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PRELIMINARY STUDY ON GREEN SYNTHESIS OF SILVER NANOPARTICLES USING CELL-FREE MICROALGAL EXTRACT

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Research Paper

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ABSTRACT

Green synthesis of silver nanoparticles using microalgal suspension has been carried through bottom-up approach using silver nitrate as a precursor. Synthesis of Silver nanoparticles was observed by visual analysis followed by characterization using UV-Visible Spectroscopy. SPR spectra recorded were in range of 500-520 nm.However, comparatively sharp peaks at lower $AgNO_3$ concentration (0.1 mM) were obtained that could offer higher homogeneity whereas broad or flat peaks obtained at higher $AgNO_3$ concentration (0.5 mM and 1mM) indicates poly dispersed AgNPs. Thus, at preliminary level screening, lower silver nitrate concentration favours synthesis of silver nanoparticles.

No. of Pages: 9 **References: 28**

Keywords: Nanoparticles, Microalgal Suspension, Surface Plasmon Resonance and UV-Visible Spectroscopy.

INTRODUCTION

Nanotechnology plays an important role in design, synthesis and manipulation of nanoparticles, nanowires, and nanomaterials in the range between 1-100nm in dimension (Jain *et al.* 2009; Senthilkumar *et al.* 2015). Nanoparticle can be metallic, ceramic, polymeric, semiconductor, fullerenes and lipid based (Khan *et al.* 2017). Different metallic nanomaterials are being produced using copper, zinc, titanium, magnesium, gold, alginate and silver (Dubchak*et al.* 2010; Hasan. S, 2015). Materials developed in the nanoscale range were applied in different fields such as solar energy conversion, catalysts, medicine, and water treatment (Henglein, 1993).

There are various methods available for the synthesis of different types of nanoparticles by chemical, physical and biological means (Vanaja *et al.* 2013). According to Soleimani and Pirkoohi, (2017) these methods, though effective in producing metal nanoparticles suffer limitations due to environmental and health considerations (Edison *et al.* 2012). Therefore, biosynthesis of nanoparticles using microorganisms, enzymes, and plant extracts has emerged as a clean, cost-effective and efficient alternative to chemical methods (Ahmed *et al.* 2016). For the synthesis of AgNPs, biological methods are both economical and environmentally benign (Dhuper*et al.* 2012). Compared to other physiochemical synthesis, biogenic synthesis has a well-defined size, shape, and morphology and is free of contamination (Kumar *et al.* 2017). Vijayaraghavan (2010) asserts that slower kinetics of biologically synthesised nanoparticles provide more control over crystal formation and cheaper manufacturing costs (Aziz *et al.* 2014). Numerous researchers have shown the use of microalgal solution in the biological production of silver nanoparticles. Microalgae are regarded as cell factories for nanoscale particle

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production because of their rapid development and high biomass output in a short duration of time during culture (Ahmad *et al.* 2011; Mata *et al.* 2010).

In the present investigation, efforts have been made to synthesize Silver nanoparticles using microalgal suspension. Literature survey confirms the presence of secondary metabolites like Phenols (Jacob *et al.* 2007), Flavonoids (Sahu*et al.* 2016), Tannins (Soliwoda*et al.* 2017), Saponins (Geethalakshmi*et al.*2013), Vitamins (Qin *et al.*2010), Amino acids (Shankar *et al.* 2015) etc. Research studies showed that biomolecules, proteins and peptides present in Algae are mainly responsible for the formation and stabilization of AgNPs (Sharma *et al.* 2015). According to Ebrahiminezhad*et al. (*2016), proteins present in cell extract of *Chlorella vulgaris* were involved in the biosynthesis by providing dual function in reduction and shape-controlling of the synthesized AgNPs (Xie*et al.* 2007). The study aims to evaluate the synthesis of Silver nanoparticles (AgNPs) at different dilutions of cell free micro algal extract using different concentrations of $AgNO_3$ as Precursor and to characterize the synthesized Silver nanoparticles at the level of Preliminary screening (Optical properties).

MATERIALS AND METHODS

Chemicals/Reagents

All the chemicals/reagents used in the present study were of analytical grade. Triple distilled water was used to prepare microalgal nutrient media.

Preparation of Cell free Microalgae extract

Microalgae cultures [Consortia of *Chlorella sp., Scenedesmus sp., and Cosmarium spp.;* maintained in BBM medium (Nichols and Bold, 1965) under 16/8-hr light/dark cycle and 3000 lux intensity at 25 ± 1°C temperature (Shaker *et al.* 2017) were procured from departmental Algal Biotechnology laboratory. Microalgae culture was centrifuged at 10,000 rpm for 10 mins and pellets were discarded. The resulting supernatant was collected in eppendorf tubes and filtered twice with Whatman filter paper No.1 to eliminate any physical contaminants and stored at 4°C.

Sunlight-induced biological synthesis of silver nanoparticles

Silver nanoparticles (AgNPs) were synthesized by solution-based photo-irradiated biologically inspired reduction process (Fig. 1). Silver Nitrate was used as precursor and cell free microalgal suspension as reducing agent. In general, the synthesis of colloidal silver nanoparticles involved simple aqueous phase mixing of Precursor (AgNO3) with Reducing agent (microalgal suspension). Experiments were designed to study the effect of micro algal suspensions (1:5 & 1:10 dilutions) on synthesis of Silver Nanoparticles at different concentrations of Silver nitrate (0.1, 0.5 & 1 mM) (Table 1). Govindaraju*et al.* (2009) conduct extracellular Synthesis of Silver Nanoparticles by a Marine Alga, *Sargassum wightii* at 1:10 dilutions with 1mM AgNO3 solution. The synthesis of silver nanoparticles was carried out at different concentrations of precursor and reducing agent at variable exposure time in order to optimize the reaction parameters for better understanding and maximizing the yield of silver nanoparticles (Phatak and Hendre, 2015). The reduction reaction was carried out in presence of sunlight. The reaction mixtures were placed in direct sunlight on bright sunny days (March to June, 2018 at Institute's premises). The maximum and minimum temperature throughout the study was recorded as 45 °C and 28 °C respectively. The variation in light intensity was monitored after every 10 mins of interval throughout the experiment and recorded between ≈60,000 to ≈1, 10,000 Lux using Digital Lux Meter. The reaction time was extended up to 180 mins and observations were recorded after periodic time intervals (i.e. 20, 40, 60, 90, 120, 150, 180 mins) using UV-Vis spectrophotometer (ELICO SL-150) in order to record the SPR and to characterize the AgNPs. All the experiments were conducted in a completely randomised design, in duplicates. Mean ± Standard error was computed from raw data.

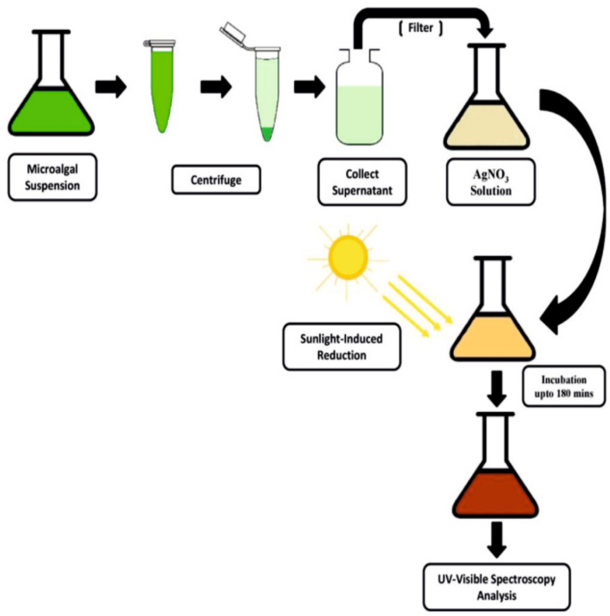

Fig 1: Schematic diagram of sunlight-mediated biological synthesis of AgNPs.

Table1: Experimental design:

Sample	Precursor	Microalgal Suspension Dilution
A_1	$AgNO_3$ (0.1mM)	1:5
A_2	$AgNO_3$ (0.1mM)	1:10
A_3	$AgNO_3$ (0.5mM)	1:5
A_4	$AgNO_3$ (0.5mM)	1:10
A_5	$AgNO_3$ (1mM)	1:5
A_6	$AgNO_3$ (1mM)	1:10

*All the treatments were performed in duplicates.

RESULTS & DISCUSSION

Observation of Colour Change

The preliminary analysis is the colour identification of the reaction mixture that confirms the synthesis of nanoparticles which is based on their optical properties for e.g., formation of characteristic brown colour indicates the synthesis of silver nanoparticles, and the acquired colour change is due to excitation of Surface Plasmon Resonance of metallic nanoparticles (Paulkumar*et al.* 2014). In the present investigation, the colour development from colourless to various shades of brown has been observed. Variations in colour of the reaction mixtures could be due to the active biochemical components present in the cell free extract of microalgal consortium. Experimental treatments performed at 0.1 mM $AgNO_3$ concentration, 1:5 (A_1) and 1:10 (A_2) dilution showed change in colour from colourless to light brown in 20 minutes of reaction time and further with the progress of reaction, not much significant colour development has been observed **(Fig. 8 &9)**. Treatments performed at 0.5 mM $AgNO_3$ conc., 1:5 (A_3) &1:10 (A_4) and at 1 mM $AgNO_3$ conc., 1:5 (A_5) & 1:10(A_6) showed distinct dark brown colours within 20 minutes of exposure time, thus indicates rapid synthesis of AgNPs **(Fig. 10 to 13)**.

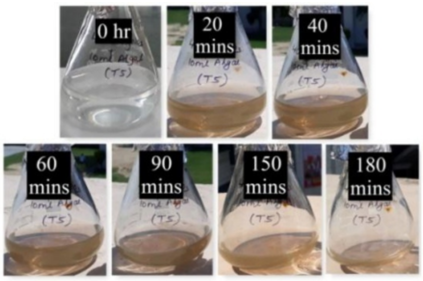

Fig 2: 0.1 mM $AgNO_3$ at 1:5 Broth Dilution (S_1)

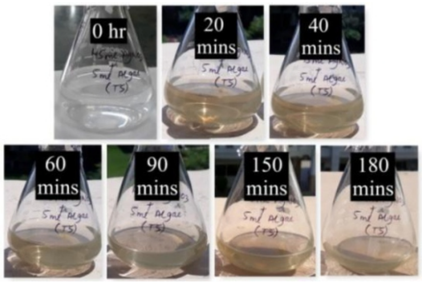

Fig 3: 0.1 mM $AgNO_3$ at 1:10 Broth Dilution (S_2)

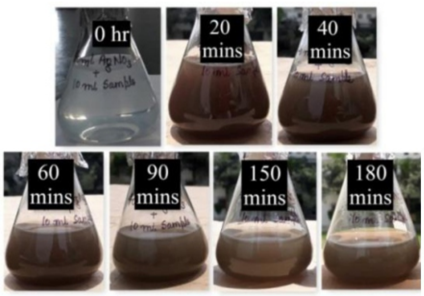

Fig 4: 0.5 mM $AgNO_3$ at 1:5 Broth Dilution (S_3)

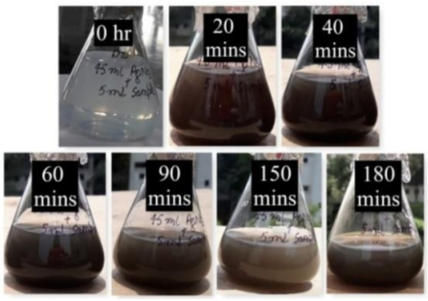

Fig 5: 0.5 mM $AgNO_3$ at 1:10 Broth Dilution (S_4)

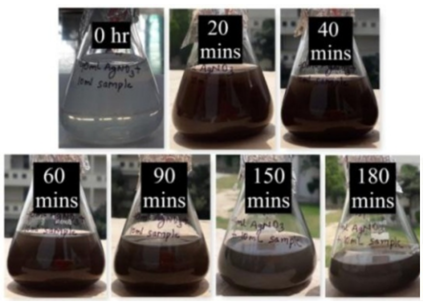

Fig 6: 1mM $AgNO_3$ at 1:5 Broth Dilution (S_5)

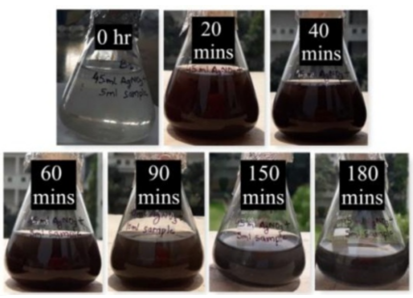

Fig 7:1mM $AgNO_3$ at 1:10 Broth Dilution(S_6)

In a similar study on AgNPs synthesis using cell-free extract of *Brevundimonas spp.*, Rajamanickam*et al.* (2013) observed appearance of dark brownish yellow colour indicating the synthesis of silver nanoparticles. Sharma *et al.* (2015) also observed appearance of dark brown colour of solution as an indicator of silver nanoparticles synthesis using cell free aqueous extracts of *S. platensis*. Visual observation of colour change in aqueous solution can also provide information about the size of synthesized AgNPs i.e., the smaller sized AgNPs results greater shift in colour towards red (Vadlapudi and Amanchy, 2017). However, observations leading to the growth and morphology of Silver nanoparticles need further investigations like SPR analysis, bandwidth analysis, etc.

Effect of Cell-free Microalgal Suspension and Silver nitrate concentrations on Synthesis of Silver Nanoparticles (AgNPs):

Green Synthesis of Silver nanoparticles (AgNPs) carried out using cell-free microalgal extract under sunlight as a reducing agent. The concentrations of Microalgal Suspensions were diluted at 1:5 & 1:10 in aqueous solution of $AgNO_3$ and reduction reaction was carried out in sunlight with periodic stirring. Continuous stirring facilitates availability of desired sites for developing silver nanoparticles (Raza *et al.* 2016). The reaction time was extended up-to 180 mins and observations were recorded periodically after 20 mins of intervals. The spectral data was recorded using UV-Vis spectrophotometer. The photo-irradiation based reduction was carried out based on the preliminary survey. Patel *et al.* (2015) in his study on different cell free microalgal and cyanobacterial cultures, observed AgNPs synthesis in light but not in dark. The Experimental design included variable concentrations of Silver nitrate (0.1, 0.5 and 1 mM) with Microalgal suspensions (1:5 and 1:10 dilutions). Total of 6 experiments were performed in duplicates (A_1 to A_6).

Observation of SPR spectra showed rapid synthesis (within 20 mins) of Silver nanoparticles in all the treatment samples (Fig 8-13). SPR spectra recorded were in the range of 500-520 nm. Variation in SPR peaks in different treatment samples indicates AgNPs of variable sizes and morphology. In treatment sample A_1 (0.1 mM $AgNO_3$, 1:5 broth dilution), peak sharpening increase with the time and maximum absorption was recorded after 180 mins of exposure time (Fig 8). There was not much variation in absorption values recorded after 40 mins of reaction time in treatment sample A_2 (Fig 9). After 20 mins of reaction, comparatively sharp peak was recorded in treatment sample A_4 (Fig 11) than A_3 (Fig 10) was observed. Experimental treatments A_5 and A_6 showed flat peaks, however maximum absorption was recorded after 20 mins of reaction in both the samples (Fig 12 & 13).

Overall, cell free microalgal suspension has played significant role in AgNPs synthesis, as rapid reduction was observed in all the treatment samples. Mi-Kyung and Jeune (2009) in his work on biochemical pool shifts analysis in *Chlorella ovalis* (cultured under different media composition) using FT-IR recorded the presence of carbohydrates, proteins, and lipid through functional group analysis. According to Mahdieh*et al.* (2012), presence of cellular reductases in *Spirulina platensis* is responsible for the synthesis of Silver Nanoparticles. Phenolic compounds present in plant extracts can be effective for the bio-reduction of silver ions to AgNPs (Baharara*et al.* 2015). Sivathanu*et al.* (2011), reported presence of bioactive compounds such as alkaloids, flavonoids, carotenoids, saponins, fatty acids, amino acids and carbohydrates in organic solvent extracts of green algae *Chlorococcumhumicola*. Makarov *et al.* (2014) underlined the role of biomolecules (like different classes of flavonoids such as flavonols, flavones, flavanones, isoflavonoids, etc.) in nanoparticle synthesis. These bioactive molecules have various functional groups which can actively chelate and reduce metal ions into NPs through tautomeric transformations of flavonoids (Makarov *et al.* 2014).

Microalgal broth conc. doesn't affect peak intensity significantly. However, comparatively sharp peaks at lower $AgNO_3$ conc. (0.1 mM) was obtained that could offer higher homogeneity whereas broad or flat peaks obtained at higher $AgNO_3$ conc. (0.5 mM and 1mM) indicates poly-dispersed AgNPs. Jena *et al.* (2014) observed increase in peak intensity with increase in exposure time and suggested poly dispersed and aggregated AgNPs (SPR 430 nm) obtained from raw extract of *Scenedesmus* microalgae at 5 mM concentration of $AgNO_3$.

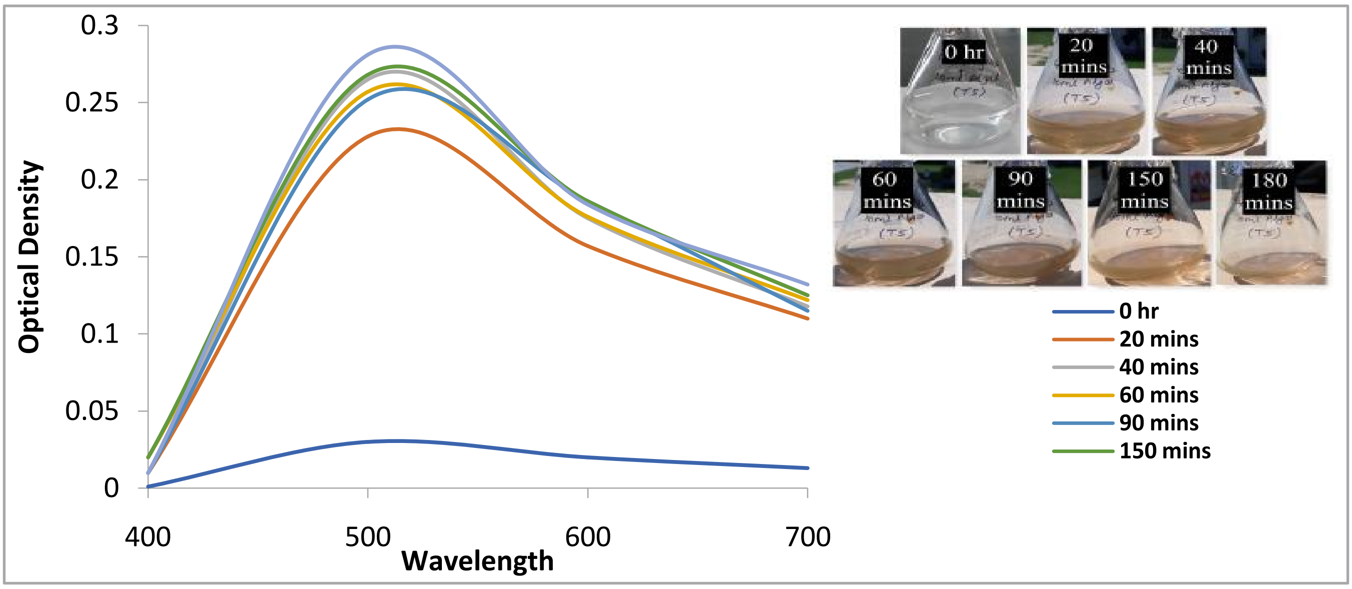

Fig 8: Visible spectra of Silver colloids at 0.1 mM $AgNO_3$ and 1:5 microalgal suspension dilution.

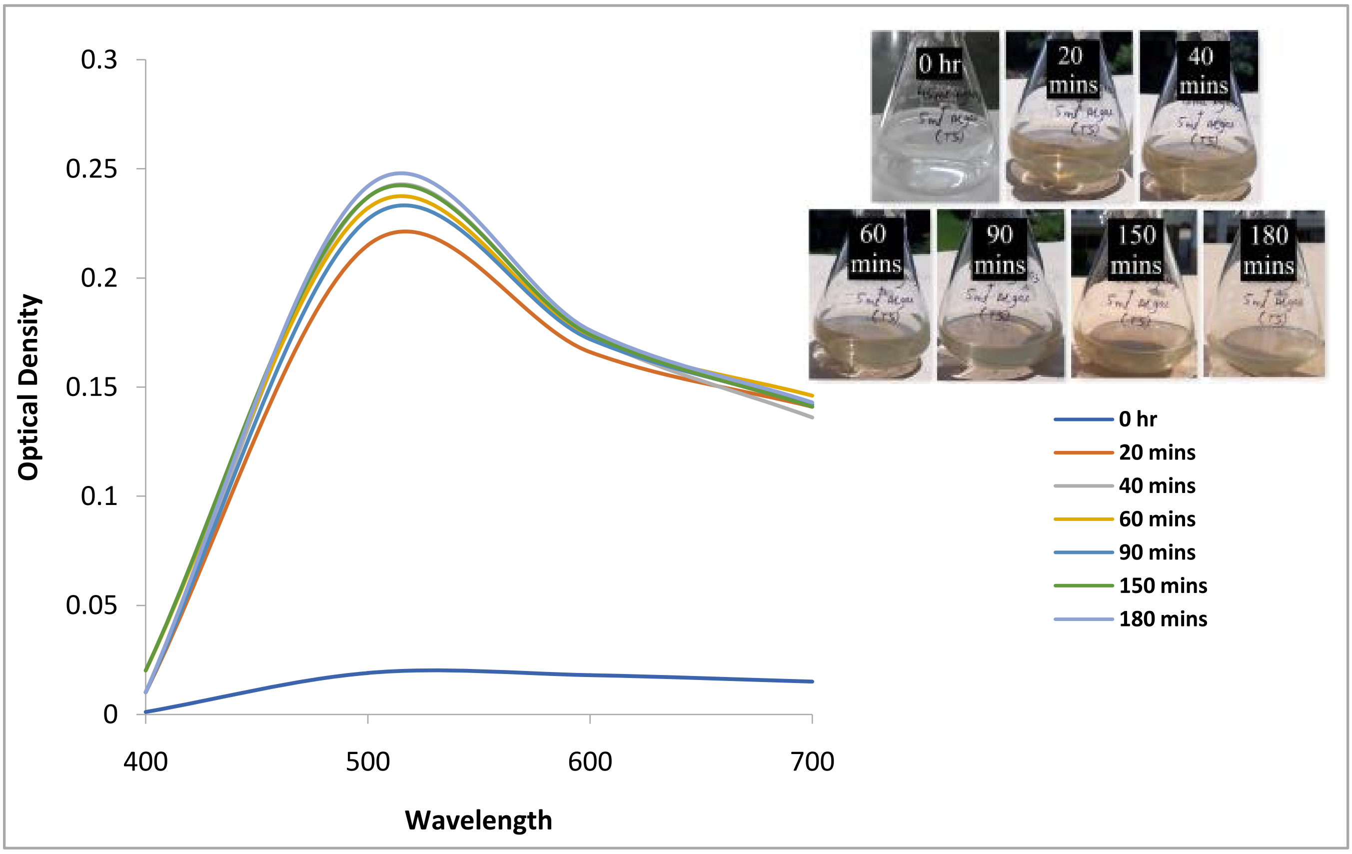

Fig 9: Visible spectra of Silver colloids at 0.1 mM $AgNO_3$ and 1:10 microalgal suspension dilution.

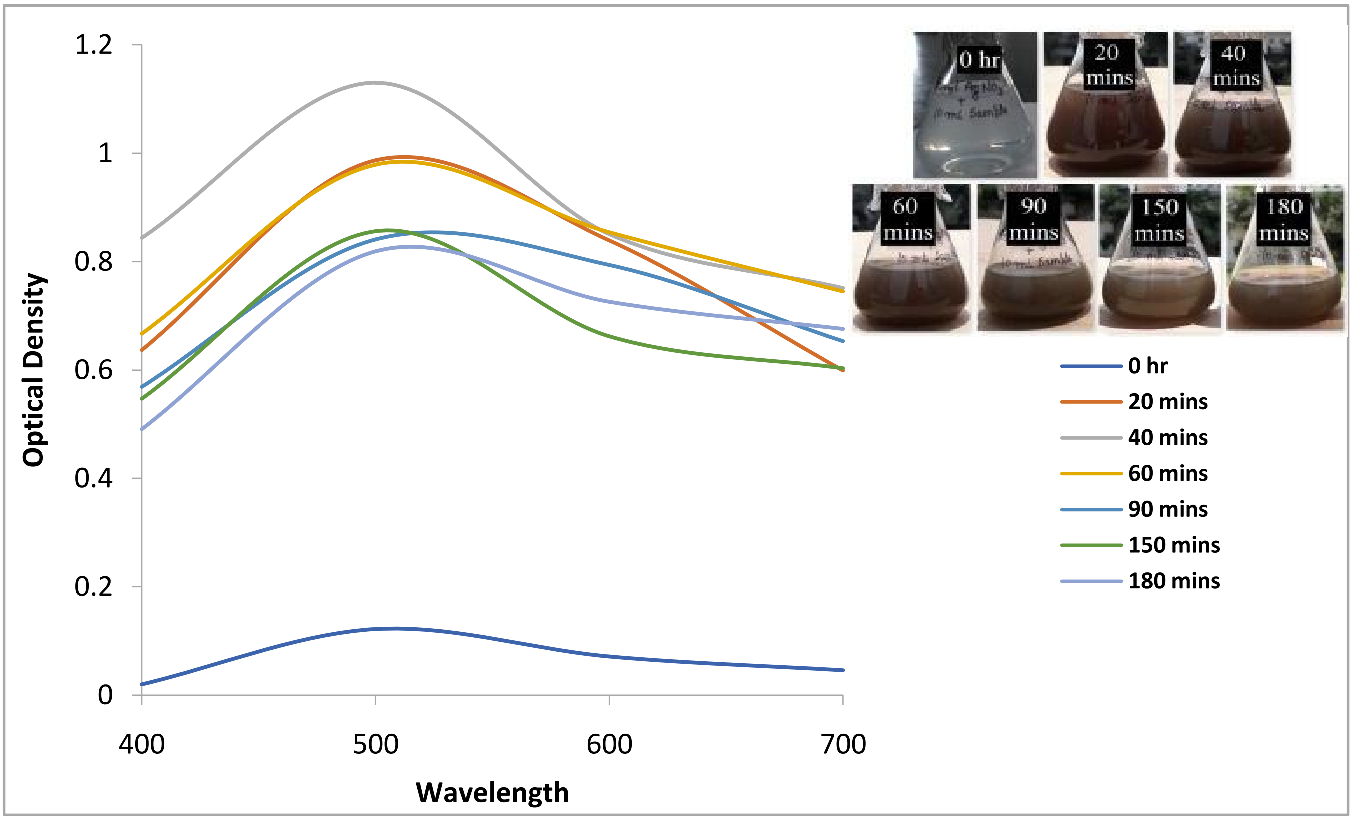

Fig 10: Visible spectra of Silver colloids at 0.5 mM $AgNO_3$ and 1:5 microalgal suspension dilution.

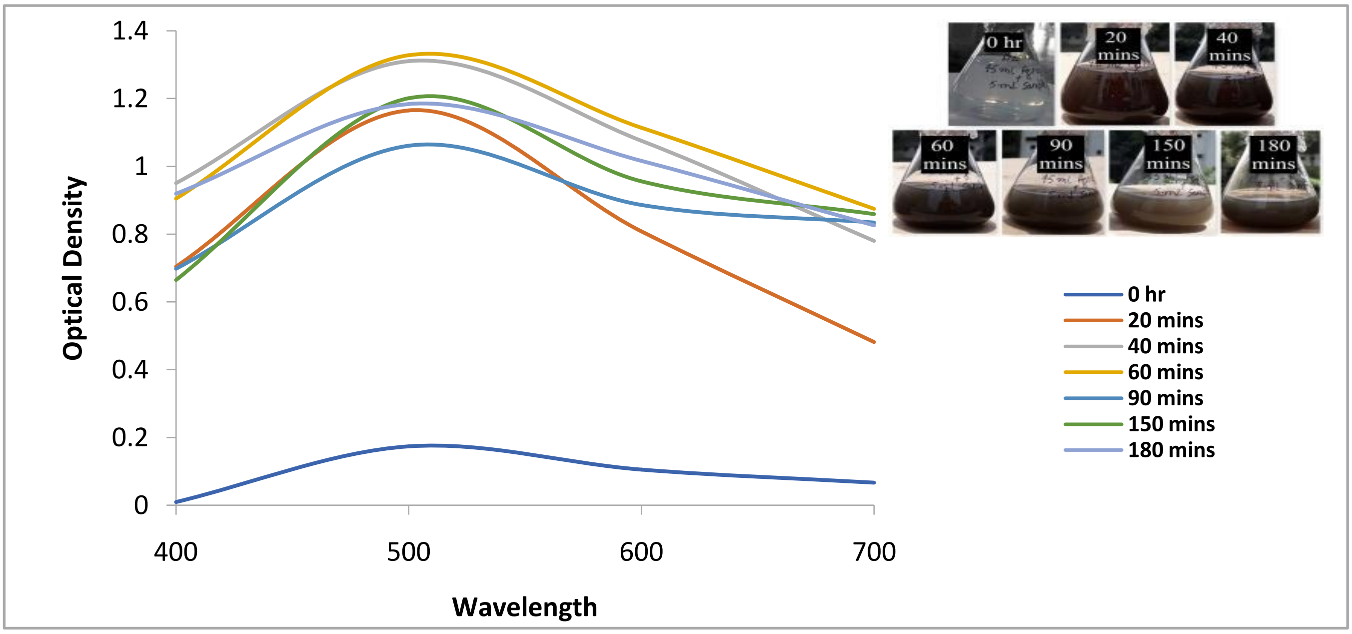

Fig 11: Visible spectra of Silver colloids at 0.5 mM $AgNO_3$ and 1:10 microalgal suspension dilution.

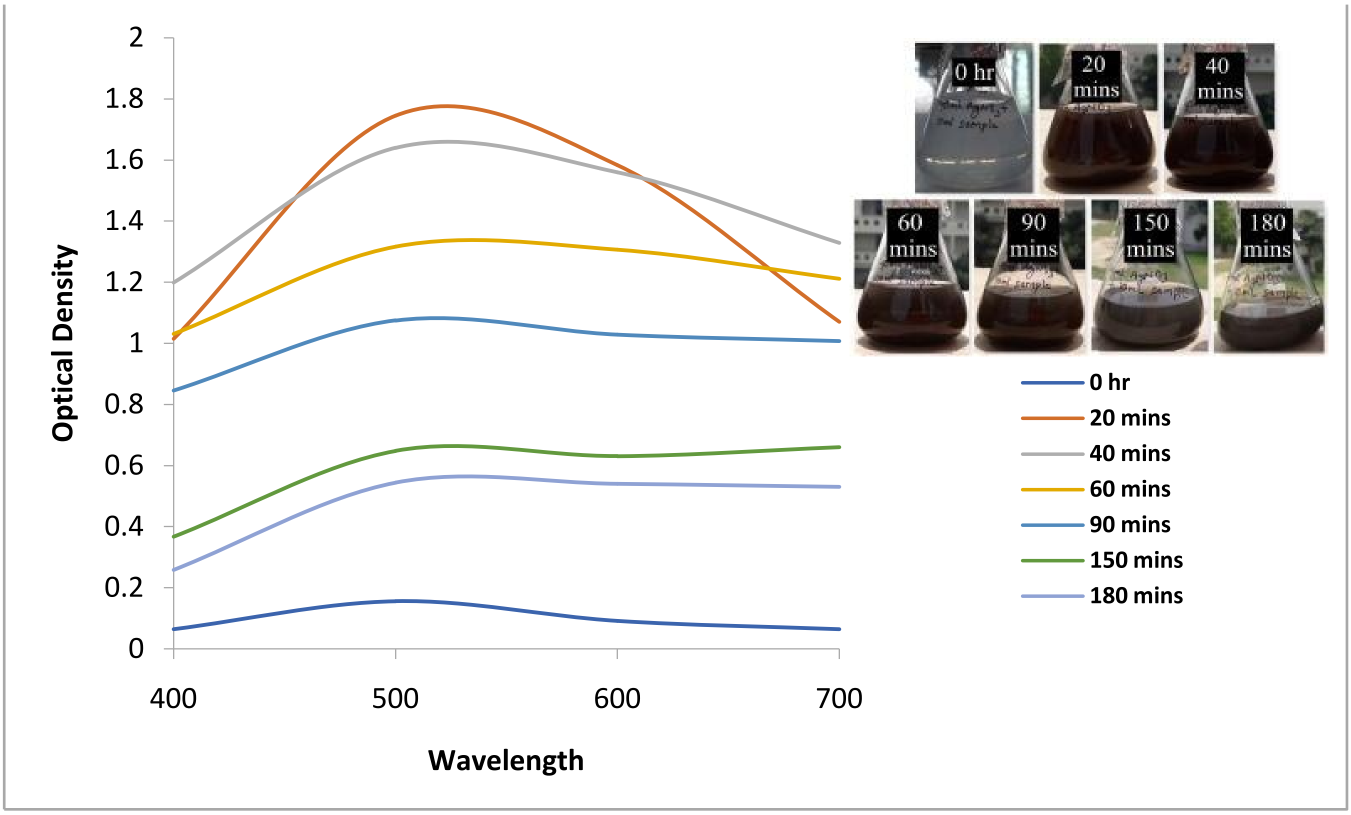

Fig 12: Visible spectra of Silver colloids at 1 mM $AgNO_3$ and 1:5 microalgal suspension dilution.

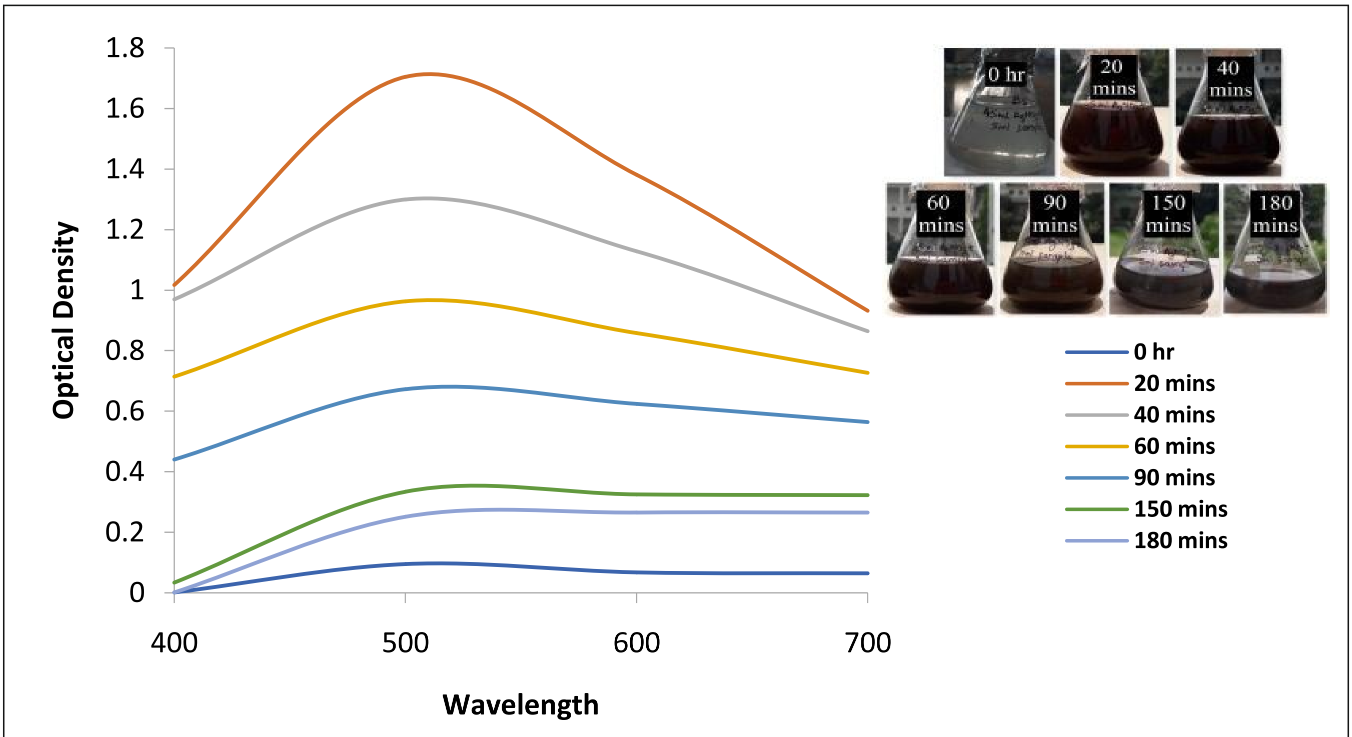

Fig 13: Visible spectra of Silver colloids at 1 mM $AgNO_3$ and 1:10 microalgal suspension dilution.

CONCLUSION

Algae are considered as an important source of carbohydrates, protein and lipids (Vincy*et al.* 2017). The use of algae in the synthesis of NPs has encouragd the designing of simple, green, cost and time effective approaches thereby, minimizing the use of chemicals and solvents (Vincy*et al.* 2017). In the present investigation, efforts have been made to synthesize silver nanoparticles in sunlight using Silver Nitrate used as precursor, cell free microalgae consortium as reducing agent and stabilizing agent. Visual analysis followed by UV-Vis Spectra results showed that the concentration of microalgal suspension and $AgNO_3$ plays a significant role in synthesis of silver nanoparticles. Observation of SPR spectra confirmed rapid synthesis of silver nanoparticles in all the treatment samples **(Fig 8-13)**. SPR spectra recorded were in the range of 500-520 nm. Although, cell free microalgal suspension has played significant role in AgNPs synthesis, as rapid reduction was observed in all the treatment samples but, microalgal broth conc. doesn't affect peak intensity significantly. However, comparatively sharp peaks at lower $AgNO_3$ conc. (0.1 mM) were obtained that could offer higher homogeneity whereas broad or flat peaks obtained at higher $AgNO_3$ conc. (0.5 mM and 1mM) indicates polydispersed AgNPs.

However, further studies pertaining to size, shape and distribution coupled with stabilization under variable conditions like stabilizing agents, different precursors, oxidizing agents, reaction time, pH, temperature, etc., to optimize the controlled synthesis of silver nanoparticles must be recommended.

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DECLARATION

CONFLICT OF INTERESTS

The authors declare no competing interests.

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