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Sunlight Irradiation Induced Synthesis of Silver Nanoparticles using Glycolipid Bio-surfactant and Exploring the Antibacterial Activity

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Abstract

In the present study, sunlight irradiation induced strategy for the rapid green synthesis of silver nanoparticles (AgNPs), is reported for the first time using glycolipid bio-surfactant. On exposing a mixture of silver nitrate solution and glycolipid bio-surfactant in the ratio 3:1 to sunlight, stable silver nanoparticles were obtained within few minutes. The glycolipid bio-surfactant acts as both reducing and stabilizing agents in the synthesis of nanoparticles. The nanoparticles synthesized were characterized using UV–Vis spectrophotometer, scanning electron microscopy and FTIR. Synthesized nanoparticles were in the range of 70-90 nm with spherical shape. The absorption spectroscopy indicates the band gap of silver nanoparticles was 3.4 eV. Further, the antibacterial activity assay of the synthesized nanoparticle exhibits as potential antibacterial agents. Hence, the present study demonstrates that biologically synthesized nanoparticle could be employed for developing antibacterial drug.

Keywords: Silver nanoparticles; Glycolipid bio-surfactant; Photo induced; Antibacterial agent

Introduction

Photo elicited reduction of nanoparticle strategies method gaining wide attention in recent years because of the subsequent advantages as the methods are more competitive and cost effective, controlled reduction of metal ions can be done without using excess of reducing agent and radiation is absorbed regardless of the radiation is absorbed in spite of the presence light absorbing solutes and products [1,2]. Biosurfactant are rising as a possible nanoparticles stabilizing agent, due to their non-toxic and eco-friendly nature. Silver nanoparticles (AgNPs), united of nanomaterial's of noble metals, have intensive applications in several fields. They are widely used as an additive for numerous materials and products including plastics, glass, cement, ceramics, lubricants, rubber, adhesives, paints, pigments, ointments etc. They can also be used for antistatic materials, antibacterial materials, biosensor materials and cryogenic superconducting materials [3,4]. Hence, in the present study attempt has been made for synthesis of silver nanoparticles using glycolipid bio-surfactant utilizing sunlight as the irradiation source.

Materials and Methods

Bio-surfactant production

Bio-surfactant used in the present study was produced by *Pseudomonas sp*. This strain was isolated from petroleum oil contaminated soil and stored in our laboratory at 4°C. For screening the bio-surfactant production different test were performed. Hemolytic assay was performed by streaking the bacterial strain onto blood agar plate [5,6]. Drop collapse test was performed in a 96-microwell plate [7]. Oil displacement test was determined by following the method of Ohno et al. [8]. For bio-surfactant production seventy two hours grown bacterial culture in mineral salt medium (composition g/l $MgSO_4$ -0.5, $(NH_4)_2SO_4$ -1, $NaNO_3$ -2.5, Na_2HPO_4 -6, K_2HPO_4-1.0, $FeCl_3$ -0.1, KH_2PO_4-1.0, $CaCl_2$ -0.01, $MnSO_4$ -0.005, dextrose -15) was centrifuged at 10,000 rpm, 4°C temperature for 20 minutes. Culture supernatant (crude bio-surfactant) obtained was transferred to empty beakers and stored at 4°C temperature.

Detection of glycolipids by phenol H_2SO_4 method

Glycolipids production was detected according to the method Dubois et al. [9]. Briefly, 1 ml of 5% phenol was added to small amount of crude bio-surfactant. To above mixture, 4 ml of concentrated H_2SO_4 was added drop by drop. Colour change was observed; development of yellow to orange color indicated the presence of glycolipids.

Synthesis of silver nanoparticles

For the synthesis of silver nanoparticles, 2ml of crude bio-surfactant was mixed with 6 ml of 0.001 M of silver nitrate solution. The reaction mixture was then placed in direct sunlight on a bright sunny day for different time periods to determine the minimum interaction time [10]. Same volume of silver nitrate solution is considered as control. The study was carried out in the summer months of May to June at, Lucknow, India having the temperature of ranging from 42°C to 45°C. After 25 to 30 minutes of reaction in sunlight changes in colour of the samples indicates the synthesis of nanoparticles. After then change in color of the solution the reaction mixture containing the nanoparticles was centrifuged for 10 min at 10,000 rpm and pellet received was re-dispersed in deionized water [10].

Characterization of Silver Nanoparticles

UV-visible spectrophotometer analysis

The optical property of Silver nanoparticles synthesized was determined by UV-Visible Spectrophotometer Analysis (Thermoscientific Evolution 201). After the addition of $AgNO_3$ to the bio-surfactant, the spectras were taken in different time intervals up to 24 hrs between 200 nm to 800 nm. Then the spectra were taken after 24 hrs of $AgNO_3$ addition. The optical band gap of silver nanoparticles is calculated using the Tauc relation [11].

Fourier transform infrared (FTIR) spectroscopy analysis

The chemical composition of the synthesized silver nanoparticles was studied by using FTIR spectrometer (Thermo scientific Nicole 6700). The solutions were characterized in the range 4000–400 cm^{-1}.

Scanning electron microscope analysis (SEM)

The morphological features and the size of synthesized silver nanoparticles through bio-surfactant were studied by Scanning Electron Microscope (Jeol 6490LV). After 24 hrs of the addition of $AgNO_3$ the SEM slides were prepared by making a smear of the solutions on slides. A thin layer of platinum coating was done to make the samples conductive. Then the samples were characterized in the SEM at an accelerating voltage of 20 KV.

Exploring Antibacterial Activity

Well diffusion method was employed for screening the antibacterial activity of synthesized silver nanoparticle [12]. Briefly, sterilized nutrient agar (20 ml) was poured into sterile petriplate, after solidification, 100 µl of 24 hr bacterial culture (*Escherichia coli*) was swabbed on the each petri plates. 10mm diameter wells were made in each petriplates and 100 µl of different prepared silver nanoparticle solution (100, 200, 300, 400 and 500 mg/ml) were added to the wells. Then the plates were incubated at 30°C for 24 hours. After incubation inhibitor zones in each plate were measured and recorded.

Results and Discussion

Screening and characterization of bio-surfactant produced by the test strains

The test strains produced bio-surfactant on Mineral Salt Medium (MSM) which was confirmed by the foam formation. The results of drop collapse test were positive for surfactant production as the drop spread and collapsed. The strain grown in blood agar media showed a clear zone around its colony. The blood agar method is often used for a preliminary screening of microorganisms for the ability to produce biosurfactants on hydrophilic media. Bio-surfactants cause lysis of erythrocytes. This principle is employed for the hemolysis assay that was developed by Mulligan et al. [6]. The bio-surfactant from *Pseudomonas* species showed good oil displacement activity by forming clear zone of large diameter.

Synthesis of silver nanoparticle

Both the samples, mixture solution (3 ml silver nitrate solution and 2 ml of crude bio-surfactant) and control solution were held at sunlight. The sample exposed to sunlight changed from colour less to brown after 15-20 mins of sunlight exposure, whereas the control sample remained colourless. The change in colour happens within the presence of light indicates that sunlight and bio-surfactant plays an important role in the synthesis of AgNPs.

Absorption spectra characterization

Formation of silver nanoparticle in presence of bio-surfactant and sunlight exposure was indicated by change of in colour owing to surface Plasmon resonance development. Nanoparticles have surface plasm on resonance absorption in the UV–visible range. This surface plasm on resonance property of metal nanoparticle is due to the combined vibration of free electrons in resonance with light wave [13-27]. Sharp bands of 410 nm was reported in case of silver nanoparticles synthesized silver nanoparticle in present study (Figure 1). The optical direct band gap of silver nanoparticles calculated by the tauc plot, which indicates band gap of synthesized silver nanoparticles are 3.4 eV. The value of band gap is more than previously reported literature and this higher value of band gap might be due to a quantum confinement effect [28-30]. To monitor the stability of prepared nanoparticles, we measured absorption spectra of solution on different weeks. This shows that prepared silver nanoparticle solution can remain stable at least 2 to 3 months at room temperature. The bio-surfactant in the solution is act as the stabilizer, which forms a steric hindrance around the particles to prevent them from aggregating greatly by electrostatic interactions [13,14].

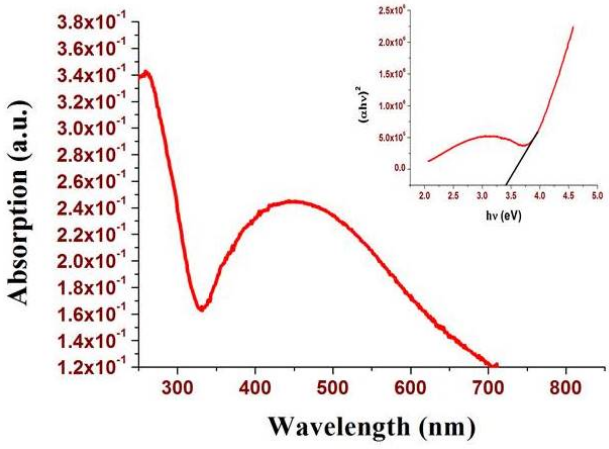

Figure 1: Absorption spectra (a) and Band gap (b) of synthesized silver nanoparticles.

SEM Analysis

Scanning Electron Microscope results demonstrated that (SEM) provided the size and morphology of the prepared nanoparticles. The result shows that a particle size in the range of 70-90 nm was obtained by using glycolipid bio-surfactant under sunlight irradiation (Figure 2). The structure of the bio-surfactant plays a vital role in determining size and shape of the synthesized nanoparticles. These micelles are spherical in shape and favored the formation of spherical nanoparticles during synthesis. One of the foremost necessary properties exploited within the nanoparticles synthesis is their micelle-forming ability. The non-covalent interactions that arise as a result of solvophobic effects of

hydrophobic tails form the basis for self-aggregation into structures like micelles and vesicles [13,14]. Micelles exist in numerous morphologies, like cylindrical, spherical and ellipsoid structures, whereas vesicles are hollow spheres enclosed by bilayers of ampiphilic surfactants [15-17].

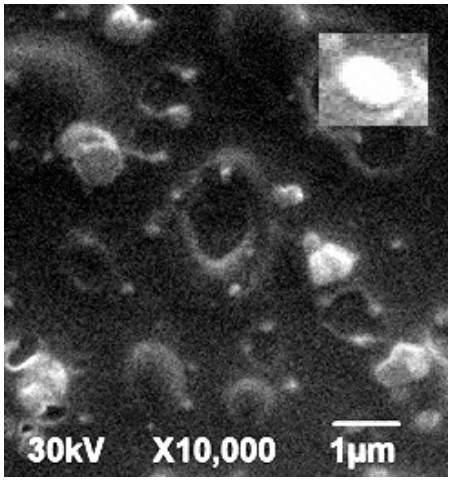

Figure 2: SEM Image synthesized of nanoparticles.

Transform infrared (FTIR) spectroscopy analysis

Fourier Transform Infrared (FTIR) peaks of the functional groups present in the prepared solution of synthesized silver nanoparticle. OH group (3363.3, 2618 cm^{-1}), C-H stretching (2884.5 cm^{-1}), carbonyl stretching (1763.8 cm^{-1}), N-H bending (1636.3 cm^{-1}) were the functional presented in synthesized nanoparticle solution.

Antibacterial Activity

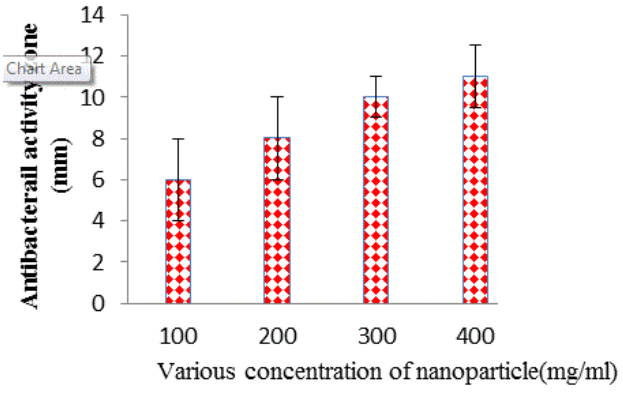

Figure 3: Antibacterial activity of synthesized silver nanoparticle against pathogenic *Escherichia coli*.

Silver ions and salts containing silver exhibit antimicrobial activity and is used in various fields as antimicrobial agent due to their inhibitory ability against microorganisms. Hence, in present study it is reported that antibacterial activity increase with increase in silver nanoparticle concentration (Figure 3). The result shows similarity with previous study of Sondi and Salopek-Sondi [18], who reported the antimicrobial activity of silver nanoparticles on Gram negative bacteria mainly depend on the concentration of silver nanoparticle. Effecting the replication ability and inactivation of cellular protein is believed to be the possible mechanism of silver ions on microorganisms [19]. Apart, from it silver ions cause denaturation of protein by binding to functional groups of proteins [18,20]. Various studies have demonstrated that electrostatic attraction between positively charged silver nanoparticle and negative charged cell membrane of microorganism is cause for antimicrobial activity of nanoparticle [21-23].

Various studies on silver nanoparticle synthesis by using glycolipid bio-surfactant

Numerous studies have shown bio-surfactant as best candidate for nanoparticle synthesis. Farias et al. reported that bio-surfactant produced by Pseudomonas aeruginosa cultivated in an exceedingly low cost substrate has the tendency to synthesize and stabilize silver nanoparticles in the liquid phase [13]. Synthesized silver nanoparticle poses a size within the range of 1.13 nm. The UV via absorption spectra planned that silver nanoparticles may be shaped within the reverse micelles and comparatively stable for a minimum of three months. The Transmission microscope (TEM) shows that the silver nanoparticles are of spherical type and comparatively uniform [13]. Kiran et al. [14] reported that a glycolipid bio-surfactant extracted from sponge-associated marine *Brevibacterium caseai* was able to synthesized uniform and stable silver nanoparticles for two months. Kumar et al. [24] synthesize silver nanoparticles from purified rhamnolipids (glycolipid bio-surfactant) *Pseudomonas aeruginosa* BS-161R. The synthesized nanoparticles was spherical in shape had a sharp adsorption peak at 410 nm, with an average particle size of 15.1 nm. Singh et al. demonstrated the production of glycolipid metal nanoparticle conjugates (sophorolipid capped silver nanoparticles) named sophorolipid capped silver nanoparticles [25]. The produced sophorolipid capped silver nanoparticles had strong absorption with a peak at 410 nm and size of 10–50 nm [25].

Various studies on silver nanoparticles synthesis utilizing sunlight radiation and reducing agent

Very few reports are available regarding the synthesis of silver nanoparticle by employing reducing agent and sunlight. Silver synthesized nanoparticles rapidly by treating silver ions with lemon extract utilizing sunlight radiation. Synthesized nanoparticle was below 50 nm with spherical and spheroidal shape. They reported effect of time period of the day on reaction rate and nanoparticle formation was investigated in details [2]. Rastogi and Arunachalam [26] synthesized highly stable silver nanoparticles using aqueous garlic extract under bright sunlight for 15 min. The garlic extract components served as both reducing and capping agents in the synthesis of silver nanoparticles while the sunlight acted as catalyst in the synthesis process. The synthesized nanoparticles were spherical in shape with 7.3 ± 4.4 nm in size and poly dispersed in nature. And the silver colloidal solutions synthesized were found to be stable for a very long period. Amaladhas et al. [27] synthesized of silver nanoparticles using aqueous leaf extract of *Achyranthes aspera*. On exposing a mixture of silver nitrate solution and aqueous leaf extract of *Achyranthes aspera* to sunlight, stable silver nanoparticles were formed within few seconds. The synthesized nanoparticles were spherical in shape with the size of 12.82 nm and mono-dispersed nature [27].

Present study and the first report of synthesizing of silver nanoparticle by sunlight irradiation using glycolipid bio-surfactant

Silver nanoparticles have been previously synthesized under normal laboratory conditions using glycolipid bio-surfactant. But in the present study we report for first time the Synthesis of silver nanoparticle using by Sunlight Irradiation using glycolipid bio-surfactant. The study was performed at Lucknow, in Uttar Pradesh, India located at latitude 2655' N and longitude 8059' E with during the summer months of May to June at, having the temperature of ranging from 42C to 45C with higher sunlight radiation. The reaction typically got over after 15-20 min of interaction under direct sunlight. Silver nanoparticles synthesized in the present study were in the range of 70-90 nm with spherical and spheroidal shape.

Conclusion

The present study signifies that synthesizing silver nanoparticles utilizing sunlight radiation and bio-surfactasnt can be best cost effective method for high elevation production. Hence, further more studies are required to determine the effect of sunlight intensity on the particle size of the nanoparticle. Also, the present findings demonstrate that silver nanoparticle can be used as antimicrobial agent in field of biomedical sciences.

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