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Green Synthesis of Silver Nanoparticles using *Ulva flexousa* from the Persian Gulf, Iran

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Abstract

The present study focused on the synthesis of silver nanoparticles using the aqueous extract of *Ulva flexousa* (green seaweed). Ten ml of the aqueous extract of alga was mixed with 90 ml of 1mM aqueous AgNO3 solution for reducing of Ag+ ions. The synthesized silver nanoparticles were characterized by UV-Vis spectra, XRD (X-ray diffraction), TEM (Transmission electron microscopy) and FT-IR (Fourier transform infrared spectroscopy) analysis. The formation of silver nanoparticles was confirmed by UV-Vis spectra showed the absorbance peak values at 430nm. XRD pattern showed particles were crystalline in nature. TEM analysis, showed silver nanoparticles were circular in shape with maximum particles in size range within 2–32 nm with mean diameter of 15 ± 1.5 nm. Therefore, *U. flexousa* exhibited the potential production of silver nanoparticles after 60 min. of incubation in illuminated room condition. Thus, the approach of algae-mediated synthesis appeared to be safe, eco-friendly and good alternative to conventional methods of silver nanoparticles synthesis.

Keywords: *Green synthesis, Silver nanoparticles, Ulva flexousa, Persian Gulf*

1. Introduction

Bio-Nanotechnology is a new and rapidly advancing field of research lies at the interface between biology and nanotechnology (Sahayaraj and Rajesh, 2011). The field of Nanotechnology is a science and technology to control at the nanoscale level (Bala and Arya., 2013). Nanotechnology and nanoscience could be used across all the other science fields, such as chemistry, biology, physics, material sciences, and engineering (Rajasekharreddy *et al*., 2009). The fabrication and application of materials at the nanometer scale in biology is a great concern in the field of nanotechnology (Safekordi *et al*., 2011). Nanomaterials are usually described particles with at least one dimension less than 100 nm (El-Sheikh *et al*., 2013).

Currently, metallic nanomaterials are widely used in various fields due to their unique potential applications such as optics, energy science, biomedicine (Singhal *et al*., 2011), biosensing, biological labeling and DNA sequencing (Thangaraju *et al*., 2012).

Out of all kinds of nanoparticles, silver nanoparticles (AgNPs) have attracted the interests of scientists because of their advantageous characteristics in antimicrobial and anticancer activities (Thangaraju *et al*., 2012), forensic science, cosmetics (Vanaja and Annadurai, 2012), paints, food packing and textile

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industry (Jagtap and Bapat., 2012). They have also vital importance as therapeutic agent for epilepsy, nicotine addiction, gastroenteritis, stomatitis and sexually transmitted diseases (Kumar *et al*., 2012).

Customarily, different approaches including chemical and physical methods, photochemical reactions in reverse micelles, electrochemical techniques and biological methods (Jegadeeswaran *et al*., 2012) have been used for the production of metal nanoparticles such as Ag, Au, Pt and Pd (Krishnaraj *et al*., 2010).

Among the various known synthesis methods, physical and chemical methods need toxic chemicals, (Ingle *et al*., 2009) high pressure, energy and temperature (Gnanadesigan *et al*., 2011).They are also expensive (Nabikhan *et al*., 2010) and flammable (Sahayaraj and Rajesh, 2011) with disadvantages effects to the environment (Jagtap and Bapat, 2012). Biological method, in comparison, is safe, clean, (Jagtap and Bapat, 2012) cost-effective (Suriya *et al*., 2012) eco-friendliness and biocompatibility (Vanaja and Annadurai, 2012). With no requirement to high pressure, energy, temperature and toxic chemicals (Rajeshkumar *et al*., 2013), green – mediated synthesis of metal nanoparticles using diatoms (Govindaraju *et al*., 2008), fungi (Krishnaraj *et al*., 2010), seaweed (Noruzi *et al*., 2011), plants and plant extracts (Bala and Arya, 2013) (for instance) could be a possible alternative over physical and chemical methods (Jagtap and Bapat, 2012).

Among the marine organisms, marine algae (in particular, seaweeds) are important source of phytochemical compounds such as, carbohydrates, alkaloids, steroids, phenols, protein and flavonoids that are used to reduce Ag+ ions for the synthesis of silver nanoparticles. (Kokabi et al., 2013; Rajeshkumar et al., 2013). There are several reports on the synthesis of silver nanoparticles using seaweeds, including *Urospora sp*. (Suriya *et al*., 2012), *Sargassum tenerrimum* (Kumar *et al*., 2012), *Sargassum polycytum* (Thangaraju *et al*., 2012), *Padina tetrastromatica* (Jegadeeswaran et al., 2012) etc. Since, there was no report on the synthesis of silver nanoparticles utilizing *Ulva flexuosa* (wulfen) J. Agardh, the synthesis of silver nanoparticles by reduction of silver ions present in the aqueous solution of silver nitrate ($AgNO_3$) using *U. flexousa* was undertaken.

Ulva flexuosa (wulfen) (class: Chlorophaceae, order: ulvales, family: Ulvaceae, Genus: *Ulva*, species: *flexuosa* has thalli form light to dark and its cells are square to rectangular in surface view. The species grows on rocky substrate attached to stones and dead corals at the intertidal zone. Thalli form thick tufts about 2-3 cm high on substratum (Gavino and Trono, 2003).

2. Materials and Methods

2.1. Sample collection and preparation

U. flexousa samples were collected from the intertidal region of Qeshm Island, the Persion Gulf, Iran and immediately transported to the laboratory in polythene bags containing natural seawater to prevent evaporation (Figure 1).

Fig 1: Image of *Ulva flexuosa* in natural habitat

They were washed several times with tap water and then twice with distilled water to remove the epiphytes and unwanted materials. After cleaning, the fresh algae were shade-dried at room temperature for a week. Dried seaweeds were powdered with the help of mixer grinder.

2.2. Preparation of seaweed extract

Ten grams of *U. flexousa* powder were mixed with 200 ml deionized water in a 500 ml Erlenmeyer flask and boiled at 100 C° for 30 min. The broth

extract was filtered through Whatman filter paper No.1 and kept at 4 C° for further use.

2.3. Synthesis of Silver Nanoparticles

In the typical synthesis of silver nanoparticles, 10 ml of the aqueous extract of alga was mixed with 90 ml of 1mM aqueous $AgNO_3$ solution to bring final volume to 100 ml for reduction of Ag^+ ions. The reaction mixture was incubated at the illuminated room condition until the color change was arisen for 24 h.

2.4. Characterization of Nanoparticles

The synthesis of silver ions in aqueous solution was confirmed both measuring the change of color through visual observation and absorption measurement by UV-Vis spectra at regular intervals (0-24h) at wavelength between 200 to 600 nm. Deionized water was used as blank for all measurements. X-ray diffractometer (XRD) [CuK_α radiation (λ=1.54 nm) with the scanning 2θ angle ranging from 20 to 80 degree by Step Size 0.0390, Generator Settings: 40 mA, 40 kV, Model: X'Pert PRO] is used for characterization of nanoparticles crystalline. Transmission Electron Microscopic (TEM) analysis was done by Philips, CM-30 for characterizing size and shape of synthesized silver nanoparticles. The sample was first sonicated for 15 min. A drop of the sample was placed on the carbon coated copper grid, making a thin film of sample on the grid and an extra sample was removed using a cone of blotting paper, and kept in a grid box sequentially. To remove any free biomass residue or compound that is not the capping ligand of the nanoparticles, the residual solution of 100 ml after reaction was centrifuged at 5000 rpm for 10 min. The supernatant was again centrifuged at 10000 rpm for 60 min and the pellet was obtained. This was followed by re-dispersion of the pellet of Ag-NPs into 1 ml of deionized water. Thereafter, the purified suspension was freeze-dried to obtain dried powder. Finally, the dried nanoparticles were analyzed by FTIR Nicolet IR100 (Nicolet, USA).

3. Results

In this regard, *U. flesuosa* (green alga) demonstrated to be a good biological constituent in the right direction for synthesis of silver nanoparticles (fig. 1). Silver nanoparticles were formed by the reduction of Ag+ ions with the addition of seaweed extraction to the solution of 1 mM $AgNO_3$. When 10 ml seaweed extract was added to 90 ml 1 mM silver nitrate solution, the reaction started after 60 min of incubation in light room condition. The color transformation from green to dark- brown of the solution was due to excitation of surface plasmon vibrations in the silver metal nanoparticles. The control $AgNO_3$ solution (without seaweed extract) showed no color change (Fig. 2).

Fig 2: Change of color by addition of silver nitrate

The results obtained with UV- Vis spectra of the silver nanoparticles synthesized from the extract of the seaweed, *U. flexousa* presented in (figure 3).

The reduction of Ag+ ions using aqueous extract *U. flexousa* was monitored by spectrophotometer in a range of wavelength from 200 to 600 nm. This detected a peak at 430 nm where indicating of the surface plasmon resonance (SPR) phenomenon. The observed band in this range has been associated with Ag nanoparticles confirming the synthesis of spherical Ag nanoparticles with narrow size distribution (Kumar *et al*., 2012).

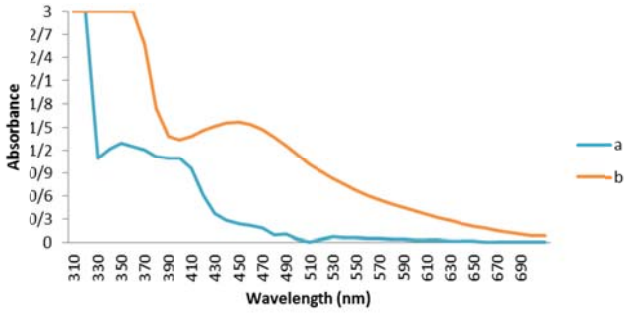

Fig 3: UV-Vis Spectra of aqueous extract alone showed SPR peak at 320-340 nm (a), aqueous extract reduced silver nanoparticles SPR peak at 430nm (b). The figure inset shows extract alone (a) and synthesized silver nanoparticles (b).

XRD is a technique widely used to estimate the size of nanoparticles in the range between 1 to 100 nm, because of the commonly used x-ray's wavelength (Suriya *et al*., 2012). XRD analysis of the nanoparticles showed intense peaks, corresponding to (111), (200), (220) and (311) Bragg reflection, based on the face-centered cubic (fcc) structure of Ag nanoparticles, with a lattice constant of a = 4.086 Å (Figure 4).

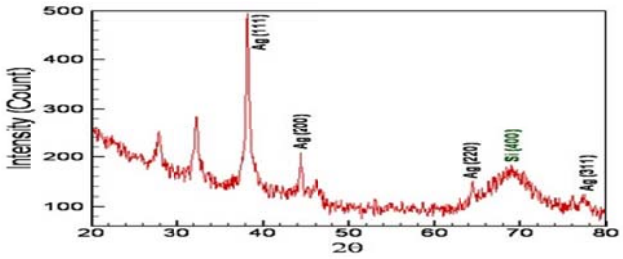

Fig 4: XRD pattern of Ag nanoparticles deposited on Si substrate

The mean particle diameter of Ag nanoparticles was calculated from the XRD pattern, according to the line width of the maximum intensity reflection peak. The size of the nanoparticles was calculated through the Scherer equation:

$D = (0.9\ \lambda)/(\beta c \cos \theta)$, and $\beta c = (\beta 2s - \beta 2r)1/2$,

Where D is the average crystal size, λ is the X-ray wavelength (λ = 1.5406 Å), °2θ is Bragg's angle, βc is the corrected full width at half maximum (FWHM) in radians, and β_s and β_r are the FWHM of the reference and sample peaks, respectively. In our experiment, the FWHM of reference was equal to 0.0899.

The average crystal size of the silver crystallites was calculated from the FWHMs of the diffraction peaks, using the Scherer equation. The size of crystallite in different planes of silver was determined as 13.8, 40.0 and 59.2 nm with the mean value of all three peaks as 37.6 nm.

TEM is powerful method to determine the size and shape of nanoparticles. The TEM images of AgNPs formed from aqueous $AgNO_3$ and seaweed extract after 24h at room temperature are showed in Figure 5. In all these spectra, there were no peaks located around 335 and 560 nm, indicating the complete absence of nanoparticles aggregation (Suriya *et al*., 2012).

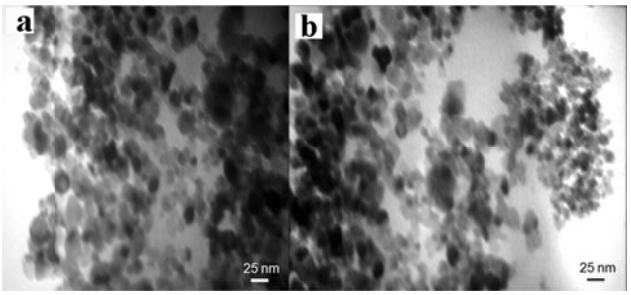

Fig 5: TEM images of Ag nanoparticles synthesized by *U. flexuosa* extract at 25 nm scale.

The nanoparticles were circular in shape with maximum particles in size range within 2–32 nm with mean diameter of 15 ± 1.5 nm. It was also observed that silver nanoparticles were evenly distributed in the sample. Particle size distribution histogram determined from TEM is shown in Figure 6.

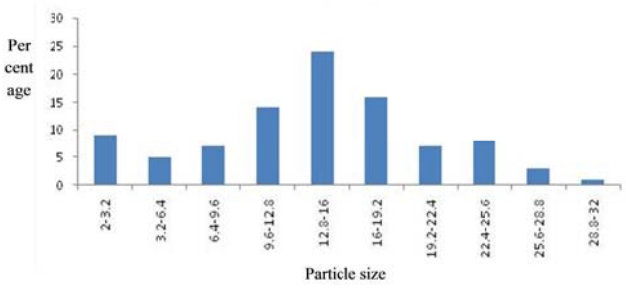

Fig 6: Particle size histogram of biosynthesized by *U. flexuosa* extract

FTIR analysis were carried out to identify the possible biomolecule responsible for the reduction of the silver ion and capping agent of bio-reduced silver nanoparticles synthesized by the seaweed *U. flexuosa*. Intense FT-IR bands were observed at 3418 cm^{-1}, 2921 cm^{-1}, 1639 cm^{-1} and 1424 cm^{-1}, which indicated the presence of molecular functional groups that are responsible for the reduction of silver ions (Figure 7).

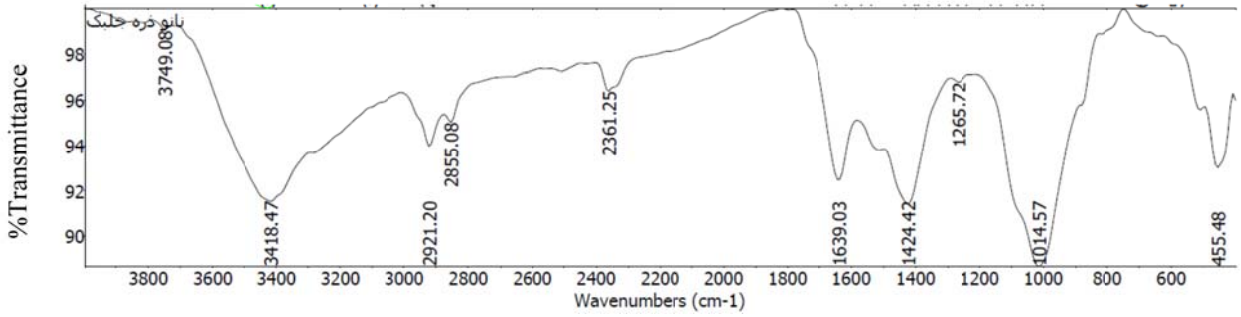

Fig 7: FTIR pattern of biosynthesized silver nanoparticles.

The spectrum of IR peak at 3418 cm^{-1} was referred as the strong stretching vibrations of N-H functional group. The corresponding peaks at 2921 cm^{-1} due to the C-H vibrational mode. The vibrational mode at 1424 cm^{-1}corresponds to C=C variables present in the plant protein. On the other hand, the shift of band from 1639 cm^{-1} was attributed to the binding of C=O group with nanoparticles. Since a member of C=O group within the cage of cyclic peptides were involved in stabilizing the nanoparticles, the peptides could play a major role for the reduction of silver ions (Ragupathi Raja Kannan *et al*., 2012).

4. Discussion

The development of ecofriendly synthesis processes for the synthesis of metal nanoparticles of different shapes and sizes is a great concern in the field of nanotechnology.

The green synthesis of silver nanoparticles using marine macro algae provides a safe and simple method for the synthesis of nanoparticles with good optical properties directed by particle size.

In this study, the production of silver nanoparticles was carried out from *U. flexuosa* at room temperature. Silver nanoparticles were formed by the reduction of Ag^{+} into Ag° with the addition of seaweed extraction to the solution of 1 mM $AgNO_3$. The colorless reaction mixture was turned into dark brown color solution after 60 min of incubation in light room condition indicating the biotransformation of ionic silver to reduced silver, as a result of the surface Plasmon resonance phenomenon. Rajeshkumar *et al*. (2013) reported gold nanoparticles synthesis using *Turbinaria conoides* that the color change occurred at 1h time of incubation.

The synthesized silver nanoparticles using *U. flexuosa* extract was demonstrated by UV- Vis spectra at 200 to 600 nm where an intense band was clearly detected at 430 nm which confirmed the formation of silver nanoparticles. According to Suriya et al. (2012), the biosynthesis of silver nanoparticles activity using seaweed *Urospora* sp. showed the UV spectra reading at 430nm.

The particles size and nature of the nanoparticles were obtained by X-ray (**P**onarulselvam *et al*., 2012). The XRD analysis showed that the Ag- NPs synthesized by the *U. flexuosa* were crystalline. A similar result was reported using *Padina tetrastromatica* (Jegadeeswaran *et al*., 2012).

TEM technique was employed to determine the internal structure of materials, either of biological or non-biological origin (Rajasekharreddy *et al*., 2009). The TEM images of *U. flexuosa* – synthesized silver nanoparticles exhibited large and small spherical particles. TEM analysis of particle size also showed maximum particles in size range of 2 to 32nm. Suriya *et al*. (2012) reported particles size between 3 to 44 nm with average of 30 nm. A similar result was reported by Saraniya Devi *et al*. (2012) using *Ulva lactuca* as reducting as well as capping agent.

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