“Scientific discovery and scientific knowledge have been achieved only by those who have gone in pursuit of it without any practical purpose whatsoever in view.”

-Louise Pasteur
Chapter I

“Research is to see what everybody else has seen, and to think what nobody else has thought.”

- Albert Szent-Györgyi

1.1. Introduction

The extraordinary progress in engineering and physical science was achieved in the 20th century. The 21st century of bio era brought these fields tremendous opportunities in the biology to engineer the biological system. Though serious efforts have been made for acquiring and addressing biomedical and healthcare problems using engineering technologies, a grand challenge is the optimization these approaches towards engineering the life science and healthcare. The science and engineering engaged in design, synthesis, characterization and application of devices and materials containing smallest particle of 1-100 nm range is ‘nanotechnology’. Nanotechnology and nanoscience have proved to bring potential benefits in various fields like water decontamination, information and communication technologies, drug development, and the production of stronger, lighter materials. Revolutionary advances in the areas of medicine, communications, genomics and robotics are thought to be possible as tremendous developments have occurred in nanotechnology from its genesis.

Though it is only in the last 5 years, a new branch of science, known as “nanomedicine,” has emerged as a distinct field, and it has since grown exponentially. Nanomedicine offers examples of how nano-technological tools are being utilized in biomedical research. By using nanometer - sized particles, the increased functional surface area per unit volume can be exploited in various ways. Some of these combine even more of the “smart” properties of nanosized material, including the enabling of drug delivery and improvement of biocompatibility.

1.2. Convergence of Biology and Nanotechnology

All man-made and biological systems have the first level of organization at the nanoscale e.g. a nanochannels, nanocrystals, nanobiomotors or nanotubes where their fundamental properties and functions are defined. Nature assembles
molecules into objects, in order of several length scales, and to disassemble objects into small molecules in living systems and in the environment. The nanotechnology is considered as following the same approach. The nanoscale principles and techniques to study and transform biosystem and to use biological principles with its materials to manufacture new devices and systems incorporated with nanoscale are termed ‘nanobiotechnology’. The amalgamation of biotechnology and nanotechnology with information technology has accelerated the evolution in the field of theoretical and applied science world. The union of nanotechnology with modern biology and medicine is a trend that should be reflected in science policy decisions. Biology and nanotechnology seems to be taking advantages from each other’s beneficial aspects. For the investigation and conversion of biosystems nanotechnology is one of essential technology and biology has become the role model to the nanotechnology for the assimilation of the molecules at nanosize (figure1.1).

![Figure 1.1](http://web67.cc.utexas.edu/)

**Figure 1.1** ‘Give and Take’ policies between nanotechnology and biology (http://web67.cc.utexas.edu/)

Figure 1.2 summarizes several such examples including Quantum dots, fullerene, protein, nanoparticles, and hemoglobin. For instance, the thickness of
human hair is 50,000 nm, while the size of a glucose molecule is less than 1 nm [1]. It is remarkable that a molecule 50,000 times smaller in size than one strand of human hair provides energy to species through metabolic activities. Figure 1.2 compares entities of bionanotechnology with matters of biology and daily life so as to give a rational picture of what it takes to be nano and to feel how small they are [2]. The nano world and bio world share the same range of sizes (1-100 nm), which created scope for researchers to explore the interaction between them.

Figure 1.2. Comparison of nanoparticles with biological materials [2]

The physical, chemical, optical, magnetic and electronic properties of nanomaterials are influenced by number of factors. These factors intern strongly modulate their properties viz size [3-11], shape [12-14], surface composition [15-20], dielectric environment of the particle [21-26] and interparticle interactions [27-32]. The factors are their dimensions comparable to that of De Broglie wavelength of the charge carriers and high surface to volume ratio responsible for such remarkable variations in their properties [33-35]. Metal nanoparticles show colors in colloidal solution due to the surface
plasmon i.e. the coherent charge density oscillations [36]. The excitation of the surface plasmons by an electromagnetic field at an incident wavelength, where the resonance occurs, results in the appearance of intense surface plasmon resonance (SPR) bands and an enhancement of local electromagnetic field [37]. Such quantum size effects are best studied with semiconductor nanoparticles where the energy level spacing for a spherical particle is predicted to be inversely proportional to R^2 [38-40]. Thus, with decreasing size the effective band gap increases, leading to relevant absorption and emission spectra blue shifts. The size and the shape of the nanoparticles are marginally depending on the synthesis processes.

1.3. Biomedical applications of metal nanoparticles

Among all versatile nanoparticles, the metal nanoparticles are considered an offspring of a modern science as the applications of nanotechnology have been studied exclusively in past few decades. The Noble metal nanoparticles such as gold, silver and platinum are particularly interesting due to their size and shape dependent unique optoelectronic properties and have very much influence on the biomedical nanotechnology which is an essential field having enormous potential to positively impact the health care system. The integration of metal nanoparticles into biological systems has left greatest impact in biomedicine and biology. In the recent past, the optical properties of metal nanoparticles have been studied in great detail. The reason for these investigations is that metal nanoparticles bridge the gap between the properties of an atom and a bulk material. Metal nanoparticles exhibit unique properties, which depend substantially on their size, shape, composition, and dielectric surroundings [41-48]. In particular, the size- and shape-dependent properties make metal nanoparticles promising candidates for a variety of applications, for example, as biosensors [49], catalysts [50], and data storage media [51], and for wave guiding [52], in all optical switching devices [53], or in thermal cancer therapy [54]. The excitation of an LSPPR is accompanied by a local field enhancement, which is exploited in many and extremely different applications, for example, in surface-enhanced Raman
spectroscopy (SERS) [55], surface-enhanced fluorescence (SEF) [56], and confocal microscopy [57], as well as to enhance the efficiency of solar cells [58], or to structure surfaces [59-61].

In recent years AgNPs has become promising material for the interest of researchers and industries [62]. AgNPs have proved their eminence in application as biomedicine and healthcare due to their characteristic bactericidal properties [62]. AgNPs are identified to induce rapid healing of wounds. They exercise antimicrobial properties that advance cosmetic appearance, reduce inflammation, and modulate cytokine expression, thus providing a novel therapeutic modality for future wound management [63, 64]. Use of AgNPs for the wound healing is a relatively under-explored area of research. Numbers of studies have been undertaken using both in vitro and in vivo approaches to explore the mechanism of wound healing mediated by AgNPs. Furthermore, medical polymers impregnated with AgNPs have been used as coatings for medical equipment so as to maintain sterility and reduce possible nosocomial infections [65]. The drug delivery potential of AgNPs is also promising as the large surface area facilitates delivery of a relatively high dose of drugs [66]. The healing properties of Ag are an ancient discovery. Monovalent Ag compounds have been applied extensively for antimicrobial treatment and prophylaxis since ancient times [67]. Many researchers agree that more wide-scale and vigorous studies are still needed to better assess the risks and hazards, associated with the use of AgNPs in the pursuit of medical advancements.

1.4. Synthesis of metal nanoparticles

The properties of nanoparticles are solely dependent on the size and shapes of the nanoparticles. So ultimately there is tremendous importance to the synthesis methods of metal nanoparticles in the nanoparticles research where the size and shape of the nanoparticles can be controlled. The various methods are being used for the synthesis of nanostructure. These methods
ultimately come under two approaches; ‘top-down’ and ‘bottom-up’ synthesis approach.

In top-down approach, materials are breakdown to sub micron level [68]. It was used for many years at early stages of nanotechnology research. The synthesis methods of this type have limitation as these methods create imperfection in the surface of the nanoparticles, precluding their applications many fields. These methods are expensive and time consuming. On the other hand, from the smaller entities the nanoparticles are constructed. First the nanostructured building blocks of the nanoparticles are formed and then assembled to produce the final particle [69].

![Synthesis of metal nanoparticles](image)

**Figure 1.3.** Schematic outlining the various synthesis methods of metal nanoparticles

The synthesis methods are broadly are divided into physical, chemical and biological methods. Further biological methods are classified in microbial synthesis, plant mediated synthesis and other different biological routes of
metal nanoparticle synthesis. Figure 1.3 gives the summary of the various routes of the synthesis process involved in nanoparticle construction.

1.4.1. Physical methods

Different physical methods are employed for the synthesis metal nanoparticles. The metal salts immersed in solvent containing surfactant are irradiated by the intense laser pulses, in the laser ablation method [70]. The high temperature created by laser pulse causes evaporation of metals and these metals are solvated by surfactants to form nanostructures. In sonochemical method, the gas bubbles are oscillated in given liquid medium under acoustic field created by ultrasonic waves. It leads to growth and collapsing of bubbles followed by generation of micro streaming in the liquid, thereby generating mass transport within same medium. High temperature is generated due to collapse of bubbles causing local heating. Due to extreme conditions, solvent and solute molecules decompose into reactive radicals which reactive radicals are used for the reduction of the metallic salts. Synthesis of metallic nanoparticles of Au and Ag has been reported by sonicating in the presence of a surfactant aqueous solutions of the corresponding metallic salts [71, 72]. In radiolytic method, free radicals produced by radiolysis reduce metal ions to nanoparticles in the presence of donor ligands [73]. Huang et al. reported the synthesis of silver nanoparticles (AgNPs) by irradiation of aqueous AgNO₃ solution with UV light of wavelength in the presence of poly (N-vinylpyrrolidone) [PVP] as a stabilizer [74]. Physical vapor deposition (PVD) and chemical vapor deposition (CVD) are widely used vapor deposition methods for the synthesis of metal nanoparticles. Using an electrical heating vessel under high vacuum and employing heat, nanometer thicknesses of metal films are produced on glass substrate. Whereas in CVD, carrier gasses with the elements of desired compound are passed over suitably heated surfaces resulting in decomposition of carrier gas causing the deposition of atoms or molecules on the substrate. Solvated metal atom deposition (SMAD) involves heating of bulk metal till it starts evaporating under vacuum and co-
condensation of its vapors with solvents to form nanoparticles in solution [75]. The major drawback of the physical methods is the cost of the process which makes them unsuitable for the mass productions.

1.4.2. Chemical methods

Chemical methods for the synthesis of metal nanoparticles are demonstrated as easiest and well known routes. Here in this method, the metal precursor salts are reduced by the suitable reducing agents to nanoparticles. The controlled shape and size metal nanoparticle synthesis using various reducing agents like, sodium borohydride [76], trisodium citrate [77], amino acids [78, 79], citrate reduction [80] etc are well reported. Major advantage of the chemical routes are easy manipulation in chemical reactions and reactivities, better control over reaction conditions during synthesis, and reaction simplicity in surface functionalization of nanoparticles. Different solvent mediums such as organic medium [81], ionic liquids [82] aqueous medium [76-79] and supercritical fluids [83] can be used for the synthesis of metal nanoparticles. The assembly of nanoparticles at various interfaces is possible using chemical routes such as at air-water interfaces [84] and liquid-liquid [85] interfaces, which allow reduction and controlled growth of metal nanoparticles and facilitates 2-D arrangement of nanoparticles. The main feature of this method is the choice of the reducing agent depending upon the application of metal nanoparticles for example biological applications in aqueous medium and catalysis in aqueous as well as organic medium. The large production of the nanoparticles with controlled size and shape is demonstrated using chemical routes. Since minimal resources are employed in the synthesis method, chemical route of synthesis is widely used and showed promising outputs in nanotechnology arena.

1.4.3. Biological methods

Traditional chemical methods of synthesis of metallic nanoparticles, in particular AgNPs, employ toxic reagents, release harmful byproducts to the
environment, consume a lot of energy, and use expensive chemicals. Moreover, chemical syntheses result in the absorbance of toxic chemicals on the surface of nanoparticles. In contrast, microorganisms and plants extracts are considered interesting nanofactories in the fabrication of metallic nanoparticles, in a novel concept of environmentally friendly synthesis of nanomaterials. Followings are various methods involved in biological route.

### 1.4.3.1. Microbial synthesis

Microbial synthesis of metal nanoparticles is one of the thrust areas of scientific community where the scientist concentrated on various microbial sources for the synthesis of nanoparticles. Microorganisms like bacteria, fungi, algae, actinomycetes are being used for the synthesis of nanoparticles. The microorganisms may synthesize nanoparticles intra- or extracellularly. The classical example of intracellular synthesis is marine magnetotactic bacterium MV-1 isolated from sulfide-rich sediments of an estuarine salt marsh anaerobically bioreduced nitrous oxide and ferric quinate to iron-rich magnetosomes which is composed of magnetite (Fe₃O₄) particles [86]. Synthesis of nanoparticles can be done extracellularly using cell free microbial growth medium [87]. *B. licheniformis* is known to secrete the cofactor NADH and NADH-dependent enzyme especially nitrates reductase. These observations are in line with the findings of Duran *et al.* who speculated that the reduction of silver ions by *Fusarium oxysporum* strains occurs by a nitrate-dependent reductase and a quinone that could act as electron shuttle in metal reduction [88]. Fungi possess some important advantages as a potent microorganism for the synthesis of metal nanoparticles. It is relatively easy to culture fungi, the NPs synthesis is mostly extracellular, and the synthesized NPs have a good polydispersivity, size, and stability [89, 90]. Compared with other microorganisms, such as bacteria and fungi, there are a few reports describing the use of yeasts to produce metal nanoparticles [91]. The unicellular algae *Chlamydomonas reinhardtii* was used as a model system to investigate the role of cellular proteins in the synthesis of AgNPs [92]. Cell-
free extract \textit{(in vitro)} of \textit{C. reinhardtii} and \textit{in vivo} cells produced AgNPs of size range 5 to 15 nm and 5 to 35 nm, respectively. The list of some microorganisms and the location of nanoparticle synthesis is given in table 1.2.

**Plausible mechanism of microbial synthesis of nanoparticles**

Nitrate reductase is an enzyme in the nitrogen cycle responsible for the conversion of nitrate to nitrite [88]. The reduction mediated by the presence of the enzyme in the organism has been found to be responsible for the synthesis.

![Diagram of intracellular synthesis of AgNPs by B. licheniformis](image)

**Figure 1.4.** Schematics of intracellular synthesis of AgNPs by \textit{B. licheniformis}; depicting the reduction of Ag$^+$ to Ag$^0$ through electron shuttle enzymatic metal reduction process having the NADH-dependent reductase as a carrier of electrons from NADH [87].

The use of a specific enzyme a-NADPH-dependent nitrate reductase in the \textit{in vitro} synthesis of nanoparticles is important because this would do away with the downstream processing required for the use of these nanoparticles in homogeneous catalysis and other applications such as non-linear optics.
Table 1.1. List of bacteria and fungi that synthesize noble metal nanoparticles [95]

<table>
<thead>
<tr>
<th>Microorganisms</th>
<th>Nanoparticles</th>
<th>Localization/Morphology</th>
<th>Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacteria-Intracellular</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Bacillus subtilis</em>168 Sulfate-reducing bacteria</td>
<td>Au</td>
<td>Octahedral inside cell wall</td>
<td>5–25 nm</td>
</tr>
<tr>
<td><em>Pleconema boryanum</em> UTEX485</td>
<td>Au</td>
<td>Cell envelope</td>
<td>10 nm</td>
</tr>
<tr>
<td><em>Corynebacterium</em> sp. SH09</td>
<td>Au</td>
<td>Membrane vesicles/Cubic</td>
<td>10 nm</td>
</tr>
<tr>
<td><em>Bacillus</em> sp</td>
<td>Ag</td>
<td>Cell wall</td>
<td>10–15 nm</td>
</tr>
<tr>
<td><em>Lactobacillus</em> sp</td>
<td>Ag</td>
<td>Periplasmic space</td>
<td>5–15 nm</td>
</tr>
<tr>
<td><em>Pseudomonas stutzeri</em> AG259</td>
<td>Ag, Ag2S</td>
<td>Periplasmic space</td>
<td>&lt;200 nm</td>
</tr>
<tr>
<td><em>Desulfovibrio desulfuricans</em> S. oneidensis MR–1</td>
<td>Pd</td>
<td>Cell surface</td>
<td>~50 nm</td>
</tr>
<tr>
<td>Bacteria-Extracellular</td>
<td>Pd</td>
<td>Periplasmic space</td>
<td>ND</td>
</tr>
<tr>
<td><em>Rhodopseudomonas capsulata</em></td>
<td>Au</td>
<td>Spherical</td>
<td>10–20 nm</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>Au</td>
<td>Spherical</td>
<td>15–30 nm</td>
</tr>
<tr>
<td><em>Aermonas</em> sp SH10 ND</td>
<td>Ag</td>
<td>Spherical</td>
<td>4–6 nm</td>
</tr>
<tr>
<td><em>Morganella</em> sp.</td>
<td>Ag</td>
<td>Spherical</td>
<td>20±5 nm</td>
</tr>
<tr>
<td><em>Rhodococcus</em> NCIM 2891</td>
<td>Ag</td>
<td>Spherical</td>
<td>10–35 nm</td>
</tr>
<tr>
<td>Fungus- Intracellular</td>
<td>Au</td>
<td>Cell wall/spherical, cytoplasmic membrane</td>
<td>20±8 nm</td>
</tr>
<tr>
<td><em>Verticillium</em> sp</td>
<td>Au</td>
<td>Spheres</td>
<td>10 nm</td>
</tr>
<tr>
<td><em>V. luteoalbum</em></td>
<td>Au</td>
<td>Spheres, cytoplasmic membrane/spherical</td>
<td>25±12 nm</td>
</tr>
<tr>
<td><em>Aspergillus flavus</em></td>
<td>Ag</td>
<td>Cell wall</td>
<td>8–10 nm</td>
</tr>
<tr>
<td>Fungus- Extracellular</td>
<td>Au</td>
<td>Spherical, triangular</td>
<td>20–40 nm</td>
</tr>
<tr>
<td><em>Fusarium oxysporum</em></td>
<td>Au</td>
<td>Spherical</td>
<td>20–40 nm</td>
</tr>
<tr>
<td><em>Colletotrichum</em> sp</td>
<td>Ag</td>
<td>Triangular</td>
<td>13–18 nm</td>
</tr>
<tr>
<td><em>Trichoderma asperellum</em></td>
<td>Ag</td>
<td>Spherical</td>
<td>20 nm</td>
</tr>
<tr>
<td><em>Aspergillus niger</em></td>
<td>Ag</td>
<td>Spherical</td>
<td>25–75 nm</td>
</tr>
<tr>
<td><em>Coriolus versicolor</em></td>
<td>Ag</td>
<td>Spherical</td>
<td>60–80 nm</td>
</tr>
<tr>
<td><em>Phoma glomerata</em></td>
<td>Ag</td>
<td>Spherical</td>
<td>8–14 nm</td>
</tr>
<tr>
<td><em>F. oxysporum</em></td>
<td>Au–Ag</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td><em>Volvariella volvacea</em></td>
<td>Au, Ag</td>
<td>Spherical, hexagonal</td>
<td>20–150 nm</td>
</tr>
</tbody>
</table>
During the catalysis, nitrate is converted to nitrite, and an electron will be shuttled to the incoming silver ions. Mukherjee et al described intracellular synthesis of gold nanoparticles by *Verticillium* sp was occurring due to enzyme activity occurring at the cell membrane [93]. Intracellular synthesis of gold nanoparticles by *Rhodococcus* sp was demonstrated by Ahmad et al stating proteins or enzymes at the cell membrane may have involved in the reduction process of gold salt [94].

This has been excellently described in the organism *Bacillus licheniformis* [87]. *B. licheniformis* is known to secrete the cofactor NADH and NADH-dependent enzymes, especially nitrate reductase that might be responsible for the bioreduction of Ag\(^+\) to Ag\(^0\) and the subsequent formation of AgNPs.

**Figure 1.5.** Image showing the role of extracellular proteins secreted by fungal isolate; 32 kDa proteins in reduction of Ag\(^+\) ions to AgNPs and 35kDa proteins as stabilizer for the AgNPs [97]

Figure 1.4 shows that the nitrate reductase presents in the bacteria may aid the synthesis of AgNPs [87]. Although all these were theories, direct
evidence was provided by Anil Kumar et al. (2007) who directly used the purified nitrate reductase from the organism Fusarium oxysporum for the synthesis of AgNPs in test tube [96]. Their reaction mixture contained only the enzyme nitrate reductase, silver nitrate and NADPH. Slowly, the reaction mixture turned brown with all the characteristics of AgNPs. This is the first direct evidence for the involvement of nitrate reductase in the synthesis of AgNPs. Navin Jain et al showed extracellular proteins from Aspergillus flavus NJP08 are involved in synthesis of AgNPs and proposed mechanisms behind reduction (figure 1.5) [97].

1.4.3.2. Plant mediated synthesis

Plants have emerged as a simple, cost-effective and eco-friendly system to rapidly synthesize NPs. In producing nanoparticles using plant extracts, the extract is simply mixed with a solution of the metal salt at room temperature. The reaction is complete within minutes. Nanoparticles of silver, gold and many other metals have been produced this way [98]. Synthesis of AgNPs using extract of Desmodium trifolium was ascribed to the presence of H⁺ ions, NAD⁺ and ascorbic acid in the extract [99]. Varma and co-workers have demonstrated [100] an effective and environmental friendly synthesis approach for Ag nanoparticles utilizing polyphenols found in tea extract and epicatechin. A tuber extract of Dioscorea bulbifera was used to produce various shaped gold and AgNPs [98].

Figure 1.6 gives the possible chemical constituents of plant extracts involved in the formation of metal nanoparticles [98]. The extract contained alkaloids, proteins, enzymes, amino acids, alcoholic compounds, and polysaccharides are said to be principally involved in the formation of nanoparticles from silver ions [101]. Other than above mentioned chemicals quinol and chlorophyll pigments present in the extract proved for conversion of silver ions and stabilization of the nanoparticles. The plant-derived polysaccharides and phytochemicals were also demonstrated as the reducing and stabilizing agents in the green synthesis of silver and gold nanoparticles.
where the oxidation of the polysaccharide hydroxyl group in the carbonyl group plays an important role in the reduction of metal salt into nanoparticles.

![Chemical constituents of plant extract](image)

**Figure 1.6.** Schematics of reducer and stabilizers of Ag\(^0\) to Ag\(^+\) present in plant extracts like alkaloids, terpenoids, polyphenols, flavonoids etc. [98]

Also the reducing end of polysaccharide can provide the site for an amino functionality capable of complexing and stabilizing metallic nanoparticles [101]. The role of aromatic amine, amide (I) group, phenolic groups, and secondary alcohols in reduction of metal ions for the synthesis of nanoparticles was demonstrated during the study on *Coleus aromaticus* leaf extract-mediated synthesis of AgNPs [101]. Various parts of the plants have been utilized for the synthesis of metal nanoparticles and some are listed in table 1.2. Hitherto due to the rich biodiversity of plants, their potential for the synthesis of noble metal nanoparticles is to be fully explored. It is very important to elucidate the mechanism for the phytosynthesis of precious noble metal nanoparticles so as to establish robust, green chemistry, and economical methods. Due to vast variability and complexity in different plant constitutes it up to this date very difficult to put forth the exact mechanism behind nanoparticle formation.
Table 1.2. List of plants and plant parts used for the synthesis of noble metal nanoparticles [98]

<table>
<thead>
<tr>
<th>Plant</th>
<th>Nanoparticle</th>
<th>Plant part used</th>
<th>Size/shape of nanoparticles</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aloe vera</td>
<td>Ag</td>
<td>leaves</td>
<td>15 nm spherical</td>
</tr>
<tr>
<td>Ocimum tenuiflorum</td>
<td>Ag</td>
<td>leaves</td>
<td>25–40 nm spherical</td>
</tr>
<tr>
<td>Azadirachta indica</td>
<td>Ag</td>
<td>leaves</td>
<td>spherical</td>
</tr>
<tr>
<td>Pelargonium graveolens</td>
<td>Ag</td>
<td>leaves</td>
<td>16–40 nm crystalline</td>
</tr>
<tr>
<td>Emblica officinalis</td>
<td>Ag</td>
<td>fruit</td>
<td>10–20 nm spherical</td>
</tr>
<tr>
<td>Cinnamomum zeylanicum</td>
<td>Ag</td>
<td>bark, powder</td>
<td>31–40 nm spherical</td>
</tr>
<tr>
<td>Ocimum sanctum</td>
<td>Ag</td>
<td>root stem</td>
<td>5–10 nm spherical</td>
</tr>
<tr>
<td>Curcuma longa</td>
<td>Ag</td>
<td>tuber powder</td>
<td>21–30 nm quasi-spherical, triangular, rod shaped</td>
</tr>
<tr>
<td>Boswellia ovalifoliolata</td>
<td>Ag</td>
<td>stem bark</td>
<td>spherical</td>
</tr>
<tr>
<td>Shorea tumbugai</td>
<td>Ag</td>
<td>stem bark</td>
<td>spherical</td>
</tr>
<tr>
<td>Cassia auriculata</td>
<td>Ag</td>
<td>leaves</td>
<td>20–40 nm spherical</td>
</tr>
<tr>
<td>Zingiber officinale</td>
<td>Ag</td>
<td>rhizome</td>
<td>6–20 nm spherical</td>
</tr>
<tr>
<td>Syzygium cumini</td>
<td>Ag</td>
<td>seed</td>
<td>3.5 nm</td>
</tr>
<tr>
<td>Jatropha curcas</td>
<td>Ag</td>
<td>seed</td>
<td>15–50 nm spherical</td>
</tr>
<tr>
<td>Cympodopogon flexuosus</td>
<td>Au</td>
<td>leaves</td>
<td>spherical, triangular</td>
</tr>
<tr>
<td>Pelargonium graveolens</td>
<td>Au</td>
<td>leaves</td>
<td>20–40 nm decahedral, icosahedral</td>
</tr>
<tr>
<td>Tanacetum vulgare</td>
<td>Au</td>
<td>fruit</td>
<td>11 nm triangular</td>
</tr>
<tr>
<td>Menta piperita</td>
<td>Au</td>
<td>leaves</td>
<td>150 nm spherical</td>
</tr>
<tr>
<td>Memecylon edule</td>
<td>Au</td>
<td>leaves</td>
<td>10–45 nm circular, triangular, hexagonal</td>
</tr>
<tr>
<td>Murraya keenigii</td>
<td>Au</td>
<td>leaves</td>
<td>20 nm spherical, triangular</td>
</tr>
<tr>
<td>Cicer arietinum</td>
<td>Au</td>
<td>bean extract</td>
<td>– triangular</td>
</tr>
<tr>
<td>Beta vulgaris pulp</td>
<td>Au</td>
<td>sugar beet</td>
<td>– spherical, rod shaped, nanowires</td>
</tr>
<tr>
<td>Nyctanthes arboristis</td>
<td>Au</td>
<td>flower extract</td>
<td>19.8 nm spherical, triangular, hexagonal</td>
</tr>
<tr>
<td>Cuminum cyminum</td>
<td>Au</td>
<td>seed</td>
<td>1–10 nm spherical</td>
</tr>
<tr>
<td>Pinus resinosa</td>
<td>Pd</td>
<td>bark</td>
<td>16–20 nm spherical</td>
</tr>
<tr>
<td>Curcuma longa</td>
<td>Pd</td>
<td>tuber</td>
<td>10–15 nm spherical</td>
</tr>
<tr>
<td>Musa paradisica</td>
<td>Pd</td>
<td>peeled banana</td>
<td>50 nm crystalline irregular</td>
</tr>
<tr>
<td>Cinnamomum camphora</td>
<td>Pd</td>
<td>leaves</td>
<td>3.2–6.0 nm</td>
</tr>
<tr>
<td>Glycine max</td>
<td>Pd</td>
<td>leaves</td>
<td>15 nm spherical</td>
</tr>
<tr>
<td>Pinus resinosa</td>
<td>Pd</td>
<td>bark</td>
<td>16–20 nm spherical</td>
</tr>
</tbody>
</table>
Plenty of reports have hypothesized the reduction mechanisms but without a systemic experiments and sophisticated instrumentations. Therefore, this is still an issue can be considered further by scientific community.

### 1.4.3.3. Other Biological routes

Other than microorganisms and plants plenty of biomolecules have been utilized for the synthesis of metal nanoparticles. Miriam Colombo et al. demonstrated protein-assisted one-pot synthesis and biofunctionalization of spherical gold nanoparticles for selective targeting of cancer cells. In this method, authors used spaBC3 as a capping agent in the colloidal reduction of a gold salt precursor, thus resulting in a controlled surface functionalization of the gold particle suitable for binding with human IgGs [102]. Gabriel Shemer et al. synthesized AgNPs using single stranded DNA [103].

Gao et al. also used bio-degradable starch to manufacture stable Ag nanoparticles [104]. They found that one of the constituents – glucose was responsible for the reduction of silver ions and AgNPs were covered by starch layer and formed spherical core–shell Ag/starch nanoparticles with diameters ranging from 5 to 20nm. AgNPs were prepared from silver nitrate in the presence of vitamin C derivative 6-palmitoyl ascorbic acid-2-glucoside (PAsAG), via a sonochemical route [105]. Chien-Junget al. fabricated Au nanorods with simple seeded mediated growth method in the presence of vitamin C [106]. Peptide-mediated reduction of silver ions on engineered biological scaffolds was demonstrated by Ki Tae Nam et al [107]. DNA has been effectively utilized to assemble metal nanoparticles like Au, Ag, Pt, and even Cu [108]. There have also been reports of formation of Ag nanoparticles by photo-reduction of the DNA-Ag⁺ complex with UV light at 254 nm [109]. Virus stands for yet another beautiful natural example of self-assembly of which tobacco mosaic virus (TMV) has been extensively dealt with. TMV contains as many as 2130 identical proteins assembled around a single strand of RNA as a coat, giving it a size-length of 300 nm and an external and internal diameter of 18 nm and 4 nm respectively. The self-assembly is highly
specific and uses non-covalent interactions [110]. Thus using these various properties nanotubular assemblies of Au, Pt, Ag have been obtained [111].

1.4.4. Advantages of Biological Methods

Different types of physical and chemical methods are employed for the synthesis of nanoparticles. The use of these synthesis methods requires both strong and weak chemical reducing agents and protective agents (sodium borohydride, sodium citrate and alcohols) which are mostly toxic, inflammable, cannot be easily disposed off due to environmental issues and also show a low production rate [112]. For example, in the seeded growth method chemical reducing agents like sodium citrate and sodium borohydride [113] are used, whereas in the polyol synthesizing process methanol and ethanol are used [114]. Moreover, these are capital intensive and are inefficient in materials and energy use. In addition, in many cases, synthesis is carried out at elevated temperatures, which generate a large amount of heat. For example, in thermal decomposition method, synthesizing process is carried out at very high temperature [115]. The biological method for the synthesis of nanoparticles employs use of biological agents like bacteria, fungi, actinomycetes, yeast and plants [95]. Thus, the biological method provides a wide range of resources for the synthesis of nanoparticles. The rate of reduction of metal ions using biological agents is found to be much faster and also at ambient temperature and pressure conditions. For instance, in case of synthesis of nanoparticles using Aspergillus niger synthesis of AgNPs was observed within 2 h of treatment of fungal filtrate with silver salt solution [89]. Thus, the biological method requires minimum time for synthesis of nanoparticles.

Shape and size controlled nanoparticles could be synthesized by modulating the pH or the temperature of the reaction mixture. Gericke and Pinches obtained different shape morphologies (triangle, hexagons, spheres, and rods) by modulating the pH of reaction mixture to 3, 5, 7 and 9 [116]. Riddin et al. (2006) also demonstrated that at 65 ºC temperature less amount of nanoparticles were synthesized, whereas at 35 ºC temperature more amount of
nanoparticles were synthesized [117]. The biological agents secrete a large amount of enzymes, which are capable of hydrolyzing metals and thus bring about enzymatic reduction of metals ions [89]. In case of fungi, the enzyme nitrate reductase is found to be responsible for the synthesis of nanoparticles [96]. The biomass used for the synthesis of nanoparticles is simpler to handle, gets easily disposed of in the environment and also the downstream processing of the biomass is much easier. Synthesis can be carried out at ambient temperature and pressure conditions that require less amounts of chemical [118]. The synthesizing process is less labor-intensive, low-cost technique, nontoxic and is more of a greener approach. Thus, considering the above points the biological method employed for the synthesis of nanoparticles proves to be superior compared with the physical and chemical methods of synthesis due to its environment friendly approach and also as a low cost technique.

1.5. Inspiration of the work and statement of problem

The intense necessity of drug development against antibiotic resistant pathogenic microorganisms is rising due to frequent use of these antibiotics. The advance technology like nanotechnology can help to solve the problem of infectious diseases. Noble metal nanoparticles like Ag, Au, and Cu etc are being used for the imaging, diagnosis, and therapeutics. Out of these noble metal nanoparticles, AgNPs have been proved to be most effective against pathogenic microorganisms and less toxic to the mammalian cells. Various chemical methods have been employed for the synthesis of the AgNPs like chemical, physical. But these methods are proved to be toxic to the ecosystem as well as components of ecosystem and precluding the use of nanoparticles in the field of medicine. As discussed earlier, plenty of biological materials have been explored but the toxicity studies of the biologically synthesized nanoparticles have not been studied extensively. Varieties of microorganisms are attempted for the synthesis of AgNPs, intracellular and extracellular. But much scope is left to demonstrate the mechanism of intracellular synthesis of nanoparticles.
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The main aim of the present work is to develop the cost effective, green synthesis processes using biological components for the synthesis of AgNPs and study of toxicity effects of the nanoparticles towards pathogenic microorganisms as well as mammalian cells. For the synthesis of desired characteristic AgNPs, various biological entities have been used like microorganisms, cell free extracts, various plant parts’ extracts and biological fermented products. The mechanism of intracellular synthesis of AgNPs is predicted through biochemical analysis and demonstrated with TEM. Waste material like phenol degraded broth, leaf and seed extracts is utilized for the synthesis of AgNPs.

The present work was planned into following objectives:

- To synthesize AgNPs, AgNP-polymer composite and core shell AgNPs using different biological materials like microorganisms, cell free extract, plant material extracts viz seeds and leaf, biological fermented products etc
- To standardize the synthesis process of AgNPs using microorganisms by changing the environmental condition like pH, temperature, growth, carbon source and test of antimicrobial activity
- To study the stability of colloidal solution of AgNPs by varying ionic strength, pH and temperature to prove the role of proteins in stability of nanoparticles
- To evaluate biomedical application by studying the antimicrobial activity of AgNPs against pathogenic bacteria and fungi and demonstration of the antimicrobial activity through micrographic techniques
- To estimate the cytotoxicity of AgNPs synthesized using microorganism and leaf extract using in-vitro cytotoxicity assays on cell line by MTT, NRU and AO/EB staining assays

For the achievement of these objectives the experiments were planned and designed. The cost effective and green chemistry methods were planned for
the synthesis of AgNPs using microorganisms, cell free extract of phenol degraded broth, plant part extracts like seeds and leaf extracts and biological fermented product i.e. whisky. To reveal the mechanisms of synthesis of AgNPs using microorganisms, various biochemical experiments were designed. The stability studies of AgNPs in colloidal solution were planned by varying the ionic strength, pH and temperature. The method to produce AgNP-polymer composite by microorganisms at ambient temperature is designed. Various analytical techniques like UV-Vis spectroscopy, FT-IR, XRD, TEM and SEM were on the map of experimentation for structural, morphological and optical properties of AgNPs. The experiments were mapped to evaluate the biomedical application through antimicrobial activity by various antimicrobial techniques like agar diffusion method, effect of growth curve on various pathogenic bacteria and fungus. For the biocompatibility studies of AgNPs prepared from microorganism and plant part, various cytotoxicity assays were designed.
References

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