Chapter III

Biosynthesis and characterizations of Selenium nanoparticles

The theme of this chapter is the synthesis of Selenium (Se) nanoparticles (NPs) by biological route. A bacterium *Pantoea agglomerans* isolated from collected soil samples was explored for the synthesis of Se NPs in our laboratory. *Pantoea agglomerans* mediate synthesis of Se NPs under aerobic conditions both intra and extra cellurally. Synthesized NPs were characterized by UV- Vis spectroscopy, XRD, SEM and EDAX. After analysis it was found that Se NPs were amorphous, sizes is less than 100 nm.

Work presented in this chapter has been published in journal:

3.1. INTRODUCTION

Se NPs are important nanomaterial’s due to its enormous applications ranging from electronics to catalysis to biomedicines (Fig 1). Se NPs attracted considerable attention in biomedicine due to their high bioavailability and lower toxicity than that of other biologically utilizable form. Recent studies have indicated that Se NPs express antibacterial, antioxidant, antipprotozoal, and anticancer properties\(^1\). Biomedical applications of Se NPs are increasing day by day so it became important to fabricate biocompatible Se NPs.

![Applications of Se NPs](image)

**Fig.3.1.** Applications of Se NPs.

Physical and chemical methods are dominant in the synthesis of Se NPs as they generate large quantity, finite sizes and shapes. But these methods are complicated, costly, time
Table 3.1. Disadvantages of physical and chemical methods used for synthesis of Se NPs.

<table>
<thead>
<tr>
<th>Method of Synthesis</th>
<th>Nanostructured material synthesized</th>
<th>Disadvantages</th>
<th>References</th>
</tr>
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<tbody>
<tr>
<td>Wet chemical reduction</td>
<td>Amorphous Se NPs</td>
<td>Capital intensive, low production rate, difficult to scale up.</td>
<td>(2; 3; 4; 5; 6; 7)</td>
</tr>
<tr>
<td>Hydrothermal</td>
<td>Amorphous Se NPs, Se nanowires, Trigonal Se nanowires and nanotubes</td>
<td>Difficult to control process reproducibility</td>
<td>(8; 9; 10; 11)</td>
</tr>
<tr>
<td>Solvothermal and aging</td>
<td>Se NPs</td>
<td>High cost</td>
<td>(7)</td>
</tr>
<tr>
<td>Sonochemical</td>
<td>Se NPs</td>
<td>Inability to Control particle size</td>
<td>(12,13,14)</td>
</tr>
<tr>
<td>Photothermal assisted synthesis</td>
<td>Se NPs</td>
<td>Low rate of production, high energy consumption, highly uneconomical</td>
<td>(7)</td>
</tr>
<tr>
<td>Photocatalytic reduction</td>
<td>Se NPs</td>
<td>High energy consumption</td>
<td>(15; 16)</td>
</tr>
</tbody>
</table>

Therefore, in current study we have used the microorganism for the synthesis of Se NPs. The Se NPs produced by biogenic processes are far superior, in several ways, than that of the chemical methods\(^1\). Micro-organisms reduce the toxic, selenate and selenite oxyions into non-toxic Se NPs by anaerobic and aerobic reduction process as follows;

1. **Anaerobic reduction of Se oxyions by microorganisms**

Some bacterial species like *Enterobacter cloacae*\(^18\), *Bacillus selenitireducens*, *Thauera selenatis*\(^19\), *Clostridium pasteurianum*\(^20\) *Sulfurospirillum barnesii*, *Shewanella sp. HN-4*\(^21\), *Selenihalanaerobacter shriftii*\(^22\) etc., grows microaerophilically but the synthesis takes place only under anaerobic conditions. *Shewanella putrefaciens* mediated synthesis of Se nanowires which react with mercury (Hg) in water to form Hg-Se core-shell structures at ambient temperatures. Reductions of Se oxyions take place at cell surface and Se NPs release through membrane-
associated efflux pumps. Recent studies on anaerobic selenite reduction and proteins associated with the Se NPs showed that outer membrane porins and elongation factor Tu principally associated with it.

2. Aerobic reduction of oxyions by microorganism

Anaerobic mode of reduction is preferred in mining, coal combustion, oil refinery, waste water treatment but large scale bioreduction process by anaerobic way is tedious and challenging. So it is highly essential to find aerobic microorganisms with high tolerance to Se oxyions and synthesised Se NPs of smaller sizes in aerobic environment. Particle sizes of Se NPs synthesized through aerobic reduction process are smaller because O₂ promotes oxidation of Se (backward reaction) as a consequence of which the redox step becomes slower by production of smaller Se NPs.

Efforts have been put forward by various researchers in this context and they got succeeded in finding microbial strains like Duganella sp, Aspergillus terreus, Bacillus sp., Klebsiella pneumonia, Agrobacterium sp., Pseudomonas sp., Bacillus selenireducens strain MLS10, Rhodospirillum rubrum, Wollinella succinogenes and Stenotrophomonas maltophilia etc. Bacillus subtilis initially produces unstable spherical Se NPs when dissolved into solution, they forms Se nanorods by self aggregation of Se atoms.

Few microorganisms are sensitive to Se, while others do not show any adverse effect but they detoxify Se oxyions and produce Se NPs. In those microorganisms, special types of reductase are present. E. coli produces specific types of proteins (AdhP, Idh, OmpC and AceA) which are associated with synthesis of Se NPs. These proteins play an important role in generating uniform and equal sized Se NPs. It was reported that AdhP proteins have a strong affinity with Se NPs.

NPs synthesized by aerobic reduction of Se oxyions are characterized using following characterization techniques:

i. UV-Vis spectroscopy

When any substance/material absorb particular wavelength of light in visible region of electromagnetic spectrum then the absorbing material appears colored to the observer. Molecular and structural changes in the materials, and leads to change in color absorbing potential in the visible region of the electromagnetic spectrum. Noble metal NPs absorb light in the visible region due to surface plasmon resonance effect. While semiconductor NPs absorb in
UV region of the electromagnetic spectrum due to single electronic excitation. Hence the UV–Visible absorption spectroscopy is a primary measurement tool to study the NPs formation\textsuperscript{19}. Absorption of light leads to a shift of electron from ground state to excited state. The intensity of light passing through a sample is given by the equation 1:

\[ I = I_0 \exp (-\alpha k x) \]  

Where \( I \) = intensity of transmitted light; \( I_0 \) = intensity of incident light; \( \alpha \) = molar absorption coefficient; \( k \) = constant; \( x \) = path length.

Beer-Lambert’s law gives exact concentration of unknown species in a mixture using UV-Vis spectroscopy. This can be done by drawing a graph of intensities of absorption for different concentrations of the sample and comparing with a standard graph\textsuperscript{39}. The Beer-Lambert’s law is given by equation 2;

\[ A = \varepsilon c l \]  

Where \( \varepsilon \) = absorptivity

\( c \) = Concentration of absorbing material

\( l \) = Path length

\textit{ii. X-ray diffractometry}

The X-ray diffraction (XRD) measurement is a very powerful and preferred tool for characterizing microstructure of thin films. It provides useful information about structure, phase, grain-size, orientation and strain state etc\textsuperscript{40}. When X-rays are bombard on material they are scattered by each set of lattice planes with a characteristics diffraction angle. The scattering intensity and diffraction angle depends on arrangement of atoms in the crystals. X-rays are generated from an X-ray tube containing copper anode. The copper anode is irradiated with a beam of high energy electrons that is accelerated by a high voltage electric field to a very high speed. X-rays in the range of a few angstroms to 0.1 Å (1 - 120 keV) are used\textsuperscript{41}. The scattering from all the different sets of planes results in a pattern unique to the crystal structure of a given compound. In 1912, Prof. W. L. Bragg described the diffraction condition as,

\[ n\lambda = 2dsin\theta \]  

Where, \( n \) = order of diffraction

\( \lambda \) = wavelength,

\( d \) = interplanar spacing

\( \theta \) = scattering angle in degrees.
Diffraction pattern corresponding to the various interplanar spacing in the crystal lattice are used for identifying the underlying structure of the material\(^4\).

**iii. Scanning Electron microscopy**

The high spatial resolution Scanning Electron Microscope (SEM) is used to illustrate a wide range of objects at their nanometer to micrometer length scales\(^4\). The SEM unit provides 2-dimensional images of objects on recording the secondary electrons that are released from the sample. Secondary electrons are most valuable for showing morphology and topography of samples. Accelerated electrons with high kinetic energy produce variety of signals when interact with sample/material. These signals includes secondary electrons (that produce SEM images), backscattered electrons (BSE), diffracted backscattered electrons (that are used to determine crystal structures and orientations of minerals), photons (characteristic X-rays that are used for elemental analysis and contineous X-rays), visible light and heat. Secondary electrons and backscattered electrons are commonly used for imaging process; backscattered electrons are most valuable for illustrating contrasts composition in multiphase samples\(^4\). X-ray generation is produced by inelastic collisions of the incident electrons with electrons in discrete shells of atoms in the sample.

**iv. Energy dispersive X-ray analysis**

Energy dispersive X-rays analysis (EDX) is a chemical microanalysis technique. The technique utilizes X-rays that are emitted from the sample, when it is bombarded by electron beam. This technique is used in combination with SEM. An electron beam (10-20 keV) strikes on the surface of a conducting sample. This causes X-ray to be emitted from the material. The energy of the X-ray emitted depends on the material under examination. EDX spectra can be obtained by focusing the beam at different regions of same sample to verify spatially uniform composition of bimetallic material if sample has any\(^4\).
3.2. MATERIALS AND METHODS

3.2.1. Culture and inoculums

The isolated bacterial strain (herein named E) was maintained on agar slants at 4°C and sub-cultured after every five days. The inoculum was cultured in 100 mL of tryptic soy broth (TSB) using 250 mL Erlenmeyer flasks\textsuperscript{27}.

3.2.2 Production of Se NPs

Inoculum 1% (v/v) was inoculated in 500 mL TSB supplemented with filter sterilized 9 mM SeO\textsubscript{2} and incubated at 37°C at 200 rpm for 48 h. \textit{Escherichia coli} K-12 was used as a negative control. After the lapse of incubation period production flask were observed for the change of color. Change in color from yellow to red was an indication of production of Se NPs\textsuperscript{27}.

3.2.3 Recovery of Se NPs from the culture broth

Cell suspensions containing Se NPs were sonicated at 100 W for 2 min and immediately centrifuge at 10,000 x g for 10 min, aqueous phage was transferred to next tube. The tube was centrifuged at 10020 × g at 4°C for 30 min. The supernatant was discarded and pellet containing red Se NPs were re-suspended in de-ionised water. The suspension was washed twice with de-ionised water by repeating the two centrifugation steps\textsuperscript{31,32}.

3.2.4 Characterization of synthesized Se NPs

The Se NPs synthesized by isolated bacterial strain \textit{Pantoea agglomerans} were characterized using following techniques:

a) UV-Vis spectroscopy

Se NPs synthesized in current study was suspended in deionised water and absorption spectrum was recorded in UV–VIS spectrophotometer (Jasco V-680) at 300-1100 nm.

b) X Ray Diffractometry

X-Ray diffraction analysis of the synthesized Se NPs was carried out by using X-Ray diffractometer (Rigaku D/MAX 2500 V, Cu Ka, k=1.5418 Å). Samples for XRD analysis were made by drop-coating the aqueous solution of Se NPs on a clean glass slide.
c) **Field-Emission scanning electron microscopy analysis**

Samples for field-emission scanning electron microscopy (FE-SEM) analysis were prepared by drop-coating Se NPs solution. Scanning electron micrographs were obtained on FE-SEM, Hitachi S-4200.

d) **Energy Dispersive X-ray analysis**

The sample which was used for SEM was also used for EDAX. The back scattered X-rays were used to chemical microanalysis.
3.3. RESULTS AND DISCUSSION

The appearance of red color in production medium at the end of incubation period supported the synthesis of Se NPs (Fig.3.2). The red color was due to excitation of surface plasmon\textsuperscript{17}. The SeO\textsubscript{2} is water soluble and is without any color but the production medium itself has light yellow color (Fig.3.2a).

![Fig 3.2. Synthesis of Se NPs by *Pantoea agglomerans*\textsuperscript{46}](image)

When SeO\textsubscript{2} reduced to nanoscale level by the action of bacterial enzymes, it gives characteristic red color and due to this, color of production medium changes from yellow to red (Fig.3.2b). The bacterial strain *Pantoea agglomerans* isolated in current study was used for synthesizing Se NPs, it has ability to synthesized Se NPs both extracellularly and intracellularly in aerobic conditions.

The UV-visible absorption spectrum of the Se NPs recovered from the culture broth gives a characteristic peak at 593 nm (Fig.3.3). Researchers working on Se NPs also reported the peak at 593 nm in UV-visible absorption spectrum\textsuperscript{27,31}.
The XRD obtained from the Se NPs is shown in Fig.3.4a. The XRD spectrum of the synthesized NPs was free from the reflection peaks, suggesting that NPs were amorphous in form.

![Absorption spectrum of Se NPs](image)

**Fig.3.3.** Absorption spectrum of Se NPs obtained from *Pantoea agglomerans* $^{46}$. 

The sizes of the Se NPs were further confirmed by SEM imaging which demonstrates that the particles were spherical and less than 100 nm (Fig.3.4b and c). EDX analysis confirmed qualitative as well as quantitative status of elements that were present. **Fig. 3.4d** shows the elemental profile of the synthesized Se NPs using bacterium *Pantoea agglomerans*. EDAX analysis revealed the highest proportion of Se in the product.
Fig. 3.4. Characterization of synthesized Se NPs; (a) XRD, (b and c) SEM images, and (d) Elemental mapping for Se$^{46}$. 
REFERENCES
