Green synthesis of silver nanoparticle and silver based chitosan bionanocomposite using stem extract of *Saccharum officinarum* and assessment of its antibacterial activity

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Abstract

Synthesis of nanoparticles and nanocomposites using green route is a major focus of modern nanotechnology. Herein we demonstrate the synthesis of silver nanoparticle and silver based chitosan bionanocomposite using the stem extract of *Saccharum officinarum*. The absorbance peak at 460 nm in the UV–Vis spectrum reveals the synthesis of silver nanoparticles using the stem extract of *Saccharum officinarum*. The size of the synthesized silver nanoparticle was in the range of 10–60 nm obtained from transmission electron microscope (TEM) analysis. The presence of silver nanoparticles on the chitosan suspension was identified by scanning electron microscope (SEM) and energy dispersive x-ray spectroscopy (EDS). The presence of possible functional group involved in the reduction of silver metal ions into silver nanoparticles was identified by Fourier transform infrared spectroscopy (FTIR) analysis. The antibacterial activity of silver based chitosan bionanocomposite was evaluated against *Bacillus subtilis* (MTCC 3053), *Klebsiella planticola* (MTCC 2277), *Streptococcus faecalis* (ATCC 8043), *Pseudomonas aeruginosa* (ATCC 9027) and *Escherichia coli* (ATCC 8739). The antibacterial activity of silver based chitosan bionanocomposite has remarkable scope in medicine, food packaging, textile and pharmaceuticals.

Keywords: *Saccharum officinarum*, silver nanoparticle, chitosan, bionanocomposite, antibacterial activity

Classification numbers: 2.0, 2.04
1. Introduction

Synthesis of metal nanocomposites is one of the emerging research areas in the arena of modern nanoscience and nanotechnology. Due to their distinct physicochemical properties, metal nanocomposites have been used in a variety of applications such as adsorption [1, 2], water purification [3, 4], antimicrobial activity [5, 6], biomedicine [7, 8] and sensors [9]. Nanocomposites are defined as the dispersion of inorganic nanoparticles in a polymeric compound [10]. Mostly, silica materials (used as polymer matrix) and inorganic nanoparticles like silver [11], gold [12], zinc oxide [13] and titanium oxide [14] are used for the synthesis of nanocomposites. Apart from silica, other materials like carbon nanotubes [15] and titanium oxide [16] are also used as polymer matrix in the nanocomposite synthesis process. Among those materials, chitosan as a non-toxic biopolymer is used for the preparation of nanocomposites. Chitosan has been used in different applications like adsorption [17], drug [18] and gene [19] delivery systems etc due to their fine inherent properties. The antimicrobial property of chitosan materials could be more beneficial in food preservation process [20]. Thereby, in the present study, the chitosan material is used as a polymer matrix for the synthesis of silver based chitosan bionanocomposites.

Due to the potent antimicrobial [21] and sensor [22] activities, silver nanoparticles are most widely used in various applications. The synthesis of nanoparticles using biological resources is gaining more attention due to its rapid, eco-friendly and non-toxic nature. In bio-route, microorganisms such as bacteria [23] and fungus [24] are mostly involved in the silver nanoparticle synthesis process. Apart from that algae are also used for the production of silver nanoparticles [25, 26]. Nowadays, plant mediated synthesis process is preferable compared to microbe mediated synthesis due to its simplicity, rapidity and avoidance of culture maintenance [27, 28]. Plant materials like leaves [29, 30], flowers [31, 32], seeds [33, 34], stems [35, 36], fruits [37–39], peels [40, 41] and weeds [42] have been used for the synthesis of silver nanoparticles. In the present investigation we have used the stem extract of Saccharum officinarum (S. officinarum) for the synthesis of silver nanoparticle and nanocomposite. The S. officinarum is a popularly known plant for sugar (sucrose) which is a daily need recipe. Apart from sucrose, the stem of S. officinarum is rich in phenolic acids and flavones [43, 44]. Herein, the presence of sugar and phenolic acids might be act as good reducing and stabilizing agents in the silver nanoparticle synthesis process. The formation of silver nanoparticles in the reaction mixture (aqueous silver nitrate solution with S. officinarum extract) was visually identified by the appearance of brown color in the reaction mixture. Further, the synthesized silver nanoparticles and silver based chitosan bionanocomposite was characterized by UV–Vis spectroscopy, XRD, SEM, TEM, and FTIR analysis. In addition, the antibacterial activity of silver based chitosan bionanocomposite was also examined.

2. Experimental

2.1. Synthesis of silver nanoparticles by using S. officinarum extract

The S. officinarum (Purple sugarcane) was purchased from local market at Ambasamudram, India. The sugarcane stem was sliced into small pieces and washed thoroughly in tape-water and followed by double distilled water. About 10 g of sliced pieces was taken and grinded in the mixer. The extract was purified by whatmann no. 1 filter paper and centrifuged at 7000 rpm for 15 min. The supernatant matter was separated and stored in brown bottle for further experiment. About 10 ml of sugarcane extract was added into 90 ml of 1 mM silver nitrate solution and the mixture was incubated at 37 °C. The silver nitrate was purchased from Hi-media laboratories, Mumbai, India. The color changes from pale yellow to brown indicate the formation of silver nanoparticles. A control was also maintained without addition of S. officinarum exhibits no color change.

2.2. Characterization of S. officinarum synthesized silver nanoparticles

The formation of silver nanoparticles was monitored by measuring the UV–Vis spectra of the reaction mixture (aqueous silver nitrate solution with S. officinarum extract). The UV–Vis spectra measurements were carried out on Perkin-Elmer double beam spectrophotometer operated with a resolution of 2 nm. Further, the reaction mixture was centrifuged at 10000 rpm for 10 min and washed with double distilled water. Then, the centrifugation process was repeated for 4–5 times with double distilled water. Finally, the pellets were dried in hot air oven and the dried powder was used for further characterization studies. For XRD analysis, the powdered nanoparticles were coated on the amorphous silica substrate. The spectra was recorded by using XDL 3000 powder x-ray diffractometer with 40 kV and a current of 30 mA with Cu-Kα (1.5405 Å) radiation. The shape of the silver nanoparticles was examined by SEM (Scanning Electron Microscope). The presence of elemental silver was analyzed by energy dispersive spectroscopy attached with SEM. For TEM analysis, the synthesized silver nanoparticles were coated on copper grids and analyzed by Philips CM200 operated at 200 kV. The FTIR experiment was carried out on Perkin-Elmer instrument at wavelength ranges from 3800 to 500 cm⁻¹ with a resolution of 4 cm⁻¹.

2.3. Preparation and characterization of silver based chitosan bionanocomposite

About 1 g of chitosan was mixed with 0.5 ml of acetic acid and made up the volume to 50 ml for dissolving the powdered chitosan. Then 1 mM of silver nitrate was added to the chitosan solution and the suspension was mixed by using magnetic stirrer. The suspension was allowed to stand for 2 h and 10 ml of S. officinarum stem extract was added to the chitosan suspension. After addition of stem extract of S. officinarum, the
colorless suspension was changed into brown color indicates the formation of silver nanoparticles in the suspension. The SEM and EDS analyses were carried out to confirm the presence of silver nanoparticles on the surface of chitosan.

2.4. Antibacterial activity of silver based chitosan bionanocomposite

The antibacterial activity of silver based chitosan bionanocomposite was analyzed by agar well diffusion method on Muller-Hinton agar plates against *Bacillus subtilis* (MTCC 3053), *Klebsiella planticola* (MTCC 2277), *Streptococcus faecalis* (ATCC 8043), *P. aeruginosa* (ATCC 9027) and *Escherichia coli* (ATCC 8739). Chloramphenicol, a standard antibiotic, is used as control. It was purchased from Himedia Laboratories, India. Individually, single colony of bacterial strain was grown in nutrient broth and maintained on a shaker at 37 °C for 24 h. After 24 h incubation, separately each bacterial strain was spread into the Muller-Hinton agar plates using sterile cotton swabs. The silver based chitosan bionanocomposite at different concentrations (20, 40 and 60 µl) was poured into the well and the plates were incubated at 37 °C for 24 h. After incubation, the zone of inhibition was measured.

3. Results and discussion

3.1. Visual observation

Synthesis of nanoparticles using plant extracts attains considerable interest due to its cheapest and eco-friendly nature. Herein we examine the silver nanoparticle synthesis property of commercially important crop *S. officinarum*. The observation of color changes is an analysis to identify the synthesis of silver nanoparticles using biological materials such as bacteria [45], fungus [46] and plant [47] materials. The formation of brown color is observed within 5 min after the addition of *S. officinarum* stem extract reveals the silver nanoparticle synthesis reaction was started and the strength of brown color was increased up to 24 h (inset (B) of figure 1). Without addition of *S. officinarum* extract, the silver nitrate solution can be treated
as control (inset (A) of figure 1). After 24 h, the aggregation of synthesized silver nanoparticles indicates the completion of silver metal ions into silver nanoparticles reduction process. The color changes from white to brown due to the excitation of surface plasmon resonance (SPR) of the synthesized silver nanoparticles [48]. Similar results were observed in the plant leaf extracts of *Acalypha indica* [49] and *Piper betle* [50].

3.2. UV–Vis spectroscopy analysis

UV–Vis spectroscopic analysis is widely used method to identify the formation of metal nanoparticles by analyzing the unique optical properties which depends on the size and the shape of the nanoparticles [29, 45]. Figure 1 exhibits the UV–Vis spectrum of silver nanoparticles synthesized by using *S. officinarum* at different times of incubation (5 min to 24 h). The UV–Vis spectrum of silver nanoparticles exhibited a strong absorption band at 460 nm (figure 1). Initially (5 and
10 min incubation), the nanoparticle synthesis process was started slowly and the SPR peak was observed at 480 nm. At 20 min incubation, the nanoparticles were synthesized vigorously and the SPR peak was achieved at 460 nm. There was no SPR peak change observed after 20 min and the peak increases gradually with the increase in time of incubation which remains constant up to 24 h incubation time. At the beginning of the reaction, the weak absorbance peak indicates the fewer amount and large sized silver nanoparticles due to the absence of redox reaction between the aqueous silver metal ions with the reducing agents [51]. But, after 20 min incubation time, the higher redox reaction may shift the peak from 480 nm to 460 nm. The observation of slight narrow peak after 20 min incubation indicates that the synthesized particles are small in size. This was also confirmed by TEM observations.

### 3.3. XRD analysis

The XRD spectra were used to verify the crystalline nature of the silver nanoparticles synthesized by *S. officinarum* and the pattern was shown in figure 2. The XRD spectra of silver nanoparticles indicate the synthesized silver nanoparticles using the stem extract of *S. officinarum* is crystalline in nature. The peaks were observed at 2θ values of 38.2°, 46.3° and 77.3° corresponding to (1 1 1), (2 0 0) and (3 1 1) planes, respectively, which was indexed for face centered cubic of silver (JCPDS file no. 04-0783). Some unassigned peaks beside crystalline peaks of silver nanoparticles indicates the presence of phytochemicals of *S. officinarum*. The present result was in good agreement with the observation in [52, 53]. The contamination of phytochemicals with the synthesized silver nanoparticles can be avoided by continuous
centrifugation with double distilled water after the nanoparticle synthesis process [54].

3.4. SEM and EDS analysis

The silver nanoparticles synthesized by *S. officinarum* were mostly spherical in shape (figure 3). In addition, elemental analysis was also performed to confirm the presence of silver nanoparticles in the solution. Figure 4 shows the EDS analysis of *S. officinarum* mediated synthesis of silver nanoparticles. The EDS analysis showing an intense signal at 3 keV indicates the presence of elemental silver.

3.5. TEM analysis

The TEM images of silver nanoparticles synthesized by using *S. officinarum* were shown in figures 5. The images signify that the synthesized silver nanoparticles were polydispersed. Figure 5(B) exhibits the formation of bioorganic materials stabilizing silver nanoparticles produced by *S. officinarum* juice. The presence of enormous amount of sucrose in *S. officinarum* juice might be responsible for the reduction and stabilization of silver nanoparticles (figure 5(B)). Vasireddy *et al* [55] have reported that the glucose molecules could act as reducing agent in the production of silver nanoparticles. In addition, Meshram *et al* [56] have demonstrated the action of gluconic acid as a capping agent in the synthesis of silver nanoparticles. In the present work we study the sucrose molecules in the *S. officinarum* extract might act as both reducing and stabilizing agent. The small spherical shaped silver nanoparticles were surrounded by the stabilizing agents (arrows marked 1 and 2 in figure 5(C)) whereas, the large nanoparticles could not be surrounded by the stabilizing agents (arrows marked 3, 4 and 5 in figure 5(C)).

One of possible reasons is following: at the beginning of the reaction large silver nanoparticles were obtained due to the low redox reaction of *S. officinarum* extract to the aqueous silver metal ions. Subsequently, when the time of incubation is increased, the small and uneven shaped nanoparticles were formed due to the high redox reaction between the *S. officinarum* extract and aqueous silver metal ions. The TEM images were well correlated with UV–vis spectra result and the representative images exhibit the presence of spherical (arrows marked 1–3 in figure 5(D)) and undefined shaped (arrows marked 4–6 in figure 5(D)) silver nanoparticles with the size ranges from 10–60 nm.

3.6. FTIR analysis

The possible functional groups of phytochemicals in *S. officinarum* extract involved in nanoparticles synthesis was identified by FTIR analysis. The FTIR spectrum of silver nanoparticles synthesized by *S. officinarum* was shown in figure 6. The observation of band at 3436 cm\(^{-1}\) and 3374 cm\(^{-1}\) exemplifies the H–OH stretching of phenols. The reducing sugars are the major compounds present in the *S. officinarum* extract might be acting as reducing agent to synthesize the silver nanoparticles. The adsorption of band at 1630 cm\(^{-1}\) denotes the C=O stretching of aldehydes and ketones. The peaks at 1388 cm\(^{-1}\) corresponds to N=O bending of nitro groups. The bands observed at 1115 cm\(^{-1}\) and 1008 cm\(^{-1}\) represent the C–O stretching of esters. The peaks observed at 845, 751, 665 and 604 cm\(^{-1}\) correspond to C–H stretching of alkenes. The aldehyde and ketone groups indicate the presence of reducing sugar compounds (glucose, fructose and sucrose) in the *S. officinarum* extract [56–58]. In addition, the redox reaction of reducing sugar compounds initiates the reduction of silver metal ions to silver nanoparticles.

3.7. Visual identification of silver nanocomposite

The formation of silver nanoparticles in the chitosan solution was visually identified by the color changes from white to brown color (figures 7(A) and (B)). The synthesis of silver nanoparticles in the chitosan solution was facilitated by the addition of reducing agent *S. officinarum* extract. Further, the synthesized silver based chitosan bionanocomposite was characterized by SEM and EDS analysis.

3.8. SEM and EDS analysis of silver based chitosan bionanocomposite

The morphology of the synthesized silver based chitosan bionanocomposite was viewed by SEM analysis. The biosynthesized silver nanoparticles, chitosan and silver based chitosan bionanocomposite synthesized by *S. officinarum* were shown in figure 8. The synthesized silver nanoparticles were strongly bound on the surface of the chitosan (figures 8(D) and (E)). Similar observations were noticed in silver/montmorillonite/ chitosan bionanocomposites synthesized using sodium borohydride [60]. The silver/chitosan/polyethylene glycol nanocomposites have been synthesized without addition of any reducing agent. Chitosan and polyethylene glycol (PEG) are stabilizing agents to stabilize the silver nanoparticles from
aggregation [59]. In addition, chitosan has a fine property that could inhibit the growth and the dispersion of inorganic metal nanoparticles [59, 61]. Furthermore, in figure 8(D), the observation of shining molecules along with the polymer suspension clearly indicates the presence of enormous amount of silver nanoparticles. The EDS analysis also confirmed the presence of silver nanoparticles on the chitosan suspension (figure 9). The formation of intense peak at 3 keV related to silver element and other signals like C, O and P indicate the presence of elemental compounds of chitosan.

3.9. Assessment of antibacterial activity

The antibacterial activity of silver based chitosan bionanocomposite synthesized by S. officinarum was examined against Bacillus subtilis (MTCC 3053), Klebsiella planticola (MTCC 2277), Streptococcus faecalis (ATCC 8043), P. aeruginosa (ATCC 9027) and E. coli (ATCC 8739) (table 1). Herein three different concentrations of 20 µl, 40 µl and 60 µl were used to check the antibacterial activity of silver based chitosan bionanocomposite. The 60 µl concentration shows strong antibacterial activity in all aforementioned bacteria. Among the bacteria, the bionanocomposite has shown high zone of inhibition against P. aeruginosa (table 1). The silver based chitosan bionanocomposite exhibits potent antibacterial activity due to the presence of nano sized silver particles on the surface of chitosan suspenosion.

Still, the exact mechanism of antibacterial activity of silver nanoparticles is not properly understood. Researchers have found some possible mechanisms and interaction between the silver ions and bacteria. The possible mechanisms are as follows, the electrostatic interaction between positive charged silver metal ions with the negative charged DNA and protein molecules could collapse the structure and function of DNA and protein [62–64]. Morones et al [65] and Panacek et al [66] have suggested that the silver metal ions interact with the cell membrane and disturb the membrane permeability, respiration and membrane proteins. The increased membrane permeability leads to poor transport and it is one of the possible ways to penetrate the silver metal ions into bacterial cell. It is well known that the bacterial cell contains phosphorus and sulfur based compounds. Due to high binding strength of silver metal ions with the DNA and proteins it could easily demolish or inactivate the functions of DNA and proteins. The free radicals also play a vital role in the bacterial cell membrane damage. The release of free radicals from silver could be identified by electron spin resonance study [67]. Kim et al [68] suggested that the free radicals might be involved in the membrane damage. They have depicted that the antioxidant (N-acetyl cysteine) could influence the antibacterial activity of free radicals which may be released from the surface of silver nanoparticles.

Another, new concept is the formation of pits in the surface of cell wall. Sondi et al [69] have resolved a query regarding the antibacterial effect of negatively charged silver ions. The silver nanoparticles on the surface of the E. coli cell surrounded by a membrane called pits. The metal depletion in the outer membrane resulted in the disturbance of membrane permeability and may form the pits. Subsequently, the membrane interruption leads to the release of lipopolysaccharides and finally the cell was totally collapsed [69, 70]. Currently, we are analyzing the release of silver ions from silver nanoparticles and nanocomposite during the antibacterial activity. The antimicrobial activity of silver based chitosan bionanocomposite will give a beneficial application in medicine, agriculture, food packing and textile technology.

4. Conclusion

The present investigation reports the synthesis of silver based chitosan bionanocomposite and its application in antibacterial activity. The S. officinarum extract was used as reducing agent to the synthesis silver nanoparticle and silver based chitosan bionanocomposite. The TEM images confirms the presence of spherical and uneven shaped silver nanoparticles with the size range of about 10–60 nm. The presence of silver nanoparticles on the surface of chitosan was confirmed by SEM analysis. Among the test bacterial culture, the nanocomposite exhibits efficient activity against P. aeruginosa at 60 µl. Now the important task is to find out the release of silver ions from silver nanoparticles and explore the mechanism of antibacterial activity of silver nanoparticles and silver based chitosan bionanocomposite.

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