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Short communication

Synthesis of silver nanoparticles using the seaweed *Codium capitatum* P.C. Silva (Chlorophyceae)

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ABSTRACT

The formation of silver nanoparticles by the reduction of aqueous silver metal ions during exposure to both fresh and dry seaweed extracts of *Codium capitatum* is reported. The silver nanoparticles obtained were characterized using UV–visible spectroscopy with characteristic absorption peaks at 422 and 425 nm. The color intensity at 422 nm increased for the duration of the incubation period. Using energy dispersive X-ray spectrometry (EDX) analysis, a distinct peak of silver was confirmed. Silver concentrations of 63.7% in the fresh and 56.0% in the dried seaweed were detected. The Fourier Transform Infrared (FTIR) spectra indicated the involvement of amine, peptide and sulfate groups in the *C. capitatum* extract for bioreduction and stabilization of AgNP. No synthetic reagents were used in this investigation, and thus it is an environmentally safe method with potential for biomedical and agriculture applications.

1. Introduction

Nanotechnology deals with the synthesis of nanoparticles of different shapes and sizes and their potential applications. Currently, there is a growing need to develop environmentally friendly and sustainable methods for the synthesis of nanomaterials that do not use toxic chemicals in the synthesis protocols so as to avoid adverse effects in medical applications. Among the nanoparticles, silver nanoparticles (AgNPs) have received considerable attention due to their attractive physicochemical properties (Elechiguerra et al., 2005). AgNPs have been widely investigated as they can be applied in label-free colorimetric assays to detect enzymatic reactions (Wei et al., 2008), as antibacterial water filters (Jain and Pradeep, 2005), in metal catalysis (Shiraishi and Toshima, 2000), as biosensors (Chen et al., 2007), as optical receptors for biolabelling (Ling et al., 2008), in antibacterial activity (Venkatpurwar and Pokharkar, 2011), in anti-HIV activity (Elechiguerra et al., 2005) and in controlling plant pathogens (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; a benign reducing agent and a non-hazardous stabilizing agent (Vigneshwaran et al., 2006).

Compared to microbe assisted synthesis, plant-mediated synthesis of nanoparticles is an under exploited field. AgNPs have been successfully synthesized using several plants (Amkamwar et al., 2005; Chandran et al., 2006; Li et al., 2007; Shankar et al., 2003; Venkatpurwar and Pokharkar, 2011). All these investigations were restricted to terrestrial plants and there are only limited reports on the synthesis of nanoparticles from marine plants (Govindaraju et al., 2009; Kannan et al., 2012; Nabikhan et al., 2010; Prasad et al., 2012; Venkatpurwar and Pokharkar, 2011; Vivek et al., 2011).

Algae are also used as "bio-factories" for synthesis of metallic nanoparticles. Among different genera of bioreductants, seaweeds have distinct advantages due to their high metal uptake capacity and low cost (Davis et al., 2003). Rapid synthesis of silver nanoparticles through extracellular biosynthesis from seaweeds is feasible (Govindaraju et al., 2009; Kannan et al., 2012; Prasad et al., 2012; Venkatpurwar and Pokharkar, 2011; Vivek et al., 2011). The genus *Codium* (Bryopsidophyceae) comprises intertidal and subtidal green seaweeds and encompasses about 130 species, that are widely distributed. The highest species diversity is in subtropical regions (Guiry and Guiry, 2011; Rindi et al., 2012). *Codium capitatum* P.C. Silva is a green seaweed found in the intertidal zone along the South African east coast. As far as we know there are only two reports on the biological activity of *C. capitatum* (Celikler et al., 2009; Stirk et al., 2007). No work has been done on any of the South African seaweeds for the synthesis of silver nanoparticles. This motivated the present investigation on the synthesis of AgNPs using *C. capitatum* biomass.

2. Materials and methods

2.1. Chemicals

Analytical grade silver nitrate ($AgNO_3$) was purchased from Merck, South Africa. All other reagents used in this investigation were of analytical grade.

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2.2. Sample collection and extract preparation

The green seaweed *C. capitatum* was collected from the intertidal region at Rocky Bay (30°23′S; 30°43′E) located on the east coast of South Africa. Collected samples were immediately brought to the laboratory in new plastic bags containing natural sea water to prevent desiccation. In the laboratory, the seaweed was washed thoroughly with tap water to remove extraneous materials. A portion of the material was freeze-dried, then ground to a fine powder using an electric blender (IKA-Labortechnik) and stored in airtight containers at 4 °C for future use. When needed, the dried finely powdered *C. capitatum* (1 g) was extracted in 100 ml double distilled water at 60 °C for 15 min. The extracts were filtered through Whatman No. 1 filter paper and used for further experiments.

In addition, fresh seaweed was washed thoroughly in running tap water in the laboratory for 10 min in order to remove extraneous materials, cut into small pieces, and then rinsed with sterile distilled water. The washed seaweed (10 g) was chopped finely and boiled in 100 ml sterile distilled water for 5 min. The extracts were filtered through Whatman No. 1 filter paper and the filtrate then used for further experiments.

2.3. Synthesis of silver nanoparticles

Flasks containing 88 ml $AgNO_3$ solution (1 mM) were separately reacted with 12 ml fresh and dry *C. capitatum* extracts. These reactions were incubated in the dark to minimize the photoactivation of $AgNO_3$ at room temperature under static conditions. A control reaction was also maintained without seaweed extract.

2.4. 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, in a range of wavelengths between 300 and 700 nm (Varian, Cary 50 Conc Spectrophotometer). After 48 h of reaction, the bioreduced reaction mixture was subjected to centrifugation at 12,000 rpm for 20 min at 4 °C. The resulting pellet was dissolved in de-ionized water and filtered through a Millipore filter (0.45 μm). The purified AgNPs were examined for the presence of biomolecules responsible for bioreduction using Fourier Transform Infrared spectral analysis. The spectrum obtained from the dried reaction mixture was recorded on a Bruker Alpha FTIR Spectrometer with an ATR platinum diamond accessory (32 scans per sample).

An aliquot of this filtrate containing AgNPs was placed on a carbon-coated copper grid. Excess solution was removed using blotting paper. SEM and EDX analyses of the formed AgNPs were done using a Carl Zeiss, EVO, MA15 instrument. For TEM studies, 25 μl of AgNPs was placed on carbon-coated copper grids and the images of nanoparticles were studied using TEM (JEOL JEM-1400 Orius SC600A camera with digital micrograph).

3. Results and discussion

Reduction of $AgNO_3$ was visually evident from the color change (brownish-yellow) of the reaction mixture after 48 h. The intensity of the brown color increased in direct proportion to the incubation period. This may be due to the excitation of the Surface Plasmon Resonance (SPR) effect and the reduction of $AgNO_3$ (Mulvaney, 1996). The control $AgNO_3$ solution (without seaweed extract) showed no color change. The silver nanoparticles obtained were characterized by UV–visible spectroscopy and the characteristic absorption peaks at 422 and 425 nm for fresh and dried *C. capitatum* in the spectrum confirmed the formation of silver nanoparticles. In the present study, the color intensity at 422 nm increased with incubation time. This is similar to the surface plasmon vibrations with characteristic peaks of silver nanoparticles prepared by chemical reduction (Kong and Jang, 2006; Petit et al., 1993).

A transmission electron microscope was employed to analyze the structure of the nanoparticles that were formed. The AgNPs coalesced into nano-clusters (Fig. 1). When the reaction mixtures were incubated for 48 h, some nanoparticles aggregated. The size of the nanoparticles synthesized in the present study by *C. capitatum* biomass varied from 3 to 44 nm with an average of ~30 nm. Jain et al. (2009) reported particle sizes of 25–50 nm in a cubic structure synthesized by a papaya fruit extract.

A scanning electron microscope with energy dispersive X-ray spectrometer (SEM-EDX) was employed to determine the silver concentration of the nanoparticles. AgNPs generally show a typical absorption peak at approximately 3 keV due to the surface plasma resonance phenomenon (Prasad et al., 2012). From the EDX analysis (Fig. 2), the distinct peak detected at 3 keV confirmed the presence of elemental silver in the nanoparticles. A silver concentration of $63.4 \pm 1.6\%$ in the fresh algae and $55.4 \pm 0.9\%$ in the dried algae was detected after incubation for 48 h.

FTIR measurements were carried out to identify the possible biomolecules responsible for the stabilization of the newly synthesized AgNPs. Fig. 3 represents the FTIR spectrum of fresh and dried *C. capitatum* extract which showed peaks at 1023, 1076, 1148, 1227, 1509, 1535, 1542, 1630, 1638, 1654 cm^{-1} and dried *C. capitatum* which showed peaks at 623, 1056, 1235, 1535, 1630, 2920 cm^{-1}. The FTIR spectra of Ag nanoparticles obtained from both fresh and dried *C. capitatum* extract showed a strong transmission band at 1535 cm^{-1} corresponding to the bending vibration of secondary amines of proteins. Another band observed at 1630 cm^{-1} was assigned to the stretching vibration of the (NH) C═O group. After reduction of $AgNO_3$ the decrease in intensity at 1535 cm^{-1} signified the involvement of secondary amines in the reduction process. The shift of the band from 1630 cm^{-1} was attributed to the binding of a (NH) C═O group with the nanoparticles. The (NH) C═O groups within the cage of cyclic peptides are involved in stabilizing the nanoparticles. The shift of (NH) C═O band was quite small. Thus the peptides may play a major role in the reduction of Ag^+ to Ag nanoparticles.

The band at 1023 cm^{-1} can be assigned to absorption peaks of –C–O–C. (Huang et al., 2007; Philip, 2009a,b; Shankar et al., 2003). A strong IR band at 1509 cm^{-1} in the spectrum of silver nanoparticle is due to the stretching vibrations of the C═C chain (Schulz and Baranska, 2007). The distinct band at 1654 cm^{-1} represents the involvement of C═N in plane vibrations of amino acids and 1023–1227 cm^{-1} represents the involvement of C–N in plane vibrations of aliphatic amines. The above bonds commonly occur in proteins indicating the presence of proteins as ligands for silver nanoparticles, which increases the stability of the nanoparticles synthesized (Jacob et al., 2012). A characteristic peak at 1227 cm^{-1} was noticed in the nanoparticles obtained from the fresh seaweed extract and this may be due to the asymmetric stretching vibration of sulfate groups commonly available in seaweeds in the form of sulfated polysaccharides which are used for the stabilization of silver nanoparticles (Kloareg and Quatrano, 1988; Witvrouw and De Clercq, 1997). This is in agreement with Venkatpurwar and Pokharkar's (2011) who reported that sulfated polysaccharides isolated from the marine alga *Porphyra vietnamensis* (Rhodophyta) had a strong ability to synthesize silver nanoparticles, coherent with earlier reports as the genus *Codium* is well known to contain sulfated polysaccharides (Matsubara et al., 2001; Shiddhanta et al., 1999).

There are several methods for the preparation of AgNPs such as citric acid reduction, electrochemical synthesis, photochemistry and radiation reaction. However, silver nanoparticles prepared from these methods can only be kept for a maximum of 3 months (Song et al., 2009). Many experiments have shown that the growth of silver nanoparticles in solution is sensitive to the presence of citric acid or sodium citrate but the exact role of citric acid is still unidentified. Recenly, Jiang et al. (2009) reported that using citric acid as a reducing agent,

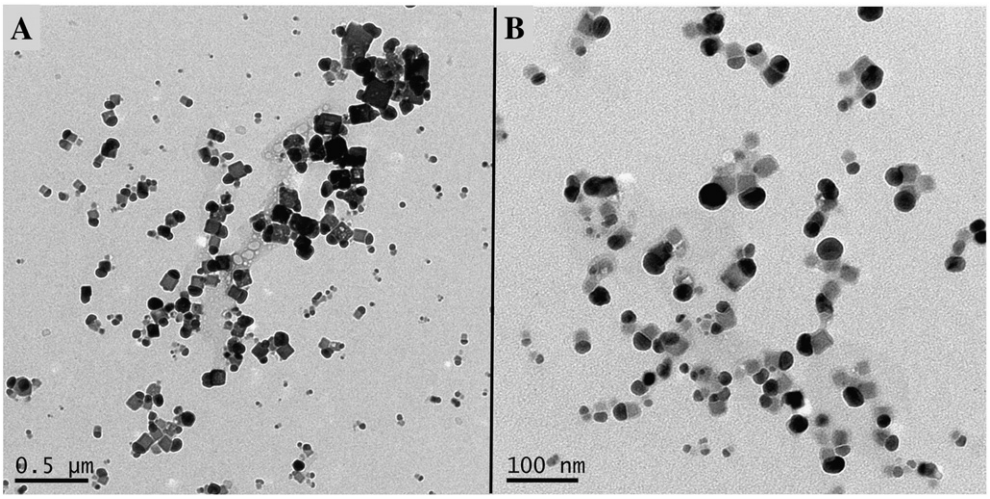

Fig. 1. TEM images of silver nanoparticles synthesized from fresh *Codium capitatum* extract at different magnifications.

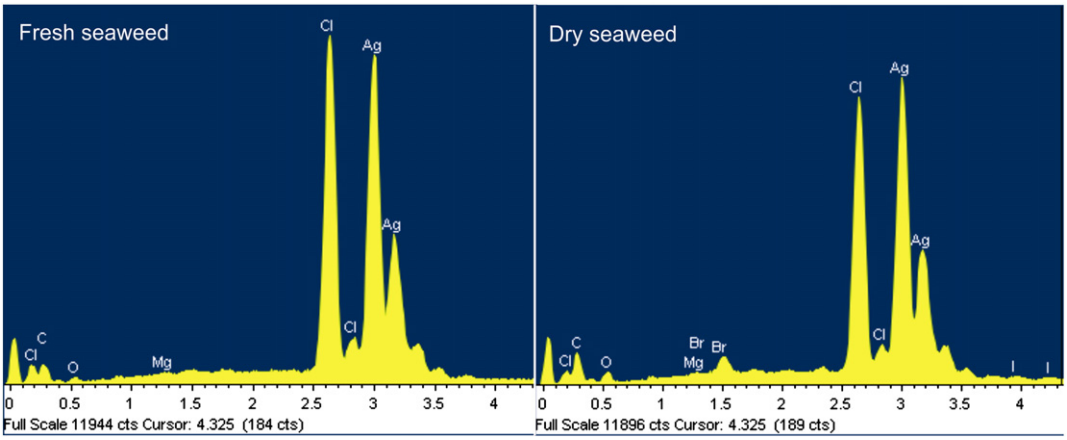

Fig. 2. EDX spectrum of synthesized silver nanoparticles using fresh and dried *Codium capitatum*.

made the reduction reaction take one week longer and led to a wide size distribution. They also reported that the use of sodium citrate did not result in the formation of silver nanoparticles. As far as stabilizers are concerned, citrate ions can bind on silver surfaces for shape control, but their ability to stabilize silver particles is weaker than thiols (Ramteke et al., 2013). Nowadays, a molecule which can act both as a reducing and capping agent is preferred so that the reaction takes place in one step and there is no need for an external reducing agent. This will reduce the number of steps involved for metal nanoparticle synthesis. In this study, amine, peptide and sulfate groups present in the *C. capitatum*

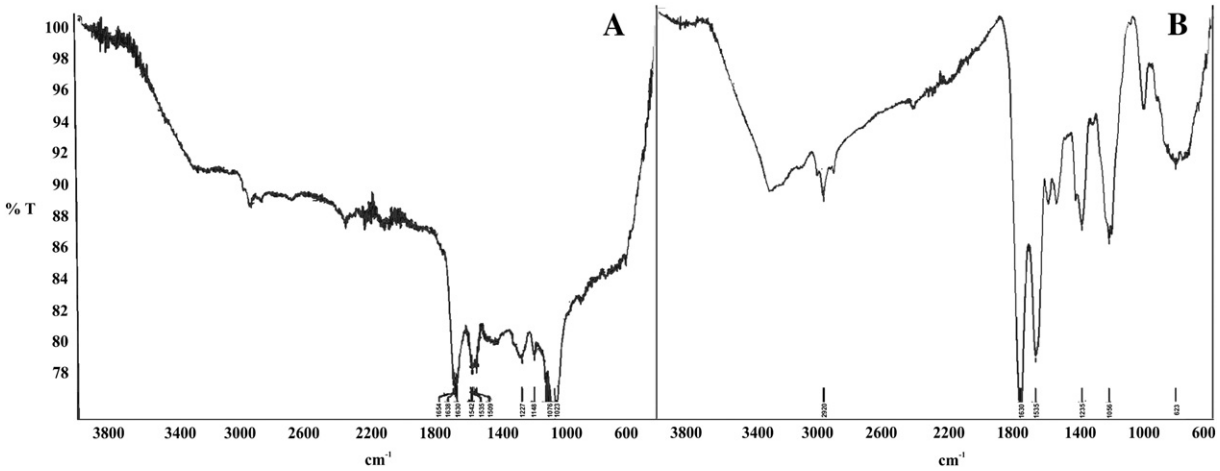

Fig. 3. FTIR spectrum of silver nanoparticles synthesized by A) fresh and B) dried *Codium capitatum* extract.

extract appeared to be involved in the bioreduction and stabilization of AgNP, and Ramteke et al. (2013) reported that the nanoparticles synthesized using plant extracts exhibited excellent stability for six months.

4. Conclusions

In this investigation, the environmental friendly synthesis of AgNPs using fresh and dried extracts of the green seaweed *C. capitatum* is described. The amine, peptide and sulfate groups present in the *C. capitatum* extract are apparently involved in the bioreduction and stabilization of AgNP. Compared to the dried extract, the fresh extract has more potential for the synthesis of nanoparticles. Owing to their abundance and ready availability, seaweeds are good and cost-effective sources for the synthesis of metallic nanoparticles. They have been less studied and exploited in comparison to terrestrial plants and microbes. The present study is the first report on using seaweed from the widespread *Codium* genus for the production of AgNPs. This method of AgNPs synthesis does not use any toxic reagents and thus has potential for use in biomedical and agricultural applications.

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