Green synthesis of silver nanoparticles using marine sponge *Axinella sinoxia*

Mahta Rezazadeh Hamed, Mohammad Hadi Givianrad* & Ali Mashinchian Moradi

Department of Marine Chemistry, Science and Research Branch, Islamic Azad University, Tehran, Iran

*Email: givianradh@yahoo.com*

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Silver nanoparticles synthesis using dry and fresh marine sponge *Axinella sinoxia*. Experimental parameters such as time duration, pH, and temperature are studied. Optimizations for silver nanoparticles were carried out. The optimum syntheses of silver nanoparticles were achieved within 4 hours at pH 8.5, 1mM AgNO$_3$. Characterizations of silver nanoparticles were applied based on UV-Vis spectrophotometry around 420 nm. The sizes of synthesized nanoparticles (23-38nm) were confirmed by scanning electron microscopy (SEM). X-ray diffraction (XRD) crystallography expounded the silver nanoparticles crystalline nature. Fourier transform infrared spectroscopy (FT-IR) shows that the functional groups are hydroxyl, carbonyl, amide, amine, alkyl halide, and phenolic compounds of extract of *Axinella sinoxia* are entangled in the reduction of aqueous silver nanoparticles. This method of Ag-NPs synthesis does not use any toxic reagents and thus has potential for use in biomedical, pharmaceutical and agricultural application.

**Keywords:** Green synthesis, Silver nanoparticles, Sponge, *Axinella sinoxia*, SEM, XRD

Introduction

Marine bio-nanotechnology is an exciting and upcoming area of investigation. The biologically various marine environment has a great promise for nanoscience and nanotechnology. Nanobiotechnology is a course that depends on both science and nanotechnology. Nanoparticles can be synthesized by chemical, physical and biological procedures. Nanoparticles generally have unique properties and changed physical and biological properties compared. Synthesis of noble metal NPs for usage such as catalysis electronics, optics, environmental, and biotechnology is an area of constant interest. Preparation of nanoparticles by green technologies is advantageous over chemical agents due to their environmental achievement. Chemical and physical procedures are very expensive and involve the use of toxic, harmful chemical, which may pose potential environmental and biological dangers. Physically and chemically mediated syntheses require high pressure, energy, temperature, high cost and toxicity. Silver an old element is being wildly used as antibacterial agent since 19th century although it has been discovered since ancient Babylonians and Greeks periods. Silver is well known to rush a wide range of bacteria’s by modified the cell membrane structure and its functions. Silver also kills bacteria less than 1-10 µm and therefore it is being used as an antiseptic agent in water purification systems. Thus silver has been extensively used for development of many biological and medicinal products. Silver nanoparticles have specification physico-chemical properties such as high electrical and thermal conductivity surface enhanced Raman scattering, chemical stability, catalytic activity and non-linear optical behavior. Extract from bio organisms may act reducing agents in silver nanoparticles synthesis. The reduction of silver ions by combination of biomolecules found in these extracts extract includes enzymes/proteins, amino acids polysaccharides and vitamins are environmentally safeness, yet chemically complex. Synthesis of silver nanoparticles using biological marine microorganisms such as bacteria, actinomycetes, yeast, fungi and plants were reported. Silver nanoparticles using marine animals such as sponges have been undiscovered which aroused our interest. Synthesis of nanoparticles may be to do several compounds includes carbonyl groups terpenoids, phenolics,
flavanones, amines, amides, proteins, pigments, alkaloids and other reducing agents present in plant extracts and microbial cell. Therefore, nowadays specific focus on green chemistry by investigators is highly created because of increasing knowledge about the environment usage of nontoxic chemicals environmentally harmless solvents and renewable materials are some the key problems that merit important consideration a green synthesis strategy.

Materials and Methods

Silver nitrate (AgNO₃) was bought from Merck, Germany. Deionized water was used for all experiment and buffer solution. Axinella sinoxia a marine sponge was collected from the intertidal region at (Lat. 26° 33/41/ Long. 26° 34/15/) located on the Persian Gulf. Thoroughly samples washed with fresh water to remove adhering debris and associated s biota then brought to the laboratory in an ice box. Marine sponges were washed with deionized water. For fresh sponge, five grams of sample was weighted and were boiled with 50 ml of deionized water at 70°C for 10 min. The crude extract was filtered by Millipore filter (0.45 µm) and maintained at 4°C for further experiment. For the dry sponge, the cleaned marine sponge with deionized water were freeze dried at -20°C and then crushed into powder and stored at 4°C for future use. Then the dried sponge powder Axinella sinoxia (5g) was boiled with 50 ml of deionized water at 70°C for 10 min. The extract obtained was followed by Millipore filter (0.45 µm) and used at 4°C further experiments.

10 ml dry and fresh sponge aqueous extract was mixed with 100 AgNO₃ solution 1mM in a 250 ml Erlenmeyer flask. The whole mixture was put in to a stirrer at 70°C (700rpm) for 4 hour and maintained in the dark. The bio reduction of AgNO₃ ions occurred within 4 hour continuous stirring after that color change to yellowish. The solution was centrifuged at 12000 rpm for 30 min in 4°C and then the pellets were suspend ed in deionized water and again centrifuged at 8000rpm for 15 min. The purified silver NPs were freeze dried and stored for further use characterization.

Characterization of Silver Nanoparticles

The reduction of AgNO₃ was showed using UV-Visible spectrophotometer in the different wavelength range of 350-600 nm (T90+UV/spectrophotometer PG Instruments Ltd). It is one of the important techniques to verify the formation of metal nanoparticles provided surface Plasmon resonance exists for the metal. For detect silver nanoparticles at the absorption range of 400-450 nm.

FT-IR spectra of functional groups in sponge extract solution was recorded by using FT-IR spectrometer (Thermo-scientific, Nicolet 8700 USA FT-IR) at a resolution of 4 cm⁻¹ range from 400 to 4000 cm⁻¹ in KBr pellet.

The presence of bioactive functional groups responsible in sponge extract and synthesized silver nanoparticles recorded with using FT-IR spectrometer (Thermo Nicolet, Nexus 870, USA FTIR) at a resolution of 4 cm⁻¹ range from 400 to 4000 cm⁻¹ in KBr pellet.

X-ray diffraction pattern indicated the crystalline structure of silver nanoparticles. The patterns of silver nanoparticles 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 the size of silver nanoparticles were characterized by using Scanning Electron Microscope (MIRA3 TESCAN).

Effect of Different Factors in Nanoparticles Biosynthesis

For exploration the effect of incubation time on nanoparticles synthesis, the reaction solution was incubated at 2, 4, 24 and 72 hours. For study the effect of pH, experiments were achieved by different pH (4.5, 5.5, 7.5 and 8.5) of the reaction solution. The reaction temperature was maintained at 35°C, 50°C, 70°C and 90°C. The absorbance of the resulting solution was measured by UV-Vis spectrophotometer at diverse wavelengths.

Results and Discussion

Biosynthesis of Silver Nanoparticles

The synthesis of silver nanoparticles was confirmed by UV-Vis spectrophotometry analysis. After 4 hours, it was observed that the color solution in Erlenmeyer flask turned to yellowish brown (Fig. 1). It confirmed the formation of silver nanoparticles in flask and color change was due to excitation of surface plasmon vibration (SPR) effect and the reduction of silver ions.
UV-Vis spectrophotometry is an important technique for identification of silver nanoparticles in biosynthesis procedure. The obtained silver nanoparticles were characterized by UV-Visible spectrophotometer at 423 nm and 427 nm for fresh and dried *Axinella sinoxia* in the spectrum confirmed of silver nanoparticles (Fig. 2). This is similar to the surface plasma vibration with characteristic peaks of silver nanoparticles.\(^\text{20}\)

**Optimization of Different Parameters**

The temperature is one of the most important factors for nanoparticle formation. As we see in Fig. 3 temperatures with range 35 to 90°C. While increasing temperature, the rapidity of formation of silver nanoparticle also increased quickly. Color change in 35°C was 72 h, while color change in 90°C was 50 min. The measure of the nanoparticle decreased originally due to the reduction in an agglomeration of the growing nanoparticles. Finally, as increasing temperature after 70°C, the absorption pattern decreased.

**pH**

The pH is significant factor for nanoparticles synthesis. The size of Ag-NPs are dependent on the pH of solution, the acidic pH 4.5 shows the week peak at 429 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 consequently was at 423 nm. Therefore, acidic pH defeats the NPs formation. At low pH, agglomeration happened since of the over nucleation and synthesis of bulky NPs. At high pH, a large number of nanoparticles with the small surface area are present due to the bioavailability of functional groups in the sponge extract\(^\text{21}\). As a result, pH 8.5 was optimum for the nanoparticles synthesis (Fig. 4).

The time duration of reaction time is an important factor. The silver nanoparticles formation was increased while increasing the time reduction. (Fig. 5)

The sponge extract and the silver nanoparticles synthesis FT-IR spectra of silver nanoparticles was recorded before and after the reduction of silver nanoparticles (Fig. 6).

Figure 6a confirmations some bands in the FT-IR spectrum of *Axinella sinoxia* extract in the range of 3421.65, 2921.78, 2851.18, 2327.10, 1737.49, 1657.32, 1542.55, 1466.96, 1384.49, and 1283.28, 1051.78, 876.48, 721.03 cm\(^{-1}\). An absorption peak at 3421.65 cm\(^{-1}\) corresponded of phenols, alcohols with free O–H group. The peak at 1051.78 cm\(^{-1}\) indicated C–O bend that verification is the O–H group. A signal at 2921.78 and 2851.18 cm\(^{-1}\) shows to C–H stretch alkanes and O–H stretch carboxylic acids\(^\text{22}\). The peak at 1657.32 cm\(^{-1}\) belongs to carbonyl groups and amides. The band 1466.96 cm\(^{-1}\) corresponds to O–H bend indicated carboxylate. The peak at 721.03 cm\(^{-1}\) was assigned for C–Cl stretching vibration of alkyl halides.

FT-IR analysis of silver nanoparticles (Fig. 6b) displayed different bands at 3422.89, 2923.25, 2852.30, 1650.13, 1525.01, 1459.39, 1380.37, 1040.14 cm\(^{-1}\), and 551.07 cm\(^{-1}\). The band at 3422.89 cm\(^{-1}\) is the characteristic of the hydroxyl functional group in alcohol and phenol compounds. Both peaks at 2923.25 and 2852.30 shows carbonyl group and secondary amines and shows C–H stretching for alkanes. The peak at 1650.13 cm\(^{-1}\) shows the group, amines and amides. The band shift in hydroxyl groups and carbonyl groups in FT-IR spectra confirm the oxidation of amides, alcohols, phenol and carboxylic acids\(^\text{23}\).

XRD indicate the typical pattern of silver
Fig. 2—UV-Vis absorbance of aqueous solution of 1mM AgNO$_3$ with *Axinella sinoxia* extract (A) fresh extract; (B) dry extract.

Fig. 3—UV-Vis spectrum of silver nanoparticles at different temperatures after 6 h of reaction time.

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

Nanoparticles synthesized using the marine sponge *Axinella sinoxia* are crystalline with small size (Fig. 7). Almost different peaks were observed in the spectra at 20 values of 27.62°, 32.23°, 46.18°, 54.59°, 58.915°, 76.56° reflection of both silver (Ag) and silver oxide (Ag$_2$O). A
peak at $2\theta = 32.23^\circ$ represents the formation of pure silver (Ag) at the start of the reaction. The formation of Ag$_2$O may be due to the coupling reaction with alcohol (O-H) groups$^{24}$.

Scanning electron microscopy has supplied further insight into morphology and size detail of silver nanoparticles. This image shows that the spherical and face centered cubic structures, as we see the particle size is between 23 and 38 nm (Fig. 8)$^{25}$.

**Fig. 5**—UV-Visible absorption spectra of silver nanoparticles with different incubation time

**Fig. 6**—FTIR spectrum for (a) the *Axinella sinoxia* aqueous extract; and (b) *Axinella sinoxia* formed Ag-NPs

**Fig. 7**—XRD patterns of synthesized silver nanoparticles

**Fig. 8**—SEM image of synthesized silver nanoparticles using *Axinellaa sinoxia*

**Conclusion**

Present results suggested that the extract of marine sponges is capable of reducing silver nanoparticles extracellularly. The formation of silver nanoparticles was observed within 4 h at 70°C and 50 min at 90°C. Our investigation showed that by increasing pH and temperature the synthesis of silver nanoparticles would be accelerated and also with raising temperature more than 90°C, agglomeration of nanoparticles will take place. Also we studied that the fresh extract has more potential for synthesis of silver nanoparticle compared to the dried extract. The SEM analysis of silver nanoparticles indicated the size, ranging from 23 to 38 nm and it showed that spherical and face centered cubic structure and the crystalline nature of nanoparticles was confirm by XRD pattern.
Consequently, this technique is simple, rapid and eco-friendly method without any physical or harmful chemicals.

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References