polyphenol oxidase and peroxidase activities | [116]     |
| Fuji apples        | Retarded enzymatic browning of cut apples, decreased respiration, transpiration and suppressed softening of tissues | [117]     |
| Papaya             | Micronized chitosan retarded water loss, inhibited the growth of microorganisms, retained the color and increased shelf life | [118]     |
| Orange juice       | Chitosan at lower optimal concentration of 1 g L− 1 extended the quality, reduced enzymatic and non-enzymatic browning, reduced spoilage, maintained ascorbic acid and carotenoid levels | [119]     |
| Mango              | Chitosan alone decreased decay of fruit during storage, weight loss, delayed coloring, pH and titratable acidity. Chitosan with teapolypheon enhanced shelf life extension | [120]     |
| Strawberries       | Delayed weight loss, decay percentage, titratable acidity, pH, total soluble solids. Maintained higher concentrations of total phenolics and total anthocyanins | [121]     |
| Fresh cut Mushrooms | Delayed discoloration associated with reduced enzyme activities of laccase, phenylalanine ammonia lyase, peroxidase, catalase and polyphenoloxidase. In addition reduced enzyme activities of cellulase, total amylase and α-amylase thereby slowed down texture | [122]     |
| Carrot shreds      | Chitosan alone could retain better color, superior sensory, lower microbial load and retain vitamin C. Chitosan with citric acid treatment retained beta carotene | [123]     |

Chitosan based nanocomposites

Modifications or alterations were attempted to improve the functional characteristics of chitosan such as the use of chitosan composites [80]. Efforts were made to prepare chitin whiskers by acid hydrolysis of chitin by various scientific teams and obtained various dimensions of whiskers ranging length from 417nm - 500nm and width 33nm - 50nm [97,98]. It is understood that nanoreinforcement is more suitable for biopolymers like chitosan for increasing their functional properties as compared to commonly used petroleum derived polymers. Such nano-reinforcement, in fact, may have more benefit in managing cost besides improving the properties of chitosan type biopolymers [99]. The chitin whiskers were found to improve tensile strength and water resistance of the matrix when added to soyprotein isolates thermoplastics [97]. Similarly, when chitosan whiskers were added to chitosan films, it was observed that whiskers enhanced the tensile strength and water resistance of the chitosan films [98]. Chitosan nanoparticles can be prepared by ionic gelation [100] and it was found that incorporation of chitosan-tripolyphosphate nanoparticles in hydroxypropyl methylcellulose increased the mechanical and barrier functions of the films [101].

Methods of using reinforcing fillers (nanofillers) with specific dimensions helped to obtain specific chitosan composites with improved mechanical and barrier functions of matrix chitosan [102-105]. The poor properties of chitosan can be improved by the incorporation of nano-reinforcement into the chitosan polymer. Nanocomposites of chitosan have many applications in various areas of human health care management such as medicine, food industry, paper industry, etc. Chitosan is known to form nanocomposites with montmorillonite (MMT) and represent an excellent sorbent material [106]. These chitosan-based composites films and coating provide...
promising advantage for food packaging industry due to their ability to support the shelf life of food products [107-109].

The functional properties of chitosan nanocomposites mainly depend on the nanofillers and the physical properties of nanofillers such as shape, size, etc. A nanofiller particle has to be at the nanometer scale of 100 and various types of nanofillers have been reported and described [80]. Hence, the types, size, surface areas and other physical and chemical properties of nanofillers can greatly enhance the functional properties of the chitosan-based nanocomposites and these depend on the ratio of particle length to thickness among particles, platelets and fibers (Figure 1). It was observed that clay loading nanocomposite films were found to have improved oxygen barrier than neat chitosan film. Hence the incorporation of clay to the chitosan matrix is known to improve the permeability and barrier properties and these clay-chitosan nanocomposite films seem suitable for food packaging and protective coatings [80]. Table 2 depicts various types of nanofillers used for chitosan polymer, various types of composites, preparation and their applications [80].

| Nanofiller type | Nanomaterial used | Nanocomposite | Preparation method for chitosan nanocomposite | Application |
|-----------------|------------------|---------------|-----------------------------------------------|-------------|
| Particles       |                  |               |                                               |             |
| Metal oxide     | ZnO, SiO<sub>2</sub>, TiO<sub>2</sub>, Al<sub>2</sub>O<sub>3</sub>, Fe<sub>3</sub>O<sub>4</sub>, CuO | Thin films, hydrogel, powder | Electrospinning, ultrasonic dispersion, electrophoretic deposition, solution mixing, and freeze drying | Biosensor for nucleic acid, drug delivery, enzyme immobilization, photocatalysis, water purification, UV protection of fabric, and water treatment |
| Metals          | Ag, Au, Pt, Pd, Co, Ni | Thin films, hydrogels | Solution mixing, spin coating, dip coating, co-precipitation, and electrochemical deposition | Cell stimulation, antibacterial coatings, biosensor, catalysis, etc. |
| Other           | Bioactive glass, CdS quantum dots | Hydrogel, powder | Solution mixing and freeze drying | Thermo-responsive injectable scaffolds, tissue engineering, and latent finger mark detection |
| Fibers          | SWCNTs, MWNTs, Fe nanowires | Composite fibers, powder, films | Solution spinning, freeze drying, pH, electrical actuators | |

Table 2: Summary of various nanofillers used in chitosan matrix, the nanocomposite types, and their preparation methods and targeted applications.

As an alternative to plastic materials, Mura et al. reported the excellent improvement in the mechanical properties of a nanostructured film composed of chitosan, methylcellulose and silica nanoparticles which may replace plastic for packaging foods thereby reducing the environmental pollution by plastics [110]. Another interesting work done by de Moura et al. show the application of chitosan nanoparticles-carboxymethylcellulose films [111]. Chitosan nanoparticles ranging from 8-110 nm were used to make chitosan nanoparticles-carboxymethyl cellulose films with better mechanical and thermal properties. This study supports, as authors argue, that carboxymethyl cellulose films with chitosan nanoparticles prepared would provide a suitable material for food and beverage packaging applications [111].

Attempt was also made to increase the hydrophobicity of chitosan by making Dodecyl succinylphthaloyl chitosan (DS-g-PHCTS) films without affecting anti-bacterial activity of chitosan and thus increasing functional properties of chitosan which appears more suitable in food packaging and coating [112]. Similar to this, Bordenave et al. carried out studies to increase water resistance of

![Figure 1: Various nanofiller shapes and typical aspect ratios. Reproduced with permission from Pillai and Ray [80].](image-url)
chitosan and paper based materials for environmentally friendly food packaging materials [113]. In this study these workers have made special chitosan-papers by coating papers with chitosan-palmitic acid emulsions or with a blend of chitosan and O,O’-dipalmitoilychitosan (DPCT) and thus improved the liquid water resistance and anti-bacterial activity suggesting the importance of these chitosan and paper based materials having much biodegradability. To conclude, considering the recent research progress, industrial demand and environmental sustainable issues, the use and application of chitosan and chitosan derived materials for active packaging for food industry are very promising.

**Future Research**

Present environmental scenario and sustainability issues force the industries to search for natural alternatives instead of synthetic polymers for various applications. Chitosan and chitosan nanoderivatives are one of the best known and studied natural polymers with versatile functions. However, the major concern with the use of macro, micro or nano chitosan material, especially in food industry is the cost and less functional performance compared to the conventionally available synthetic polymers.

The future research relating to chitosan and nanochitosan containing materials should be based on the current caveats and pitfalls in using chitosan. There are a few concerns that need to be addressed on top priority to have a wide acceptance of applications of chitosan materials viz. i) quality of the chitosan particles needs to be established ii) oligosaccharide engineering for chitosan applications as food additives needs to be studied in detail, iii) stability of chitosan composites especially used for transfer of biomolecules such as transfection process needs to be improved, since current operating temperature are in a narrow range, iv) improvement in the mechanical, thermal and barrier properties compared to synthetic polymers and v) reduction of inconsistency of raw material specification such as the molecular weight and the acetylation content.

Albeit chitosan compositions are known to be nontoxic and non-allergenic, supporting evidences may be made available to support the fact that chitosan and chitosan derivatives have least effect on humans under long term exposure and consumption. The limited exposure is due to the differential regulatory approval regimes across the globe. Only few regulatory agencies or countries have proposed the use of chitosan compositions for multiple applications such as US FDA (GRAS status), approval for use in water purification upto 10 mg/L of potable water by US EPA and as food additive in Japan and Korea. Uniform globally acceptable criteria shall be established to determine the quality and characters of modified chitosan biopolymer for human use.

Future research in the areas of food, food packaging and personal care needs to focus on the “value added packaging” or active packaging. Vargas and Gonzalez-Martinez proposed that chitosan inclusion into recycling composite materials for food packaging [15]. In addition, attempts may be made to make multilayer packaging where in the synthetic polymer may act as liquid barrier, while the nanochitosan layer would impart antioxidant benefit as oxygen barrier. Furthermore, blends of chitosan with other chemicals or molecules such as chitosan silver, chitosan pectin and chitosan natural biomolecules towards antimicrobial efficacy may be studied in detail with an approach to make such composites at industry level. Development of chitosan biosensors for purification of water or for clarifying juices will be a useful tool for screening and packaging. It is also necessary to establish the compatibility and feasibilities of these chitosan nanomaterials with different types of food items. It also remains to study how the nature and characteristics of food items stored would affect the stability and functional characteristics of chitosan nanomaterials used for packaging these foods.

It is a paramount importance that the chemical methods used for preparations of chitosan and its nanoparticle varieties shall be well established, validated and which are scalable at industrial level. Therefore, the simple and low cost production process of chitosan derived nanoparticles having valuable application in human health care management need to be established. Even chemical, physical and other qualities and characteristics of such chitosan derived materials should be well tested and analytically established. Industry supported private and academic research amalgamated with regulatory agencies would accelerate the development of such naturally available chitosan and chitosan derivatives for human use. These combined efforts to develop chitosan derived products would certainly support environmental sustainability for human health and existence of other living system.

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