International Journal of Science and Research (IJSR)
ISSN (Online): 2319-7064
Index Copernicus Value (2013): 6.14 | Impact Factor (2013): 4.438

Eco-Friendly Synthesis and Characterization of Silver Nanoparticles Using Marine Macroalga *Padina Tetrastromatica*

K. F. Princy[1], Anu Gopinath[2]

[1]Research Scholar, Department of Chemistry, St. Albert's College, Ernakulam, Kerala, India
[2]Assistant Professor in Chemical Oceanography, Kerala University of Fisheries and Ocean Studies, Cochin, India

*Corresponding Author

Dr. Anu Gopinath
+919446838319
anugowri@hotmail.com

Abstract: ***Green synthesis of noble metal nanoparticles is a developing area of current nanotechnology research .In this paper we report the eco-friendly biosynthesis of Silver nanoparticles (AgNPs) using the aqueous extract of brown seaweed Padina Tetrastromatica as reducing agent and stabilising agent.. The reduction of silver ions occurred when silver nitrate solution was treated with aqueous extract of seaweed at 60 ˚C. The AgNPs obtained were characterized by UV-Visible Spectroscopy, HR-TEM, SAED, EDAX, XRD and FTIR techniques. The formation of silver nanoparticles is confirmed by the appearance of signatory brown colour of the solution and a characteristic peak at 424 nm in the UV–Vis spectra. The high crystallinity of nanoparticles is evident from bright circular spots in the SAED pattern and peaks in the XRD pattern. TEM images revealed that the synthesised nanoparticles are spherical withsize in the range 5–35 nm.FTIR spectrum indicates the presence of different functional groups in capping the nanoparticles.***

Keywords: Eco-friendly synthesis, Silver Nanoparticles, *Padina Tetrastromatica*, Characterization

1. Introduction

Nanotechnology is emerging as a rapidly growing field in modern researchwith its application in science and technology. It involves the synthesis of nanoparticles of size ranging from 1 to 100 nm [1] .Nanoparticles have attracted considerable attraction due to their unusual and fascinating properties, with various applications, over their bulk counterparts [2].Nanoparticles can be synthesised through different methods. Chemical method of nanoparticle synthesis is the most popular one but this will leads to the presence of some toxic chemical species, adsorbed on the particle surface that may have adverse effects in its applications. Sothere is a growing need to develop environmentally friendly method for the synthesis of metal nanoparticles that do not use toxic chemicals in the synthesis protocols [3]. Biological methodsof nanoparticle synthesis using microorganisms or plant extracts can potentiallyeliminate this problem by making the nanoparticles more biocompatible [4, 5].

Compared to microbe assisted synthesis, plant- mediated synthesis of nanoparticles isinnovative, cheaper, and environmentally benign process by eliminating the elaborate process of maintaining cell cultures [6]. Metal nanoparticles have been successfully synthesized using several terrestrialplants [7-11]; however utilization of renewable marine resources such as marine macroalgae in the synthesis is yet to be pursued.The biologically diverse marine environment has a great promise for nanoscience and nanotechnology. The utilization of marine macroalgae in the synthesis of nanoparticles is quite novel leading to truly green chemistry which provide advancement over chemical and physical method as it is cost effective and environment friendly and easily scaled up for large scale synthesis.

Among noble metal nanoparticles, silver nanoparticles (AgNPs) have received considerable attention due to their attractive physicochemical properties [12].They have been extensively used as antibacterial water filters [14],in metal catalysis [15],as biosensors [16], as optical receptors for biolabelling [17],in antibacterial activity [18], label-free colorimetric assays to detect enzymatic reactions [19], in anti-HIVactivity [12] and in controlling plant pathogens [20].

Hence the present study aims to establish a novel environmentally safe method for preparation of silver nanoparticles using aqueous extract of brown seaweed *Padina Tetrastromatica*. The seaweed extract has a dual effect as it act as reducing agents of silver ions and as stabilizing agents for the formed silver nanoparticles.

2. Materials and Methods

2.1 Sample Collection:
The seaweed *Padina Tetrastromatica* [Figure.1] was collected from the coast of Thirumullavaram ($8^0$53'38''N ; $76^0$33'14''E), Kollam district of Kerala. To maintain the freshness, the seaweed samples were instantly kept in a polythene bag with natural seawater.

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Figure 1: *Padina Tetrastromatica*

2.2 Preparation of Seaweeds Extract:

The samples were washed thoroughly with Milli Q water to remove extraneous materials and 10 g of the washed seaweed was finely cut into small pieces and stirred with 100mL sterile Milli Q water for 1min and kept in a water bath at 60^{o} C for 20 minutes. Finally, the extract was filtered with Whatman No. 1 filter paper. The filtrate is used as reducing agent and stabilizer.

2.3 Biosynthesis of AgNPs

For the biosynthesis of Ag nanoparticles, 50 ml seaweed extract was mixed with 50 ml of 1 mM $AgNO_3$ solution, stirred well for 1 minute, kept in a water bath at 60^{o} C for 1 hour and then incubated in dark at room temperature under static condition. A control setup was also maintained without seaweed extract. The bioreduction of $AgNO_3$ into AgNPs can be confirmed visually by the change in colour from yellow to brown.

2.4 Characterization techniques

The bioreduction of $AgNO_3$ was confirmed by sampling the reaction mixture at regular intervals and the absorption maximum was scanned by UV–Vis spectra, in a range of wavelengths between 300 and 700 nm (Thermo scientific Evolution 201 Spectrophotometer). After 48 h of reaction, the bioreduced reaction mixture was subjected to centrifugation at 15,000 rpm for 20 min at 4 °C. The resulting pellet was redispersed in sterile Milli Q water and freeze dried. The process of centrifugation and redispersion was repeated thrice to obtain better separation of AgNPs. The freeze driedAgNPs were examined for the presence of biomolecules responsible for bioreduction using FTIR Spectrometer (Perkin Elmer Spectrum 100) at the wavelength range of 4000-550cm^{-1}. Crystalline nature of the purified silver nanoparticles was analysed by powder XRD (XPERT-PRO) operated at a voltage of 40 kV and a current of 30 mA with CuKα radiation (λ= 0.1542 nm).HRTEM images, EDAX pattern and SAED patterns have been obtainedwith a Tecnai G^2 30 transmission electron microscope.

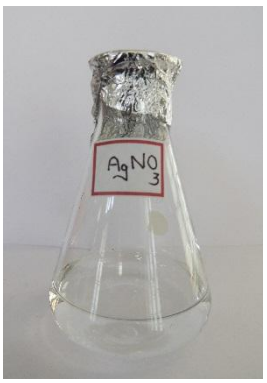
3. Results and Discussion

3.1 Visual and UV-Vis Spectrophotometer Analysis

The formation of AgNPs was visually evident from the colour changeof reaction mixture from light yellow to dark brown [Figure.2]The color change was observed within 15 min indicating the formation of AgNPs. Intensity of brown colour increased in direct proportion to the incubation period. It may be due to the excitation of surface Plasmon resonance (SPR) effectarising due to the collective oscillation of free conduction electrons induced by an interacting electromagnetic field and reduction of $AgNO_3$[21].The control $AgNO_3$ solution (without seaweed extract) showed no colour change.



(a)(b)



(c)

Figure 2: Colour intensity of the *Padina Tetrastromatica* extract incubated with Silver ions (a) at the beginning of reaction, (b) after 48 hours, (c) control 1 mM$AgNO_3$

UV–visible spectroscopy is an important technique to ascertain the formation of metal nanoparticles.A sharp intense peak at 424 nm in the UV–Visible spectra [Figure 3]confirmed the formation of AgNPs.Similarly Kannan et al, 2012 [22] reported that the SPR band was located at 422 nm for silver nanoparticles synthesised using green algae *Chaetomorpha linum.*

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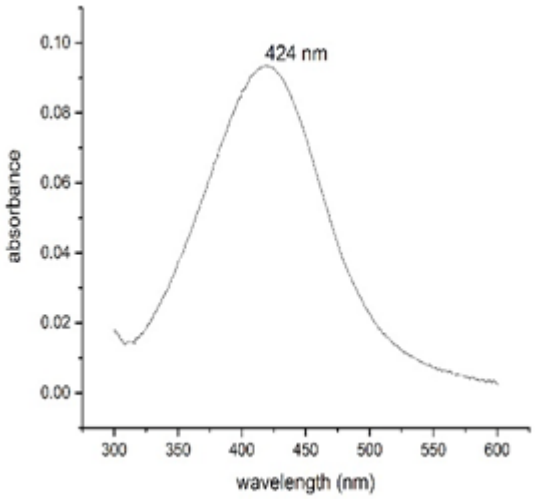
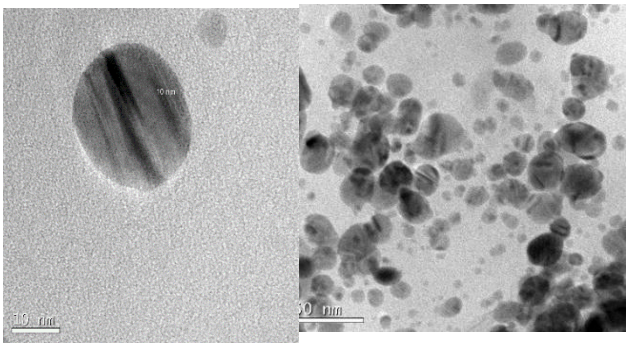


Figure 3: UV-Visible Spectrum of AgNPs formed using *Padina Tetrastromatica*

3.2 Transmission Electron Microscopy (TEM)

TEM has been used to identify the size, shape and morphology of nanoparticles. HR-TEM image at different magnification [Figure.4 (a-b)] revealed that the nanoparticles AgNPs were nearly spherical in shape with a size range of 5-35 nm. Further, no aggregation of silver nanoparticles particles is observed in the images. Kumar et al, 2012, reported AgNPs of analogous structure synthesized from *Acanthophora spicifera*. This may be due to the presence of similar reducing agents like polyphenols with free O-H groups. The bright circular spots in the selected area electron diffraction (SAED) patterns revealed that the particles are crystalline in nature [Figure.5]. The histogram [Figure.6] obtained using TEM image revealed that majority of particles were in the range of 15-20 nm in diameter.



(a) **(b)**

Figure 4 (a-b): TEM images of AgNPs at different magnifications

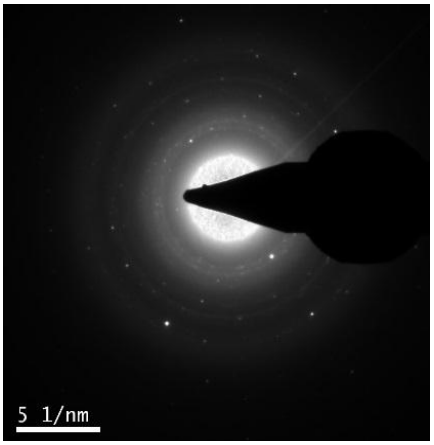


Figure 5: SAED pattern of AgNPs

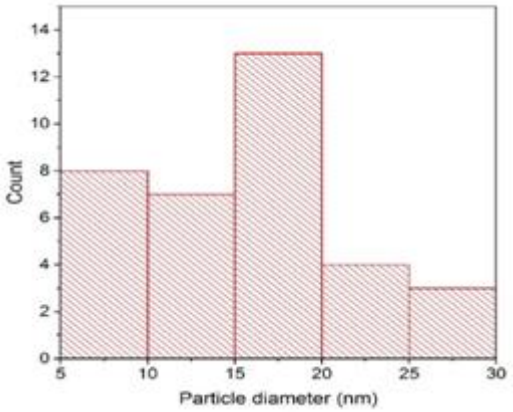


Figure 6: Particle size histogram of AgNPs

3.3 EDXanalysis of Silver Nanoparticle

The elemental analysis of the silver particle was performed using the EDX on the TEM.The presence of an optical absorption band at 3 KeV confirmed the presence of pure metallic silver nanoparticles [Figure: 7].The carbon and the copper peaks correspond to the holding grid which is a carbon-coated copper grid.

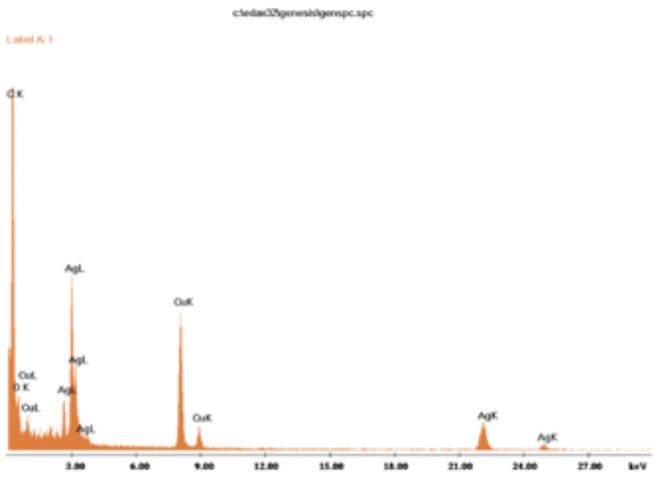


Figure 7: EDX spectrum of synthesized AgNPs

3.4 XRD analysis of silver nanoparticle

The crystalline nature of AgNPs was further confirmed from X-ray diffraction (XRD) analysis. The XRD spectrum [Figure.8] gave characteristic peaks at 2θ values of 38.26°,

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44.33°, 64.69° and 77.58° which can be indexed to the (1 1 1), (2 0 0), (2 2 0) and (3 1 1) reflections of fcc structure of metallic silver, respectively revealing that the synthesized silver nanoparticles are composed of pure crystalline silver (JCPDS file no. 04-0783).The average crystalline size of silver nanoparticles can be calculated using Debye–Scherrer equation:

$D=k \lambda /\beta \cos\theta$

where K is the Scherrer constant and its value is .094, λ is the wavelength of the X-ray, β is the full width at half maximum and θ is the Bragg angle. From the Scherrer equation the average crystalline size of silver nanoparticles is found to be 16 nm which is in agreement with the TEM results.

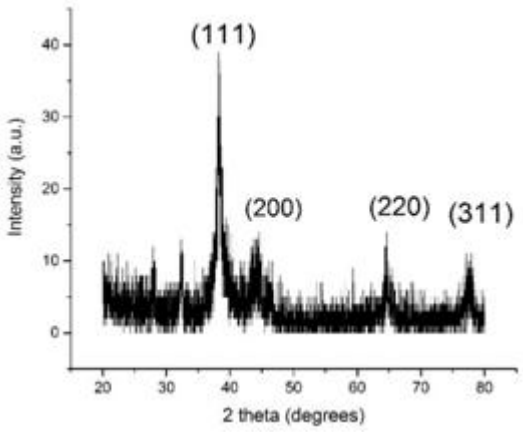


Figure 8: XRD pattern of AgNPs

3.5 FTIR Analysis of Silver Nanoparticle

FTIR measurements were carried out to identify the possible biomolecules in Padina Tetrastromatica extract which are responsible for capping leading to efficient stabilization of the AgNPs. The IR spectrum (Figure. 9) of AgNPs synthesised using *Padina Tetrastromatica* manifests prominent absorption bands located at 3211, 2922,2095,1627, 1535, 1333, and 1032cm^{-1}. The broad spectrum at 3211 cm^{-1} shows the O-H bond stretching of alcohols and phenols. The very strong absorption band at 1627 cm^{-1} corresponds to the amide I band and that at 1535 cm^{-1} corresponds to the amide II band. These bands arises due to the carbonyl stretching vibrations in the amide linkages of the proteins [24]. The band located at 1333cm^{-1} arises due to the C–N stretching or the O–H bending vibrations .The absorption peak at 1032 cm^{-1} illustrates the presence of carboxylic acid functional group. It is well-known that proteins can bind to AgNPs through free carboxylate group [26]. The presence of bands at 1627, 1333 and 1032 cm^{-1} indicates that AgNPs are possibly bound to proteins through carboxylate group.IR spectroscopic study confirmed that the *Padina Tetrastromatica* extract has the ability to perform dual functions of reduction and stabilization of silver nanoparticles.

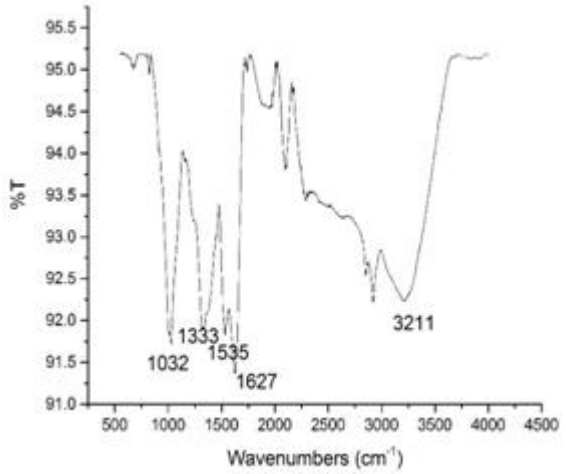


Figure 9: FTIR spectrum of AgNPs

4. Conclusions

Through the study we have confirmed that the aqueous extract of seaweed *Padina Tetrastromatica* is capable of producing AgNPs by reducing $AgNO_3$ solution and is proved to be an efficient, eco-friendly and simple method. We have characterized the synthesised AgNPs using several techniques. The characteristic absorption peak at 424 nm in UV-Visible spectrum confirmed the formation of AgNPs. Crystalline nature of AgNPs is evident from the characteristic peaks in the XRD pattern, bright circular spots in the SAED pattern and clear lattice fringes in the HRTEM images. TEM images revealed that the synthesised nanoparticles are nearly spherical with size in the range 5–35 nm. The characteristic peaks in the FTIR spectrum revealed the presence of functional bio active metabolites in seaweed extractwhich is responsible for the formation of AgNPs. Seaweeds are cost – effective, renewable marine resources. Their abundance and ease of availability also make them good green reagents for the green synthesis of novel silver nanoparticles.

Acknowledgments

The authors are pleased to acknowledge STIC, KOCHI, School of Pure and Applied Physics, M.G University and Marine Biotechnology Centre, Chemical Oceanography Department, CUSAT for characterization studies.

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Author Profile

Dr. Anu Gopinath, Assistant Professor in Chemical Oceanography, Kerala University of Fisheries and Ocean Studies, Cochin, India

Awards / Recognitions

- Dr. Anu Gopinath – selected from India to participate in UNESCO/IOC/WESTPAC training programme in Sanya Island China from 8-11 June, 2011. Topic – Water Pollution Monitoring In Coral Reefs.
- Commonwealth Academic Staff Fellowship in UK for the year 2012. National Oceanography Centre, Southampton, UK. 1.10.2012 – 26.12.2012
- Department of Science and Technology, GOVT of India - Fast Track Scheme for Young Scientists – 2012 – Biogeochemical factors and Ecosystem Dynamics of Coral Reefs of Lakshadweep Archipelago.
- Department of Science and Technology, GOVT of India Young Women Scientists' Fellowship-2006.
- ISAS Award for Outstanding Contribution in Teaching and Research – 2014.
- Participated in Indian Arctic Expedition -2014.

Current Research Interests:

- Green synthesis of nanoparticles from marine resources
- Studies related to ocean acidification – Dissolved Inorganic Carbon and Alkalinity Measurements of Ocean focusing on Lakshadweep Archipelago.
- Biogeochemical factors and Ecosystem Dynamics of Coral Reefs of Lakshadweep Archipelago, Indian Ocean.
- Trace metal and nutrient biogeochemistry of Oceans – focusing on Coral Reefs of Lakshadweep Archipelago.
- Biogeochemistry of Arctic Fjords.

Publications - Research Papers – International 21
Text books – 03.
Research projects - Major -03.
Number of ongoing PhD Programmes – 07

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