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Synthesis of silver nanoparticles using Phytoplankton and its characteristics

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Abstract

Green synthesis of silver nanoparticles (SNPs) using microalgae is environmentally benign and emerging technique. In the present investigation, the extracts of green microalgae, *Chlorella vulgaris* and the diatom, *Chaetoceros calcitrans* were examined for synthesis of SNPs. The absorbance spectrum of aqueous medium containing SNPs showed a peak at 436 and 420 nm by *C. vulgaris* and *C. calcitrans* respectively. The shape and size of SNPs were studied by Scanning Electron Microscope (SEM). The spherical shaped SNPs were observed and their size was ranged from 50-70 nm and 30-35 nm from the extracts of *C. vulgaris* and *C. calcitrans* respectively. To identify the possible biomolecule responsible for the reduction of Ag⁺ ions to SNPs, the Fourier Transform Infrared Resonance (FTIR) measurements were investigated that the functional groups like Amines, Phenols and Alcohols, Ethers and Aromatic rings were found to be responsible for the reduction of silver ion to SNPs. From the results of the present study showed that the green microalgae and diatom could be employed for the green synthesis of nanoparticles and also suggested that the algal biomass can be produced using wastewater in order to treat various wastewaters.

Keywords: Green synthesis, Silver nanoparticles, *Chlorella vulgaris*, *Chaetoceros calcitrans*

1. Introduction

Silver nanoparticles (SNPs) have been known to be used for numerous physical, biological and pharmaceutical applications. They have diverse properties and uses like magnetic and optical polarizability, electrical use due to the highest electrical and thermal conductivity among metals, catalysis, surface enhanced Raman scattering (SERS), textile engineering, biotechnology and bioengineering, water treatment, electronics, antibacterial/antifungal agents in a diverse range of consumer products etc [1-5]. Due to the fluorescence and surface Plasmon resonance characteristics of SNPs, it is used in DNA sequences, mass spectrometry of peptides, colorimetric determination of histidine and ammonia [6]. In biochemistry, they are considered to be a better catalyst [7]. And good biological and chemical sensors [8-9].

Though the NPs have synthesized by chemical, electrical methods, the biosynthetic methods have many advantages and eco-friendly. Green synthesis of metal nanoparticles (NPs) using microalgae and plants is an emerging science of the intersection of nanotechnology and biotechnology has received greater attention due to increasing need to develop environmentally benign technologies in nanomaterial production [10-12]. Biosynthesized nanoparticles has great significance due to their unusual optical [13]. Chemical, photo electrochemical and electronic properties [13-16]. The synthesis of highly stable gold, silver, platinum, selenium, titanium and other metal NPs have been successfully synthesized by microorganism such as actinomycetes, bacteria, fungi, yeast, algae and plants [17-20]. Green synthesis of SNPs by algae and algal extract shows more advantageous over other biological processes are Bacteria and fungi, because it eliminates the cell culture, maintaining process, and also it is more suitable for large scale production of SNPs [21]. Due to its abundance and ready availability, marine algae are good and cost-effective sources of phytochemicals that can be exploited for the synthesis of metallic nanoparticles. However the marine seaweeds were studied extensively for the synthesis of NPs [22-23]. The reports on microalgae especially marine diatoms are largely restricted. Hence the present study, the marine green microalgae, *Chlorella vulgaris* and diatom, *Chaetoceros calcitrans* was examined for its potential for synthesis of SNPs. The characteristics of synthesized NPs were also studied by UV-Vis Spectrophotometer, FTIR and SEM.

2. Materials and Methods

2.1. Algal Strain and Extract

Marine green algae, *Chlorella vulgaris* and diatom, *Chaetoceros calcitrans* were isolated from Vellar estuary (11.4900° N, 79.7600° E), Parangipettai, India and their stock cultures were maintained in Conway and F/2 media respectively. The mass cultures were performed with 40 L of culture media in 50L glass aquarium tank under 24 °C temperature, 30 salinity and 4500 ± 500 Lux light intensity with 16 ± 8 Light and Dark Photoperiod for seven days. The biomass was collected by centrifugation at 6000 rpm for 10 min under 4 °C and lyophilized. The dried algal biomass was powdered using a pestle and mortar. For the preparation of algal extracts, 10 mg of algal powders were suspended in 100 ml of double distilled water and filtered using Whatman No.1 filter paper. The filtrates were used for the synthesis of SNPs.

2.2. Synthesis and characterisation

The reduction reaction of silver nitrate was started by mixing of 10 ml 10 mM AgNO₃ solution with 100 ml of algal extract. The reaction mixtures were heated at 60 °C in hot air oven for one hour. Absorption of reaction mixtures were carried out using UNICAM UV 300 Spectrophotometer at a resolution of 1 nm between 300 and 800 nm. For FTIR analysis, the reaction mixtures were centrifuged at 8000 rpm for 5 min., pellets were washed with double distilled water and centrifuged again. These pellets of algal colloid with SNPs were freeze-dried and powdered using a micro pestle. FTIR spectrums of the samples were recorded on a Shimadzu IR Affinity-1 model in the range of 500–4000 cm⁻¹ at a resolution of 4 cm⁻¹. To examine mean particle size and morphology of NPs, TESCAN, SEM machine was used. Briefly, the freeze dried sample of SNPs solution was sonicated with distilled water, thin films of the sample were prepared on a carbon coated copper grid by just dropping a very small amount of the sample on the grid, extra solution was removed using a blotting paper and then the film on the SEM grid were dried under a mercury lamp for 5 min and examined in SEM.

3. Results and Discussion

Synthesis of SNPs from silver nitrate is one of the most widely used methods for the synthesis of silver colloids. The color of the reaction mixtures were changed from yellowish green to dark brown after an hour of incubation at 60 °C (Fig. 1) during the green synthesis using the extracts of *C. vulgaris* and *C. calcitrans* due to surface plasmon resonance. This occurs due to the collective oscillation of the conduction electrons confined to metallic NPs [24-25]. The absorption of reaction mixture was observed between 350 and 600 nm by UV-Vis spectrometer has proven to be very useful for analyzing nanoparticles [24, 26]. As illustrated in Fig. 2 & 3, a strong surface plasmon resonance was centered at 436 and 420 nm by *C. vulgaris* and *C. calcitrans* respectively. Polydispersed NPs were observed to generate broad absorption peak [27]. This sturdy and wide surface Plasmon peak has also been well renowned for various metal NPs, with sizes ranging widely from 2 to 100 nm [28-31]. The variations in absorption peaks at 420 and 436 nm of *C. calcitrans* and *C. vulgaris* were due to the difference in the fraction of diameter of the agglomeration state of SNPs [32]. Similarly Rajesh Kumar *et al.* was demonstrated that the biogenic synthesis of silver nanoparticles using the extract of marine brown algae *Turbinaria conoides* on adding 1 mM of silver nitrate at room temperature and obtained a broad peak at 420 nm [33]. The role

of temperature in the synthesis of SNPs was explained that the agglomeration of the nanoparticles was observed at high temperature [34].

In order to study the size distribution and shape of synthesized SNPs, the SEM analysis was executed and Spherical shaped SNPs were observed as deposition on algal extract (Fig 4 & 5). Likewise, Rashmi *et al.* was demonstrated the deposited gold NPs on algal mycelium of *Phanerochaete chrysosporium* [35]. The size of SNPs was ranged from 50-70 nm and 30-35 nm from the extracts of *C. vulgaris* and *C. calcitrans*, respectively. The spherical nature of Ag nanoparticles is probably due to some capping or stabilizing agent bound on it [27]. Evenly, Vivek *et al.* has demonstrated the biological synthesis of silver nanoparticles by using the aqueous extract of the red seaweed *Gelidiella acerosa* as the reducing agent [27]. The synthesized nanoparticles were predominantly spherical in shape, poly dispersed and had an average mean size of 22 nm. The SEM image of SNPs in the present study, showed highly aggregated particles and the increased aggregation tendency of silver nanoparticles in algae, which could be attributed its relatively high cation concentration that could neutralize the negatively charges adsorbed on the surface of silver nanoparticles and reduce the electrostatic repulsion forces between nanoparticles [28].

Shivshankar *et al.* was reported that the formation of pure metallic nanoparticles and bimetallic nanoparticles by reduction of metal ions is possibly facilitated by reducing sugars and/or terpenoids present in the neem leaf broth [37]. Similarly Vivek *et al.* also reported the possible involvements of aromatic compound or alkane or amine has a capping ligand of silver nanoparticles [28]. FTIR analysis in the current study shows the involvement of functional groups like Amines, Phenols and Alcohols, Ethers and Aromatic rings in the reduction of silver ion to silver nanoparticles and stabilization of silver nanoparticles.

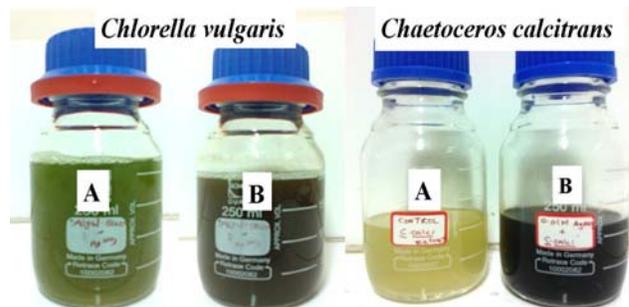


Fig 1: Extracts of *C. vulgaris* and *C. calcitrans* before (A) and after (B) exposure to AgNO₃

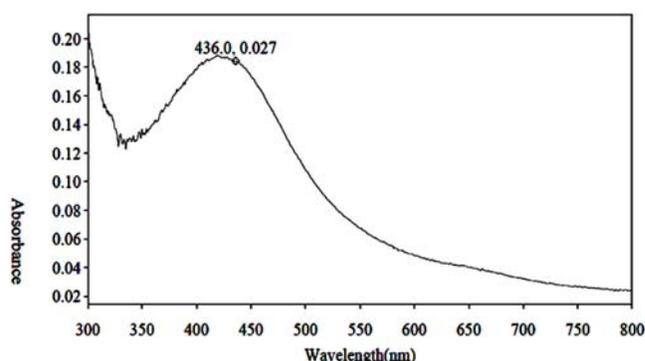


Fig 2: UV-Vis absorption spectra of silver nanoparticles by exposure of *C. vulgaris* extract with 0.01M AgNO₃ solution.

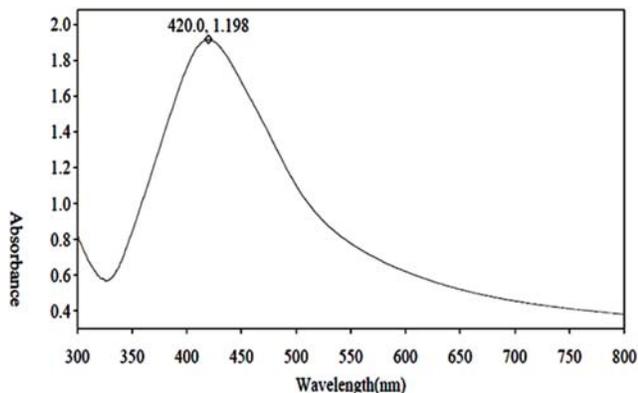


Fig 3: UV-Vis absorption spectra of silver nanoparticles by exposure of *C. calcitrans* extract with 0.01M $AgNO_3$ solution.

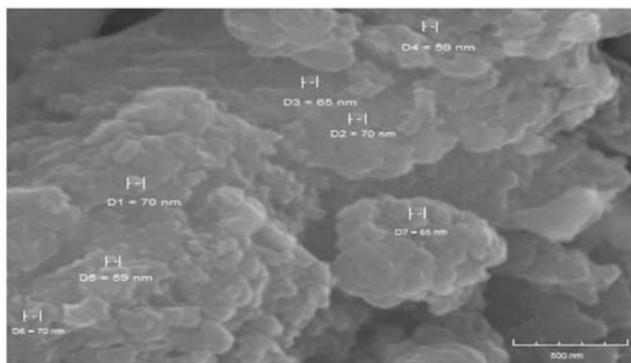


Fig 4: Scanning Electron Microscopy image of polydispersed AgNPs synthesized by *C. vulgaris* extract.

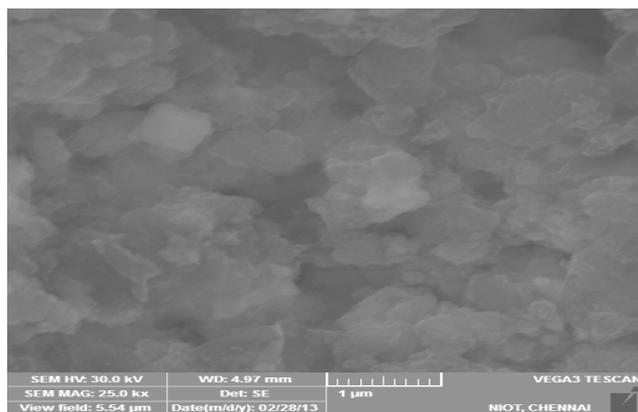
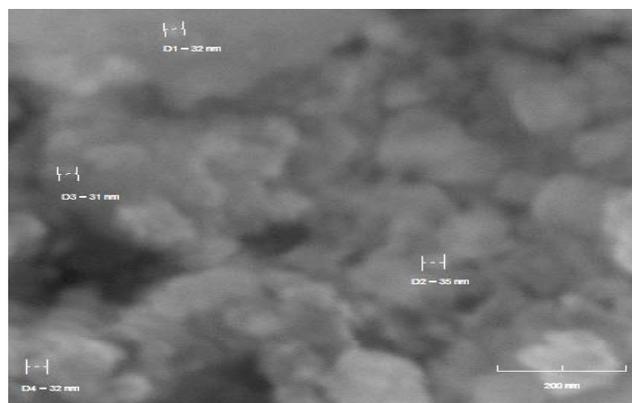


Fig 5: Scanning Electron Microscopy image of polydispersed AgNPs synthesized by *C. calcitrans* extract.

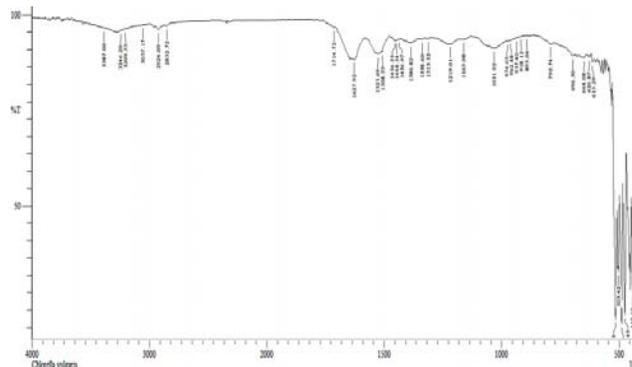


Fig 6: FTIR spectra of AgNPs of *C. vulgaris* extract

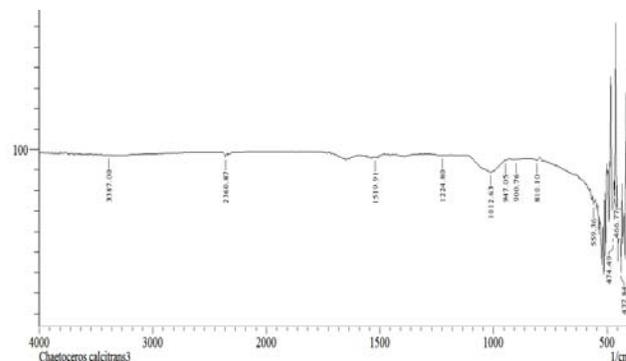


Fig 7: FTIR spectra of AgNPs of *C. calcitrans* extract

4. Conclusion

Synthesis of silver nanoparticles by the aqueous extract of *C. vulgaris* and *C. calcitrans* has been demonstrated while exposed with 0.01 M of silver nitrate in the reaction mixture. The absorbance spectrum and SEM analysis were confirmed the synthesis of SNPs. It is proved that the potential of diatom and green algal extracts for the green synthesis of nanoparticles. Also, it is suggested that the future studies are focused on green synthesis of NPs using the algal biomass produced by waste water treatment.

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