Green synthesis of polysaccharide-based inorganic nanoparticles and biomedical aspects

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8.1 Introduction

In recent years, there has been an increasing interest in the development of green and environmentally safe products. Subsequently, green chemistry has fast become the primary focus of researchers to devise green and nonhazardous chemical processes using nontoxic solvents, biodegradable, and sustainable materials.

The green synthesis of metallic NPs using polysaccharides has become an important field of research in bio-nanotechnology. The synthesis of nanomaterials using polysaccharide-based metallic NPs with specific magnetic, optoelectronic, and physiochemical properties, as well as their applications in biomedical and biochemical sciences is an increasingly important area in green chemistry [1]. The green methods to synthesize NPs have evoked a great amount of attention; especially as the conventional methods for the production of NPs have a number of limitations. The conventional methods rely on the application of toxic chemicals and are not energy efficient, which cause environmental concerns [2, 3]. Polysaccharides are chain polymers that composed of repeated saccharides units linked together through glycosidic bonds. Polysaccharides are generally more stable than proteins, and an irreversible denaturation will not normally occur in these polymeric carbohydrate biomolecules, which make them favorable in materials science. In addition, the isolation and recovery of polysaccharides are usually cost effective. Polysaccharides vary depending on a number of parameters such as molecular weight, chain structure and structural conformation, chirality level, number of hydroxyl groups, solubility properties, etc. This variation in physicochemical properties of carbohydrates offers biological and molecular advantages for their applications in the fabrication of nanocomposites and nanomaterials [4]. The stabilization and reduction of NPs is the major role of polysaccharides in the synthesis process of metallic NPs.

Various types of polysaccharides such as microalgae, macroalgae, micro seaweed, or bacteria-based polysaccharides have been introduced as the stabilizing and reducing agents in the production of the metallic NPs, which has been discussed in

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detail in the following sections. Furthermore, a various number of metals such as gold (Au), silver (Ag), zinc (Zn), copper (Cu), and palladium (Pd) have been employed for the production of polysaccharide-based metallic NPs. However, among all different types of metallic NPs, gold nanoparticles (AuNPs) and silver nanoparticles (AgNPs) are the most commonly used, as they have a wide range of applications due to their biocompatibility, shape, size, low toxicity, antimicrobial characteristic, and high stability. This chapter focuses on polysaccharide-mediated AuNPs and AgNPs, with their preparation, characterization, and biomedical applications. This chapter begins with introducing the preparation and characterization methods of NPs. It will then go on to review and summarize the biomedical applications of polysaccharide-based AuNPs and AgNPs. The final section of this chapter summarizes the potential risk of polysaccharide-based metallic NPs. Finally, the conclusion provides a brief summary, followed by the identification of areas for future research.

8.2 Polysaccharide-mediated synthesize of NP versus conventional techniques

Synthesizing NPs can be classified into sol–gel technique, colloidal process, water-oil microemulsion technique, polyol method, and hydrothermal synthesis. Conventional synthesis methods of metallic NPs such as sol-gel, chemical deposition, and physical methods are not environmentally safe, as they result in high-energy consumption and are not environmental friendly [5]. In addition, conventional methods of producing NPs usually use toxic solvents and generate toxic residuals [6], which make them inappropriate for biomedical applications [7]. For example, synthesized NPs need to be precipitated or removed from the solution, usually by adding a reducing agent. Reducing agents such as *N*,*N*-dimethyl-formamide, sodium borohydride, and trisodium citrate are some of the commonly used chemicals, which are toxic to both human and environment [8]. To limit the hazardous chemicals for synthesizing NPs, eco-friendly processes without the use of toxic chemicals have been developed. With this regard, green synthesis techniques using polysaccharides as a stabilizing/reducing agent have been suggested as an alternative to the conventional methods [9].

8.3 Preparation of polysaccharide-based metal NPs

Different strategies have been used to synthesize polysaccharide-based metallic NPs; top-down and bottom-up approaches [10, 11]. In the top-down process, starting material is subjected to pretreatments for reducing the material's size using various treatments such as milling, chemical or plasma etching, or thermal ablation. However, the application of high pressure and or temperature during the top-down process may affect the physicochemical properties of NPs [11]. Thus, the bottom-up process known as self-assembly process is the preferred method in the preparation of NPs. In this technique, the metallic precursor is first reduced to a zero valent state to form a building block and then

the process continues with nucleation and growth of nanocrystals [12, 13]. Furthermore, polysaccharides can attach to metallic ions and NPs through noncovalent bonding that changes the order of free energy. This process can, therefore, stabilize the NPs and affect the morphological properties and growth of NPs crystals [14]. Polysaccharides with stereo genic centers can also help the attachment of metallic NPs [15]. In addition, the NPs synthesized through the bottom-up process are more homogeneous compared to the top-down process. Furthermore, as the bottom-up process can be performed in either bulk or droplet solutions, makes it easy to control the experimental condition [16].

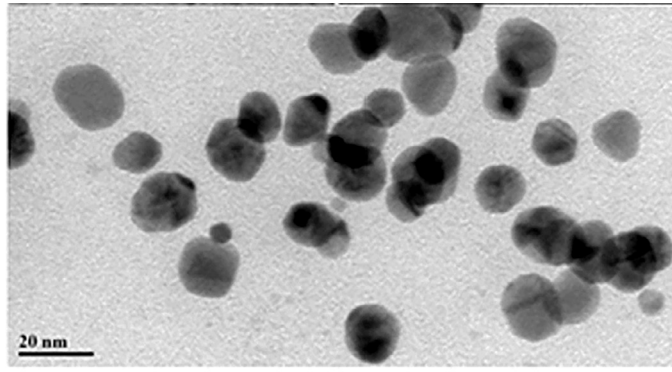
Since the method of preparation of NPs determines their physicochemical and biological properties, selection of a proper method is essential [17, 18]. Several ways including adjusting the reaction time, temperature, and molar ratios of ions and polysaccharides are available to synthesize metallic NPs [19]. However, there have been always attempts to improve characteristics of synthesized NPs. In this regard, methods such as microwave-assisted heating [20], radiolytic reduction [21], electrochemical synthetic techniques, microemulsion, and photo-induced reduction have also been introduced to the field to improve the properties of NPs [22].

The microwave-assisted synthesis of NPs is a popular method in the area of nanotechnology, as often causes faster chemical reactions, produces higher yields and fewer side products compared to the conventional heating methods (e.g., oil bath) [23, 24]. The microwave-assisted synthesis can also give an excellent control over reaction mixing, with excellent reproducibility from reaction to reaction [25]. Using microwave irradiation, metal nuclei are formed in the solution, with the ability to control the generation of nuclei and consequently particle growth. This has a very important implication, as allows the precise control of the size of metal NPs and their size distribution [26]. It is shown that a microwave-assisted heating procedure used in the production of chitosan sulfate-coated AuNPs reduces Gibb's free energy and induces the reaction activation [20]. In another study AgNPs synthesized using xylan as the reducing and stabilizing agent under microwave irradiation were highly stable, uniformly distributed, and mostly spherical. The best microwave-related reaction conditions were microwave radiation temperature of 60–70°C, microwave power of 800 W, and microwave time of 30 min [27].

The radiolytic reduction is also a convenient and environmental-friendly method to prepare metallic NPs. The method is shown to be useful in nucleation, growth, and the aggregation of NPs and large-scale production of NPs [21, 28]. Radiolytic reduction method provides highly pure NPs that are free from by-products and chemical reducing agents. It is also possible to control the size and structure of particles using this method [21]. The electrochemical method has also been used in a number of studies to produce polysaccharide-based metallic NPs [29]. Using this method, a multistep process of NPs production will be replaced by a single step, which is considered as the biggest benefit of the method. It is shown that electrochemical method produces metallic NPs of larger size, whereas electrochemical complexation/UV reduction produces smaller size metallic NPs with an increased antimicrobial activity [30]. The potential beneficial effects of methods such as microemulsion and photo-induced reduction on the characteristics of polysaccharide-based metallic NPs needs to be considered in the future studies [22].

8.4 Characterization of NPs

NPs physical properties such as diameter, morphology, dispersibility, and molar volume determine their unique characteristics. There is a variety of techniques such as scanning electron microscopy (SEM) and transmission electron microscopy (TEM) to evaluate the characteristics of the NPs (Fig. 8.1A–D). This section discusses the current approaches that have been used to characterize NPs. Size and morphological properties of NPs are major parameters that have been considered in many reports. The size, shape, and distribution of NPs can be evaluated using SEM [31]. However, for SEM analysis, samples need to be dried before evaluation. This drying process might alter the samples characteristics, resulting in faulty data that are not true representative of the sample [32]. In that regard, TEM can be considered to characterize the NPs properties such as size and distribution aqueous solution [33]. Nevertheless, polysaccharide-based NPs normally do not have a regular shape and size and may agglomerate which make it hard to define the particles' size using TEM [34]. In some cases, such as drug-loaded NPs, TEM might not be useful in determining the size of the NPs due to the presence of a drug [31]. Dynamic light scattering (DLS) is another technique that can be used to define the particle size and their distribution in an aqueous environment [35]. DLS utilizes a red laser as a light source, which is focused on the particles. The scattering of the light source by the particles results in a change in light intensity collected by a detector and is converted to pulses. This technique provides a quantitative assessment of particle size distribution and can complement the primary particle size measurements using TEM. However, it is limited by the inability to differentiate between particle shapes and is reliant on a high-quality dispersion of particles. This quality is also dependent on the polydispersity, which is the relative number of primary (single) particles in comparison to agglomerates (bound clusters of particles held together by weak interparticle forces) or aggregates (chemically bound clusters of particles). The optical properties of the polysaccharide-based NPs can be evaluated using UV-visible spectroscopy. In UV-spectroscopy technique, particles response to the electromagnetic waves in the range of 190–700 nm [36] to determine their size. Moreover, using UV-visible spectroscopy, it makes possible to evaluate the effect of pH and solution concentration on the NPs stability and possible aggregation over time [11]. The chemical and functional groups of the NPs can be defined using Fourier-transform infrared spectroscopy (FTIR). FTIR provides information about the stability of the NPs and illustrates the coupling and conjugation between polysaccharide and metallic NPs [37]. The crystal structure of the NPs can be determined using X-ray diffraction (XRD). The analysis of XRD intensity reveals information on the structure, orientation, shape, and distribution of NPs. The crystal phase of NPs is identified by a comparison of the obtained pattern with the known reference patterns from the International Center for Diffraction Data (ICDD). This method can be used in samples with spherical shape crystals [38] and submicron particles. The application of XRD is however limited due to the requirement of single conformation and high atomic number of crystals [39]. Nevertheless, using small-angle X-ray scattering, it is possible to obtain information about either crystalline or amorphous NPs [33].



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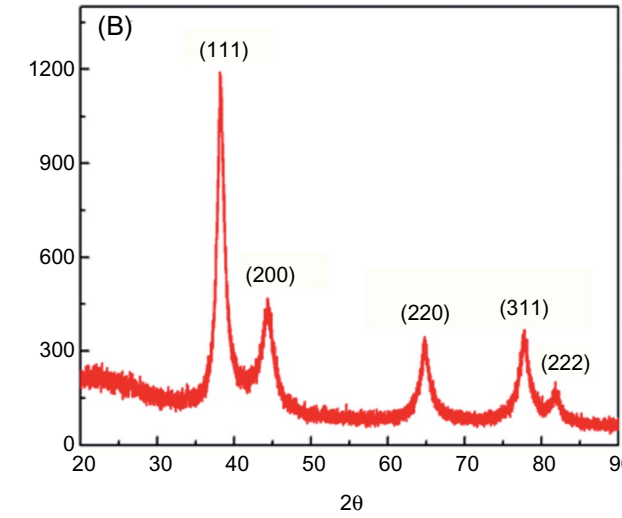

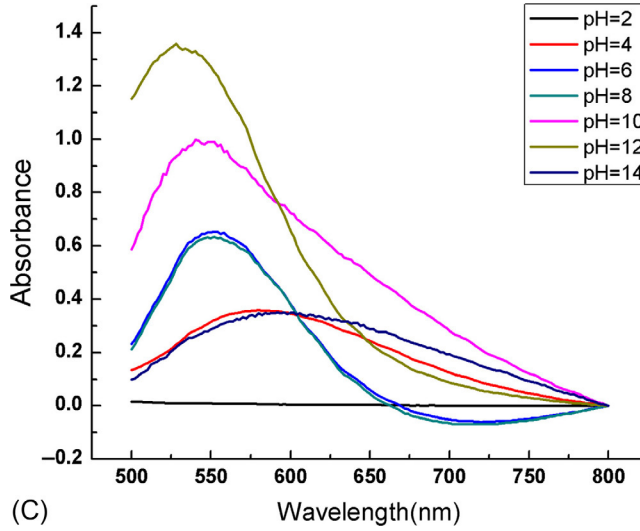

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Fig. 8.1 See figure legend on next page

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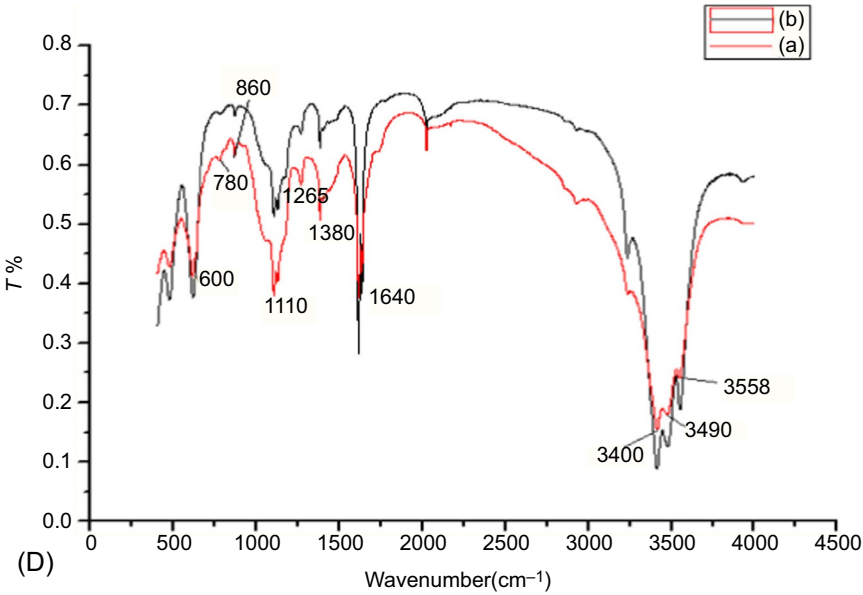


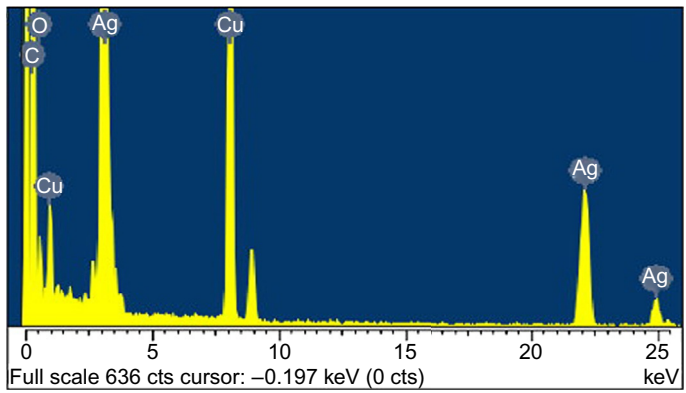


Fig. 8.1, Cont'd Illustration of some of the techniques that have used for the characterization of polysaccharide-based nanoparticles: (A) TEM analysis of AuNPs. (B) XRD pattern of AuNPs [1]. (C) UV-visible absorption spectrum of gold nanoparticles as a function of pH values. (D) FTIR spectra of the polysaccharide (a) (dried pepper) and (b) gold nanoparticles [5]. (E) EDS elemental composition analysis of Ag-NPs [3].
Adapted from: Tagad CK, Rajdeo KS, Kulkarni A, More P, Aiyer RC, Sabharwal S. Green synthesis of polysaccharide stabilized gold nanoparticles: chemo catalytic and room temperature operable vapor sensing application. RSC Adv 2014;4:24014–9; Medina-Ramirez I, Bashir S, Luo Z, Liu JL. Green synthesis and characterization of polymer-stabilized silver nanoparticles. Colloids Surf B 2009;73:185–91; Yuan C-G, Huo C, Yu S, Gui B. Biosynthesis of gold nanoparticles using *Capsicum annuum* var. grossum pulp extract and its catalytic activity. Physica E Low 2017;85:19–26, with permissions of The Royal Society of Chemistry and Elsevier.

The aggregation of crystals and the presence of NPs with polycrystalline structure affect the reliability of the result of crystal size [40]. In case of magnetic NPs, the magnetization property of the particles can be evaluated using a superconducting quantum interference device (SQUID) magnetometer [41]. Other available techniques such as atomic force microscopy (AFM), Raman scattering, and scanning tunneling microscopy (STM) have also used for the characterization of polysaccharide-based NPs [11].

8.5 Biomedical applications of polysaccharide-based AuNPs and AgNPs

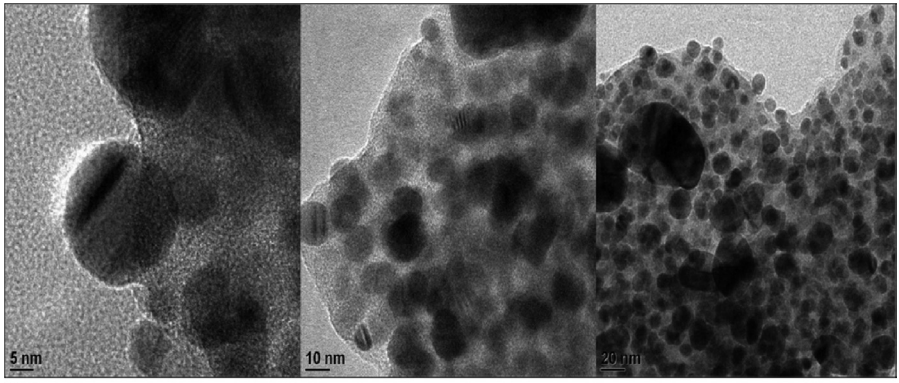
Polysaccharide-based NPs with unique physical, chemical, and biological properties have been widely used in various fields of technology. In this section, the biomedical application of the polysaccharide-based NPs, including antimicrobial, anticancer, and wound healing are discussed.

8.5.1 Antimicrobial properties of AuNPs

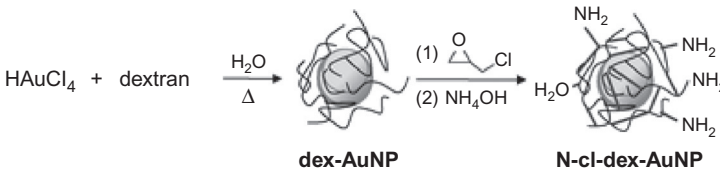
A wide range of polysaccharide-based NPs with antimicrobial and antiviral properties have been synthesized and documented in the literature [42]. Infections caused by bacteria, viruses, parasites, or fungi are major human health risks. Over the years, there has been a growing increase in the resistance of microbes to current available antimicrobial agents such as antibiotics and antifungals. Nevertheless, the research is also widely continuing to find novel and effective antimicrobial agents [43]. In that regard, polysaccharide-based NPs containing different phytocompounds with rapid synthesis process and a wide range of biological activities have attracted a significant attention.

AuNPs with diverse shape and size have different biomedical applications, including drug and gene delivery or photothermal therapies [44–47]. *Enterococcus faecalis* is a frequently isolated bacteria from infection sites and is a major cause of the surgical wound, urinary tract infection, and nosocomial bacteria that have become resistant to major conventional therapies [48]. AuNPs synthesized using the aqueous extract of *Musa paradisiaca* peels showed antibiofilm properties against pathogenic, antibiotic-resistant gram-positive *E. faecalis* and reported as nontoxic, eco-friendly biomaterial for biomedical applications [49]. *Bacillus megaterium* exopolysaccharide (EPS) was used as reducing and stabilizing agent for the synthesis of AuNPs. The EPS encrusted NPs had a spherical shape with an average diameter of 10 nm. The average diameter of 5–35 nm and spherical shape of AuNPs have commonly been reported in the literature for NPs synthesized using dextran, hyaluronic acid and heparin [50–52]. The type and concentration of polysaccharide used for reducing/stabilizing the NPs play an important role in determining the diameter of the final synthesized particles that can also avoid the aggregation of the NPs. AuNPs stabilized by other polysaccharides such as chitosan, bacterial polysaccharides alginate, or carrageenan have also been evaluated as antimicrobial agents for the treatment of bacterial infections [53, 54].

The ESP mediated synthesized AuNPs has been reported as a green and safe alternative method to chemical route for the synthesizing of AuNPs [53]. As shown in Fig. 8.2A the AuNPs were grown inside the ESP network and capped/stabilized by the ESP matrix. In polysaccharide-based NPs, the moieties help the reduction and stabilization of the NPs and form a coating around the NPs which stabilize the particles [53]. The presence of electrostatic forces between the amine and sulfonic groups of polysaccharides (e.g., chitosan and heparin) with $AuCl_4^-$ can play as an efficient driving force, which results in the stabilization and formation of the NPs [55]. Despite the stability of the synthesized NPs due to the formation of chemical bonds between the polysaccharide and NPs, the stability of NPs under extreme conditions such as high temperature, a high concentration of salts or extreme pH condition have not been evaluated extensively. AuNPs utilized by dextran (Fig. 8.2B) were further cross-linked using epichlorohydrin and aminated by ammonium hydroxide. The cross-linking



(A)



(B)

Fig. 8.2 (A) TEM image of EPS molecule packed with gold nanocrystals, different sizes of gold nanoparticles (5–20 nm) were capped by the thin faint layer of exopolysaccharide (EPS) matrix extracted from *Bacillus megaterium* MSBN04 [53]. (B) Synthesis of aminated, cross-linked dextran-gold nanoparticles AuNPs [52].
Adapted from Jang H, Kim Y-K, Ryoo S-R, Kim M-H, Min D-H. Facile synthesis of robust and biocompatible gold nanoparticles. Chem Commun 2010;46:583–5; Sathiyanarayanan G, Vignesh V, Saibaba G, Vinothkanna A, Dineshkumar K, Viswanathan MB, et al. Synthesis of carbohydrate polymer encrusted gold nanoparticles using bacterial exopolysaccharide: a novel and greener approach. RSC Adv 2014;4:22817–27, with permission of The Royal Society of Chemistry.

enhanced the stability of the AuNPs by inhibiting the dissolution of the dextran at the extreme condition; also the amination reaction introduced primary amines on the AuNPs surface and made them more amenable to bioconjugation [52].

8.5.2 Antimicrobial properties of polysaccharide-based AgNPs

Antimicrobial properties of AgNPs depend on factors such as size, shape of the AgNPs (e.g., spherical, rod, or a mixed shape) [56], microbial strain, the composition of the medium, effluent circulation, temperature, light [57], capping agent, and oxidizer [6, 57]. The positive charge of silver ions in AgNPs has an important role in their antimicrobial activity [58]. The positive charge of the NPs causes an electrostatic attraction with negatively charged gram-negative bacterial cells (e.g., *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella planticola*, *Klebsiella pneumoniae*, *Vibrio parahaemolyticus*, *Proteus vulgaris*, *Salmonella typhimurium*, *Aeromonas hydrophila*, and *Vibrio cholerae*). AgNPs can accumulate inside cell membrane and therefore penetrate into the bacteria and damage the cell walls or membranes and intercellular structure [59]. The disintegration of bacterial cells could be one reason for the antibacterial property of the AgNPs [6]. Evidence has shown that AgNPs have also an antimicrobial activity against gram-positive bacteria such as *Staphylococcus epidermidis*, *Staphylococcus aureus*, *Bacillus subtilis*, *Lactobacillus fermentum*, *Enterococcus faecium*, *Bacillus licheniformis*, *Bacillus cereus*, *Kocuria rhizophila*, *Streptococcus pyogenes*, *Actinomycetes*, *Staphylococcus*, *Streptococcus pneumoniae*, *Listeria monocytogenes*, and *E. faecalis* [15]. However, the susceptibility of gram-positive bacteria to silver ion (Ag^+) is less than that of the gram-negative bacteria. This has been attributed to the presence of a thicker layer of negatively charged peptidoglycan molecules in gram-positive than gram-negative bacteria [60]. Thus, there is a higher chance that more silver ions get attached to the peptidoglycan layer of the gram-positive than that of the gram-negative bacteria and therefore lower chance to penetrate inside the gram-positive bacteria [6]. AgNPs can also impart their antibacterial activity through the interaction and interruption of the macromolecules function such as DNA and enzyme by mechanism such as a free-radical production or electron release. AgNPs produced using the EPS secreted from *B. subtilis* has shown an effective inhibition against bacterial growth, with greater efficiency toward gram-negative than gram-positive bacteria [61]. These types of AgNPs synthesized from bacteria secreted ESP have shown successful inhibition activity against microorganisms such as *P. aeruginosa*, *S. aureus*, *B. cereus*, *E. coli*, and *P. vulgaris* [62, 63]. AgNPs synthesized using sulfated polysaccharide extracted from marine red algae (*Porphyra vietnamensis*) were also more effective to inhibit gram-negative bacteria, that is, *E. coli* compared to gram-positive bacteria (*S. aureus*). The AgNPs successfully inhibited the *E. coli* growth at a low concentration of 5 µg/mL, while a higher dose of the AgNPs (15 µg/mL) was required to inhibit 60% of *S. aureus* growth [64]. Authors have suggested that internalization of NPs from the cell wall of bacteria may inactivate protein structure and consequently leads to cell death. Similarly, algal polysaccharide-based AgNPs have shown greater antimicrobial effects on *E. coli* (gram-negative) than *S. aureus* (gram-positive) [60]. AgNPs synthesized using an aqueous solution of carboxy methyl

tamarind (CMT) was examined for their antimicrobial activity. CMT-capped AgNPs inhibited the growth of gram-negative (*E. coli* and *S. typhimurium*) and gram-positive (*B. subtilis*) bacteria at 175 µM concentration. A low concentration (10 µM) of CMT-capped AgNPs inhibited biofilm formation by preventing bacterial cell division and membrane damage (e.g., *Bacillus* and *E. coli*). Researchers also found that the CMT-capped AgNPs can successfully inhibit the growth of bacteria such as *Staphylococcus haemolyticus*, *S. epidermidis*, *E. coli* C19, *K. pneumoniae Kp52*, and *Enterobacter cloacae Ec18* at low concentrations (about 1.5–6 µg/mL). Thus, the AgNPs might be a good alternative for MDR bacterial strains [65]. The majority of AgNPs reported spherical shape [15, 65] varied in size, between 1.1 [62] and 65.1 nm [66]. Nevertheless, shapes such as polygonal, oval-shaped, face-centered-cubic [67], irregular shape [68], rod/oval-shaped structures [69], triangular [70], and uneven shape [71] have also been documented for AgNPs.

8.5.3 Antiviral properties of polysaccharide-based AuNPs

In addition to antibacterial properties, AuNPs have also been proposed as antiviral agents. AuNPs complex has been used in the development of sensors for diagnosis, detection, and differentiation of viruses such as influenza. In order to cause infection, influenza virus requires binding to host receptors. This binding process is facilitated by the interaction between the viral protein, hemagglutinin (HA) and the glycan receptors at the host surface which contain terminal sialic acid (SA) [72]. AuNPs functionalized with different glycan structures can aggregate and bind on the virus surfaces resulting in plasmon resonance shifts and color change, which is detected with spectroscopy. This assay is based on the distance-dependent optical properties of AuNPs. The application of AuNPs for sensing colorimetric changes dated back to 1980 and has been used for the identification of proteins, nucleic acid, and metal ions [73–75]. In this process, the surface of NPs is coated with different SA receptors that allow the virus to bind to the particle detected by color change (Fig. 8.3). Due to surface plasmon resonance (SPR) of gold probes, the glycan functionalized NPs can absorb a narrow band of light at maximum absorbance of 522 nm that changes the solution color into red. After interaction/binding of the NPs with the virus, the AuNPs aggregate and result in a shift in SPR absorbance and the solution color changes to purple [72]. One major advantage of glycan-coated AuNPs is the positive and amplifying effect of these particles on the weak and low-affinity interaction of protein and glycan [76]. Fourteen strains of influenza virus were identified and differentiated using the glycan functionalized AuNPs in a simple colorimetric process [77].

8.5.4 Antiviral properties of polysaccharide-based AgNPs

Viruses are infectious agents and the breakout of viruses such as HIV, influenza, Zika, and severe acute respiratory syndrome virus during the past decades has caused public health issues [78]. Although antibodies produced by the immune system might be a useful treatment to eliminate viruses, the diversity and rapid mutability of viruses make the diagnosis and prevention difficult [77]. The characteristics of AgNPs have made

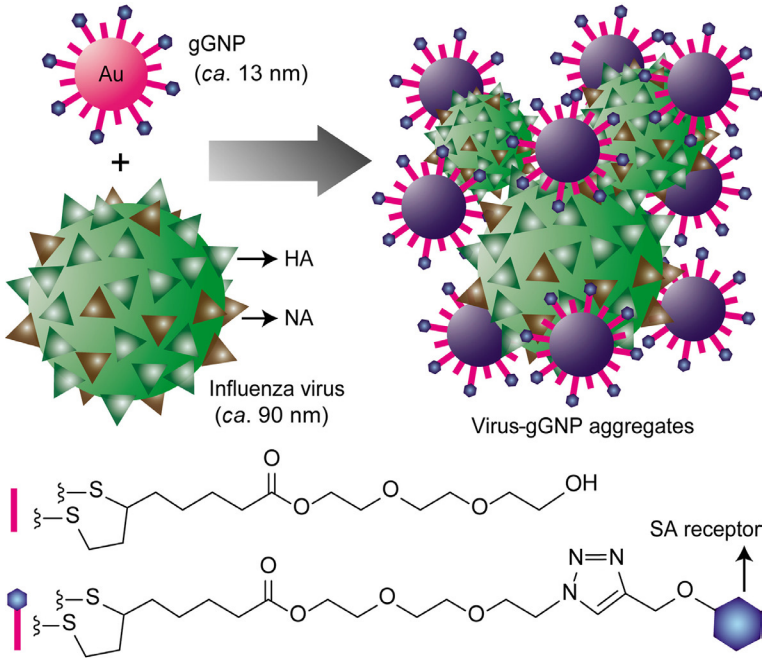


Fig. 8.3 Glycan functionalized gold nanoparticles for the detection of influenza virus through binding between terminal sialic acid (SA) receptors and virus resulted in the nanoparticle aggregation on the surface of the virus. This aggregation of nanoparticles resulted in color change that was measured using UV-visible spectroscopy.
Adapted from Zheng L, Wei J, Lv X, Bi Y, Wu P, Zhang Z, et al. Detection and differentiation of influenza viruses with glycan-functionalized gold nanoparticles. Biosens Bioelectron. 2017;91:46-52, with permission from Elsevier.

them as a potential alternative for the treatment of virus-caused complications. The oxidized curdlan-based AgNPs (Oc-AgNPs), with a mean diameter of 15 nm have been used for fluorescence-enhanced nucleic acid detection. The Oc-AgNPs can absorb and quench dye-labeled single-stranded DNA efficiently and therefore might be useful for detecting HIV virus [79]. Furthermore, polysaccharide-based AgNPs (10 nm) at the nontoxic level of 10, 25, and 50 µg/mL inhibited *Tacaribe* virus replication. Bigger-size polysaccharide-coated AgNPs (25 nm) also inhibited viral replication at 50 µg/mL and caused a significant reduction in progeny virus production at 25 µg/mL in vitro. It should be noted that although preinfection treatment with AgNPs is very effective, the postinfection use of AgNPs is only effective if administered within the first 2–4 h of virus replication [80]. In another study, polysaccharide-based AgNPs (10 nm) showed a decreasing dose–response effect (12.5, 25, 50, and 100 µg/mL) on plaque-forming units of *Monkeypox* virus in vitro, with no cytotoxicity toward Vero cell monolayer sloughing [81]. However, NPs are used for therapeutic purposes to understand their in vivo toxicity and long-term impact is crucial. Future research is needed to address and clarify these questions and determine the in vivo biocompatibility of NPs.

8.5.5 AgNPs as a potent alternative for the treatment of multidrug resistance pathogens

The overuse and poor effectiveness of antimicrobial drugs such as quaternary ammonium salt, metal salt solutions, and antibiotics have led to the growing multidrug resistance (MDR) [9, 66]. Nowadays, the MDR pathogens are serious threats to public health [82]. To address this problem, there is a need to develop alternative antimicrobial agents that are effective and have low levels of toxicity. Of available antimicrobial agents, metal NPs, in particular, AgNPs, have gained attention because of their small size, large surface/volume ratio, and tunable plasmon resonance characteristics [83, 84]. Silver ions interact with disulfide (S–S) bonds of the glycoprotein/protein contents of microorganisms such as viruses, bacteria, and fungi. During the interaction, silver ions interfere with S–S bonds, affects the three-dimensional structure of proteins, and thus occludes the functional operations of the microorganism [6, 85]. The AgNPs synthesized using *Lactobacillus rhamnosus* produced EPS can effectively inhibit MDR pathogens such as *P. aeruginosa* and *Klebsiella pneumoniae*. The nano-size, shape, chemical, physical, or optical properties of the AgNPs are responsible for their antimicrobial activity [70].

8.5.6 Anticancer properties of polysaccharide-based AuNPs

Despite all the advancement, research and investment, cancer remains an important health risk. Chemo and radiotherapy treatments are widely used; however, these approaches are followed by severe side effects resulting from chemical and radiation toxicity. Therefore, there is an urging need for the development of accurate preventive/diagnostic and nontoxic therapeutic methods for cancer treatment [47, 86]. There has been an increasing trend in designing and development of nanotechnology-based drugs for cancer therapy. Currently, available chemotherapeutic agents are nonselective and usually have side effects. It is expected that functional nano-based drugs such as magnetic, gold, and silver NPs will overcome this nonselectivity and will improve and enhance the result of cancer therapy [87, 88].

Having unique thermal, optical, and magnetic characteristics, NPs are alternative methods with high potential for the detection and treatment of cancer. Biocompatible AuNPs have photothermal properties, which make them an interesting candidate for possible localized heating and therefore localized drug release [89]. Furthermore, AuNPs with a large surface area, simple functionalization, and stability at microenvironment have gained attention for the diagnosis and treatment of cancer [90].

A major potential of metallic NPs for biomedical application is related to the presence of SPR, which can be addressed via different photonic and spectroscopic methods. A noble metal NPs such as AuNPs with a diameter smaller than the light wavelength can be resonated by an electromagnetic field at a specific frequency. This resonates, which is the oscillation of metal-free electrons across the NPs is known as the SPR [91]. This oscillation of electrons gives the NPs unique optical properties because of the adsorption and scattering of electromagnetic radiation. The AuNPs can penetrate and accumulate inside of the tumor cell and with scattering brought light act as selective photothermal agent for targeting cancer cells [92]. The NPs composition, shape, size,

surrounding medium, and the presence of interparticle interaction affect the frequency and cross section of SPR adsorption and scattering [47]. AuNPs can be synthesized in a size range of 4–80 nm through the reduction of gold ions in a solution. Nevertheless, the AuNPs need to be safe and nontoxic in order to be useful for the biomedical application including diagnosis and treatment of cancer. With this in mind, the conventional method using toxic chemicals are no longer useful. Natural polysaccharides such as chitosan and cellulose are safe nontoxic alternative materials for the stabilization and synthesis of AuNPs. AuNPs synthesized using pectin (pectin-AuNPs) and its anticancer properties have been evaluated against mammary cell lines of MDA-MB-231 and MCF-7 [90]. The pectin-AuNPs showed toxicity toward tested cancerous cell lines and induced cell apoptosis and damaged the DNA cells. This was evident from the loss of asymmetry in the plasma membrane, determined by dual staining and comet assay. The pectin-AuNPs aggregated and adsorbed on the surface of the cells and interrupted the cell integrity [90]. Pectin is a biocompatible, low toxicity anionic polysaccharide with diverse biomedical applications including drug delivery to decrease cholesterol levels, fabrication of scaffolds, and hydrogels [93, 94]. The presence of functional groups such as amide, carboxyl, and ester on pectin chain made it an ideal candidate for functionalization and attachment of other compounds. In particular, the carboxyl groups present in the backbone chain of pectin gives it the required stability to act as capping agent in the synthesis of AuNPs [90]. Polysaccharide has usually used as capping and stabilizing agent for synthesis of AuNPs for cancer treatment/diagnosis. However, the polysaccharide itself may also have anticancer properties such as polysaccharide isolated from seed kernels of *Tamarindus indica* (Ti) [95]. The Titanium-polysaccharide-mediated AuNPs (Ti-AuNPs) with an average diameter of 20 nm were highly stable with good pH resistance over a pH range of 1–14, antitumor, and immunomodulatory [95]. The AuNPs showed their anticancer properties through inducing apoptosis of tumor cells while they were not toxic to the normal tested tissues. With the stability of up to 1 year, the Ti-AuNPs were reported as more stable than commercially available AuNPs reduces by citrate or borohydride [96]. The interaction of polysaccharides with the receptors on the cell surface is required for a cellular response. Therefore, grafting of polysaccharides such as fucoidan with a diverse range of biological activity on a polymeric structure can result in enhanced therapeutic efficacy. To enhance the anticancer properties, AuNPs have been synthesized using modified polysaccharide. Tengdelius and colleagues [97] coated AuNPs with synthesized fucoidan-mimetic glycopolymers, which showed promising anticancer properties. Despite natural polymers, synthetic functional polymers can be prepared in a controlled procedure that can transform and improve the natural polysaccharide into a product with biomedical applications. The AuNPs coated with fucoidan-mimetic glycopolymers showed a selective cytotoxicity toward colon cancer cells and they were not toxic to a tested fibroblast cell line.

8.5.7 Anticancer properties of polysaccharide-based AgNPs

Given the antiproliferative properties of AgNPs, they have been used in anticancer drug development; however, there is limited information on the anticancer activity of the AgNPs. An in vitro study examined the cytotoxicity of chitosan-based AgNPs on

cancer cell lines including hepatocellular carcinoma (*HepG2*), human lung carcinoma (LU), human epidermic carcinoma (KB), and human breast carcinoma (MCF-7) [98]. Researchers found an elevated dose–response cytotoxicity effect of AgNPs on cancer cell lines. The required concentration of AgNPs leading to 50% death in HepG2, LU, KB, and MCF-7 cells was 6.09, 5.20, 5.71, 3.74 μg/mL, respectively [98]. In addition, the minimum AgNPs concentration of 6.25 μg/mL could significantly cause morphological changes on cancer cell lines by inhibiting their growth. Similar results were reported in the concentrations of 25 and 100 μg/mL [98]. The localization of AgNPs inside the leukemia cell mitochondria might induce oxidative stress levels and by acidification of the internal environment of the cell may inhibit the viability of the cancer cell lines [99]. The release of AgNPs inside the cell and generation of reactive oxygen species could damage the DNA cell and mitochondria and consequently lead to cancer cell damage [99].

Prostate cancer is the most common cancer in men, contributing to the 25% of all newly diagnosed cancer cases [100]. The aqueous extract of a pink oyster mushroom (*Pleurotus Djamor* var. *roseus*) used as a platform for the synthesis of AgNPs. The antiproliferative potential of the AgNPs was tested on human prostate carcinoma PC3 cells. With an IC_{50} of 10 μg/mL, the green synthesized AgNPs reduced the synthesize of PC3 cell DNA, suppressed the cell growth, and inhibited the cell proliferation [100].

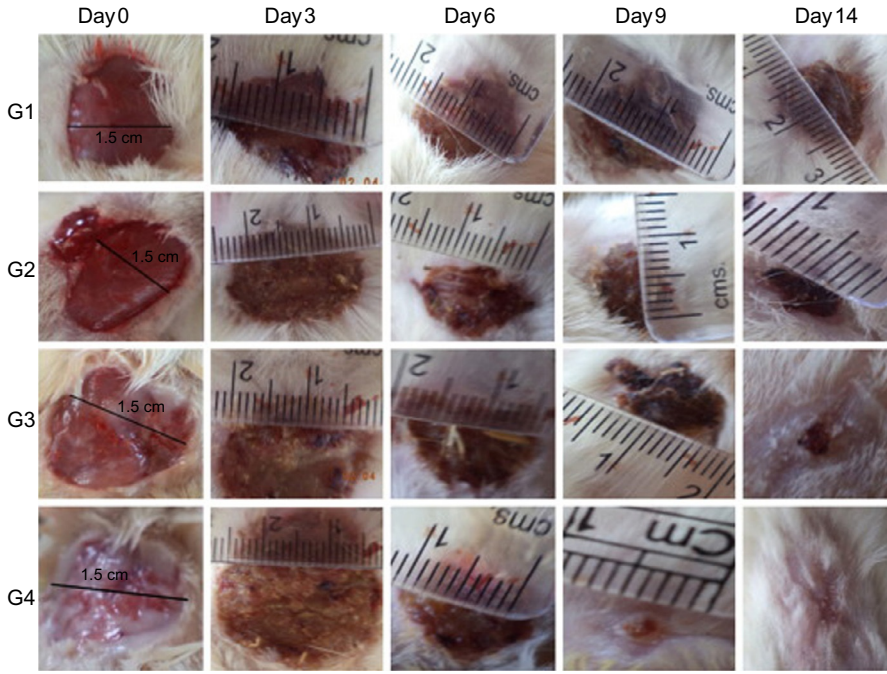
The poor performance of the current antibreast cancer drugs such as doxorubicin and bleomycin motivated researchers to develop alternatives for the treatment of the breast cancer. The bio-reduction activity of fungi has made them good candidates for the synthesis of metallic NPs [101]. The reduction of silver ions using fungi can be due to the secretion of extracellular proteins by the fungus. The oyster mushroom (*Pleurotus ostreatus*) extract used for the reduction of silver ions and synthesize of AgNPs by Yehia and coworkers [101]. The viability of breast carcinoma cells (MCF-7) decreased by 78% on exposure to 640 μg/mL of the green synthesized AgNPs. Similarly, the AgNPs synthesized by seaweed extracts was reported effective against the MCF-7, the human laryngeal Hep-2, and colon cancer HT29 cell [102]. The cytotoxicity of the green synthesized AgNPs showed a direct dose-response relationship and the cytotoxicity increased by increasing the concentration of NPs [103, 104]. The irregular shape of AgNPs and their interaction with the organic moieties effects on the anticancer and cell apoptosis properties of the green synthesized AgNPs.

8.5.8 Wound healing and antiinflammatory properties of polysaccharide-based AuNPs

Wound healing is a multiphasic overlapping process comprises of hemostasis, inflammation, and proliferation, followed by maturation. During hemostasis, platelets form clots while the dead cells are removed during the inflammatory phase that follows by the proliferation and remodeling of the tissue. The rate of each healing phase depends on the size and depth of the wound and the presence of bacteria, which may cause infection and hamper the healing process. With this regard, gauze dressing as the most readily available dressing promotes the wound healing by providing a barrier against an external microorganism and therefore reducing the infection. Nevertheless,

the traditional dressing has no direct influence on the healing process. Metallic NPs including AuNPs with easy synthesize process, binding affinity to biological molecules and diverse biomedical applications are good candidates for wound healing. Polysaccharides such as heparin and heparan sulfate are known to have a positive effect on wound healing, angiogenesis, and inflammation [50]. Heparin as the most sulfated glycosaminoglycan has been used for reducing and stabilization of AuNPs. The synthesized particles can provide localization for antiinflammatory applications and deliver a more prolong effect and therefore prevent the rapid release of the drug which may reduce the drug activity. Due to electrostatic repulsion, the heparin-bonded NPs were more stable at a tested physiological solution than nonbonded NPs.

The AuNPs synthesized using *Coleus forskohlii* root extract greatly increased the deposition of collagen in the wound, 14 days posttreatment (Fig. 8.4A and B) [105]. Wound epithelization of a 1.5 × 1.5 excision treated with the AuNPs was completed after 14 days while the control wound was only partially epithelialized. The high antioxidant and antimicrobial activity of the AuNPs reported effective in the healing process [105, 106]. Combination of AuNPs with polyphenolic compounds with high antioxidant properties can also improve the wound healing efficacy of AuNPs. The 3–5 nm AuNPs prepared with epigallocatechin gallate and an alpha lipoic acid as an



(A)

Fig. 8.4 (A) Wound healing process in control (G1), standard drug (G2), silver nanoparticle treated (G3), and gold nanoparticle treated groups (G4) at 0, 3, 6, 9, and 14 days of postwounding.

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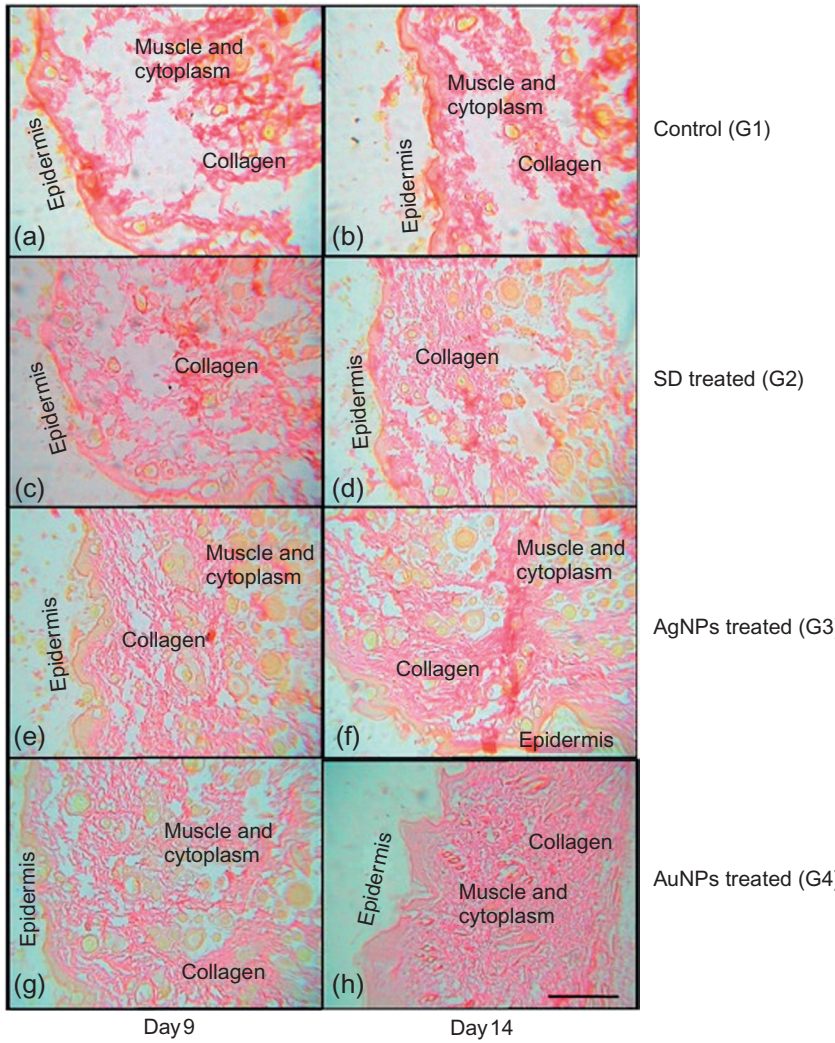


(B)

Fig. 8.4, Cont'd (B) Stained histological sections of wound area at 9 and 14 days of postwounding. Compared to the control and standard treatment, wounds treated with gold (AuNPs) and silver (AgNPs) nanoparticles showed higher collagen and lower macrophage. (The scale bar in the image is 300 μm at a magnification of 10×.)
Adapted from Naraginti S, Kumari PL, Das RK, Sivakumar A, Patil SH, Andhalkar VV. Amelioration of excision wounds by topical application of green synthesized, formulated silver and gold nanoparticles in albino Wistar rats. Mater Sci Eng C 2016;62:293–300, with permission from Elsevier.

oxygen scavenger significantly enhanced diabetic wound healing in mice through angiogenesis regulation and reduction of glycation end-products expression [106].

8.5.9 Wound healing properties of polysaccharide-based AgNPs

Polysaccharide-based AgNPs have been proved to be useful in wound healing. AgNPs may inhibit the colonization of pathogenic bacteria and fungus due to their antimicrobial activity or might help the host by affecting repair mechanisms that are involved in wound healing [107]. AgNPs synthesized using pectin extracted from citrus peel were examined for their wound healing potential (Fig. 8.5). Findings showed a significant wound healing process in contact with pectin-based AgNPs (8 nm), a decreasing trend was observed in width of the wound; 503 ± 5.98 µm at time zero, 424 ± 28.8 µm after 24 h, 239 ± 17.5 µm after 48 h, and completely closed wound gap after 72 h [108]. A study on excision and incision wound models in vivo also exhibited that mannan sulfate polysaccharide-based AgNPs (MS-AgNPs) (spherical shape, 20 nm) can be effectively used in wound healing process. MS-AgNPs significantly reduced the width of wound area in early stages of treatment, that is, within 4 days [109]. In another study, xanthan-based film incorporated in AgNPs (10 µg/mL) effectively reduced the level of inflammatory cytokines and cells such as IL-6, TNF-α, and macrophages. Furthermore, the AgNPs successfully stimulated angiogenesis and therefore granulation of tissue regeneration. Authors have suggested that the polysaccharide-based film incorporated in AgNPs has promising potential for the treatment of wound infections [110]. In addition, hydrogels containing bamboo cellulose nanocrystals impregnated with AgNPs were used to assess their wound healing characteristics in vivo using diabetic mice. The treatment showed promising results with almost 100% wound healing

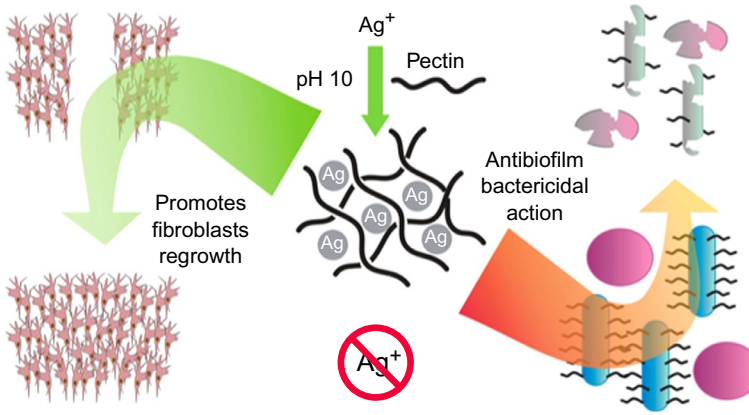


Fig. 8.5 Pectin-mediated silver nanoparticles and their wound healing application. Adapted from Pallavicini P, Arciola CR, Bertoglio F, Curtosi S, Dacarro G, D'Agostino A, et al. Silver nanoparticles synthesized and coated with pectin: an ideal compromise for antibacterial and antibiofilm action combined with wound-healing properties. J Colloid Interface Sci 2017;498:271–81, with from Elsevier.

after 168 days and accelerated wound closure rate as compared to untreated control group. The effectiveness of treatment has been partly attributed to the antibacterial activity of the AgNPs in hydrogels [111]. In another study, AgNPs (spherical shape, 6 nm) were synthesized using triple helical schizophyllan (SPG) as a reducing and stabilizing agent. The hydrogel-mediated SPG-AgNPs were nontoxic when used for mouse fibroblast (NIH-3 T3) and human keratinocyte cell lines (HaCaT) and did not inhibit cells growth and proliferation. Thus, it was suggested that these particles have potential wound healing applications [112]. Other studies using hydrogel-mediated polysaccharide-based (e.g., linseed, chitosan, or Glucuronoxylan isolated from seeds of *Mimosa pudica*) AgNPs have also shown wound healing capabilities, mainly due to their antimicrobial characteristics and stimulation of the reepithelialization and granulation tissue formation [15, 113].

8.5.10 Drug delivery and release properties of polysaccharide-based AuNPs

Conventional chemotherapy suffers from lack of delivering the required dose of the drug to a specific area. Instead, it distributed therapeutic agents in a large area, which causes damage to the healthy tissues [86]. Nanocarriers such as NPs proved to be a great platform for target drug delivery applications. With nano-size diameters the particles can also travel across biological membranes without being trapped by the macrophage system [114]. AuNPs with the ability to efficiently deliver a diverse range of payloads such as small drug molecules to large proteins has attracted scientists. The release of the payload can be activated by internal or external stimulants. The internal stimulant such as pH functions in a biological control process while the external stimulant such as light controls the release based on a spatial-temporal process [45]. AuNPs with specific light-sensitive physical characteristics can release their payload at a remote space and provide a nontoxic and inert core, which can be synthesized in a diverse diameter range of 1–150 nm [115, 116].

Therapeutic agents such as insulin are susceptible to degradation in the gastrointestinal tract before delivery to the target organ. Chitosan-mediated AuNPs with improved surface properties were successfully loaded with insulin which resulted in improved delivery of insulin and enhanced the pharmacodynamic activity. The chitosan functioned as a reducing agent in the synthesizing process of the AuNPs and improved the uptake of insulin across mucosa [117]. Considering that tumor cells have different receptors on their surface, many studies have focused on developing NPs with specific moieties such as ligands, antibiotics, and peptides on the surface that can uptake by the tumor cells. Nevertheless, the chemical bonding strategies to attach the moieties on the particle surface may negatively affect the configuration of the binding site and consequently decrease the binding affinity [118]. Considering this, hyaluronic acid as a natural linear polysaccharide can bond to the surface of AuNPs, which could be correlated and recognized by the tested cancer cell CD 44 receptors [118]. Therefore, polysaccharide conjugated AuNPs may be considered as potential candidates for targeted delivery of therapeutic agents.

Considering that the pH of the cancer cell endosome is acidic and the normal cell has a neutral pH, pH-dependent release behavior of AuNPs makes them of particular interest for cancer therapy. Green AuNPs synthesized using a naturally occurring sulfated polysaccharide, fucoidan as reducing and capping agent for the delivery of doxorubicin to human breast cancer cells. The NPs induced apoptosis of cancer cells in a dose-dependent manner and showed higher release in pH 4.5 than neutral pH 7.4 [119].

Due to possessing of optical and electrochemical properties, green synthesized AuNPs are promising candidates in optoacoustic tomography which combine the benefits of both ultrasonic imaging and optical imaging techniques. This tomography technique has a diverse range of biomedical applications including the detection of a breast tumor and monitoring of blood oxygenation [120]. In light of this electrochemical property of AuNPs, fucoidan-mediated AuNPs successfully used as a contrast agent for imaging and detection of breast cancer cells using photoacoustic-imaging method [119].

In another study, the chitosan oligosaccharide-mediated biocompatible AuNPs demonstrated a controlled pH-dependent release of the drug (paclitaxel) that showed a strong cytotoxic effect against human breast cancer cells of MDA-MB-231 [121]. This pH dependency property of AuNPs increases the efficiency of the therapeutic agent against the targeted cancer cells and also reduces the possible toxic effect on the healthy tissue which is an ideal characteristic for cancer drug delivery applications. In spite of promising results, green synthesized NPs have not widely used in the field of drug delivery and more studies required to explore further the potential of green synthesized NPs for the delivery of therapeutic agents.

8.5.11 Drug delivery and release properties of polysaccharide-based AgNPs

Polysaccharide (e.g., starch)-based AgNPs significantly inhibited rat liver mitochondrial ATPase activity, with their strongest effect at the concentration of 25 mg/L. AgNPs increased the level of ATP hydrolysis and consequently permeability of the intact mitochondrial membrane. AgNPs were able to enter mitochondria and interact directly with ATPase [122]. In another in vivo study, mannan sulfate-based AgNPs (MS-AgNPs) reduced the period of epithelization and increased the rate of wound contraction, suggesting a potential use of MS-AgNPs for site-specific wound treatment [109].

8.5.12 Biosensing properties of polysaccharide-based AuNPs

Advances in the field of nanoscience have resulted in the development of sophisticated bio diagnostic assays/sensors based on NPs for the detection of biomolecules. The functionalized NPs-based sensors can convert specific biological phenomenon to certain physicochemical signals [123]. NPs with special optical, chemical, and electrical properties have been widely studied for the development of bio-sensing probes. Due to sensitivity and application flexibility, optical properties of NPs have attracted a significant

attention. The major principles of optical biosensors are based on chemical events including absorbance, reflectance, scattering luminescence, and changes in light and refractive index [124]. Melamine with a high nitrogen content (66%) is a food adulterant additive that has widely practiced and used in infant formula and pet food resulted in contamination and toxicity. The available method for detection of melamine in food is based on GC-MS or HPLC, which are usually expensive and time consuming. In this regard, chitosan-mediated AuNPs were capable of detecting melamine with a concentration of as low as 6×10^{-6} g/L. This fast and sensitive method can function based on a color change in the presence of melamine in samples (Fig. 8.6A and B) [125].

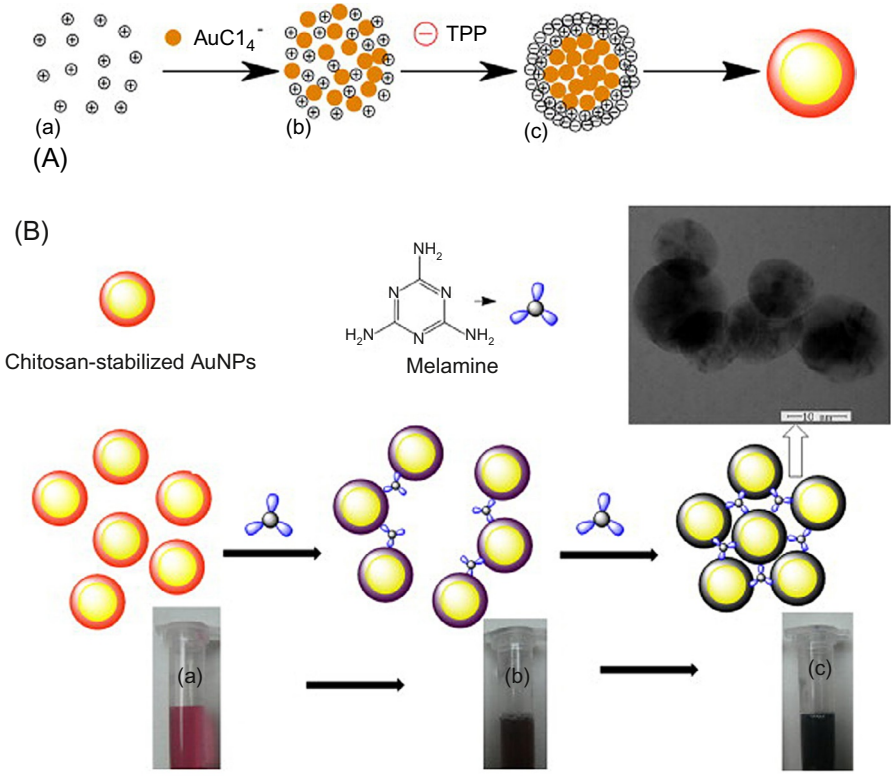


Fig. 8.6 (A) Chitosan-stabilized AuNPs through the formation of ion pairs from polycationic chitosan (a) with (b) $AuCl_4^-$. (B) Colorimetric detection of melamine through reaction of chitosan stabilized gold nanoparticles and melamine after 1 min at 25°C reaction temperature. The solution of the tube (a) contains chitosan-stabilized AuNPs, tube (b), and (c) contain chitosan-stabilized AuNPs with melamine (1×10^{-3} g/L) and (5×10^{-3} g/L), respectively. TEM image also shows chitosan-stabilized AuNPs with melamine (5×10^{-3} g/L). [125].
Adapted from Pandey S, Goswami GK, Nanda KK. Green synthesis of polysaccharide/gold nanoparticle nanocomposite: an efficient ammonia sensor. Carbohydr Polym 2013;94:229–34, with permission from Elsevier.

Ammonia hydroxide as a toxic gas has a safe allowed limit of 20 ppm, while at a concentration of above 500 ppm, ammonia can cause serious damages to various human organs including lung, skin, and eyes. AuNPs synthesized with guar gum demonstrated effective for detection of up to 1 ppb of ammonia at room temperature with a response time of about 10 s [126, 127].

Nitrogen dioxide is another toxic pollutant affecting human health including respiratory infections. Gattu and coworkers [128] were able to synthesize Au doped SnO_2 NPs using the gram beans waste extracts. The AuNPs were found to be a highly sensitive detector for nitrogen dioxide and reported efficient for monitoring the level of nitrogen dioxide [128]. Green synthesized AuNPs can also be used for recognition of amino acids and proteins. An example of this is the study carried out by Lee et al. [129] in which dextran encapsulated AuNPs were used for the detection of insulin. Turning to the experimental evidence, it was demonstrated that the sequence numbers of 1–22 on the B chain of insulin can interact with the dextran encapsulated AuNPs. Colorimetric measurements of enzyme activity such as cellobiase and hyaluronidase are other applications of the green synthesized AuNPs [130, 131].

It is also possible to develop ratio metric fluorescent biosensors using green synthesized AuNPs. In a study by Li and colleagues, alginate dialdehyde, diphenylalanine, and AuNPs were used for the encapsulation of a fluorescent dye. The synthesized nano-sphere was then used successfully for the detection of intracellular bio thiols such as glutathione [132]. This approach may be used in future for the development of more efficient biosensors.

8.5.13 Biosensing properties of polysaccharide-based AgNPs

Given specific characteristics of AgNPs such as optical, electronic, and chemical properties researchers have shown interest in using AgNPs for bio-sensing applications [133, 134]. AgNPs-based biosensors can be used to detect chemicals, gas, metal ions, and proteins. Toxic pollutants such as ammonia and hydrogen peroxide (H_2O_2) can be detected using polysaccharide-based AgNPs. H_2O_2 has various applications and is usually used for wood, pulp and paper bleaching purposes, as well as in pharmaceuticals and food industries. However, H_2O_2 is highly toxic which can impose health and environmental issues [135]. Thus, green-based NPs have been used for the detection of H_2O_2. Locust bean gum (LBG; as a reducing and stabilizing agent)-based AgNPs (LBG-AgNPs) have been used for developing a fiber optic sensor to monitor the concentration of H_2O_2. LBG-based AgNPs were able to successfully monitor H_2O_2 at the concentrations between 10^{-2} and 10^{-6} M (Fig. 8.7A and B) [124]. Polysaccharide-based AgNPs have also been used in the development of optical sensors for ammonia detection. Ammonia is widely used in industry and its toxic effects can cause serious health-related issues in human [136]. Thus, using sensors that are able to detect ammonia concentrations before it causes health complications is crucial. AgNPs synthesized using *Cyamopsis tetragonoloba* (guar gum) were used in the production of an optical sensor for ammonia detection. The optical sensor was able to detect low concentrations of ammonia (1 ppm) after a short period of time (2–3 s) at room temperature. Authors have suggested that this optical sensor can be used in the medical diagnosis of low concentrations of ammonia in various biological fluids such as saliva, sweat, and plasma [134].

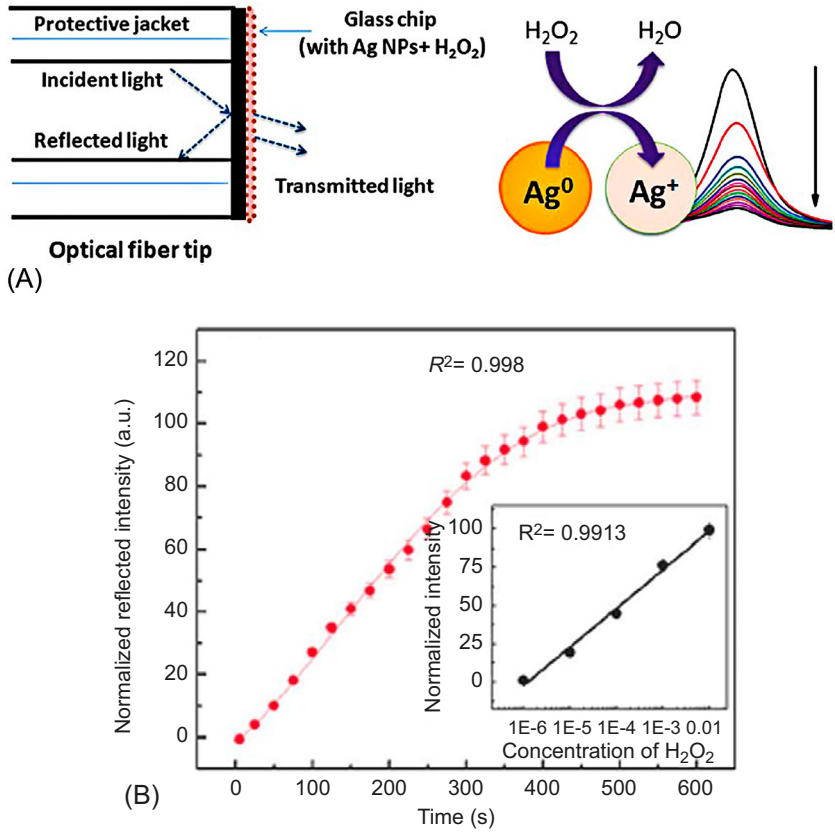
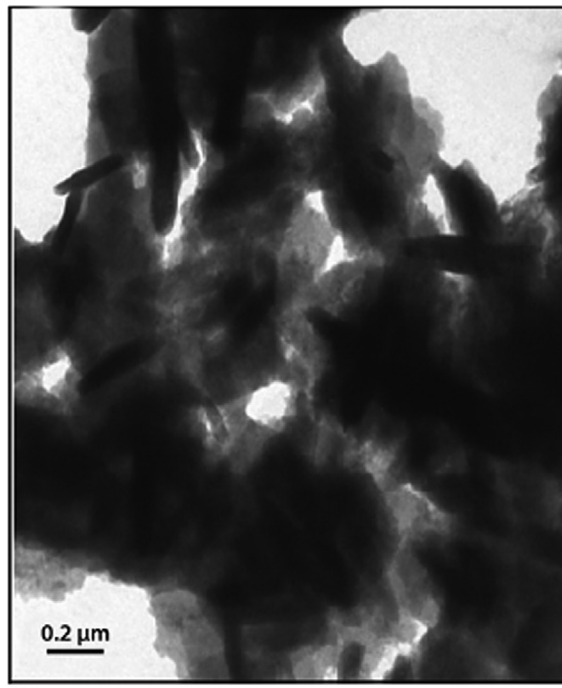


Fig. 8.7 (A) Locust bean gum-based AgNPs (LBG-AgNPs) used in a fiber optic sensor for monitoring the concentration of H_2O_2. H_2O_2 induced the degradation of AgNPs and a concomitant reduction in the surface plasmon resonance (SPR) peak of Ag NPs, and LBG-AgNPs could detect H_2O_2 at concentrations between 10^{-2} and 10^{-6} M. (B) Sensor response for hydrogen peroxide at a concentration of 10^{-2} M as a function of time; inset figure also shows the sensor response for various H_2O_2 concentration as a function of time.
Adapted from Tagad CK, Kim HU, Aiyer RC, More P, Kim T, Moh SH, et al. A sensitive hydrogen peroxide optical sensor based on polysaccharide stabilized silver nanoparticles. RSC Adv 2013;3:22940–3, with permission of The Royal Society of Chemistry.

Hydrogen sulfide, a toxic gas is the by-product of oil-processing industry and waste water treatment [137], as well as a metabolic product of living species. Thus, accurate measurement of hydrogen sulfide can be important from both biomedical and food quality control point of view [138, 139]. Chitosan/silver-coated nanocomposites have been introduced as potential optical sensors for detecting hydrogen sulfide molecules. Evidence has shown a high sensitivity and irreversible sensor response to the presence of hydrogen sulfide at various concentrations (5 and 50 ppm). Due to its high sensitivity, it is suggested that the silver-coated polysaccharide-based nanocomposites can be used in monitoring food freshness [139].

AgNP-based biosensors can be also used to detect toxic metal ions in biological and environmental samples. Alginate-stabilized AgNPs have been used as a label-free colorimetric sensor for the quantification of manganese(II) metal ions (Mn^{2+}). This biosensor can be therefore used to detect Mn^{2+} in environmental or biological samples [140]. The interaction between AgNPs and Mn^{2+} can cause agglomeration, which is detectable based on color changes (from pale yellow to brownish yellow). Also, based on microscopy images, Mn^{2+} leads to aggregation of AgNPs that suggests cross-linking aggregation due to decreases in the surface charge of AgNPs [140]. In another study, dextrin (as reducing and stabilizing agent)-based AgNPs have been used to detect copper ions (Cu^{2+}). The method used by Bankura and colleagues to synthesize AgNPs using dextrin (Fig. 8.8A) was in an aqueous medium without a need for the addition of alkali [141]. Findings showed that dextrin-based AgNPs played a colorimetric sensor role in detecting Cu^{2+} through two mechanisms of Cu^{2+}-induced aggregation and/or direct deposition of Cu^{2+} onto AgNPs (Fig. 8.8B) [141]. In addition, AgNPs (spherical shape, 20–35 nm) prepared in the aqueous solutions of xylan (as reducing and stabilizing agent) under microwave irradiation were also suggested as a highly sensitive and selective sensor for detecting mercury ions (Hg^{2+}) in environmental water. In the presence of Hg^{2+}, AgNPs were aggregated and accumulated in big blocks.



(A)

Fig. 8.8 (A) The schematic representation illustrating the formation and stabilization of AgNPs, and AuNPs using dextrin.

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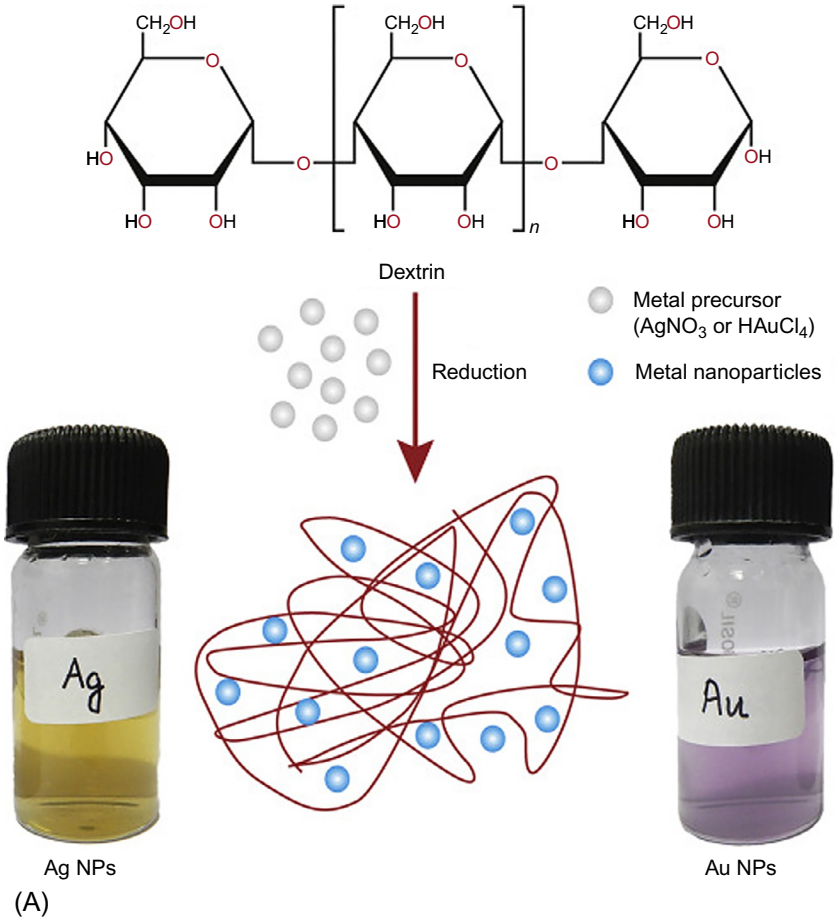

Fig. 8.8, Cont'd (B) TEM images of aggregated dextrin-based Ag NPs (rod-like structure) after addition of Cu^{2+} ion. The presence of Cu^{2+}-induced aggregation of AgNPs that result in the solution color changes and detected using colorimetric measurement methods. Adapted from Bankura K, Rana D, Mollick MMR, Pattanayak S, Bhowmick B, Saha NR, et al. Dextrin-mediated synthesis of Ag NPs for colorimetric assays of Cu^{2+} ion and Au NPs for catalytic activity. Int J Biol Macromol 2015;80:309–16, with permission from Elsevier.

The formation of Hg^{2+}-xylan complex, due to the affinity between Hg^{2+} and carboxyl/hydroxyl groups in xylan can explain in part the aggregation of AgNPs. This was detectable from color changes, that is, light yellow to colorless in the AgNPs solutions with increasing the Hg^{2+} concentration [27].

Polysaccharide-based AgNPs are also used as sensors for the detection of amino acids (e.g., cysteine) and phenols. Dextran-coated AgNPs (spherical shape, 12 nm) can be used in the selective detection of amino acid cysteine. Distinct color changes of silver colloids that are induced by the agglomeration of AgNPs allow the detection of the low concentrations of cysteine (100 μM) in aqueous solutions. Thus, this method might have the potential for on-the-spot detection of cysteine even at low

concentrations [142]. Chitosan-capped AgNPs have been used as a colorimetric sensor to detect ortho-trihydroxy phenols. Findings have shown that chitosan-capped AgNPs could be used as a highly selective colorimetric sensor to visually inspect the presence of ortho-trihydroxy phenols such as Gallic acid, pyrogallol, and tannic acid in an aqueous medium without any sophisticated instruments [143]. The hydrogen bonds formed between phenols and AgNPs led to intensified plasmon absorbance bands and thus color changes from yellow to orange (Fig. 8.9A and B) [143].

8.6 Catalysis of polysaccharide-based AuNPs

Catalysis provides an efficient and economically sustainable approach for the transformation of materials and production of various chemicals required for different industries. Production of traditional catalyst normally involves the application of toxic chemicals in a harsh chemical reaction condition. In addition, the utilization of traditional catalyst usually results in by-products with a negative impact on the environment. Synthesize of green, environmentally safe catalysts without application of toxic and hazardous chemicals are of particular interest. In this regard, green synthesized NPs catalyst with the high surface area and surface energy catalyst have attracted lots of attention and introduced a new horizon in the field of green catalysts [144]. In synthesize of metallic NPs catalyst, polysaccharides can stabilize the particles and have a positive effect on the dispersion of the particles. Polysaccharide-based AuNPs catalyst for the removal of 4-nitrophenol (4-NP) is one major application of these green catalysts.

4-NP as a phenolic compound is a by-product of industrial manufacturing and processing such as drug, fungicide, and dye industries. 4-NP can be found in the air,

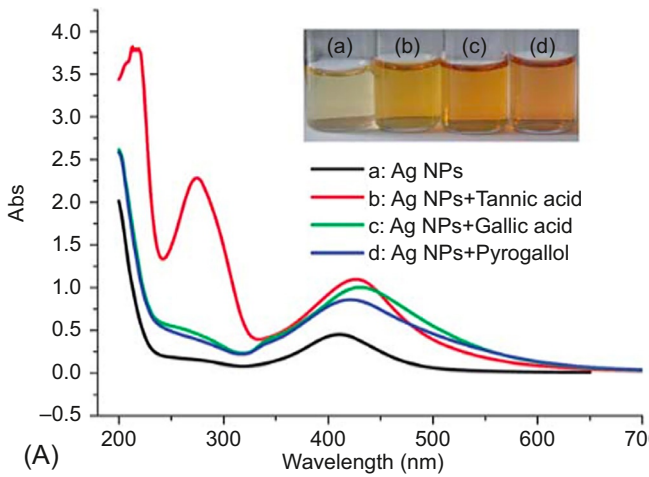

Fig. 8.9 (A) Schematic diagram of chitosan capped silver nanoparticles (Ch-Ag NPs) for visual sensing of gallic acid as a model of aromatic ortho-trihydroxy phenols. [143].

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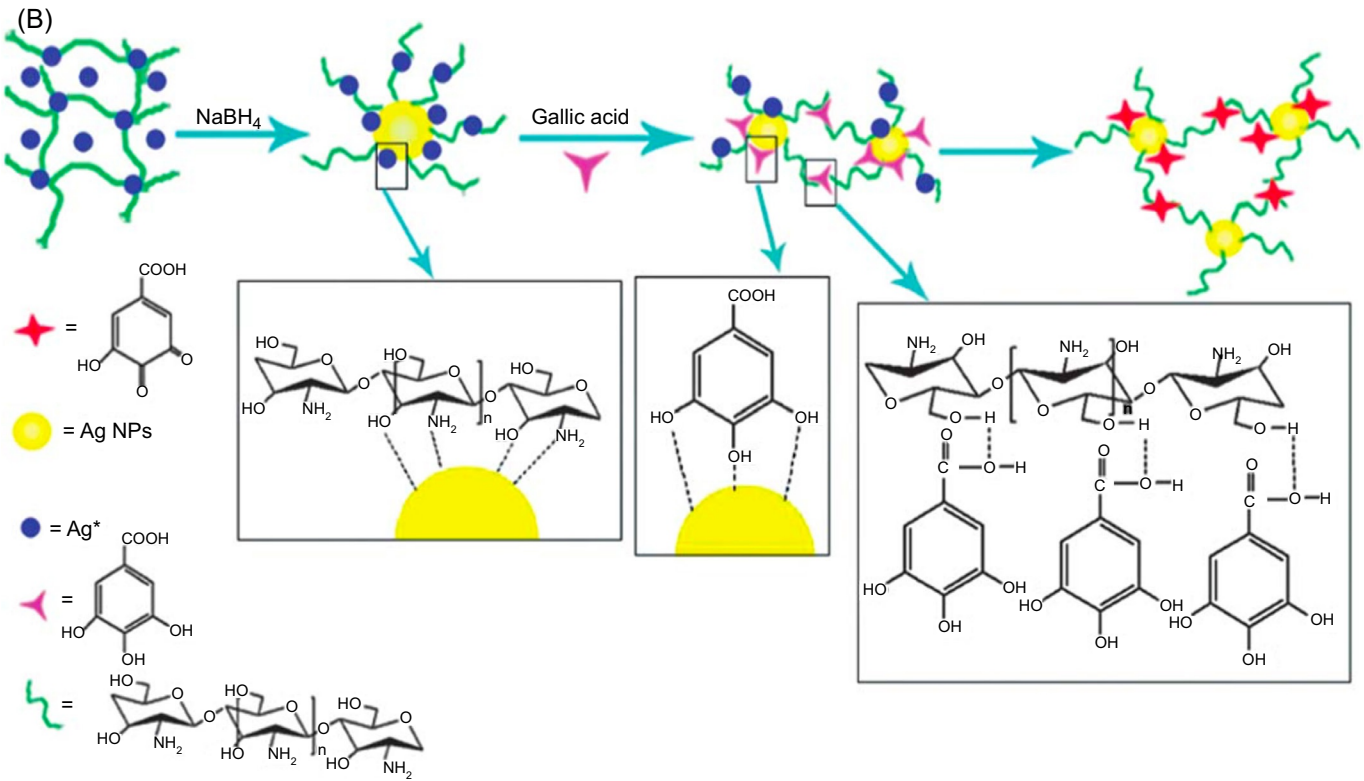

Fig. 8.9, Cont'd (B) The UV-visible absorption spectra of Ch-Ag NPs colloidal solution with and without target phenols. Inset: Photographic images [143]. Adapted from Chen Z, Zhang X, Cao H, Huang Y. Chitosan-capped silver nanoparticles as a highly selective colorimetric probe for visual detection of aromatic ortho-trihydroxy phenols. Analyst 2013;138:2343–9, with permission of The Royal Society of Chemistry.

water, and soil. Therefore, breathing air, drinking water, and eating foods that have been polluted by 4-NP may cause a number of health complications for the central nervous system, kidney, and liver, as well as skin irritation [145].

Due to the toxic effect of 4-NP and its potential danger to the human or animal organs, there has been an interest for the development of an efficient method for the reduction of 4-NP to 4-aminophenol (4-AP) as a safe intermediate [146]. AuNPs synthesized with various polysaccharides from different sources including katira gum (*Cochlospermum religiosum*) [146], konjac glucomannan (KGM) [147], and hydroxyethyl starch-g-poly (acrylamide-*co*-acrylic acid) [148] were reported effective for the safe conversion of 4-NP to 4-AP.

8.7 Catalysis properties of polysaccharide-based AgNPs

AgNPs stabilized by different kinds of polysaccharides have been suggested as the most effective nanocatalysts for the conversion of 4-NP to 4-AP. *Cordyceps sinensis* EPS-based AgNPs (5 nm) can be used as a suitable catalyst for the reduction of 4-NP to 4-AP [149]. AgNPs (5–20 nm) synthesized using xanthan gum as reducing and capping agent also showed an excellent catalytic capability of 4-NP reduction [150]. In addition, AgNPs produced from arabinogalactan mucilage of *portulaca* (a food grade natural polysaccharide complex) successfully converted 4-NP to 4-AP. This has been attributed to the role of AgNPs in enabling electron transmission from BH_4^- to 4-NP and therefore the production of 4-AP [151]. The large surface area of AgNPs is served as a suitable area for electron donor (BH_4^-) and acceptor (4-NP) to interact. As such AgNPs can act as a suitable catalyst for the reduction of 4-NP. Other studies have also found that polysaccharide-based AgNPs have a significant catalytic activity to reduce pollutants such as 4-NP, methyl orange, methylene blue, and rhodamine B in the presence of $NaBH_4$ medium [15, 152].

8.8 Toxicity of polysaccharide-based AuNPs

Polysaccharide-based NPs have been studied intensively, which resulted in a development of NPs with significant properties for a wide range of biomedical applications. Nevertheless, less attention has been paid to evaluate the toxicity of these polysaccharide-based NPs toward human or animal health. Many studies highlighted NPs as safe with low toxicity, but an in-depth evaluation of the toxicity of NPs and their possible health risks have not been provided extensively. Therefore, a careful examination of the toxicity and potential side effects of NPs used in the biomedical applications is needed [153]. Although properties of NPs such as large surface area, small size, and specific shape and structure have made them interesting for the biomedical applications, it should be noted that these properties might actually contribute to their toxic properties after entering into a biological system. In particular, smaller particles with the larger surface area may show a higher level of reactivity and toxicity

toward biological systems [153]. Yah and colleagues [154] in their review paper on the toxicological significance of NPs have discussed health risks of NPs. NPs may target the respiratory system, blood, skin, central nervous system, and gastrointestinal tract, which may cause a complicated and diverse range of side effects [153, 154]. Despite the low or no toxicity of polysaccharides, their possible health risks need to be considered and evaluated [155].

Once NPs entered the body through lungs they can pass through the respiratory tract and reach the bloodstream that resulted in distribution and access to different organs [153]. The access of NPs to bloodstream can occur in a very short time. In a study, AuNPs of 30 nm detected by microanalysis in the bloodstream just 1 min after being introduced into the rat's trachea [156]. Therefore, AuNPs that have not stabilized/coated with a nontoxic agent such as polysaccharide can be dangerous to healthy organs. Another example is the experiments of Tsoli and coworkers on 1.4 nm AuNPs that demonstrated a strong toxicity of the AuNPs toward 13 different healthy or cancerous human cell lines [157], whereas larger particles of 18 nm were not inherently detrimental to functions of human cell lines [115]. The extreme toxicity of small NPs could be related to their ability to interact and clustering with DNA [157]. Obviously, the size of NPs and dose of the administration can affect the toxic properties of the AuNPs [105].

Gum karaya (GK) as an anionic natural polysaccharide used for the synthesis of AuNPs for drug delivery and tested for biocompatibility. The NPs with an average diameter of 15–20 nm demonstrated very low hemolytic toxicity of 1.4% at the highest tested concentration of 250 μg/mL. The cell cytotoxicity studies also indicated to the biocompatibility of the gum synthesized AuNPs, which was associated with the presence of GK on the surface of the AuNPs [158]. Similar low toxicity reported for xanthan-mediated AuNPs [159]. In some studies, the main aim was the toxicological profile of the polysaccharide synthesized NPs. AuNPs (14 ± 2 nm) synthesized using polysaccharide isolated from marine red algae (porphyran) and tested for in vitro and in vivo toxicity. The particles were nontoxic when tested in vitro on monkey kidney cell lines. Additionally in vivo suboral uptake of the NPs by Wistar rats were also safe even at a high dose of 1500 ppm/kg/day [160]. A mixture of natural polysaccharide polymers and chemically synthesized polymers has also been tested for synthesizing AuNPs. In one of these studies [161], extract of *Leucas aspera* plant and poly lactic acid-*co*-poly ethylene gly*co*l-*co*-poly lactic acid were used to synthesize AuNPs with an average diameter of 7–13 nm. The GNPs-LAE-loaded polymer NPs had no significant negative effect on monkey's kidney cell lines at the maximum tested concentration of 100 μg.

8.9 Toxicity of polysaccharide-based AgNPs

The effects of AgNPs synthesized using sulfated polysaccharide extract from a brown alga; *Sargassum siliquosum* on blood biochemical parameters and liver enzyme parameters of rats have been examined. A high dose of AgNPs (2000 mg/kg BW) was responsible for a reduction on blood creatinine level but the increased levels of blood

ureic nitrogen (BUN), uric acid, alanine amino-transferase (ALT), and aspartate amino-transferases (AST). Furthermore, administration of AgNPs (2000 mg/kg BW) caused moderate toxicity to kidney cells; that is, diffuse tubular epithelial necrosis, a number of atrophied glomeruli, and foci of lymphoid infiltrates in the interstitium of the cortex. However, using AgNPs did not cause any toxic effect on liver cells and a normal organization of hepatocytes, intact cell membrane without necrotic evidence was observed. Furthermore, it has been shown that the pretreatment of rats using AgNPs can prevent hepatocellular damage caused by drug toxicity such as intake of a toxic level of paracetamol (2000 mg/kg BW). AgNP treated rats have shown near normal levels of ALT and AST. Authors concluded that AgNPs synthesized from *S. siliquosum* have hepatoprotective effects that can keep liver enzymes near to normal in intoxicated groups. However, the toxic effects of AgNPs on the kidney are still not clear and further studies needed to clarify their safety [162].

8.10 Conclusion

A large variety of polysaccharide-based NPs has been developed for many different possible biomedical applications including drug delivery, biomarkers, diagnostic, and the treatment of certain diseases. The abundance of polysaccharides with diverse physiochemical properties makes them valuable green alternatives to oil-based materials for the fabrication of various green NPs. AuNPs and AgNPs are the most commonly used nanomaterials in the field of biomedical applications, due to their antimicrobial properties; nevertheless, one major challenge for the biomedical application of NPs is their possible undesirable effect, as NPs may possess certain toxicological properties that limit their applications. Nanotoxicology studies have been therefore developed to study the toxicity of nanomaterials, in particular, to evaluate the possible negative effects of NPs. Thus, the use of NPs in the field of biomedicine needs to consider toxicological properties of NPs. In this regard, polysaccharide synthesized NPs may be an alternative safe option for the development of NPs with biomedical applications.

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Further reading

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