

Chitosan nanoparticles: Preparation, food applications and health benefits

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ABSTRACT: Chitosan (CS) is a biodegradable and nontoxic natural polymer extensively employed in food and biomedical industries pertaining to its excellent bioactivities and biocompatibility. Superior gelling characteristic of CS, due to its polycationic nature, is achieved in the presence of anionic substances. The ionic gelation method with the aid of mechanized stirring or ultrasound has been popularly used to produce CS nanoparticles (CSNPs) of various sizes (84–600 nm) with encapsulation efficiency of 23–97%. In addition, other techniques, including emulsion cross-linking, coacervation and reverse micelle have been implemented. Moreover, nanoparticles (NPs) of various sizes (70–1000 nm) have been produced by coating nanoliposome with the help of CS to enhance their stability and activities. The CSNPs loaded with active compounds have improved the stability during storage, sustained release, higher bioactivities such as antioxidant and antimicrobial activities than the free form. This review also aims to cover the major applications of various CSNPs as an encapsulating agent, food additives and edible coatings. Finally, the efficacy of CSNPs is also addressed for medical uses, such as reducing glucose or fat levels, wound healing and cancer treatments.

KEYWORDS: chitosan, ionic gelation, nanoparticles, edible coating, human health

INTRODUCTION

Chitosan (CS) is a deacetylated (DA) product of chitin, which is broadly used in medicinal and pharma industry due to its biodegradable, biocompatible and less toxic nature [1]. It also has a wide array of usages such as drug delivery, packaging material, antibacterial agent, tissue engineering, antiaging agent, antibody response enhancer as well as the remedy of ailments (such as cancer treatment) [2, 3]. In general, CS is an aqueous acid soluble biomolecule. Low MW CSs (2.8–87.7 kDa) possessed higher solubility and functional activities than high MW counterparts (604–931 kDa) [2, 4]. To better exploit CS, it has been reduced to low MW using various methods, such as hydrolysis by chemical, enzymatic and physical methods. The conjugation with various compounds to enhance the potential applications of CS has been documented [4]. Apart from various bioactivities, cationic nature of CS associated with the amino group at C-2 shows a potential to form gel or acts as a crosslinking agent with unique biophysical and biochemical properties. Those properties of CS in conjunction with the use of nanotechnology have led to the formation of CS nanoparticles (CSNPs).

Nanoparticles (NPs) possess unique properties

such as color, diffusivity, toxicity, solubility, strength as well as magnetic, optical, and thermo-dynamic properties due to high surface ratio [5]. It can interact with atoms, molecules or macromolecules for the targeted reaction. CSNPs have both characteristics of CS and NPs, such as surface and interface effect, small size and quantum size effects. Applications of CSNPs as food preservative, active packaging material, drug carrier, and encapsulating agent for bioactive compounds have been gaining interest for the last few decades [6]. Therefore, CSNPs' preparation and their applications in various sectors are revisited in this review.

PREPARATION OF CSNPs

Various methods have been developed to formulate CS particulates as a delivery system. Those methods are mainly dependent upon the nature of the bioactive compounds, particle size, release kinetic, stability, residual toxicity, and mode of delivery of the final products [7]. In general, ionic gelation, emulsion cross-linking and coacervation or precipitation methods were widely employed in the preparation of CSNPs [7–9]. In addition, spray drying, emulsion-droplet coalescence, reverse micellar, and sieving are also known for the preparation of NPs,

but are still rarely used [7, 10].

Ionic gelation (IG)

IG, also called ion-induced gelation, is a non-toxic, organic solvent-free, easy, and regulated method to produce the desired particles. For preparation of CSNPs via IG method, CS (0.1–2%, w/v) solution is firstly added to the tripolyphosphate (TPP) solution with ranged concentrations (0.1–0.5%, w/v) via drop-wise process and constant stirring. Sizes and encapsulation efficiency (EE) of the CSNPs varied in the ranges of 84–600 nm or higher and 23–97%, respectively (Fig. 1A and Table 1). The catechin-loaded CSNPs prepared by IG method (0.05 or 0.1% of CS and 0.1% TPP) have the size range of 110–130 nm and EE of 90% when CS/TPP ratio of 2:1 and pH 5.5 were used [11]. The positively charged amino groups of CS at the C-2 position interact with negatively charged phosphoric groups of TPP [12]. During this process, three kinds of phenomena occur, involving (1) solution, (2) aggregation and (3) opalescent suspension, in which the last one determines the formation of NPs [13]. Various factors, such as concentration, MW and ratios of ingredients used, mixing style, and pH of the system, have the influence on the formation of NPs. Generally, with increasing MW of CS, the particle size of NPs was increased, which was more likely due to the formation of long chain beaded structure of NP prepared using IG method [14]. Similarly, EE was influenced by the MW of CS. For example, when MW of CS (1.5 mg/ml) was increased from 50 to 150 kDa, the EE of tea catechin augmented from 26 to 47%, respectively, and then decreased to 36%, when MW was increased further to 300 kDa [15]. The addition rate of TPP modified the shape and chain length of NPs beads. This was more likely due to competition between phosphate ion and hydroxyl group to interact with the amino group of CS. Various bioactive compounds, including polyphenols, have been encapsulated in CSNPs prepared by IG methods (Table 1). Particles produced via this method possessed high dispersibility index but showed non-uniform surface morphology, lack of active surface site for attachment as well as poor mechanical properties [13, 14].

Liposomes

Liposomes are colloidal particulates with lipid bilayer membranous structure (Fig. 1B). Liposomes can be used in both lipid and aqueous phases as a delivery agent for various pharma drug; and they have also been used as vector for various

macromolecules, such as proteins, DNA and RNA [16, 17]. Generally, nanoliposomes (NLs) formation is based on hydrophilic and hydrophobic interaction between phospholipids and water molecules. The sizes of NLs are varied widely (70–600 nm), based on their applications [18]. Various methods, such as thin film evaporation, sonication, reverse phase evaporation, melting, and freezing-thawing, have been employed to obtain different types of liposomes, multilamellar, large or small unilamellar, etc [16]. The different structures of NLs were governed by the nature and concentration of core material, phospholipids and stabilizers. Those factors affect size, EE and release of the active compounds [19]. The cationic nature of CS forms an ionic bond with negatively charged phospholipids, leading to the formation of CS-coated NLs. Quercetin was loaded into CS-lecithin NLs (Q-NPs) through the electrodeposition method [20]. The Q-NPs possessed smaller particle size (350–600 nm) and high EE (71.14%). The zeta potential of Q-NPs was 35.12 mV, suggesting that vesicle prepared using this method had high stability. In general, zeta potential greater than 30 mV or less than –30 mV can be dispersed stably in medium [20]. In another study, when vitamin-C-NLs were prepared using ethanol as a solvent along with phosphatidylcholine and cholesterol ratios of 40:60 and 60:40, NLs having sizes of 97.4 and 95.8 nm were produced, respectively. When those NLs were coated with CS at various concentrations (0.1–1.0%), mean particle diameter was increased (100–460 nm) with increasing CS concentrations [21]. This was more likely associated with the augmenting number of layers or interactions of CS chains over the NLs resulting in a higher viscosity of the suspension.

Emulsification method

The emulsification process is implemented to produce both nano- and micro-particles using water-in-oil or oil-in-water nanoemulsion (NE) with and without a cross-linking agent such as glutaraldehyde or TPP [22, 23]. The cross-linking agents induced hardening of the droplets formed during emulsification [24]. CSNPs, having an average diameter of 250 nm, were used for encapsulation of 5-fluorouracil (5FU) or immobilization of aminopentyl-carbamoyl-5FU or aminopentyl-ester-methylene-5FU in the presence of glutaraldehyde using emulsion method. In this method, 10 ml of glutaraldehyde was saturated with toluene (11 mg/ml) containing 10% (v/v) Span 80 [25]. Generally, the water-in-oil NE is prepared by in-

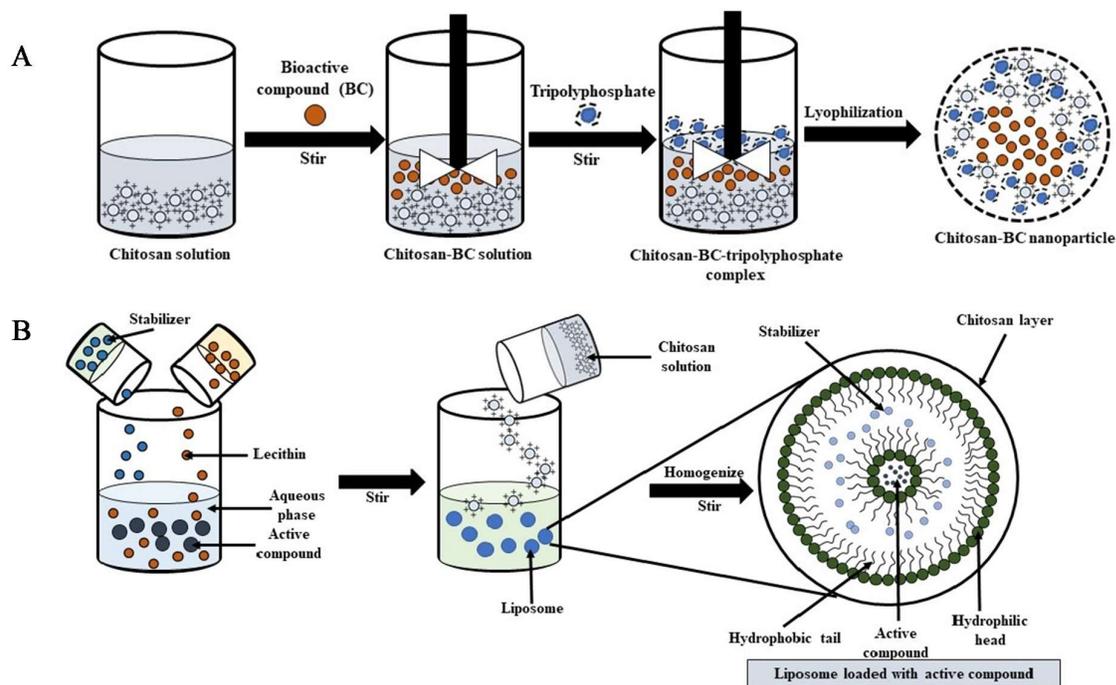


Fig. 1 Preparation of chitosan nanoparticles loaded with active compounds through ionic gelation (A) and liposome (B) methods.

Table 1 Characteristics and applications of chitosan nanoparticles loaded with active compounds prepared using various methods.

Shell	Method	Core	EE (%)	Size (nm)	Activity/application	Ref.
Chitosan	Oil-in-water emulsion and ionic gelation	Carvacrol	14–81	40–80	Antimicrobial activity against <i>S. aureus</i> , <i>B. cereus</i> and <i>E. coli</i>	[62]
	Ionic gelation	<i>Elsholtzia splendens</i> extract	23–31	350–520	Antioxidant and lipid peroxidation inhibitory activity	[8]
	Ionic gelation	Polyphenols	59–90	300–600	Antimicrobial activity	[9]
	Ionic gelation	Catechins	90	130	Enhance muco-adhesivity and controlled release of catechin in GIT	[11]
Chitosan	Ionic gelation	Curcumin	75	160	Antimicrobial activity against <i>S. aureus</i> and <i>P. aeruginosa</i>	[63]
	Ionic gelation	Ellagic acid	94	176	Enhanced cytotoxicity in human oral cancer cell line	[64]
	Ionic gelation	Gallic acid		453	Antimicrobial activity	[65]
	Ionic gelation	Green tea catechins and EGCG		432–440	Increase intestinal absorption	[66]
	Ionic gelation	Oxaliplatin	89	103	Mucoadhesive topical treatment of oral tumors	[67]
	Self-assembly	Catechins	74	130–150	Enhance the transport of tea catechins across Caco-2 cell monolayers	[43]
	Coprecipitation	Doxorubicin	99–81	3–8	Anticancer	[68]
	Emulsion-ionic gelation	Clove essential oil	46	100	Enhanced antifungal property of CEO due to controlled release	[37]
	Coacervation	Iron casein succinate		830–1070	Enhanced delivery of iron in Caco-2 cell model	[31]
	Electrospray	Tea polyphenols	66.7	205	Incorporated in gelatin film	[44]
Liposome	Quercetin	71	350–600	Enhanced inhibitory effect of nanoliposome on HepG2 cells	[37]	
β -chitosan	Ionic gelation	Catechins and catechins-Zn	50–84	208–590	Antibacterial activity against <i>L. innocua</i> and <i>E. coli</i>	[41]
	Ionic gelation	Tea polyphenols-Zn	97	84	Antioxidant activity	[69]

jecting an organic phase loaded with an emulsifier containing oil, into a mixture of bioactive compound and CS with the aid of mechanical stirring (Fig. 2A) [26]. The oil-in-water NE is composed of minute lipid droplets containing lipophilic active compounds, which were dispersed in an aqueous medium. In both water-in-oil and oil-in-water NEs, the final size of the particles mainly depends on the cross-linking agent and stirring speed [24]. The cross-linked agents undergo reaction with CS via amino groups to form an emulsion followed by the formation of nano- or micro particles [24]. Moreover, CS can be used to coat the emulsion, via electrostatic adsorption, without a cross-linking agent (chitosomes) [27]. However, the emulsification method is tedious and requires toxic cross-linking agents, thus limiting the use of this method for preparation of NPs.

Other methods

Besides the aforementioned methods, CSNPs have been prepared by simple and complex coacervation, reverse micelles, self-assembly, and spray drying methods [7, 10, 26]. In general, a homogeneous solution of charged CS and cross-linking agents underwent liquid to liquid phase separation, which results in the formation of polymer-rich dense and transparent phase (Fig. 2B) [28]. They are of mainly two types, simple and complex coacervations. For simple coacervation, the electrostatic forces occur between opposite-charge molecules of a single biopolymer, whereas the complex coacervation is made by a complex set of electrostatic forces between negative-charge biopolymers (e.g., gum Arabic) and positive-charge biopolymers (e.g., chitosan) [29, 30]. For CS, Min, Cho [31] produced CSNPs from CS (degree of deacetylation < 90%) for immobilizing iron casein succinylate (ICS) using the complex coacervation method. The mean diameter (830–1070 nm) of CS-ICS NPs was increased with augmenting concentrations of both biomolecules. Overall, these formulations have substantial potential to deliver various bioactive compounds, not only for drug delivery but also for food systems [7, 26]. For the reverse micelles technique, surfactant dissolved in organic solvent was mixed with CS-bioactive compound solution and followed by constant stirring [22]. Thereafter, the mixture was centrifuged or sonicated to obtain the desired particle size [7]. The schematic diagram of the reverse micelles process is given in Fig. 2C. For self-assembly, the organization of new structures from initial components including atoms, molecules, or NPs mostly

undergo non-covalent interactions [32, 33]. Therefore, the process can be simply affected by pH, temperature, and mechanical stresses. In spray drying method, the sample solution is vaporized in a hot-air drying chamber, in which the NPs can be obtained as dried powder within an electrostatic collector [34]. The major limitation of the method is associated with viscosity of biopolymer suspension, which may block the vaporizer nozzle and resulted in lower the yield [35]. Owing to the difficulty in operation or chemicals used, these methods have been implemented rarely [7, 26].

Overall, CSNPs have been served as a vector for various drugs and bioactive compounds associated with their capability of stabilization and release of active core compounds.

APPLICATIONS OF CSNPs

Encapsulating agent

Generally, labile compounds, such as tea polyphenols, essential oils, vitamins, enzymes, etc., are unstable and can be altered or lose their bioactivities during the formulation, food processing, digestion, and delivery. Therefore, to tackle this problem, encapsulation technology has been introduced widely, in which CSNPs are one of the major encapsulating agents. For example, CSNPs prepared by the emulsion-electrostatic interaction method with the help of TPP reduced the formation of hydroperoxide formation in krill oil stored for two weeks at 45 °C [36]. Similarly, clove essential-oil (CEO) encapsulated into CSNPs by emulsion-IG method showed lower degradation of volatile compounds as well as improved *in vitro* antifungal activity due to the controlled release of CEO [37]. Furthermore, Hadidi, Pouramin [38] observed the enhanced antioxidant potential of entrapped CEO. Higher inhibition of *Staphylococcus aureus* and *Listeria monocytogenes* was also achieved by entrapped CEO when compared with free CEO. The higher antimicrobial activity was more likely due to the round shape of NP, in which polycationic CSNPs can interact with the negatively-charged surface of bacteria to a higher extent. Since the large surface area of CSNPs can absorb onto the bacterial surface and cause the leakage of intracellular compounds and subsequent cell death [39]. For smaller particles, penetration into cytosol of bacterial cell can cause failure of metabolic pathways of bacteria [4]. The particle size of NPs has been known to influence its antimicrobial activity, smaller particles possessing higher activity [40]. The minimum inhibitory

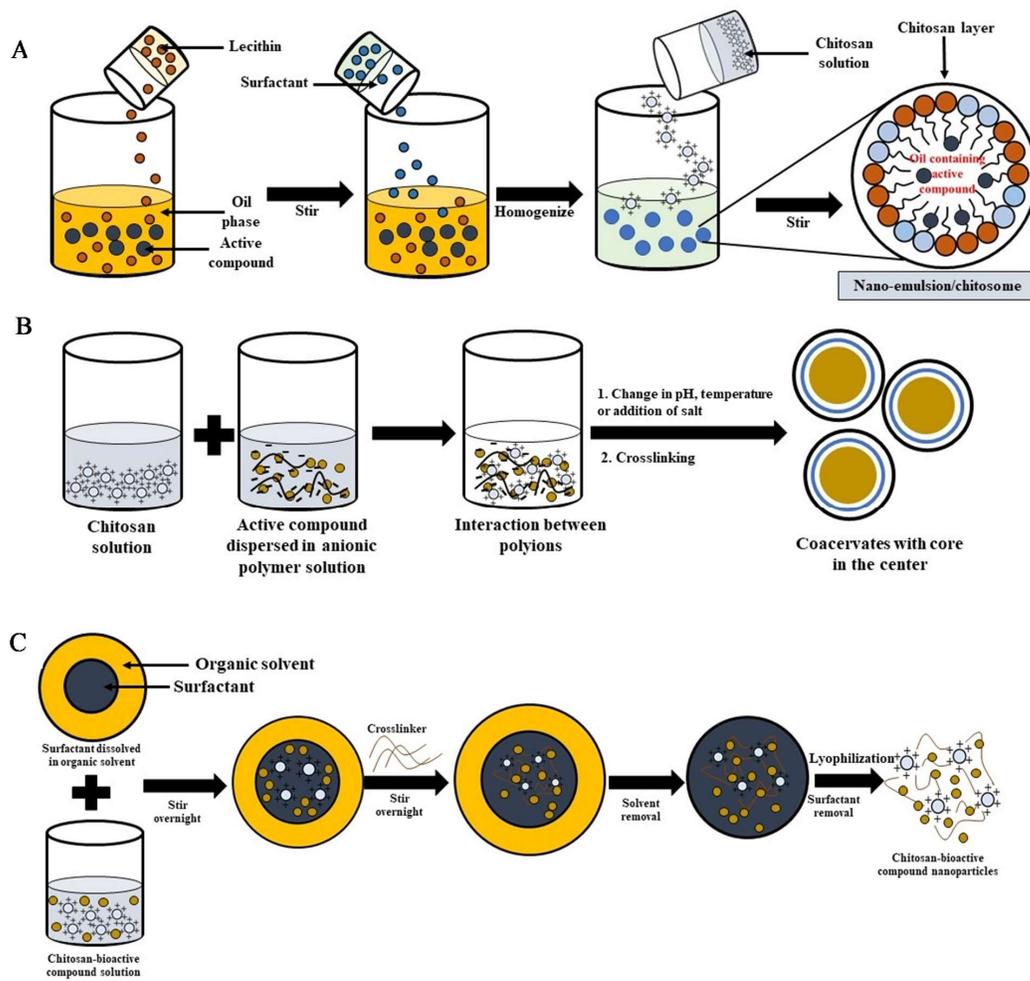


Fig. 2 Preparation of chitosan nanoparticles loaded with active compounds through nano-emulsion (A), coacervation (B), and reverse micelle (C) methods.

concentration (MIC) and minimum bactericidal concentration (MBC) values of the smallest particle size (208 nm) of catechins-Zn complex encapsulated by β -CSNPs against *Listeria innocua* and *Escherichia coli* were 0.03 and 0.06 mg/ml, and 0.06 and 0.13 mg/ml, respectively [41]. Conversely when particle size was increased to 590 nm, the MIC and MBC values of 0.13 and 0.03 mg/ml and 0.3 and 0.13 mg/ml against aforementioned bacteria were obtained. It was noted that MIC and MBC values were increased against both bacteria, indicating lower antibacterial activity. However, MIC for *L. innocua* was not changed with increasing size. This might be associated with lower ability of larger particles to penetrate through the bacteria cell wall, thus lowering its antimicrobial effect. In addition, CS possesses an excellent antimicrobial

activity, which synergistically enhances the potential effect of CSNPs [2–4]. The samples encapsulated using NLs had been determined for various activities. For example, Q-NLs showed improved stability and higher antioxidant activity compared with free quercetin, which might be due to the enhanced solubility of quercetin NLs [20]. Vitamin-C NLs coated with CS and stored at 4 °C for 15 weeks, could maintain its antioxidant activity indicating higher stability of the coated samples in comparison to the free form [21]. Additionally, CSNLs has been used as a carrier for several drugs. Docetaxel, a popular anticancer drug, was loaded into NLs, which was further encapsulated inside folate grafted thiolated-CS. It effectively inhibited MD-MB-231 breast cancer cells, showing IC_{50} value of 200-times lower than that of pure docetaxel [42]. This indicated the

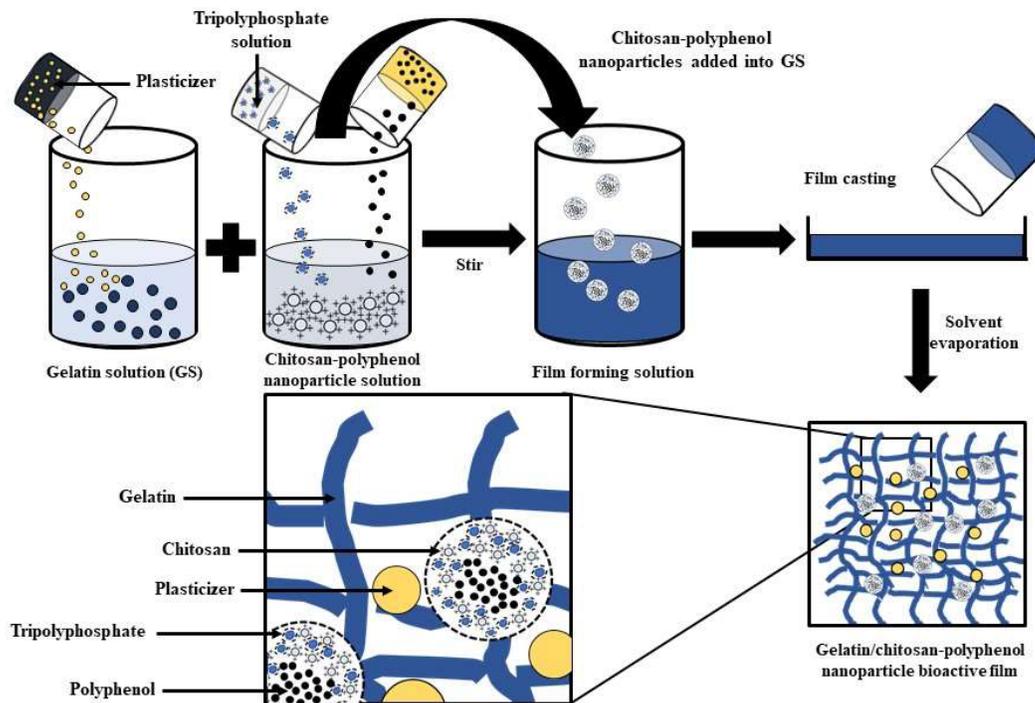


Fig. 3 Preparation of gelatin film incorporated with chitosan-polyphenol nanoparticles.

enhanced oral bioavailability, which was confirmed by pharmacokinetics study. Similar observations were obtained for berberine hydrochloride when loaded into CS coated NPs [18].

Oral administration of sensitive drugs is limited by the harsh conditions of the gastrointestinal tract (GIT). Therefore, self-assembled NPs, prepared by CS and an edible polypeptide, were used to deliver the tea catechins into GIT [43]. The increase in paracellular transport of tea catechins was due to positive surface charge of NPs, which opened tight junctions between Caco-2 cells. Similarly, CS and sodium TPP was used for synthesis of catechin-loaded CSNPs with the size of 110–130 nm and possessing the controlled release of catechin in GIT [11].

Edible coating or film

The shelf-life of a wide array of foods has been increased by the application of CS and its derivatives [26]. Hence, the ability of CSNPs to control the release of various bioactive components can enhance the antimicrobial/antioxidant properties of food packaging materials. The gelatin film incorporated with tea polyphenol loaded CSNPs was generated through electrospray (Fig. 3) [44]. Beluga (*Huso huso*) fish fillets treated with an ed-

ible coating containing CSNPs loaded with fennel essential oils in combination with modified atmospheric packaging showed lower value of peroxide, total volatile nitrogen and thiobarbituric acid [45]. Also, lower mesophilic, psychotropic, *Pseudomonas* spp., and lactic acid bacteria counts were noted in fillets coated with CSNPs as compared to those without CSNPs [45]. Similarly, the shelf-life of *Litopenaeus vannamei* (whiteleg shrimp) stored at 4°C was increased up to 10 days, when coated with CSNPs [46]. The retarded lipid oxidation of silver carp (*Hypophthalmichthys molitrix*) fillets was observed up to 12 days at 4°C when coated with CSNPs in combination with orange or pomegranate peel extract [47]. However, CSNPs of pomegranate peel extract was more effective than orange peel extract. In another study, Albooghbeish and Khodanazary [48] compared quality characteristics of *Carangoides coeruleopinnatus* fillets coated with CS and CSNPs for 12 days at 4°C. NPs coating on fillets retarded lipid oxidation, as well as growth of microorganisms, more efficiently than CS coated fillets. Recently, the lowest peroxide value of soybean oil on day 14 was documented when packaged in gelatin film incorporated with 30% (w/v) tea polyphenol-loaded CSNPs [44].

Despite the shelf-life extension of various foods,

CSNPs also improved the mechanical and physicochemical properties of films. Tara gum films were developed using the incorporation of bulk CS and CSNPs. Films added with CSNPs showed higher mechanical strength, antimicrobial activity, water solubility and lower hydrophilicity than CS added film [49]. In another study, solubility of CS-ZnO-NPs composite coating was increased by 42%, whereas degree of swelling was reduced by 80% when compared to the CS coating [50]. Gelatin films have been developed for food packaging; but still their mechanical properties are low, and preservative effects are poor on various food products. Hosseini, Rezaei [51] developed fish gelatin films using CSNPs as a filler, which resulted in augmented mechanical properties and increased water vapor barrier by 50%. Furthermore, the composite films based on gelatin/CSNPs containing oregano essential oil were more flexible, with decreased surface hydrophilicity and water vapor permeability. Thus, CSNPs can be potentially used in bio-composite films to enhance their mechanical properties and bioactivities.

Other applications in foods

CSNPs were prepared by IG process and used as a cryoprotectant in grey mullet surimi [52]. The incorporation of NPs in surimi efficiently impeded the microbial load and lipid oxidation by lowering the formation of thiobarbituric acid substances and conjugated dienes. Moreover, CSNPs were also able to inhibit oxidation of fish myoglobin via lowering transformation of heme iron. Besides, CSNPs were used as a glazing material for cryogenically frozen shrimp [53]. Glazing materials containing CSNPs could suppress lipid oxidation with no negative effects on color and texture of frozen shrimps during storage [53].

Human health benefits

CS plays an important role in maintaining stability and sustaining release of bioactive components. Moreover, its ability to act as antioxidant, antitumor, antiviral agent, etc. synergistically enhanced the efficacies of various drugs and therapeutic compounds. Various studies have been performed *in vivo* or *in vitro* conditions to elucidate the CS's properties.

Antitumor activity

CSNPs, in combination with various derivatives, have been used at a large scale in the pharma or medicinal industry as wound healing, anticancer,

drug delivery agents, etc. CS-folate conjugated NPs loaded with EGCG (mean diameter of 400 nm) showed higher *in vitro* antitumor activity against various cell lines, such as HeLa, H1299 and Capan-1 cells, than free EGCG [54]. The inhibitions of NPs towards HeLa, H1299 and Capan-1 cells were 85, 56 and 64%, respectively. Similarly, CS-folate conjugated NPs encapsulated with catechin (average diameter of 255 nm) showed antitumor activity against MCF-7 and HepG-2 cells [55]. In both cells, the loaded CSNPs showed a higher anti-proliferation effect than the blank NPs. Moreover, MCF-7 cells were more sensitive to catechin-loaded folate-conjugated CSNPs than HepG-2 cells, indicating that the CSNPs had a higher cytotoxic effect on folate receptor-positive tumor cells. CSNPs possessed positive charges on their surface and high affinity toward negatively charged tumor cell membrane, thereby causing endocytosis via releasing active components into the cytosol. As a result, various cellular mechanisms were altered and ultimately led to cell death.

Wound healing (WH) activity

The natural origin and biocompatibility of CS promoted its use for WH because CS fuels haemostasis and expedites tissue renewal. The hydrophilic character and homogenous structure of CS, relative to glycosaminoglycans, serve as an attractive material for a tissue engineering scaffold [56]. CSNPs of average diameter 208.5 nm and positive zeta potential about 24.2 mV, prepared by IG method and loaded with calcium alginate hydrogel, exhibited remarkable antibacterial and pro-inflammatory activity, as indicated by the inhibition of generation of reactive oxygen species. Subsequently, the process promoted vascular endothelial cell invasion, metastasis and neovascularization, resulting in a higher rate of WH. The rate of wound closure was increased to 85.6% within 7 days and 98.7% within 14 days [57]. Amputation of limbs due to diabetes wounds is a major concern in patients. CS-curcumin NPs of size 196.4 nm with a positive zeta potential of 30.3 mV were impregnated into collagen/alginate scaffolds, and able to treat wounds at a higher rate than the control (sterile gauze) and placebo scaffolds [58]. Synergistic action of curcumin (antiinflammatory or antioxidant), CS (carrier, WH activity) and collagen (stimulant for WH activity) was persuaded.

Antiobesity activity

Obesity affects the quality of human life and is an important cause of cardiovascular, cerebrovascular,

and metabolic diseases, which made it a major criterion in medicine and pharmacology. Alone, CS has been considered as a dietary fiber due to its indigestible, viscous nature and protection of oil droplets from digestive enzymes, especially lipase [1]. CS-insulin NPs prepared using IG method, with sizes of 296–688 nm by filtration, showed strong affinity to intestinal epithelium after 3 h of postoral administration and could lower the serum glucose level [59]. CS more likely acted as a co-factor for better absorption of insulin. Similarly, CS microparticles loaded with capsaicin having 600–1000 nm in size were prepared through the IG method for evaluation of antiobesity effects using high fat diet (HFD) in the rats [60]. Capsaicin-encapsulated NPs showed a superior ability to regulate body weight, fat and serum lipids as compared to native capsaicin and commercial agent orlistat. Similar results were obtained in the treatment of HFD-induced obese rats between water insoluble and soluble CSNPs [61]. It can be inferred that CS and its derivatives, such as NPs, show an excellent capability for treatment of diabetes associated with high efficiency in drug delivery as well as effectiveness on diabetic WH.

CONCLUSION

Chitosan nanoparticles (CSNPs) have been prepared using several methods, in which various bioactive components could be loaded for their sustained release with increased bioavailability and biological activities. Ionic gelation is the most exploited method for preparation of CSNPs. CSNPs also have the potential to enhance the absorption or availability of bioactive components by opening epithelial tight junctions and/or by direct uptake into epithelial cells via endocytosis. The augmented antimicrobial activity of CSNPs can enhance the shelf-life of various foods. Moreover, the ability of CSNPs to stabilize sensitive antioxidants, such as tea polyphenols, vitamins, etc., can help reduce the lipid or protein oxidation of foods. Hence, nanostructured forms of CS can be considered as profitable value-added products, which are used in a wide array of foods, packaging, encapsulation, biomedical remedy, and health promotion.

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