Biosynthesis of Silver Nanoparticles from Eucalyptus Corymbia Leaf Extract at Optimized Conditions

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Abstract. This study reports the biosynthesis of narrow range diameter silver nanoparticles at optimum conditions using \textit{Eucalyptus corymbia} as a reducing and stabilizing agent. Optimal conditions for biosynthesis of silver nanoparticles (AgNPs) were found to be; an extraction temperature of 90\degree C, pH of 5.7 a Silver Nitrate concentration of 1mM and AgNO\textsubscript{3} to plant extract ratio of 4:1. UV-Visible spectroscopy monitored the formation of colloidal AgNPs. The UV-Visible spectrum showed a peak around 425 nm corresponding to the Plasmon absorbance of the AgNPs. The size and shape characterization of the AgNPs was done using Transmission Electron Microscopy (TEM) techniques which revealed narrow range diameter (18-20 nm), almost monodispersed AgNPs, spherical in nature and with minimal agglomeration. Energy Dispersive X-ray (EDX) results showed the presence of two peaks at 3.0 and 3.15 keV in the silver region. The Fourier Transform Infrared-Spectra (FTIR) of the plant extract and the AgNPs gave rise to vibrational peaks at 3260 and 1634 wavenumbers which are due to the presence of OH and \textit{–C=C–} functional groups respectively.

Introduction

Nanoparticles are nanosized structures in which at least one of its phases has one or more dimensions (length, width or thickness) in nanometer range i.e. 1-100nm. Research in metal nanoparticles has attracted significant attention in recent years owing to unique properties and practical applications of nanoparticles [1]. Their optical, electrical, magnetic, physical and chemical properties differ significantly from those of bulk materials [2]. Metal nanoparticles size and shape dependent properties are of interest due to the broad applications of nanoparticles such as catalyst, optical sensors, in data storage and antimicrobial applications [3]. Antibacterial property of silver nanoparticles is reported to be size and shape dependent [4]. Elsewhere, cooperatively nitrogen-doped ordered mesoporous carbon/iron oxide nanocomposites [5] and bimodal porous carbon-silica nanocomposites [6] have exhibited large surface areas thus enabling them to be employed as anode materials for lifetime batteries.

There are two approaches for the synthesis of nanomaterials and the fabrication of nanostructures; Top-down approach and Bottom-up approach. Top-down approach involves making smaller and smaller structures or particles through etching from the bulk material while bottom-up approach involves building up nanoscale structures from atoms and molecules [7]. The biggest problem with the top-down approach is the imperfection of surface structure and significant crystallographic damage to the processed patterns, and therefore bottom up approach is preferred. Nanoparticles exist naturally or the can be fabricated in laboratory. Those that are made in laboratory are said to be engineered nanoparticles. Engineered nanoparticles are synthesized through different methods; chemical, physical and biological methods. Chemical and physical methods may successfully produce pure, well-defined nanoparticles, but these techniques are more expensive, energy consuming and potentially toxic to the environment [8].
Ideally metallic nanoparticles should be prepared by a method which is reproducible, which allow control of the shape of the particles, yields monodispersed nanoparticles, cheap and easy, uses fewer toxic precursors with fewer solvents, with few synthetic steps (one-pot reaction), produces less toxic waste products and uses a reaction temperature close to room temperature [9]. Nanoparticles coalesce to give thermodynamically favoured bulk particle in absence of counter-active repulsive force between the nanoparticles due to their large surface energy [10]. The van der waals forces act as the driving force leading to coagulation. For this reason, spatial confinement of the particles in nano range is essential to stabilize the particles. This can be achieved by electrostatic or steric stabilization using capping agent such as polymer, surfactant or ligands [10]. Electrostatic stabilization is weak and sensitive to ionic strength of the dispersion medium and works only in polar solvents that can dissolve electrolytes. Steric stabilization in solution is achieved by binding of polymers or surfactant molecules with long alkyl chains to the particle surface [10]. The long alkyl chains prevent the particles from coming close to each other. Chemical method uses toxic organic solvents as such as hydrazine hydrate, sodium borohydride and dimethylformamide as reducing, stabilizing or capping agents. Capping and stabilizing agents are used prevent aggregation which may hinder the production of small sized silver nanoparticles [8]. The biological method offers a better synthetic protocol devoid of toxic reducing, capping and stabilizing agents. Bio-inspired methods utilize plant extracts and microorganisms to reduce the metal precursor to nanoparticles and in some cases help in stabilization or capping of the formed nanoparticles [11]. The bioreduction of the metal ions is accomplished by combinations of biomolecules found in plant extracts (e.g., enzymes/proteins, amino acids, polysaccharides, and vitamins) in an environmentally benign, yet chemically complex process [12]. Microbe mediated synthesis is not industrially feasible due to the requirements of highly aseptic conditions and maintenance of cell culture [13]. Plant-mediated synthesis is preferred over use of microorganisms (bacteria or fungi) due to ease of improvement; it eliminates the elaborate process of maintaining cell culture and it’s a single step process (one pot reaction) [14]. Plants are also readily available, widely distributed, safe to handle with a full range of metabolites and other active groups which can be used as reducing, capping and stabilizing agents [14].

Synthesis of gold, silver and palladium nanoparticles from plant extracts such as geranium leaves, Lemongrass, Neem leaves, Aloe Vera [15-18] among others has been reported. These researchers have not only been able to synthesize nanoparticles but also obtained particles of exotic shapes and morphologies. These impressive successes have opened up avenues to develop “greener” methods of synthesizing metal nanoparticles with perfect structural properties using mild starting materials [19].

The present study reports the synthesis of silver nanoparticles from Eucalyptus corymbia maculata leaf extract at optimized conditions. Corymbia maculata contains many chemical compounds that play several roles in the plant. These include defense against insect and vertebrate herbivores and protection against UV radiation and cold stress. The best-known compounds are the terpenoids, which form most of the essential oil giving eucalyptus foliage its characteristic smell. However, eucalyptus is also a rich source of phenolic constituents such as tannins and simpler phenolics [20]. The fundamental characteristics of metallic nanoparticles strongly depend on their size and their morphology [21]. In order to achieve a control of the size, morphology, stability and rate of biosynthesis, nanoparticles are prepared at optimized conditions.

Materials and Methods

Reagents and Graphical Methodology for Nanoparticle Preparation. Silver nitrate (AgNO₃) crystals, HPLC grade Methanol, Ethanol and Diethyl Ether were purchased from Fischer Scientific Chemicals (United Kingdom). Distilled de-ionized water (DDW) was used in all the experiments. Folin-ciocalteu’s phenol reagent (2N), NaOH, FeCl₃, and Gallic Acid of analytical grade were purchased from Sigma-Aldrich (Germany). The instruments used were UV-1700 Pharmaspec spectrophotometer, FTIR-JAS-CO 4100, Philips Technai-FE 12 TEM, thermo-shaker (Gallenkamp).
A leaf extract of Eucalyptus corymbia was prepared by weighing 5g of green leaves. The leaves were properly washed in distilled de-ionised water, chopped into small pieces and transferred to 250ml Erlenmeyer flask containing 100ml of distilled water. The mixture was boiled at 90°C for 5 minutes before filtering using whatman filter paper. The filtrate obtained was centrifuged at 15000rpm for 10 minutes and stored at 4°C for subsequent use within the next seven days after extraction. The whole procedure is summarized in Fig. 1, below.

Confirmatory Test for Phenolic Compound in the Leaf Extract.
An aliquot of Folin-ciocalteus’s phenol reagent (2N) was added to 5mLs of the leaf extract and the colour change recorded [22] to verify presence or absence of phenolic compounds in the extract. Absence of these substances would signify that the extract could not play the reductant/stabilizing role during the process of nanostructurization.

Synthesis of Silver Nanoparticles (AgNPs).
Exactly 1.7g of silver nitrate salt was dissolved in 10mL of distilled de-ionized water to make a 1M solution. An aqueous solution of 1mM AgNO₃ was prepared from 1 ml of 1M AgNO₃ in a litre of distilled de-ionized water. Different volumes of the leaf extracts were added slowly to varying amounts of aqueous silver nitrate solutions in a 250ml Erlenmeyer flask and stirred vigorously. This procedure was repeated for preparation of 0.8mM, 0.6mM, 0.4mM and 0.2mM of silver nanoparticles. An analysis was done on the resulting solutions.

UV-Vis Procedures
Preparation of Solutions for Optimization using UV-Vis. All solutions prepared for UV-Vis analysis were diluted ten times before the analysis was done. All the UV-Vis analysis were done using UV-VIS double beam spectrophotometer [UV-1700 pharmaSpec UV-Vis spectrophotometer (shimadzu)]. Scanning of the spectra was done between 200-700nm at a resolution of 1 nm using quartz cuvette. Baseline correction was done using de-ionized water as the blank.
Extraction Temperatures of Plant Extract. 5g of eucalyptus corymbia leaves were cut into fine pieces and transferred into 250ml Erlenmeyer flask containing 100ml of distilled water then put in a water bath, and the temperature maintained at 25°C, 50°C, 70°C and 90°C by heating. 20ml of each plant extract was added to 80ml of 1mM silver Nitrate solution and the UV-Vis spectra of the resulting diluted solution taken.

Optimization of Different Parameters pH. The pH of plant extract (5.7) was adjusted using 1M HCl and 1M NaOH to a final pH of 3.7, 7.7, 8.7. Exactly 20ml of the plant extract with adjusted pH, was added to 80ml of 1mM Silver Nitrate and analysed for the size and position of the surface plasmon (SPR) peak using UV-Vis spectrophotometer.

Concentration Ratio of Silver Nitrate to Plant Extract. Different volumes of silver nitrate and plant extract solution were added together in the ratio (1:1, 4:1, 7:3, 9:1). The absorbance of the resulting solution measured spectrophotometrically to study the influence of degree of acidity on the SPR peak.

Time and Stability Study. An extract of Eucalyptus corymbia obtained at 90°C and 1mM silver nitrate were mixed in the ratio 4:1. Absorbance of the resulting solution measured spectrophotometrically every 24 hours for 30 days to study the stability of the SPR with time.

Effect of Concentration of Silver Nitrate Solution Procedure. Different concentrations of silver nitrate (0.1mM, 0.2mM, 0.4mM, 0.6mM, 0.8mM, and 1.0mM) were reacted with plant extract and the UV-Vis analysis done.

Fourier Transform Studies of the Nanoparticles
Pelletization of the AgNPs. A dry powder of the samples was obtained by centrifuging at 15000rpm a solution of silver nitrate and plant extract at optimized conditions. This powder was mixed with a small amount of potassium bromide (KBr) and pulverized in a crucible. The mixture was then pressed in a mechanical press transforming into a thin transparent pellet. The collar and the pellet were put onto the sample holder for Fourier transform infra-red study. The nanoparticles pellets were analyzed using FTIR-JAS-CO 4100 spectrophotometer in the range of 4000–400 cm⁻¹. FTIR spectroscopy of plant extracts was obtained by dropping a sample between two plates of sodium chloride (salt) and analyzed in a liquid cell.

Transmission Electron Microscopy (TEM) Studies
TEM analysis was done by drop coating biologically synthesized silver nanoparticles solution onto carbon-coated copper TEM grids. The AgNPs were dispersed in acetone. TEM images were acquired on Philips Technai-FE 12 TEM instrument operated at an accelerating voltage of 120 kV equipped with an Energy Dispersive X-ray (EDAX) detector (Oxford LINK-ISIS 300) for elemental composition analysis and the EDAX spectra was measured at an accelerating voltage of 10 kV.

Percent Yield of Silver Nanoparticles
A 10 ml aliquot of the mixture of plant extract and silver nitrate solutions were centrifuged at 15000rpm and washed in distilled de-ionized water and oven dried at 60°C for 24 hours. The resultant AgNPs powder weights were noted. The percentage yields were obtained from Eq. 1, where \( Ag^0 \) is the weight of the synthesized silver nanoparticles and \( Ag^+ \) is the mass of silver ions in 10 ml of 1mM AgNO₃.

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\text{% yield} = \frac{\text{mass of } Ag^0}{\text{mass of } Ag^+ \text{ ion}} \times 100
\]

Eq. 1
Results and Discussion

Testing for Phenolic Compounds from the Plant Extract. Two tests were used to establish the presence of polyphenolic materials in the plant extracts namely the Ferric chloride and the Folin-ciocalteus’s phenol reagent test. On addition of FeCl₃ solution (30 mM) to the aqueous leaf extract, a change of color from reddish-brown to blue-black color indicated the presence of hydrolysable tannins – a class of astringent polyphenolic biomolecules, Fig. 2. Condensed tannins give brownish-green colour while forming ferrous compound [23].

Figure 2: A Photograph Showing (a) A Positive Reaction for the Presence of Phenols in Plant Extract (b) Ferric Chloride without Plant Extract.

Similarly, when an aliquot of Folin-ciocalteus’s phenol reagent (2N) was added to 5mLs of the leaf extract the colour changed from yellow to black, Fig. 3. This Folin-ciocalteus’s phenol reagent test also called the Gallic acid equipment method (GAE) measures not only the presence of phenols but also reacts with any reducing substance [24]. It, therefore, measures the total reducing capacity of a sample. Change of its color from yellow to black (Fig. 3), confirmed the presence of reducing compounds in Eucalyptus corymbia leaf extract.

Figure 3: A Photograph showing (a) Folin-ciocalteus’s Phenol Reagent Without Plant Extract (2N). (b) Folin-ciocalteus’s Phenol Reagent (2N) With the Plant Extract.

Though no effort was made whatsoever to isolate the biomolecules form the plant extracts, these tasts show that *Eucalyptus corymbia* leaf extract contains a variety of bio-molecules which can play a reduction and stabilizing role towards nanoparticle [25], among these bio-molecules are polyphenols and water-soluble heterocyclic compounds [26].
Silver Nanoparticles Synthesis. Formation of silver nanoparticles (AgNPs) from the plant extract and AgNO₃ was noted by visual observation. A gradual colour change, which took less than ten minutes from a colorless solution to yellow then deep red/brown on the addition of the leaf extract of Eucalyptus corymbia, indicating the formation of AgNPs (Fig. 4), which was further confirmed by UV-Vis analysis.

The appearance of the brown color was due to collective oscillation of the conduction electrons in resonance with the wavelength of irradiated light [27]. Typical AgNPs have surface plasmons resonance peaks with λₓᵧ values in the visible range of 400–500 nm [28]. The biosynthesized silver nanoparticles were found to have an absorbance peak at around 425nm as shown in figure 5. The appearance of surface plasmon resonance (SPR) peak with a maximum at 430 nm provides a convenient spectroscopic signature for the formation of silver nanoparticles indicating a reduction of silver ions and formation of stable nanoparticles [29]. Various factors are known to influence nanoparticles synthesis. These include volume of the biological material and metal salt concentration, degree of acidity [29] among others and were therefore studied for optimization.

Optimization

Effect of Metal ion Concentration on the Silver Nanoparticles Synthesis

The influence of metal ion concentration on the synthesis of AgNPs by green synthetic method was investigated by measuring UV–visible spectra of the reaction solutions at different silver nitrate concentration. Plasmon peak for AgNPs became more distinct with an increasing concentration of AgNO₃ indicating the formation of a higher number of AgNPS which is in agreement with Beer-Lamberts’s law. Silver nitrate concentration of 1mM was adopted as the optimum concentration for the synthesis of AgNPs. Figure 5 below shows an absorbance spectrum of silver nanoparticles synthesized with varying silver nitrate concentrations (0.2mM-1mM) and plant extract.
Figure 5: a) The Absorbance Spectrum of Silver Nanoparticles Synthesized with Varying Silver Nitrate Concentrations from 0.2 mM to 1mM with a Wavelength Maximum at Around 425nm (b) A plot of Absorbance Against Concentration at the $\lambda_{\text{max}}$.

Figure 5 (a), shows a slight peak absorbance shift towards higher wavelengths. The Plasmon bands are not very broad indicating a near monodispersity of nanoparticles [30]. The calibration curve Fig. 5b, indicates the nanoparticle formation process obeys Beer Lambert’s law. An increase in silver nitrate concentration enhances the number of silver ions which are consequently reduced and stabilized to form nanoparticles.
Effect of Plant Extract pH on Silver Nanoparticles Synthesis
The influence of pH on the synthesis of AgNPs was investigated by determining UV–Visible spectra of the reaction mixtures prepared with the plant extract at various pH values. Figure 6 shows an absorbance spectrum of silver nanoparticles synthesized with 1mM silver nitrate concentration and varying pH of plant extract i.e. 3.7, 5.7 (plant extract pH), 7.7, 8.7 and 10.7.

Figure 6 indicates that the peak area and height of the UV–visible spectrum obtained for the reaction solutions at pH 10.7 were considerably higher than those at other pH values, meaning AgNPs formation is pH dependent and that among the various pH studied a pH of 10.7 was optimal. It seems nanoparticle formation is favoured by an alkaline environment. Studies reveal that addition of NaOH to silver nitrate solution favours the formation of silver oxide (Ag₂O) precipitate which is reduced to pure silver (Ag) in the presence of a suitable reducing agent i.e. reduction of Ag⁺ to Ag⁰ occurs on the surface of existing colloids in the system [31].

Nanoparticles formation is however not favoured in an acidic environment as is indicative of the large absorbance gap between the spectrums due to pH 3.7 and 7.7. Reaction pH has ability to change the electrical charges of biomolecules which might affect their capping and stabilizing capacity and the subsequent growth of nanoparticles. Phenols are considered to be weak acids, and therefore, react with and dissolve in strong bases; like sodium hydroxide [32]. However because of the bathochromic shift seen in the 10.7 pH solution surface plasmon peak and the intended end use of the nanoparticles in antimicrobial activity studies (which could be pH sensitive), a pH of 5.7 was adopted for all synthesis.

Figure 6: Absorbance Spectrum of Silver Nanoparticles Synthesized Using 1mM Silver Nitrate and Plant Extract at Varying pH. A Wavelength Range of 200 (nm) to 700 (nm) Was Used.
Effect of Extraction Temperature of Plant Extract on Silver Nanoparticles Synthesis

The influence of extraction temperature of plant extract on the synthesis of AgNPs was investigated. This was done by measuring UV–visible spectra of the reaction solutions prepared using plant extract obtained at different extraction temperatures; at (25°C) room temperature, 50°C, 70°C and 90°C Fig. 7, below. The color intensity of the resulting solutions deepened with increasing extraction temperature indicating the formation of more AgNPs [33].

![Figure 7: Absorbance Spectrum of Silver Nanoparticles Synthesized Using 1mM Silver Nitrate and Plant Extract at pH (5.7) at Various Extraction Temperatures.](image)

There was a corresponding increase in the absorbance (peak) with increase in the extraction temperature. The peak area and height of the UV–Visible spectra obtained for the reaction solutions at an extraction temperature of 90°C were considerably higher than those at other extraction temperatures. Higher concentration of nanoparticles was formed when extraction was done at 90°C. This implies that at higher temperatures the solubility of the reducing compounds in *Eucalyptus corymbia* is high. Studies reveal that increase in temperature increases the solubility of water soluble bio-molecules mostly polyphenols and heterocyclic compounds [34] which increase the reducing and stabilizing power of the plant extract. Furthermore, such observation is in line with the Arrhenius theory of reaction rates which states that an increase in the temperature of the reactants increases the kinetic energy of reacting particles leading to a greater portion of the collisions meeting the minimum energy threshold requirements for a reaction to occur. This consequently translates into increased yield (AgNPs formation in this case).

**Silver Nitrate to Plant Extract Volume Ratio Nanoparticle production**

The plant extract contains bio-molecules responsible for the reduction of metal ions to their respective atoms and stabilization of nanoparticles formed [35]. Also according to Beer’s law there is a direct correlation between the absorbance of a chromophore and its concentration in solution. Figure 8 below shows absorbance responses of various AgNPs synthesized at different silver nitrate to plant extract ratios at the optimal 90°C temperature.
From Fig. 8, AgNPs production is seen to dependent on the silver nitrate to plant extract ratio. The highest absorbance and the most well behaved peak was obtained at a ratio of 4:1. It probably means this biomaterial is rich in phenolic reductants with multiple metal ion reducing sites. Therefore, amount of aliquot used has no much influence on AgNPs formation meaning that probably the number of silver ions are the limiting factor. Beyond a silver nitrate to plant extract ratio of 4:1 probably other factors like site saturation would explain why AgNPs production at higher ratios would be lower.

**Effect of Time on Stability of Nanoparticles**

The AgNPs stability was assessed after certain duration of storage using UV–vis spectroscopy. The absorbance of the AgNPs was studied between 0 and 30 days on AgNPs prepared under optimal conditions, Fig. 9, below. In the figure, immediately means just after adding the precursors.
Figure 9: Absorbance Spectrum of Silver Nanoparticles Synthesized using 1mM Silver Nitrate and a Plant Extract pH (5.7) Extracted at 90°C and Silver Nitrate to Plant Ratio 4:1.

The position of the Plasmon peak shifts to lower wavelength indicating the formation of bigger nanoparticles [36]. The intensity of the SPR peak increased with increase in reaction time, indicating increasing concentration of the silver nanoparticles. Prepared nanoparticles were found to be stable over the time period and with little aggregation as confirmed by TEM studies below. The stability results from a potential barrier that develops as a consequence of the competition between weak van der Waals forces of attraction and electrostatic repulsion [36].

Transmission Electron Microscopy and Energy Dispersive Spectroscopy Results
Transmission electron microscopy results, Fig. 10, for the biologically synthesized AgNPs revealed that the mean average AgNPs diameter was 18-20nm indicating monodispersity. The particles were spherical in shape and exhibited minimal agglomeration implying that the Eucalyptus corymbia extracts were well suited for the reducing/stabilizing roles.
Energy Dispersive X-ray Spectroscopy was used to verify the presence of silver in the sample. As reported in our previous work, Sila et al., (2014), two peaks at 3.0 keV and 3.15 keV were formed in the silver region. The typical optical absorption band peaked nearly at 3 KeV confirms the formation of metallic silver nanoparticles [38].

Fourier Transform Infra-Red Spectroscopy Analysis
The FT-IR spectra of Eucalyptus corymbia leaf extract and synthesized nanoparticles were done to identify the possible biomolecules responsible for the reduction of the Ag⁺ ions and capping of the bio-reduced Ag-NPs. According to our previous work, Sila et al., (2014), the FT-IR results showed the presence of (–C=C) and hydroxyl (–OH) groups in Eucalyptus corymbia leaf extracts.

Calculating Percent Yield of Silver Nanoparticles
When three replicates of 10ml AgNO₃-plant extract solution were centrifuged, the mass of silver nanoparticles produced were 0.000879g, 0.000881g, 0.000884g. The percentage yield was calculated using Eq. 1. The percent yield of AgNPs synthesized was 81.6 ± 0.3%, implying that 81.6 ± 0.3 % of the silver ions were converted to atomic state hence forming silver nanoparticles.

Conclusion
The use of Eucalyptus corymbia leaf extract offers a simple synthetic protocol devoid of toxic chemicals either as reducing, stabilizing or capping agents in line with green chemistry principles. Ferric chloride and Folin-ciocalteus’s phenol reagent tested positive for the presence of reducing compounds. FT-IR spectra of the plant extract reveal that functional groups OH and –C=C– were responsible for reduction and stabilization of the nanoparticles. The particles were almost monodispersed with an average mean size of 18-20 nm and were spherical in shape without significant agglomeration as revealed by the TEM analysis. EDX spectrum showed a strong signal in the silver region hence confirming the formation of silver nanoparticles. At optimum conditions, a yield of 81.6 ± 0.3% was obtained.
Conflict of Interest
No conflict of interest.

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References


