A COMPREHENSIVE REVIEW ON BIOGENIC SYNTHESIS, CHARACTERIZATION AND APPLICATION OF METAL NANOPARTICLES

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CANDIDATE'S DECLARATION

I, Arkopratim Chakraborty, 2K20/NST/02 of M.Tech. Nanoscience and Technology, hereby declare that the project Dissertation titled "***A Comprehensive Review On Biogenic Synthesis, Characterization And Application Of Metal Nanoparticles***" which is submitted by me to the Department of Applied Physics, Delhi Technological University, Delhi in partial fulfillment of the requirement for the award of the degree of Master of Technology, is original and not copied from any source without proper citation. This work has not previously formed the basis for the award of any Degree, Diploma Associateship, Fellowship or other similar title or recognition.

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ABSTRACT

Metallic nanoparticles have unique properties that depend on their composition and structure. Achieving mastery over the synthesis of bi/trimetallic nanoparticles has been one of the greatest accomplishments in recent years. This is a matter of interest because of the fact that, altering the properties of these nanoparticles can prove beneficial for wide arrays of technological use. The goal of this thesis has been to present a comprehensive review of the latest research and advancements made regarding bi and trimetallic nanoparticles. This begins with a brief introduction describing the need of the synthesis of nanoparticles from eco-friendly methodologies, followed by which the process of synthesis from plants, microorganisms and waste material is discussed taking reference from selected articles that best represent the idea. The properties of these alloy nanoparticles are also discussed in terms of their optical, catalysis and magnetism. Finally, concluding this review by discussing their applications in medical fields.

CONTENTS

LIST OF ABBREVIATIONS

1. **NPs** – Nanoparticles.
2. **UV-Vis** – Ultraviolet-Visible.
3. **SPR** – Surface Plasmon Resonance.
4. **TEM** – Transmission Electron Microscopy.
5. **XRD** – X-Ray Diffraction.
6. **FTIR** – Fourier Transform Infrared .
7. **LSPR** – Localized Surface Plasmon Resonance.
8. **MRI** – Magnetic Resonance Imaging.
9. **CT-Scan** – Computed Tomography Scan.
10. **MCAs** – Multimodal Contrast Agents.
11. **SERS** – Surface Enhanced Raman Scattering.
12. **EPR** – Enhanced Permeability and Retention.
13. **E.coli** – Escherichia coli.

1. INTRODUCTION

Metal nanoparticles, due to their unique catalytic, optical, and structural properties have attracted a lot of attention. These bimetallic/trimetallic nanoparticles can be used extensively in a wide spectrum of technological applications such as – nanoelectronic devices, sensors, catalysts, etc. All the various methods of synthesizing these nanoparticles have faced similar drawbacks of toxicity of reactants and generation of potentially hazardous by-products. Biogenic Synthesis is one of the major alternative routes available for the synthesis of nanomaterials in the present day, since it uses non-toxic reactants that are derived from biological sources ranging from unicellular organisms to complex plants. Usage of the biogenic process for synthesis purposes is advantageous because of its easy availability and requires a very rudimentary setup. These methods also have the least probability of generation of toxic by-products.

The aim of the thesis has been to highlight the advances made in recent years regarding the synthesis of bi/trimetallic nanoparticles, their properties and their application in medical fields. This thesis has been completed by noting recent developments by selecting articles that best represent the concept.

2. SYNTHESIS OF NANOPARTICLES

2.1 SYNTHESIS FROM PLANTS

Recently, plants are chosen as a viable choice for the synthesis of nanoparticles. This method uses different parts of the plants such as roots, stems, seeds, leaves, flowers and their extract for manufacturing the nanoparticles. The method is stable and eco-friendly, and the nanoparticles thus created have different biomedical and environmental applications. Primary constituents including proteins, organic acids, polysaccharides and other metabolites like polyphenols, flavonoids, alkaloids and others that are present in plants acts like reducing agents and stabilizing factors.[1,2] Nanoparticles such as Ag-Ni, Ag-Co, Pt-Cu, and Au-Ag have been produced from the leaf extract of African arrowroot, Alchornea-laxiflora (Lowveld bead-string), Azadirachta-indica, Cacumen-Platycladi, Palm and Mirabilis jalapa, whereas trimetallic nanoparticles such as Au-Ag-Sr and Fe-Ag-Pt were synthesized from the root extract of coriander and Platycodon.[3,4,5] Also, gold-silver alloy nanoparticles were produced from the fruit extract of Chinese wolfberry.[6,7]

The plant extract preparation begins with cleaning and sanitizing the plant of any dust or foreign materials present with distilled water. They may be dried and crushed to form a coarse powder [8,9], or the clean plants are boiled or may be subjected to some sort of irradiation. The widely used solvent for preparation of extract is water, but methanol can be more beneficial.[10]

The synthesis of alloy nanoparticles involves the simultaneous reduction of all the metal ions by the plant extract. Equimolar solutions of all the metal salts are added into a certain amount of extract and is subjected to different excitation like stirring or irradiation. For instance, Au-Ag NPs were synthesized by adding R.hypocrateriformis extract (20 ml) to an equimolar mixture of $HAuCl_4$ and $AgNO_3$. Again, NPs were produced by the aqueous mixture of $AgNO_3$ and $HAuCl_4$ and aqueous extract of mahogany, which was continuously stirred.[10]

The formation of NPs can be confirmed by the colour changes in the solution over time. Another method is by studying absorbance and Surface Plasmon Resonance of the solution at different intervals [8,10].

2.2 SYNTHESIS FROM MICROORGANISMS

For reasons like swift development, affordable costs of culturing and easy control and manipulation of the growth environment, and few species even having the ability to to suppress the toxicity of heavy metals, bacteria have clearly been the target choice for producing nanoparticles. The synthesis process begins with cleaning the collected biomass, drying and storing it under optimal conditions for future use. An appropriate amount of biomass is taken and a solution is made with distilled water, to which metal salts are added.

Ag-Au NPs were produced by mixing wet biomass of Fusarium oxysporum to some quantity of $HAuCl_4$ and $AgNO_3$ to make the overall concentration of Ag^+ and Au^{3+} ions equal to 1mm in an aqueous solution. Again, Au-Ag NPs were prepared by taking washed biomass of F. semitectum with distilled water and aqueous $HAuCl_4$ and $AgNO_3$[11].

The synthesis of nanoparticles is done with both extra-cellular as well as intracellular enzymes with the use of simply-cultured and fast-breeding eukaryotes like yeast, where the size of the nanoparticles depends on the conditions for incubation and solutions of the metallic ion[12,13,14].

The method for the formation of nanoparticles varies with respect to the microorganisms. But, they usually follow these steps; first the metal ions get trapped over the surface or inside the cell. Then the trapped metallic ion undergoes a reduction in the presence of enzymes. Microbes impact the formation of minerals in two different ways. Firstly, the microbes are able to modify the composition of the solution such that it becomes supersaturated than it previously was with respect to the specific phase. And secondly, it can impact the formation of minerals by producing organic polymers, which in turn affects the nucleation process by promoting the stabilization of the mineral seeds [15].

The metal ions are bio- reduced by the microbial extract and the formation of nanoparticles is initially seen from the changes in the hue of the solution. The UV-Vis spectrum is also studied at different intervals to see the absorbance and hence the formation of the NPs[16].

2.3 SYNTHESIS FROM WASTE

Nanoparticles are mostly been synthesized from both agricultural and industrial waste material. Waste is generated after harvesting rice husks, and fruits form about 80% of the biomass present in the fields. Wild weeds, sugarcane bagasse, timber dust ,etc. These are generally burned and fall under the category of industrial wastes that are used as biological sources for nanoparticle synthesis. The advantages of using waste over other methods include a decrease in the use of harmful chemicals, low cost and energy needed and reusing of the waste materials. Both monometallic and alloy nanoparticles were prepared form sugarcane bagasse, bamboo leaves, peels of citrus fruits, and coconut and eggshells.[17-21]

The process of synthesis depends on various factors like pH, temperature, etc, and different wastes have a unique route of synthesis. These tend to have complicated parameters of synthesis depending upon the precursor waste material.[22] Ge and C composite nanoparticles were synthesized by mixing precursors GeO_2 (99.998% Sigma Aldrich) with coffee waste ($C_{25}H_{28}N_6O_7$) and milk powder.[23] The precursors were crushed and mixed with ethanol and finally dried in the atmospheric gas mixture. Also, PCBs powder was leached using ammonium chloride and ammonium solution. This was leftover time and CuO powder was formed. They were centrifuged and finally washed with water and ethanol, this way copper nanoparticles were formed from printed circuit boards.[24].

3. CHARACTERIZATION AND PROPERTIES OF ALLOY NANOPARTICLES

3.1 CHARACTERIZATION

This section makes an attempt to discuss the characterization process of the nanoparticles that are synthesized by different methodologies. Although the process of synthesis may vary, the method of characterization follows a similar path in all cases. Firstly, we see a change in the colour of the aqueous solution(e.g. light brown to dark brown). These colours are seen due to the excitation of the surface plasmon vibrations present in the metallic nanoparticles.[9]. The light brown colour suggests the incomplete reduction due to the less concentration in the plant extract in the solution, whereas the dark brown colour formation is suggestive of the completion of the reduction reaction at high plant extract concentrations in the solution.[8] The formation of nanoparticles can also be monitored by studying the SPR peak of the UV-Vis Spectrometer at regular intervals during the entire course of the reaction. The optical absorption spectrum is reported to show only one band rather than two distinct bands, suggesting the formation of metallic alloy nanoparticles and not bimetallic core/shell nanoparticles.[8,9]. Also a gradual shift in the red direction in the surface plasmon bands is also seen which infer the formation of larger nanoparticles as the reaction progresses.[8,11]. TEM analysis suggests that the particles are predominantly spherical in shape and range 10-50nm in diameter. TEM images also show the nanoparticles to have uniform contrast and hence suggesting the possibility of homogenous electron density. This infers the formation of bimetallic alloy nanoparticles, which is in coherence with the conclusion drawn from the UV-Vis study.[8-11] .XRD studies reveals the bimetallic/alloy nanoparticles have similar structures as their constituent metals.[10].Most of them have a fcc structure. Since the biomolecules and functional groups responsible for capping and stabilizing and bio-reduction of metal ions varies between plants, microbes and waste materials , the FTIR spectra helps us to identify them . Fungi uses proteins for the process and hence FTIR shows peaks at 1643, 1543, 1405, and 1075 cm−1, at regions of amide I and amide II groups which are characterized by the presence of proteins and enzymes.[11].In the case of plants, there is an abundance of polyphenols and polyols that are present in the extract which are entirely responsible for bio-reduction of the metallic ions and hence shows peaks in that region suggesting the presence of C-H, C-O and OH bonds.[8,11].

3.2 PROPERTIES OF ALLOY NANOPARTICLES

In the case of alloy nanoparticles, two or more metals combine to show unique properties which are the combination of all the constituent metals, which makes these nanoparticles very useful for industrial and technological use. Another advantage of alloy nanoparticles over their monometallic counterparts is the extra degree of freedom present in them. They also have a grater surface area thereby increasing the absorption power.[25]

3.2.1 OPTICAL PROPERTIES

When loosely bound electrons present inside the metallic NPs are exposed to an external electromagnetic radiation, they start to oscillate in a collective fashion. If the frequency of oscillation matches with the source, it gives rise to localized surface plasmon resonance(LSPR).[26]

Studies have shown that the peak position of LSPR depends on the geometry and composition of the nanoparticles. The constituent metals in the nanoparticles have a very strong effect on the optical properties of the nanoparticles. When plasmonic metals which are resonant in the visible region are combined, LSPR is seen to increase, which quenches when metals from the UV region are added.[27] A redshift was seen in the LSPR peak when alloying Cu into Au nanoparticles. It has also been observed that when the geometry of the nanoparticles becomes more complex than spherical, the LSPR shows more peaks, and the nanoparticles became more sensitive.[26, 28]

3.2.2 CATALYTIC PROPERTIES

Alloy nanoparticles have been reported to be more reactive than the monometallic nanoparticles made with the same metal. These depend on the structure and composition of the said nanoparticles.[27] The addition of Au-Ag alloy NPs increases the reaction time for the reduction of Methyl Blue with $NaBH_4$, and almost 45% reduction was observed as compared to little or no reduction that occurred before adding the nanoparticles.[29] The oxygen reduction reaction was reported to be ten times faster with Pt-Ni alloy nanoparticles as compared to when using only Pt nanoparticles.[30]

3.2.3 MAGNETIC PROPERTIES

After reducing the NPs to a critical size, they start showing single magnetic domain as compared to bulk magnets that have multiple magnetic domains. This limit of size is known as the single-domain limit which is dependent on the geometry and composition of the nanoparticles, and identifies the maximum size possible so that the magnetization only induces unidirectional spin alignment.[28] It has been reported that mixing metals like Fe and Ni, which have big local magnetic moments with metals with strong spin-orbit coupling like Pd and Pt. A bimetallic nanopaticle has a higher magnetic moment and anisotropy.[28,31]

4. APPLICATION OF ALLOY NANOPARTICLES

4.1 IMAGING

A better understanding of the human body has been a long wish of researchers and medical professionals. This comes in hand with the requirement of high-quality imaging tools form macro to the cellular level. For a long time, various imaging techniques like microscopy, MRI and CT-Scan have been used for imaging. As an improvement, the optical properties of nanoparticles are put into use for bioimaging.[32,33,34] It has been reported that multimodal contrast agents or MCAs which are based on Au-Fe alloys are good for MRI and CT-Scan, as well as SERS imaging simply by administering a biocompatible contrast drug that reportedly does not have any cytotoxicity, and accumulates in tumors by EPR effect as compared to regular contrast agents which comprise of metals like Fe and Au. Such kind of MCAs requires a simple method of synthesis without the involvement of any toxic chemicals.(magneto-plasmonic). Another report suggests that nanoparticles that are used as probes for bioimaging have properties such as better dispersion in solutions or intracellular environments, higher degree of stability , better analytical signals and decline in cytotoxicity.[35,36,37] Alloy nanoparticles such as Fe-Ni, which demonstrate superior magnetic properties are used in MRI for better brightness in the results.[38]

4.2 PHOTOCATALYTIC APPLICATIONS

Metals like Pd, Rh and Pt when hybridized with Au and Cu which are plasmonic in the visible region gives us a unique opportunity to create novel photocatalysts. This has allowed to successfully derive bimetallic heterostructures that serve specific functions.

The advantages of hybridization are two folds, firstly, it allows the unique combination of light absorbing properties of Au or Ag and the catalytic activity of Pd or Pt. Secondly, hybridization of cheaper metals like Copper with Gold has shown to outperform both Au and Cu in reactions like oxidation of benzyl alcohol.[39,40] Furthermore, trimetallic nanoparticles have been seen to possess even higher catalytic properties as compared to their mono and bimetallic counterparts. Ag-Au-Pt and Au-Pd-Pt nanoparticles when used instead of Pd-Pt or Au-Pt for the oxidation of methanol, the trimetallic nanoparticles showed much greater catalytic activity.[41]

4.3 DRUG DELIVERY

This refers to transferring of drugs into the target site accurately, without having any effect on the healthy cells in proximity to the target cells. Systems that are used lack specificity and sometimes have a certain degree of toxicity. Newly developed drug carriers, which are also known as Smart carriers demonstrate an impressive arrays of characteristics, such as specific targeting, controlled release of drug and lower toxicity.[42,43,44] These drug carriers can be customized as per the requirement, by altering the composition and geometry of the nanoparticles.[44] The effects of Fe-Pt core/shell nanoparticles were researched for drug delivery in the thoracic and gastrointestinal regions by filling the hollow part with anti-cancer drug doxorubicin and coating it with a lipid membrane to avoid any kind of leakage. These capsules were guided under the effect of a magnetic field. The final result showed the destruction of almost 70% of the cancer cells. [45]. Quantum dots having fluorescence properties are surface modified with the application of tumor-recognized drugs and molecules to be used for drug delivery.[46] Building on nanoparticles as a drug delivery system, they have shown great potential for novel vaccine carriers since the activation effect of metal nanoparticles are higher against foreign bodies.[47]

4.4 CANCER THERAPY

Inorganic carriers also serve as therapeutic agents for the treatment of cancer through photothermal therapy, hyperthermia therapy, etc. There has been a study of photothermal therapy by targeting cancerous cells of the mammary glands wherein Fe-Pt nanoparticles were funtionalised with folic acid. Upon irradiation with lasers the photothermal effects from the nanoparticles destroyed the plasma membrane of the cancerous cells.[48] Gold nanoparticles also is a great alternative for cancer treatment, since it is a radiosensitizer and is able to convert light into heat.[46]

The therapeutic process of hyperthermia is entirely based on using heat as an agent to destroy malignant or cancerous cells. Studies have shown that tungsten oxide-coated alloy nanoparticles are used for hyperthermia treatment. Magnetic nanoparticles are heated under a magnetic field and channeling them to kill cancer cells. In the study done by Seeman et al.[49], Fe-Pt nanoparticles were annealed at 700°C, to get an amorphous tungsten-oxide layer on the core/shell nanoparticles. Annealing showed to increase the magnetic moment of the nanoparticles, which increased the heating effect which is the most important factor for hyperthermia.[49]

4.5 ANTIBACTERIAL ACTIVITY

Antibiotics, from their genesis were used to treat infections and diseases caused by bacteria and other organisms. With time, along with antibiotics so did the microbes changed and gave rise to newer drug resistant bacteria. Fortunately, with improving technology novel methods to fight bacteria were created. It was seen that metals like silver, copper, gold depending upon their morphology showed antibacterial properties. A study was done with an aqueous solution of metallic nanoparticles with E.coli, which is a gram-negative bacteria. The results showed a reduction in the number of colonies when nanoparticles of different compositions were applied. This study also showed that the alloy of Au-Ag exhibited higher antibacterial activity with respect to pure metals. The positive metal particles get attached to the membrane of the negatively charged bacteria and disintegrate them. The abundance and presence of sulfur and phosphorus inside the bacteria and the affinity of sliver towards them is the main reason for the antibacterial activity of such metals.[50]. This was also confirmed by another research with Cu-Ag nanoparticles against E.coli, which also came to the conclusion that the silver or copper ions that were released by the nanoparticle, attach themselves to the cell wall of the negatively charged bacteria and rupture it, finally resulting in denaturation of the protein and eventually cell death.[51] The overall process of antibacterial activities solely lies in the degradation of the bacterial cell wall, which was supported by the studies mentioned above.[52]. Although, silver has shown a higher degree of antibacterial activity, other metallic alloys such as Cu-Pt also demonstrated bacterial resistance by generating hydroxyl radicals.[53]

CONCLUSION AND FUTURE PRESPECTIVE

The discussion above gives us an abundance of evidence of the superiority of alloy nanoparticles over mono-metallic nanoparticles due to their unique properties that arise from the composition of two or more metals. These properties have shown great potential for industrial applications. The use of metallic alloy nanoparticles in medical fields has also been discussed in this review, and their use to create novel methods for cancer treatment and drug delivery has been acknowledged. These nanoparticles can be further modified by tuning their morphology and composition to create better antibiotics and MCAs, which remains a prospect of future research.

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