

Biomimetic Synthesis of Nanoparticles: Science, Technology & Applicability

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1. Introduction

Nanotechnology emerges from the physical, chemical, biological and engineering sciences where novel techniques are being developed to probe and manipulate single atoms and molecules. In nanotechnology, a nanoparticle (10^{-9}m) is defined as a small object that behaves as a whole unit in terms of its transport and properties. The science and engineering of nanosystems is one of the most challenging and fastest growing sectors of nanotechnology.

This review attempts to explain the diversity of the field, starting with the history of nanotechnology, the physics of the nanoparticle, various strategies of synthesis, the various advantages and disadvantages of different methods, the possible mechanistic aspects of nanoparticle formation and finally ends with the possible applications and future perspectives. Though there are a few good reviews dealing with the synthesis and applications of nanoparticles, there appears to be scanty information regarding the possible mechanistic aspects of nanoparticle formation. This review attempts to fill the void.

The review is organized into five sections. In section 2, we discuss about the early history of nanotechnology and the significant contributions made by eminent scientists in this field. In the next section we describe about the unique properties of nanoparticles, their classification and significance of inorganic nanoparticles. The next section discusses about the various methods of synthesis of nanoparticles and the possible mechanistic aspects. The last section highlights the recent advances and possible applications of nanoparticles.

2. Early history

The concept of nanotechnology though considered to be a modern science has its history dating to as back as the 9th century. Nanoparticles of gold and silver were used by the artisans of Mesopotamia to generate a glittering effect to pots. The first scientific description of the properties of nanoparticles was provided in 1857 by Michael Faraday in his famous paper "Experimental relations of gold (and other metals) to light" (Faraday, 1857).

In 1959, Richard Feynman gave a talk describing molecular machines built with atomic precision. This was considered the first talk on nanotechnology. This was entitled "There's plenty of space at the bottom".

The 1950's and the 1960's saw the world turning its focus towards the use of nanoparticles in the field of drug delivery. One of the pioneers in this field was Professor Peter Paul Speiser. His research group at first investigated polyacrylic beads for oral administration, then focused on microcapsules and in the late 1960s developed the first nanoparticles for drug delivery purposes and for vaccines. This was followed by much advancement in developing systems for drug delivery like (for e.g.) the development of systems using nanoparticles for the transport of drugs across the blood brain barrier. In Japan, Sugibayashi *et al.*, (1977) bound 5-fluorouracil to the albumin nanoparticles, and found denaturation temperature dependent differences in drug release as well as in the body distribution in mice after intravenous tail vein injection. An increase in life span was observed after intraperitoneal injection of the nanoparticles into Ehrlich Ascites Carcinoma-bearing mice (Kreuter, 2007).

The nano- revolution conceptually started in the early 1980's with the first paper on nanotechnology being published in 1981 by K. Eric Drexler of Space Systems Laboratory, Massachusetts Institute of Technology. This was entitled "An approach to the development of general capabilities for molecular manipulation".

With gradual advancements such as the invention of techniques like TEM, AFM, DLS etc., nanotechnology today has reached a stage where it is considered as the future to all technologies.

3. Unique properties of nanoparticles

A number of physical phenomena become more pronounced as the size of the system decreases. Certain phenomena may not come into play as the system moves from macro to micro level but may be significant at the nano scale. One example is the increase in surface area to volume ratio which alters the mechanical, thermal and catalytic properties of the material. The increase in surface area to volume ratio leads to increasing dominance of the behaviour of atoms on the surface of the particle over that of those in the interior of the particle, thus altering the properties. The electronic and optical properties and the chemical reactivity of small clusters are completely different from the better known property of each component in the bulk or at extended surfaces. Some of the size dependant properties of nanoparticles are quantum confinement in semiconductors, Surface Plasmon Resonance in some metallic nanoparticles and paramagnetism in magnetic nanoparticles.

Surface plasmon resonance refers to the collective oscillations of the conduction electrons in resonance with the light field. The surface plasmon mode arises from the electron confinement in the nanoparticle. The surface plasmon resonance frequency depends not only on the metal, but also on the shape and size of the nanoparticle and the dielectric properties of the surrounding medium (Jain *et al.*, 2007). For example, noble metals, especially gold and silver nanoparticles exhibit unique and tunable optical properties on account of their Surface Plasmon Resonance.

Superparamagnetism is a form of magnetism that is a special characteristic of small ferromagnetic or ferromagnetic nanoparticles. In such superparamagnetic nanoparticles, magnetization can randomly change direction under the influence of temperature.

Superparamagnetism occurs when a material is composed of very small particles with a size range of 1- 10nm. In the presence of an external magnetic field, the material behaves in a manner similar to paramagnetism with an exception that the magnetic moment of the entire material tends to align with the external magnetic field.

Quantum confinement occurs when one or more dimensions of the nanoparticle is made very small so that it approaches the size of an exciton in the bulk material called the Bohr exciton radius. The idea behind confinement is to trap electrons and holes within a small area (which may be smaller than 30nm). Quantum confinement is important as it leads to new electronic properties. Scientists at the Washington University have studied the electronic and optical changes in the material when it is 10nm or less and have related it to the property of quantum confinement.

Some of the examples of special properties that nanoparticles exhibit when compared to the bulk are the lack of malleability and ductility of copper nanoparticles lesser than 50nm. Zinc oxide nanoparticles are known to have superior UV blocking properties compared to the bulk.

3.1. Classification of nanoparticles

Nanoparticles can be broadly grouped into two: namely organic and inorganic nanoparticles. Organic nanoparticles may include carbon nanoparticles (fullerenes) while some of the inorganic nanoparticles may include magnetic nanoparticles, noble metal nanoparticles (like gold and silver) and semiconductor nanoparticles (like titanium dioxide and zinc oxide).

There is a growing interest in inorganic nanoparticles as they provide superior material properties with functional versatility. Due to their size features and advantages over available chemical imaging drugs agents and drugs, inorganic nanoparticles have been examined as potential tools for medical imaging as well as for treating diseases. Inorganic nanomaterials have been widely used for cellular delivery due to their versatile features like wide availability, rich functionality, good biocompatibility, capability of targeted drug delivery and controlled release of drugs (Xu *et al.*, 2006). For example mesoporous silica when combined with molecular machines prove to be excellent imaging and drug releasing systems. Gold nanoparticles have been used extensively in imaging, as drug carriers and in thermo therapy of biological targets (Cheon & Horace, 2009). Inorganic nanoparticles (such as metallic and semiconductor nanoparticles) exhibit intrinsic optical properties which may enhance the transparency of polymer- particle composites. For such reasons, inorganic nanoparticles have found special interest in studies devoted to optical properties in composites. For instance, size dependant colour of gold nanoparticles has been used to colour glass for centuries (Caseri, 2009).

4. Strategies used to synthesize nanoparticles

Traditionally nanoparticles were produced only by physical and chemical methods. Some of the commonly used physical and chemical methods are ion sputtering, solvothermal synthesis, reduction and sol gel technique. Basically there are two approaches for nanoparticle synthesis namely the Bottom up approach and the Top down approach.

In the Top down approach, scientists try to formulate nanoparticles using larger ones to direct their assembly. The Bottom up approach is a process that builds towards larger and

more complex systems by starting at the molecular level and maintaining precise control of molecular structure.

4.1. Physical and chemical methods of nanoparticle synthesis

Some of the commonly used physical and chemical methods include:

- a) Sol-gel technique, which is a wet chemical technique used for the fabrication of metal oxides from a chemical solution which acts as a precursor for integrated network (gel) of discrete particles or polymers. The precursor sol can be either deposited on the substrate to form a film, cast into a suitable container with desired shape or used to synthesize powders.
- b) Solvothermal synthesis, which is a versatile low temperature route in which polar solvents under pressure and at temperatures above their boiling points are used. Under solvothermal conditions, the solubility of reactants increases significantly, enabling reaction to take place at lower temperature.
- c) Chemical reduction, which is the reduction of an ionic salt in an appropriate medium in the presence of surfactant using reducing agents. Some of the commonly used reducing agents are sodium borohydride, hydrazine hydrate and sodium citrate.
- d) Laser ablation, which is the process of removing material from a solid surface by irradiating with a laser beam. At low laser flux, the material is heated by absorbed laser energy and evaporates or sublimates. At higher flux, the material is converted to plasma. The depth over which laser energy is absorbed and the amount of material removed by single laser pulse depends on the material's optical properties and the laser wavelength. Carbon nanotubes can be produced by this method.
- e) Inert gas condensation, where different metals are evaporated in separate crucibles inside an ultra high vacuum chamber filled with helium or argon gas at typical pressure of few 100 pascals. As a result of inter atomic collisions with gas atoms in chamber, the evaporated metal atoms lose their kinetic energy and condense in the form of small crystals which accumulate on liquid nitrogen filled cold finger. E.g. gold nanoparticles have been synthesized from gold wires.

4.2. Biosynthesis of nanoparticles

The need for biosynthesis of nanoparticles rose as the physical and chemical processes were costly. So in the search of for cheaper pathways for nanoparticle synthesis, scientists used microorganisms and then plant extracts for synthesis. Nature has devised various processes for the synthesis of nano- and micro- length scaled inorganic materials which have contributed to the development of relatively new and largely unexplored area of research based on the biosynthesis of nanomaterials (Mohanpuria *et al.*, 2007).

Biosynthesis of nanoparticles is a kind of bottom up approach where the main reaction occurring is reduction/oxidation. The microbial enzymes or the plant phytochemicals with anti oxidant or reducing properties are usually responsible for reduction of metal compounds into their respective nanoparticles.

The three main steps in the preparation of nanoparticles that should be evaluated from a green chemistry perspective are the choice of the solvent medium used for the synthesis, the

choice of an environmentally benign reducing agent and the choice of a non toxic material for the stabilization of the nanoparticles. Most of the synthetic methods reported to date rely heavily on organic solvents. This is mainly due to the hydrophobicity of the capping agents used (Raveendran *et al.*, 2002). Synthesis using bio-organisms is compatible with the green chemistry principles: the bio-organism is (i) eco-friendly as are (ii) the reducing agent employed and (iii) the capping agent in the reaction (Li *et al.*, 2007). Often chemical synthesis methods lead to the presence of some toxic chemical species adsorbed on the surface that may have adverse effects in medical applications (Parashar *et al.*, 2009). This is not an issue when it comes to biosynthesized nanoparticles as they are eco friendly and biocompatible for pharmaceutical applications.

4.2.1. Use of organisms to synthesize nanoparticles

Biomimetics refers to applying biological principles for materials formation. One of the primary processes in biomimetics involves bioreduction. Initially bacteria were used to synthesize nanoparticles and this was later succeeded with the use of fungi, actinomycetes and more recently plants.

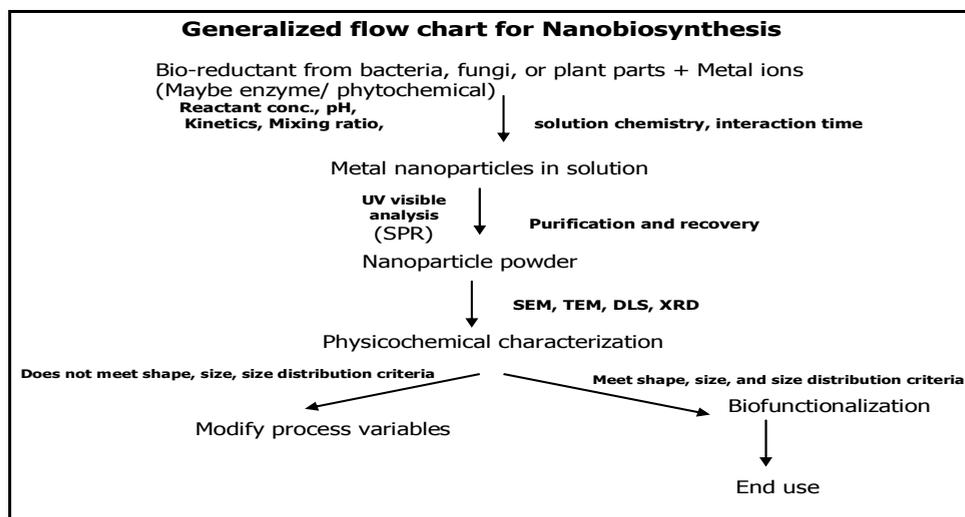


Fig. 1. Flowchart denoting the biosynthesis of nanoparticles

4.2.2. Use of bacteria to synthesize nanoparticles

The use of microbial cells for the synthesis of nanosized materials has emerged as a novel approach for the synthesis of metal nanoparticles. Although the efforts directed towards the biosynthesis of nanomaterials are recent, the interactions between microorganisms and metals have been well documented and the ability of microorganisms to extract and/or accumulate metals is employed in commercial biotechnological processes such as bioleaching and bioremediation (Gericke & Pinches, 2006). Bacteria are known to produce inorganic materials either intra cellularly or extra cellularly. Microorganisms are considered as a potential biofactory for the synthesis of nanoparticles like gold, silver and cadmium

sulphide. Some well known examples of bacteria synthesizing inorganic materials include magnetotactic bacteria (synthesizing magnetic nanoparticles) and S layer bacteria which produce gypsum and calcium carbonate layers (Shankar *et al.*, 2004).

Some microorganisms can survive and grow even at high metal ion concentration due to their resistance to the metal. The mechanisms involve: efflux systems, alteration of solubility and toxicity via reduction or oxidation, biosorption, bioaccumulation, extra cellular complexation or precipitation of metals and lack of specific metal transport systems (Husseiny *et al.*, 2007). For e.g. *Pseudomonas stutzeri* AG 259 isolated from silver mines has been shown to produce silver nanoparticles (Mohanpuria *et al.*, 2007).

Many microorganisms are known to produce nanostructured mineral crystals and metallic nanoparticles with properties similar to chemically synthesized materials, while exercising strict control over size, shape and composition of the particles. Examples include the formation of magnetic nanoparticles by magnetotactic bacteria, the production of silver nanoparticles within the periplasmic space of *Pseudomonas stutzeri* and the formation of palladium nanoparticles using sulphate reducing bacteria in the presence of an exogenous electron donor (Gericke & Pinches, 2006).

Though it is widely believed that the enzymes of the organisms play a major role in the bioreduction process, some studies have indicated it otherwise. Studies indicate that some microorganisms could reduce silver ions where the processes of bioreduction were probably non enzymatic. For e.g. dried cells of *Bacillus megaterium* D01, *Lactobacillus sp.* A09 were shown to reduce silver ions by the interaction of the silver ions with the groups on the microbial cell wall (Fu *et al.*, 1999, 2000). Silver nanoparticles in the size range of 10- 15 nm were produced by treating dried cells of *Corynebacterium sp.* SH09 with diammine silver complex. The ionized carboxyl group of amino acid residues and the amide of peptide chains were the main groups trapping $(Ag(NH_3)_2^+)$ onto the cell wall and some reducing groups such as aldehyde and ketone were involved in subsequent bioreduction. But it was found that the reaction progressed slowly and could be accelerated in the presence of OH⁻ (Fu *et al.*, 2006).

In the case of bacteria, most metal ions are toxic and therefore the reduction of ions or the formation of water insoluble complexes is a defense mechanism developed by the bacteria to overcome such toxicity (Sastry *et al.*, 2003).

4.2.3. Use of actinomycetes to synthesize nanoparticles

Actinomycetes are microorganisms that share important characteristics of fungi and prokaryotes such as bacteria. Even though they are classified as prokaryotes, they were originally designated as ray fungi. Focus on actinomycetes has primarily centred on their exceptional ability to produce secondary metabolites such as antibiotics.

It has been observed that a novel alkalothermophilic actinomycete, *Thermomonospora sp.* synthesized gold nanoparticles extracellularly when exposed to gold ions under alkaline conditions (Sastry *et al.*, 2003). In an effort to elucidate the mechanism or the processes favouring the formation of nanoparticles with desired features, Ahmad *et al.* (2003), studied the formation of monodisperse gold nanoparticles by *Thermomonospora sp.* and concluded that extreme biological conditions such as alkaline and slightly elevated temperature

conditions were favourable for the formation of monodisperse particles. Based on this hypothesis, alkalotolerant actinomycete *Rhodococcus sp.* has been used for the intracellular synthesis of monodisperse gold nanoparticles by Ahmad *et al.* (2003). In this study it was observed that the concentration of nanoparticles were more on the cytoplasmic membrane. This could have been due to the reduction of metal ions by the enzymes present in the cell wall and on the cytoplasmic membrane but not in the cytosol. The metal ions were also found to be non toxic to the cells which continued to multiply even after the formation of the nanoparticles.

4.2.4. Use of fungi to synthesize nanoparticles

Fungi have been widely used for the biosynthesis of nanoparticles and the mechanistic aspects governing the nanoparticle formation have also been documented for a few of them. In addition to monodispersity, nanoparticles with well defined dimensions can be obtained using fungi. Compared to bacteria, fungi could be used as a source for the production of large amount of nanoparticles. This is due to the fact that fungi secrete more amounts of proteins which directly translate to higher productivity of nanoparticle formation (Mohanpuria *et al.*, 2007).

Yeast, belonging to the class ascomycetes of fungi has shown to have good potential for the synthesis of nanoparticles. Gold nanoparticles have been synthesized intracellularly using the fungi *V.luteoalbum*. Here, the rate of particle formation and therefore the size of the nanoparticles could to an extent be manipulated by controlling parameters such as pH, temperature, gold concentration and exposure time. A biological process with the ability to strictly control the shape of the particles would be a considerable advantage (Gericke & Pinches, 2006).

Extracellular secretion of the microorganisms offers the advantage of obtaining large quantities in a relatively pure state, free from other cellular proteins associated with the organism with relatively simpler downstream processing. Mycelia free spent medium of the fungus, *Cladosporium cladosporioides* was used to synthesise silver nanoparticles extracellularly. It was hypothesized that proteins, polysaccharides and organic acids released by the fungus were able to differentiate different crystal shapes and were able to direct their growth into extended spherical crystals (Balaji *et al.*, 2009).

Fusarium oxysporum has been reported to synthesize silver nanoparticles extracellularly. Studies indicate that a nitrate reductase was responsible for the reduction of silver ions and the corresponding formation of silver nanoparticles. However *Fusarium moniliformae* did not produce nanoparticles either intracellularly or extracellularly even though they had intracellular and extracellular reductases in the same fashion as *Fusarium oxysporum*. This indicates that probably the reductases in *F.moniliformae* were necessary for the reduction of Fe (III) to Fe (II) and not for Ag (I) to Ag (0) (Duran *et al.*, 2005).

Instead of fungi culture, isolated proteins from them have also been used successfully in nanoparticles production. Nanocrystalline zirconia was produced at room temperature by cationic proteins while were similar to silicatein secreted by *F. oxysporum* (Mohanpuria *et al.*, 2007).

The use of specific enzymes secreted by fungi in the synthesis of nanoparticles appears promising. Understanding the nature of the biogenic nanoparticle would be equally

important. This would lead to the possibility of genetically engineering microorganisms to over express specific reducing molecules and capping agents and thereby control the size and shape of the biogenic nanoparticles (Balaji *et al.*, 2009).

Microbiological methods generate nanoparticles at a much slower rate than that observed when plant extracts are used. This is one of the major drawbacks of biological synthesis of nanoparticles using microorganisms and must be corrected if it must compete with other methods.

4.2.5. Use of plants to synthesize nanoparticles

The advantage of using plants for the synthesis of nanoparticles is that they are easily available, safe to handle and possess a broad variability of metabolites that may aid in reduction.

A number of plants are being currently investigated for their role in the synthesis of nanoparticles. Gold nanoparticles with a size range of 2- 20 nm have been synthesized using the live alfalfa plants (Torresday *et al.*, 2002). Nanoparticles of silver, nickel, cobalt, zinc and copper have also been synthesized inside the live plants of *Brassica juncea* (Indian mustard), *Medicago sativa* (Alfalfa) and *Helianthus annuus* (Sunflower). Certain plants are known to accumulate higher concentrations of metals compared to others and such plants are termed as hyperaccumulators. Of the plants investigated, *Brassica juncea* had better metal accumulating ability and later assimilating it as nanoparticles (Bali *et al.*, 2006).

Recently much work has been done with regard to plant assisted reduction of metal nanoparticles and the respective role of phytochemicals. The main phytochemicals responsible have been identified as terpenoids, flavones, ketones, aldehydes, amides and carboxylic acids in the light of IR spectroscopic studies. The main water soluble phytochemicals are flavones, organic acids and quinones which are responsible for immediate reduction. The phytochemicals present in *Bryophyllum sp.* (Xerophytes), *Cyprus sp.* (Mesophytes) and *Hydrilla sp.* (Hydrophytes) were studied for their role in the synthesis of silver nanoparticles. The Xerophytes were found to contain emodin, an anthraquinone which could undergo redial tautomerization leading to the formation of silver nanoparticles. The Mesophyte studied contained three types of benzoquinones, namely, cyperoquinone, diethequinone and remirin. It was suggested that gentle warming followed by subsequent incubation resulted in the activation of quinones leading to particle size reduction. Catechol and protocatechaldehyde were reported in the hydrophyte studied along with other phytochemicals. It was reported that catechol under alkaline conditions gets transformed into protocatechaldehyde and finally into protocatecheuic acid. Both these processes liberated hydrogen and it was suggested that it played a role in the synthesis of the nanoparticles. The size of the nanoparticles synthesized using xerophytes, mesophytes and hydrophytes were in the range of 2- 5nm (Jha *et al.*, 2009).

Recently gold nanoparticles have been synthesized using the extracts of *Magnolia kobus* and *Diopyros kaki* leaf extracts. The effect of temperature on nanoparticle formation was investigated and it was reported that polydisperse particles with a size range of 5- 300nm was obtained at lower temperature while a higher temperature supported the formation of smaller and spherical particles (Song *et al.*, 2009).

While fungi and bacteria require a comparatively longer incubation time for the reduction of metal ions, water soluble phytochemicals do it in a much lesser time. Therefore compared to

bacteria and fungi, plants are better candidates for the synthesis of nanoparticles. Taking use of plant tissue culture techniques and downstream processing procedures, it is possible to synthesize metallic as well as oxide nanoparticles on an industrial scale once issues like the metabolic status of the plant etc. are properly addressed.

4.2.6. Work on the biomimetic synthesis of nanoparticles in India

There has been considerable significant research in India in the field of biomimetic synthesis of nanoparticles. More research has been found to be concentrated in the area of biomimetic synthesis using plants.

It has been observed that a novel alkalothermophilic actinomycete, *Thermomonospora sp.* synthesized gold nanoparticles extracellularly when exposed to gold ions under alkaline conditions (Sastry *et al.*, 2003). The use of algae for the biosynthesis of nanoparticles is a largely unexplored area. There is very little literature supporting its use in nanoparticle formation. Recently stable gold nanoparticles have been synthesized using the marine alga, *Sargassum wightii*. Nanoparticles with a size range between 8nm to 12nm were obtained using the seaweed. An important potential benefit of the method of synthesis was that the nanoparticles were quite stable in solution (Singaravelu *et al.*, 2007).

Yeast, belonging to the class ascomycetes of fungi has shown to have good potential for the synthesis of nanoparticles. *Schizosaccharomyces pombe* cells were found to synthesize semiconductor CdS nanocrystals and the productivity was maximum during the mid log phase of growth. Addition of Cd in the initial exponential phase of yeast growth affected the metabolism of the organism (Kowshik *et al.*, 2002). Baker's yeast (*Saccharomyces cerevisiae*) has been reported to be a potential candidate for the transformation of Sb₂O₃ nanoparticles and the tolerance of the organism towards Sb₂O₃ has also been assessed. Particles with a size range of 2- 10 nm were obtained.

Aspergillus flavus has been found to accumulate silver nanoparticles on the surface of its cell wall when challenged with silver nitrate solution. Monodisperse silver nanoparticles with a size range of 8.92+/- 1.61nm were obtained and it was also found that a protein from the fungi acted as a capping agent on the nanoparticles (Vigneshwaran *et al.*, 2007).

Aspergillus fumigatus has been studied as a potential candidate for the extracellular biosynthesis of silver nanoparticles. The advantage of using this organism was that the synthesis process was quite rapid with the nanoparticles being formed within minutes of the silver ion coming in contact with the cell filtrate. Particles with a size range of 5- 25nm could be obtained using this organism (Bhainsa & D Souza, 2006).

In addition to the synthesis of silver nanoparticles, *Fusarium oxysporum* has also been used to synthesize zirconia nanoparticles. It has been reported that cationic proteins with a molecular weight of 24- 28 kDa (similar in nature to silicatein) were responsible for the synthesis of the nanoparticles (Bansal *et al.*, 2004).

Recently, scientists in India have reported the green synthesis of silver nanoparticles using the leaves of the obnoxious weed, *Parthenium hysterophorus*. Particles in the size range of 30- 80nm were obtained after 10 min of reaction. The use of this noxious weed has an added

advantage in that it can be used by nanotechnology processing industries (Parashar *et al.*, 2009). *Mentha piperita* leaf extract has also been used recently for the synthesis of silver nanoparticles. Nanoparticles in the size range of 10-25 nm were obtained within 15 min of the reaction (Parashar *et al.*, 2009). Table 1 denotes the use of various organisms for the synthesis of nanoparticles.

Biological entity	Nanoparticles synthesized	Size	Intra/ Extracellular	Reference
Bacterium				
<i>Pseudomonas aeruginosa</i>	Au	15-30nm	Extracellular	Husseiny <i>et al.</i> , (2007)
<i>Bacillus subtilis</i>	Ag	5-60nm	Extracellular	Saiffudin <i>et al.</i> , (2009)
<i>Pseudomonas stutzeri</i>	Ag	Upto 200nm	Periplasmic	Joerger <i>et al.</i> , (2000) Klaus <i>et al.</i> , (1999)
Fungi				
<i>Coriolus versicolor</i>	Ag	10-75nm	Extracellular	Sanghi & Verma (2008)
<i>Fusarium semitectum</i>	Ag	10-60nm	Extracellular	Basavaraja <i>et al.</i> , (2007)
<i>Fusarium oxysporum</i>	Ag	5-15nm	Extracellular	Ahmad <i>et al.</i> , (2003)
<i>Phaenerochaete chrysosporium</i>	Ag	-	Extracellular	Vigneshwaran <i>et al.</i> , (2006)
<i>Aspergillus flavus</i>	Ag	8.92+/- 1.62nm	Intracellular	Vigneshwaran <i>et al.</i> , (2007)
Plants				
<i>Azadirachta indica</i>	Ag, Au, Ag/Au bimetallic	50-100nm	Extracellular	Shankar <i>et al.</i> , (2003), Tripathy <i>et al.</i> , (2009)
<i>Pelargonium graveolens</i>	Ag	16-40nm	Extracellular	Shankar <i>et al.</i> , (2003)
<i>Capsicum annum</i>	Ag	10-40nm	Extracellular	Li <i>et al.</i> , (2007)

Table 1. Use of biological entities for the synthesis of various nanoparticles

Azadirachta indica leaf extract has also been used for the synthesis of silver, gold and bimetallic (silver and gold) nanoparticles. Studies indicated that the reducing phytochemicals in the neem leaf consisted mainly of terpenoids. It was found that these reducing components also served as capping and stabilizing agents in addition to reduction as revealed from FT IR studies. The major advantage of using the neem leaves is that it is a commonly available medicinal plant and the antibacterial activity of the biosynthesized silver nanoparticle might have been enhanced as it was capped with the neem leaf extract.

The major chemical constituents in the extract were identified as nimbin and quercetin (Shankar *et al.*, 2004, Tripathy *et al.*, 2009). Figure 2 and 3 show the TEM micrograph of the biosynthesized silver nanoparticles (unpublished data, Prathna T.C. *et al.*, 2009).

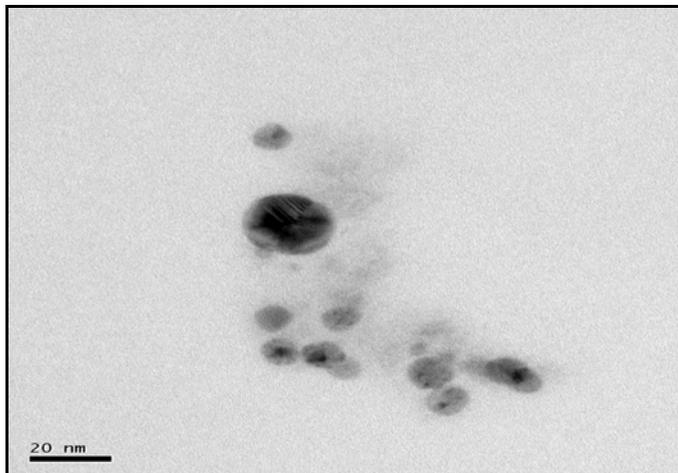


Fig. 2. Transmission electron micrograph showing silver nanoparticles synthesized using neem leaf extract (unpublished data, Prathna T.C. *et al.*, 2009)

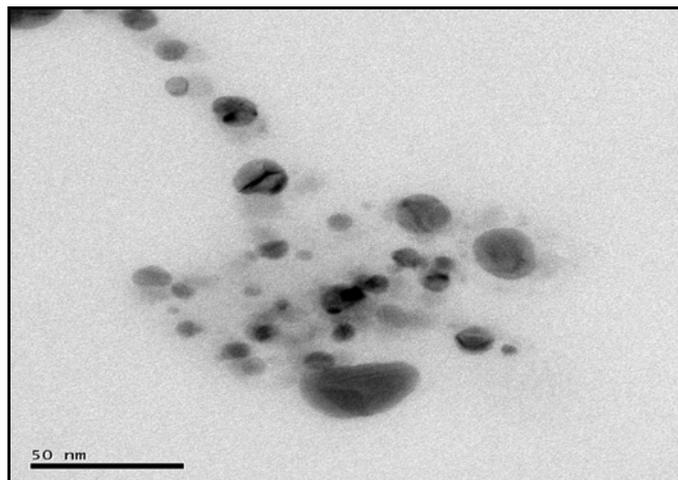


Fig. 3. Transmission electron micrograph showing silver nanoparticles synthesized using neem leaf extract (unpublished data, Prathna T.C. *et al.*, 2009).

4.2.7. Some of the mechanistic aspects of nanoparticle formation

Though there are many studies reporting the biosynthesis of various nanoparticles by bacteria, there is very little information available regarding the mechanistic aspects of nanoparticle production.

The mechanisms of gold bioaccumulation by cyanobacteria (*Plectonema boryanum* UTEX 485) from gold (III) chloride solutions have been studied and it is found that interaction of cyanobacteria with aqueous gold (III) chloride initially promoted the precipitation of nanoparticles of amorphous gold (I) sulfide at the cell walls and finally deposited metallic gold in the form of octahedral (III) platelets near cell surfaces and in solutions (Lengke *et al.*, 2006).

Scientists in Iran have investigated the extracellular biosynthesis of silver nanoparticles by the cells of *Klebsiella pneumoniae*. They hypothesize that the reduction of the metallic ions in the solution by the cell free supernatant is most likely due to the presence of nitroreductase which is produced by some members of Enterobacteriaceae. It has been widely studied that nitrate reductase is necessary for some metallic reduction.

Recently cadmium sulfide nanoparticles have been biosynthesized using the photosynthetic bacteria, *Rhodospseudomonas palustris*. The work indicated that the cysteine desulfhydrase (C-S lyase) could control crystal growth, because cysteine rich proteins can produce S²⁻ through the action of C-S lyase. The content of C-S lyase in *R.palustris* was suggested to be responsible for nanocrystal formation. C-S lyase is found to be an intracellular enzyme located in the cytoplasm and it was indicated that *R.palustris* synthesized CdS nanoparticles intracellularly, later discharging it (Bai *et al.*, 2009).

Schizosaccharomyces pombe cells were found to synthesize semiconductor CdS nanocrystals and the productivity was maximum during the mid log phase of growth. Addition of Cd in the initial exponential phase of yeast growth affected the metabolism of the organism (Kowshik *et al.*, 2002). A possible mechanism for this could be that when Cd is initially added, it causes stress to the organism triggering a series of biochemical reactions. Firstly, an enzyme phytochelatin synthase was activated to synthesize phytochelatins (PC) that chelated the cytoplasmic Cd to form a low molecular weight PC- Cd complex and ultimately transport them across the vacuolar membrane by an ATP binding cassette type vacuolar membrane protein (HMT- 1). In addition to Cd, sulfide could also be added to this complex in the membrane and this could result in the formation of high molecular weight PC- CdS complex that allows it to be ultimately sequestered into the vacuole (Mohanpuria *et al.*, 2007).

Baker's yeast (*Saccharomyces cerevisiae*) has been reported to be a potential candidate for the transformation of Sb₂O₃ nanoparticles and the tolerance of the organism towards Sb₂O₃ has also been assessed. Particles with a size range of 2- 10 nm were obtained. It has been hypothesized that membrane bound oxido reductases and quinones may have played a role in the biosynthesis. The oxidoreductases are pH sensitive and work in alternative manner. At a lower pH, oxidase gets activated while a higher pH value activates reductase. This along with a number of simple hydroxy/ methoxy derivatives of benzoquinones and toluquinones mainly found in lower fungi (and hypothesized to be present in yeast) may facilitate the redox reaction due to its tautomerization. The transformation appears to be negotiated at two levels, one at the cell membrane level immediately after the addition of SbCl₃ solution which triggers tautomerization of quinones and low pH sensitive oxidases which thereby makes molecular oxygen available for transformation. Also when Sb³⁺ enters

the cytoplasm, it might trigger the family of oxygenases harboured in the ER, meant for cellular level detoxification by a process of oxidation/ oxygenation (Jha *et al.*, 2009).

The synthesis of gold and silver nanoparticles has also been reported using black tea leaf extracts. Black tea leaf extracts are known to contain more amounts of flavonones and polyphenols. It was found that the reduction of metal ions was accompanied by oxidation of polyols (Begum *et al.*, 2009). Table 2 gives a summary of some aspects of nanoparticle formation.

Biological entity	Nanoparticle	Enzyme/ phytochemical	Reference
<i>Rhodospseudomonas palustris</i>	CdS	C-S lyase	Bai <i>et al.</i> (2009)
<i>Schizosaccharomyces pombe</i>	CdS	Phytochelatin synthase/ phytochelatins	Mohanpuria <i>et al.</i> (2007)
<i>Schizosaccharomyces cerevisiae</i>	Sb ₂ O ₃	Oxidoreductases/ quinones	Jha <i>et al.</i> ,(2009)
<i>Fusarium oxysporum</i>	Ag	NADH dependant reductase	Duran <i>et al.</i> , (2005)
Black tea leaf	Ag/ Au	Polyphenols/ flavonoids	Begum <i>et al.</i> , (2009)
<i>Azadirachta indica</i>	Ag/ Au	Terpenoids	Shankar <i>et al.</i> , (2004), Tripathy <i>et al.</i> ,(2009)
<i>Jatropha curcas</i>	Ag	Curcain, curacycline curacycline B	A, Bar <i>et al.</i> , (2009)

Table 2. Mechanistic aspects of nanoparticle formation

The latex of *Jatropha curcas*, a plant whose seeds are used to extract biodiesel has also been used for the synthesis of silver nanoparticles. Some of the major components in the latex of *Jatropha curcas* were identified as curcain, curacycline A and curacycline B. The silver nanoparticles obtained using this source had two broad distributions- one having particles in the range of 20- 40 nm and the other having larger and uneven particles. Molecular modeling studies of the peptides in the latex revealed that the silver ions were first entrapped in the core structure of the cyclic structure of the protein and were then reduced and stabilized insitu by the amide group of the peptide. This resulted in particles with radius similar to the peptides. It was also found that the larger particles with uneven shapes were stabilized by the enzyme curcain (Bar *et al.*, 2009).

Li *et al* (2007) synthesized silver nanoparticles using the *Capsicum annum* L. extract. *Capsicum annum* L. extract is known to contain a number of biomolecules such as proteins, enzymes, polysaccharides, amino acids and vitamins. These biomolecules could be used as bioreductants to react with metal ions and they could also be used as scaffolds to direct the formation of nanoparticles in solution. The mechanism responsible for the reduction was postulated as follows: the silver ions were trapped on the surface of proteins in the extract via electrostatic interactions. This stage was the recognition process. The silver ions were

then reduced by the proteins leading to changes in their secondary structure and the formation of silver nuclei. The silver nuclei subsequently grew by the further reduction of silver ions and their accumulation of the nuclei.

5. Applications of Nanoparticles

Nanotechnology has a wide range of applications in the fields of biology, medicine, optical, electrical, mechanical, optoelectronics etc.

Silver nanoparticles have also been used for a number of applications such as non linear optics, spectrally selective coating for solar energy absorption, biolabelling and antibacterial activities.

Silver nanoparticles have shown promise against gram positive *S. aureus*. Nanoparticles have also been incorporated in cloth which has shown promise to be sterile and thus helping in minimizing infections. Metal nanoparticle embedded paints have been synthesized using vegetable oils and have been found to have good antibacterial activity (Kumar *et al.*, 2008).

Current research is going on regarding the use of magnetic nanoparticles in the detoxification of military personnel in case of biochemical warfare. It is hypothesized that by utilizing the magnetic field gradient, toxins can be removed from the body. Enhanced catalytic properties of surfaces of nano ceramics or those of noble metals like platinum and gold are used in the destruction of toxins and other hazardous chemicals (Salata, 2005).

Photocatalytic activity of nanoparticles has been utilized to develop self- cleaning tiles, windows and anti- fogging car mirrors. The high reactivity of Titania nanoparticles either on their own or when illuminated by UV light have been used for bactericidal purposes in filters.

An important opportunity for nanoparticles in the area of computers and electronics is their use in a special polishing process, chemical-mechanical polishing or chemical-mechanical planarization (CMP), which is critical to semiconductor chip fabrication. CMP is used to obtain smooth, flat, and defect-free metal and dielectric layers on silicon wafers. This process utilizes slurry of oxide nanoparticles and relies on mechanical abrasion as well as a chemical reaction between the slurry and the film being polished. CMP is also used in some other applications, such as the polishing of magnetic hard disks.

Nanoscale titanium dioxide and zinc oxide have been used as sunscreens in cosmetics. The primary advantage of using these nanoparticles is that they are well dispersed and transmit visible light, acting as transparent sunblocks. On the other hand, inorganic sunscreens appear white on the skin- a potential drawback.

The interaction of silver nanoparticles with HIV I has been demonstrated in vitro. It was shown that the exposed sulfur binding residues of the glycoprotein knobs were attractive sites for nanoparticle interaction and that the silver nanoparticles had preferential binding to the gp 120 glycoprotein knobs. Due to this interaction, it was found that the silver nanoparticles inhibited the binding of the virus to the host cells in vitro (Elechiguerra *et al.*, 2005).

Magnetic nanoparticles are also used in targeted therapy where a cytotoxic drug is attached to a biocompatible magnetic nanoparticle. When these particles circulate in the bloodstream, external magnetic fields are used to concentrate the complex at a specific target site within the body. Once the complex is concentrated in the target, the drug can be released by enzymatic activity or by changes in pH or temperature and are taken up by the tumour cells (Pankhurst *et al.*, 2003).

Porous nanoparticles have also been used in cancer therapy where the hydrophobic version of a dye molecule is trapped inside the Ormosil nanoparticle. The dye is used to generate atomic oxygen which is taken up more by the cancer cells when compared to the healthy tissue. When the dye is not entrapped, it travels to the eyes and skin making the patient sensitive to light. Entrapment of the dye inside the nanoparticle ensures that the dye does not migrate to other parts and also the oxygen generating ability is not affected.

Alivisatos (2001) reported the presence of inorganic crystals in magnetotactic bacteria. The bacterium was found to have about 20 magnetic crystals with a size range of 35- 120nm diameter. The crystals serve as a miniature compass and align the bacteria with the external magnetic field. This enables the bacterium to navigate with respect to the earth's magnetic field towards their ideal environment. These bacteria immobilize heavy metals from a surrounding solution and can be separated by applying a low intensity magnetic field. This principle can be extended to develop a process for the removal of heavy metals from waste water.

Bioremediation of radioactive wastes from nuclear power plants and nuclear weapon production, such as uranium has been achieved using nanoparticles. Cells and S layer proteins of *Bacillus sphaericus* JG A12 have been found to have special capabilities for the clean up of uranium contaminated waste waters (Duran *et al.*, 2007).

Biomaterials have been formulated by using several bacteria such as *Pseudomonas aeruginosa*, *E. coli* and *Citrobacter sp.* Metal sulfide microcrystallites were formulated using *S. pombe* which could function as quantum semiconductor microcrystallite. These crystals have properties like optical absorption, photosynthetic and electron transfer.

Magnetosome particles isolated from magnetotactic bacteria have been used as a carrier for the immobilization of bioactive substances such as enzymes, DNA, RNA and antibodies (Mohanpuria *et al.*, 2007).

Gold nanoparticles are widely used in various fields such as photonics, catalysis, electronics and biomedicine due to their unique properties. *E. coli* has been used to synthesize gold nanoparticles and it has been found that these nanoparticles are bound to the surface of the bacteria. This composite may be used for realizing the direct electrochemistry of haemoglobin (Du *et al.*, 2007). p- nitrophenol is widely used in pesticides, pharmaceutical industries, explosives and in dyes and is known to be a carcinogenic agent. Gold nanoparticles have been synthesized using the barbated skullcap extract. The nanoparticles synthesized by this method have been modified to the glass electrode and this has been used to enhance the electronic transmission rate between the electrode and p- nitrophenol (Wang *et al.*, 2009).

Tripathy *et al.*, (2008) reported the antibacterial applications of the silver nanoparticles synthesized using the aqueous extract of neem leaves. The nanoparticles were coated on cotton disks and their bactericidal effect was studied against *E.coli*. Duran *et al.*, (2005) reported the significant antibacterial activities of the silver nanoparticles synthesized using *Fusarium oxysporum*.

Table 3 gives a list of a few companies which have utilized nanoparticles in their products.

Company	Product	Advantage
Air quality		
NanoStellar	Nanocomposite catalyst for use in automotive catalytic converters	Reduced cost due to less platinum use
AmericanElements	Catalyst composed of MnO ₂ nanoparticles to remove volatile organic compounds (VOC)	Capable of destroying VOC down to parts per billion level
Batteries		
Zpower	Ag-Zn battery using nanoparticles in the silver cathode	Higher power density, low combustibility
Cleaning products		
Samsung	Ag nanoparticles used in household appliances like clothes washer and refrigerator	Kills bacteria and reduces odour
Nanotec	Spray-on liquid containing nanoparticles	Repels water and dirt
Fabrics		
Aspen Aerogel	Fabric enhanced with nanopores	Insulates against heat
Nano horizons	Fabric enhanced with silver nanoparticles	Reduces odours
Sporting goods		
InMat	Nanocomposite barrier film	Prevents air loss from tennis balls
Easton	Bicycle components strengthened with C nanotubes	Strong, light weight components

Table 3. A list of few companies utilizing nanoparticles in their products (Courtesy: www.understandingnano.com)

Though the applications of nanoparticles are exhaustive, an effort has been made in this review to highlight specific applications

6. Conclusion

An important challenge in technology is to tailor optical, electric and electronic properties of nanoparticles by controlling their size and shape. Biomimetic synthesis of nanoparticles has opened its doors to a world of nanoparticles with easy preparation protocols, less toxicity and a wide range of applications according to their size and shape. Nanoparticles of desired size and shape have been obtained successfully using living organisms- simple unicellular organisms to highly complex eukaryotes. The field of nano biotechnology is still in its infancy and more research needs to be focused on the mechanistics of nanoparticle formation which may lead to fine tuning of the process ultimately leading to the synthesis of nanoparticles with a strict control over the size and shape parameters.

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Nature's evolution has led to the introduction of highly efficient biological mechanisms. Imitating these mechanisms offers an enormous potential for the improvement of our day to day life. Ideally, by bio-inspiration we can get a better view of nature's capability while studying its models and adapting it for our benefit. This book takes us into the interesting world of biomimetics and describes various arenas where the technology is applied. The 25 chapters covered in this book disclose recent advances and new ideas in promoting the mechanism and applications of biomimetics.

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