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Padina gymnospora derived a linear polysaccharide "alginate" mediated synthesis of silver nanocomposite and its antibacterial activity

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ABSTRACT

Sodium alginate extract of *Padina gymnospora* was used for the synthesis of silver (Ag) nanoparticles. UV-visible spectroscopy studies were carried out to assess the formation of Ag nanoparticles. Reductions of Ag+ ions on sodium alginate were conformed to FTIR. Scanning Electron Microscopic (SEM-EDX) was used to characterize the Ag nanoparticles. SEM image divulges that silver nanoparticles were quite polydispersed, the size ranging from 54nm to 78nm. EDX spectroscopy also confirmed the presence of silver ions. AFM analysis did analyze the morphology of the nanocomposite. Biosynthesized silver nanoparticles showed the antibacterial activity against multi-drug resistance human pathogens such as *Staphylococcus aureus* and *Pseudomonas aeruginosa*. These results suggest that Ag nanoparticles can be used as effective growth inhibitors in various microorganisms, making them applicable to various medical devices and antimicrobial control systems.

Keywords: *Antibacterial activity; Biosynthesis; Padina gymnospora; Seaweed; Sodium alginate.*

1. INTRODUCTION

The improper and uncontrolled use of many antibiotics resulted in the occurrence of antibiotic resistance, which became a significant health problem worldwide. Due to the outbreak of the infectious diseases caused by different pathogenic bacteria and the development of antibiotic resistance, the pharmaceutical companies, and the researchers are searching for new antibacterial agents. New treatments based on antibody technology have been proven to be efficacious. Although antibodies are effective, they are not efficient. Large amounts of antibodies are needed to suppress the targeted areas, and the inhibitory effects of antibodies are transient unless these high doses are administered repeatedly. Nowadays, bio-based polymers are highly effective and crossing over the borderline of multi-drug resistance in treating infectious diseases like malaria, tuberculosis (TB), diarrheal diseases, cancer [1].

In the present scenario, nanoscale materials have emerged up as novel antimicrobial agents owing to their high surface area to volume ratio and the unique chemical and physical properties [2]. Nanotechnology can be defined as research for the design, synthesis, and manipulation of the structure of particles with dimensions smaller than 100nm. Polysaccharide based derivatives and nanomaterials have received considerable attention in recent years because of their unique properties and potential applications in catalysis [3], plasmonics [4], optoelectronics [5], biological sensor [6] and therapeutics delivery and tissue engineering [7]. Currently, efforts are being made to investigate the use of nanomaterials in various therapeutic applications, where the NPs could act as the active component or could just be the physical support for the functional moieties. In addition, the importance of augmenting the performance of conventional drugs by incorporating the NPs cannot be overstated as the synergistic effect may offer valuable alternatives with minimization of harmful consequences [8].

Biological synthesis protocols leading to the formation of nanostructures have been reported using bacteria [9], fungi [10], and plants [11]. Extensive research has been done to unveil the synthesis of nanoparticles from live marine organisms (free-living and symbiotic bacteria and fungus, seaweeds, and plants), and the results are amazingly diverse and productive [12-13]. In this paper, we report on the synthesis of Ag nanoparticles by the reduction of aqueous Ag+ ions with the help of seaweeds. However, to date, there have been no reports on the synthesis of Ag nanoparticles with sodium alginate of *Padina gymnospora.*

2. MATERIALS AND METHODS

2.1. Alginate extraction from seaweed.

Padina gymnospora was collected from the intertidal rocky shore region of Pudhumadam coast in the Gulf of Mannar, India, and identified using keys provided according to Ganesapandianand Kumaraguru [14]. The homogenized powder sample of *Padina gymnospora* (10 g) mixed with 3g of sodium carbonate in 400ml of distilled water. The sample was continuously boiled in a water bath for 3-4hrs and stirred frequently. The material was filtered through bolting silk cloth, and the residue was discarded. The addition of hydrochloric acid (4ml) to the filtrates was made to achieve complete precipitation of alginic acid, and to overcome acidity, sodium bicarbonate was added. The applied procedure helped

alginic acid getting converted to sodium alginate. The sample was dried at 35˚C for 5hrs. The dried sodium alginate was powdered with an electronic grinder.

2.2. Synthesis of silver nanoparticles.

The derived sodium alginate (0.5 mg) was dissolved to 100ml of an aqueous solution of silver nitrate with difference concentration (0.25, 0.5, 1, 2mM). The aqueous solutions were kept on a water bath at 80°C for 60min. The reduction of silver nitrate to silver ion was confirmed by a color change from colorless to brown color. The formation of silver nanoparticles was recorded using a UV visible spectrophotometer at a resolution of 1 nm between 300 to 700 nm in a 10-mm-path-length quartz cuvette.

2.3. Optimization of physico-chemical parameters.

Different parameters were optimized, including temperature, pH, and reaction time with 1mM $AgNO_3$. The temperature was maintained at 50, 60, 70, and 80˚C using a water bath; pH was maintained 6, 7, and 8 adjusted with 0.1N HCl and 0.1N NaOH, and reaction time was maintained 1hr-30days respectively. The absorbance was measured through a UV visible spectrophotometer.

2.4. Characterization of silver nanoparticles.

Sodium alginate aqueous solution of *Padina gymnospora* before and after the reduction of Ag+ ions was centrifuged at 5000rpm for 10min. The pellet was resuspended in 10ml sterile distilled water, and the centrifugation process was repeated for three times. Thereafter, the purified suspension was freeze-dried to obtain a dry powder. The dried powder was analyzed using FTIR (model no 8400s SHIZAMAZU). A disk of 50mg of KBr was prepared with a mixture of 2% finely dried samples and then examined under IR-Spectrometer. Infrared spectra were recorded in the region of 500 to 4000 cm^{-1}.

Synthesized silver nanoparticle solution was centrifuged at 12000rpm for 30 min. The pellet was resuspended in 10ml sterile deionized water, and the centrifugation process was repeated for three times. The lyophilized sample was used to analyze the size and basic configuration of AgNPs using SEM-EDX (JEOL model-L6390) and atomic force microscopy.

2.4. Antibacterial activity

The silver nanoparticles synthesized using sodium alginate of *Padina gymnospora*weretested for antimicrobial activity by agar disks-diffusion method against multi-drug resistant (MDR) human pathogenic bacteria, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*.. Each strain was uniformly swabbed onto the surface of Mueller Hinton agar plates using sterile cotton swabs (Himedia Labs, Mumbai, India). The lyophilized nanoparticle (1mg) was dissolved in 1ml of deionized water. Different concentrations of nanoparticles solution (10µl, 20µl, 40µl) was poured onto each disk and placed on Mueller Hinton agar plates. After incubation at 37°C for 24 hours, the different levels of the zone of inhibition were measured.

3. RESULTS

Several approaches have been employed to obtain a better synthesis of silver nanoparticles, such as chemical and biological methods. Yusheng *et al.*, [15] synthesized silver nanoparticles using seaweeds extract after 24hrs of incubation. Similarly, in the present study, silver nanoparticles were synthesized within 60min using sodium alginate extract of *Padina gymnospora*. Interestingly sodium alginate extract, when mixed with an aqueous solution of the silver nitrate, started to change the color from watery to brown due to the reduction of silver ion, which indicated the formation of silver nanoparticles whereas the control $AgNO_3$ solution did not produce any color (Fig. 1). The intensity of brown color increased in direct proportion to the incubation period. It may be due to the excitation of surface Plasmon resonance (SPR) effect and the reduction of $AgNO_3$ [16]. The characteristic absorption peak at 417nm in UV–vis spectrum confirmed the formation of silver nanoparticles. Absorption spectra of silver nanoparticles formed in the reaction media had absorbance peak at 417 nm, and broadening of peak indicated that the particles are polydispersed. It is generally recognized that UV–Vis spectroscopy could be used to examine the size and shape-controlled nanoparticles in aqueous suspensions [17].

3.1. Fixation and Synthesis of silver nanoparticles

Different parameters were optimized, including the concentration of silver nitrate temperature, pH, and reaction time, which had been identified as factors affecting the yields of silver nanoparticles. Different concentration of silver nitrate solution was used (Fig. 2). The ratio of silver nitrate solution (1mM) and the sodium alginate extract was altered to investigate the optimum composition to maximize the yield of silver nanoparticles.

Figure. 1. Reduction of silver nitrate to silver ion.

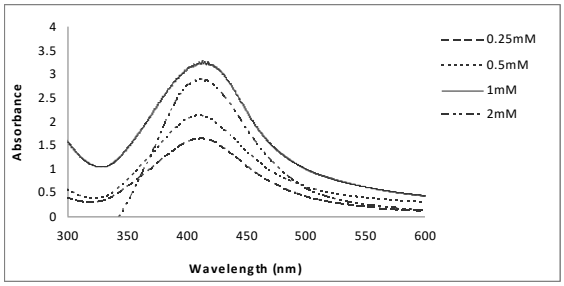

Figure. 2. Effect of reaction concentration on the formation of silver nanoparticles.

The highest plasmon peaks were recorded for 1mM.$AgNO_3$. The study involved the function of temperature for the maximum rate of synthesis. Increasing the temperature above 80ºC aids the growth of the crystal around the nucleus (Fig. 3). Large nanoparticles were formed at lower pH 7, whereas small and highly dispersed nanoparticles were formed at high pH 8. At low pH, the aggregation of silver nanoparticles to form larger nanoparticles was believed to be favored over the nucleation. At higher pH, however, the large number of functional groups available for silver binding facilitated a higher number of silver nanoparticles to bind and subsequently form a large number of nanoparticles with smaller diameters. However, at higher pH agglomeration of nanoparticles took place (Fig. 4). This result confirmed the significant role played

by pH in controlling the shape and size of the Ag nanoparticle synthesis. Andreescu *et al.* [18] reported a similar pH effect, in addition to the rapid and complete reduction of the silver species at elevated pH. Acidic condition suppresses the formation of silver nanoparticles, but the essential condition enhances the formation of silver nanoparticles [19].

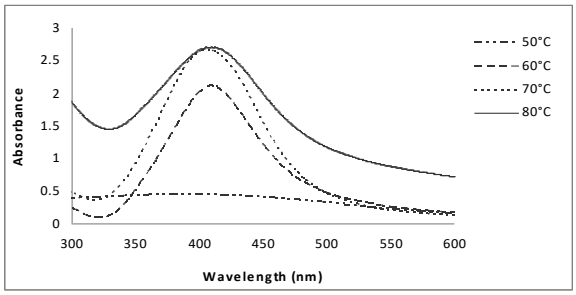

Figure. 3. Effect of reaction temperature on the production of silver nanoparticles.

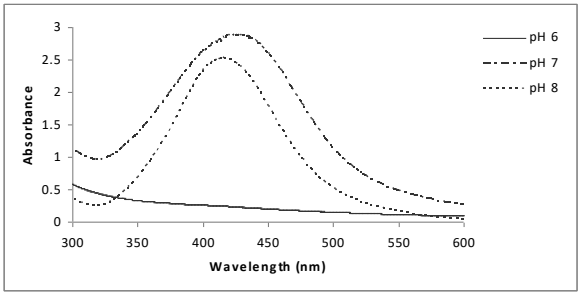

Figure. 4. Effect of reaction pH on the production of silver nanoparticles.

The kinetics of the reduction process was monitored. It can be seen that the intensity of the plasmon resonance peak increases with time; during initial reaction times, no significant increase in the absorption peak was found. The sample was vigorously stirred at room temperature for different time periods, and the SPR band of AgNPs was observed (Fig. 5). The absorbance of AgNPs was obtained at 24hrs, and it maintained up to 30days. The findings were in agreement with other reports on the time duration required for the complete formation of nanoparticles synthesized using dried seaweed at ambient temperature [20]. SPR patterns, characteristics of metal nanoparticles strongly depend on particle size, stabilizing molecules, or the surface adsorbed particles and the dielectric constant of the medium. The single SPR band in the early stages of synthesis corresponds to the absorption spectra of spherical nanoparticles. Many SPR bands resulted later; with an increase in the pH and temperature, it indicates the formation of anisotropic molecules that later stabilized in the medium.

3.2. Characterization of silver nanoparticles.

FTIR absorption spectra of sodium alginate of *Padina gymnospora* before and after the reduction of Ag are shown in Fig. 6. Absorbance bands were observed in the region of 500- 4000 cm^{-1}. Among them, the band at 1024cm^{-1} was corresponding to the bending vibration of carboxylate asymmetric stretching. After the reduction of $AgNO_3$ the changes in intensity at 1037.63cm^{-1} signify the involvement of the carboxylate asymmetric stretching, which is following the high uronic content [21] the reduction process. The bands can be assigned to C-C and C-O stretching as well as C-C-H and C-C-O bending modes in the low wavenumber region, which are characteristic for alginate. The broad glycosidic ring breathing mode at approx. 1100 cm^{-1} can be found in the spectra of several polysaccharides. Since alginate or alginic acid, respectively, is a polyuronic acid there are symmetric and asymmetric stretching modes of the carboxylate groups found in the spectral range above 1200 cm^{-1} Chourpa *et al.*, [22] and Chourpa *et al*., [23].

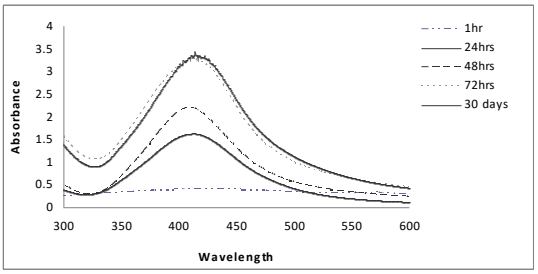

Figure. 5. Effect of incubation period on the production of silver nanoparticles.

SEM analysis of silver nanoparticles was clearly distinguishable owing to their size difference. Silver nanoparticles have around 54-78nm in size (Fig. 7). Most of the nanoparticles were roughly circular in shape with smooth edges. Further, although most of the particles appear in spheroidal shape, there are quite a few with a pronounced anisotropic morphology, like nanoprisms.

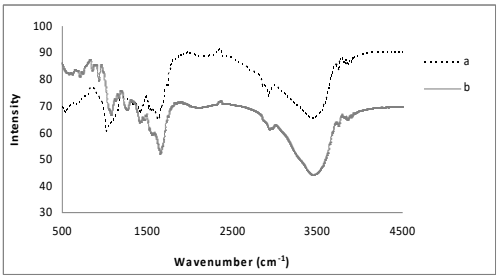

Figure. 6. FTIR spectra of $AgNO_3$ and AgNPs from sodium alginate (a) before reduction, (b) after reduction of Ag ion.

In a few cases, the nanoparticles form small aggregates. This clearly indicates that silver nanoparticles entrapment along with sodium alginate. It was quite common that the control or alignment of nanoparticles could be achieved by modifying the sodium alginate network architectures. EDX spectroscopy results confirmed the significant presence of pure 100% silver with no other contaminants. The EDX spectrum recorded from silver nanoparticles showed a strong signal of silver (Fig. 8). The optical absorption peak is observed at approximately 3 keV, which is typical for the absorption of metallic silver nanocrystallites due to surface plasmon resonance [24]. The AgNPs were characterized by AFM for detailed size, morphology, and agglomeration of silver. AFM analysis was performed in the dry state. Upon drying, Ag NPs underwent surface aggregation, and uniformity was lostSmrati Gupta *et al.*, [25]. The wet synthesized silver had a distinct spheroid-like structure under AFM; the topographical image of irregular AgNPs is shown in Fig.9, where it can be clearly seen that the particle size was in the range from 103-110 nm and cannot be controlled by varying the synthesis condition.

3.3. Antibacterial activity.

The nanoparticle syntheses by sodium alginate are found to be highly effective against multi-drug resistant human pathogenic bacteria. The novel techniques are being applied for treating bacterial infection by forming different nanocarriers [26]. Antibacterial activity of silver nanoparticles against MDR human pathogenic bacteria of *Pseudomonas aeruginosa* and

Staphylococcus aureus was investigated. The zone of inhibition of silver nanoparticles (10, 20, and 40µl) for *Staphylococcus aureus* was 2, 3, and 4mm (Fig. 10), whereas *Pseudomonas aeruginosa* was 2, 4, 6mm. The antibacterial activity produced by the silver nanoparticles prepared by using sodium alginate of *Padina gymnospora* was similar antibacterial activity exhibited by silver nanoparticles prepared by using mangosteen leaf extract [19].

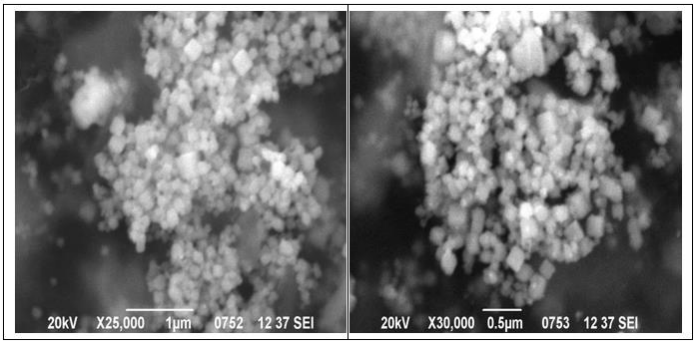

Figure. 7. SEM images of the synthesized silver nanoparticles.

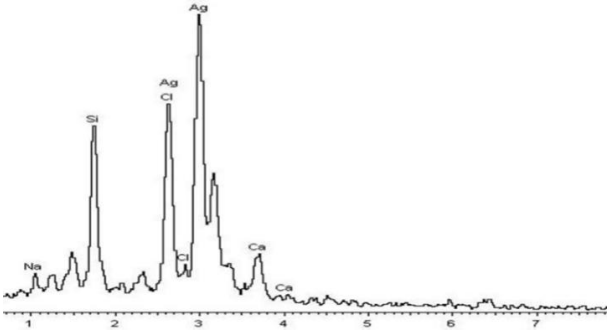

Figure. 8. EDX spectrum for synthesized silver nanoparticles.

Silver is probably the most potent antimicrobial that exhibits strong cytotoxicity toward a broad range of microorganisms, and simultaneously remarkably low human toxicity compared to other heavy metal ions. It has an oligodynamic effect, that is, silver ions are capable of causing a bacteriostatic (growth inhibition), or even a bactericidal (antibacterial) impact.

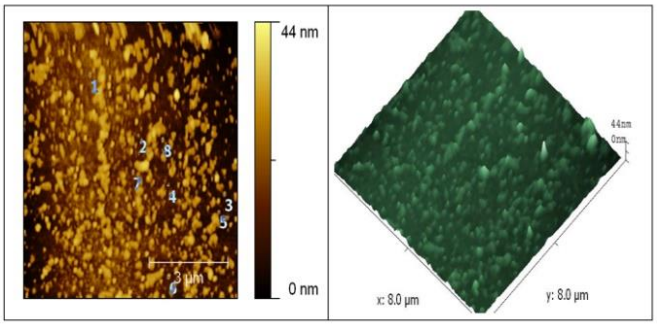

Figure. 9. AFM recorded the topographical image of synthesized silver nanoparticles.

The development and characterization of polymeric-based nanoparticles for sustained release of amoxicillin – an antimicrobial drug also supported the finding for developing alginate-based nanoparticles [27]. In recent years it has been used in a variety of medical applications ranging from wound dressings to urinary catheters [28]. Therefore, there is a necessity to develop micro- and nano bio-based delivery systems for various applications [29].

Figure 10. Antibacterial activity of silver nanoparticles against MDR *strains.*

4. CONCLUSIONS

The silver nanoparticles were biosynthesized using sodium alginate extract of *Padina gymnospora.* Further, the above silver nanoparticle revealed to possess an effective antibacterial property against multi-drug resistance human pathogens such as *Staphylococcus aureus* and *Pseudomonas aeruginosa.* The present studies have shown that there is a small range of nanoparticle sizes and shapes that allow optimal antibacterial activity. The antibacterial efficacy of silver nanoparticles is dependent upon particle size and shape, as well as on surface properties. The present study emphasizes the medicinal importance of seaweeds for the synthesis of silver nanoparticles with antibacterial activity.

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