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BIOSYNTHESIS OF SILVER NANOPARTICLES USING FRESHWATER GREEN ALGA AND THEIR CHARACTERIZATION

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Abstract:

Researchers have been driven to create biologically active nanoparticles as a result of the expansion of infectious diseases and the rise in treatment resistance among bacteria. In the field of nanobiotechnology, progress is being made daily in developing an environmentally friendly method for creating nanoparticles. The present paper on green synthesis of silver nanoparticles and their uses are important because of their antimicrobial, antifungal, anticancer, and wound healing activities. Algal-synthesized AgNPs are increasingly being used in biomedicine. Targeted drug delivery techniques employ silver nanoparticles. Nanoparticle production is one of the many uses for nanoparticles and is a fascinating study topic. Due to its numerous applications across numerous industries, green synthesis of nanoparticles is a growing field. Several techniques, including physical, chemical, and biological ones, are used to create silver nanoparticles. The synthesis of silver nanoparticles is done by Green filamentous algae, *Rhizoclonium* sp. Aqueous Algal biomass extract (5ml) was added to 100 ml of 0.01M of aqueous silver nitrate at room temperature, the reaction will occur and the silver nanoparticles were formed and its characterization was fulfilled by the UV-visible spectrophotometer. UV-visible absorption spectrum revealed that the formation of silver nanoparticles (AgNPs) was increased by the addition of *Rhizoclonium* sp. alga and the spectral peak observed between a wavelength of 400 nm and 500 nm. The synthesized silver nanoparticles show the size and shapes of the nanoparticles that are made. The production of silver nanoparticles through algal biosynthesis is quick, simple, non-toxic, and beneficial to the environment. The characterization of silver nanoparticles was carried out by using UV-Vis Spectroscopy and SEM.

Keywords: Biosynthesis, Green algae, Silver Nitrate, Nanoparticles, UV-Visible Spectroscopy, SEM

Introduction:

In addition to architecturally exhibiting much better physical, chemical, and biological properties, features, and functionality during the past few decades, nanoparticles at their nanometric scale (1-100 nm) have attracted tremendous interest [Arya et al. 2018; Singh, et al., 2023; Singh, et al., 2023]. Nanomaterials have distinctive qualities that are not present in traditional macroscopic materials, making them stand out as having superior, unique, and important qualities. Its increased surface-to-volume ratio is specifically what gives them their distinctiveness [Thangaraju N, 2012; Sahayaraj K, 2012]. They symbolize the role of atomic-scale matter control and manipulation in the creation of innovative devices with potential utility in a range of physical,

biological, biomedical, and pharmacological applications [Anjum S, 2016]. Nanophasic and nanostructured materials have demonstrated a high potential for achieving specialized processes and selectivity in recent years of study, notably in biological and pharmaceutical applications, which have attracted a significant deal of interest [Kannan RRR, 2013].

The ability of silver to exert a substantial potential for a variety of biological applications, including preventing infections, healing wounds, and acting as an anti-inflammatory even at very low concentrations, has led to the description of silver as being oligodynamic. Due to their antibacterial properties, silver ions (Ag+) are used in the manufacture of dental resin composites, bone cement, ion exchange fibers, and coatings for medical equipment [Elechiguerra JL, 2005]. Recent research has demonstrated that silver nanoparticles strongly inhibit and operate as a barrier against the majority of microorganisms, including viruses, fungi, gram-positive and gram-negative bacteria, drug-resistant bacteria, and even some pathogens [Sahayaraj K, 2012; Bhuyar, P. et al., 2020].

According to studies, silver nanoparticles made from algae can be produced cheaply and without utilizing harmful chemicals, making them both cost-effective and environmentally benign [Abdel-Raouf N, 2018].

The goal of the current study is to biosynthesize silver nanoparticles utilizing an aqueous extract of the green alga *Rhizoclonium* sp.

2. Materials:

Algal sample – *Rhizoclonium* sp. Were collected from Ishwar Sharan Degree college Prayagraj, Microscope – True Vision BLISCO India; Centrifuge machine -Remi R-4C, Centrifuge tube, Water bath – impact ICON INSTRUMENTS COMPANY; MODEL NO. 11C104C- digital; purchased from- Z-67, Okhla industrial area, phase -II, New Delhi-110020, Conical flask -100ml, 250ml; Borosil company, Thermometer, Pestle Mortar, Double Distilled Water, Sieve Plate, Butter Paper, Aluminum Foil, Pipette 2ml- Borosil company, Weighing machine, 0.01M $AgNO_3$ solution – LOBA CHEMIEPVT. Ltd. Company, Magnetic Stirrer - LABQUEST Borosil company, Spectrophotometer – SYSTRONICS AU- 2701 UV- Vis DOUBLE BEAM SPECTROPHOTOMETER, Cuvette, Refrigerated Centrifuge (8000rpm, 30min,30degree)- M-Labs company, Eppendorf tube 2ml (12in no.) -, Micropipette (20-200µl) – LABQUEST Borosil company, Beaker 500ml - Borosil company, Measuring cylinder (10ml) – Borosil company; purchased from – 1101, Crescenzo, G-Block, Opp. MCA Club, Bandra (E), Mumbai – 400051, Maharashtra, India, EPMA – JEOL JXA-8100 Electron Probe Micro Analyzer, Gloves were used.

3. Methods:

3.1 Collection of alga sample:

The green alga was collected by handpicking method as depicted from Ishwar Sharan Degree College Prayagraj and Phaphamau Prayagraj area, India. The green alga samples which were collected in large amounts were repeatedly surface-sterilized using sterile seawater followed by distilled water to get rid of extraneous materials such as sand, dust, and salt content as well.

A B

Fig. 1 A and **B** are the samples of Alga *Rhizoclonium* sp.

3.2 Identification of green alga:

Identification of algae is done by microscopic Blisco India trinoculor (True Vision) study.

3.3 Preparation of aqueous green alga extract:

The algal sample was crushed and milled into a powder form and then the powder sample was weighed by the weighing machine. The 5 gm of dry weight of algal biomass was added into the 100 ml of DDW (double Distilled Water). The sample was kept in the water bath (– impact ICON INSTRUMENTS COMPANY; MODEL NO. 11C104C- digital) at 100º C for 15 minutes. After this, the mixture was allowed to cool at room temperature, and then the mixture was centrifuged at 5000 rpm for 30 minutes. After centrifugation the pellet was discarded and the yellow-green color supernatant was kept, and the algal extract was prepared.

Fig. 2 Green algal Extract

3.4 Synthesis of silver nanoparticles (AgNPs):

In the typical synthesis of silver nanoparticles, 5 ml of the aqueous extract of *Rhizoclonium* sp. was added to 100 ml of 0.01 M of aqueous silver nitrate (AgNO3, LOBA CHEMIEPVT. Ltd. Company) solution which was prepared using deionized water in 250 ml conical flask. The synthesis medium was gradually and indirectly heated at 60ºC temperature and stirred using a magnetic stirrer for 48 hours. at 300 rpm to ensure a complete reduction of metal ions to occur [Kumar P, 2013; Suriya, J. et al., 2012]. The synthesis medium was carried out in triplicate and the setup of the experiment was done. Besides that, the setup was carried out in dark conditions to minimize the photoactivation of silver nitrate. A control setup was also maintained without the aqueous green alga extract. The change in color from yellowish-green to a concentrated dark red color solution was observed as a visible confirmation of the formation of silver nanoparticles before the sample was subjected to further characterization process.

Fig 3A-C Algal biomass, **D** algal extract, **E -G** formation of nanoparticle

After 48 h completed, the synthesis medium was centrifuged at 8000 rpm at 30 °C for 30 min. The silver nanoparticles which deposited at the bottom formed a pellet upon centrifugation and the supernatant was discarded. And the pellets were collected.

Fig. 4: Formation of Silver Nanoparticles

3.5 Characterization of silver nanoparticles synthesized:

The UV-Vis spectrophotometer (SYSTRONICS AU- 2701 UV- Vis DOUBLE BEAM SPECTROPHOTOMETER) was used to measure the wavelength between 300 and 700 nm to monitor the formation of silver nanoparticles using marine alga extract with regular intervals of 0 min (before heating), 15 min, 30 min, 1 h, 24 h and then followed by 48 h. Dilutions of samples were made if the sample was too concentrated. The UV-visible reading was recorded and then analyzed using the Origin Pro or Microsoft Excel analysis tool. The JEOL JXA-8100 Electron Probe Micro Analyzer to measure the nanoparticles' size and shape. A tiny drop of nanoparticle powder was applied on a copper grid that had been coated with carbon before being transported to the microscope. The morphology of the silver nanoparticles was further studied, and high-resolution photographs of the silver nanoparticles were captured.

4. Result:

4.1 Characterization of silver nanoparticles:

The synthesis of AgNPs was performed with 0.01 M of silver nitrate solution with green alga extract in Erlenmeyer flask in the ratio of 1:10, respectively, at the initial time point of the synthesis reaction, and 0.01 M of silver nitrate solution without green alga extract was maintained as a control. The solution remained colorless and showed no color change even after 48 h upon heating as shown in Fig. 5a. The reduction of silver nitrate (AgNO3) was visually confirmed by the change of color from yellowish-green to reddish-brown after 30 min of reaction as shown in Fig. 5b. The color of the green alga extract became turbid after the addition of aqueous AgNO3 solution signifying the initiation of the reaction. The intensity of brown color increased in direct proportion to the heating period of 2 h followed by 18 h as shown in Fig. 5c.

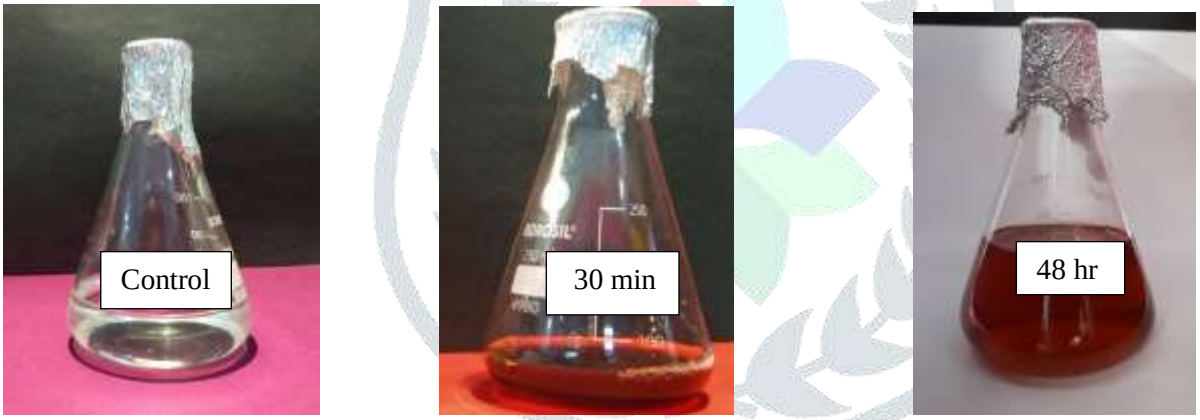


Fig.5 a0.01M of silver Nitrate solution **b** silver nanoparticles synthesis at 30 min of heating **c** silver nanoparticles synthesis at 18 hr of heating

4.2 Ultraviolet-visible spectroscopy (UV-Vis) analysis:

The characterization of silver nanoparticles based on surface plasmon resonance (SPR) vibration observed at 445 nm confirmed the synthesis of AgNPs using marine alga extract. The silver SPR band that occurred at 400 nm to 445 nm showed a steady increase in absorbance until 18 h as shown in Fig. 6. The broadening of peak signified that the particles are polydispersed. Furthermore, the peak intensity increased as the samples were treated longer.

4.3 Scanning electron microscopy analysis of silver nanoparticles:

The SEM image in Fig. 7 showing the high-density silver nanoparticles synthesized by treating *Rhizoclonium* sp. extract further confirmed the development of silver nanostructures. The silver nanoparticles seem to be distributed with an average mean size of 90-100 nm.

Table 1: UV-Vis absorption spectrum of silver nanoparticles synthesized from *Rhizoclonium* extract treated with 0.01M silver nitrate during different time intervals

Wavelength	0 hr	15 min	30 min	1 hr	24 hr	48 hr	Control
300	0.957	1.168	1.1629	1.725	1.026	1.075	-0.222
400	0.991	1.168	1.64	1.68	0.45	0.674	-0.174
500	0.995	1.171	1.624	0.967	0.219	0.487	-0.176
600	0.992	1.174	1.636	0.873	0.075	0.142	-0.178
700	0.976	1.172	1.597	0.867	0.036	0.051	-0.178

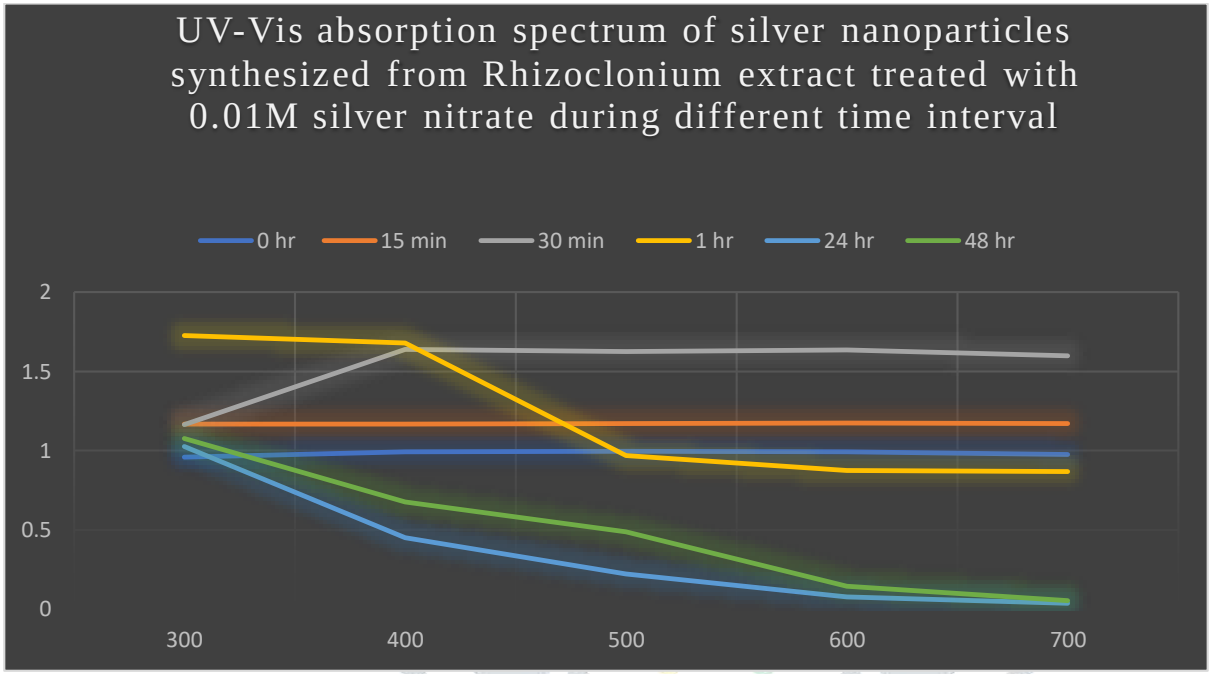
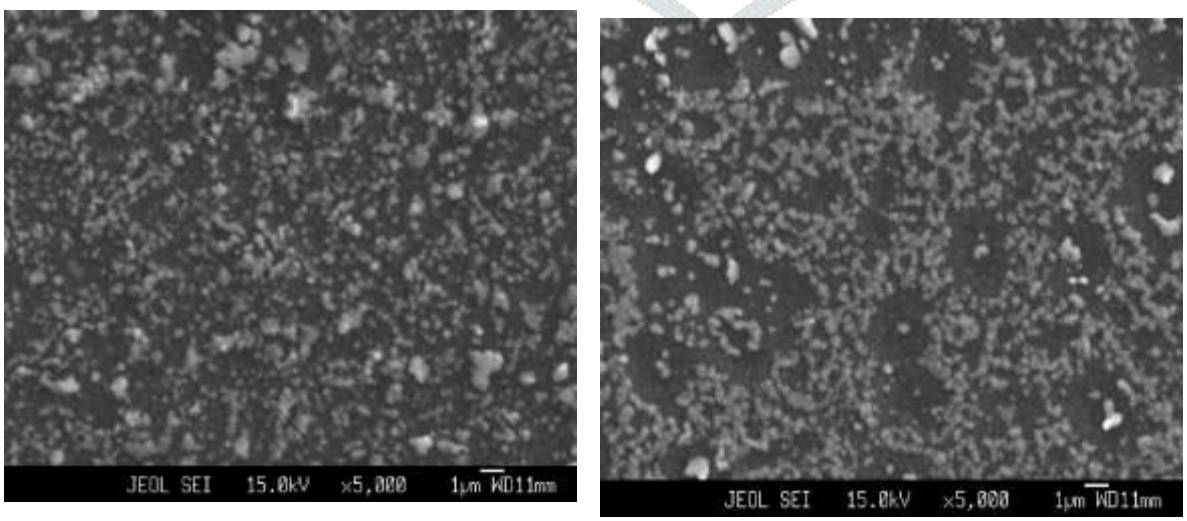


Fig. 6: UV visible absorption spectrum of silver nanoparticles



A **B**

Fig 7: A- B EPMA images of silver nanoparticles (AgNPs) synthesized by the reaction of *Rhizoclonium* sp. extract with 0.01 M of silver nitrate (AgNO3) solution

5. Discussion

5.1 Characterization of silver nanoparticles:

The result indicated the formation of AgNPs by reduction of the aqueous Ag^+ during the exposure to the aqueous extract of *Rhizoclonium* sp. showed a reddish-brown color upon 48 h of incubation period. It is well known that AgNPs exhibit reddish-brown in water [Patil V. et al1997] due to the excitation of surface plasmon resonance (SPR) effect of silver metal nanoparticles. It indicates that silver nanoparticles were formed by the reduction of Ag^+ into Ag^0 upon the addition of green alga extract to the solution of 0.01 M of AgNO3 solution [Suriya, J. et al., 2012]. Particularly, even after 48 h, there is no remarkable deepening of color indicating the saturation of the reaction. This indicates the particles might be well dispersed throughout the synthesis medium with mild agglomeration [Nabikhan A 2010].

5.2 Ultraviolet-visible spectroscopy (UV-Vis) analysis:

The frequency and width of the surface plasmon absorption depend on the size and shape of the metal nanoparticles as well as on the dielectric constant of the metal itself and the surrounding medium [Mukherjee P et al (2001)]. Similar SPR vibrations have been observed in the previous report using marine alga *Urospora* sp. for the biosynthesis of AgNPs [Suriya, J. et al., 2012].

5.2 Scanning electron microscopy analysis of silver nanoparticles:

The SEM micrographs of nanoparticles showed that the nanoparticles synthesized were polydispersed spherical shaped, and highly distributed with aggregation [Puchalski M 2007].

6. Conclusion:

In summary, silver nanoparticles produced using *Rhizoclonium* sp. alga extract were stable; as a result, this unique green synthesis technique may serve as a substitute for conventional chemical synthesis processes since it is cheap, efficient, and environmentally friendly. After 30 minutes of reaction, the color changed from yellowish-green to reddish, providing visible evidence of the reduction of silver nitrate (AgNO3). SEM and a UV-visible spectrophotometer were used to characterize the silver nanoparticles. The creation of silver nanoparticles was confirmed by the silver SPR band, which was present between 400 and 445 nm, and continuously grew in absorbance over time until 48 h without any movement in peak position (wavelength).

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