Sargassum Polycystum C. Agardh Mediated Synthesis of Gold Nanoparticles Assessing its Characteristics and its Activity against Water Borne Pathogens

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Abstract

This paper reports on the eco-friendly synthesis of gold nanoparticles (AuNPs) using the seaweed Sargassum polycystum C. Agardh extract. Biological synthesis for nanoparticle using plants is gaining considerable interest among researchers as an eco-friendly alternative to conventional physical and chemical methods, as this approach eliminates the use of toxic chemicals. Synthesized AuNPs was monitored by UV-Vis spectroscopy and was found to be complete within 30 min. Confirmation of elemental gold was carried out by elemental mapping using different physical techniques such as Fourier transform infrared (FT-IR) spectroscopy, X-ray diffraction (XRD) and energy dispersive X-ray spectroscopy (EDX). Scanning electron microscopy (SEM). The bio reduced AuNPs exhibited remarkably good anti-bacterial activity against pathogens specifically Pseudomonas aeruginosa (20 mm) which is more susceptible. The elaborate experimental evidences support that the seaweed Sargassum polycystum C. Agardh extract can provide an environmentally benign rapid route for synthesis of AuNPs that can be applied for various purposes.

Keywords: X-ray diffraction; Optical properties; Nano structures

Introduction

Synthesis of gold nanoparticles (AuNPs) has gained immense significance during the last few years due to their catalytic, optical, and electrical properties [1]. Nanoparticles exhibit atom-like behaviours due to high surface energy resulting from high and large specific surface area, high fraction of surface atoms and wide gap between valence and conduction band when divided to near atomic size. The synthesis of AuNPs using an eco-friendly method is important to address the growing concerns on the overall toxicity of nanoparticles for medical and biotechnological applications. AuNPs have been considered important due to their unique and tunable surface plasmon resonance (SPR) property and their applications in biomedical science including drug delivery, tissue/tumour imaging, photo thermal therapy, immune chromatography, and identification of pathogens in clinical specimens [2]. Most of the available chemical processes for synthesis of gold nanoparticles (AuNPs) involve toxic chemicals that get adsorbed on the surface, leading to adverse effects in medical applications. Presently there is a growing need to develop environmentally benign process for rapid synthesis of nanoparticles [3]. The choice of an environmentally compatible solvent system, an eco-friendly reducing agent, and non-hazardous capping agents for the stabilization of the nanoparticles are three main criteria for a totally “green” nanoparticle synthesis. Concerning the biological application of nanoparticles it has been emphasized that methods of synthesis through biological system via, micro-organisms include bacteria, yeasts, fungi and diatoms synthesizing inorganic materials either intra or extracellularly would make the nanoparticles more biocompatible [4]. The uses of marine algae include traditional cosmetics, as antipyretic and antiseptic compounds, vermifuges and as treatments for sunstroke, coughs, haemorrhoids, stomach ailements, nose-bleeds, goitre and urinary diseases [5]. Sargassum polycystum belongs to Phaeophyta species. It is reported to be the most abundant brown algae and chief source of alginic acid. It has been suggested that environmental factors such as temperature, salinity, rainfall and pH influences the seasonal growth in Sargassum species. During recent years Sargassum from coastal water have been subjected to repeated annual [6]. Herein, we report the biogenic synthesis of AuNPs using aqueous extract of S. polycystum for reduction of Au ions. We demonstrated its antibacterial activity against various water borne pathogens.

Materials and Methods

Collection of seaweed and extract preparation

Sargassum polycystum was collected from the Gulf of Mannar, Rameshwaran (9.2800° N, 79.3000° E), Tamil Nadu, India. The seaweed sample was washed in seawater and then fresh water to remove the epiphytes and other contamination. The sample was authenticated by Botanical Survey of India (BSI), Coimbatore (BSI/SRC/5/23/2014-15/Tech.169). The dry leaves were ground into fine powder in electric blender (Bajaj Model GX 11, Mumbai, India), 5 g of this powder was suspended in 100 mL of distilled water in a 300 mL Erlenmeyer flask followed by boiling for 15 min. The reaction obtained was filtered through Whatman filter paper No.1 (50 mm; Sigma, Bangalore, India). The filtrate was collected and stored at 4°C which was used throughout all the experiments. The reaction was carried out at room temperature [7]. The reagents were of analytical grade obtained either from Merck (Mumbai, India) and Sisco Research Laboratories (Mumbai, India).

Synthesis of AuNPs

The reaction mixture was initially light brown and there was no...
turbidity formation during the course of time. Synthesis of AuNPs was carried out at the concentration of 50% against 1 mM HAuCl₄ in a total volume of 100 ml made up with double distilled water. Reduction of Au³⁺ ions was initiated by addition of 5 ml of extract to 95 ml of 10⁻² M aqueous chloro auric acid solution in a 500 ml Erlenmeyer flask. The resulting mixtures were placed on a magnetic stirrer at the rate of 150 rpm for 25 min and initial colour change was observed from light brown to ruby red colour.

**Characterization of AuNPs**

The color change in reaction mixture (metal ion solution+ algae extract) was recorded through visual observation. The bioreduction of gold ions in aqueous solution was monitored by periodic sampling subsequently measuring UV-Vis spectra of the solution at 500-600 nm using a 3-5 mm quartz cuvette. UV-Vis spectra of these aliquots were monitored as a function of time of the reaction. All the measurements were carried out at room temperature. All UV-visible (UV–vis) spectroscopic measurements of the synthesized AuNPs were carried out on a Cary 100 BIO UV–vis spectrophotometer (Varian, Palo Alto, CA, USA). AuNPs synthesized after 20 min of reaction between 1 mm chloroauric acid solution and extract were centrifuged at 10,000 rpm for 15 min at room temperature, following which the pellet was re-dispersed in sterile distilled water to remove any uncoordinated biological molecules. The chemical composition of the gold nanoparticles was characterized by FT-IR (Bruker Tensor 27). The phase formation of bio-reduced AuNPs was studied with the help of XRD. Bio reduced gold chloride solution was air dried. XRD patterns were recorded by a SEIFERT X-ray diffractometer with Cu Kα radiation. The samples were scanned in the 20 range of 10°C-70°C. Energy-dispersive X-ray (EDX) spectrometry confirmed the presence of elemental gold signals of the Au-NPs (Figure 2). The phase formation of the synthesized AuNPs was analysed employing X-ray diffraction which confirmed that the bio reduced metal nanoparticles are of elemental gold existence of peaks (111), (200), (220) and (311) matched with the standard Joint Committee for Powder Diffraction Set (JCPDS). In similar works, the presence of intense peaks of nanoparticles (111), (200) and (221) appeared which are indexed as crystalline silver centered cubic phase, the XRD pattern thus clearly shows that the silver nanoparticles formed by the reduction of Ag⁺ ions by S. polycystum extract are crystalline [11] (Figure 3). The XRD patterns of green-synthesized Turbinaria conoides nanoparticle revealed that AuNPs corresponded to the crystalline gold fcc phase. The diffraction peaks obtained at 2θ=38.36° (111), 44.13° (200), 64.78° (220), and 77.98° (311) are identical with those reported for standard gold metal (Joint Committee on Powder Diffraction Standards-JCPDS, USA) [12]. Similarly, the XRD patterns of green-synthesized Guinida glauca flower extract revealed existence of peaks (111), (200), (220) and (311) matched with the standard Joint Committee for Powder Diffraction Set (JCPDS) data-04784. This confirmed face centered cubic structured AuNPs formation. Peak broadening indicated restricted particle size. Enlarged pattern of (111) peak is shown in the inset of XRD plot [13]. Seaweed extract alone does not bring any effects in the shape and size of the particles, the phytochemicals present in the extracts may have the role of synthesizing the nanoparticles. Using the Scherrer’s formula the size of the nanoparticle has been calculated. 

\[ d = \frac{0.9\beta}{\cos \theta} \]

Here 0.9 is the shape factor, generally taken for a cubic system, λ is the X-ray wavelength, typically 1.54 Å, β is the full width at half the maximum intensity (FWHM) in radians, and θ is the Bragg angle. Using the above formula the crystallite size calculated is ~15 nm. The surface of gold nanoparticles and the elements present in the extract were analysed by EDX (Figure 2). The AuNPs were mono dispersed as spherical shaped. The average size of the particles is between 30-60 nm (Figure 4). A few agglomerated particles were also observed due to the spectral shift. Antibacterial activity of S. polycystum extract, gold nanoparticles and their combination were tested against E.coli and P.aeruginosa organism and the activity is shown in Figure 5. Antibacterial activity of S. polycystum extract, silver nitrate and their combination were tested against human pathogens also.

The crude extract of S. polycystum and silver nanoparticles showed cytotoxicity against MCF-7 breast cancer cell line in dose dependent manner. The IC50 for seaweed extract at 300 mg mL⁻¹ for 72 h was higher than other concentration and the IC50 for Silver nanoparticles at 135 mg mL⁻¹ for 72 h was higher than other concentration [14,15].
Conclusion

Green synthesis of spherical shaped AuNPs has been achieved using extract of *S. polycystum* thus bringing into light yet another use
of the plant, besides its usual utilities. The method stands out primarily due to the fact that it is eco-friendly and shuts down the demerits of conventional physical and chemical methods. These particles are anticipated to have an extensive application in various biomedical applications. Thus this rapid, eco-friendly and economical route can be used to synthesize AuNPs with wide biotechnological and chemical applications.

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