Herein, we described the modification of chitosan with cyanuric chloride as a mediator for preparation of chitosan-s-triazinyl-bis(2-aminomethylpyridine) and chitosan-s-triazinyl-bis(8-oxyquinoline) derivatives to be used as reagents for preparation of silver nanoparticles under ecofriendly conditions. These two reagents are convenient and effective for reduction of silver ions to silver nanoparticles with particle size less than 10 nm that might be suitable for industrial and medicinal applications. The formation and particle size of AgNPs are characterized by transmission electron microscopy (TEM), X-ray diffraction (XRD), scanning electron microscope (SEM), and energy-dispersive X-ray analysis (EDX). The antimicrobial activity of the two modified chitosan-s-triazine-AgNPs was evaluated against activities against Gram-positive bacteria (M. luteus ATCC 10240 and MRSA ATCC 43300), Gram-negative bacteria (E. coli ATCC 25922 and P. aeruginosa ATCC 75853), and C. albicans. The results showed that chitosan-s-triazinyl-bis(2-aminomethylpyridine) AgNPs showed high antimicrobial activities against all the tested microorganisms, while their analogous chitosan-s-triazinyl-bis(8-oxyquinoline) AgNPs showed moderate activities.

1. Introduction

In recent years, efficacy of nanoparticles has been the subject of great interest due to their unique physical and chemical properties, which make them significantly different from their counterpart in the bulk form [1–5]. In particular, silver nanoparticles have gained special interest in recent years because of their outstanding physical, chemical, and biological properties [6–11]. Several methods were used for the synthesis of AgNPs [12–17]; among them, the chemical reduction methods were considered as the most common methods used for the synthesis of AgNPs. Generally, the reduction of silver ions in the chemical methods occurred in the presence of a reducing agent such as hydrazine, ammonium formate, and sodium borohydride [17–22]. These reducing agents are somewhat considered as potentially dangerous to the environment and biological system. Numerous methods have been reported for the synthesis of AgNPs using green, cost-effective, and biocompatible, among them is using extracts of medicinal plants [23–26].
On the contrary, chitosan has excellent biodegradability, antibacterial effect, hemostatic action, and anti-inflammatory effect. Because of these properties, it gains extensive use in pharmaceutical, food, biotechnology, nanobiocatalyst, and agricultural applications [9, 27–34], eliminating heavy minerals, phosphorus, and oil from water [35–39]. There are several publications regarding chitosan modifications through amino groups. However, chemical modifications in the fundamental skeleton of chitosan may lose its original physicochemical and biochemical properties [40–43].

Recently, we reported the synthesis of 2,4,6-tris(quinolin-8-yloxy)-1,3,5-triazine and N trimming of the active groups. The reactions were carried out using hydrazine hydrate and hydrazine hydrate, respectively. The yields were reported in almost quantitative yields.

2.2. Synthesis of Chitosan-4,6-dichloro-1,3,5-triazine (3).

The reaction was carried out by modification of the reported method [27] as follows: chitosan (20.0 gm) was suspended in toluene (200 mL) followed by the slow addition of cyanuric chloride 1 (27.60 gm; 150 mmol) and sodium carbonate (16.0 gm, 150 mmol) at room temperature with vigorous stirring. The reaction temperature was raised gradually to 70–80°C and kept at this temperature for 24 hours (ninhydrin test was negative as shown in Figure S1, Supplementary Materials). The solid product was separated by filtration, washed with toluene and mixture of water-ethanol (1 : 1), and then dried at 50°C for 6 hours to afford modified chitosan Ch-TC2, 3.

2.3. Synthesis of Chitosan-4,6-disubstituted-1,3,5-triazine, 6 and 7.

8-Hydroxyquinoline 4 or 2-aminoethylpyridine 5 (100 mmol) was added to a suspended solution of 3 (5.0 gm) in tetrahydrofuran (THF, 50 mL) followed by addition of triethylamine (200 mmol). The reaction mixture was refluxed overnight, and the solid product was separated by filtration washed with dichloromethane and ethanol, and then dried at 60°C for 12 hours to afford the target products Ch-TQ 6 and Ch-TAMPy 7 in almost quantitative yields.

2.4. Synthesis of Silver Nanoparticles (AgNPs).

Modified chitosan (0.1 gm, Ch-TQ or Ch-TAMPy) was suspended in 50 mL water, and then solution of AgNO3 in water (50 mL, 10–3 M) was added dropwise with stirring at room temperature; after complete addition, the mixture was kept overnight at room temperature. Color of the solution was changed from colorless to brownish black with no further color observed for longer time (Figure S2, Supporting Information). The AgNPs immobilized on modified chitosan were collected by centrifugation at 8000 rpm for 5 minutes, then washed with water, and dried at 60°C for 12 hours.

2.5. Characterization of Silver Nanoparticles (UV-Vis, XRD, SEM, and TEM).

UV-Vis analysis was performed in quartz cuvettes using double distilled water as a reference solvent, and scanning was performed after the reaction was completed in the range 250–870 nm absorbance spectrum in steps of 1 nm. XRD analysis was performed at 40 kV and 40 mA. The scans were typically performed over a 2θ range from 10° to 85° at a scan rate of 0.02°/s, with an aperture slit, an anticscatter slit, and a receiving slit of 2 mm, 6 mm, and 0.2 mm, respectively. X-ray Cu Kα wavelength was 1.54056 Å. The surface morphology was examined using the scanning electron microscope (SEM). The elemental compositions of AgNPs were measured using the high-energy beam of electrons. X-rays were detected using an energy-dispersive spectrometry analyzer (EDS) combined with SEM. The formation of nanoparticles was confirmed by using compact high-performance transmission electron microscopy (TEM).

2.6. Thermogravimetric Analysis (TGA) for Ch-TQ and Ch-TAMPy.

Thermal properties of modified chitosan (Ch-TQ and Ch-TAMPy) were examined using TA-Q500, and the data were collected under nitrogen with a flow rate of 60 mL/min in the temperature range 25°C–800°C with a different heating rate of 5, 10, 20, and 25°C/min.
2.7. Antibacterial Activity. Antibacterial activities of two modified chitosan-AgNPs (Ch-TQ 6 and Ch-TAMPy 7) against the test organisms, *E. coli* ATCC 25922, *P. aeruginosa* ATCC 75853, *M. luteus* ATCC 10240, MRSA ATCC 43300, and *C. albicans* ATCC 10145, were determined by the modified Kirby–Bauer disk diffusion method [45] (as described in Method S1, Supplementary Materials).

3. Results and Discussion

Chitosan was modified by using cyanuric chloride (TCT, 1) in the presence of Na₂CO₃ as the HCl scavenger to ensure complete capping for the amino groups in the chitosan skeleton as observed from the ninhydrin test (Figure S1, Supporting Information). Modified chitosan 3 was reacted with 8-hydroxyquinolone 4 or 2-aminomethylpyridine 5 in the presence of triethylamine to afford Ch-TQ 6 and Ch-TAMPy 7 as shown in Scheme 1.

Figure 1 exhibits the TGA degradation curves of Ch, Ch-TQ, and Ch-TAMPy. The thermal degradation parameters are summarized in Table S1 (Supporting Information). The results showed that modified chitosan (Ch-TQ and Ch-TAMPy) have slight change in the thermal stability with two main degradation steps. In case of chitosan, the first step starts in a range of 36°C to 167°C, with mass loss of 7.7%.

The second step starts in a range of 287°C to 449°C with mass loss of 7.8% and 9.5% for Ch-TQ and Ch-TAMPy, respectively, which could be due to the presence of the hydroxyl group in the chitosan skeleton which prevents agglomeration of AgNPs [48–50].

EDS analysis established the presence of the elementary silver signal of the prepared AgNPs (Figures S4A and S4B, Supporting Information), where strong signal peaks in the range of 2.5–4 keV were observed; these peaks correspond to the binding energies of crystalline silver [51]. A strong signal peak near 0.2 keV was observed which is corresponding to carbon in the ligand connected to AgNPs.

The XRD patterns (Figures 3(a) and 3(b)) showed peaks at about 2θ = 38.10°, 44.09°, 64.36°, 77.29°, and 81.31° for both prepared modified chitosan; these peaks correspond to 111, 200, 220, 311, and 420 planes, respectively. This indicates a typical face-centered cubic structure of silver as per the available literature (Powder Diffraction File, PDF file no. 04–0783) [52, 53].

The lattice parameters, unit cell volume, and space group were calculated with the help of the FullProf and Chekcell programs [R]. The observed and calculated crystallographic data Miller indices (hkl), d spacing (the interplanar spacing), and 2θ (the diffraction angle) were also calculated and are matched with the standard values of Ag [53]. The average grain size (D), dislocation density (δ), and the strain (ε) of the AgNPs are calculated, and the average grain size (D) for both samples is found to be in the range 40–45 nm (Figure S5, Tables S2–S4, Supplementary Information).

The TEM (Figure 4) for samples from Ch-TQ-AgNPs and Ch-TAMPy-AgNPs showed a uniform structure and a homogenous distribution of AgNPs over the modified chitosan surface as shown in Figures 4(a) and 4(b).

The SEM image of AgNPs formed by using 6 and 7 is represented in Figures 5(a) and 5(b), respectively. Analysis of TEM imaging showed that prepared AgNPs are mono-dispersed spheres in the range of 2–10 nm in size for both samples prepared by using 6 and 7. AgNPs were formed after reduction by the lone pair of electrons of the nitrogen atom at 2-aminomethylpyridine and 8-hydroxyquinolone side chains and triazine moiety in the ligand structure. AgNPs have diameters more than 10 nm were formed due to aggregation through preparation of the TEM holding grid.

Finally, both synthesized AgNPs from Ch-TQ and Ch-TAMPy exhibit good antimicrobial activity against all the test organisms in the Kirby–Bauer assay. The diameters of the zone of inhibition for all these compounds against Gram-positive bacteria (*M. luteus* and MRSA), Gram-negative (*E. coli* and *P. aeruginosa*) bacteria, and against *C. albicans* with various concentrations of the test compounds are listed in Table 1. It is interesting to note that these zones of inhibition were obtained even with very low concentration (10–40 μg/well) of the test compounds. Chitosan derivatives are used as the control and did not show any bioactivity against the tested pathogenic microbes, while the synthetic silver nanoparticles have bioactivity against the tested microbes. The data presented in Table 1 showed that Ch-TAMPy-AgNPs exhibited higher antimicrobial activities against the tested microorganisms specially at higher concentrations.
concentration (30 \text{μg/well} and 40 \text{μg/well}) than Ch-TQ-AgNPs, which is in agreement with the reported data for pyridine derivatives [54, 55].

In addition, as shown in Table 1, Gram-negative bacteria were found to be less sensitive than Gram-positive; this may be due to the outer membrane components of Gram-negative bacteria, which may absorb the AgNPs and prevent them from penetration of the bacterial cell wall. This is also in agreement with the findings report on silver nanoparticles synthesized using P. glutinosa plant extracts [56].

![Scheme 1: Synthesis of modified chitosan Ch-TQ 6 and Ch-TAMPy 7.](image)

![Figure 1: TGA degradation curves for Ch, Ch-TQ, and Ch-TAMPy.](image)
Figure 2: SEM images of chitosan/modified chitosan: (a) chitosan; (b) Ch-TQ; (c) Ch-TAMPy.

Figure 3: (a) X-ray diffraction pattern of the silver nanoparticles produced by employing Ch-TQ; (b) X-ray diffraction pattern of the silver nanoparticles produced by employing Ch-TAMPy.

Figure 4: High-resolution electron microscope (SEM) micrograph: (a) silver-Ch-TQ composite using the secondary electron imaging mode (SEI); (b) silver-Ch-TAMPy composite using the secondary electron imaging mode (SEI).
4. Conclusion

The two modified chitosan-s-triazinyl-bis(2-amino-methylpyridine) (Ch-TAMPy) and chitosan-s-triazinyl-bis(8-oxyquinoline) (Ch-TQ) derivatives showed good thermal stability compared with chitosan itself as observed from TGA degradation. The two derivatives were used for preparation of AgNPs under ecofriendly conditions (water medium) without using any reducing agent or stabilizer with a particle size of AgNPs less than 10 nm as observed from TEM. Silver nanoparticles have good stability at room temperature for 5-6 months which indicated that chitosan protects the AgNPs from agglomeration. The AgNPs immobilized with new modified chitosan (Ch-TAMPy and Ch-TQ) showed high antimicrobial activity against the tested Gram-positive bacteria (*M. luteus* and *MRSA*), lowest activity against *C. albicans*, and had a moderate activity against Gram-negative bacteria (*E. coli* and *P. aeruginosa*). These results could be useful for development of biocompatible polymers with antimicrobial activity and can be used in the textile industry or in wound dressing.

Data Availability

The data used in this study to support the findings are included in the article and supporting information.

Conflicts of Interest

The authors declare no conflicts of interest.

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Supplementary Materials

Method S1: methodology of the antibacterial activity of the modified chitosan-AgNPs against the tested organism; Figure S1: ninhydrin test; Figure S2: preparation of AgNPs using modified chitosan Ch-TQ; Figure S3: UV-Vis spectra of silver nanoparticle solutions; Figures S4A and S4B: energy-dispersive X-ray spectra (EDX) of the prepared silver nanoparticles from Ch-TQ and Ch-TAMPy; Figures S5A and S5B: AgNPs from Ch-TQ and Ch-TAMPy and their particle diagram; Table S1: information derived from TG curves for thermal degradation of copolymers; Table S2: 2θ and d spacing for AgNPs calculated and observed by Ch-TQ and Ch-TAMPy; Table S3: lattice parameters, unit cell volume, and space group for AgNPs by Ch-TQ and Ch-TAMPy; and Table S4: average grain size (D), dislocation.
density (ρ), and the strain (ε) of the AgNPs. (Supplementary Materials)

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