Silver nanorod: green synthesis and their electronic, optical and antibacterial properties

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Abstract: Synthesis of nanomaterials has been the subject of a lot of interests due to its commercial demands and wide applicability in various fields such as chemistry, physics, biology, material science, engineering, medicine etc. This paper reports the green synthesis of silver nanorod. For synthesis, silver nitrate solution was used as a metal precursor and aqueous extract of root of *Beta vulgaris* was used as a reducing agent. Four different sets of samples were synthesized by varying the reduction time to study the size dependent electronic and optical properties. The formation of nanostructure was confirmed by presence of surface plasmon resonance in absorption spectra. Optical band gap calculation was done by using absorption spectroscopy. Absorption spectroscopy showed that the maximum absorption was red shifted when the reaction time was increased, indicating the increasing size of material. Similarly, it was found that the optical band gap was decreased with increasing reduction time. Moreover, the antimicrobial activity of synthesized nano silver was studied against both the gram negative (*Pseudomonas aeruginosa*, *Escherichia coli*) as well as gram positive bacteria (*Staphylococcus aureus*).

Keywords: Silver nanorod; Electronic and Optical property; Antibacterial activity.

Introduction

Nanotechnology is the science of the small things but having big potential that is revolutionizing our world. Nanotechnology involves the production, manipulation and use of materials having size less than micron. The term "nanotechnology" was first defined by Tokyo Science University, Norio Taniguchi in a 1974 as follows: 'Nano-technology' mainly consists of the processing, separation, consolidation, and deformation of materials[1] with a size in the range of 10-100 nm. The development of nanotechnology is rapidly involving as an integrative science with endless applications in the developments in different applications such as: alternative energy, development of sensors, biomedical device, electronic applications, environmental restoration etc. Various nanostructures such as nanorods, nanospheres, nanoscaffolds and a variety of metallic and non-metallic nanoparticles are the candidates increasingly contributing to several applications[2]. Metallic nanomaterials are of great interest due to their unique physical and chemical properties different from their bulk properties. As the material approaches nanoscale dimensions, it shows new behavior and properties that are different from its bulk counterparts. The small size of the nanomaterial leads to an increased surface area to volume ratio, which ensures an increase in the number of atoms at the surface in comparison to those in interior, recent advancements in understanding the properties of nanomaterials have enabled researchers to create new materials showing novel behavior for various applications[3].

Silver as antimicrobial agent

For centuries silver has been in use for the treatment of burns and chronic wounds. As early as 1000 B.C. silver was used to make water potable. In the 1940s, after penicillin was introduced the use of silver for the treatment of bacterial infections minimized.

Silver again came in picture in the 1960s when Moyer

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introduced the use of 0.5 % silver nitrate for the treatment of burns. He proposed that this solution does not interfere with epidermal proliferation and possess antibacterial property against *Staphylococcus aureus, Pseudomonas aeruginosa and Escherichia coli*[1]. Nanoparticles are now considered a viable alternative to antibiotics and seem to have a high potential to solve the problem of the emergence of bacterial multidrug resistance[4]. The silver nanoparticles have been reported to have strong antibacterial activity. For a long time ago, the researchers reported that silver nanoparticles can kill more than 650 pathogen microorganism, including bacteria, viruses, parasites, fungi etc[5].

Optical properties of Ag NPs

Optical properties occur when electron transition occurs between conduction and valence band. Optical properties of nanomaterials are dynamic and may differ significantly from properties exhibited by the same bulk material. The optical properties of nanomaterials are some of the most important, and can be identified using various spectroscopic techniques. Silver is the best plasmonic material in terms of optical performance and they have ability to support strong surface plasmon resonance over the entire visible to near infrared spectral region[6]. Silver nanoparticles absorb and scatter light with extraordinary efficiency. Their strong interaction with light occurs because the conduction electrons on the metal surface undergo a collective oscillation when they are excited by light at specific wavelengths. Optical properties of NPs determine potential applications in different fields such as: imaging, sensor, display, solar cell, photocatalysis, biomedicine, lasers, etc. A wide range of optical effects may be produced for a variety of applications by simply manipulating its shape, size, and surface functionality[7, 8].

Various methods are reported to synthesize the silver nanoparticles such as physical, chemical, and green synthesis method. All of these synthesis methods involve the use of reductants and stabilizers and the precursors. The physical method has several drawbacks, for example, it usually requires high energy consumption and high cost due to the use of some modern instruments. On the other hand, the chemical method requires inorganic chemicals which are harmful to the environment and expensive.

In this paper, we have reported green synthesis method for the synthesis of nano silver. The green synthesis method uses biological template to obtain the nano silver. The biological templates are plant extract, fungi, algae etc. This is environmentally benign method and produces fewer impurities. In green synthesis method, the main functional groups that have strong effect to reduce silver ion to metallic silver are carboxyl, aldehyde, hydroxyl etc, where as the active functional groups for stabilizing the nanoparticles is usually peptide from proteins[9]. In this study, we use aqueous extract of root of *Beta vulgaris* to synthesize nano silver. Four different types of samples were prepared to study the effect of reaction time on the size and optical properties of nano silver.

Experimental Methods

Preparation of Plant Extract

For the preparation of extract, the root of *Beta vulgaris* was collected from a local market, then washed properly and cut down. These beet root pieces (10 g.) were soaked in distilled (100 mL) water for 1 night. Then filtered and filtrate was used for synthesis of nanosilver.

Synthesis of Silver Nanorod

In this method, silver nitrate solution (50 mL, 5 mM) and aqueous root extract of *Beta vulgaris* (5 mL) were mixed and heated in magnetic stirrer bar with hot plate at temperature 100 ºC. The UV-Visible spectrophotometer was used to monitor the color changes of the mixture at different interval of time. The reaction mixture slowly changed from light pink to a dark brown suspension after 60 minutes reaction time. Four different sets of samples were synthesized namely G_1, G_2, G_3 and G_4 with reduction time 15, 30, 45 and 60 minutes respectively.

Measurements

The solution state UV-Vis spectra were carried out in USB2000, Photonics in range 300-1100 nm. The surface morphology of the sample was investigated by scanning electron microscope (SEM, HITACHI, S-4300). Energy Dispersive Spectroscopy (Powder form, Horiba Model EMAX 7593-H) was used for elemental characterization.

Antibacterial Test

Antibacterial activity of nano silver was studied by Muller-Hinton Agar (MHA) test[10] against one gram positive bacteria *Staphylococcus aureus* (SA) two gram-negative bacteria *Escherichia coli* (EC) and *Pseudomonas aeruginosa* (PA). The MHA plates were left overnight at room temperature to check for any contamination to appear. The bacteria test organisms were grown in nutrient broth. Standard organisms were swabbed on MHA plates. Then the holes were made with the help of borer to put the sample. Filter discs were impregnated with synthesized silver nanorods and placed on the plates. Antibiotic chloramphenicol served as the standard for measuring the antibacterial activity. The plates were then incubated at 37 ^{o}C for 24 hours and the zone of inhibition was measured.

Results and Discussion

Formation of Nano Silver

Figure 1: Green Synthesis of Nano Silver with Different Reduction Time.

Aqueous extract of root of *Beta vulgaris* was used for the reduction of silver nitrate to form nano silver. Beet root contains valuable active components such as minerals, amino acid, phenolic acid, flavonoid, betaxanthn[11] etc. Flavonoids contain various functional groups which can actively chelate and reduce metal ions to metal nanoparticles[12]. When silver nitrate solution was added in aqueous extract of root of *Beta vulgaris*, color start to change from light pink to dark (Figure 1) indicating the formation of nano silver begins[13]. The color of the solution became darker with time and turned into dark brown after 60 minute. The color of nanoparticles originates from the excitation of the surface plasmon resonance that generally depends on the size, shape of the nanoparticles, chemical surrounding adsorbed species, dielectric constant[14] etc.

Electronic and Optical Properties

The reaction time can have a significant impact on the size of nanoparticles formed during synthesis. To study this effect, UV-Vis spectra were recorded in different interval of reduction time.

Figure 2 shows the UV-Visible spectra of Ag NPs synthesized at various reduction times and their corresponding band gap energies form Tauc plot is shown in Figure 3.The Figure 2 shows that each absorption spectrum consists of a peak that corresponds to characteristic surface plasmon resonance peak of nano silver. The surface plasmon resonance is the unique optical properties of metal nanoparticles originates from the collective oscillations of conduction electrons, which, when excited by electromagnetic resosnanc[15]. It is clear from the absorption spectra that the maximum absorbance wavelength (λ_{max}) is red shifted from 484 to 575 nm by increasing the reaction time. This showed that this red shifting may due to the increasing in size of the nanoparticles by increasing the reaction time[16]. After nucleation, the nanoparticles continue to grow by the addition of atoms from the reaction solution. The duration of reaction time can influence the growth rate of the nanoparticles. Longer reaction time may lead to more extensive growth resulting in larger sized particles.

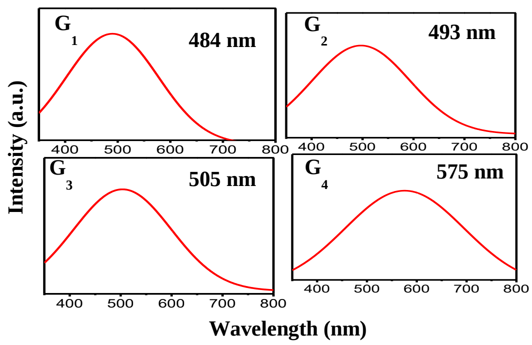

Figure 2: UV-Vis Spectra of Samples G_1, G_2, G_3 and G_4.

The gap between valence band and conduction band is called band gap. The band gap is different in conductor, semiconductor and insulator. In the same matter band gap is changed with change in size of materials. The optical absorption of metal nanoparticles has been described by Mie theory[17] as the localized surface plasmon resonance (LSPR). There is evidence that the optical absorption of metal nanoparticles can be described quantum mechanically due to intra-band excitations of conduction electrons[18, 19] due to the interactions of light on metal surface via the photoelectric absorption and Compton scattering. In metal nanoparticles, the conduction electrons are not entirely free as in the bulk structure, but instead some are held by the individual atoms and some are free and moved between atoms to form metallic bonds that cement the metal nanoparticles. Upon receiving photon energy having the maximum absorbance wavelengths (λ_{max}), the conduction electrons experience intra-band quantum excitations beyond the Fermi energy level, from which the conduction band of metal nanoparticle is defined. For smaller particle size, fewer numbers of atoms form the particle and so reduce the potential attraction between the conduction electrons and metal ions of the particle. In this way, the conduction band energy increases for the smaller particle. Conversely, for larger particle size, large numbers of atoms form the particle, thus increasing the potential attraction between conduction electrons and metal ions and therefore reduce the conduction band energy of the metal nanoparticles[18].

From the absorption spectroscopy plot, the band gap of materials can be estimated using the Tauc formula[20]:

$$\alpha h\nu = A(h\nu - E_g)^m \text{……………..(1)}$$

Where $h\nu$ is the incident photon energy (eV), A is energy independent constant, E_g is the bandgap (eV) of the material and m is an exponent which has value ½, and 2 for direct and indirect band gap. The value of α is obtained from spectra. The Figure 3 shows the graph plotted between $(\alpha h\nu)^2$ and $h\nu$ of samples G_1, G_2, G_3 and G_4. From graph the direct band gap was found by extrapolating the linear portion of the curve to $(\alpha h\nu)^2 = 0$ and values of direct optical band gap energy, E_g was determined.Graphs showed that the band gap decreasing in order with increasing the reduction time. This showed that the size of nanoparticles increase with increase of reaction time.

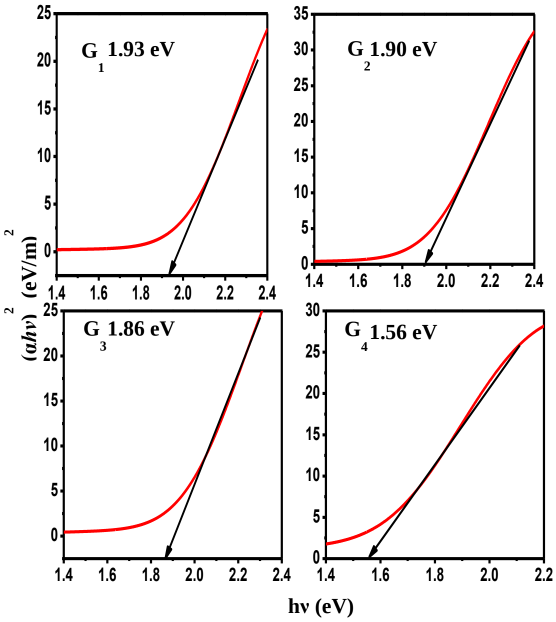

Figure 3: The Plots of $(\alpha h\nu)^2$ *vs.* $h\nu$ of Samples G_1, G_2, G_3 and G_4.

EDS/SEM Analysis

Energy Dispersive Spectroscopy (EDS) analysis was carried out to investigate the chemical characterization of sample. The EDS showed that the synthesized sample contain 48 % metallic Silver.

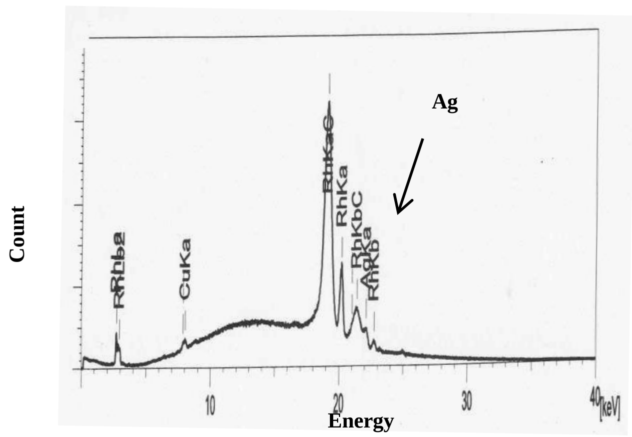

Figure 4: Energy Dispersive Spectrum of Nano Silver.

The morphology of the silver nanoparticles is characterized by using a scanning electron microscope with an acceleration voltage of 15 kV. Figure 5 below shows that the synthesized nano silver (Sample G_4) seems to be rod shaped.

Figure 5: SEM image of Sample G_4.

FTIR Analysis

FTIR spectroscopy was carried out to identify the possible biomolecules present in *Beta vulgaris* which is responsible for capping and reducing agent for the nanoparticles synthesis. Figure 6 showed the FTIR spectrum of aqueous extract of root of *Beta vulgaris*. In FTIR spectrum the peak centered at 3315 cm^{-1} is attributed to the N–H stretching of amines and amides, O–H stretching of alcohols and phenols[21]. The medium intense peak appeared at 1640 cm^{-1} is due to amino groups present in alcohol, phenol, and amines in the extract involved in the NPs synthesis[22].

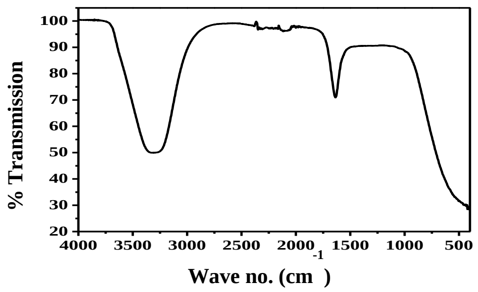

Figure 6: FTIR spectra of aqueous extract of root of *Beta vulgaris*.

Antibacterial Analysis

The synthesized silver nanorods were screened for *in vitro* antibacterial activity against two Gram-negative (*E. coli and P. aeruginosa*) and one Gram-positive (*S. aureus*) bacterial strains by the Muller-Hinton Agar (MHA) test method[10]. The results obtained were compared with that of the standard drug Chloramphenicol and are reported in Table 1 and zone of inhibition are shown in Figure 7 and % inhibition are shown in Figure 8. The results support the findings of Pak et. al.[13]. The activity of synthesized compounds (in %) was compared with the activity of the standard drug considering its activity as 100%.

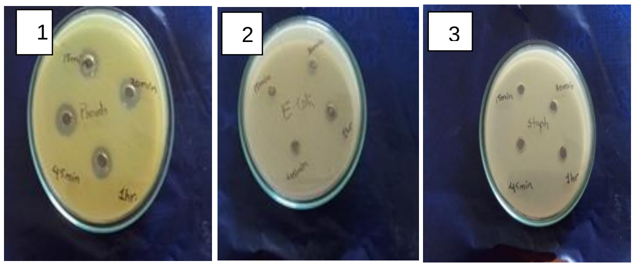

Figure 7: Antibacterial Activity howing Zone of Inhibition against Pathogenic Bacteria.

1) *Pseudomonas aeruginosa*
2) *Escherichia coli*
3) *Staphylococcus aureus*

Table 1: The measurement of Zone of Inhibition against.

Samples	Bacteria	Zone of inhibition (mm)
G_1, G_2, G_3, G_4	*S. aureu* *E. coli* *P. aeruginosa*	NO ZOI 0, 0, 6, 7.5 13, 14, 15, 17
Antibiotics (chloramphenicol)	*S. aureus* *E. coli* *P. aeruginosa*	23 22 21

Different Types of Bacteria

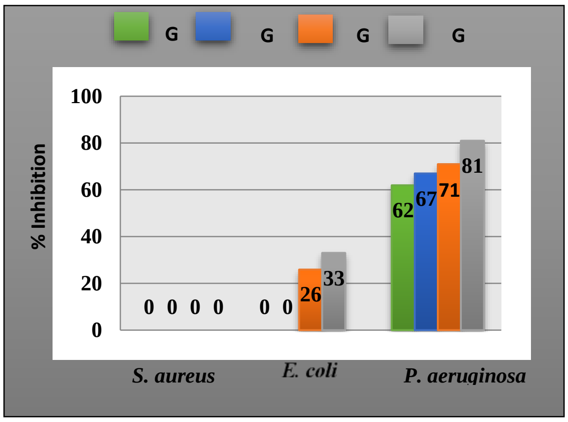

Figure 8: The Measurement of Percentage (%) Inhibition.

It is clear from the above table and figure that green synthesized silver nanoparticles have shown greater antimicrobial activity against *P. aeruginosa*. The variation in sensitivity to Gram positive and gram negative bacteria could be due to the different cell structure. Gram-positive bacteria possess a thick peptidoglycan layer while Gram-negative bacteria have a thin peptidoglycan layer. However, Gram-negative cell wall is composed of complex membrane with two cell membranes, a plasma membrane, and an outer membrane whereas, only plasma membrane appear in Gram-positive bacteria. The addition of the outer membrane in Gram-negative bacteria cells affects the permeability of molecules[22]. The synthesized silver nanoparticles with sodium dodecyl sulphate (SDS) show enhanced antimicrobial activity[23] demonstrates that the mechanism of this activity could be by forming the reactive oxygen species (ROS). Because SDS is acapping as well as oxidizing agent, which oxidizes the silver from Ag(0) to Ag(I).

Conclusions

Due to the wide applicability and commercial demands, the synthesis of nano metallic silver has been increased. It can be synthesized through physical, chemical and biological methods. Among them green synthesis are the best methods because of its accuracy, good product yield, economical and eco-friendly. In green synthesis, aqueous extract of root of *Beta vulgaris* was used as a reducing as well as capping agent. The characterization techniques such as UV-Vis, EDS, and SEM characterized the synthesized silver nanorod. The electronic spectroscopy shows that the shift of band to a higher along with increase in reaction time and it also shows the increase in size of silver nanoparticles. The EDS showed that the synthesized sample contain 48 % metallic Silver. The SEM shows the synthesized nano silver are rod shaped. The antimicrobial activity of green synthesized silver nanoparticles against both gram positive and gram negative bacteria have been studied and it gives the better results for gram negative bacteria *P. aeruginosa.*

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