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ORIGINAL ARTICLE

Green synthesis of silver nanoparticles using marine macroalga *Chaetomorpha linum*

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Abstract The present investigation demonstrates the formation of silver nanoparticles by the reduction of the aqueous silver metal ions during exposure to the seaweed (*Chaetomorpha linum*) extract. The silver nanoparticles obtained were characterized by UV–visible spectrum, FTIR and scanning electron microscopy. The characteristic absorption peak at 422 nm in UV–vis spectrum confirmed the formation of silver nanoparticles. The colour intensity at 422 nm increased with duration of incubation. The size of nanoparticles synthesized varied from 3 to 44 nm with average of ~30 nm. The FTIR spectrum of *C. linum* extract showed peaks at 1,020, 1,112, 1,325, 1,512, 1,535, 1,610, 1,725, 1,862, 2,924, 3,330 cm^{-1}. The vibrational bands corresponding to the bonds such as –C=C (ring), –C–O, –C–O–C and C=C (chain) are derived from water-soluble compounds such as amines, peptides, flavonoids and terpenoids present in *C. linum* extract. Hence, it may be inferred that these biomolecules are responsible for capping and efficient stabilization. Since no synthetic reagents were used in this investigation, it is environmentally safe and have potential for application in biomedicine and agriculture.

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Introduction

Recently, silver nanoparticles (AgNPs) are widely investigated to owing their broad range of applications as antibacterials (Venkatpurwar and Pokharkar 2011; Wang Yh et al. 2008), biosensors (Chen et al. 2007) and in plant growth metabolism (Krishnaraj et al. 2012). The green synthesis of inherently safer AgNPs depends on the adoption of the basic requirements of green chemistry: the solvent medium, the benign reducing agent and the non-hazardous stabilizing agent (Vigneshwaran et al. 2006). Compared to microbe-assisted synthesis, plant-mediated synthesis of nanoparticles is a relatively underexploited field and is recently gaining wide attention. AgNPs have been successfully synthesized using several plant extracts (Shankar et al. 2003; Amkamwar et al. 2005; Chandran et al. 2006; Li et al. 2007; Venkatpurwar and Pokharkar 2011). All these investigations are restricted to terrestrial plants, but only limited reports are available for synthesis of nanoparticles from marine plants (Govindaraju et al. 2009; Nabikhan et al. 2010; Venkatpurwar and Pokharkar 2011). Marine macroalgae produce a great variety of secondary metabolites that showed therapeutic potential (Tierney et al. 2010; Mayer et al. 2011). *Chaetomorpha linum* (Muller) Kutzing is a green seaweed mainly acknowledged for its ecological role as possible regulator of nutrient availability in estuarine habitats (Krause-Jensen et al. 1999). Many researchers (Heo et al. 2005; Patra et al. 2009; Prabhahar et al. 2011) have reported that *C. linum* exhibits medicinal properties with high-value phytochemicals, which motivated us to carry out the present investigation on the synthesis of AgNPs using *C. linum* biomass.

Materials and methods

Chemicals

Silver nitrate ($AgNO_3$) was purchased from Merck, India. All other reagents used in this present investigation are of analytical grade.

Sample collection and preparation

The green seaweed *C. linum* (Muller) Kutzing was collected from the intertidal region of Kanyakumari coast located at southeast coast of India. Collected sample was immediately brought to the laboratory in new plastic bags containing natural sea water to prevent evaporation. Plants were washed thoroughly with tap water to remove extraneous materials and shade-dried for 5 days and oven-dried at 60 °C until constant weight was obtained, then ground into fine powder using electric mixer and stored at 4 °C for future use.

Preparation of seaweed extract

Dried finely powdered *C. linum* (10 g) was boiled with 100 ml of distilled water at 60 °C for 15 min. The extract was filtered through nylon mesh, followed by Millipore filter (0.45 μm) and used for further experiments.

Synthesis of silver nanoparticles

The Erlenmeyer flask containing 100 ml of $AgNO_3$ (1 mM) was reacted with 20 ml of the aqueous extract of *C. linum*. This setup was incubated in dark (to minimize the photoactivation of silver nitrate) at 37 °C under static condition. A control setup was also maintained without *C. linum* extract.

Characterization of silver nanoparticles

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, at the wavelength of 300–700 nm in Perkin Elmer Lamda 25 spectrophotometer. Further, the bioreduced reaction mixture was subjected to centrifugation at 75,000×*g* for 30 min, resulting pellet was dissolved in de-ionized water and filtered through Millipore filter (0.45 μm). An aliquot of this filtrate containing AgNPs was used for FTIR and SEM studies. For electron microscopic studies, 25 μl of the sample was sputter-coated on copper stub and the images of nanoparticles were studied using SEM (JEOL, Model JFC-1600).

Results and discussion

Reduction of $AgNO_3$ was visually evident from the colour change (brownish-yellow) of reaction mixture after 30 min of reaction (Fig. 1). Intensity of brown colour increased in direct proportion to the incubation period. It may be due to the excitation of surface plasmon resonance (SPR) effect and reduction of $AgNO_3$ (Mulvaney 1996). The control $AgNO_3$ solution (without seaweed extract) showed no colour change. Absorption spectrum of reaction mixture at different wavelengths ranging from 400 to 700 nm revealed a peak at 420 nm (Fig. 2). The characteristic absorption peak at 422 nm in UV–vis spectrum (Figs. 2, 3) confirmed the formation of AgNPs. In the present study the

Fig. 1 Colour intensity of the *Chaetomorpha linum* extract incubated with silver ions at the beginning of reaction and after 48 h reaction

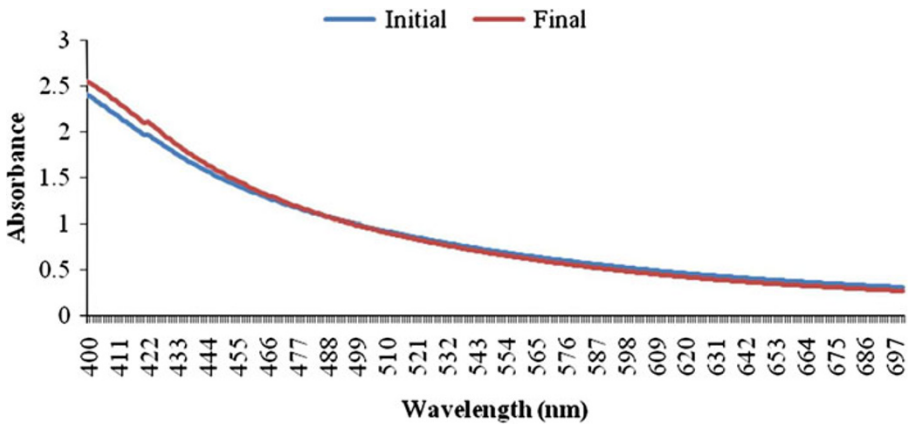

Fig. 2 UV–visible spectrum of *C. linum* extract treated with and without AgNPs during different wavelengths

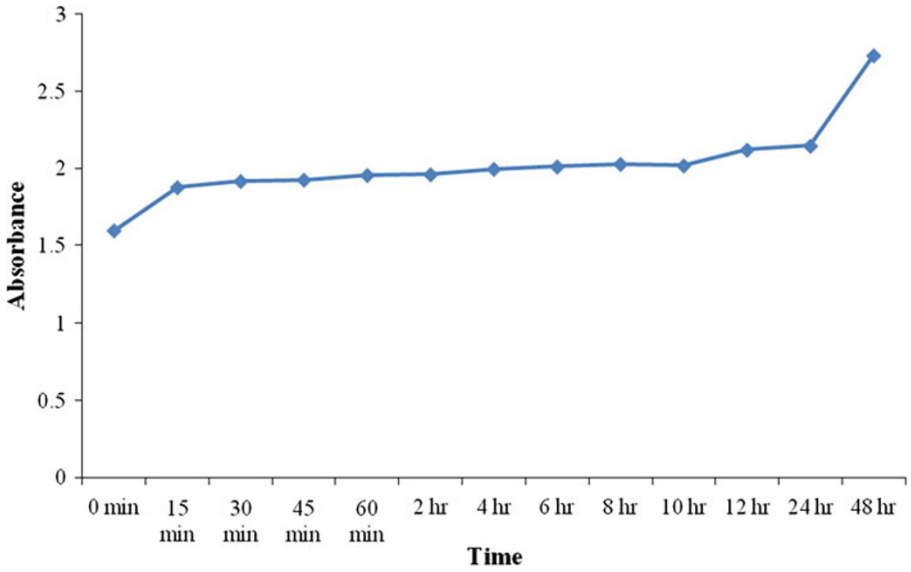

Fig. 3 Colour intensity at 422 nm for AgNPs synthesis by *C. linum* during different time intervals

colour intensity at 422 nm increased with duration of incubation. This is similar to the surface plasmon vibrations with characteristic peaks of silver nanoparticles prepared by chemical reduction (Petit et al. 1993; Kong and Jang 2006).

A scanning electron microscope was employed to analyse the structure of the nanoparticles that were formed. From Fig. 4 it is evident that the AgNPs coalesced to nano-clusters (white marking). When reaction mixtures were incubated for 48 h some nanoparticles aggregated. The size of nanoparticles synthesized in the present study by *C. linum* biomass varied from 3 to 44 nm with average of ~30 nm. Jain et al. (2009) reported particle size between 25 and 50 nm in cubic structure synthesized by papaya fruit extract and Cataleya (2006) reported macrophages in comparison to larger nanoparticles (i.e. 30–55 nm).

FTIR measurements were carried out to identify the possible biomolecules responsible for the stabilization of the newly synthesized AgNPs. Figure 5 represents the FTIR spectrum of *C. linum* extract shows peaks at 1,020, 1,112, 1,325, 1,512, 1,535, 1,610, 1,725, 1,862, 2,924, 3,330 cm^{-1}. The FTIR spectra of dried *C. linum* extract showed strong absorption band at 1,535 cm^{-1} corresponding to bending vibration of secondary amine of proteins. Another band observed at 1,610 cm^{-1} is assigned to the stretching vibration of (NH) C=O group. After reduction of $AgNO_3$ the decreases in intensity at 1,535 cm^{-1} signify the involvement of the secondary amines in the reduction process. On the other hand, the shift of band from 1,610 cm^{-1} is attributed to the binding of (NH) C=O group with nanoparticles. Since a member of (NH) C=O group within the cage of cyclic peptides is involved in stabilizing the nanoparticles, the shift of (NH) C=O band is quite small. Thus, the peptides play a major role for the reduction of Ag* to Ag nanoparticles. Bands at 1,020 cm^{-1} can be assigned as absorption peaks of –C–O–C. The bands at 1,325 cm^{-1} in silver nano may be attributed to –C–O stretching mode (Shankar et al. 2003; Huang et al. 2007; Philip 2009a, b). Strong IR band at 1,512 cm^{-1} in the spectrum of silver nanoparticle arise from the stretching vibrations of C=C

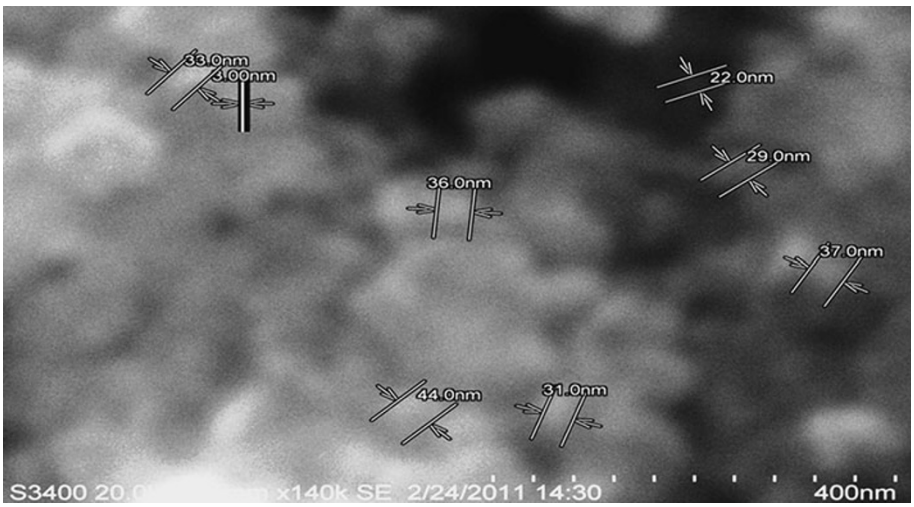

Fig. 4 SEM image of AgNPs synthesized by *C. linum*

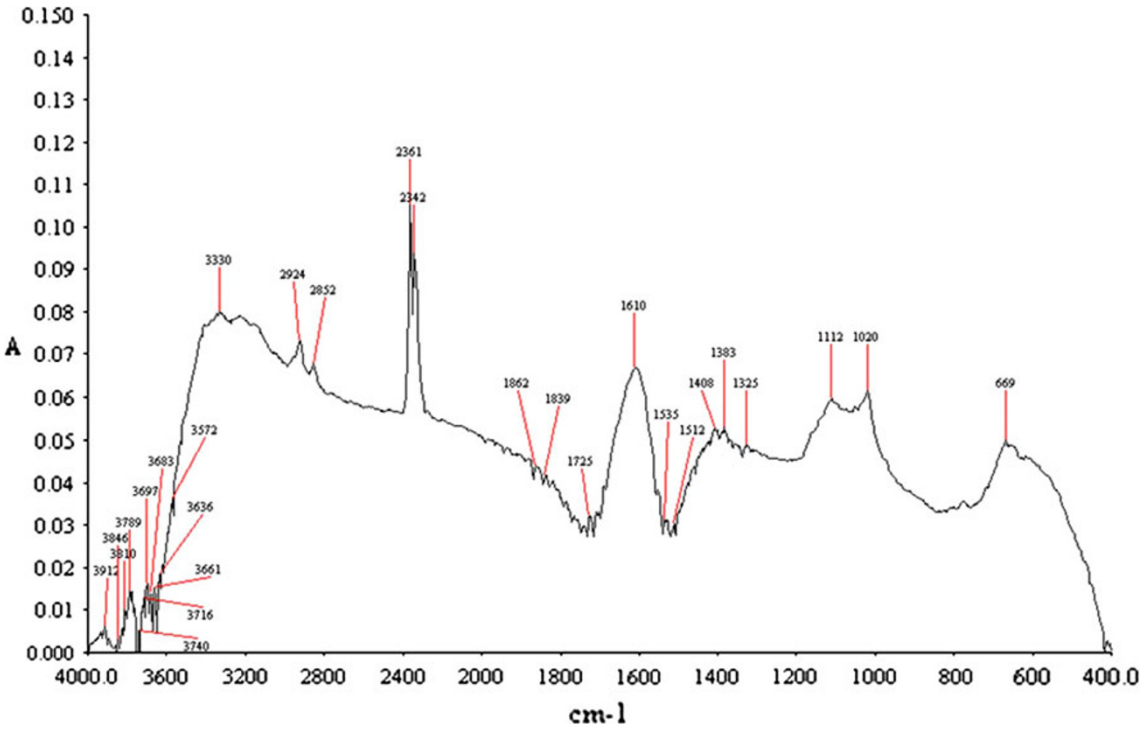

Fig. 5 FTIR spectrum of AgNPs synthesized by *C. linum* extract

chain (Schulz and Baranska 2007). The characteristic peak at 1,725 cm^{-1} was noticed in the *C. linum* extract and this may be due to the free amine groups which are used for the stabilization of silver nanoparticles (Philip 2010). The vibrational bands corresponding to the bonds such as –C=C (ring), –C–O, –C–O–C and C=C (chain) are derived from water-soluble compounds such as flavonoids and terpenoids present in *C. linum* extract. Hence, it may be inferred that these biomolecules are responsible for capping and efficient stabilization.

Conclusion

In this present investigation, we described environment-friendly synthesis of AgNPs using marine macroalgae

C. linum. The amines, peptide groups and secondary metabolites flavonoids and terpenoids present in the *C. linum* extract were involved in the bioreduction and stabilization of AgNP. This method of AgNPs synthesis does not use any toxic reagents and thus has the potential for use in biomedical and agricultural applications.

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