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# Studies on Biologically Synthesized Nano-Particles; Their Anti-Bacterial, Antifungal & Anticancer Activities and Applications in Nano-Medicine

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## INTRODUCTION

Nanotechnology is a branch of technology associated with the synthesis, characterization, and application of materials in the nano-scale range of 1–100 nm) term “nanotechnology” was defined in 1974 by Norio Taniguchi of Tokyo University as the ability to manipulate materials at the nanometer scale. Since then, nano-particles have been used for various applications in science and technology. Application of nanotechnology in health sciences is in the field of nano-medicine. Unique property of nano-particles is that they have a considerably high surface area, which facilitates binding with different functional ligands. Small size of nano-particles enables their use for various applications, including development of new devices or tools used in the biomedical and pharmaceutical fields. In the recent years, compared to bulk metals or metal ions, metallic nano-particles, including silver (Ag), gold (Au), platinum (Pt), and palladium (Pd), have been extensively studied because of their unique properties, particularly the effect of quantum size and large surface area. In addition, metallic nano-particles are compatible with the biological system, and therefore, they have been used for drug delivery, diagnostic imaging, labelling, and as biosensors.

Metallic nano-particles are synthesized by various techniques, including a chemical method. )e process of synthesis via a chemical method is divided into two steps: the first step involves reduction by a reducing agent followed by stabilization of the formed nano-particles by using a suitable stabilizing agent; the first step is crucial in avoiding particle agglomeration that leads to the disappearance of the formed nano-particles. A nontoxic stabilizing agent or a capping agent should be selected during the synthesis of nano-particles because the toxicity of the nano-particles depends on the properties of the capping agent rather than the nano-particle itself. Metallic nano-particles stabilized using a biodegradable polymer such as chitosan can be used for the development of drug delivery systems because chitosan acts as an effective adjuvant owing to its efficient interaction with and permeation across the cellular membranes. Chitosan is widely used because of its low toxicity and high biocompatibility. )e presence of a stabilizer such as chitosan in the synthesis of metallic nano-particles can facilitate modification of the surface physical absorption, specific recognition, and electrostatic interaction and thus improve stability, which is important for determining the potential use of metallic nano-particles as therapeutic agents.

Biosynthesis of metallic nano-particles is gaining popularity because of the eco-friendly and cost-effectiveness nature of this method, whereas the chemical method is associated with biological hazards and environmental toxicity. Metallic nano-particles synthesized using biological agents, including fungi, plants, bacteria, and other microorganisms, have high stability. Many studies have investigated the applications of silver nano-particles (AgNPs) and AgNPs in health sciences; therefore, in this article, we have reviewed the mechanism of biosynthesis and the potential applications of silver nano-particles (AgNPs) and AuNPs and their chitosan-mediated nano-composites.

The microbial-mediated biosynthesis of nano-materials has been extensively explored showing different advantages and features including the following: (i) synthesized nano-materials have defined chemical composition, size and morphology, (ii) biosynthesis is performed at mild physico-chemical conditions, (iii) easily handling and cultivation of microbial cells and possibility of cell culture scale-up, (iv) possibility of in vivo tuning nano-material characteristics by changing key parameters of cell culture operational set up or through genetically engineering tools. In order to enable a broad applicability of microbial-mediated biosynthesis of nano-materials as a real alternative to 'traditional' synthetic approaches to nano-manufacturing, many hurdles still need to be overcome: a reduction of polidispersity of nano-particles, a more complete characterization of biocapping layer agents, effectiveness of removal procedures of biocapping layer and nano-materials purifications, standardization of microbial cell culture protocols for reproducibility of nano-synthesis processess, as well as production costs and yields. Overreaching the challenge for the development of reliable eco-friendly nanotechnologies for nano-material synthesis is of utmost importance for future exploitations of broad-impact nano-structured-based technologies and applications, like innovative

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optical and electrochemical (bio) sensoristic devices and therapeutic and diagnostic applications of nano-structured materials e.g., for drug delivery, in vivo/in vitro imaging and development of antimicrobial and antitumoral drugs.

### 1.1 STATEMENT OF THE PROBLEM

In recent years, physical and chemical techniques have been used to produce nanoparticles [37, 40]. These techniques are costly and utilize toxic chemicals during the synthesis of nanoparticles under ultraviolet irradiation, laser ablation and aerosol spray [36, 40]. While these methods are extensively used, but use of toxic chemicals is subject of concern. To overcome this problem, microbial synthesis of nanoparticles is being explored.

Cancer is a complex, multifactorial disease which has the characteristic feature of the uncontrolled growth and spread of abnormal cells caused by several factors, including a combination of genetic, external, internal, and environmental factors [25], and it is treated by various treatments including chemotherapy, hormone therapy, surgery, radiation, immune therapy, and targeted therapy [25]. Therefore, the challenge is to identify effective, cost-effective, and sensitive lead molecules that have cell-targeted specificity and increase the sensitivity. Recently, AgNPs have been shown much interest because of their therapeutic applications in cancer as anticancer agents, in diagnostics, and in probing. Taken literature into consideration, in this review we focused on recent developments in synthesis, characterization, properties, and bio-applications mainly on the antibacterial, antifungal, antiviral, anti-inflammatory, anti-cancer and anti-angiogenic properties of AgNPs in a single platform. This review also emphasizes mechanism of anticancer activity, therapeutic approaches and the challenges and limitations of nanoparticles in cancer therapy. Finally, this review ends with conclusion and the future perspective of AgNPs.

### 1.2 RATIONALE OF THE STUDY

Nanotechnology deals with the synthesis of materials, structures and/or devices having dimensions up to 100 nm with new properties. When the particle size of the materials is reduced below a certain value is found to be exhibiting superlative chemical and physical properties. Nano-structured materials exhibit a host of interesting new phenomena directly related to their reduced

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dimensionality. Not only the electronic, magnetic and optical properties but also chemical, electrochemical and catalytic properties of nano-structured materials are very different from those of the bulk form and depend sensitively on size, shape and composition. Nano-structured metal oxides have been extensively used as catalysts or catalytic supports for a variety of industrial reactions. In the past decade, nickel oxide (NiO), as an important functional material, has received intensive focus due to their novel properties, specific and potential applications in diverse fields. This study aims to synthesize NiO nano-particles by using a simple, eco-friendly and novel solution method. The properties of the synthesized nano-particles are confirmed by various characterization techniques. As the doping leads some novel applications, the synthesized NiO is doped by transition metals.

## 1.3 GEOGRAPHICAL LOCATION OF RESEARCH

Nano-science and nanotechnology can solve many of the problems in the scientific world; it is already proven that many unanswered questions in material science has got solution from nano-science and nanotechnology. This study will be done in the geographical area of West Bengal.

## 1.4 LIMITATION

- The biological activity of AgNPs depends on the morphology and structure of AgNPs, controlled by size and shape of the particles.
- Insensitive correlation to size fractions
- Limited size resolution

## 1.5 HYPOTHESIS

- Structural, morphological and magnetic properties of the as synthesized nano-particles
- Comparative study on the photocatalytic behavior under three different light sources
- Synthesis of transition metal doped NiO nanoparticles

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## 1. LITERATURE REVIEW

Sang Hun Lee (2019) Over the past few decades, metal nanoparticles less than 100 nm in diameter have made a substantial impact across diverse biomedical applications, such as diagnostic and medical devices, for personalized healthcare practice. In particular, silver nanoparticles (AgNPs) have great potential in a broad range of applications as antimicrobial agents, biomedical device coatings, drug-delivery carriers, imaging probes, and diagnostic and optoelectronic platforms, since they have discrete physical and optical properties and biochemical functionality tailored by diverse size- and shape-controlled AgNPs. In this review, we aimed to present major routes of synthesis of AgNPs, including physical, chemical, and biological synthesis processes, along with discrete physiochemical characteristics of AgNPs. We also discuss the underlying intricate molecular mechanisms behind their plasmonic properties on mono/bimetallic structures, potential cellular/microbial cytotoxicity, and optoelectronic property. Lastly, we conclude this review with a summary of current applications of AgNPs in nano-science and nano-medicine and discuss their future perspectives in these areas.

Gerardo Grasso (2019) Nano-materials are increasingly being used in new products and devices with a great impact on different fields from sensoristics to biomedicine. Biosynthesis of nano-materials by microorganisms is recently attracting interest as a new, exciting approach towards the development of ‘greener’ nano-manufacturing compared to traditional chemical and physical approaches. This review provides an insight about microbial biosynthesis of nano-materials by bacteria, yeast, molds, and microalgae for the manufacturing of sensoristic devices and therapeutic/diagnostic applications. The last ten-year literature was selected, focusing on scientific works where aspects like biosynthesis features, characterization, and applications have been described. The knowledge, challenges, and potentiality of microbial-mediated biosynthesis was also described. Bacteria and microalgae are the main microorganism used for nano-biosynthesis, principally for biomedical applications. Some bacteria and microalgae have showed the ability to synthesize unique nanostructures: bacterial nano-cellulose, exopolysaccharides, bacterial nano-wires, and bio-mineralized nano-scale materials (magnetosomes, frustules, and coccoliths). Yeasts and molds are characterized by extracellular synthesis, advantageous for possible reuse of cell cultures and reduced purification processes of nano-materials. The intrinsic variability of the microbiological systems requires a greater protocols standardization to obtain nano-materials with increasingly uniform and reproducible chemical-physical characteristics. A deeper knowledge about biosynthetic pathways and the opportunities from genetic engineering are stimulating the research towards a breakthrough development of microbial-based nano-synthesis for the future scaling-up and possible industrial exploitation of these promising ‘nano-factories’.

Priyanka Singh (2017) Nanotechnology has become one of the most promising technologies applied in all areas of science. Metal nanoparticles produced by nanotechnology have received global attention due to their extensive applications in the biomedical and physiochemical fields. Recently, synthesizing metal nanoparticles using microorganisms and plants has been extensively studied and has been recognized as a green and efficient way for further exploiting microorganisms as convenient nano-factories. Here, we explore and detail the potential uses of various biological sources for nano-particle synthesis and the application of those nanoparticles. Furthermore, we highlight recent milestones achieved for the biogenic synthesis of nanoparticles by controlling critical parameters, including the choice of biological source, incubation period, pH, and temperature.

Nikolaos Pantidos (2014) over the past few decades’ interest in metallic nanoparticles and their synthesis has greatly increased. This has resulted in the development of numerous ways of producing metallic nanoparticles using chemical and physical methods. However, drawbacks such as the involvement of toxic chemicals and the high-energy requirements of production make it difficult for them to be widely implemented. An alternative way of synthesizing metallic nanoparticles is by using living organisms such as bacteria, fungi and plants. This “green” method of biological nano-particle production is a promising approach that allows synthesis in aqueous conditions, with low energy requirements and low-costs. This review gives an overview of some of these environmentally friendly methods of biological metallic nano-particle synthesis. It also highlights the potential importance of these methods in assessing nano-particle risk to both health and the environment.

Kaushik N.ThakkarMS (2010) the synthesis of metallic nanoparticles is an active area of academic and, more importantly, “application research” in nanotechnology. A variety of chemical and physical procedures could be used for synthesis of metallic nanoparticles. However, these methods are fraught with many problems including use of toxic solvents, generation of hazardous by-products, and high

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energy consumption. Accordingly, there is an essential need to develop environmentally benign procedures for synthesis of metallic nanoparticles. A promising approach to achieve this objective is to exploit the array of biological resources in nature. Indeed, over the past several years, plants, algae, fungi, bacteria, and viruses have been used for production of low-cost, energy-efficient, and nontoxic metallic nanoparticles. In this review, we provide an overview of various reports of synthesis of metallic nanoparticles by biological means.

Xiangqian Li (2011) the development of eco-friendly technologies in material synthesis is of considerable importance to expand their biological applications. Nowadays, a variety of inorganic nanoparticles with well-defined chemical composition, size, and morphology have been synthesized by using different microorganisms, and their applications in many cutting-edge technological areas have been explored. This paper highlights 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 as well. The conditions to control the size/shape and stability of particles are summarized. 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). The current limitations and future prospects for the synthesis of inorganic nanoparticles by microorganisms are discussed.

Ratul Kumar Das (2017) the green synthesis (GS) of different metallic nanoparticles (MNPs) has re-evaluated plants, animals and microorganisms for their natural potential to reduce metallic ions into neutral atoms at no expense of toxic and hazardous chemicals. Contrary to chemically synthesized MNPs, GS offers advantages of enhanced biocompatibility and thus has better scope for biomedical applications. Plant, animals and microorganisms belonging to lower and higher taxonomic groups have been experimented for GS of MNPs, such as gold (Au), silver (Ag), copper oxide (CuO), zinc oxide (ZnO), iron ($Fe_2O_3$), palladium (Pd), platinum (Pt), nickel oxide (NiO) and magnesium oxide (MgO). Among the different plant groups used for GS, angiosperms and algae have been explored the most with great success. GS with animal-derived biomaterials, such as chitin, silk (sericin, fibroin and spider silk) or cell extract of invertebrates have also been reported. Gram positive and gram

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negative bacteria, different fungal species and virus particles have also shown their abilities in the reduction of metal ions. However, not a thumb rule, most of the reducing agents sourced from living world also act as capping agents and render MNPs less toxic or more biocompatible. The most unexplored area so far in GS is the mechanism studies for different natural reducing agents expect for few of them, such as tea and neem plants. This review encompasses the recent advances in the GS of MNPs using plants, animals and microorganisms and analyzes the key points and further discusses the pros and cons of GS in respect of chemical synthesis.

Xi-Feng Zhang (2016) recent advances in nano-science and nanotechnology radically changed the way we diagnose, treat, and prevent various diseases in all aspects of human life. Silver nanoparticles (AgNPs) are one of the most vital and fascinating nano-materials among several metallic nanoparticles that are involved in biomedical applications. AgNPs play an important role in nano-science and nanotechnology, particularly in nano-medicine. Although several noble metals have been used for various purposes, AgNPs have been focused on potential applications in cancer diagnosis and therapy. In this review, we discuss the synthesis of AgNPs using physical, chemical, and biological methods. We also discuss the properties of AgNPs and methods for their characterization. More importantly, we extensively discuss the multifunctional bio-applications of AgNPs; for example, as antibacterial, antifungal, antiviral, anti-inflammatory, anti-angiogenic, and anti-cancer agents, and the mechanism of the anti-cancer activity of AgNPs. In addition, we discuss therapeutic approaches and challenges for cancer therapy using AgNPs. Finally, we conclude by discussing the future perspective of AgNPs.

Ill-Min Chung (2016) Interest in “green nanotechnology” in nano-particle biosynthesis is growing among researchers. Nanotechnologies, due to their physicochemical and biological properties, have applications in diverse fields, including drug delivery, sensors, optoelectronics, and magnetic devices. This review focuses on the green synthesis of silver nanoparticles (AgNPs) using plant sources. Green synthesis of nanoparticles is an eco-friendly approach, which should be further explored for the potential of different plants to synthesize nanoparticles. The sizes of AgNPs are in the range of 1 to 100 nm. Characterization of synthesized nanoparticles is accomplished through UV spectroscopy, X-ray diffraction, Fourier transform infrared spectroscopy, transmission electron microscopy, and scanning electron microscopy. AgNPs have great potential to act as antimicrobial agents. The green synthesis of AgNPs can be efficiently applied for future engineering and medical concerns. Different types of cancers can be treated and/or controlled by phytonanotechnology. The present review provides a comprehensive survey of plant-mediated synthesis of AgNPs with specific focus on their applications, e.g., antimicrobial, antioxidant, and anticancer activities.

Haliza Katas (2018) Biosynthesized or biogenic metallic nanoparticles, particularly silver and gold nanoparticles (AgNPs and AuNPs, respectively), have been increasingly used because of their advantages, including high stability and loading capacity; moreover, these nanoparticles are synthesized using a green and cost-effective method. Previous studies have investigated reducing and/or stabilizing agents from various biological sources, including plants, microorganisms, and marine-derived products, using either a one-pot or a multistep process at different conditions. In addition, extensive studies have been performed to determine the biological or pharmacological effects of these nanoparticles, such as antimicrobial, antitumor, anti-inflammatory, and antioxidant effects. In the recent years, chitosan, a natural cationic polysaccharide, has been increasingly investigated as a reducing and/or stabilizing agent in the synthesis of biogenic metallic nanoparticles with potential applications in nano-medicine. Here, we have reviewed the mechanism of biosynthesis and potential applications of AgNPs and AuNPs and their chitosan-mediated nano-composites in nano-medicine.

### 2.1 Research Gap

The authors reported that nanoparticles are produced from nitrate as well as acetate salt precursor solution when propane–oxygen diffusion flame is used to decompose aqueous aerosol droplets. This investigation work focuses on the synthesis of a wide-band gap nickel (II) oxide nanoparticles by a simple and economical solution method using nickel sulphate as the raw material.

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## 2. OBJECTIVES

1. Synthesis of Nickel oxide nanoparticles using a low cast and simple solution method.
2. Synthesis of transition metal doped NiO nanoparticles. Studies on the effect of doping on the properties.
3. Comparative study on the photo-catalytic behavior under three different light sources.
4. Evaluation of Antimicrobial activity of the NiO nanoparticles on various pathogens.
5. Synthesis of Anticancer activity of nano-medicine

## 3. RESEARCH METHODOLOGY

**Research Design**

Biological nanoparticles will be applied in many biomedical contexts, including anticancer and antimicrobial applications because of the higher efficacy of biological nanoparticles compared with physiochemical nanoparticles for biomedical applications.

**Material Required**

Nanoparticles of transition metal oxides will be investigated by several researchers in the last few years; Oxide nanoparticles particularly that of transition metals represent a very important class of materials contributing a variety of functions; further, many transition metal oxides are biocompatible and the most stable materials due to the high oxygen content of earth's atmosphere. The nano sized silver particle is an interesting material due to its useful electronic and magnetic properties. The properties are enhanced if the metal oxide nanostructures are intentionally doped with foreign elements with respect to the attuned of the chemical nature, because doping is an efficient and simple process to modify the physical properties like optical and magnetic properties of nano-materials. Though the elements in the periodic table can be doped in the metal oxide nanoparticles, the transition metal elements like Cu, Al, Fe etc., have definite benefits when used as dopant.

**Solution Method**

Analytical grade (AR) chemicals were used for this synthetic work and hence they were used as received without further purification. Silver particle was used as the starting material for the preparation of Ag precursor material. It was prepared using 1g of nickel sulphate dissolved in 200ml of DD water; liquid ammonia (NH3, 18%) solution was added, drop wise, a pale green precipitate was formed. Further addition of ammonia solution, the precipitate was completely dissolved and a clear blue coloured solution was obtained. At the time of colour change the pH of the content was measured using a pH meter and the observed value was 10.2. The color change was due to the formation of the aqueous nickel-ammonia complex ion ([Ni (NH3)4] 2+).

**Analysis Tools and Techniques**

X-Ray Diffraction Studies: The primary characterization technique most commonly used in nanoparticles research is X-ray diffraction Studies (XRD).When X-rays interact with a crystalline substance diffraction patterns are formed known as XRD patterns. This XRD pattern of a pure substance is like a fingerprint of the substance and hence the features like crystal structure, crystallite size, lattice constants, particle size distribution and strain can be inferred. Thus the powder diffraction method is perfectly suited for characterization and identification of nanoparticles.

Scanning Electron Microscope (SEM): The SEM is a type of electron microscope which images a sample surface by scanning with a high-energy beam of electrons. The main application of SEM is to study the surface topography and morphology. The electrons interact with the atoms in a sample producing signals that has the information about the sample's morphology and composition.

Transmission Electron Microscopy (TEM): The direct tool for the determination of the size and shape of the nano-structured materials is Transmission Electron Microscopy (TEM). It also provides the structural information. In TEM analysis, the electrons are accelerated to 100KeV or higher projected on to a thin specimen through a condenser lens system.

Fourier Transform Infrared Spectroscopy (FT-IR): FTIR spectroscopy is an analytical tool provides information about the chemical bonding or molecular structure of organic and/or inorganic materials.

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The greatest advantage of FT-IR is used to identify unknown materials present in a sample. The principle of IR is the bonds and groups of bonds vibrate at characteristic frequencies.

UV-Visible Spectroscopy (UV-VIS): Ultraviolet-visible spectroscopy is also known as ultraviolet-visible spectro-photometry is one of the essential techniques for the analysis. It involves the photons in the UV-Visible region. In this region of the electromagnetic spectrum, molecules undergo electronic transitions.

## 4. EXPECTED OUTCOME

The use of nanoparticles in the field of medicine is proving to be a novel and promising technique in order to treat various infections. Among various NPs, silver NPs have proved to possess therapeutic alternative that can be exploited in diagnostic and treatment of certain bacterial infections and cancer. To date, various fungi have been reported to biosynthesize the silver nanoparticles. A few among them have been evaluated for the treatment of infection(s) caused by bacterial pathogens providing evidence for their potential role as a new generation antimicrobial agents against a broad spectrum of Gram-positive and Gram-negative bacteria including multidrug-resistant human pathogens. In addition, mono-dispersed silver NPs can be synthesized by controlling various parameters to avoid toxic effects on human cells. Furthermore, biosynthesized silver NPs have successfully exhibited the anticancer activities against different cancer cell lines through inhibiting cell progression (cell proliferation), ROS formation, blockage of DNA synthesis, and apoptosis. However, further research work is required to understand possible mechanism of action of antibacterial and anticancer activities of silver NPs on microbial cell. Various factors (physiochemical and biological) affecting bioavailability, biocompatibility, and cellular toxicity of silver NPs at molecular level should also be addressed in future research that will open new insight for their application alone or in combination with other bioactive agents to control and treat the microbial infections and cancer.

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