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Review Article

Biosynthesis of Metal Nanoparticles: A Review

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The synthesis of nanostructured materials, especially metallic nanoparticles, has accrued utmost interest over the past decade owing to their unique properties that make them applicable in different fields of science and technology. The limitation to the use of these nanoparticles is the paucity of an effective method of synthesis that will produce homogeneous size and shape nanoparticles as well as particles with limited or no toxicity to the human health and the environment. The biological method of nanoparticle synthesis is a relatively simple, cheap, and environmentally friendly method than the conventional chemical method of synthesis and thus gains an upper hand. The biomineralization of nanoparticles in protein cages is one of such biological approaches used in the generation of nanoparticles. This method of synthesis apart from being a safer method in the production of nanoparticles is also able to control particle morphology.

1. Introduction

The way we see, feel, and touch things is about to change. In fact, the change has already begun and though it has not touched our lives in any significant manner, the day when that happens is around the corner. From self-cleaning windows to super energy efficient lighting, nanotechnology is revolutionizing the way we live. Lighting has been an important aspect of our lives, of our existence. There is hardly any doubt that nanotechnology is very beneficial to man. With all the applications this new frontier of knowledge has been seen from the human body to industries and chemicals; thus far, nanotechnology has lived up to its name in enhancing the wealth of knowledge possessed by man.

Li et al., in 2011 [1], highlighted the recent developments of the biosynthesis of inorganic nanoparticles including metallic nanoparticles, oxide nanoparticles, sulfide nanoparticles, and other typical nanoparticles. Different formation mechanisms of these nanoparticles will be discussed with the conditions to control the size/shape and stability of particles. The applications of these biosynthesized nanoparticles in a wide spectrum of potential areas are presented including targeted drug delivery, cancer treatment, gene therapy and DNA analysis, antibacterial agents, biosensors, enhancing reaction rates, separation science, and magnetic resonance imaging (MRI).

Iron oxide nanoparticles (Fe_3O_4-NPs) were synthesized using a rapid, single-step and completely green biosynthetic method by reduction of ferric chloride solution with brown seaweed water extract containing sulfated polysaccharides as a main factor which acts as reducing agent and efficient stabilizer. The structural and properties of the Fe_3O_4-NPs were investigated by X-ray diffraction, Fourier transform infrared spectroscopy, field emission scanning electron microscopy (FESEM), energy-dispersive X-ray fluorescence spectrometry (EDXRF), vibrating sample magnetometry (VSM), and transmission electron microscopy. The average particle diameter as determined by TEM was found to be 18 ± 4 nm. X-ray diffraction showed that the nanoparticles are crystalline in nature, with a cubic shape. The nanoparticles synthesized through this biosynthesis method can potentially be useful in various applications. A critical need in the field of nanotechnology is the development of reliable and ecofriendly processes for synthesis of metal oxide nanoparticles. Fe_3O_4-NPs with an average size of 18 ± 4 nm and cubic shapes were synthesized by bioreduction of ferric chloride solution with a green method using brown seaweed (*Sargassum muticum*) aqueous extract containing sulfated polysaccharides as the

reducing agent and efficient stabilizer. The hydroxyl, sulphate, and aldehyde group present in the BS extract are apparently involved in the bioreduction and stabilization of Fe_3O_4-NPs. The involvement of these groups in biosynthesis is revealed by FTIR analysis. The characteristics of the obtained Fe_3O_4-NPs were studied using FTIR, XRD, UV-visible, FESEM, EDXRF, TEM, and VSM techniques. Biosynthesis of Fe_3O_4-NPs using green resources is a simple, environmentally friendly, pollutant-free, and low-cost approach. Functional bioactivity of Fe_3O_4-NPs (antimicrobial) is comparably higher than particles which were synthesized by chemical method. This green method of synthesizing Fe_3O_4-NPs could also be extended to fabricate other, industrially important metal oxides [2].

Gautama and van Veggel in 2013 [3] reviewed an overview of two classes of nanoparticles, namely iron oxide and NaLnF4, and synthesis methods, characterization techniques, study of biocompatibility, toxicity behavior, and applications of iron oxide nanoparticles and NaLnF4 nanoparticles as contrast agents in magnetic resonance imaging. Their optical properties will only briefly be mentioned. Iron oxide nanoparticles show a saturation of magnetization at low field; therefore, the focus will be $MLnF_4$ (Ln = Dy3+, Ho3+, and Gd3+) paramagnetic nanoparticles as alternative contrast agents which can sustain their magnetization at high field. The reason is that more potent contrast agents are needed at magnetic fields higher than 7 T, where most animal MRI is being done these days. Furthermore we observe that the extent of cytotoxicity is not fully understood at present, in part because it is dependent on the size, capping materials, dose of nanoparticles, and surface chemistry, and thus it needs optimization of the multidimensional phenomenon. Therefore, it needs further careful investigation before being used in clinical applications [3].

Silver has always been an excellent antimicrobial and has been used for this purpose for ages. The unique physical and chemical properties of silver nanoparticles only increase the efficacy of silver. Though there are many mechanisms attributed to the antimicrobial activity shown by silver nanoparticles, the actual and most reliable mechanism is not fully understood or cannot be generalized as the nanoparticles are found to act on different organisms in different ways. Chemical and physical methods of silver nanoparticle synthesis were being followed over the decades, but they are found to be expensive and the use of various toxic chemicals for their synthesis makes the biological synthesis the more preferred option. Though bacterial, fungal, and plant extract sources can be used for nanosilver synthesis, the easy availability, the nontoxic nature, the various options available, and the advantage of quicker synthesis make plant extracts the best and an excellent choice for nanosilver synthesis. The uses of silver nanoparticles are varied and many, but the most exploited and desired aspect is their antimicrobial capacity and anti-inflammatory capacity. This has been utilized in various processes in the medical field and has hence been exploited well. However, the downside of silver nanoparticles is that they can induce toxicity at various degrees. It is suggested that higher concentrations of silver nanoparticles are toxic and can cause various health problems. There are also studies that prove that nanoparticles of silver can induce various ecological problems and disturb the ecosystem if released into the environment. Hence, care has to be taken to utilize this marvel well and in a good, effective, and efficient way, understanding its limitations and taking extreme care that it does not cause any harm to an individual or to the environment. It can be believed that if utilized properly, silver nanoparticles can be a good friend, but if used haphazardly, they can become a mighty foe (Sukumaran Prabhu et al., 2012 [4]).

The biosynthesis of metal nanoparticles by marine resources is thought to be clean, nontoxic, and environmentally acceptable "green procedures." Marine ecosystems are very important for the overall health of both marine and terrestrial environments. The use of natural sources like Marine biological resources essential for nanotechnology. Seaweeds constitute one of the commercially important marine living renewable resources. Seaweeds such as green *Caulerpa peltata*, red *Hypnea* valentiae, and brown *Sargassum myriocystum* were used for synthesis of zinc oxide nanoparticles. The preliminary screening of physicochemical parameters such as concentration of metals, concentration of seaweed extract, temperature, pH, and reaction time revealed that one seaweed *S. myriocystum* was able to synthesize zinc oxide nanoparticles. It was confirmed through the initial colour change of the reaction mixture and UV-visible spectrophotometer. The extracellular biosynthesized clear zinc oxide nanoparticles size of 36 nm through characterization technique such as DLS, AFM, SEM-EDX, TEM, XRD, and FTIR. The biosynthesized ZnO nanoparticles are more effective antibacterial agents against gram-positive than the gram-negative bacteria. Based on the FTIR results, fucoidan water soluble pigments present in *S. myriocystum* leaf extract are responsible for reduction and stabilization of zinc oxide nanoparticles. This approach is quiet stable and no visible changes were observed even after 6 months. These soluble elements could have acted as both reducing and stabilizing agents preventing the aggregation of nanoparticles in solution and extracellular biological synthesis of zinc oxide nanoparticles of size 36 nm [5].

Mittal et al., in 2013 [6], reviewed that biomolecules present in plant extracts can be used to reduce metal ions to nanoparticles in a single-step green synthesis process. This biogenic reduction of metal ion to base metal is quite rapid, readily conducted at room temperature and pressure, and easily scaled up. Synthesis mediated by plant extracts is environmentally benign. The reducing agents involved include the various water soluble plant metabolites (e.g., alkaloids, phenolic compounds, and terpenoids) and coenzymes. Silver (Ag) and gold (Au) nanoparticles have been the particular focus of plant-based syntheses. Extracts of a diverse range of plant species have been successfully used in making nanoparticles. In addition to plant extracts, live plants can be used for the synthesis. The use of plant extracts for making metallic nanoparticles is inexpensive, easily scaled up, and environmentally benign. It is especially suited for making nanoparticles that must be free of toxic contaminants as required in therapeutic applications. The plant extract based synthesis can provide nanoparticles of a controlled size

and morphology. In medicine, nanoparticles are being used as antimicrobial agents, for example, in bandages. Applications in targeted drug delivery and clinical diagnostics are developing.

Biological synthesis of silver nanoparticles using *Murraya koenigii* leaf extract was investigated and the effect of broth concentration in reduction mechanism and particle size is reported. The rapid reduction of silver (Ag^+) ions was monitored using UV-visible spectrophotometry and showed formation of silver nanoparticles within 15 minutes. Transmission electron microscopy (TEM) and atomic force microscopy (AFM) analysis showed that the synthesized silver nanoparticles are varied from 10 to 25 nm and have the spherical shape. Further the XRD analysis confirms the nanocrystalline phase of silver with FCC crystal structure. It was found that increasing broth concentration increases the rate of reduction and decreases the particle size. The biological synthesis of silver nanoparticles using *Murraya koenigii* extract was shown to be rapid and to produce particles of fairly uniform size and shape. Following the addition of curry leaf broth to the silver nitrate solution, silver nanoparticles began to form within 15 minutes and the reaction neared completion at 2 h, as shown by the UV-visible spectrophotometry. It was found that increasing broth concentration increases the rate of reduction and reduces the particle size as well as their agglomeration. The reduction of silver ions to silver nanoparticles was found to be optimized at a ratio of 1 : 20 of leaf broth to 10^{-3} M silver nitrate solution. The synthesized particles ranged in size from 10 to 25 nm and were spherical in shape, as shown by the TEM and AFM analysis. The particles also tended to aggregate which suggests they may be useful in applications requiring the coating of materials [7].

Correa-Llantén et al., 2013 [8], mentioned that the use of microorganisms in the synthesis of nanoparticles emerges as an ecofriendly and exciting approach, for production of nanoparticles due to its low energy requirement, environmental compatibility, reduced costs of manufacture, scalability, and nanoparticle stabilization compared with the chemical synthesis. The production of gold nanoparticles by the thermophilic bacterium *Geobacillus* sp. strain ID17 is reported. Cells exposed to Au^{3+} turned from colourless into an intense purple colour. This change of colour indicates the accumulation of intracellular gold nanoparticles. Elemental analysis of particles composition was verified using TEM and EDX analysis. The intracellular localization and particles size were verified by TEM showing two different types of particles of predominant quasihexagonal shape with size ranging from 5 to 50 nm. The majority of them were between 10 and 20 nm in size. FT-IR was utilized to characterize the chemical surface of gold nanoparticles. This assay supports the idea of a protein type of compound on the surface of biosynthesized gold nanoparticles. Reductase activity involved in the synthesis of gold nanoparticles has been previously reported to be present in other microorganisms. This reduction using NADH as substrate was tested in ID17. Crude extracts of the microorganism could catalyze the NADH-dependent Au^{3+} reduction. Results strongly suggest that the biosynthesis of gold nanoparticles by ID17 is mediated by enzymes and suggest NADH as a cofactor for this biological transformation.

The use of biologically derived metal nanoparticles for various proposes is going to be an issue of considerable importance; thus, appropriate methods should be developed and tested for the biological synthesis and recovery of these nanoparticles from bacterial cells. In this research study, a strain of *Klebsiella pneumoniae* was tested for its ability to synthesize elemental selenium nanoparticles from selenium chloride. A broth of *Klebsiella pneumoniae* culture containing selenium nanoparticles was subjected to sterilization at 121°C and 17 psi for 20 minutes. Released selenium nanoparticles ranged in size from 100 to 550 nm, with an average size of 245 nm. Our study also showed that no chemical changes occurred in selenium nanoparticles during the wet heat sterilization process. Therefore, the wet heat sterilization process can be used successfully to recover elemental selenium from bacterial cells. Selenium possesses several applications in medicine, chemistry, and electronics. In recent years, there has been an increasing interest in synthesizing metal particles using chemical and biological methods. The use of "green" synthesis of metal nanoparticles is going to be of considerable importance; thus, appropriate methods should be developed and tested, especially for the recovery of these nanoparticles from natural resources such as bacterial cells. In the present research, an oxidation-reduction titrimetric assay involving $KMnO_4$ was first used for determination of the reduction properties of different culture supernatants of *K. pneumoniae*. The highest reduction ability was observed for the culture supernatant of *K. pneumoniae* grown in TSB. Therefore, this culture medium was chosen for the biological synthesis of selenium nanoparticles. The biorecovery of selenium nanoparticles from a selenium chloride supplemented TSB was further investigated by *K. pneumoniae*. A wet heat sterilization process was used for disrupting the bacterial cells containing the selenium particles. The released nanoparticles showed nanoparticles in the range of 100–550 nm, with an average size of 245 nm. These nanoparticles were chemically stable during the sterilization process, suggesting a possible utilization of this process (wet heat sterilization) for recovering selenium nanoparticles from the cell mass of bacteria or for recovering other intracellular metal nanoparticles generated by microorganisms. On the other hand, the strong EDS signals from the atoms in the nanoparticles confirmed the reduction of selenium ions to elemental selenium and its chemical stability during cell disruption using the sterilization process [9].

Saklani et al., in 2012 [10], reviewed that interesting facts about silver nanoparticles synthesis can be very well understood when the real mechanism involved in the antimicrobial activity of silver ions is known. Silver ions are very reactive and are known to bind to the vital cell components, inducing cell death. However, generation of silver nanoparticles through chemical method is very tedious, whereas, through microbes such as *E. coli*, it is a fast and an eco-friendly approach. These can further be useful in various industrial and many biomedical applications. The nanoparticles can be artificially synthesized in vitro using chemical method via ethanol. But, here the synthesis was done through *E. coli*

at room temperature. The supernatant was taken from the nutrient broth, incubated overnight, and inoculated with *E. coli*. Then 1 mM of $AgNO_3$ (1% v/v) was added to the supernatant. The formation of silver nanoparticles was observed within 10 minutes. The color change was noticed from fine yellow color to reddish brown with time. After keeping for a long time, there was a transition of color change, from fine yellow to deep reddish brown. They are four different series of color change, namely, "fine yellow" as delta (δ), "light brown" as gamma (γ), "light reddish brown" as beta (β), and "deep reddish brown" as alpha (α). Alpha consisted of maximum nanoparticles concentration. Beta consisted of lesser number of nanoparticles as compared to alpha. Gamma consisted of less nanoparticles concentration than beta. Delta consisted of the least nanoparticle concentration of all. Therefore, one can conclude that with time nanoparticles production from *E. coli* increases and hence their antibacterial property varies accordingly. The intensity of color is directly proportional to nanoparticles production. To prove their efficacy, their zone of inhibition (MIC) was tested against *E. coli*. And for these, wells were created in a petri plate spread with microdiluted culture (25 μL). The created wells were filled with α, β, γ, and δ samples, 50 μL each. Alpha (α), reddish brown color, showed the maximum inhibition, with no growth in its zone. Delta (δ), fine yellow color, showed the minimum inhibition of growth. Alpha and beta show the maximum inhibition.

Silver nanoparticles are increasingly used in various fields of biotechnology and applications in medicine. Korbekandi et al. in 2013 studied the optimization of production of silver nanoparticles using biotransformations by Fusarium oxysporum, and a further study on the location of nanoparticles synthesis in this microorganism. The reaction mixture contained the following ingredients (final concentrations): $AgNO_3$ (1–10 mM) as the biotransformation substrate, biomass as the biocatalyst, glucose (560 mM) as the electron donor, and phosphate buffer (pH = 7, 100 mM). The samples were taken from the reaction mixtures at different times, and the absorbance (430 nm) of the colloidal suspensions of silver nanoparticles hydrosols was read freshly (without freezing) and immediately after dilution (1 : 40). SEM and TEM analyses were performed on selected samples. The presence of $AgNO_3$ (0.1 mM) in the culture as enzyme inducer and glucose (560 mM) as electron donor had positive effects on nanoparticle production. In SEM micrographs, silver nanoparticles were almost spherical and single (25–50 nm) or aggregates (100 nm), attached to the surface of biomass. The reaction mixture was successfully optimized to increase the yield of silver nanoparticles production. More details of the location of nanoparticles production by this fungus were revealed, which support the hypothesis that silver nanoparticles are synthesized intracellularly and not extracellularly [11].

Jha et al. in 2010 [12] reported that green, low-cost, and reproducible *Lactobacillus*-mediated biosynthesis of metal and oxide nanoparticles is reported. Silver and titanium dioxide nanoparticles are synthesized using *Lactobacillus* sp. procured from yoghurt and probiotic tablets. The synthesis is performed akin to room temperature in the laboratory ambience. X-ray and transmission electron microscopy analyses are performed to ascertain the formation of metallic and oxide nanoparticles. Individual nanoparticles having the dimensions of 10–25 nm (n-Ag) and 10–70 nm (n-TiO_2) are found.

Usha et al., in 2010 [13], demonstrated the synthesis of metal oxide nanoparticles by a *Streptomyces* sp. isolated from a site Pichavaram mangrove in India. Extracellular production of metal oxide nanoparticles by *Streptomyces* sp. was carried out. It was found that copper sulphate and zinc nitrate when exposed to *Streptomyces* sp. are reduced in solution, thereby leading to the formation of metal oxide nanoparticle. The metal oxide nanoparticles were in the range of 100–150 nm. The possibility of the reduction of metal ions may be by reductase enzyme. The antibacterial and antifungal activity of nanoparticle-coated fabric was evaluated according to the procedures of AATCC147 method and AATCC30 method, respectively. A 100% reduction of viable *E. coli*, *S. aureus*, and *A. niger* was observed in the coated fabric materials after 48 h of incubation. Nanoparticle shows promise when applied as a coating to the surface of protective cloth by reducing the risk of transmission of infectious agents.

Antibacterial nanopackaging can create a modified atmosphere in packaging with controlled gaseous exchange, so that the shelf life of vegetables may be increased to weeks. The surface of an ordinary packaging material such as plastic or paper can be adapted to make it suitable for food by coating it with one or more sharply defined layers of tens of nanometers thickness. Developing coated paper with antibacterial properties of zinc nanoparticles could be an alternative to other food preservation methods. Nanocoatings for glass bottles are used for better preserving the quality of fruit and vegetable products by shielding them from light waves and thereby improve their shelf life. The simplicity of the process means it would be easy to be scaled up to meet industry need. It was also noted that its technology could be used as another option to preservation processes [14].

Dhoondia and Chakraborty, in 2012 [15], reported the synthesis of silver oxide nanoparticles using *Lactobacillus mindensis*, isolated using fixer solution from an X-ray photographic laboratory. Nanoparticles obtained were characterized by means of UV-visible spectroscopy, transmission electron microscopy (TEM), and X-ray diffraction (XRD). The UV-visible spectrum shows the absorbance maximum at 430 nm, which is a characteristic of surface plasmon resonance of silver. Further, the presence of stable nanoparticles in the range of 2–20 nm was determined using TEM analysis. Silver nanoparticles in the form of silver oxide were confirmed in the XRD study. In conclusion, *Lactobacillus mindensis* serves as a promising candidate in the quest to synthesize silver oxide nanoparticles through green chemistry.

Pleurotus sp. was allowed for biosynthesis of iron nanoparticles in culture media. The fungus was allowed to grow in 2×10^{-4} M $FeSO_4$ solutions for 72 hours. UV-visible spectra analysis helps us to know about formations of nanoparticles at 226 nm and 276 nm wavelength. Involvement of proteins in culture was determined by spectral

analysis of broth culture at different intervals of time at 265 nm. FTIR analysis detects the presence of functional groups in binding of particles with biomass. There was a shift in case of treated cells indicating participations of proteins in more quantity. TEM images reflect depositions of particles both inside and outside indicating the biosynthesis process. However such depositions are absent in case of control sample. XRF of control samples does not show any iron elements but was present in treated mycelium. Some others elements were also detected, it may be due to present in culture media. The transport of iron particles may be due to presence of siderophores. Iron transport molecules like hydroxamates are mainly present in fungi. They bind the complex molecules and transport them inside of cells [16].

Vidya et al. in 2013 [17] studied that green synthesis of ZnO nanoparticles by zinc nitrate and utilizing the bio components of leaves extract of *Calotropis Gigantea*. The ZnO nanocrystallites of average size range of 30–35 nm have been synthesized by rapid, simple, and ecofriendly method. Zinc nanoparticles were characterized using scanning electron microscopy (SEM) and X-ray diffraction (XRD). The particles obtained are spherical in nature and are agglomerates of nanocrystallite. The X-ray patterns show hexagonal crystal type for ZnO. The results coincide with literature XRD pattern for hexagonal wurtzite ZnO. The size of nanocrystallites is calculated by considering XRD data by Debye-Scherrer's formula. The rapid biological synthesis of zinc nanoparticles using leaf extract of *Calotropis gigantea* provides an environmentally friendly, simple, and efficient route for synthesis of nanoparticles. The use of plant extracts avoids the usage of harmful and toxic reducing and stabilizing agents. The synthesized nanocrystallites of ZnO are in the range of 30–35 nm. Zinc nanoparticles can exist in ions only in the presence of strong oxidizing substances. The environmental conditions will affect the stability of nanoparticle and agglomerates are formed. The synthesis of ZnO nanoparticles is still in its infancy and more research needs to be focused on the mechanism 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 [18].

The development of techniques for the controlled synthesis of nanoparticles of well-defined size, shape, and composition, to be used in the biomedical field and areas such as optics and electronics, is a big challenge. The use of the highly structured physical and biosynthetic activities of microbial cells for the synthesis of nanosized materials has recently emerged as a novel approach for the synthesis of metal nanoparticles. A number of different organisms, including bacteria, yeast, and fungi, were screened for their ability to produce gold nanoparticles and examples of results demonstrating the formation of gold nanoparticles of different sizes and shapes are presented. The cellular mechanism leading to the reduction of the gold ions and formation of gold nanoparticles is not well understood and therefore the potential to manipulate key parameters, which control growth and other cellular activities, to achieve controlled size and shape of the nanoparticles, was investigated. The results provided some insight as to which parameters may impact on the cellular mechanism involved in the reduction of gold ions and formation of gold nanoparticles. In their study they demonstrated the intracellular synthesis of gold nanoparticles of various morphologies and sizes in two fungal cultures, *V. luteoalbum* and isolate 6–3. 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 to $AuCl_4^-$. In chemical nanoparticle synthesis procedures shape control of the particles is still an issue and an ongoing area of research. A biological process with the ability to strictly control the shape of the particles would therefore be a considerable advantage. An immediate objective of further research is therefore to use the highly structured physical and biosynthetic activities of microbial cells to achieve controlled manipulation of the size and shape of the particles. Issues that need to be addressed include development of a fundamental understanding of the process mechanism on a cellular and molecular level, including isolation and identification of the compounds responsible for the reduction of gold ions [19].

Sagar and Ashok, in 2012 [20], reported that antibiotic resistance is one of the world's most pressing public healthcare problems. People who become infected with drug-resistant microorganisms usually spend more time in the hospital and require a form of treatment that uses two or three different antibiotics and is less effective, more toxic, and more expensive. Silver nanoparticles (AgNPs) are attractive option because they are nontoxic to the human body at low concentrations and have broad spectrum antibacterial actions. The biosynthesis of nanoparticles has received increasing attention due to the growing need to develop safe, cost-effective, and environmentally friendly technologies for nanomaterials synthesis. Silver nanoparticles (AgNPs) were synthesized using a reduction of aqueous Ag^+ ion with the culture supernatants of *Aspergillus niger*. The reaction occurred at ambient temperature and in a few hours. The bioreduction of AgNPs was monitored by ultraviolet-visible spectroscopy, and the AgNPs obtained were characterized by transmission electron microscopy and X-ray diffraction. The synthesized AgNPs were polydispersed spherical particles ranging in size from 1 to 20 nm and stabilized in the solution. Furthermore, the antimicrobial potential of AgNPs was systematically evaluated. The synthesized AgNPs could efficiently inhibit various pathogenic organisms, including bacteria and fungi.

A low-cost, green, and reproducible probiotic microbe (*Lactobacillus sporogenes*) mediated biosynthesis of ZnO nanoparticles is reported. The synthesis is performed akin to room temperature in five replicate samples. X-ray and transmission electron microscopy analyses are performed to ascertain the formation of ZnO nanoparticles. Rietveld analysis to the X-ray data indicated that ZnO nanoparticles have hexagonal unit cell structure. Individual nanoparticles having the size of 5–15 nm are found. A possible involved mechanism for the synthesis of ZnO nanoparticles has been proposed. The H_2S adsorption characteristic of ZnO nanoparticles has also been assayed [21].

The biological process with the ability to study the shape of particles produced would therefore be a limelight

of modern nanotechnology. The bacterial strain *Escherichia coli,* used for the biosynthesis of silver nanoparticles, were investigated. These silver nanoparticles were characterized by means of UV-visible spectroscopy and particle size analyzer. The nanoparticles exhibited maximum absorbance at 400 nm in UV-visible spectroscopy corresponding to the plasmon resonance of silver nanoparticles [22].

Forough and Khalil, in 2010 [23], investigated the synthesis of stable silver nanoparticles by the bioreduction method aqueous extracts of the manna of Hedysarum plant and the soap-root (*Acanthe phylum bracteatum*) plants were used as reducing and stabilizing 16 agents, respectively. UV-visible absorption spectroscopy was used to monitor the quantitative formation of silver nanoparticles. The characteristics of the obtained silver nanoparticles were studied using X-ray diffraction analysis (XRD), energy-dispersive spectroscopy (EDX), and scanning electron microscopy (SEM). The EDX spectrum of the solution containing silver nanoparticles confirmed the presence of an elemental silver signal without any peaks of impurities. The average diameter of the prepared nanoparticles in solution was about 29–68 nm.

Researchers worked on the Biosynthesized antimicrobial potent silver nanoparticles using whole plant aqueous extract of *Andrographis paniculata*. The synthesized silver nanoparticles were confirmed by color transformation and ultraviolet-visible (UV-visible) spectrophotometery. The size and morphology of the silver nanoparticles were characterized by scanning electron microscope (SEM) and transmission electron microscope (TEM). The stability of silver nanoparticles was detected by Fourier transform infrared spectroscopy (FTIR). The effect of silver nanoparticles over bacterial strains such as *B. subtilis, E. coli, P. aeruginosa, P. fluorescence, S. aureus, S. typhii,* and *V. parahaemolyticus* and pathogenic fungi such as *A. flavus* and *A. niger* was examined. The appearance of dark brown color and UV absorption range at 430 nm confirmed the synthesized silver nanoparticles. The silver nanoparticles showed spherical structure and their sizes were ranging from 14 to 80 nm under SEM and TEM observations. FTIR spectra of silver nanoparticles showed the peaks for functional groups, N–H, C=O, and –C=C, and monosubstituted ring which indicate the stability of synthesized silver nanoparticles. The obtained nanoparticles showed good inhibitory activity on all bacterial species, whereas they showed antifungal activity only on *A. niger* and had no effect on *A. flavus*. The synthesis of silver nanoparticles using whole plant aqueous extract of *A. paniculata* would be helpful for the preparation of pharmaceutically useful drugs to destroy pathogenic microbes [24].

Copper nanoparticles synthesized in electrolysis method are showing antibacterial activities against both gram (−) and gram (+) bacteria. Changes in surface area to volume ratio of copper are enhancing its antibacterial activities. Copper nanoparticles synthesized in electrolysis method are showing more antibacterial activities (for *E. coli* bacteria) than copper nanoparticles synthesized in chemical reduction method. Using electrical power while Synthesizing copper nanoparticles using electric power increases its antimicrobial activity. The chemicals involved in the synthesis of nanoparticles are commonly available, cheap, and nontoxic. The technology can be implemented with minimum infrastructure. The experiments suggest the possibility to use this material in water purification, air filtration, air quality management, antibacterial packaging, and so forth [25].

Malarkodi et al., in 2013 [26], reported that the microbe-mediated biosynthesis of gold nanoparticles is nontoxic compared to all other reported methods. Also, the method has advantages: the process is easy since it can be scaled and it is also economically possible. Reduction and surface accretion of metals may be processed, by which bacteria keep themselves from the toxic effects of metallic ions. In the present investigation, the gold nanoparticles were synthesized using *K. pneumoniae* which was isolated from saltpan soil and identified by MTCC. The synthesis of gold nanoparticles was confirmed by colour change of the liquid medium from yellow to intense dark purple, and it exhibited its maximum absorbance at 560 nm which played a prominent role in the reduction of gold chloride to gold nanoparticles. The X-ray diffractometer showed the crystalline nature of nanoparticles. The Fourier transform infrared spectroscopy showed that the functional groups of gold nanoparticles can bind to proteins through free amine groups or carboxylate groups in the protein. The morphology of the gold nanoparticles is found to be spherical in shape and stable in water for 3 months that can be attributed to the surface binding of stabilization materials secreted by the bacteria. Gold colloidal solution is biologically well suited and has the potential to be used in medical and pharmaceutical applications due to their homologous size distribution.

Biological synthesis of silver nanoparticles using microorganisms has received profound interest because of their potential to synthesize nanoparticles of various sizes, shape, and morphology. In the current study, synthesis of silver nanoparticles by a bacterial strain (CS 11) isolated from heavy metal contaminated soil is reported. Molecular identification of the isolate showed it as a strain of *Bacillus* sp. On treating the bacteria with 1 mM $AgNO_3$, it was found to have the ability to form silver nanoparticles extracellularly at room temperature within 24 h. This was confirmed by the visual observation and UV-visible absorption at 450 nm. Further characterization of nanoparticles by transmission electron microscopy confirmed the size of silver nanoparticles in 42–92 nm range [18].

2. Enzymatic Synthesis of Nanoparticles

Enzymes are extensively used as biocatalysts in numerous industries like food, chemical, pharmaceutical, and fermentation industries. These biocatalysts are known for their selective substrate specificity and catalytic activity in most of the chemical reaction. Hence biosensors advancements in the field of food are expected to yield substantial returns. However, till date, biosensor for medical applications continues to dominate the market; the major reason behind the dormancy of biosensor for other applications is the high fluctuations in operating environment, that is, exposure to temperature and pH farther away from optimum conditions for the enzymes used in these biosensors. The high temperature and

pH variability and hence instability of enzyme, leading to low shelf life, are a major concern for commercial viability. The low free energy difference (~40 kJ/mol) between the native and denatured structure of enzyme makes it a fragile molecule to deal with and limits its unlimited applications. The appropriate folding of proteins leading to the complex three-dimensional structure allows high catalytic activity of biocatalyst. Thus, any destruction or damage to this three-dimensional structure results in loss of catalytic activity of the biocatalyst. This crucial conformation of the protein is stabilized by a variety of interactions based on hydrophobic effects, electrostatic interactions, coordinative complexes, and at times even covalent disulfide bonds. Different factors are known such as temperature and pH that may disturb the balance of weak noncovalent forces responsible for preserving the native conformation of enzyme. It is well-known fact that thermal denaturation of many enzymes begins at temperatures exceeding 35°C, making the usage of enzyme difficult in any reaction system, where the substrate has a high melting point. The presence of flexible regions in the structure of enzymes results in high kinetic energy of these regions from vibrations even at room temperature. This enhanced kinetic energy of some region in structure of the enzyme molecule can rearrange weak noncovalent interactions and thereby inactivating the enzyme. Moreover, the enzyme is known to be highly unstable in solution. Chemical modifications can also lead to changes in the conformation of the protein. As reported by Manning et al. in 2009 [27], the solvent water for instance may react with proteins, resulting in deamidation of glutamine and asparagine residues, whereas oxidation modifies thiol groups. Therefore, it is a must to provide an appropriate environment to enzyme in order to sustain their catalytic activity. Research efforts to analyze genetic makeup of thermophilic and hyperthermophilic microorganism in order to elucidate the factors responsible for their thermal stability like codon usage or specific amino acid sequence and so forth are in progress. However, not much success has been achieved in converting a thermally labile enzyme into thermally stable enzyme.

Various available reports claim that different natural or synthetic substances like an amino acid, a polyol, sucrose, and dextran are often added to an enzyme solution for enhancing its thermal stability. However, the enzyme obtained by the conventional methods described above is stable only under very narrow temperature range and thus is relatively unstable over broad temperatures fluctuations on either side of optimum values. Hence, it is imperative to develop methods to enhance the stability of enzymes.

3. Effect of Desolvating Agent

Different desolvating agents were used for the purpose of protein desolvation. Alcohols (ethanol and propanol) and ketones (acetone) were added to the enzyme solution at a constant concentration ranging from 50 to 100% at a constant rate of addition.

4. Effect of Crosslinking Agent Concentration

Following desolvation, the effect of concentration of crosslinking agent (glutaraldehyde) on the protein was studied. Varying concentration of glutaraldehyde from 0.001% to 0.005% was added to the desolvated protein. The crosslinking process was performed under continuous stirring of the suspension over a time period of 24 hours to ensure complete crosslinking of the particles.

5. Effect of Functionalization Agent on Stability of Enzyme Nanoparticles

Functionalization of synthesised nanoparticles was performed using different functionalizing agents in order to analyze the effect of functionalizing agents on stability, functionality, and biocompatibility of synthesized enzyme nanoparticles. Functionalizing agents such as L-cysteine and chemical X were added with constant stirring for 5-6 hours to the solution containing enzyme nanoparticles.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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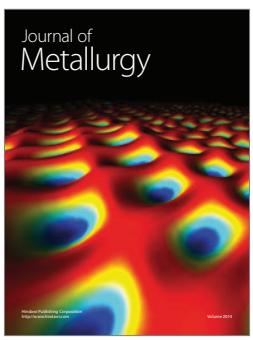

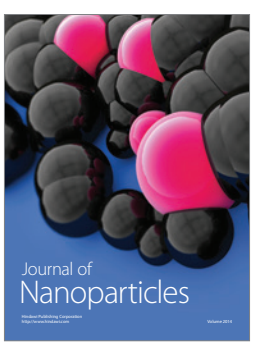

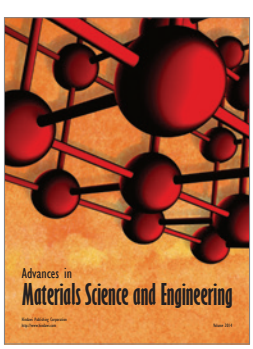

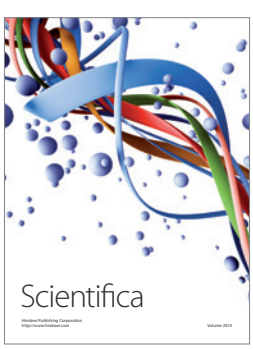

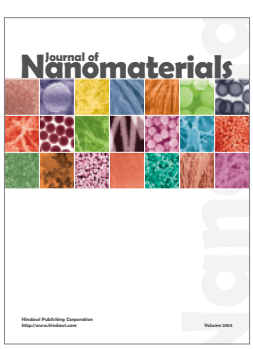

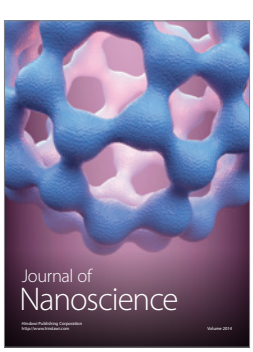

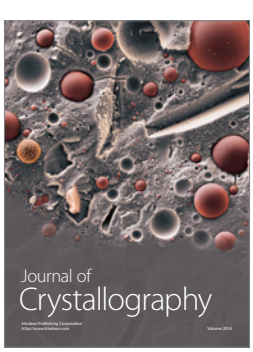

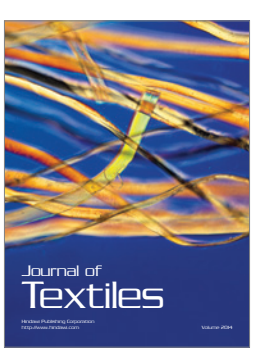

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