Chapter 6

Green synthesis and characterization of silver nanoparticles using Jatropha seedcake extract

This part has been communicated as:
6.1. Introduction

Nanoparticles (structures with at least one dimension approximately within 1 to 100 nm) have received considerable attention in both scientific and technological areas due to their unique and unusual physico-chemical properties compared with that of bulk materials (Bhushan 2004). Further, metal nanoparticles are particularly interesting because they can be easily synthesized, modified chemically and applied for device fabrication (Feldheim and Foss 2002).

Amongst various metal nanoparticles, silver nanoparticles (AgNPs) are perhaps most widely recognized owing to its broad range of applications such as, in photonics (Gould et al 2000), micro-electronics (Maekawa et al 2010), photocatalysis (Chen et al 2010), lithography (Hulteen et al 1999), biosensing (Ngece et al 2011), etc. The properties and applications of nanoparticles particularly depend on their morphology i.e. crystal structure and dimensions. Consequently, significant research has been concentrated on developing techniques to control their size, shape and distribution (Ozin 1992, Feldheim and Foss 2002). Different surface passivator reagents such as thiophenol, thiourea, mercapto-acetate etc. have been employed in order to prevent the agglomeration of particles during and upon their synthesis as well as to subsequently control their size (Li et al 2003, Stewart et al 2012). Unfortunately, these passivators are toxic (HSDB, TOXNET, U.S. NLM, NIH), would raise environmental issues, if they are to be used in processes for large scale synthesis of nanoparticles.

In addition, AgNPs have also been recognized as novel therapeutic agents extending its use as antibacterial, antifungal, antiviral, anti-inflammatory and anti-cancerous agents (Vaidyanathan et al 2009). The broad-spectrum antimicrobial properties of AgNPs encourage its use in a large number of biomedical and environmental applications as well as in cosmetics, clothing and different consumer products (Rai et al 2009). The conventional synthesis of AgNPs involve number of chemical and physical methods that are energy and capital intensive, employ toxic chemicals, thus limiting its biomedical applications. Consequently, there has been growing need for synthesis of bio-compatible metal nano-particles, without employing toxic chemicals in the synthesis protocols. In this respect, use of microorganisms in biosynthesis of nano-particles has been demonstrated in last two decades, particularly owing to their facile assembly of the nano-dimensional...
particles. However, slow reaction rates, difficulty in maintaining culture and aseptic conditions during synthesis, directed research towards the use of plant part and extract for the synthesis of metal nanoparticles. Gardea-Torresdey and co-workers first reported the formation of AgNPs by living plants (Gardea-Torresdey et al 2003). Later on, the synthesis of AgNPs has been reported using plants (Iravani 2011) and plant products like green tea (Camellia sinensis) (Vilchis-Nestor et al 2008), natural rubber (Abu Bakar et al 2007), Aloe-vera plant extract (Chandran et al 2006), latex of Jatropha curcas (Bar et al 2009), mesocarp of coconut (Roopan et al 2013) etc.

In the present chapter, we discussed the use of Jatropha seedcake as reducing and capping agent for biogenesis of AgNPs. The AgNPs thus obtained were characterized by Transmission Electron Microscopy (TEM), X-Ray Diffraction (XRD), and Fourier Transform Infra-Red (FTIR) spectroscopy.

6.2. Materials and Methods

6.2.1. Materials

Jatropha seedcake (JSC) was obtained as gratis from the Food Processing and Bioenergy division, Anand Agriculture University, Anand, Gujarat, India. The seedcake was sun dried and powdered. 10 g powdered JSC was boiled in 100 mL distilled water for 1 h and then filtered to get the extract. This extract was used as reducing agent and stabilizer. Silver nitrate (AgNO$_3$) analytical grade was purchased from Hi-Media, India.

6.2.2. Synthesis of Silver nanoparticles

In a typical reaction procedure, a measured volume of JSC extract was added to appropriate volume of aqueous AgNO$_3$ solution to get a final volume of 10 mL, the mixture was heated at 90°C and the resulting solution became reddish in color after 15 min of heating. The reaction was stopped by plunging the tubes in ice-cold water.

Reactions were performed to determine the effect of varying concentrations of JSC extract and AgNO$_3$ on the AgNPs synthesis.
6.2.3. Characterization of the nanoparticles

Formation and stability of AgNPs in aqueous colloidal solution was confirmed by UV-Visible spectrophotometry (UV-Visible spectrophotometer, Shimadzu UV-1601) in the wavelength range of 300-700 nm operated at resolution of 1 nm.

The size and morphology of the nanoparticles was determined by transmission electron microscopy (Techai 20; Philips, Holland). AgNPs were mounted on TEM grids by placing a drop of the particle solution on a carbon-coated copper grid and dried overnight. The SAED was obtained by directing the electron beam perpendicular to one of the nanoparticle. The crystalline nature of the metal nanoparticles was confirmed XRD using Powder X-ray diffractometer (X’pert MPD; Philips, Holland) with Cu-Kα radiation and data were obtained over the range of 0 to 100° (2θ) with a scanning rate of 0.005°s⁻¹ and step size of 0.02°. The XRD peaks were indexed and crystallographic lattice parameters were determined by Powder X-diffraction analysis software (Dong 1999).

6.2.4. FTIR analysis

The functional groups responsible for reduction and stabilization of the bioreduced AgNPs were analyzed using FTIR spectroscopy (Spectrum GX; Perkin-Elmer). Samples were prepared by grinding 1 to 2 mg of nanoparticles along with 250 mg KBr and pelletized using hydraulic press at 20,000 prf.

6.2.5. Evaluation of antibacterial activity of the bioreduced silver nanoparticles

The antibacterial activity of AgNPs was measured by agar gel diffusion method using following bacterial test cultures: *Escherichia coli* MTCC 40, *Salmonella para-typhi* MTCC 735, *Bacillus subtilis* ATCC 6051 and *Staphylococcus aureus* MTCC 87.

To evaluate the minimum inhibitory concentration (MIC), 50 µL of 10⁸ cfu/mL test culture was added to nutrient broth supplemented with varying concentrations (25 to 500 µg/mL) of silver nanoparticles. Control tubes contained only inoculated broth. The tubes were incubated at 37°C for 24 h. The turbidity of the tubes was measured using visible spectrophotometer (Systronics, Ahmedabad, India) at 600nm.
6.3. Results and Discussion

6.3.1. Synthesis of silver nanoparticles

The formation of AgNPs in aqueous colloidal solution was marked by the change in the colour of the reaction solution from pale yellow to deep red (Mulvaney 1996). The characteristic surface plasmon resonance (SPR) band of colloidal silver was observed between 424 to 438 nm, which is in good agreement with the earlier reports of bioreduced AgNPs (Bar et al 2009, Kumar and Mamidyala 2011, Roopana et al 2013). Furthermore, it was observed that with increase in volume fraction of JSC extract upto 0.1, the intensity of SPR band increased and further increase in JSC extract volume fraction seized the reaction (Fig. 6.1). The increase in the intensity of characteristic SPR band may be attributed to higher concentration of AgNPs (Bar et al 2009). However, at higher volume fraction the reaction mixture became hazy, probably due to the presence of excess biomaterial (Bar et al 2009).

![Figure 6.1 UV-Visible absorption spectra of silver nanoparticles at varying volume fractions of JSC aqueous extract.](image)

Figure 6.2 shows the effect of varying AgNO₃ concentrations on AgNPs synthesis when the reaction was carried out using fixed volume fraction (0.1) of JSC extract. The
intensity of SPR band, which corresponds to concentration of AgNPs, increased with increasing concentration of AgNO$_3$ up to 1 mM. At concentration of AgNO$_3$ above 1 mM, no further increase in intensity of SPR band was observed, indicating the saturation of biomolecules for the reduction of silver ions to silver. Such effect has been earlier reported for synthesis of nanoparticles employing the extract of *Cinnamon zeylanicum* bark (Satishkumar et al 2009) and Banana peel (Bankar et al 2010).

![Figure 6.2 UV-Visible absorption spectra of silver nanoparticles at varying AgNO$_3$ concentration.](image)

**6.3.2. Characterization of the bioreduced silver nanoparticles**

The bioreduced AgNPs were found to be predominantly spherical in shape, well dispersed with no agglomeration (Fig. 6.3). The average particle size of AgNPs observed in TEM image was computed to be 10.48 ± 2.74 nm (Fig. 6.4). The similar size of AgNPs was reported by Philip (2010) and Valodkar et al (2011) using leaf extract of *Hibiscus rosa sinensis* and latex of *Euphorbia nivulia*, while AgNPs of larger size have been reported by Mude et al (2009) and Santhoshkumar et al (2011) using callus extract of *Carica papaya* and leaf extract of *Nelumbo nucifera* as reducing and stabilizing agents. Such size variation
of metal nanoparticles synthesized using plant parts and products could be attributed to
difference in reductive potential and capping biomolecules from different plant origin.

Figure 6.3 TEM micrograph of uniformly distributed silver nanoparticles.

Figure 6.4 Particle size distribution of silver nanoparticles synthesized using JSC aqueous extract

The SAED pattern (Fig. 6.5) indicated the polycrystalline nature of bioreduced
AgNPs. Inter-planar spacing (also known as $d$-spacing) was calculated using Bragg
equation (Bragg and Bragg 1931):

$$d_{hkl} = \frac{\lambda L}{R}$$

(1)
where, $R$ (in mm) is the radius of diffraction pattern, $L$ (320 mm) is the distance between specimen and photographic film and $\lambda$ (0.02736 Å) is the wavelength of the electron based on the accelerating voltage (200 kV). The calculated values for $d$-spacings ($d_{hkl}$) (2.068 and 1.245 Å) were found consistent with the values from standard JCPDS File No. 04-0783 for silver nanocrystals (Table 6.1). Subsequently, two distinct diffraction planes were indexed as (200) and (311) facets which correspond to the cubic phase of silver.

![Figure 6.5 Selected Area Electron Diffraction pattern of bioreduced silver nanoparticles](image)

Table 6.1: Comparison of Inter-planer spacings ($d_{hkl}$) from standard silver diffraction data (JCPDS file no. 04-0783) with the calculated and experimentally observed values from SAED and XRD diffractogram

<table>
<thead>
<tr>
<th>JCPDS No:04-0783</th>
<th>SAED</th>
<th>XRD</th>
</tr>
</thead>
<tbody>
<tr>
<td>$d_{hkl}$ (Å)</td>
<td>h k l</td>
<td>Calculated $d_{hkl}$ (Å)</td>
</tr>
<tr>
<td>2.359</td>
<td>1 1 1</td>
<td>ND</td>
</tr>
<tr>
<td>2.044</td>
<td>2 0 0</td>
<td>2.068</td>
</tr>
<tr>
<td>1.445</td>
<td>2 2 0</td>
<td>ND</td>
</tr>
<tr>
<td>1.231</td>
<td>3 1 1</td>
<td>1.245</td>
</tr>
<tr>
<td>1.1796</td>
<td>2 2 2</td>
<td>ND</td>
</tr>
</tbody>
</table>

*ND denotes ring pattern not detected
Further, the crystalline nature of the newly synthesized AgNPs was confirmed using XRD analysis (Fig. 6.6). The presence of five intense diffraction peaks at 2θ values 38.148°, 44.383°, 64.524°, 77.666° and 81.949° correspond to (111), (200), (220), (311), and (222) diffraction facets, respectively, for face centered cubic structures of silver (JCPDS, File No. 04-0783). The presence of other peaks at 2θ values of 27.752°, 32.358° and 46.172° corresponds to (211), (122), and (231) diffraction facets for orthorhombic structure of AgNO$_3$ (JCPDS, File No. 43-0649) which indicated the presence of the unreacted AgNO$_3$ as impurity. The XRD pattern thus clearly illustrates that the AgNPs synthesized by the present green method were crystalline in nature.

![Figure 6.5 X-Ray Diffraction pattern of bioreduced silver nanoparticles (* denotes diffraction facets for AgNO$_3$)](image)

For FCC structure, the lattice parameter ($a$) can be calculated from measured values for the $d$-spacing of the (111) diffraction facet using the following equation,

$$d_{hkl} = \frac{a}{\sqrt{h^2 + k^2 + l^2}}$$

(2)

where, $a$ is the lattice parameter, $h$, $k$ and $l$ are the Miller indices of the Bragg plane. The calculated value for lattice parameter $a$ was found to be 4.083 Å, which is in good
agreement with the standard lattice parameter value (a=4.086 Å) from standard JCPDS File No. 04-0783 for silver nanocrystals.

The crystallite size of the bioreduced AgNPs was calculated from broadening of the diffraction peaks according to Scherrer equation (Scherrer 1981):

\[
T = \frac{K\lambda}{\beta_{2\theta} \cos \theta} \]

where, \(T\) is the crystallite size, \(K\) (~1) is the Scherrer’s constant related to the shape and index \((h.k.l)\) of the crystal, \(\lambda\) is the wavelength of the X-ray used (Cu-K\(\alpha\), 1.54056 Å), \(\theta\) is the Bragg’s diffraction angle (in degree), and \(\beta_{2\theta}\) is broadening of diffraction lines measured at half of its maximum intensity (in radian). The average crystallite size was calculated to be 5.58 ± 1.29 nm (Table 6.2).

### Table 6.2: Calculation for crystallite size of bioreduced silver nanoparticles from XRD data using Scherrer equation

<table>
<thead>
<tr>
<th>FWHM (\beta_{2\theta}) (°)</th>
<th>FWHM (\beta_{2\theta}) (radians)</th>
<th>(2\theta) (°)</th>
<th>(\theta) (radians)</th>
<th>(\cos \theta) (radians)</th>
<th>(T^*) (Å)</th>
<th>(h k l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.8</td>
<td>0.0314159</td>
<td>27.752</td>
<td>0.242181682</td>
<td>0.970817072</td>
<td>50.51166761</td>
<td></td>
</tr>
<tr>
<td>1.9</td>
<td>0.03316123</td>
<td>32.358</td>
<td>0.282376581</td>
<td>0.960395943</td>
<td>48.37240705</td>
<td></td>
</tr>
<tr>
<td>1.9</td>
<td>0.03316123</td>
<td>38.148</td>
<td>0.33290382</td>
<td>0.94597394</td>
<td>49.15542442</td>
<td>1 1 1</td>
</tr>
<tr>
<td>1.1</td>
<td>0.01919861</td>
<td>44.383</td>
<td>0.387314414</td>
<td>0.925926752</td>
<td>86.66271677</td>
<td>2 0 0</td>
</tr>
<tr>
<td>1.9</td>
<td>0.03316123</td>
<td>46.172</td>
<td>0.402926371</td>
<td>0.919917469</td>
<td>50.5090366</td>
<td></td>
</tr>
<tr>
<td>2.2</td>
<td>0.03839721</td>
<td>64.524</td>
<td>0.563077648</td>
<td>0.845616297</td>
<td>47.44656408</td>
<td>2 2 0</td>
</tr>
<tr>
<td>1.95</td>
<td>0.03403389</td>
<td>77.666</td>
<td>0.67763136</td>
<td>0.778977297</td>
<td>58.10883986</td>
<td>3 1 1</td>
</tr>
<tr>
<td>2.1</td>
<td>0.03665188</td>
<td>81.949</td>
<td>0.71513933</td>
<td>0.755001887</td>
<td>55.67167461</td>
<td>2 2 2</td>
</tr>
</tbody>
</table>

\(T^* = 55.80378601\)

6.3.3. **FTIR analysis**

It was observed that the AgNPs in aqueous solution remained stable over one year with little aggregation. It has been reported that the carbonyl groups from the amino acid residues and peptides have a strong affinity to bind with metals and act as encapsulating agent, thus preventing the nanoparticles from agglomeration (Mitra and Das 2008). In FTIR spectrum of AgNP, the bands at 1650.42, 1452.36 and 1241.89 cm\(^{-1}\) (Fig. 6.7) corresponded to characteristic amide I, II and III bands (Coates 2000). The amide band I was assigned to the stretch mode of the carbonyl group coupled to the amide linkage (NH)-
C=O. The band at 1452.36 cm\(^{-1}\) was assigned to the methylene scissoring vibrations from the proteins. The occurrence of these three bands indicated the presence of peptide moiety probably as capping agent in bioreduced AgNPs.

In addition, the decrease in intensity at 3426.22, 1452.36 and 1406.13 cm\(^{-1}\) after reduction of AgNO\(_3\) may be attributed to the involvement of polyphenols and amines in the reduction process. The strong absorption band at 3426.22 cm\(^{-1}\) may be due to presence of polyphenolic hydroxyl group while the bands at 1452.36 and 1406.13 cm\(^{-1}\) are characteristic of amide II and are assigned to N-H stretching modes of vibration in the amide linkage (Coates 2000).

This suggests that the biological molecules could possibly perform dual functions of reduction and stabilization of AgNPs in the aqueous medium.

6.3.4. Application as antimicrobial agent

Silver is well known as one of the most universal antimicrobial substances. Silver ion and silver-based compounds are highly toxic to microorganisms, exhibiting strong biocidal effect against them (Rai et al 2009). However, silver ions or salts have limited usefulness as antimicrobial agents due to interfering effects of salts and/or discontinuous release of inadequate concentration of silver ions from metals (Dipankar and Murugan 2012). In
contrast, these kinds of limitation can be overcome using AgNPs because they are highly reactive species due to their extremely large surface area, which provides better contact with micro-organisms. The AgNPs produced biologically are known to exhibit potent antimicrobial activity (Sharma et al 2009).

Figure 6.8 Anti-bacterial activity of silver nanoparticles against (a) *E. coli* MTCC 40, (b) *S. paratyphi* MTCC 735, (c) *B. subtilis* ATCC 6051 and (d) *S. aureus* MTCC 87. (CON indicates reaction control and AgNP indicates bioreduced silver nanoparticles)

The AgNPs described in present study were found to exhibit antibacterial activity against both Gram positive and Gram negative bacteria tested (Fig. 6.8). Similar observations regarding antibacterial activity of AgNPs have been reported by Sondi and Salopek-Sondi (2004), Satishkumar et al (2009), Kora et al (2010) and Soo-Hwan et al (2011). In addition, the AgNPs exhibited lower MIC against the Gram-positive bacteria
than the Gram-negative (Table 6.3). This difference in sensitivity of Gram-positive and Gram-negative bacteria towards AgNPs may be attributed to the difference in their cell wall structure. The cell wall of Gram-negative bacteria consists of an outer membrane composed of lipids, proteins and lipopolysaccharides (LPS) which may act as a barrier and provide higher protection against silver nanoparticles. But the cell wall of Gram-positive bacteria do not consist of an outer membrane hence are more susceptible to silver nanoparticles in comparison to Gram negative bacteria (Maneerung et al 2008, Xu et al 2009).

Table 6.3: Antibacterial activity of bioreduced silver nanoparticles against bacterial strains

<table>
<thead>
<tr>
<th>Test organism</th>
<th>Inhibition Zone (mm)</th>
<th>MIC* (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Escherichia coli</em> MTCC 40</td>
<td>18.5</td>
<td>100</td>
</tr>
<tr>
<td><em>Salmonella paratyphi</em> MTCC 735</td>
<td>17.5</td>
<td>100</td>
</tr>
<tr>
<td><em>Bacillus subtilis</em> ATCC 6051</td>
<td>1.9</td>
<td>50</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em> MTCC 87</td>
<td>1.9</td>
<td>50</td>
</tr>
</tbody>
</table>

* MIC, minimum inhibitory concentration

Although a number of studies have tried to fully elucidate the mechanism behind the biocidal action of AgNPs, no universal conclusion has been drawn so far. There is no doubt that the antibacterial activity of AgNPs is a complex process, and several possible modes of action are proposed, involving the direct attachment to cell membrane, disruption of membrane integrity (Sondi and Salopek-Sondi 2004), increased membrane permeability (Soo-Hwan et al 2011), formation of free radicals (Soo-Hwan et al 2011) or interaction with DNA and/or proteins thus interrupting cell replication and metabolism (AshaRani et al 2009).

6.4. Conclusion

The present work demonstrates the competence of using biomaterial toward the synthesis of AgNPs, by adopting the principles of green chemistry. The experiments were carried out using the aqueous extract of Jatropha seedcake as reducing and capping agents. The bioreduced AgNPs thus produced were predominately spherical in shape with a narrow particle size distribution ranging from 3 to 19 nm. The average particle size, as obtained from TEM analysis was found to be 10.48 ± 2.74 nm. The X-ray diffraction pattern of
AgNPs indicated a face-centred cubic crystalline phase of silver nanoparticles with lattice parameter estimated to be 4.083 Å. AgNPs prepared through this route were quiet stable and remained intact for over one year at 4°C. The AgNPs synthesized in present study exhibited potential antibacterial activity, suggesting its prospective applications in antibacterial formulations.
References

HSDB (Hazardous Substances DataBank), TOXNET (TOXicology Data Network), U.S. National Library of Medicine, National Institute of Health.


