Chapter 2
Strategies for Synthesis and Chemical Modifications of Chitosan-Based Nanocomposites: A Versatile Material with Extraordinary Potential for Diverse Applications

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Abstract Chitosan is a bio-functional polysaccharide that has a great potential for applications in various fields owing to its chemical functional groups which can be easily modified to achieve specific goals. Chitosan-based nanomaterials are gaining immense interest from researchers due to their versatile physicochemical and biological properties. In the present chapter, we give a complete overview of the preparation strategies of chitosan nanoparticles, including both novel and green methods. Moreover, we have systematically summarized the modification strategies of chitosan for improving their water solubility, biocompatibility, mechanical properties, and antimicrobial activity, which will help the researchers pick the most appropriate strategy for its particular application.

Keywords Chitosan · Chemical modifications · Cross-linking · Chitin · Nanoparticles · Synthesis

Abbreviations

BNNT	Boron nitride nanotube
Ch	Chitosan
COS	Chito oligosaccharide
GTA	Glutaraldehyde
HMW	High molecular weight
LCC	N lauryl-carboxymethyl-chitosan
LMW	Low molecular weight
NPs	Nanoparticles
PCDHN	Physically crosslinked hydrogel double-network
PCL	Polycaprolactone

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PEG	Polyethylene glycol
QAS	Quaternized ammonium salt
TEMPO	(2,2,6,6-tetramethylpiperidin-1-yl)oxyl
TPP	Sodium tripolyphosphate

1 Introduction

The research interest in natural polymers has been rising for the last three decades. The worldwide market for chitosan derivatives has grown at a rate of approximately 6.5% approximately over a period of 5 years, and in 2024, it is anticipated to reach 53 million USD, owing to the increasing investments in newer drug developments, applications in the biomedical field, as well as applications like detoxification of water and wastewater. There is also a growing interest in the use of bio-degradable chitosan products such as fertilizers. Key areas of interest include physical moderation of polysaccharides to increase their applications in the mechanisms of biological activity of the enzymatic methods, polymers, chemical, and products of their physical, enzymatic, chemical, or degradation; and the molecular and biochemical characterization of chitosanolytic and chitinolytic enzymes synthesized by numerous organisms. Chitin deacetylases can be used to bio-convert chitin to chitosan. The aforementioned enzymatic reaction has many advantages over the traditional process used in the conversion, namely, the production of chitosan with the desired degree of deacetylation and higher molecular weight. Chitosanases and chitinases are two other enzymes involved in chitosan and chitin conversion. The various functional groups of chitosan can be moderated using a large number of ligands. The amino group functional group within ligands can be used in a variety of chemical reactions, including metal chelation, sulfonation, alkylation, carboxymethylation, grafting acetylation, quaternization, and so on. Hydroxyethylation, carboxymethylation, sulfonation, and phosphorylation can also be used to modify hydroxyl groups. A plethora of effective strategies for chitosan and its derivatives have been created in order to broaden their applications by increasing their antimicrobial activity and water solubility. Betaine, nitric oxide (NO) releasing donors, a quaternary phosphonium salt, and metal ions, for example, have been added to chitosan derivatives to improve their antimicrobial activity. Various solid materials with good adsorptive properties have been used to improve the heat resistance, strength, and adsorptive activity of chitosan. Chitosan in combination with activated carbon performs well in the removal of some inorganic and metallic pollutants from an aqueous medium. This chapter aims to present recent research on a wide range of chitosan modification strategies [30].
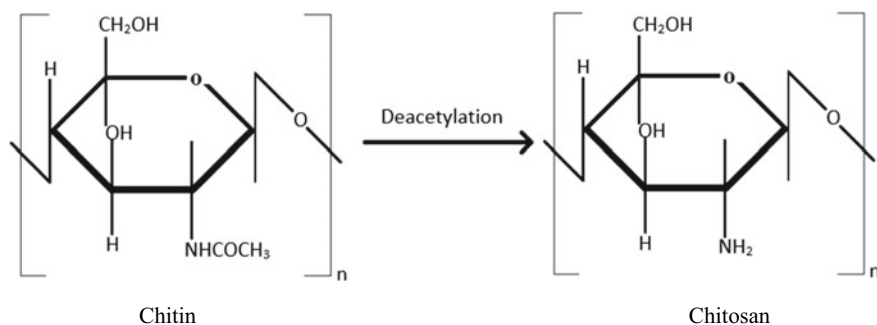
Fig. 1 Structures of chitin and chitosan

2   Structures and Properties of Chitosan

2.1   Structure

Chitosan's structure (Fig. 1) is very similar to that of cellulose, which is made up of hundreds to thousands of –(1-4) linked D-glucose units [57]. The hydroxyl group at the C-2 position of cellulose has been replaced by an acetamide group in the chitosan structure. Chitosan, also known as –(1-4) linked 2-amino-2-deoxy—D-glucopyranose, is an *N*-deacetylated chitin derivative obtained by converting the acetamide groups into primary amino groups [2].

However, chitin deacetylation is never complete, so some chitosan or deacetylated chitin still contains acetamide groups. Chitosan and chitin both contain 5–8% nitrogen, in the form of acetylated amine groups in chitin and primary aliphatic amine groups in chitosan [11] On each repeat unit of chitosan, there are primary and secondary hydroxyl groups, as well as an amine group on each deacetylated unit. These reactive groups can easily change the physical and mechanical properties of chitosan [26].

The presence of amine groups in chitosan is advantageous because it allows for various biological functions as well as the use of modification reactions [3]. Chitosan's excellent properties, such as biocompatibility, biodegradability, bioactivity, non-toxicity, and good absorption, make it an excellent alternative to synthetic polymers [12, 67].

2.2   Physiochemical Properties

Chitosan is well-known for its chelation, solubility, viscosity, mucoadhesive nature, film formation, polyoxysalt formation, and polyelectrolyte behavior. The unbranched and linear forms of chitosan have high viscosity and can be customized by adjusting

the deacetylation conditions. Chitosan is distinguished by its low crystalline region content and a high degree of deacetylation. Several factors influence the physicochemical properties of chitosan, including the degree of deacetylation (DD), crystallinity, MW, and degradation methods [19]. Commercial chitosan is classified into two types based on its MW: high MW chitosan and low MW chitosan. High MW chitosan has a molecular weight (MW) of 190–375 kDa and a DD of >75%, whereas low MW chitosan has a MW of 20–190 kDa and a DD of 75%. It was discovered that the rate of chitosan degradation is inversely proportional to DD and is affected by the order and distribution of acetyl groups. Every glycosidic residue contains three reactive positions, one amino group, and two hydroxyl groups. The amino group is very important because it is responsible for the cationic nature of chitosan as well as the regulation of its various physiochemical properties. It is also pH sensitive. Chitosan dissolves and forms soluble cationic polysaccharides due to the pH-responsive amino groups protonated at lower pH. At a pH greater than 6, the amino group is deprotonated, rendering chitosan insoluble. Its solubility is also affected by acetyl group positioning along the chain, deacetylation methods, and ionic strength. Chitosan exhibits chelating properties for several metal ions at an acidic pH, which occurs at a pH $= 7$ or by electrostatic attraction on protonated ionic groups.

2.3 Biological Properties

According to studies, chitosan is a non-toxic, biocompatible, and biodegradable polymer [4, 36, 62]. Chitosan and its derivatives showcase exceptional biological properties like anti-inflammatory [10, 37], anti-tumor, anti-bacterial [23, 72], antifungal [72], hemostatic [76], and analgesic [5, 76]. Chitosan has a powerful antibacterial effect on a wide variety of pathogenic bacteria due to its cationic nature, which allows negatively charged proteins and lipids in the bacterial cell wall to interact. Chitosan diffusion into the cell membrane causes membrane permeability to expand and disrupt, resulting in cytoplasmic portion leakage and bacterial cell death. Various in-vitro experiments revealed that chitosan's antibacterial function may be due to its DNA binding capacity. When chitosan comes into contact with bacteria nuclei, it binds to DNA and prevents mRNA synthesis [17, 38].

Chitosan's antifungal properties are due to an electrostatic reaction with the negatively charged phospholipids in the cell membrane. Chitosan can penetrate the cell and prevent DNA/RNA synthesis, resulting in cell death when the cell membrane is disrupted. The DD and MW play an important role in regulating chitosan's antifungal properties. Chitosan's antifungal effect is generally enhanced when the DD is higher and the MW is lower [71]. Chitosan has anti-inflammatory properties because it inhibits the release of interleukin-8 (IL-8) and tumor necrosis factor (TNF) from mast cells [53]. Chitosan is superior to NSAIDs (the conventional medications for various inflammatory conditions). It has no gastric side effects because the free amino groups form a defensive shield over the stomach. Chitosan can also be used to heal connective tissues. When chitosan is acid hydrolyzed, glucosamine monosaccharides

are formed, which form the proteoglycan structural units of connective tissues and cartilage, assisting in tissue repair. Chitosan derivatives with anti-tumor activity are known as chit oligosaccharides. According to some researchers, the anti-tumor effects were caused by the increased activity of natural killer lymphocytes. Chit oligosaccharides are thought to cause T-cell differentiation and proliferation by stimulating lymphocyte-activating factors. Some research suggests that chit oligosaccharides effectively boost immune responses and modulate the functions of immunocompetent cells [47]. Chitosan (poly-N-acetyl glucosamine) exhibits a hemostatic effect by accelerating erythrocyte accumulation [63]. Chitosan's positive charge promotes erythrocyte adhesion, fibrinogen absorption, and platelet adhesion and activation. Chitosan's hemostatic property is due to its polycationic content and non-specific plasma membrane binding [73]. Several in-vitro studies have revealed that the main analgesic effect of chitosan is achieved by lowering the concentration of inflammatory mediators (bradykinin) at the site of the injury. It also absorbs protons released at the site of inflammation to control pain [50].

3 Some Common Chitosan Derivatives

A. Mono-Carboxymethylated Chitosan (MCC)

MCC is a polyampholyte capable of forming viscous-elastic gels with anionic macromolecules at neutral pH or in aqueous environments. MCC appears to be less potent than the quaternized derivative of chitosan. It improves the permeation and absorption of low molecular-weight heparin (LMWH; an anionic polysaccharide) across intestinal epithelial cells. Because they cause cell membrane damage, chitosan derivatives have no effect on the viability of intestinal epithelial cells. Permeation increases due to an increase in the number of pores formed in the cell membrane, and there is no need to alter epithelial cell viability [29, 69].

B. N-Succinyl Chitosan

It is made by inserting succinyl groups into the chitosan terminals of glucosamine units. In a succinyl chitosan molecule, $-NH_3^+$ and $-COO^-$ form polyionic complexes. At various pH levels, this derivative is water-soluble [60]. N-succinyl-chitosan exhibits distinct properties in vivo and in vitro, including low toxicity, biocompatibility, and long-term retention in the body. N-succinyl-chitosan is useful as a drug carrier because it can be conjugated with various drugs to avoid complications during chemotherapy [29, 32, 33, 51, 65].

C. N-Acetylated Chitosan

N-acetylated chitosan is used as a gene delivery carrier to overcome the gastrointestinal tract's morphological and physiological barriers to target gene expression. Gene delivery to the intestine via N-acetylated chitosan is more efficient than chitosan alone. This finding is especially significant in the duodenum, where the LacZ gene

is most effectively expressed using *N*-acetylated chitosan. The IL-10 gene is also successfully transferred to the intestines when mixed with *N*-acetyl chitosan. As a result, plasmid DNA can be delivered to the intestines orally using *N*-acetylated chitosan as a carrier. As a result, we can create a dietary dose system to deliver a DNA vaccine for the treatment of gastrointestinal diseases [28, 29].

D. *N*, *N*-Di carboxymethyl Chitosan

This derivative is produced by the alkylation of chitosan with monochloroacetic acid at 90 °C and pH 8–8.5. This water-soluble derivative is a chelating agent that can be used to treat osteogenesis and other chelating applications [25, 29].

E. Thiolated Chitosan Conjugate

This chitosan derivative was created by covalently attaching isopropyl-S-acetylthioacetimidate to chitosan. This derivative has in situ gelling properties, making it a promising novel tool for a variety of drug delivery systems [15, 29].

F. Polyethylene Glycol-Crosslinked *N*-Methylene Phosphonic Chitosan (NMPC)

By reductive animation, NMPC is modified with polyethylene glycol-aldehyde (PEG-CHO) of varying molecular weight. The cross-linking of NMPC with PEG-COH chains increases hygroscopicity and water swelling. This cross-linked NMPC derivative is suitable as a medical material item due to its film-forming capacity and swelling properties [15, 29].

G. Graft-Copolymerization of Chitosan

Radical polymerization is used to create this derivative with 4-(6-methacryloxyhexyloxy)-4′-nitrobiphenyl. Graft-copolymerization is carried out in a homogeneous environment using Azobisisobutyronitrile (AIBN) as an initiator and 2% acetic acid as a solvent [13, 13].

H. *N*-lauryl-Carboxymethyl-Chitosan

The hydrophobic moieties are provided by chitosan with lauryl groups attached to amino groups, and the hydrophilic moieties are provided by carboxymethyl groups attached to hydroxy groups (N lauryl-carboxymethyl-chitosan (LCC)). Taxol is found to solubilize in LCC by forming micelles with particle sizes less than 100 nm. In vitro hemolysis testing revealed that LCC is less likely to cause membrane damage than polysorbate 80 as an intravenous surfactant [29, 42].

I. Galactosylated, Chitosan-Graft-Poly (Vinyl Pyrrolidone)

GCPVP (galactosylated chitosan-graft-poly (vinyl pyrrolidone)) has been identified as a potential hepatocyte-targeting gene carrier. GCPVP alone and GCPVP/DNA complex have negligible cytotoxicity regardless of GCPVP concentration or charge ratio, but GCPVP/DNA complex has a slightly cytotoxic effect on HepG2 cells when

a higher charge ratio and Ca2+ are used. It is established using confocal laser scanning microscopy that the ASGPR of hepatocytes and endocytosis by an interaction between galactose ligands of GCPVP is the major route of transfection of GCPVP/F plasmid complexes [29, 48].

J. Chitosan–Glutathione (GSH) Conjugate

This derivative improves the mucoadhesive and permeation properties of chitosan. The novel thiolated chitosan appears to be a promising multifunctional excipient for various drug delivery systems due to the strong permeation enhancing effect of the chitosan–GSH conjugate/GSH system and the improved cohesive and mucoadhesive properties [29, 44].

4 Methods of Synthesis of Chitosan Nanoparticles

In 1994, Ohya et al., for the first time, described the classification of chitosan NPs for the systemic administration of a chemotherapeutic drug [49]. Following that, the chitosan NPs have been extensively explored by the researchers leading to the development of different methods for their preparation. Various important preparation strategies are mentioned in Fig. 2, and their advantages and drawbacks are depicted in Fig. 3. A brief description of these methods is given below.

4.1 Emulsification and Cross-linking

It is the first method documented in the literature for the preparation of chitosan NPs in which the amino group of chitosan and the aldehyde group of the crosslinking agent has been employed. In this method, an emulsion and an oil phase are prepared. The emulsion consists of an aqueous solution of chitosan while the oil phase comprises span 80 as a stabilizer, toluene, and glutaraldehyde as a crosslinker [27, 49]. On intense mixing of these two phases, the droplets are formed after crosslinking which forms the basis of NPs. The NPs can be separated from the emulsion by centrifugation, multiple washings, and vacuum drying. This approach is, however, no longer employed because of the evident toxicity of glutaraldehyde [75].

4.2 Reversed Micelles Method

It is another method based on covalent crosslinking in which the production of chitosan NPs is assisted by water-in-oil reverse micelle structures (Fig. 4). This method resolved the inconveniences associated with the conventional methods based

Fig. 2 Various methods of preparation of chitosan NPs

on glutaraldehyde as a non-harmful solvent and a crosslinker are employed in this strategy [20, 31]. The aqueous phase consisting of chitosan and glutaraldehyde is mixed with the organic phase which comprises an organic solvent and a lipophilic surfactant. The chitosan NPs are formed via crosslinking within the chitosan-containing core of the micelle.

4.3 Precipitation-Based Methods

NPs formed by precipitation methods are generally larger than 600–800 nm. Two precipitation-based methods which have been described in the literature are:
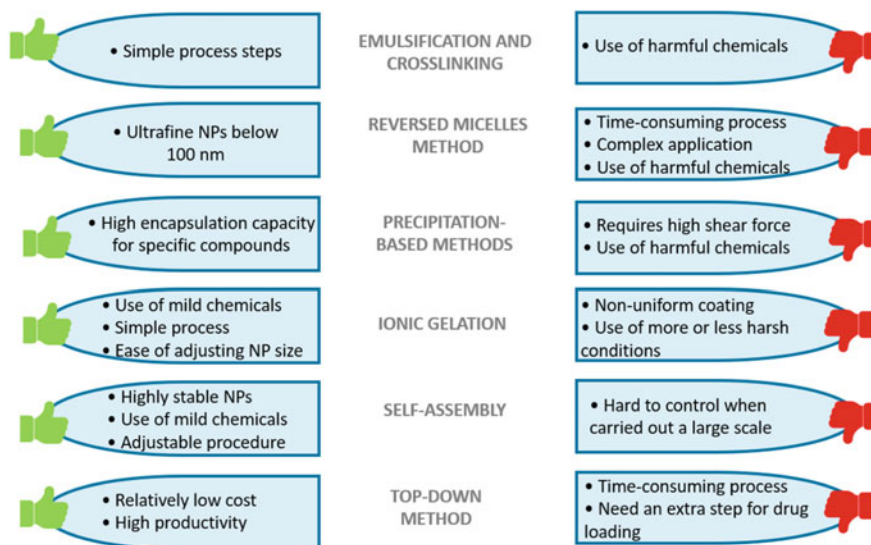
👍 • Simple process steps	EMULSIFICATION AND CROSSLINKING	• Use of harmful chemicals 👎
👍 • Ultrafine NPs below 100 nm	REVERSED MICELLES METHOD	• Time-consuming process • Complex application • Use of harmful chemicals 👎
👍 • High encapsulation capacity for specific compounds	PRECIPITATION-BASED METHODS	• Requires high shear force • Use of harmful chemicals 👎
👍 • Use of mild chemicals • Simple process • Ease of adjusting NP size	IONIC GELATION	• Non-uniform coating • Use of more or less harsh conditions 👎
👍 • Highly stable NPs • Use of mild chemicals • Adjustable procedure	SELF-ASSEMBLY	• Hard to control when carried out a large scale 👎
👍 • Relatively low cost • High productivity	TOP-DOWN METHOD	• Time-consuming process • Need an extra step for drug loading 👎

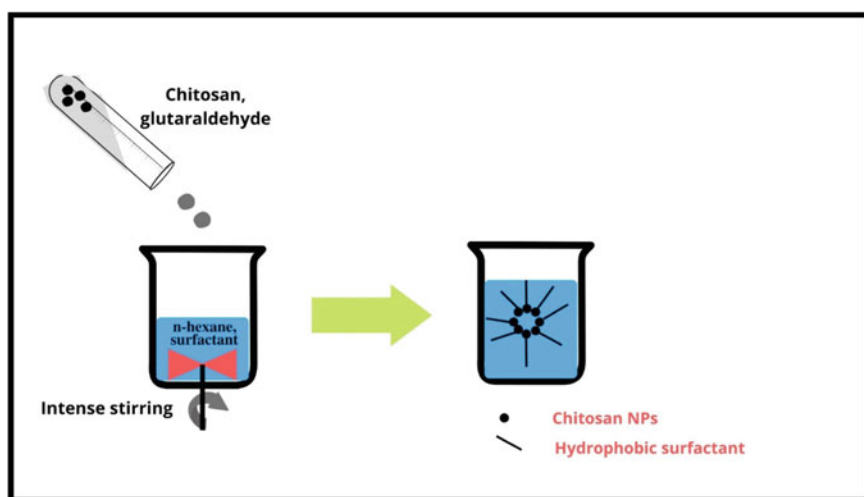**Fig. 3** Advantages and drawbacks of various preparation methods of chitosan NPs

Fig. 4 Schematic illustration of formation of chitosan NPs via reversed micelles method

- The phase inversion precipitation method: This method is based on the combination of emulsification and precipitation. In the presence of a stabilizer, an oil-in-water emulsion is prepared using an organic phase and an aqueous solution of chitosan. After the application of high-pressure homogenization, the emulsion is

separated from the methylene chloride by evaporation, causing acetone to diffuse out of the droplets and NPs to precipitate simultaneously [75].

- The emulsion-droplet coalescence method: In this process, the precipitation of NPs is induced by the coalescence of two water-in-oil emulsions. The continuous phase for two emulsions, one with chitosan and another with NaOH, is prepared by mixing liquid paraffin and sorbitan sesquiloleate. The chitosan-containing emulsion is prepared by applying high-speed homogenization. Following the mixing of two emulsions, NaOH diffuses into the ultrafine droplets which lower the solubility of chitosan, inducing NP formation and precipitation [24].

4.4 Ionic Gelation

It is one of the most preferred strategies for the preparation of chitosan NPs primarily because it does not involve the use of toxic crosslinkers or solvents. This method was first described by Calvo et al. in 1997 [8]. This technique follows the principle of ionic crosslinking, which occurs when the oppositely charged groups are present. Firstly, chitosan is added to the aqueous solution of an acid (generally acetic acid) followed by the addition of an aqueous solution of sodium tripolyphosphate (TPP) under intense stirring [75]. The diffusion of anionic molecules and the cationic chitosan molecules takes place leading to crosslinking and ultimately the formation of NPs (Fig. 5). Although various anionic crosslinkers like glutaraldehyde can be used to synthesize chitosan NPs, TPP is preferred because of its biocompatibility and biodegradability [59].
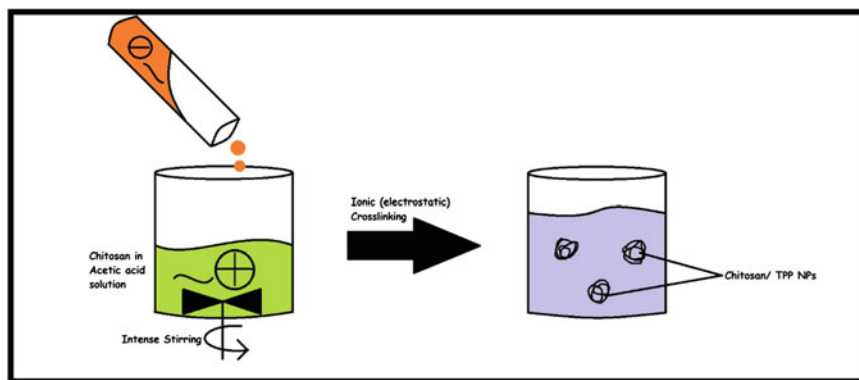

Fig. 5 Schematic illustration of production of chitosan NPs via ionic gelation method

4.5 Self-assembly

This method is extensively employed for the formation of NPs. It is based on numerous interactions which take place at the same time. The nature of interactions can be any of the following [56, 78]:

- Electrostatic
- Hydrophobic
- Hydrogen bonding
- Van der Waals forces

The self-assembly approach may either involve complex formation between chitosan and the natural anionic molecules or the modification of hydrophobicity of chitosan via grafting. NPs produced by this approach is highly suitable for the encapsulation of lipophilic and hydrophilic drugs [56].

4.6 Top-Down Approach

In this approach, two steps are involved in the preparation of nanoparticles:

 (i) Acid hydrolysis: Acid hydrolysis of chitin is carried out in the presence of a strong acid like HCl to form chitin nanocrystals.
(ii) Deacetylation: This step involves treatment with alkali (NaOH) to form chitosan NPs, which are basically the chitin NPs with a deacetylation level of more than 60% [74].

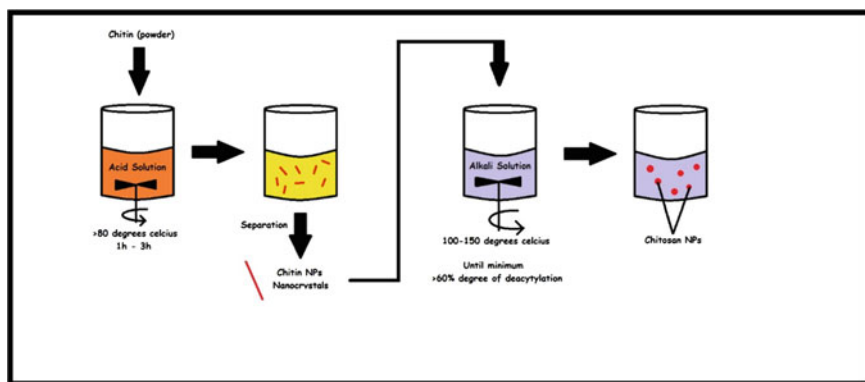The formation of chitin NPs via the top-down method is illustrated in Fig. 6.

Fig. 6 Schematic illustration of formation of chitosan NPs via top-down method

5   Green Synthesis of Chitosan Nanoparticles

The biological/green methods for the extraction of chitosan are becoming highly prevalent due to the various drawbacks of chemical extraction techniques such as [64]:

- Alteration of the physico-chemical properties of chitin
- High cost of purification processes
- Presence of some chemicals in the wastewater effluents

In biological synthesis, enzymes and micro-organisms are employed for the extraction of chitin and the recovery of chitosan. The various advantages of the biological extraction of chitin are highlighted in Fig. 7. Khanafari and co-workers compared the chemical and biological methods of extraction of chitin from shrimp shells and showed the superiority of the biological method over the chemical one because the structural integrity of chitin was preserved when it was extracted through the biological method [35]. In a study, the extraction of chitin from shrimp shells and fungi was investigated by Teng et al. in which they used a one-pot fermentation process where the proteins were hydrolyzed into amino acids by fungal proteases [68]. Younes et al. extensively reviewed the methods of preparation of chitosan from marine sources and their future outlooks [79].

Spray drying is one of the green preparation routes for the preparation of chitosan NPs. In this method, chitosan is dissolved in the aqueous acetic acid and the resulting solution is passed through a nozzle, keeping the temperature range between 120 and 150 °C, leading to the formation of chitosan NPs. Spray drying can also be used to obtain magnetic chitosan NPs [22].

Supercritical-CO_2-assisted solubilization and atomization (SCASA) is a green method in which only water and CO_2 are used during the preparation process. This technique is devoid of any acid or harmful solvents. After about 48 h of the dissolution of CO_2 in water, the chitosan solution is fed to a fluidized bed, and NPs are formed which are collected by a filter placed on the fluidized bed [75]. The publications associated with the "green synthesis" of chitin/chitosan-based nanomaterials in the literature are very few. As discussed earlier, chitosan nanoparticles are frequently prepared by the ionic gelation method in which the ionic crosslinking takes place between chitosan and TPP. Recently, Gadkari et al. reported the green synthesis of antimicrobial chitosan NPs, which were prepared by chemical crosslinking between chitosan and a cinnamaldehyde, an eco-friendly bactericidal agent [18]. Purified chitin films and nano fibres with high molecular weight were successfully obtained by Qin et al. by employing eco-friendly ionic liquids [55]. Green synthesis of Ag-Ch nanocomposites was reported in which chitosan served as a reducing agent as well as a stabilizing agent, using NaOH as an accelerator. The as-prepared nanocomposite was shown to have antimicrobial activity against *E. coli* and *S. aureus* bacteria [66].
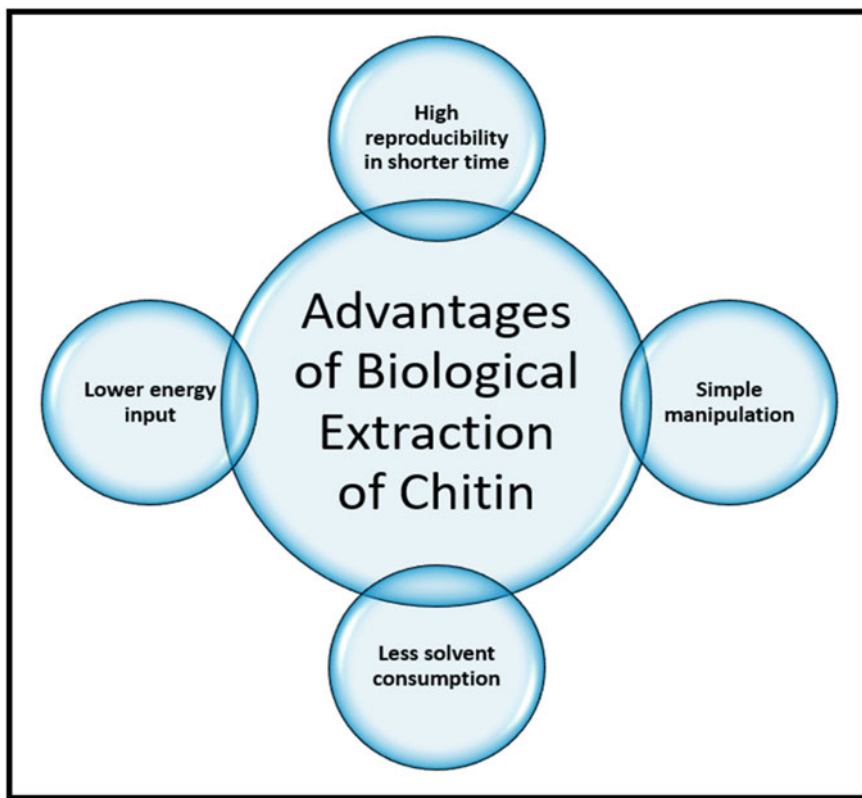
Fig. 7 Advantages of biological extraction of chitin

6 Modification Strategies of Chitosan

There are different scopes of modifications of chitosan that are depicted in Fig. 8.

6.1 Chitosan Modification to Produce Functional Derivatives

There are various types of chitosan derivatives that are produced by modification and are presented in Fig. 9.

6.1.1 Quaternized Chitosan Derivatives

According to numerous publications, it is possible to modify the positive (NH_3^+) charge of chitosan in order to make it soluble over a wide pH range and in a neutral or

Fig. 8 A schematic diagram depicting a few scopes of modification of chitosan
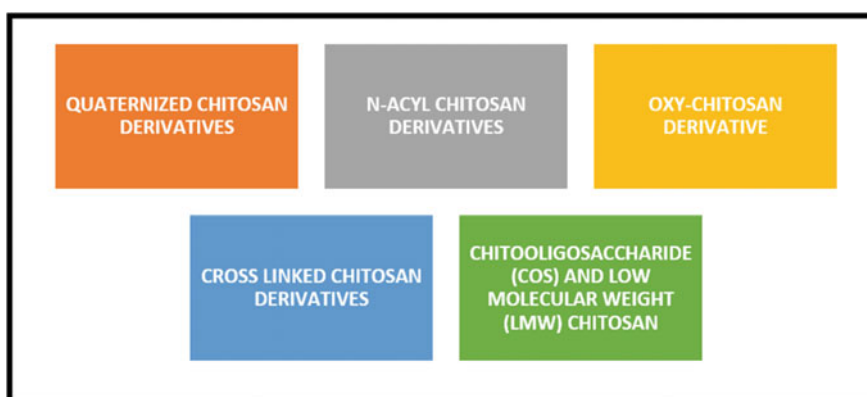

Fig. 9 A schematic representing various types of modified chitosan for the generation of functional derivatives

a little alkaline medium. This is one method for increasing the solubility of chitosan in water. At pH 6.5, chitosan is positively charged, whereas quaternized chitosan remains permanently positively charged at pH > 6.5. Under basic conditions, a quaternization reaction occurs between chitosan and alkyl iodide. The most well-known quaternized chitosan is N,N,N-trimethyl chitosan chloride (TMC), which has been described for a variety of applications. TMC is produced through two consecutive reactions. The first involves N-methyl-2-pyrrolidinone (NMP), which is used as a solvent in alkaline conditions (NaOH) in the presence of methyl iodide and chitosan, and the second involves the replacement of iodide ions with chloride ions using anionic exchange resin. By varying the carbon length of alkyl halides, different types of quaternized chitosan can be easily obtained [7].

6.1.2 *N*-Acyl Chitosan Derivatives

Chitosan becomes aquaphobic through the N-Acylation process, which involves implanting various fatty acids. The reaction is an addition of amide to fatty acid –COOH groups and chitosan –NH$_2$ groups. Acyl halide or acid anhydride is the chemical reagents used in N-Acylation. This acylation is typically carried out in pyridine/chloroform, pyridine, and methanol/acetic acid/water. Nonetheless, because the chitosan repeating unit contains two reactive –hydroxyl groups, this reaction can produce O-alkyl chitosan. Many researchers recommend replacement with trityl groups, in place of chitosan's primary –OH groups to avoid O-acylation. Because of the formation of chitosan chloroacyl, this process improves the N-Acylation step. N-Acyl chitosan has been produced using a variety of acid anhydrides [7].

6.1.3 Oxy-Chitosan Derivatives

Many researchers have looked into the production of chitouronic acid sodium (carboxylated chitin or chitosan) which is soluble in water using TEMPO-an organic catalyst used in the oxidation of –OH functions into –CHO in NaOCl and NaBr conditions. TEMPO is well-known for its use in regioselectively oxidizing the primary hydroxyl groups of various polysaccharides [7]. TEMPO is used to make oxy-chitosan derivatives, specifically 6-oxy-chitosan. Chitouronic sodium salts are typically made from shrimp or fungal cell chitin that has been pre-treated (chemically or enzymatically). A new class of carboxylated chitosan with high biocompatibility on human keratocytes was produced from Trichoderma and Aspergillus fungal biomass. C6-oxy-chitosan, a novel bioactive derivative, has shown to be efficient against activity Leishmania. Recently, an environmentally friendly process has been developed for the C$_6$ oxidation of chitosan using a TEMPO system to produce chitosan soluble in water has been developed [7].

6.1.4   Chitosan's Cross-linked Derivatives

The cross-linking of chitosan requires the use of very particular chemical agents to get the chains linked together, which ultimately results in a 3-D macromolecular network. Chitosan is cross-linked in the presence of –CHO derivates, such as glyoxal, glutaraldehyde (GTA), or formalin with covalent bonds, to produce chitosan-based hydrogels. The cross-linking reaction with chitosan involves the formation of a Schiff base (imine). The most researched cross-linking agent is GTA [7]. It is cheap, readily available, and synthetic. In the presence of labile hydrogen, the hired response is a condensation response among the aldehyde group and a primary amine group from the chitosan chain. However, GTA is toxic, and natural GTA substitutes are being investigated for use in the production of chitosan hydrogel. Figure 3 depicts the chitosan cross-linking reactions. Citric acid was used as a cross-linking agent in the preparation of chitosan/polyvinyl alcohol (PVA) membranes. This cross-linking strategy was investigated in order to create biomaterials for hemodialysis membranes [7]. Cross-linking citric acid and chitosan was expected to incorporate carboxylate groups (COO) into biomaterials, increasing the bioactive sites on the chitosan membrane for biomolecule transport (urea, creatinine, etc.). PVA was used to improve the crosslinked chitosan membrane's mechanical efficiency and hydrophobicity. Figure 10 depicts the main cross-linking chitosan strategies.

6.1.5   Chitosan with Low Molecular Weight (LMW) and Chito Oligosaccharide (COS)

Because of its excessive viscosity, HMW chitosan is difficult to be applied commercially. Because of the production of COS and the low molecular weight of chitosan, a reduction in its molecular weight is an effective idea for addressing viscosity issues and improving its biological properties [7]. Oligosaccharides are defined as oligomers with a degree of polymerization ranging from 2 to 10, but some higher degrees of polymerization are classified as LMW. COS and LMW chitosan are primarily produced using enzymatic, chemical, and physical methods. The reduction of MW via enzymatic, chemical, or physical processes is linked to improved chitosan water or acetic acid solubility. Depolymerization of chitosan is primarily accomplished through chitosanolysis of acid, which is the most used method for producing COS and LMW chitosan. Using HCl, HNO_2, H_2O_2, and potassium persulfate in chitosanolysis are examples of chemical processes. Sonication, microwave irradiation, gamma irradiation, or a thermal procedure are examples of physical processes. Enzymatic processes employ both specific enzymes and nonspecific enzymes like chitinase, chitosanase and pepsin, cellulase, pronase, lysozyme, papain, hemicellulase, or pectinase, respectively. However, the main issues with enzymatic depolymerization are the costs [7]. High-pressure homogenization (HPH), plasma, and the use of zeolites as adsorbents to purify acid hydrolysis COS and LMW chitosan have all been described as new methods for reducing the molecular mass of chitosan. Furthermore, electrochemical processes for depolymerizing chitosan have been developed.
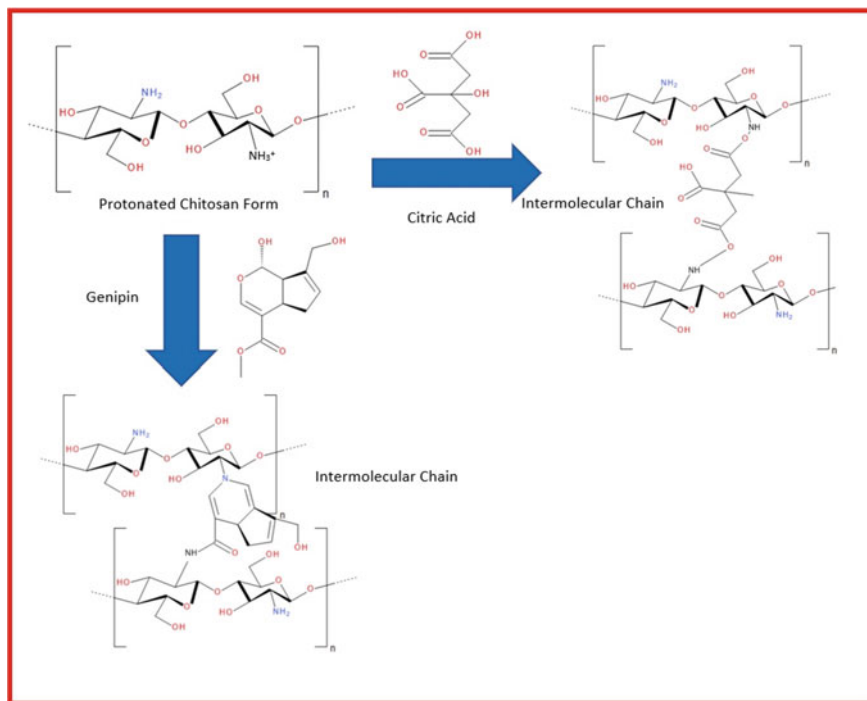
Fig. 10 The cross-linking reactions of chitosan

6.2   Enhanced Anti-microbial Performance

6.2.1   Introducing Cationic Compounds

Although the degree of de-acetylation, molecular weight, and content of the NH_2 residual group can influence the anti-microbial activity of chitosan derivatives, the same of chitosan derivatives is primarily dependent on the positively charged NH_2 group, which can interfere with and destroy the negative bacterial membrane, bind bacterial DNA, and block protein synthesis [14]. The higher the positive charge, the greater the microbial activity. Increasing positive electricity can improve the antimicrobial activity of chitosan derivatives:

A. NH_2 Direct Quaternization

Quaternary ammonium salt is the most commonly used anti-microbial group in chitosan derivatives [1]. NH_2's direct quaternization group was carried out primarily to improve the anti-microbial activity of the chitosan derivates, which can be tuned by varying the ratio of the cationic groups present. Following research, it was discovered that the N-trimethyl group was the most important factor in improving the anti-microbial activity of chitosan derivatives [14]. By varying the trimethylation,

acetylation, and acylation, the chitosan derivative with the best anti-microbial activity, better than *S. aureus* and *E. coli*, could be obtained.

B. Grafting onto Chitosan

By grafting QAS, and betaine, chitosan derivatives' antimicrobial activity can be improved. Combining acetate chitosan and C_{12}–C_{18} alkyl aminopropyl betaine demonstrated a broader anti-microbial spectrum and superior anti-microbial activity than either compound alone. Despite the fact that glycine betaine contains QAS, its anti-bacterial mechanism differs from traditional QAS due to chelation between the NH_2 group and the trace amount of metal ions (Ca^{2+}, Na^+) that are required for bacteria to maintain their stability and metabolism of the cell, which can destroy the stability and inhibit the growth of cell membrane [16]. As a result, retaining the necessary amounts of NH_2 and OH groups during the chemical modification of chitosan is required [14].

C. Grafting Polymerization from Chitosan

Through grafting copolymerization of chitosan acrylamide and methacrylamido-propyl trimethyl ammonium chloride, PQAS can be incorporated into the chitosan skeleton. The produced chitosan derivatives can destroy the bacteria membrane at just a concentration of 14 mg/L. Similarly, anti-microbial chitosan-based polymer-ized nanoparticles with positive surface charges were synthesized by grafting *N*-trimethyl aminoethyl methacrylate chloride or methyl methacrylate and *N*-dimethyl aminoethyl methacrylate hydrochloride from chitosan [14].

6.2.2   Neutral Anti-microbial Compound Conjugation

A. Polypeptide

Another way of producing chitosan peptide conjugate (CPC) with improved antimi-crobial activity is to integrate it with an antimicrobial peptide (CGGG(KLAKLAK)2) and an enzyme-cleavable peptide (GPLGVRGC). The morphology of CPC can be enhanced due to the peeling off of the protective PEG layer induced by the pres-ence of gelatinase from bacteria, resulting in excellent anti-microbial activity with a minimum inhibitory concentration value of about 7×10^{-6} M based on the disruption of hydrophobic/hydrophilic balance.

B. NO Donor

Chitosan can be used as a loading matrix for NO, an anti-biofilm agent [58] to create a derivative that can slowly release NO by sparging chitosan derivatives with NO gas Planktonic cells treated with NO-loaded chitosan show a decrease in colony-forming units, which could be useful in wound dressing. NO can be loaded on chitosan-poly(amidoamine) via reaction with its secondary NH_2 group, yielding chitosan-poly(amidoamine)/NONOate with a loading amount of 1.7 mol/mg and excellent antimicrobial and wound healing activity.

6.2.3 Metal Coordination

By coordinating chitosan with Ag^+ ions, a construction based on the synergistic impact of chitosan and Ag^+ ions was recently done [9], Moldable hydrogel with an antibacterial action against *S. aureus* and *E. coli*. The Ag^+ ions can be complexed with chitosan and then photo reduced or electro reduced in situ [77]. The anti-microbial effect was greatly enhanced by the synergistic effect of chitosan and metal ions, which stabilized the metal through coordination and by increasing its positive charge, which further promoted its interaction with negatively charged bacteria. Cu(II) complexes formed by chelating O-carboxymethyl chitosan (CMCS) Schiff bases with Cu^{2+} ions have significantly higher antifungal activity against Phytophthora capsici than the original chitosan [54]. The easy release of copper cations from the complex, caused by the benzene ring's space steric hindrance, results in an effective anti-fungal performance.

6.2.4 Physical Integration

The physical blending of chitosan derivatives with antibiotics is another common method to increase the anti-microbial activity of chitosan derivatives. Through the solgel transition process, a polyelectrolyte composite hydrogel based on chitosan/CMchitosan/AgNPs demonstrates long-term antibacterial efficacy against *S. aureus* and *P. aeruginosa* [77]. Even while direct combination with antibiotics can enhance chitosan's anti-microbial performance, slow-release antibiotics may increase the probability of microorganism resistance, which requires careful consideration in future research. Based on the synergistic impact of CNC rod and polycationic CS, chitosan can be mixed with cellulose nanocrystals (CNCs) to generate a hydrophobic spray-coating composite with increased antimicrobial action. CNC can cause bacterial membrane rupture, allowing chitosan derivatives with protonated NH_2 groups to enter the cell, resulting in bacterial death [70].

6.3 Improved Water Solubility

For the improvement of solubility in the water of chitosan under a basic and neutral environment, hydrophilic compounds with reactive groups can be conjugated with chitosan through amide and hydroxyl groups [14].

6.3.1 Modification of the Amine Group

Hydrophilic compounds with –COOH, –CHO, –OCN, and epoxy easily bind to the backbone of the chitosan with the aid of coupling reactions with its very reactive amide group. By grafting Brij-S20-succinic anhydride to chitosan, the solubility

in the water of chitosan can be enhanced. Brij-S20-succinic anhydride disrupts the inter/intra molecular bonds, lowering crystallinity and increasing solubility. Chitosan complex 1-(4-(2-aminoethyl) phenoxy) zinc(II) phthalocyanine (ZnPcN) [46] on conjugation with acid groups of CMCS, and amide groups of phthalocyanine, improves solubility in the water because the conjugate's absorption intensity ratio is double than that of ZnPcN used alone, and agglomeration in water is reduced. When the amide group of chitosan reacts with the acid group of gluconic acid, the generated sugar-carrying chitosan is soluble under various pH settings due to the hydrophilicity of the gluconyl group and the *N*-acetyl group [14].

6.3.2   Hydroxyl Group Modification

The OH group of chitosan reacts with halo hydrocarbon or sulfonyl chloride to produce CMCS, which has better solubility in the water [40]. The solubility in the water of chitosan can also be improved by the reaction of its hydroxyl group with *N*-acetyl ethylenediamine. It is evident that solubility in water of chitosan can be enhanced by the replacement of the hydrophilic group chemically [14].

6.4   Better Biocompatibility

Biocompatibility is very important, especially in cases where biomaterials have a direct contact with human organs or tissues [14]. It relates to how specific tissues react to different elements.

6.4.1   Organic Modification

A.   Introduction of Carboxyl Group

By inserting a carboxyl group into the molecular structure of chitosan derivates, the toxicity of protonated cationic amide or groups in chitosan derivates can be minimized, resulting in CMCS, which is non-toxic, biocompatible, and biodegradable [14].

B.   Introduction of Betaine

Zwitterionic betaine can combine huge amounts of water to form a hydration layer through hydrogen bonding and electrostatic contact [21, 34, 80]. The water of hydration between proteins and zwitterionic molecule imposes substantial repulsions against nonspecific protein adsorption because it restricts the movement of water adsorbed at the surface of glycine betaine. With the addition of betaine, the derivative exhibits effective antimicrobial characteristics, anti-nonspecific protein adsorption, and anti-platelet adhesiveness [14].

C. PEGylation

Because of its high hydrophilicity and chain flexibility, PEG has been used to alter chitosan derivatives to minimize toxicity and nonspecific protein adsorption, resulting in a volume exclusion effect [14]. Crosslinking chitosan with aldehyde terminated PEG or grafting activated monomethoxy poly(ethylene glycol) to chlorinated *N*-phthaloyl chitosan can be used to make the PEG-modified chitosan derivative [43].

D. Introduction of Polycaprolactone

Biocompatible polycaprolactone (PCL) can promote cell proliferation and adhesion [41], as a result, it can be utilized to improve the rate of healing and the quality of neonatal tissue. In comparison to $PHBHH_x$ or chitosan-g-PCL/$PHBHH_x$, the chitosan-g-PCL and poly(3-hydroxybutyrate-co-3-hydroxyhexanoate) ($PHBHH_x$) hybrid have a rougher surface and smaller grain sizes, which can increase cell adhesion and proliferation, resulting in higher initial cell survival.

E. Introduction of Gelatin

Mixed –OH groups modified biocompatible boron nitride nanotubes (BNNTs) with chitosan to form a slow degradative chitosan/BNNT-OH scaffold that showed improved cellular proliferation and adhesion compared to chitosan, based on the increased mechanical performance and pore size of biocompatible boron nitride nanotubes (BNNTs) [6]. The discontinuousness and hydrophilicity of water-swollen gelatin nanospheres are responsible for the deposited derivatives with controlled wettability and surface roughness. The ratio of chitosan and harmless gelatin nanospheres in the derivative can be changed to alter protein adsorption and cell adhesion [14].

6.4.2 Inorganic Modification

A. Carbon/Boron Materials

Some inorganic compounds, such as graphene oxide, can improve chitosan biocompatibility by enhancing its surface shape, roughness, and pole size, which encourages cell attachment and proliferation at the surface of chitosan derivatives [14].

Mixed OH groups modified biocompatible boron nitride nanotubes (BNNTs) with chitosan to form a slow degradative chitosan/BNNT-OH scaffold that showed improved cellular proliferation and adhesion compared to chitosan, based on the increased mechanical performance and pore size of biocompatible boron nitride nanotubes (BNNTs) [14].

B. Metal Ions

By combining biocompatible Zn^{2+} ions with chitosan, the resulting porous CS_2ZnAlg microspheres can further increase [Zn^{2+}] [52]. When compared with the control

group, the hemostasis time of the chitosan/Zn^{2+} derivative was drastically reduced (134 5 s) (over 600 s) [14]. Following the termination of bleeding, the chitosan/zinc derivative shapes dark red aggregates of blood cells, platelets, fibrins, and microspheres near the wound site. Chitosan's positive charge can reendow its mucoadhesive activity because it merges with negatively charged red blood cells.

Enhanced Mechanical Property

Chitosan's mechanical performance can be bettered if it forms a great number of networks either by physical or by chemical crosslinking [61] or in combination with metal nanoparticles [39]. The physically crosslinked hydrogel double-network (PCDHN) of chitosan/Na alginate (SA)/calcium ions (Ca^{2+}) is much better than a physical single network of hydrogel cross-linked via electrostatic interactions, and 10 times better than chitosan/SA hydrogel. The creation of a double network through the use of hydrogen bonding can also strengthen chitosan derivatives. Poly(vinyl alcohol) CS [45] (CPH) DN hydrogel has exceptional tensile strength, elongation at break, and compressive strength thanks to physical crosslinking. The adsorption of fracture energy by chitosan's first physical network results in CPH's outstanding excellent mechanical quality, while the second poly vinyl alcohol network maintains the hydrogel's shape. Furthermore, the biocompatible chitosan-based hydrogel with surface mineralization can enhance the differentiation of bone marrow stem cells [14].

7   Conclusions

Chitosan, one of the most available biopolymers, has been extensively studied and used in diverse fields due to its biodegradability, biocompatibility, non-toxicity, antimicrobial, and antifungal properties. However, its broad applications are limited by its poor solubility and insufficient mechanical strength. To this, chemical modification has been demonstrated to be an effective approach. After their first description over two decades ago, Chitosan NPs have acquired a significant position in various industries like pharma and agriculture. Various methods for the modification and preparation of chitosan NPs have been developed. Sometimes, the NP synthesis requires the use of toxic substances which may adversely affect the environment. To overcome this issue, green preparation techniques stand out as an alternative. These methods are eco-friendly as well as cost-effective. Also, these are devoid of toxic chemicals and high energy, pressure, etc., are not required. Moreover, the synthesis of chitosan NPs using biological methods (using microorganisms like bacteria, fungi, and algae) is even more appealing since it is rapid, non-toxic, and environment-friendly. However, there are scarce reports available on the green synthesis of NPs. The development of novel and innovative green methods for the synthesis of chitosan NPs is a domain where more research and developments are expected in the future.

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