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Biosynthesis of silver nanoparticles using brown marine seaweed *Padina boeregeseni* and evaluation of physico-chemical factors

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Present investigation consists the green nanoparticles prepared by using both fresh and dry marine macroalga of *Padina boeregeseni* was reported and the physical and chemical factors such as time duration, pH, various mixing ratios of aqueous extract to $AgNO_3$ solution and temperature that play vital role in the nanoparticles synthesis were assess. Maximum synthesis of silver nanoparticles was attained within 30 min at pH 8.5, 70°C and 1mM $AgNO_3$. Characterization of silver nanoparticles was carried out based on UV-Vis spectrophotometry (418 nm). The size of nanoparticles synthesis with average of 43.3 nm was confirmed by scanning electron microscopy (SEM). X-ray diffraction (XRD) crystallography illustrated the silver nanoparticles crystalline nature. Fourier transform infrared spectroscopy (FTIR) shows that the function groups are hydroxyl, carbonyl, amine and phenol compounds of extract *P.boeregeseni* are involved in the reduction of aqueous $AgNO_3$. This method of Ag-NPs synthesis does not use any toxic reagents and thus has potential for use in biomedical and agricultural application.

[**Keywords:** Green synthesis, Silver nanoparticles, *Padina boeregeseni*, SEM, XRD]

Introduction

Nanotechnology is one of the most striking areas of investigation in modern material science[1]. Bionanotechnology has emerged as the integration between biotechnology and nanotechnology for developing biosynthetic and environmental-friendly technology for synthesis of nanomaterials[2-4]. There is a growing need to environmentally benign nanoparticle synthesis process that does not use toxic chemicals in the synthesis protocols[5]. The nanoparticles exhibit large surface volume ratio and to the properties of nanoparticles better than bulk particles[6]. The metallic nanoparticles are mostly prepared from metal such as Au, Pt, Pd and Ag synthesized by various methods such as physical, chemical and biological methods[7]. Physically and chemically mediated syntheses necessitate high pressure, energy, temperature, high cost and toxicity[8].

Synthesis of silver nanoparticles by biological method can use bacteria[9], fungi[10], algae[11], enzymes[12] and plant extract[13]. Among nanoparticles, silver nanoparticles have received major attention due to their unique and tunable surface Plasmon resonance (SPR). Also silver nanoparticles are owing to their distinctive optical, electrical, thermal and electromagnetic properties[14]. Compared to physical and chemical synthesis, plant-mediated synthesis of nanoparticles has attracted the attention of researches extensively. All these investigation are restricted to terrestrial plants, but only some reports are available for synthesis of nanoparticles from marine plants[15]. Green synthesis of silver nanoparticles by algae extract provides more advantageous over other biological processes which use bacteria and fungi, because it eliminates the cell culture maintaining stage, and also it is more suitable for large scale production of silver nanoparticles[16]. Silver has long been recognized as an antimicrobial agent in medical and industrial processes[17]. The most important application of silver and silver nanoparticles is in medical industry such as in topical ointments to prevent infection of burns and open wounds[18]. Also medical devices and implants can be prepared with silver-impregnated polymers[19] and antibiotics[20].The antimicrobial activity of silver nanoparticles mainly depends on the size property. The small-sized nanoparticles have large surface area to improve the antimicrobial activity in addition to increase chemical stability[21].

The biosynthesis of $AgNO_3$ using marine algae *P.boeregeseni* extract was implemented in this study. Amongst different field of bioreductants, seaweeds have distinct advantages due to their high metal uptake capacity, low cost and macroscopic structure[22]. Fast formation of Ag-NPs through extracellular biosynthesis has become possible red, brown and green seaweeds (*Sargassum muticum*) [23], (*Gracilaria dura*) [24], *(Kappaphycus alvarezii*) [25]. The major components were found include amines groups, alcohols, phenols, carbonyl and secondary metabolites[26]. Nanoparticles of metal have potential applications in catalysis, biological labeling, and detection of genetic disorders. Research is a crucial stage to combat against new and emerging diseases[27]. A sustained forward-thinking applied research programme would microbial foes, and evaluates the preventive power of new approaches. Seaweeds may be an answer to unsolved and growing problem of antibiotic resistance, and a potential source to cure infectious diseases[28].

Materials and Methods

P.boeregeseni a brown marine algae, was collected from the intertidal region at (Lat. 25° 17′: Long. 60°39′) located on the Iran's southern coast in sea of Oman. Thoroughly washed in tap water to remove epiphytes and detritus attached to the fronds and then brought to the laboratory in an ice box. The cleaned marine algae with Deionized water were freeze dried at -20°C and then crushed into powder and stored at 4°C for future use. When needed, the dried finely powdered *P.boeregeseni* (10 g) was boiled with 100 ml of deionized water at 70°C for 20 min. The extract obtained was followed by Millipore filter (0.45 µm) and used within a week at 4°C for further experiments.

In addition, fresh seaweed was washed thoroughly with distilled water and then washes with deionised water for 5 min in order to remove extraneous materials. 40 g fresh *P.boeregeseni* boiled in 100 ml deionised water for 15 min. The extracts was filter through mesh, followed by Millipore filter (0.45 µm), and stored at 4°C before use. Silver nitrate ($AgNO_3$) was purchased from Merck, Germany. Deionized water was use for all working and standard solution.

20 ml fresh and dry seaweed aqueous extract was mixed with 100 ml $AgNO_3$ solution 1mM in a 250 ml Erlenmeyrer flask and incubated in the dark at temperature room for 24 h. Thereafter, the solution were centrifuged at 12000 rpm for 13 min at 4°C and then redispersed with same aliquot of deionised water and the purified silver particles were freeze dried and stored and stored at 4°C for further use characterization.

The reduction of $AgNO_3$ was monitored using UV-Visible spectrophotometer in the different wavelength range of 350- 600 nm (Cary 100 cone Varian USA UV-Visible spectrophotometer). It is one of the important techniques to verify the formation of metal nanoparticles provided surface Plasmon resonance exists for the metal[29]. Appearance of color arises from the property of the colored material to absorb selectively within the visible region of the electromagnetic spectrum. To detect silver nanopaticles at the absorption range of 400-450 nm[30]. The presence of bioactive functional groups responsible in seaweed extract and synthesized Ag-NPs Recorded by using FTIR spectrometer (Thermo Nicolet, Nexus 870, USA FTIR) at a resolution of 4.0 cm^{-1} range from 400 to 4000 cm^{-1} in KBr pellet. The crystal nature of the silver nanoparticles was determined using XRD. The patterns of Ag-NPs were recorded using STADI MP STOE diffractometer with Cu-Kα radiation (λ=1.5406 A°) at a voltage of 40 kV and a current 30 mA. Morphology and size of Ag-NPs were characterized by using Scanning Electron Microscope (Vega Π XMU Tescam SEM).

To research the effect of incubation time on nanoparticles synthesis, the reaction solution was incubated at 30, 60, 90 and 120 min. To study the effect of pH, experiments were carried out by varying the pH (4.5, 5.5, 7.5 and 8.5) of the reaction solution. The reaction temperature was maintained at 25°C, 40°C ,70°C and 90°C. *P.boeregeseni* aqueous extract was mixed with 1mM silver nitrate ($AgNO_3$) solution at different mixing ratio (1:4, 3:7, 1:9 and 1:5). Absorbance of the resulting solution was measured by UV-Vis spectrophotometer at different wavelengths.

Results and Discussion

Synthesis of silver nanoparticles was confirmed by visual inspection of color changing in aqueous solution. The reaction mixture turned to brown color from brownish-yellow color within 24 h at 25°C indicated demonstrated the synthesis of silver nanoparticles (Fig. 1). The appearance of brown color was due to excitation of surface Plasmon vibration (SPR) effect and the reduction of $AgNO_3$[31].

The silver nanoparticles obtained were characterized by UV-Visible spectrophotometer at

418 and 413 nm for fresh and dried *P.boeregeseni* in the spectrum confirmed of Ag-NPs (Fig. 2).

This is similar to the surface plasma vibration with characteristic peaks of silver nanoparticles.

Nanoparticles formation was dependent on the different physical and chemical factors such as metal ion concentration, incubation time, pH and temperature[32]. In present investigation, we report effect factors: time duration, pH, temperature and aqueous extract mixed with $AgNO_3$ solution at ratio different that play vital role in the nanoparticles synthesis. The first factor is time duration of reaction time, the nanoparticles formation was increased while increasing time reduction (Fig. 3). Similar results are obtained using marine brown algae *Sargassum wightii* with different time[33].

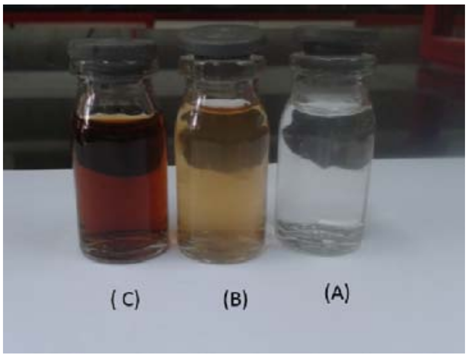


Fig. 1—(A) silver nitrate ($AgNO_3$) solution, (B) aqueous extract of *P.boeregeseni* and (C) after the 24 h reduction of Silver nitrate.

The pH is important factor for nanoparticles synthesis. Morphology of Ag-NPs are dependent on the pH of solution, the acidic pH 4.5 shows the peak week at 407 nm and absorbance band was increased while increasing pH up to 8.5 due to the excitation of surface Plasmon resonance. The alkaline pH 8.5 absorbance peak was at 418 nm. Therefore acidic pH defeats the nanoparticle formation. The color change was at pH 4.5 in 40 min and at pH 8.5 in 20 min. At low pH, accumulation happened since of the over nucleation and formation of large nanoparticles. At high pH, a large number of nanoparticles with the small surface area are present due to the bioavailability of functional groups in the seaweed extract[34]. As a result pH 8.5 was optimum for the nanoparticles formation (Fig. 4).

Temperature is one of the most important factors for nanoparticle formation. Fig. 5 showed temperature with range 25 to 90°C. While increasing temperature, the rate of formation of silver nanoparticle also increased rapidly. Time range color at 25°C for 24 h, while at 90°C was 10 min. The measure of the nanoparticle decreased initially due to the reduction in accumulation of the growing nanoparticles. As rising temperature after 70°C, the absorption pattern decreased.

In this study, different mixtures of seaweed and AgNO3 solution with the ratios of 1:4, 1:5, 1:9 and 3:7 were evaluated according to the color change and UV-Vis absorption in the similar time and temperature condition. Figure 6 shows that the 1:5 ratio has the maximum of absorption. Therefore the volume ratio of concentrated seaweed and decreasing solution of AgNO3 can significantly affect production of silver nanoparticles.

The spectra of the biomolecules involved in the reduction and of silver nanoparticles were studied by FTIR analysis of the biomass before and after reduction of nanoparticles (Fig. 7).

Figure 7a shoes some bands in the FTIR spectrum of *P.boeregeseni* extract in the range of 3332.07, 2936.86, 2339.86, 2094.85, 1936.28, 1651.68, 1421.17, 1082.14, 863.20, 795.15, 616.86 cm^{-1}. An absorption peak at 3332.07 cm^{-1} corresponded of phenols, alcohols with free O−H group. The peak at 1082.14 cm^{-1} indicated C−O bend that verification is the O−H group. A signal weak at 2936.56 cm^{-1} shows to C−H stretch alkanes and O−H stretch carboxylic acids[35]. The peak at 1651.68 cm^{-1} belongs to carbonyl groups and amides. The band 1421.17 cm^{-1} corresponds to O−H bend indicated carboxylate[36]. The peak at 616.86 cm^{-1} was assigned for C−Cl stretching vibration of alkyl halides.

FTIR analysis of silver nanoparticles (Fig. 7b) showed different bands at 3439.58, 2963.33, 2854.92, 1641.62, 1262.70, 1093.49, 1025.38, 803.21 cm^{-1}. The band at 3439.58 cm^{-1} is the characteristic of the hydroxyl functional group in alcohol and phenol compounds. Both the peaks at 2854.92 and 2916.38 cm^{-1} can be due to carbonyl

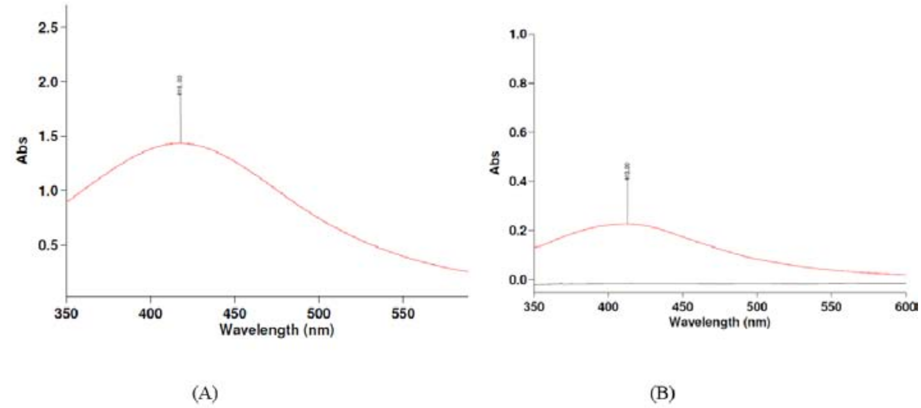


Fig. 2—UV-Vis absorbance of aqueous solution of 1mM $AgNO_3$ with *P.boeregeseni* extract (A) fresh extract; (B) dry extract

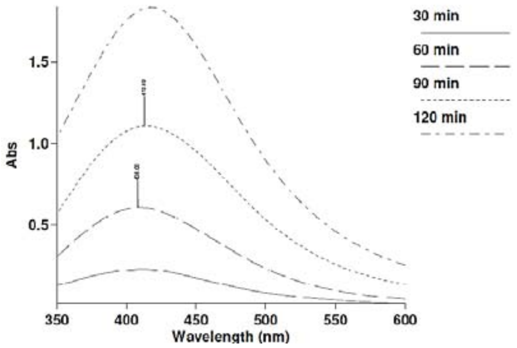


Fig. 3—UV-Visible absorption spectra of silver nanoparticles with different incubation time

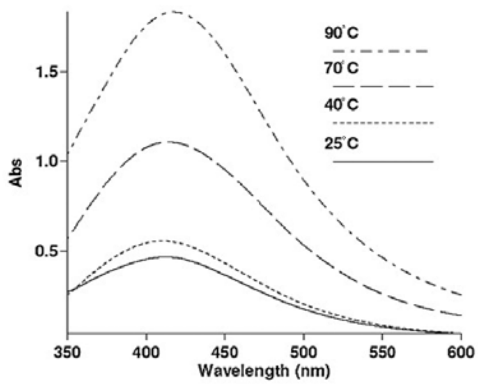


Fig. 5—UV-Vis spectrum of silver nanoparticles at different temperatures after 2 h of reaction time

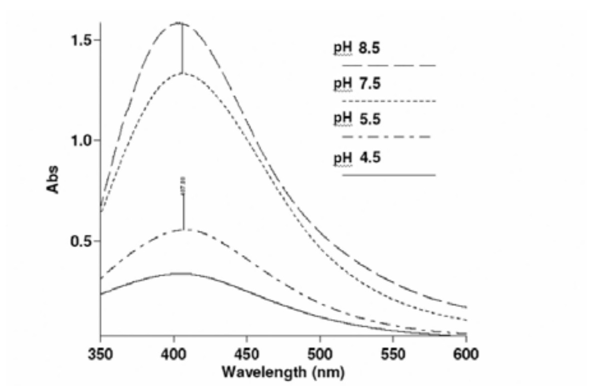


Fig. 4—Effect of reaction pH on the production of silver nanoparticles

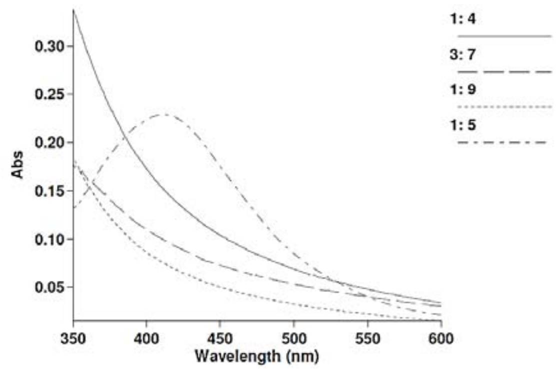
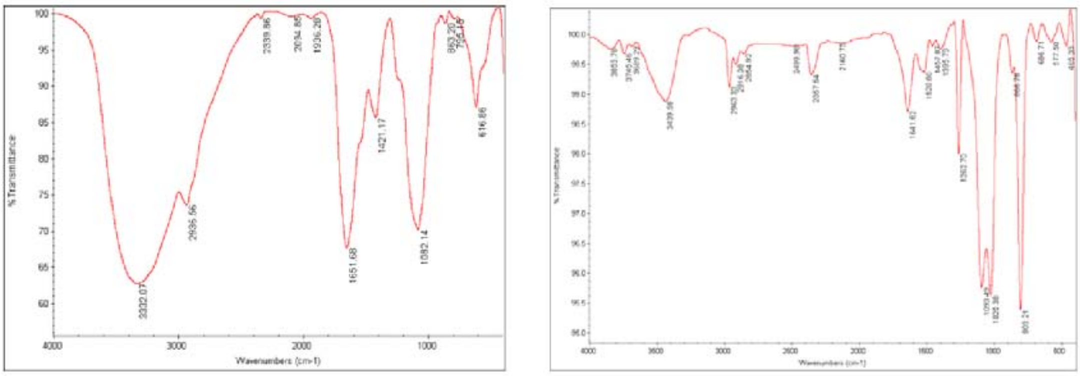


Fig. 6—Effect of various mixing ratios of aqueous extract to $AgNO_3$ solution at 40°C, 2 h



a b

Fig. 7—FTIR spectrum for (a) the *P.boeregeseni* aqueous extract; and (b) *P.boeregeseni* formed Ag-NPs

group and secondary amines. It also shows C−H stretching for alkanes. The peaks at 1641.62 cm^{-1} indicate the carbonyl group, amines and amides. The band shift in the hydroxyl groups and carbonyl groups in FTIR spectra confirm the oxidation of amines, amides, alcohols, phenol and carboxylic acids. Our report is in similar with Manoj Singh[37].

XRD shows the typical pattern of silver nanoparticles synthesized using the marine algae *P.boeregeseni* are crystalline with small size (Fig. 8). Almost eleven different peaks were observed in the spectra at 2θ values of 27.5°, 33.1°, 38.2°, 46.5°, 54.8°, 57.6°, 77.2° reflection of both silver (Ag) and silver oxide (Ag_2O). A peak at 2θ = 38.2° represents the formation of pure silver (Ag) at the start of the reaction. The formation of Ag_2O may be due to the coupling reaction with alcohol (O-H) groups. A similar result was recorded by Kumar et al. 2013[14]. The size of formed silver nanoparticles was estimated by using Scherrer's equation[39] by determining the width of the 33.1° Bragg reflection.

Scanning electron microscopy has provided further insight into the morphology and size detail of silver nanoparticles. This image shows that the spherical structures and particle size between 34.62 and 54.33 nm (Fig. 9) with average size of 43.3 nm. The SEM analysis of silver nanoprticles of *P.boeregeseni* is similar to the results of silver nanoparticles synthesized from the seaweed *Codium capitatum*[40].

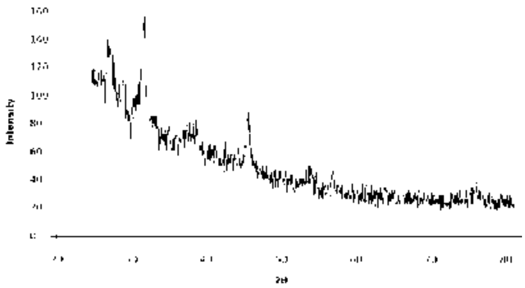


Fig. 8—XRD patterns of synthesized silver nanoparticles

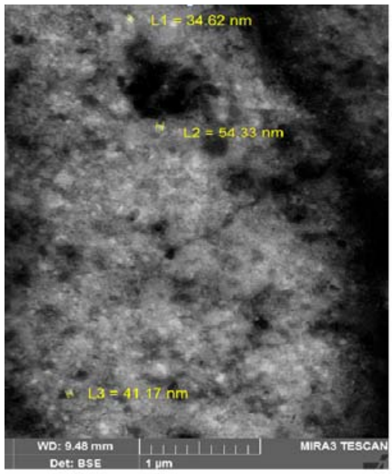


Fig. 9—SEM image of synthesized silver nanoparticles using *P.boeregeseni*

Conclusion

The present study is the first report which used seaweed from *P.boeregeseni* for the production of Ag-NPs. The formation of nanoparticles was observed within 30 min at 70°C and 10 min at 90°C. Our research showed that by increased pH and temperature influence the rapid synthesis of Ag-NPs and also with increasing temperature more than 90°C, accumulation of nanoparticles will take place.

In addition this research determined that, the fresh extract has more potential for the synthesis of nanoparticles compared to the dried extract. SEM images showed spherical structures and the average size of 43.3 nm for nanoparticles and the crystalline nature of nanoparticles was confirmed by XRD pattern.

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