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A REVIEW ON GREEN SYNTHESIS OF GOLD NANOPARTICLES

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ABSTRACT

Nanoparticle technology plays a key role in providing opportunities and possibilities for the development of a new generation of sensing tools. Nanoparticles can be integrated with ligands, imaginable and therapeutic agents and other functionalities for site specific drug delivery and cellular uptake. A simple, green method was developed for the synthesis of gold nanoparticles (GNPs) by reducing chloroauric acid ($HAuCl_4$) with different plant extracts and microbes (such as fungus, bacteria). Gold nanoparticles can be functionalized with protein, peptides and nucleic acidwhich have a great application in drug, gene and protein delivery. The review provides a brief description of green synthesis of gold nanoparticles and its applications.

KEYWORDS: AFM, FTIR, Gold nanoparticles, HPLC, SEM, TEM, , , UV-visible spectroscopy, , plant extract.

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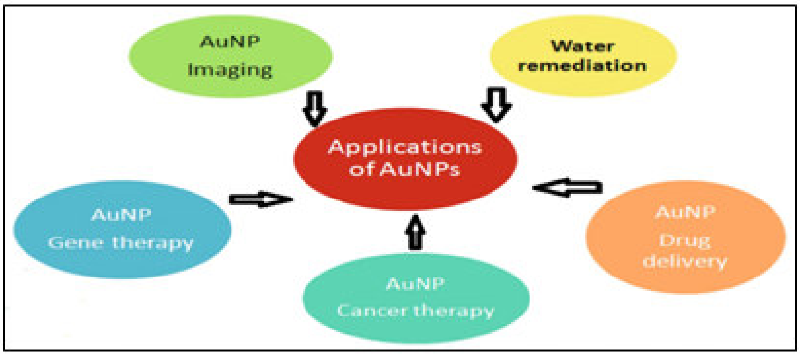
INTRODUCTION

Nanotechnology emerges from the physical, chemical, biological and engineering sciences where new techniques are being developed to probe and maneuver single atom and molecules for multiple applications in different field of the scientific world. The science and engineering technology of nanosystems is one of the most exigent and fastest growing sectors of nanotechnology[1]. Nanotechnology is a manipulation of matter on an atomic, molecular and supramolecular scale. The idea and concept behind nanoscience and nanotechnology started with a talk entitled "There's Plenty of Room at the Bottom"_by physicist Richard Feynman at an American Physical Society meeting at the California Institute of Technology (Caltech) on December 29, 1959, long before the term nanotechnology was used. In his talk, Feynman described a process in which scientists would be able to manipulate and control individual atoms and molecules. Over a decade later, in his explorations of ultra precision machining, Professor Norio Taniguchi coined the term nanotechnology. Nanotechnology is now widely used throughout the pharmaceutical industry, medicine, electronics, robotics, deliver drugs,tissue engineering and increase stability against degradation by enzymes. The use of nanoparticle (NP) materials offers many advantages due to their unique size and physical properties and one of the nanoparticles ,which can be manipulated by an external magnetic field to lead it to the target tissue.

Figure 1
Application of nanoparticles

Metallic nanoparticles have fascinated scientist for over a century and are now heavily utilized in biomedical sciences and engineering. Today these materials can be synthesized and modified with various chemical functional groups which allow them to be conjugated with antibodies, ligands, and drugs of interest and thus opening a wide range of potential applications in biotechnology, magnetic separation, preconcentration of target analytes, targeted drug delivery, vehicles for gene, drug delivery and more importantly diagnostic imaging. Over the years, nanoparticles such as magnetic nanoparticles (iron oxide), gold and silver nanoparticles, nanoshells, and nanocages have been continuously used and modified to enable their use as a diagnostic and therapeutic agent. Thus, in this particular review article we have introduced iron oxide, gold and silver nanoparticles along with newer nanoshells and nanocages. The development of green processes used for the synthesis of NP is evolving into an important branch of nanotechnology [2, 3]. The reason for selecting plants for Biosynthesis is because they contain reducing agents like Citric acid, Ascorbic acids, flavonoids, reductases and dehydrogenases and extracellular electron shuttlers that may play an important role in biosynthesis of metal nano particles[4]. Green synthesis of nanoparticles is an eco-friendly approach which might pave the way for researchers across the globe to explore the potential of different herbs to synthesize nanoparticles. Biological approaches using microorganisms and plants or plant extracts for metal nanoparticle synthesis have been suggested as valuable alternatives to chemical methods. A number of living organisms are already well-known to elaborate nanostructures composites such as cyanobacteria, bacteria, fungi, actinomycetes, biomolecules and various plant materials such as *Cinnamomum camphora*, *Medicago sativa*, *Pelargonium graveolens*, *Avena sativa*, *Azardirachta indica*, *Tamarindus india*, *Emblica offcinalis*, *Aloe vera*, *Coriandrum sativum*, *Carica papaya*, *Parthenium hysterophorus*, *Tritium vulgare*, *Acanthella elongata*, *Sesuvivm potulacastrum* [5-20]. The use of plants for the preparation of nanoparticles could be more advantageous because it does not require elaborate processes such as intracellular synthesis and multiple purification steps or the maintenance of microbial cell cultures. Many reports have been published in the literature on the biogenesis of gold nanoparticles using several plant extracts, particularly Neem leaf broth (*Azadirachta indica*), alfa alfa (*Medicago sativa*), *Eucalyptus camaldulensis*, *Pelargonium roseum* and green tea. Biological methods for nanoparticles synthesis using microorganism, enzyme and plant or plant extract have been suggested as possible ecofriendly alternatives to chemical and physical methods. The mechanism of plant-mediated synthesis of nanoparticles is a very promising area of research. The biosynthetic method employing plant extracts has received attention as being simple, eco-friendly and economically viable compared to the microbial systems like bacteria and fungi because of their pathogenecity and also the chemical and physical methods used for synthesis of metal nanoparticles. Synthesis of nanoparticles using biological entities has great interest due to their unusual optical, chemical, photoelectro-chemical and electronic properties [21-24] . The synthesis & assembly of nanoparticles would benefit from the development of clean, nontoxic and environmentally acceptable green chemistry procedure, probably involving organisms ranging from bacteria to fungi and even plants [25, 26]. Hence, both unicellular and multicellular organisms are known to produce inorganic materials either intra or extracellular [27]. Gold nanoparticles of different sizes (1 to 8 nm) and shapes including spherical, octahedral, sub-octahedral, decahedral multiple twinned, icosahedral multiple twinned, irregular shape, nanotriangles and nanoprisms, tetra hedral, hexagonal platelets and nanorods were synthesized by earlier researchers [28]. Gold nanoparticles are used for the development of biosensors, DNA labeling, vapour sensing [29-31]. Gold nanoparticles have tremendously high molar absorptivity in the visible region. Biological methods for nanoparticle synthesis using microorganisms, enzymes, and plants or plant extracts have been suggested as possible ecofriendly alternatives to chemical and physical methods. Gold nanoparticles (GNPs) are an interesting object of study due to their particular special properties: high chemical stability, capacity for self-assembly, specific optical properties, resistance to oxidation, biocompatibility, as well as for their applications in different areas such as catalysis, biology, optics, electronics and medicine. GNPs prepared by green synthesis are biocompatible and non-toxic. Major applications of GNPs were in diagnostics and therapeutics field.Gold nanoparticles are widely used in biomedicalscience including tissue or tumor imaging, drug delivery , photo thermal therapy and immunochromatographic identification of pathogens in clinical specimens due to the surface plasmon resonance (SPR)[32].

Applications of gold nanoparticles



1. Electronics

Gold nanoparticles are designed for use as conductors from printable inks to electronic chips. As the world of electronics becomes smaller, nanoparticles are important components in the chip design. Nanoscale gold nanoparticles are being used to connect resistors, conductors, and other elements of an electronic chip[33].

2. Photodynamic Therapy

Near-IR absorbing gold nanoparticles (including gold nanoshells and nanorods) produce heat when excited by light at wavelengths from 700 to 800 nm. This enables these nanoparticles to eradicate targeted tumors.When light is applied to a tumor containing gold nanoparticles, the particles rapidly heat up, killing tumor cells in a treatment also known as hyperthermia therapy[34,35].

3. Therapeutic Agent Delivery

Therapeutic agents can also be coated onto the surface of gold nanoparticles. The large surface area-to-volume ratio of gold nanoparticles enables their surface to be coated with hundreds of molecules (including therapeutics, targeting agents, and anti-fouling polymers[36].

4. Sensors

Gold nanoparticles are used in a variety of sensors. For example, a colorimetric sensor based on gold nanoparticles can identify if foods are suitable for consumption. Other methods, such as surface enhanced Raman spectroscopy, exploit gold nanoparticles as substrates to enable the measurement of the vibrational energies of chemical bonds. This strategy could also be used for the detection of proteins, pollutants, and other molecules label-free [37].

5. Probes

Gold nanoparticles also scatter light and can produce an array of interesting colors under dark-field microscopy. The scattered colors of gold nanoparticles are currently used for biological imaging applications. Also, gold nanoparticles are relatively dense, making them useful as probes for transmission electron microscopy.

6. Diagnostics

Gold nanoparticles are also used to detect biomarkers in the diagnosis of heart diseases, cancers, and infectious agents. They are also common in lateral flow immunoassays, a common household example being the home pregnancy test [38].

7. Catalysis

Gold nanoparticles are used as catalysts in a number of chemical reactions. The surface of a gold nanoparticle can be used for selective oxidation or in certain cases the surface can reduce a reaction (nitrogen oxides). Gold nanoparticles are being developed for fuel cell applications. These technologies would be useful in the automotive and display industry[39].

Characteristics of Gold Nanoparticles

- Gold nanoparticles are chemically inert.
- These have greater biological compatibility.
- Optical properties like plasmon resonance are fluorescence exhibited by gold nanoparticles.
- These exhibit versatility because of their ready functionalizationthrough thiol linkages.
- Gold nanoparticles provide microscopic probes for the study of the cancer cell.
- Gold nanoparticles accumulate in the cancerous cell and show the cytotoxic effect i.e. apoptosis or necrosis of the specific cell and cell specific receptor.
- These have high stability due to the gold-sulphur bonds.
- Their photo physical properties can be exploited for drug release at remote place

Types of Gold Nanoparticles

- Gold nanorods
- Gold nanoshells
- Gold nanocages
- Gold nanosphere
- SERS nanoparticles

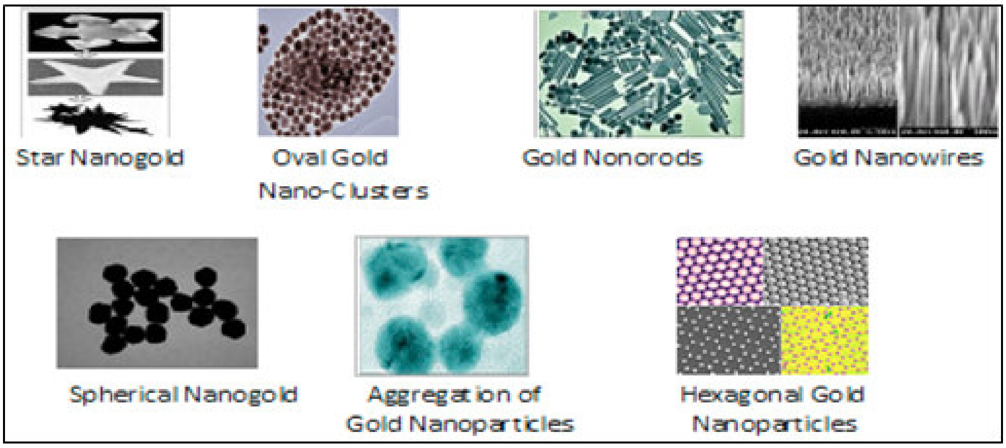


Figure 3
Types of Nanoparticle

Gold Nanorods

These are synthesized by template method. These are prepared by electrochemical deposition of gold within the pores of nanoporous polycarbonate template membranes. Gold nanorods diameter is according to the diameter of pore of the template membrane .

Gold Nanoshells

Surface plasmon resonance peaks (ranging from visible to near I.R. region) is used for thedesigning and fabrication of gold nanoshells. The core of gold nanoshells is made up of silica and outer surface is made up of gold. Gold controls the thickness of the shell.

Gold Nanocage

Through galvanic replacement reaction between truncated silver nanocubes and aqueous HAuCl gold nanocage is synthesized.

SERS Nanoparticles

SERS is an optical technique like fluorescence and chemiluminescence having better sensitivity, high levels of multiplexing, robustness and greater performance in blood and biological.

Sold Nanospheres

These are synthesized by reduction of an aqueous HAuCl by 4 using citrate as reducing agent. Through citrates / gold ratio the size of nanospheres can be controlled. By two-phase ratio, the size of nanospheres can be affected by thiol / gold molar ratios.

Optical Properties of Gold Nanoparticles

The field of Au NPs research has received tremendous attention due to their unique optical properties. The unique optical properties of Au NPs arise from their size confinement effect . The size confinement effect on the Au NPs provides new electronic and optical properties. A distinct feature of Au NPs is the strong vibrant color of their colloidal solution that is caused by the surface plasmon resonance (SPR) absorption . For the last two decades, many research groups have studied the optical characteristic features of the Au NPs with various sizes. It is worth noting that Au NPs were used as a coloring agent for staining church's glass dating back to the seventeenth century. The stained glasses with Au NPs are ruby red in color. Colloidal Au NPs sample is ruby red in color and Mie explained this phenomenon theoretically by solving Maxwell's equation for the absorption and scattering of electromagnetic radiation by spherical particles. Clear extracts of *Momordica charantia* was used for the biosynthesis of gold nano particles (GNPs). A stock solution of 50,000 ppm aurochlorate was prepared and diluted as per the pre-requisite of the experiment. The required amount of aurochlorate salt was added in a boiling solution of reaction vessel containing plant extract. In recent years, Mukherjee et al. reported a novel biological method for the synthesis of gold nanoparticles using the fungus Verticillium. Pineapple extract is used to obtain phytochemically-derived reducing agents for the production and stabilization of gold nanoparticles. The nanoparticles were examined for their consistency in Surface Plasmon Resonance (SPR) properties and reduction rate by varying the concentration of the pineapple extract. The same plasmon resonance band was observed at 600 nm at various concentrations indicating uniformity in the formation of gold nanoparticles. In a typical experiment, dark conditions and a preincubation at 90°C were applied separately to a 0.002M AuCl4 aqueous solution and the pineapple extract to achieve temperature equilibrium and the final total reaction mixture volume was 20 mL. Biosynthesis of gold nanoparticles (Pineapple-AuNPs) was begun by adding pineapple extract at 50% (v/v) with a final concentration of 0.002M AuCl4. The formation of nanoparticles was monitored by UV-Vis spectroscopy. The leaves of M. piperita were washed thoroughly thrice with distilled water and were shade dried for 5 days. The fine powderwas obtained from the dried leaves by using kitchen blender. The leaf powder was sterilized at 121 ◦C for 15 min. 20 g of powder was taken and mixed with 200 ml of Milli Q water and kept in boiling water bath at 60 ◦C for 10 min. The extracts were filtered with Whatman filter paper No 1. The filtered extract was stored in refrigerator at 4 ◦C for further studies.The Angelica extract was prepared from fragments of roots and the Hamamelis solutionfrom bark fragments, both by maceration in 60% v=v aqueous ethanol solution, for 7 days, followed by filtration. Healthy and fresh leaves of *A. spinosus* were collected from a local farm,The collected leaves were washed with double distilled water and shadow dried before being grinded to fine powder and sieved to remove coarse particles. One gram leaf powder was mixed with 100 ml of ethanol and the mixture was left in a shaking incubator operating at 200 rpm, 25°C for 24 h. The extract was then filtered and the filtrate was used for AuNPs synthesis. Various concen-trations (1% - 5%, v/v) of the ethanolic leaf extract of *A. spinosus* were mixed with aqueous solution of HAuCl4 (1 mM) and the reaction volume was made upto 2 ml with distilled water. The mixture solution was left on constant magnetic stirring at room temperature (25°C) and observed for change in colour.The plant *A. indica* was used. Leaves were dried at room temperature. The plant leaf broth solution was prepared by taking 5g of thoroughly washed and finely cut leaves in a 300mL Erlenmeyer flask with 100mL of sterile distilled water and then boiling the mixture for 5 min. They were stored at 4oC and used within a week.For the synthesis of Au- NPs (Goldnanoparticles), two boiling tubes were taken, one containing 10ml of 1*m*M Hydrogen tetra chloro aurate (Himedia, Mumbai) solution as controland the second flask containing 9ml of 1mM Hydrogen tetra chloro aurate solution and 1ml of plant leaf extracts as test solution were incubated at room temperature for 1-2 hours. The gold nanoparticle solution thus obtained was purified by repeated centrifugation at 15,000 rpm for 20 min. Supernatant is discarded and the pellet is dissolved in deionised water. The gold nanoparticules were confirmed by colour changes and qualitatively characterized by UVvisible spectrophotometer on a Perkin Elmer.Clear extracts of *Momordica charantia* was used for the biosynthesis of gold nano particles (GNPs). A stock solution of 50,000 ppm aurochlorate was prepared and diluted as per the pre-requisite of the experiment. The required amount of aurochlorate salt was added in a boiling solution of reaction vessel containing plant extract. In order to optimise the nanoparticle formation, the impacts of pH (4,6,8,10 & inherent) on synthesis of GNPs were studied at low temperature (300C) and high temperature (1000C). The parameters obtained from the above two experiments were kept constant to comprehend the impact of temperature and salt concentration on the optical as well as morphological features of GNPs.

Characterization

UV-visible spectroscopy analysis

The colour change in reaction mixture (metal ion solution + fruit extract) was recorded through visual observation. The bioreduction of gold ions in aqueous solution was monitored by periodic sampling of aliquots (1 ml) and subsequently measuring UV-vis spectra of the solution. UV-vis spectra of these aliquots were monitored as a function of time of reaction on Elico UV-vis spectrophotometer (Model SL 164 double beam) operated at a resolution of 1 nm.

SEM analysis

A scanning electron microscopy (SEM) image was obtained using JEOL JSM 7500F Field Emission Scanning Electron Microscope with a back scattered electrons (BSE) detector (marked as COMPO). K575X Turbo Sputter Coater was used for coating the part of the sample with chromium (deposited film thickness – 20 nm). The microstructure of samples was supported by chemical analysis carried out using energy dispersive X-ray spectroscope (EDX) at 20.0 kV and 15.0 mA.

FT-IR analysis

The FT-IR investigations were carried out with a Scimitar Series FTS 2000 Digilab spectrophotometer in the range of middle infrared of 4000-400 cm–1. 0.0007 g sample was pressed with 0.2000g of KBr for IR spectroscopy Uvasol® purchased from Merck, Germany. The number of scans 16 and the resolution of 4 cm-1 characterized these measurements. The plant extracts were vacuum dried, and the GNPs were separated by centrifugation and then dried out.

Atomic force microscopy(AFM)

Atomic force microscopy (AFM) samples were prepared from the gold dispersions by vertical adsorption for 2 min on glass plates, and dried at room temperature. All samples were investigated by AFM in tapping mode, on JEOL 4210equipment. Standard cantilevers, non-contact conical shaped tips of silicon nitride coated with aluminum, were used. All AFM experiments were carried out under ambient laboratory conditions (about 20_C).

High-Resolution Transmission Electron Microscopy(HRTEM)

TEMmeasurements were conducted with a Philips CM200FEG,working at an accelerating voltage of 200 kV. The point-to-point resolution was 0.24 nm. Images were recorded by aGATANslowscan CCD camera. Samples were prepared as follows. A certain amount of the gold particles was dispersed in methanol and sonicated for an hour. Then the dispesions were highly diluted with methanol, and the samples were subsequently sonicated for an hour. The well-dispersed particles were placed on a copper grid by drop-casting.

Representing plant reported synthesis of gold nanoparticles(fill the table using references)

SL.NO	**Plant extract**	**Nano particles**	**Size**	**Reference**
1	***Cinnamoum comphora***	**Ag&Au**	**5-8nm**	**5**
2	***Medicago sativa***	**Ag**	**2-3nm**	**6**
3	***Avena sativa***	**Au**	**5-2nm**	**10**
4	***Azadirachta indica***	**Ag&Au**	**50-100nm**	**11**
5	***Tomarindus indica***	**Au**	**20-40-nm**	**12**
6	***Emblica offcinalis***	**Ag&Au**	**15-20nm**	**13**
7	***Aloe vera***	**Ag&Au**	**15.2-4.2nm**	**14**
8	***Coriandrum sativum***	**Au**	**6.7-5.91**	**15**
9	***Carica papaya***	**Ag**	**60-80nm**	**16**
10	***Parthenium hysterophorus***	**Ag**	**52-80nm**	**17**
11	***Avena sativa &Tritium vulgare***	**Au**	**<20nm**	**18**
12	***Acanthella elongate***	**Au**	**7-20nm**	**19**
13	***Sesuvium portulacastrum***	**Ag**	**5-20nm**	**20**

Future perspectives

Biologically synthesized nanoparticles are an important aspect of nanotechnology. Plants and their product employed reliable with one step process towards production of nanoparticles at the same time harvesting of endangered species may pose a risk towards plant kingdom which forms a major challenge. Hence in this regard bio-template synthesis can form an immense impact in coming decades where in biomolecules present in the plant responsible for mediating the nanoparticles production can be identified and employed in rapid single step protocol which can overcome the above said disadvantageous and can give a new facelift towards green principle nanoparticles production. Today, nanotechnology and nanoscience approaches to particle design and formulation are beginning to expand the market for many drugs and are forming the basis for a highly profitable niche within the industry, but some predicted benefits are hyped. This article will highlight rational approaches in design and surface engineering of nanoscale vehicles and entities for site-specific drug delivery and medical imaging after parenteral administration. Gold nanocages represent a novel class of biocompatible vectors with potential applications in drug delivery, tumor/tissue imaging and photothermal therapy. They are prepared through the galvanic-replacement reaction between Ag nanostructures and $HAuCl_4$. By controlling the amount of $HAuCl_4$ added, we can tune the surface-plasmon resonance peaks of the Au nanocages into the near-infrared, where the attenuation of light by blood and soft tissue is relatively low. Here, we highlight recent advances in the synthesis and utilization of Au nanocages for cancer detection and treatment. We have tailored the optical properties of Au nanocages for use as contrast agents in optical coherence tomography and as transducers for the selective photothermal ablation of cancer cells. Our results show improved optical coherence tomography image contrast when Au nanocages are added to tissue phantoms as well as the selective photothermal destruction of breast cancer cells *in vitro* when immunotargeted Au nanocages are used.

CONCLUSION

Nanobiotechnology is an emergingfield that has made its contribution to all spheres of human life**.** The biological synthesis of nanoparticles has paved for better methodologies and approach in the mechanical field. Gold nanoparticles have been widely used as a novel therapeutic agent extending its use as antibacterial, antifungal, anti-viral and anti inflammatory agent. So far the chemically synthesized nanoparticles have been used for these approaches, but the recent reports suggest that biologically synthesized nanoparticles exercise numerous advantages over the chemically synthesized ones. The nanoparticles synthesized from microbes are exceptionally stable and the stability is likely to be due to capping with proteins secreted by the microbe. Using metal-accumulating microorganisms as a tool for the production of nanoparticles, and their assembly for the construction of new advanced materials, is a completely new technological approach. Biological synthesis of nanoparticles has upsurge in the field of nano-biotechnology to create novel materials that are ecofriendly, cost effective, stable nanoparticles with a great importance for wider applications in the areas of electronics, medicine and agriculture. Biosynthetic route of nanoparticles synthesis will emerge as safer and better alternative to conventional methods. With the huge plant diversity much more plant species are in way to be exploited and reported in the future era towards rapid and single step protocol with green principle.

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