Antimicrobial activity of chitosan nanoparticles

Samiyah Saeed Al-Zahrani\textsuperscript{a,b}, Roop Singh Bora\textsuperscript{c} and Saleh Mohammed Al-Garni\textsuperscript{a}

\textsuperscript{a}Department of Biology, Faculty of Science, King Abdulaziz University, Jeddah, Kingdom of Saudi Arabia; \textsuperscript{b}Department of Biology, Faculty of Arts and Science, Albaha University, Albaha, Kingdom of Saudi Arabia; \textsuperscript{c}Department of Biotechnology, Dr. Khem Singh Gill Akal College of Agriculture, Eternal University, Baru Sahib, Himachal Pradesh, India

**ABSTRACT**

Chitosan is a deacetylated chitin which is found naturally, particularly in fungal cell walls and crustacean shells. Chitosan is biocompatible and fully biodegradable and is extensively analyzed for antimicrobial property. Chitosan has also been explored as a drug carrier due to its biocompatible properties. Some studies have demonstrated that use of chitosan to coat nanoparticles made of other materials would help in reducing their impact on the body and also increase their bioavailability. The molecular weight of chitosan and the degree of deacetylation can be modified to derive different physicomechanical properties. Chitosan exhibits potent antifungal activity against several fungal strains including \textit{Rhizopus oryzae}, \textit{Aspergillus niger} and \textit{Alternaria alternata}. Various factors such as molecular weight, dose and functional groups attached to the chitosan have been shown to modulate the antifungal activity of chitosan. Chitosan is known to exert antifungal activity without the need for any chemical modification, however, new derivatives of chitosan can be created to target specific microbial pathogens. The development of novel and ecofriendly methods of chitosan nanoparticles (CSNPs) preparation is in progress for developing chitosan as an efficient antimicrobial agent and in drug delivery system. This review is to focus on recent application of CSNPs as antibacterial and antifungal agent and to highlight the effectiveness of employing chitosan with silver and other metal nanoparticles.

**Introduction**

Chitosan, \((\text{1-4})\) 2-amino-2-deoxy\(\beta\)-D glucan, obtained by chitin deacetylation, is found in the exoskeleton of crustacean and several other organisms including insects and fungi [1]. Chitosan has favorable properties which have attracted the interest of researchers over the past two decades. For instance, it is non-toxic, biocompatible, edible and also possess antimicrobial properties [2–5]. For years, chitosan has been profoundly inspected as a carrier for drug delivery and biomedical applications [6–8]. Much of the research up to now has been focused on studying the antimicrobial activity of chitosan in form of solutions, gels, fibers and films [9–15]. The interest in employing chitosan nanoparticles (CSNPs) arises mainly due to its well-founded polymeric and cationic properties [16]. Various techniques for chitosan nanoparticles synthesis have been investigated by several research groups such as preparation of CSNPs by: ionic gelation method [17], emulsion crosslinking [18], spray drying [19], emulsion-droplet coalescence [20], reverse micellar method [21], nanoprecipitation [22], desolvation [23], modified ionic gelation with radical polymerisation [24] and emulsion solvent diffusion method [25]. CSNPs is considered as a highly promising candidate for utilisation as biomaterial in food related applications due to its accessibility, lack of toxicity and antimicrobial properties. Due to their small size and large surface to weight ratio, employing CSNPs in biomedical field has been examined extensively. Chitosan is one of the bio-polymers that is utilised as a reducing agent and a protecting polymer in the formation of metallic nanoparticles.

**Chitosan nanoparticles as antibacterial agents**

In the last 25 years, bacterial resistance has emerged as a major threat to humanity which could lead to a situation in the near future, where even trivial infections could become life threatening. Due to the
emergence of multidrug-resistant microbes and lack of new antimicrobial drugs in market, there is an urgent need to discover and develop novel and more potent antimicrobial compounds. Chitosan-based nanoparticles have shown a tremendous potential as an antibacterial agent. In a recent study, CSNPs were prepared using varying concentration of chitosan and tripolyphosphate (TPP) by using ionic gelation method. The CSNPs prepared by employing 0.25% chitosan and 0.1% TPP exhibited an efficient antibacterial activity against Staphylococcus aureus and Pseudomonas aeruginosa [26]. TPP, a non-toxic polyanion, is very safe and have been used for food industry applications. The protonated-NH$_2$ groups in chitosan interact with the negatively charged counterion of TPP via an electrostatic interaction, and thus, ionic cross-linked networks are formed [27, 28]. A similar study investigated the antibacterial potency of CSNPs against Staphylococcus aureus and Escherichia coli. CSNPs in the presence of TPP exhibited potent inhibition of both E. coli and S. aureus, compared to the CSNP without TPP which showed less activity [29]. Chitosan derivatives exhibit a pronounced antibacterial potency toward various bacterial strains. In a recent study, CSNPs obtained from a chitosan derivative, i.e. betaine, was tested for antibacterial activity. Chitosan derivatives with medium molecular weight and high degree of substitution showed greater antibacterial activity than commercial antibiotics. It was also revealed that increasing the degree of substitution led to an increase in antibacterial activity of chitosan with different molecular weight [30]. CSNPs obtained from the most effective chitosan heterocyclic derivative with moderate molecular mass, enhanced the antibacterial activity approximately 3-folds than the pure chitosan [31]. CSNPs at 10% and 20% inhibited the growth of Streptococcus mutans, Pseudomonas aeruginosa and Enterococcus faecalis completely [32].

CSNPs were loaded with antibiotics including tetracycline, gentamycin or ciprofloxacin to enhance antibacterial properties after its application on cotton fabrics. Fabric samples of cotton and cotton/polyester fabrics were treated with different concentrations of antibiotic-CSNPs. Cotton fabrics treated with CSNPs and with antibiotic-CSNPs inhibited the growth of S. aureus and E. coli with zone of inhibition of 10–26 mm and from 8.5 to 24 mm, respectively. This study also revealed that gentamycin-CSNPs exhibited higher antibacterial activity as compared to ciprofloxacin-CSNPs and tetracycline-CSNPs at the same concentration and the zone of inhibition (ZOI) increased with the increase of antibiotic concentration. It was also observed that, the fiber structure of cotton fabrics makes them more effective than polyester fabrics due to the number of functional groups of cotton reacting with a sufficient amount of antibiotic-CSNPs and thus enhancing antibacterial activity [33].

CSNPs additionally show synergistic activity to improve antimicrobial activity. However, using high concentration of CSNPs suspensions in industrial application might not be cost-efficient and might make visual or textural changes to commodities being targeted. To overcome this issue, the blend of an efficient antimicrobial agents with CSNPs would have the option to decrease the effective concentration of CSNPs.

Recent study reported an innovative strategy for CSNPs production using chemical cross-linking with cinnamaldehyde and replacing the traditional method of using TPP as ionic cross linker. Synergistic antibacterial activity was observed and the inhibitory effect of chitosan was significantly increased from 65% to 98% against S. aureus and from 62% to 96% against E. coli [34]. Another study examined the synergistic action of CSNPs with the antimicrobial alkaloid berberine against B. subtilis and S. aureus. The evaluation of synergistic effect of CSNPs and berberine was performed by using the broth dilution and the agar diffusion methods. Both CSNPs and berberine showed remarkable inhibitory effect against bacterial pathogens which was improved by their synergistic action [35].

Silver nanoparticles (AgNPs) have garnered the great interest from research community due to their superior properties, as well as conductivity, chemical stability, catalytic properties and antimicrobial activity. The modified CS in AgNPs-chitosan/montmorillonite nanocomposite films may control the morphological characteristics of AgNPs besides the significant inhibition of E. coli and B. subtilis [36].

Chitosan/tea polyphenols-silver nanocomposite film (CS/TP-AgNPs) was tested for antibacterial potency. Tripolyphosphate was added to the chitosan film as a crosslinker and a reducing agent of the silver nanoparticles agent. CS/TP-AgNPs film inhibited S. aureus and E. coli more efficiently compared to the effect of chitosan films alone [37]. The bactericidal action of silver nanoparticle-loaded microspheres (ChM-Ag) was very efficient in inhibiting E. coli and S. aureus [38]. The antibacterial activities of chitosan nanoparticles and copper-loaded nanoparticles against E. coli, Salmonella choleraesuis, Salmonella typhimurium and S. aureus have been reported [39]. An eco-friendly CS-AgNPs hybrid was developed from AgNPs biologically prepared using T. portulacifolium leaf extract as a reducing agent and the inhibitory effects of these hybrid NPs were tested.
against two bacterial strains: *E. coli* and *Serratia marcescens*. These hybrid CS-AgNPs inhibited the growth of *E. coli* and *S. marcescens*. The antibacterial activity of CS-AgNPs increased with the increase in the concentration of CS-AgNPs [40].

Fungal chitosan (FCS) from *Cunninghamella elegans*, encapsulated with green silver nanoparticles from *Gynura procumbens* (GP-AgNPs) were developed to improve the antibacterial activity. The CS-AgNPs inhibited *Bacillus cereus*, *S. aureus*, *L. monocytogenes*, *E. coli* and *Salmonella enterica* [41]. Green AgNPs from *Prosopis juliflora* (PJ) leaf extract encapsulated with chitosan showed a significant inhibition zone against *E. coli* compared to streptomycin [42]. Similar study investigated green AgNPs from *Syzygium aromaticum* bud extract encapsulated with chitosan for antibacterial activity. CS-AgNPs inhibited vancomycin resistance *Staphylococcus aureus* with a zone of inhibition of 23.2 ± 0.51 mm and methicillin resistant *Staphylococcus aureus* with a zone of inhibition of 25.8 ± 0.32 mm. The results revealed their more prominent antibacterial potential compared to other antibacterial agents [43]. CS-AgNPs were synthesised via green method using chitosan, glucose and ethylene glycol. The antibacterial tests implied that CS-AgNPs prepared using glucose (G-AgNPs-CS) inhibited the growth of *E. coli* and *S. aureus*. 100 μg/mL of G-AgNPs-CS inhibited the growth of *E. coli* completely, while a much higher concentration i.e. 200 μg/mL of chitosan C-AgNPs-CS and ethylene glycol E-AgNPs-CS were required for complete inhibition of *E. coli* growth. *S. aureus* was inactivated by 300 μg/mL of all nanocomposites. The results revealed that the inhibitory effects against *E. coli* and *S. aureus* were enhanced with increasing concentration of AgNPs [44]. The antibacterial efficiency of green chitosan-PVA-silver nanoparticles (chitosan and polivinyl alcohol polymers, used as stabilising agent) was evaluated against *B. cereus*, *E. coli*, *E. faecalis*, *Micrococcus luteus*, *P. aeruginosa*, *S. enterica*, *Salmonella typhi* and *S. aureus*. This study revealed that the increase of AgNO₃ used in AgNPs synthesis enhances the antibacterial activity of CS-AgNPs [45].

Recently, the anti-biofilm activity of silver nanoparticles capped with chitosan (CS-AgNPs) was tested against pathogenic bacteria including *S. aureus* and *P. aeruginosa*. CS-AgNPs inhibited the growth of *S. aureus* (85%) and *P. aeruginosa* (95%) at 100 μg/mL which was confirmed using confocal- laser scanning microscopy. The anti-biofilm potency of CS-AgNPs enhanced with the increase in concentration from 25 μg/mL to 100 μg/mL [46].

Layer by layer (L-B-L) nanocoating process was used for deposition of polystyrene sulfonate (PSS) and CS-Ag and the formation of body layers of PSS/CS-Ag on fabric. The antibacterial activity of coated fabrics was evaluated against *S. aureus*, *B. subtilis* and *E. coli*. The coated fabrics with 0.1% of PSS/CS and 0.1% (PSS/CS-Ag) showed 100% bactericidal activity against *S. aureus* and *E. coli*, whereas PSS/CS coated fabric led to inhibition of 72% and 68% against *S. aureus* and *E. coli*, respectively. Additionally, PSS/CS-Ag showed zones of inhibition of 4.8 mm, 3.2 mm and 2.5 mm against *B. subtilis* *S. aureus* and *E. coli*, respectively [47].

Gold nanoparticles (AuNPs) capped with chitosan (CS-AuNPs), glycol-chitosan (GC-AuNPs) and poly-γ-glutamic acid (PA-AuNPs) were applied on fabrics and evaluated for antibacterial activity. PA-AuNPs showed a higher antibacterial activity against *S. enterica* and *E. coli*-O157:H7 compared to gentamycin. While CS-AuNPs and GC-AuNPs exhibited maximum antibacterial activity against *Listeria monocytogenes*, followed by *S. enterica*, *E. coli*-O157:H7, MRSA and *S. aureus*. Transmission electron microscope images revealed that glycol-chitosan altered MRSA cell permeability by attaching on the surface of the cell and blocked nutrient flow and disrupt cell membrane, while PA-AuNPs entered *S. enterica* to cause pore formation, plasmolysis and dissolution [48].

**Chitosan nanoparticles as an antifungal agent**

CSNPs prepared using low molecular weight (LMW) and high molecular weight (HMW) of chitosan have been evaluated for their antifungal activity against *Candida albicans*, *Fusarium solani* and *Aspergillus niger*. The nanoparticles prepared with different concentrations of chitosan showed an inhibitory effect against the three fungal species. While *A. niger* exhibited a strong resistance to CNPs which was fabricated with low concentrations of chitosan [49]. The CSNPs were also evaluated as a controlling agent for various plant diseases caused by *Rhizoctonia solani*, *Fusarium oxysporum*, *Collectotrichum acutatum* and *Phytophthora infestans*. Moreover, CNP shave been shown as an ideal coating agent for the coated vegetables by improving the shelf life of tomato, chilly and brinjal. CSNPs exhibited significant antifungal activity against all fungal species. Vegetables samples treated with different concentrations that ranged from 1% to 5% of CSNPs prevented the weight loss compared to uncoated vegetables samples [50].

Application of CSNPs prepared with TPP as a cross-linker had significantly reduced the loss of wood mass, when the samples were exposed to the white rot
fungus (Trametes versicolor) and brown rot fungus (Gloeophyllum trabeum). Their study clearly suggested that CSNP-TPP could provide the treated wood a resistance to fungal decay [51].

The addition of guar gum to chitosan nanoparticle (CGNP) preparation was used to enhance the effect of CSNPs as a protective agent against microorganisms. This study examined the inhibitory effect of CGNP against the rice blast fungus Pyricularia grisea using the disc diffusion method. CGNPs exhibited antifungal effect (71% inhibition) at 0.01 mg/mL concentration by inhibiting the mycelial growth. In-vitro application of 0.01% CGNP to healthy rice leaves followed by inoculation with P. grisea spores after 48 h, showed no symptom of rice blast compared to rice leaves, sprayed with distilled water as controls, which exhibited the blast symptoms. Moreover, the CGNPs enhanced seed growth and seed germination [52]. Tissue conditioner containing CSNPs at 5% and 2.5% concentration was found to completely inhibit the growth of a C. albicans strain [32]. In a recent study, CSNP-hydrogels with crosslinker TPP were prepared to improve the antimicrobial properties. A modified chitosan hydrogel showed higher antifungal activity with an inhibition zone of 25 mm for Candida albicans and 26 mm for A. niger, while chitosan and CSNPs showed no inhibition against the selected species [53].

Antifungal activity of different chitosan derivatives were analysed against Aspergillus fumigatus and Geotrichum candidum. Chitosan, highly substituted with moderate molecular weight molecules, exhibited the highest zone of inhibition (15.3 mm ± 0.5) for G. candidum while chitosan, highly substituted with high molecular weight molecules showed the highest zone of inhibition (17.9 mm ± 0.3) for A. fumigatus [54]. A recent study evaluated the use of plant extracts or plant essential oils to enhance the inhibitory effects of CSNPs against fungi and especially, pathogenic fungi. It has been observed that P. dactylifera plant extract loaded CSNPs exhibited significant inhibitory effect against C. albicans [55]. Chitosan-based nanocomposite films loaded with plant essential oil mixtures (i.e. thyme-oregano, thyme-tea tree and thyme-peppermint) exhibited significant antifungal activities towards A. niger, A. flavus, A. parasiticus and Penicillium chrysogenum [56]. The encapsulated clove essential oil with CSNPs showed a potent antifungal activity against A. niger compared to CSNPs and clove essential oil, used alone [57].

Another study evaluated the inhibitory effect of encapsulated anethole-chitosan nanomulsion on the fungal contamination and aflatoxin B1 production. Ten moulds, i.e. Aspergillus flavus strain, Aspergillus fumigatus, A. niger, Aspergillus luchuensis, Aspergillus repens, Penicillium italicum, P. chrysogenum, Fusarium oxysporum, Alternaria alternata and Cladosporium cladosporioides were selected for evaluating the efficacy of encapsulated anethole-based chitosan nanomulsion (Ant-eCsNE). The result indicated the efficacy of Ant-eCsNE against the chosen molds by inhibiting the fungal growth at 0.8 μl/mL dose and inhibiting aflatoxin B1 biosynthesis at dose of 0.4 μl/mL. In-vivo field trial data revealed that Ant-eCSne has a great potential in preserving the stored maize samples from fungal infestation and aflatoxin B1 contamination which make it a good food preservative [58].

AgNPs, produced using the extract of plant pathogenic fungus Colletotrichum gloeosporioides and conjugated with chitosan, showed an antibiofilm activity and a potential to inhibit Candida species at the dose of 50 μg/mL [59]. In a recent study the antifungal properties of Au-chitosan nanoparticles (CS-AuNPs) with different concentrations have been evaluated against two strains of Fusarium oxysporum. CS-AuNPs exhibited antifungal activity against both strains of F. oxysporum by reducing the colony diameter [60]. The CS-AuNPs exhibited the most effective fungicidal activity against C. albicans. Chitosan strongly improved antifungal properties of AuNPs [61].

In addition, nano-encapsulated chitosan in combination with the essential oils of Ocimum sanctum, Ocimum basilicum, and Ocimum canum exhibited a significant inhibitory activity against the pathogenic fungus Aspergillus flavus and aflatoxin B1. This chitosan nano-matrix enhanced the antifungal and aflatoxin B1 inhibition, compared to the free essential oils, and protected seeds samples from fungal contamination and mycotoxin production [62].

Recently, in drug delivery field considerable research has been carried out using chitosan and its derivatives. It has been observed that antifungal activity of CS-AgNPs composites against A. niger, Cryptococcus neoformans and Candida tropicalis is due to the synergistic effect between AgNPs and a chitosan derivative. These derivatives-AgNP composites have superior antimicrobial activities to pure chitosan and analogues and are also superior to antibiotic Ampicillin B [63]. Metal nanoparticles showed an improvement in antimicrobial activity of chitosan, which were prepared by doping chitosan film with silver nanoparticles, i.e. CS-AgNP. Chitosan thin film were doped with (100–400 μg) of biogenic AgNPs to enhance its antimicrobial potency. CS-AgNPs (300 and 400 μg) showed superior inhibition of S. aureus growth over non-doped chitosan films. CS-AgNPs at 300 μg concentration exhibited significant antifungal activity against C. albicans [64].
Conclusions
In conclusion, chitosan and its derivatives are safe and effective antimicrobial agents. Lately, the focus has been on the capability of chitosan in reducing and capping metal nanoparticles to develop novel and more potent anti-microbial compounds. New techniques are being developed for synthesizing nanoparticles from natural resources such as bacterial and plant extracts. These studies of combination of metal nanoparticles stabilised with chitosan may provide a synergistic antimicrobial activity which may facilitate the development of a new class of antimicrobial agents.

Data availability statement
The data that support the finding of this study are openly available in public domain.

Declaration of conflicting interests
The authors declared no potential conflicts of interest with respect to the research, authorship and/or publication of this article.

Funding
The authors received no financial support for the research, authorship and/or publication of this article.

Funding
The author(s) reported there is no funding associated with the work featured in this article.

References
[1] Toan NV, Ng Ch, Aye KN, et al. Production of high-quality chitin and chitosan from preconditioned shrimp shells. J Chem Technol Biotechnol. 2006;81(7):1113–1118.
[2] No HK, Park NY, Lee SH, et al. Antibacterial activity of chitosans and chitosan oligomers with different molecular weights. Int J Food Microbiol. 2002;74(1-2):65–72.
[3] Devlieghere F, Vermeulen A, Debevere J. Chitosan: antimicrobial activity, interactions with food components and applicability as a coating on fruit and vegetables. Food Microbiol. 2004;21(6):703–714.
[4] Larsen MU, Seward M, Tripathi A, et al. Biocompatible nanoparticles trigger rapid bacteria clustering. Biotechnol Prog. 2009;25(4):1094–1102.
[5] Azadi G, Seward M, Larsen MU, et al. Improved antimicrobial potency through synergistic action of chitosan microparticles and low electric field. Appl Biochem Biotechnol. 2012;168(3):531–541.
[6] Lehr CM, Bouwstra JA, Schacht EH, et al. In vitro evaluation of mucoadhesive properties of chitosan and some other natural polymers. Int J Pharm. 1992;78(1-3):43–48.
[7] Dodane V, Vilivalam VD. Pharmaceutical applications of chitosan. Pharm Sci Technol Today. 1998;11(6):246–253.
[8] Paul W, Sharma CP. Chitosan, a drug carrier for the 21st century: a review. STP Pharma Sci. 2000;10:5–22.
[9] Goy RC, Britto DD, Assis OBG. A review of the antimicrobial activity of chitosan. Polímeros. 2009;19(3):241–247.
[10] Kong M, Chen XG, Xing K, et al. Antimicrobial properties of chitosan and mode of action: a state of the art review. Int J Food Microbiol. 2010;144(1):51–63.
[11] Hafdani FN, Sadeghnia N. A review on application of chitosan as a natural antimicrobial. World Acad Sci Eng Technol. 2011;50:252–256.
[12] Ahmed S, Ahmad M, Ikram S. Chitosan: a natural antimicrobial agent – a review. J Appl Chem. 2014;3:493–503.
[13] Xing K, Zhu X, Peng X, et al. Chitosan antimicrobial and eliciting properties for pest control in agriculture: a review. Agron Sustain Dev. 2015;35(2):569–588.
[14] Sahariah P, Masson M. Antimicrobial chitosan and chitosan derivatives: a review of the structure–activity relationship. Biomacromolecules. 2017;18(11):3846–3868.
[15] Abd El-Hack ME, El-Saadony MT, Shaﬁ ME, et al. Antimicrobial and antioxidant properties of chitosan and its derivatives and their applications: a review. Int J Biol Macromol. 2020;164:2726–2744.
[16] Zhang H, Oh M, Allen C, et al. Monodisperse chitosan nanoparticles for mucosal drug delivery. Biomacromolecules. 2004;5(6):2461–2468.
[17] Kawashima Y, Handa T, Kasai A, et al. Novel method for the preparation of controlled-release theophylline granules coated with a polyelectrolyte complex of sodium polyphosphate-chitosan. J Pharm Sci. 1985;74(3):264–268.
[18] Ohy A, Shiratami M, Kobayashi H, et al. Release behavior of 5-fluorouracil from chitosan-gel nanospheres immobilizing 5-fluorouracil coated with polysaccharides and their cell specific cytotoxicity. J Macromol Sci Pure Appl Chem. 1994;31(5):629–642.
[19] Wang SL, Hiep DM, Luong PM, et al. Preparation of chitosan nanoparticles by spray drying, and their antibacterial activity. Res Chem Intermed. 2014;40:2165–2175.
[20] Tokumitsu H, Ichikawa H, Fukumori Y. Chitosan-gadopentetic acid complex nanoparticles for gadolinium neutron-capture therapy of cancer: preparation by novel emulsion-droplet coalescence technique and characterization. Pharm Res. 1999;16(12):1830–1835.
[21] Melo EP, Aires-Barros MR, Cabral JMS. Reverse micelles and protein biotechnology. Biotechnol Ann Rev. 2001;7:87–129.
[22] Luque-Alcaraz AG, Lizardi-Mendoza J, Goycoolea FM, et al. Preparation of chitosan nanoparticles by nanoprecipitation and their ability as a drug nanocarrier. RSC Adv. 2016;6(64):59250–59256.
[23] Alonso MJ. Nanoparticulate drug carrier technology. Drugs Pharm. Sci. 1996;77:203–242.
[24] Hu Y, Jiang X, Ding Y, et al. Synthesis and characterization of chitosan–poly (acrylic acid) nanoparticles. Biomaterials. 2002;23(15):3193–3201.

[25] El-Shabouri MH. Positively charged nanoparticles for improving the oral bioavailability of cyclosporin-A. Int J Pharm. 2002;249(1-2):101–108.

[26] Bangun H, Tandiono S, Arianto A. Preparation and evaluation of chitosan-tripolyphosphate nanoparticles suspension as an antibacterial agent. J Appl Pharm Sci. 2018;8:147–156.

[27] Mi FL, Shyu SS, Lee ST, et al. Kinetic study of chitosan-tripolyphosphate complex reaction and acid-resistant properties of the chitosan-tripolyphosphate gel beads prepared by in-liquid curing method. J Polym Sci B Polym Phys. 1999;37(14):1551–1564.

[28] Mi FL, Sung HW, Shyu SS, et al. Synthesis and characterization of biodegradable TPP/genipin co-crosslinked chitosan gel beads. Polymer. 2003;44(21):6521–6530.

[29] Pan C, Qian J, Fan J, et al. Preparation nanoparticle by ionic cross-linked emulsified chitosan and its antibacterial activity. Colloids Surf A Physicochem Eng Asp. 2019;568:362–370.

[30] Kritchenkov AS, Egorov AR, Artemjev AA, et al. Ultrasound-assisted catalyst-free thiol-ynie click reaction in chitosan chemistry: antibacterial and transfection activity of novel cationic chitosan derivatives and their based nanoparticles. Int J Biol Macromol. 2020a;143:143–152.

[31] Kritchenkov AS, Egorov AR, Artemjev AA, et al. Novel heterocyclic chitosan derivatives and their derived nanoparticles: catalytic and antibacterial properties. Int J Biol Macromol. 2020b;149:682–692.

[32] Mousavi SA, Ghotasliou R, Kordi S, et al. Antibacterial and antifungal effects of chitosan nanoparticles on tissue conditioners of complete dentures. Int J Biol Macromol. 2018;118(Pt A):881–885.

[33] El-Alfy EA, El-Bisi MK, Taha GM, et al. Preparation of biocompatible chitosan nanoparticles loaded by tetracycline, gentamycin and ciprofloxacin as novel drug delivery system for improvement the antibacterial properties of cellulose based fabrics. Int J Biol Macromol. 2020;161:1247–1260.

[34] Gadkari RR, Suwalka S, Yogi MR, et al. Green synthesis of chitosan-cinnamaldehyde cross-linked nanoparticles: characterization and antibacterial activity. Carbohydr Polym. 2019;226:115298.

[35] Dash S, Kumar M, Pareek N. Enhanced antibacterial potential of berberine via synergism with chitosan nanoparticles. Mater Today Proc. 2020;31:640–645.

[36] Gabriel JS, Gonzaga VAM, Poli AL, et al. Photochemical synthesis of silver nanoparticles on chitosans/montmorillonite nanocomposite films and antibacterial activity. Carbohydr Polym. 2017;171:202–210.

[37] Zhang W, Jiang W. Antioxidant and antibacterial chitosan film with tea polyphenols-mediated green synthesis silver nanoparticle via a novel one-pot method. Int J Biol Macromol. 2020;155:1252–1261.

[38] Pereira AKDS, Reis DT, Barbosa KM, et al. Antibacterial effects and ibuprofen release potential using chitosan microspheres loaded with silver nanoparticles. Carbohydr Res. 2020;488:107891.

[39] Qi L, Xu Z, Jiang X, et al. Preparation and antibacterial activity of chitosan nanoparticles. Carbohydr Res. 2004;339(16):2693–2700.

[40] Senthilkumar P, Yaswant G, Kavitha S, et al. Preparation and characterization of hybrid chitosan-silver nanoparticles (Chi-Ag NPs); a potential antibacterial agent. Int J Biol Macromol. 2019;141:290–298.

[41] Sathiyaseelan A, Saravana KK, Mariadoss AVA, et al. Biocompatible fungal chitosan encapsulated phytoeniglic silver nanoparticles enhanced anti-diabetic, antioxidant and antibacterial activity. Int J Biol Macromol. 2020;153:63–71.

[42] Malini S, Kumar SV, Harirahan R, et al. Antibacterial, photocatalytic and biosorption activity of chitosan nanocapsules embedded with Prosopis juliflora leaf extract synthesized silver nanoparticles. Mater Today Proc. 2020;21:828–832.

[43] Asghar MA, Yousuf RI, Shoai MH, et al. Antibacterial, anticoagulant and cytotoxic evaluation of biocompatible nanocomposite of chitosan loaded green synthesized bioinspired silver nanoparticles. Int J Biol Macromol. 2020;160:934–943.

[44] Chen J, Fan L, Yang C, et al. Facile synthesis of Ag nanoparticles-loaded chitosan antibacterial nanocomposite and its application in polypropylene. Int J Biol Macromol. 2020;161:1286–1295.

[45] Haji S, Khedir SB, Hamza-Mnif I, et al. Biomedical potential of chitosan-silver nanoparticles with special reference to antioxidant, antibacterial, hemolytic and in vivo cutaneous wound healing effects. Biochim Biophys Acta Gen Subj. 2019;1863(1):241–254.

[46] Parthasarathy A, Vijayakumar S, Malaiakozhundan B, et al. Chitosan-coated silver nanoparticles promoted antibacterial, antibiofilm, wound-healing of murine macrophages and antiphagocytosis of human breast cancer MCF 7 cells. Polym Test. 2020;90:106675.

[47] Gadkari RR, Ali SW, Joshi M, et al. Leveraging antibacterial efficacy of silver loaded chitosan nanoparticles on layer-by-layer self-assembled coated cotton fabric. Int J Biol Macromol. 2020;162:548–560.

[48] Inbaraj BS, Chen BY, Liao CW, et al. Green synthesis, characterization and evaluation of catalytic and antibacterial activities of chitosan, glycol chitosan and poly (γ-glutamic acid) capped gold nanoparticles. Int J Biol Macromol. 2020;161:1484–1495.

[49] Ing LY, Zin NM, Sarwar A, et al. Antifungal activity of chitosan nanoparticles and correlation with their physical properties. Int J Biomater. 2012;2012:632698.

[50] Divya K, Smitha V, Jisha MS. Antifungal, antioxidant and cytotoxic activities of chitosan nanoparticles and its use as an edible coating on vegetables. Int J Biol Macromol. 2018;114:572–577.

[51] Khademibami L, Barnes HM, Jeremic D, et al. Antifungal activity and fire resistance properties of nano-chitosan treated wood. BioResources. 2020;15(3):5926–5939.

[52] SathiyaBama M, Muthukumar S. Chitosan guar nanoparticle preparation and its in vitro antimicrobial activity towards phytopathogens of rice. Int J Biol Macromol. 2020;153:297–304.

[53] Ahmed ME, Mohamed HM, Mohamed MI, et al. Sustainable antimicrobial modified chitosan and its
nanoparticles hydrogels: synthesis and characterization. Int J Biol Macromol. 2020;162:1388–1397.

[54] Kritchenkov AS, Zhaliaczniak NV, Egorov AR, et al. Chitosan derivatives and their based nanoparticles: ultrasonic approach to the synthesis, antimicrobial and transfection properties. Carbohydr Polym. 2020c;242:116478.

[55] Sahyon HA, Al-Harbi SA. Antimicrobial, anticancer and antioxidant activities of nano-heart of phoenix dactylifera tree extract loaded chitosan nanoparticles: in vitro and in vivo study. Int J Biol Macromol. 2020;160:1230–1241.

[56] Hossain F, Follett P, Salmieri S, et al. Antifungal activities of combined treatments of irradiation and essential oils (Eos) encapsulated chitosan nanocomposite films in in vitro and in situ conditions. Int J Food Microbiol. 2019;295:33–40.

[57] Hasheminejad N, Khodaiyan F, Safari M. Improving the antifungal activity of clove essential oil encapsulated by chitosan nanoparticles. Food Chem. 2019;275:113–122.

[58] Chaudhari AK, Singh VK, Das S, et al. Antimicrobial, aflatoxin B1 inhibitory and lipid oxidation suppressing potential of anethole-based chitosan nanoemulsion as novel preservative for protection of stored maize. Food Bioprocess Technol. 2020;13:1462–1477.

[59] Vijayan S, Divya K, Varghese S, et al. Antifungal efficacy of chitosan-stabilized biogenic silver nanoparticles against pathogenic Candida spp. isolated from human. BioNanoScience. 2020;10(4):974–982.

[60] Lipşa FD, Ursu EL, Ursu C, et al. Evaluation of the antifungal activity of gold–chitosan and carbon nanoparticles on Fusarium oxysporum. Agronomy. 2020;10(8):1143.

[61] Hussein MAM, Baños FGD, Grinholc M, et al. Exploring the physicochemical and antimicrobial properties of gold-chitosan hybrid nanoparticles composed of varying chitosan amounts. Int J Biol Macromol. 2020;162:1760–1769.

[62] Kumar A, Singh PP, Gupta V, et al. Assessing the antifungal and aflatoxin B1 inhibitory efficacy of nanoencapsulated antifungal formulation based on combination of Ocimum spp. essential oils. Int J Food Microbiol. 2020;330:108766.

[63] Elmehbad NY, Mohamed NA. Designing, preparation and evaluation of the antimicrobial activity of biomaterials based on chitosan modified with silver nanoparticles. Int J Biol Macromol. 2020;151:92–103.

[64] Mohamed N, Madian NG. Evaluation of the mechanical, physical and antimicrobial properties of chitosan thin films doped with greenly synthesized silver nanoparticles. Mater Today Commun. 2020;25:101372.