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Research Article

Rapid Biological Synthesis of Silver Nanoparticles from *Ocimum sanctum* and Their Characterization

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With development of nanotechnology, the biological synthesis process deals with the synthesis, characterization, and manipulation of materials and further development at nanoscale which is the most cost-effective and eco-friendly and rapid synthesis process as compared to physical and chemical process. In this research silver nanoparticles (AgNPs) were synthesized from silver nitrate ($AgNO_3$) aqueous solution through eco-friendly plant leaf broth of *Ocimum sanctum* as reactant as well as capping agent and stabilizer. The formation of AgNPs was monitored by ultraviolet-visible spectrometer (UV-vis) and Fourier transform infrared (FTIR) spectroscopy. X-ray diffraction (XRD) and scanning electronic microscopy (SEM) have been used to characterize the morphology of prepared AgNPs. The peaks in XRD pattern are in good agreement with that of face-centered-cubic (FCC) form of metallic silver. Thermal gravimetric analysis/differential thermal analysis (TGA/DTA) results confirmed the weight loss and the exothermic reaction due to desorption of chemisorbed water. The average grain size of silver nanoparticles is found to be 29 nm. The FTIR results indicated that the leaf broths containing the carboxyl, hydroxyl, and amine groups are mainly involved in fabrication of silver AgNPs and proteins, which have amine groups responsible for stabilizing AgNPs in the solution.

1. Introduction

Nanotechnology is a branch of science and technology which concerns with the development of process for the design, synthesis, and manipulation of particle structure, different shape, size, and controlled disparity. Due to their unique physicochemical properties, metal nanoparticles with a dimension of approximately 1–100 nm have received considerable attention in last few decades [1]. Silver nanoparticles (AgNPs) have attracted significant interest due to their wide variety of applications and their unique optical, electrical, and thermal properties [2–4]. In the synthesis of AgNPs, numerous chemical, biological, and physical methods have been developed. However, conventional physical and chemical methods are expensive as well as resulting in low yield and poor size distribution [1].

Over the recent years, biosynthesis method has been widely studied for stable metal nanoparticles synthesis with controlled size and shape and considered as a "green" approach [5–8]. Among them, plant leaf extract mediated biological process has attracted much attention due to the simple and inexpensive protocol [9–12]. In addition, the proteins or polysaccharides or secondary metabolites found in leaf extracts can reduce the Ag^+ ions to Ag^0 state and form silver nanoparticles [1].

Traditional chemical methods of synthesizing silver nanoparticles include the use of ethylene glycol, pyridine, and sodium borohydride. The chemicals used in these methodologies can be toxic and highly reactive posing a risk to the environment and humans, or the procedures are too expensive to be feasible at an industrial scale. Therefore there has been a search for inexpensive, reliable, safe, and "green" approach to the synthesis of stable metal nanoparticles with controlled size and shape. As a result, some novel methods have recently developed using (i) biologically derived reducing agents such as chitosan, glucose, and polysaccharides, (ii) microbes such as bacteria and fungus, and (iii) a variety of

(a) (b)

Figure 1: (a) *Ocimum sanctum* leaves and (b) leaf broth.

plant (seed and leaf as well as tuber) extracts for the synthesis of metal nanoparticles. Among them, plant leaf extract mediated biological process has been widely investigated due to the inexpensive and simple protocol.

We have used *Ocimum sanctum* leaf broth to synthesize AgNPs. The used plant is wild herbaceous with medicinal values and available in all tropical countries [13, 14] and traditionally thought to have strong antimicrobial and antioxidant activity and be widely used to stimulate the appetite and ease stomach upset. Recently, Singhal et al. [15] and others [16–18] synthesized silver nanoparticles using *Ocimum sanctum* leaf extract which showed significant antibacterial activity against *E. coli* and *Staphylococcus aureus*.

The objective of this study was to find out a cost-effective and eco-friendly technique for biological synthesis of AgNPs using *Ocimum sanctum* leaf broth. It was also aimed at tackling the optical, structural, and thermal characteristics of synthesized nanoparticles.

2. Materials and Methods

2.1. Preparation of Leaf Broth. The *Ocimum sanctum* plant leaves were collected from Jessore district in Bangladesh. About 20 gm of fresh leaves was thoroughly washed three times with deionized water and chopped into small pieces. Then 100 ml of deionized water was added in 250 ml conical flask, stirred, and boiled for 20 min at 60°C. During boiling a condenser was used as a vapor recovery device. After boiling, the leaf broth was cooled and filtered yielding transparent yellow color leaf broth and these were stored at 4°C. The plant material and leaf broth are showed in Figure 1.

2.2. Synthesis of Silver Nanoparticles. In a typical synthesis of AgNPs, the leaf extract (10 ml) of *Ocimum sanctum* was added to 90 ml of 0.001 M $AgNO_3$ and 0.01 M $AgNO_3$ (99.99%) aqueous solution, respectively, and kept at 33°C. The experiment was done in triplicate for reproducibility. After 10 minutes the color of the solution changed from colorless to yellow indicating the formation of AgNPs. The bioreduced AgNPs solution was collected and monitored by periodic sampling of aliquots (5 ml) of aqueous component and measuring UV-visible spectra of the solution. Due to high optical density of the nanoparticles solution, it was diluted to 10 times with DI water to avoid errors.

2.3. Characterization. The bioreduction of AgNPs from $AgNO_3$ ions in solution was carried out using UV-visible (UV-vis) spectrophotometer (Shimadzu UV-1800, Japan) by recording absorption spectra of the samples in the wavelength range 190–800 nm. To identify the presence of potential biomolecule and functional groups, Fourier transform infrared spectra (FTIR) study was used. FTIR spectra were measured by Perkin Elmer spectrometer having a resolution 4 cm^{-1} in the wavenumber range 500–4000 cm^{-1}. The formation, crystalline behavior, and quality of synthesized silver nanoparticles powder were investigated by X-ray diffraction (XRD) spectrum with Cu-Kα radiation of 0.154187 nm wavelength. The scanning was performed in the region of 2θ from 30° to 80° at 0.02°/min and the time constant was 2 s. The Debye-Scherer equation was used to calculate the size of silver nanoparticles. Scanning electronic microscope (SEM) was used to investigate the surface morphology and particle size of synthesized AgNPs powder. Thermal gravimetric analysis/differential thermal analysis (TGA/DTA) thermal system was used to examine the reaction type and weight loss of synthesized silver nanoparticles powder. The spectrum of TGA/DTA has been recorded in temperature range from room temperature to 1000°C where Al_2O_3 was used for heating and measurement carried out in air atmosphere at the heating rate 20.0°C/min.

3. Results and Discussion

3.1. UV-Vis Study. The formation of the AgNPs was confirmed by change in the color of the solution mixture by the bioreduction of Ag^+ to Ag^0, which is presented in Figure 2. A color change occurred from transparent yellow to reddish brown which indicates the formation of AgNPs during stirring. UV-visible spectra of synthesized AgNPs show the characteristic band at 440–455 nm which indicates crystalline spherical nature of AgNPs. Here we investigate the effect of reaction time on the synthesis of AgNPs.

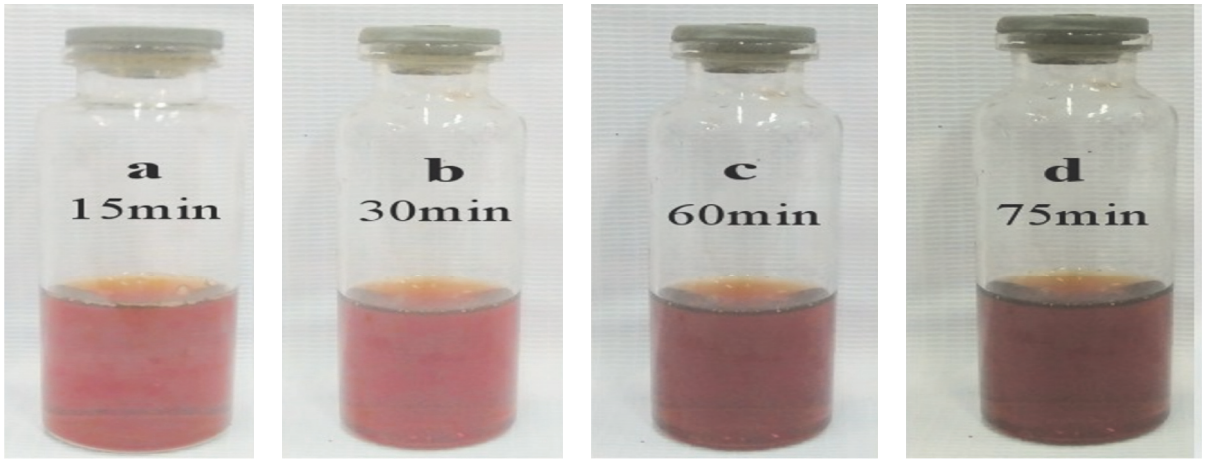

FIGURE 2: Image of synthesized AgNPs solution over reaction times (a) 15 min, (b) 30 min, (c) 60 min, and (d) 75 min, using *Ocimum sanctum* leaf broth.

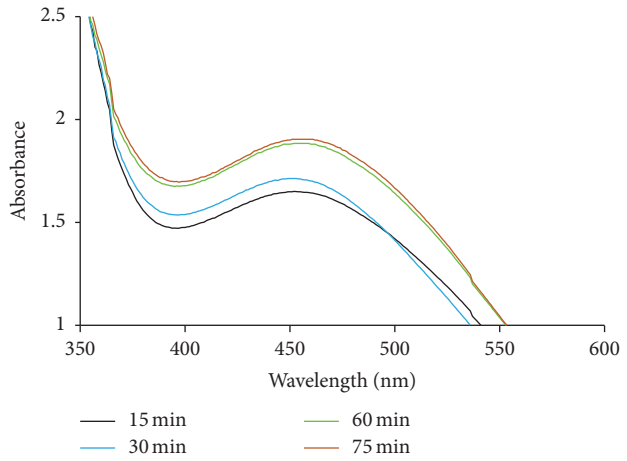

FIGURE 3: Absorption peak of synthesized AgNPs solution using *Ocimum sanctum* leaf broth.

During stirring of mixer of $AgNO_3$ solution and leaf broth the possible chemical reactions for synthesizing the AgNPs are as follows:

$$Ag^{+}\,(aq) + Ocimum\ sanctum \longrightarrow [Ag\,(Ocimum\ sanctum)]^{+} \quad (1)$$

$$[Ag\,(Ocimum\ sanctum)]^{+} + \text{R-CHO} \longrightarrow [Ag\,(Ocimum\ sanctum)] + \text{R-COOH} \quad (2)$$

After mixing of $AgNO_3$ solution and leaf broth, the dispersion of silver ion in the *Ocimum sanctum* aqueous solution mixer (1) reacted with the Ag to form $[Ag(Ocimum\ sanctum)]^{+}$ complex, which reacted with aldehyde in the molecular structure to form $[Ag(Ocimum\ sanctum)]$ due to reduction of silver ions (2).

Figure 3 shows the UV-visible spectra that were recorded at different time intervals for monitoring the reaction of silver nitrate reduction by the leaf extract. The appearance of the surface plasmon resonance (SPR) band increased in intensity with time. Due to the unique optical properties of silver nanoparticles, a great deal of information about the physical state can be obtained by analyzing the spectra. The spectra clearly show the increase in intensity of silver solution with time, indicating the formation of increased number of AgNPs in the solution. The sharp bands of silver colloids were observed at ~450 nm. The intensity of absorption band increases with increasing time period of aqueous component and consequent color changes were observed from yellow to reddish brown, shown in Figure 2. This characteristic color variation is due to the excitation of the SPR in the metal nanoparticles. The broadening of peak indicated that the particles are spherical and polydispersed. The SPR band in the AgNPs solution remains close to 450 nm throughout the reaction period indicating that the particles are dispersed in the aqueous solution, with no evidence for aggregation.

The optimum temperature required for the completion of reaction was investigated to be 60°C. Upon a further increase in temperature (up to 75°C), no further absorbance increase was observed and shows an increase in AgNPs size. Further increase in temperature caused the broadening of the peak revealing the increased size of nanoparticles. This temperature dependent increase in the peak intensity showed the dependence of the silver ion reduction on the reaction temperature. It was observed that reduction rate of silver ions increased by increasing temperature.

3.2. FTIR Study. FTIR measurements were responsible for identifying the functional group which were responsible for the capping and efficient stabilization of synthesized silver nanoparticles by the leaf broth. Absorption peaks of synthesized AgNPs using *Ocimum sanctum* were observed at 670.27 cm^{-1} assigned to C-H stretch in alkenes, 950.92 cm^{-1} assigned to C-H bending in alkenes, 1062.08 cm^{-1} assigned to

Table 1: Distribution of particle size of nanopowder using *Ocimum sanctum* leaf broth.

2θ values (degree)	hkl	FWHM (β) in radians	Particle size (nm)
32.2	(110)	0.005765	25
38.12	(111)	0.00433	34
44.34	(200)	0.00433	35
46.24	(200)	0.005765	26
54.82	(211)	0.00742	21
57.52	(211)	0.007343	22
64.56	(220)	0.00433	38
67.34	(221)	0.005765	29
74.44	(310)	0.00742	23
76.8	(311)	0.00433	41

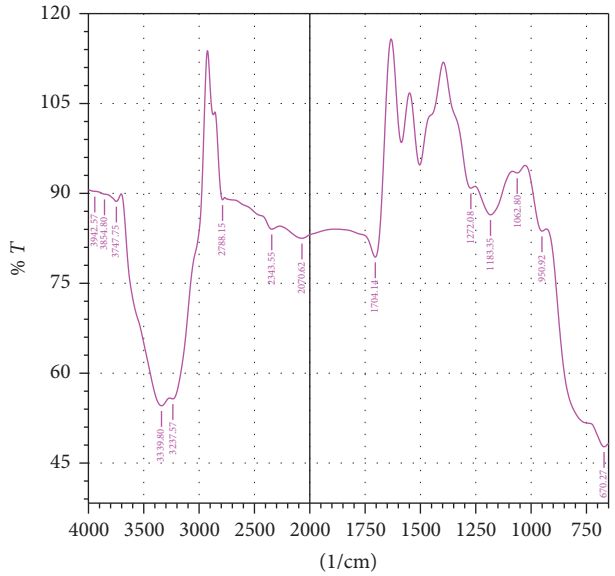

Figure 4: FTIR spectrum of synthesized AgNPs using *Ocimum sanctum* leaf broth.

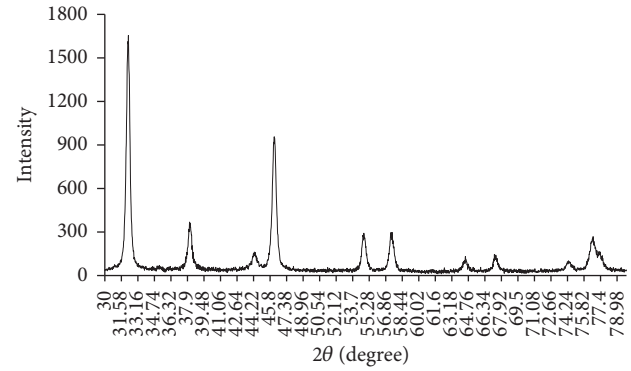

Figure 5: XRD pattern of synthesized silver nanoparticles using *Ocimum sanctum* leaf broth.

C-O stretch in ester, 1183.35 and 1272.08 cm^{-1} assigned to C-N stretch in amines, 1704.14 cm^{-1} assigned to C=O stretch in carbonyl, 2788.18 cm^{-1} assigned to O-H stretch in carboxylic acid, and 3237.57 and 3393.8 cm^{-1} assigned to O-H stretch in alcohol (Figure 4). This analysis strongly supported the capping behavior of synthesized silver nanoparticles by *Ocimum sanctum* leaf broth to stabilize the silver nanoparticles [19].

Still up to date there is no proper mechanism for the synthesis of silver nanoparticles. The proposed hypothetical mechanism behind the synthesis of nanoparticles is an enzymatic reaction in which the plant extract contains the complex of reducing enzymes which reduce the chemicals such as silver nitrate into silver ions and nitrate ions. Plants contain a complex network of antioxidant metabolites and enzymes that work together to prevent oxidative damage to cellular components. It was reported that plants extracts contain biomolecules including polyphenols, ascorbic acid, flavonoids, sterols, triterpenes, alkaloids, alcoholic compounds, polysaccharides, saponins, β-phenylethylamines, glucose and fructose, and proteins/enzymes which could be used as reductant to react with silver ions and therefore used as scaffolds to direct the formation of AgNPs in the solution. Hypothetically, biosynthetic products or reduced cofactors play an important role in the reduction of respective salts to nanoparticles.

3.3. XRD Study. Figure 5 shows the XRD pattern of synthesized silver nanoparticles using *Ocimum sanctum* leaf broth. The d-spacing is calculated by Bragg's law; that is, $d = \lambda/2\sin\theta$, where λ is wavelength in nm. The intense peaks, face-centered-cubic (FCC) plans, full width at half maximum (FWHM), and particle size are calculated mathematically and shown in Table 1. The intense peaks at 2θ degree values 38.12°C, 44.34°C, 64.56°C, and 76.8°C correspond to (111), (200), (220), and (311) for FCC plans of silver nanoparticles using *Ocimum sanctum* [20]. The particle size of the nanopowder has been calculated by *Debye Scherrer* formula $D = k\lambda/\beta\cos\theta$, where D is particle diameter size, k is Scherrer constant (0.9), λ is wavelength of X-rays (0.1541 nm), β is width at half of reflection at Bragg's angle 2θ, and θ is Bragg angle. The average particle size is 29 nm for using of *Ocimum sanctum* leaf broth.

3.4. SEM Study. The surface morphological and nanostructural studies of synthesized silver nanoparticles using

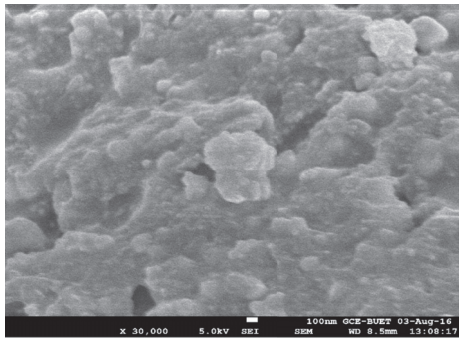

Figure 6: SEM image of synthesized silver nanoparticles using *Ocimum sanctum* leaf broth.

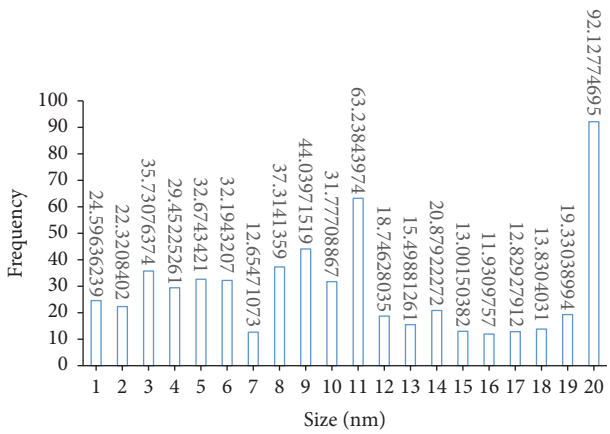

Figure 7: Particle size distribution of nanopowder using *Ocimum sanctum*.

Ocimum sanctum leaf broth were investigated by SEM. In SEM image of synthesized silver nanoparticles using *Ocimum sanctum* is shown in Figure 6. This indicates that the monodispersive and crystalline silver nanoparticles are obtained. The spherical size of synthesized silver nanoparticles using *Ocimum sanctum* leaf broth is 29 nm as estimated from the SEM picture similar to the particle size calculated from *Debye Scherrer* formula (Figure 7). Some nanoparticles size is larger because silver nanoparticles have the tendency to agglomerate due to their high surface energy and high surface tension of the ultrafine nanoparticles.

3.5. DT/TGA Study. A ceramic crucible (Al_2O_3) was used for heating and measurements were carried out in air atmosphere at the heating rate of 20°C/min. Curves of TGA and DTA of synthesized silver nanoparticles using *Ocimum sanctum* leaf broth are shown in Figure 8. The weight loss of silver nanoparticles using *Ocimum sanctum* is observed in TGA curve which occurs from 200°C to 400°C and again a loss occurs from 700°C to 1000°C which is shown in figure. DTA curve of synthesized silver nanoparticles using *Ocimum sanctum* leaf broth shows intense endothermic peak when temperature increases. DTA curve shows the thermal decomposition and crystallization of synthesized silver nanoparticles.

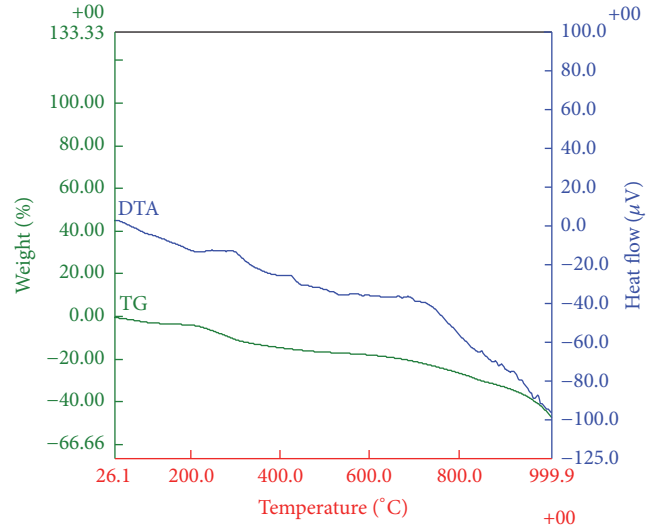

Figure 8: TGA and DTA curve of synthesized silver nanoparticles using *Ocimum sanctum* leaf broth.

4. Conclusion

The biological synthesis process is a reliable, eco-friendly, and cost-effective process for the synthesis of nanoparticles. In UV-vis spectrometer detected peaks at 450 nm confirm the formation of AgNPs. FTIR spectrum confirms the functional group of organic compound which is responsible for capping, formation, and stabilization of AgNPs. The XRD analysis confirms the FCC plans of the synthesized nanoparticles and the particles size of 29 nm using *Ocimum sanctum* leaf broth. SEM images confirm the morphological and nanostructural characteristics of nanoparticles with average size distribution calculated by *Debye Scherrer* formula. DTA/TGA curve shows the weight loss of AgNPs with temperature which also confirms the thermal decomposition and crystallization of nanoparticles. The resulting synthesized AgNPs were capped by a thin layer of proteins and metabolites having functional groups of amines, alcohol, ketones, aldehydes, and carboxylic acids which are responsible for formation and stabilization of nanoparticles. Prepared nanoparticles can be used as bactericidal, wound healing, and water purification agents and in medicine field. Due to these applications, this method is potentially exciting for the large-scale synthesis of nanoparticles.

Conflicts of Interest

The authors declare that there are no conflicts of interest.

Acknowledgments

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and Ceramic Engineering, BUET, Bangladesh, is greatly appreciated.

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