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REVIEW ARTICLE

Synthesis of Biogenic Gold Nanoparticles and its Applications as Theranostic Agent: A Review

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ABSTRACT

The tremendous potential offered by living organisms in biosynthesis of gold nanoparticles has spurred encouraging interest. This article comprehends the studies done in the role of living cells (be it microbes, fungi, actinomycetes, algae or plants) in biosynthesizing nanometals, both intra as well as extracellularly. The capability of living cells to sequester metal ions and meticulously define the dimensions via fetter like capping proteins such as glutathione, phytochelatins and metallothioneins is intriguing giving it a monodispersed size. The role of extracellular electron shuttlers in formation of nanoparticles is also the subject of the present review. These biogenic gold nanoparticles have shown better compatibility than chemically synthesized gold nano particles. Hence, are being investigated for their significance in the field of cancer therapeutics such as targeted drug delivery vehicle. This aspect has also been touched upon in this review.

Introduction

Gold is a transition metal which is known to possess many intriguing properties. Its lustrous attributes has lead to avalanche of applications. “Soluble” and stable colored gold that appeared around 4th century B.C. in Egypt and China led to its use as a pigment for coating glasses, enamel and chinaware among others. The emergence of Nanoscience is a versatile branch of material science, which deals with the properties of material at nano-metric dimensions of 1-100 nm. Nanoscience has given enough evidence to show that at macro-scale and micro-scale levels the objects behave differently than that at nanoscale. The behavioural as well as the structural properties of the nano object changes. During the reduction the size of a solid from micro-level to nano-level, the appearance gets completely altered, especially with metals. Gold appears lustrous yellow at macro-scale, but when brought to nano-level its color turns to various shades of red, depending on the size and shape of the nanoparticles (Figure 1). The magical confrontation between GNPs and light led to understanding the Surface Plasmon Resonance.

Nanotechnology is about designing the material at nanoscale of 1-100 nm in two or three dimensions, confirming their unique characteristics, possible conjugation with different particles to make nano-composites for various applications. Innumerable applications of gold nanoparticles have boosted the

nanotechnologists all over the world to find easier, cheaper and liable methodologies that could be practically applied. To fabricate gold nanoparticles of controlled shape, size and monodispersity is an important aspect of the structures, which helps in its use for various devices.

Scientific interest in colloidal gold having beautiful ruby red solution had intrigued many alchemists and now nanotechnologists. The unusual medicinal properties of gold nanoparticles were realized much earlier and were expressed as the elixir of life. Michael Faraday [1] much before the existence of scanning electron microscopy experimented on synthesis and color of ruby gold. He obtained unstable colloidal sol relatively with colors varying from purple red and sometimes blue (Figure 1).

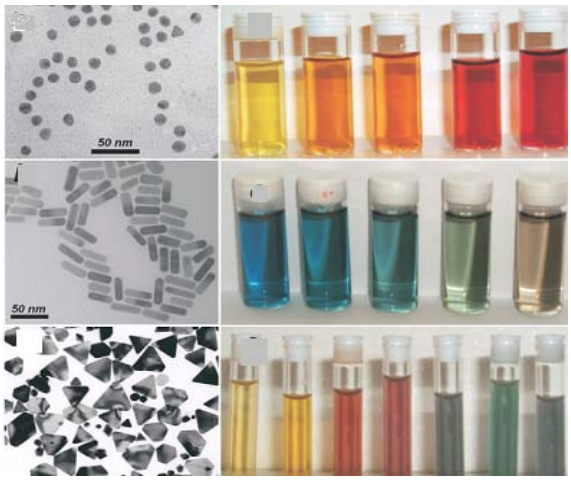

Figure 1: Colloidal gold nano particles exhibiting variation in color based on size and shape (Based on Faraday's concept).

He threw light on the relation between light and gold which was presented in the article named "The Bakerian Lecture". He also determined that the ruby glass was colored so, due to the presence of fine colloidal dispersion of gold particles and carried out reactions to ascertain that 'Purple of Cassius' could be obtained by adding tin chloride to gold sol. It must be mentioned here that Purple of Cassius was known for over two centuries as stable colorant for ceramics and glasses.

Development of Contemporary Concept of Gold NanoParticles (GNPs)

For nearly forty years, Faraday's work remained unnoticed, even the scientists who worked on the ruby glass and Purple of Cassius were not aware of it. Zsigmondy [2] began his investigations into the color of ruby glass and formulated a method for preparing colloidal gold by reducing dilute, slightly alkaline solution of gold chloride with boiling formaldehyde. After becoming aware of Faraday's methods, especially reduction using phosphorus, he combined both the synthesis techniques to arrive at a two-step synthesis method. This method is referred to as the seed-mediated method in the contemporary literature and was called 'nuclear method' in the early days. Also the nanoparticles were typically described as ultramicroscopic particles and the in place of nanometers (nm) as a unit, the equivalent unit used was ultra-microns (μμ). gmondy invented the ultra-microscope which allowed Siedentopf and Zsigmondy to visualize the colloidal gold particles (i.e. nanoparticles), showing that colloidal matter consisted of dispersion of particles of measurable size. Zsigmondy was able to make some of the first particle tracking studies to determine the diffusion behaviour of the nanoparticles. Zsigmondy was awarded Nobel Prize in 1925 "for his demonstration of the heterogeneous nature of colloidal solutions and for the methods he used, which have since become fundamental in modern colloid chemistry3.

Svedberg [4,5] played a central role in early studies of gold sols and he pioneered the use of electrochemical methods for the synthesis of gold particles. In his text, he reports the use of every conceivable reducing agent available at his time to produce colloidal gold from hydrochloroauric acid. To quote him, "The best known reduction process is the reduction of chloroauric acid (HAuCl4) to gold". Svedberg studied the particle size distribution of colloidal gold sol initially using centrifuge. The centrifugal force attained was only approximately 150 times gravity, so that it was possible to study only colloids of relatively low degree of dispersion. The particle-size of gold colloids down to ca. 20μ radius was determined with this apparatus [6]. He built a centrifuge having a field of force up to 7,000 times gravity to be produced (maximum speed of about 12,000 rpm). With this ultracentrifuge he could determine particle-sizes and the distribution of particle-

sizes, which were invisible in the ultramicroscope. The synthesis and physical behaviour, including hydrodynamics of gold particles, was central to the studies of Svedberg, who later earned the Nobel Prize for his work on disperse systems.

Since then, the varied colors exhibited by the metal in colloidal state were explained in many different ways by different researchers and was soon attributed to the optical resonance. The colloidal suspension of fine metal particles having dimensions of the particles smaller than

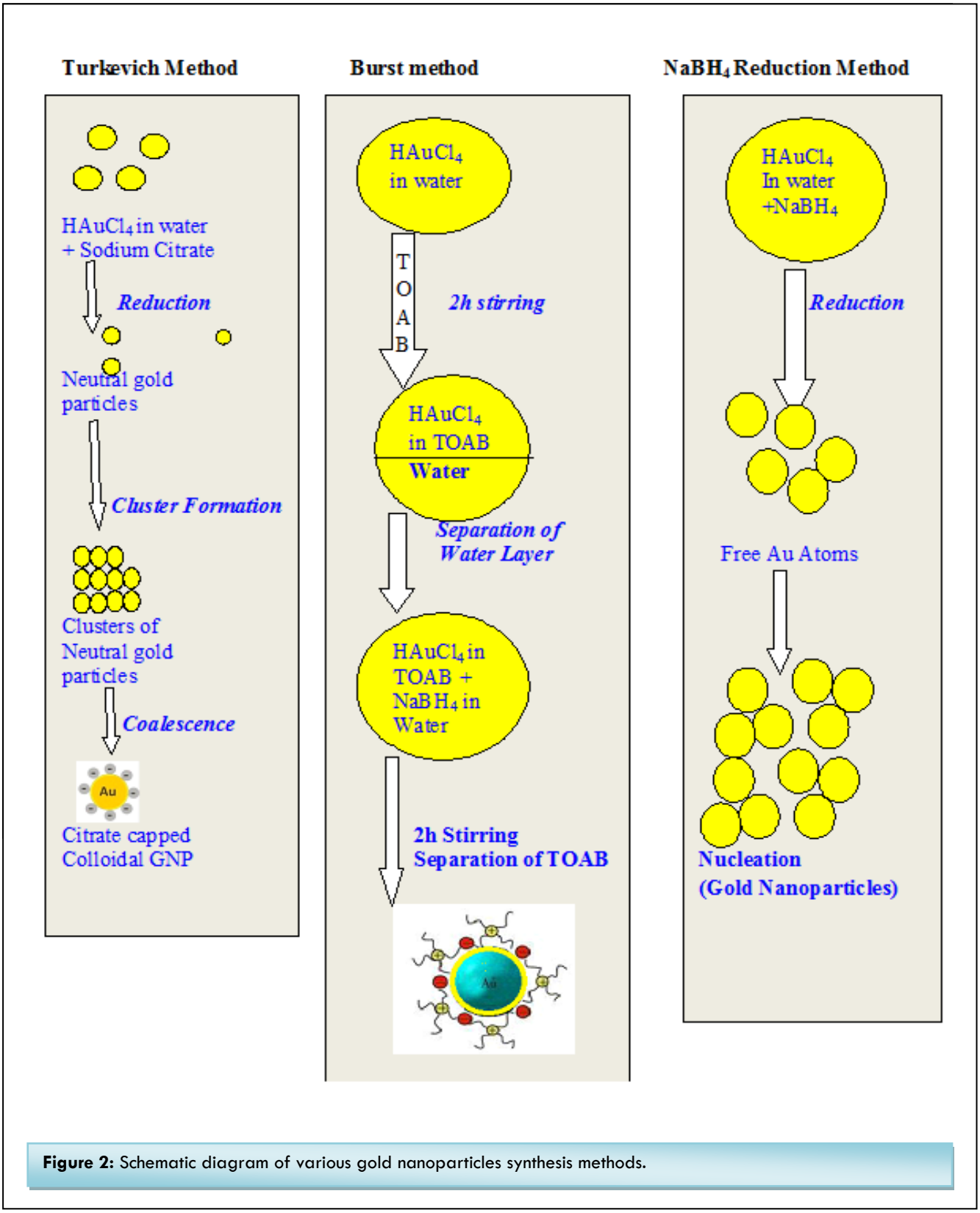

Figure 2: Schematic diagram of various gold nanoparticles synthesis methods.

the wavelength and the distance between the particles, which gives a well-defined absorption curve that can be obtained from the optical constants of the metal. Mie gave a theoretical explanation for the color of colloidal gold [7].

GNPs possess few atoms, hence exhibit dominantly statistical mechanical principles in which the energy is well quantized and discrete. This consequently leads to the dominion of surface energies due to the excited electrons of the surface atoms of nanomaterials and thus gold nanoparticles exhibits specific surface energy

Chemical Synthesis of Gold NanoParticles (GNPs)

Based on the concepts and the methodologies, various chemical methods of syntheses of GNPs evolved. Some of the common chemical syntheses methods of colloidal GNPs are mentioned are hereby briefly discussed. All these methods are based on reducing the gold ions to nanoparticles using chemical reducing agents.

1. Turkevich Method

The synthesis of colloidal gold nanoparticles by reduction of chloroauric acid with sodium citrate (Figure 2).

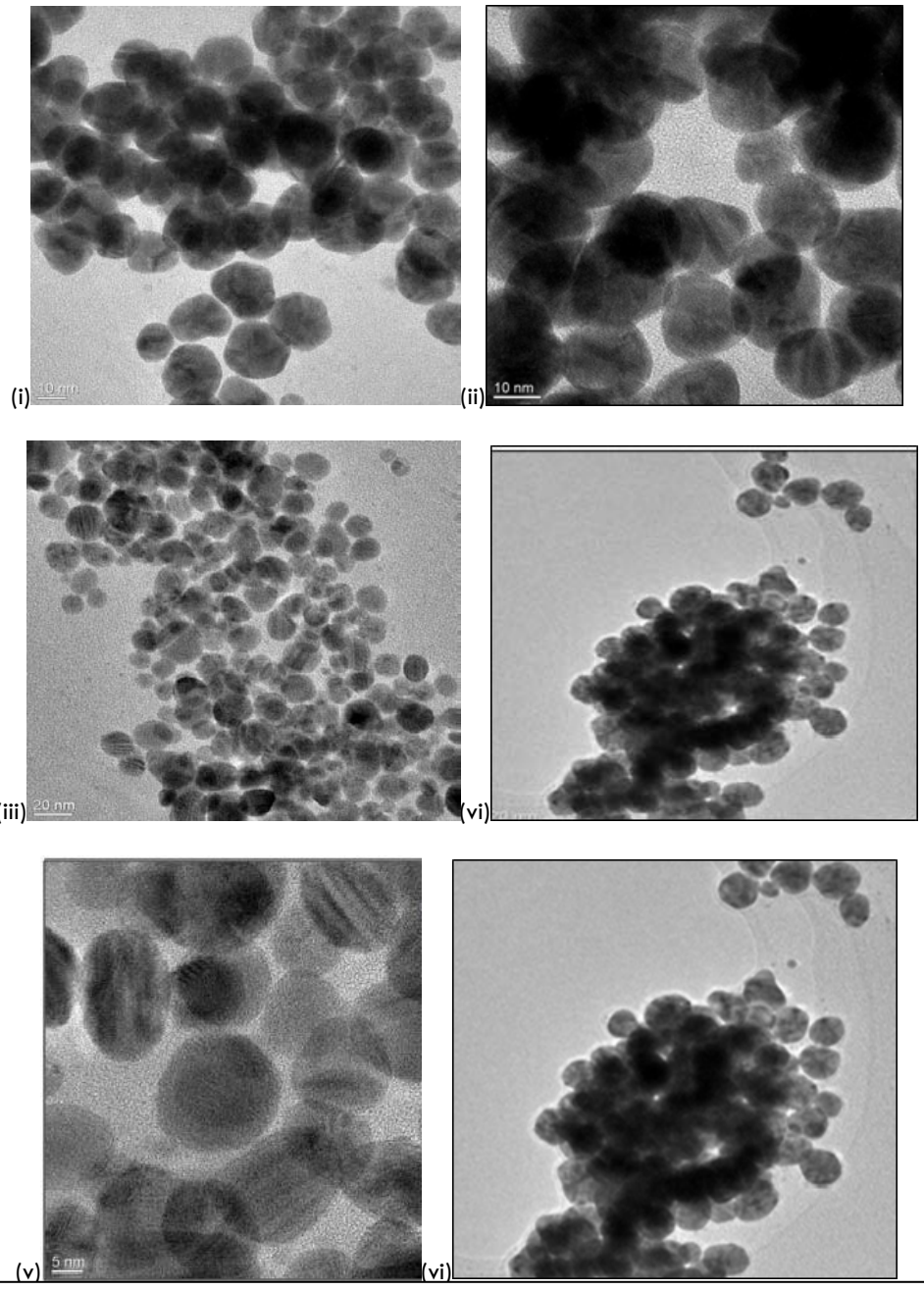

Figure 3: TEM of Biologically synthesized GNP using (i) Nocardia (ii) Candida albicans(iii) Dunaliella salina (iv) Chlorella pyrenoidusa(v) Aloe barbedensisand (vi) Adhatodavasica (Figures taken from Thesis of Goldie Oza and Sunil Pandey [64,65].

The citrate ion acts as a reducing agent as well as a capping agent thereby producing monodispersed gold nanospheres. Turkevich and his co-workers explored the properties of color, coagulation, adhesion, alloying and other catalytic properties of colloidal gold [8].

2. Brust Method

This is a method for synthesizing GNPs from HAuCl4 in non-aqueous solution using tetraoctylammonium bromide as a phase-transfer catalyst and sodium borohydride to reduce Au(III) to Au(0) (Figure 2). There is no doubt to say the Brust process of gold nanoparticle synthesis is a valuable technique for preparing thiolstabilized nanoparticles, but the functional groups are limited by the compatibility of thiols, the identification of a unique set of reaction conditions is often required for the preparation of each functionalized target, and most of the method in these reports are always accompanied with hazardous synthesis process [9].

3. Martin Method

Martin Method was brought to light much later in 2010 by the Eah's group [10], this technique generates "naked" GNPs in water by reducing HAuCl4 with NaBH4. Even without any other stabilizer like citrate, these GNPs are stably dispersed. The size distribution is nearly monodispersed and the diameter can be precisely and reproducibly tuneable from 3.2 to 5.2 nm. The key is to stabilize HAuCl4 and NaBH4 in the aqueous stock solutions with HCl and NaOH for >3 months and >3 hours respectively. In addition, the ratio of NaBH4-NaOH ions to HAuCl4-HCl ions has to be precisely controlled in the "sweet zone"

4. NaBH4 Reduction Method

In NaBH4 reduction process, NaBH4 is the reducing agent and the citrate acts only as a stabilizing agent (Figure 2). The reaction rate in the single aqueous system is controlled by the reaction conditions. Different reaction parameters (e.g. reaction temperature, reactant concentration, addition rate for NaBH4) are studied to get GNPs with uniform size distribution [11].

Biosynthesis of Gold NanoParticles (GNPs)

Different physical and chemical methods for gold nanoparticles synthesis are known but these methods are either expensive or are not eco-friendly due to use of some hazardous chemicals. These drawbacks necessitate the development of nonhazardous and greener methods for GNPs synthesis. This involves using either living being as a whole or using the extracts/ secretions (metabolites) from the living organisms. It is also known as Biogenic method. Biological method is more facile, eco-friendly and results in more monodispersed nanoparticles. The nanoparticles synthesized by chemical methods are unstable and tend to clump or agglomerate quickly and are rendered useless. The nanoparticles synthesized by various living systems have been shown to be coated with peptides or proteins. This leads to a similar charge distribution all over the surface of nanometals which results in repulsion between them. These inter particle repulsive forces prevent aggregation and so, nanometals solutions synthesized by living beings have been shown to be extremely stable even after a period of six months. This green chemistry approach for nanoparticle biosynthesis is simple and scaling up is also possible

1. Living Organisms as Nano-Assembler

To save itself from the clutches of wild and irresistible power of nature, living organisms have developed various means to survive in unfavourable environment. One of them is to protect itself from deleterious effects of heavy metals in high concentrations, by altering the redox state of the metal ions through reduction. The ultimate fate of these metal ions is their conversion into neutral oxidation state and then fabrication of each atom into particles of nanoscale dimension. In the forthcoming paragraphs all divisions of living system that have been tried as a factory for biosynthesis of nano-metals i.e. Bacteria, Fungi, Algae, and Plants are discussed (Figure 4).

2. Biosynthesis of GNPs by Bacteria

One of the powers of living system is to trigger various strategies to dilute the catastrophic conditions such as deleterious effects of heavy metals in high concentrations, which is combated by altering the redox state of the metal ions through reduction or formation of non-toxic complexes viz. sulfides and oxides. The ultimate fate of these metal ions is their conversion into

neutral oxidation state and then fabrication of each atom into particles of nanoscale dimension [12].

bacterial cell and metal clusters which leads to the formation of nanoclusters.

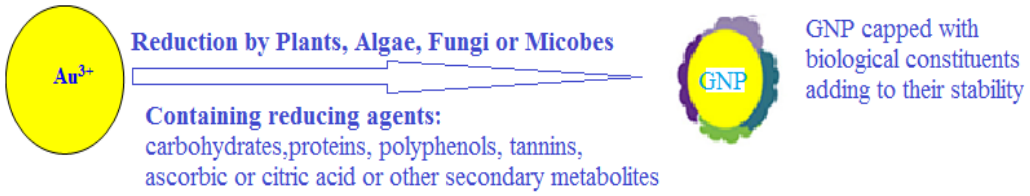

Figure 4: Schematic diagram of biogenic synthesis of gold nanoparticles

Unicellular as well as multi-cellular organisms can synthesize metal nanoparticles intracellularly or extracellularly. Such bio-mimetic actions have led to the growth of biosynthesis of nano-materials. Prokaryotes (are unicellular organism that lacks a membrane-bound organelle such as nucleus, mitochondria etc.) exhibit tolerance and resistance against metals [13].

GNPs can be readily precipitated within bacterial cells if the cells are incubated with Au+3 ions. The mechanisms which are considered for the biosynthesis of nanoparticles includes active efflux systems, redox changes i.e. alteration of solubility and toxicity via reduction or oxidation, bioabsorption, bioaccumulation, extracellular complexation or precipitation of metals, and lack of specific metal transport systems [14].

The cell wall of the microorganisms also plays a major role in the intracellular synthesis of nanoparticles. The cell wall being negatively charged interacts electrostatically with the positively charged metal ions. The enzymes present within the cell wall, bioreduce the metal ions to nanoparticles, and finally the smaller sized nanoparticles get diffused of through the cell wall. Mukherjee et.al. [15] have reported stepwise mechanisms for intracellular synthesis of nanoparticles using Verticillium species. The mechanism of synthesis of nanoparticles was divided into trapping, bioreduction and synthesis. Moreover, in the case of bacteria Lactobacillus sp, Nair and Pradeep [16] observed that during the initial step of synthesis of nanoparticles, nucleation of clusters of metal ions takes place, and hence there is an electrostatic interaction between the

Recently from extra cellular secretion of a novel bacterial strain, identified as Bacillus niabensis GNPs was synthesized. The mechanism on rapid biosynthesis of GNPs using the peptide from B. niabensis 45 may be proposed as follows: the amphiprotic peptide will present the negative charge of COO^- group, which was in favour of the formation of Au3+-peptide complex. Then tyrosine or tryptophan in peptides possibly provides electrons to reduce Au3+ ions to Au0 atoms. Furthermore, GNPs may be synthesized by the aggregation of Au0 atoms [17].

3. Biosynthesis of GNPs by fungi and actinomycetes

An actinomycetes S. hygroscopicus, have ability to synthesize intracellular gold nanoparticles. The effect of pH and metal concentration plays a vital role in this. It was observed that better biosynthesis of gold nanoparticles occurred when cell biomass treated with HAuCl4 solution. The results demonstrate that spherical gold nanoparticles in the range of 10 to 20 nm were observed at pH value of 7.0. The actinomycetal biomass and various concentration of aqueous HAuCl4 solution were incubated; it was found that 10-4 concentration shows excellent color of the actinomycetal biomass [18].

Eukaryotic organisms like fungi are successfully used in the synthesis of nanoparticles with different chemical composition and size as they display the ability to secrete large amounts of enzymes. Additionally, the fungi are found to display intracellular uptake of metals and also show high tolerance towards metals [19].

The use of microorganism especially the endophytic fungi for the synthesis of GNPs is a valuable alternative

for the existing physical and chemical methods. The particles are stabilized by the proteins released by the fungi in the medium thus making this a viable option for the synthesis of nanomaterials. The GNPs synthesized were in the size range of 15-30 nm with spherical shape and partial aggregation and didn't display significant antibacterial activity or cytotoxicity thus making them potential candidates in various medical applications [20].

4. Biosynthesis of GNPs by algae

Synthesis of metal nanoparticles using algae has been comparatively unexplored, though it has been found to be more biocompatible method than the other biological methods. However, success has been achieved using both marine and fresh water Algae with their reducing properties exploited for nanoparticle synthesis. GNP formation is a stress related mechanism which is comprehended by giving plethora of nitrogen, salt and light-induced stress by algae . Many algae are kown to have high levels of reductases and dehydrogenases. Marine algae are considered to possess very high reducing property since they grow in very high salt concentration and can be extremophilic in nature.

Singaravelu's group [21] exploited Sargassumwightii for extracellular synthesis of GNP. They achieved rapid formation of GNPs in a short duration. The UV-Vis spectrum showed peak at 527 nm corresponding to the plasmon absorbance of gold nano particles. Transmission Electron Micrograph showed formation of well dispersed gold nano particles in the range of 8 - 12 nm. An important potential benefit of the described method of synthesis of nano particles using marine algae is that they are quite stable in solution which is advantageous over other physical and chemical methods. Marine brown algae Fucusvesiculosus also has the capacity to reduce Au (III) to Au (0). It was instigated by Mata et. al. [22] that at pH 7, reduction potential is maximum. Such an environmentally useful process can be used for recovering gold from dilute hydrometallurgical solutions and leachates of electronic scraps.

Sharon's group have orchestrated crystalline GNP at 300C and pH 10 using marine algae, Sargassumwightii. They have shown involvement of nitrate reductase in formation of isotropic nanospheres along with anisotropic GNPs. Their size varied from 30–100 nm. The crystalline nature of GNP was found to be face centered cubic [23]. The same group biosynthesized stable spherical GNPs using fresh water alga Chlorella pyrenoidusa extract at alkaline pH 8 and 1000C. The SPR peaks of UV-Vis spectra showed that the size of the GNPs ranged from 25-30 nm, whereas pH 4 showed formation of anisotropic nanostructures. Here also the Nitrate reductase activity was found to be involved in the formation of GNPs [24].

Metal nanoparticle synthesis using algae extract shows rapid and non-toxic process which resulted to nano sizes having the greatest potential for biomedical applications. The green synthesis of GNPs using the algae extract of Turbinariaconoides was preliminarily confirmed by color changing from yellow to dark pink in the reaction mixture, and the broad surface plasmon resonance band was centered at 520 to 525 nm which indicates polydispersed nanoparticles. Variation in the color change was observed; it mainly depends on the reaction time and phytochemical components of the algae extract [25].

The rapid biological synthesis of gold nanoparticles can be witnessed using a novel marine brown alga Ecklonia cava (Family: Lessoniaceae) by the reduction of chloroauric acid. The formation of GNPs reaction was within 1 min at 80°C and physiochemically characterized with different analytical techniques. The newly synthesized gold nanoparticles were spherical in shape with diameter range of 20–50 nm. This method is green and environmentally friendly. Thus, the synthesized gold nanoparticles could have a high potential for use in biomedical applications. This method is inexpensive and highly recommended to be used in large-scale production of gold nanoparticles [26].

5. Biosynthesis of GNPs by plants

The phytosynthesis of nanoparticles is emerging as the intersection of nanotechnology and biotechnology. Due to a growing need to develop environmentally benign technologies in material synthesis, it has received increased attention [27]. This has motivated the researchers to synthesis the nanoparticles using this route

that allow better control of shape and size for various applications. Synthesis of gold nanoparticles using plant extract is useful not only because of its reduced environmental, but also because it can be used to produce large quantities of nanoparticles (Figure 3). Plant extracts may act both as reducing agents and stabilizing agents in the synthesis of nanoparticles.

Two medicinally important plants Cucurbitapepo and Malvacrispa were also reported for synthesis of GNPs with potent antibacterial agent against common food spoilage pathogens [28]. GNPs of size 20-30 nm were rapidly synthesized using aqueous leaves extract of Acalyphaindica as novel source of bio-reductants [29]. There is an exhaustive list of plants that have been used for biosynthesis of GNPs such as Zingiberofficinale [30], MomordicaCharantia, Citrus limonii, Asparagus racemosus, Adhatodavasica, Aloe vera [31-35], Azadirachtaindica [36], Cymbopogon citrates [37], Neem-Gum [38] etc.

The stable GNPs of variable size were obtained by using extract of leaves of Pelargonium graveolens and its endophytic fungus as extracellular synthesis [39].

In biogenic synthesis protocol there is a demand for separating anisotropic nanoparticles from a mixture of spherical and anisotropic particles. For this purpose density gradient centrifugation technique was optimized for achieving maximal NIR absorbance. The biocompatibility and efficacy of these anisotropic GNPs for photo thermal therapy of cancer cells was evaluated. Further, the suitability of the nanoparticles for X-ray contrast applications was also analyzed. A facile green route for preparing anisotropic GNPs using cocoa as the reducing and stabilizing agent has been tried, which displayed characteristic NIR absorption. Owing to the polyphenolic coating imparted by cocoa, these anisotropic GNPs showed high colloidal stability and cyto-compatibility in vitro [40].

Role of Different Enzymes in Reduction of Gold Ions to GNPs

Assay of various enzymes have indicated the role of enzyme as a reducing and shape directing agent. Laccase, an enzyme found in fungi have a very important role in extracellular synthesis of GNPs. For example Ligninase was responsible for the intracellular formation of GNPs on the fungal mycelium. The stabilization of the GNPs via protein layer was evident by Atomic Force Microscopy (AFM) which revealed the nanoparticles to be spherical in the range of 10-100 nm [41].

Another facile synthesis of ultra-stable GNPs is demonstrated using fruit peel extract of Momordicacharantia. The best parameters for the synthesis of GNPs were pH10, high temperature (1000C) and 100 ppm aurochlorate salt. The results were verified using UV-Vis spectroscopy, XRD and Transmission electron microscopy. The GNPs were monodispersed and found to be 10-100 nm in size. The stability of the GNPs synthesized using biological protocols was found to be extremely high than the chemically synthesized GNPs when tested using 5M NaCl solution. The Nitrate reductase activity was found to be 0.1667 mmole/min/gram of plant tissue which got reduced to 0.0132 mmole/min/gm in the solution after the formation of gold nanoparticles. The protein content got depleted after the formation of GNPs in the solution from 214.12 mg/ml to 64.42 mg/ml 31.

Bio-Molecules Involved in Synthesis of Gold Nanoparticles

Mechanism or the modus operandi involved in size regulation of nano particles have been an intriguing complexity. One of the most justifiable mechanisms appears to be the role of stress proteins such as Glutathione (GSH), PhytoChelatins (PCs) and MetalloThioneins (MTs) Super oxide dismutases, catalases and anti-oxidants like Vitamin E and Vitamin C also leads to the efficient synthesis of nanoparticles. GSH and metallothioneins occur in animals, several fungi, algae, some prokaryotes and perhaps in plants, which are induced by metals.

The discharge of heavy metals due to agricultural, industrial, and military operations has serious adverse effects on the environment [42,43]. Higher organisms respond to the presence of heavy metals with the production of cysteine-rich peptides such as GSH, PCs

and MTs that bind metal ions (such as cadmium, lead, mercury, copper) and sequester them in biologically inactive forms [44,45]. PCs are short, cysteine-rich peptides with the general structure (γGlu-Cys) [46,47]. PCs possess unique structural characteristics, particularly the continuously repeating γGlu-Cys units. PCs have higher metal-binding capacity (on a per cysteine basis).

Advantages and Various Applications of Biogenic GNPs

Biogenic GNPs have various potential applications due to their small size and large surface area. Anisotropic GNPs have attracted the interest of with the synthesis of numerous 1D, 2D, and 3D shapes as well as hollow GNPs structures. The anisotropy of these non-spherical, hollow, and nanoshell GNPs structures is the source of the plasmon absorption in the visible region as well as in the near infrared (NIR) region. This NIR absorption is especially sensitive to the GNP shape and medium and can be shifted towards the part of the NIR region in which living tissue shows minimum absorption. This has led to crucial applications in medical diagnostics and therapy ("theranostics"), especially with Au nanoshells, nanorods, hollow nanospheres, and nanocubes. In addition, Gold nanowires can be synthesized with longitudinal dimensions of several tens of micrometers and can serve as plasmon waveguides for sophisticated optical devices. The application of anisotropic GNPs has rapidly spread to optical, biomedical, and catalytic areas [48].

1. Biogenic GNPs for cancer therapy

Cancer can be defined as a condition in which a healthy cell of the human body gets modified due to genetic defects and starts to grow in an uncontrolled way, invading the adjacent tissues and spreading throughout the body as metastasis. Currently available treatments include chemotherapy, radiation therapy and surgery. These techniques have seen improvement over the past few decades but they are still far from optimal. Hence, more innovations in these treatments as well as diagnostic methods remain the main goal of current cancer research.

Nanotechnology has shown tremendous promising potential in cancer diagnostics and treatment which can revolutionize this field in near future. The solubility of nanoparticles in water, ease of functionalization, biocompatibility to normal cells is important factors which make these nanomaterials effective agent for cancer therapy. The effectiveness of cancer therapeutic device is measured by its ability to reduce and eliminate tumors without damaging the surrounding healthy tissues. Therefore, targeting tumors becomes essential for efficient working of therapeutic device. An increased site specificity and internalization can improve the efficacy of treatment and decrease the possibility of serious side effects that cancer patients often experience in conventional therapy protocol.

The most important attributes of GNPs is their size tunability, easy absorption by body fluids and passage through the body circulation without rejection. GNPs can pass through leaky capillaries and directly reach tumor surfaces. Also, GNPs display negligible toxicity owing to its biological precursors. These properties can be exploited for designing molecular armadas for ferrying therapeutic moieties to solid tumors. GNPs can be targeted passively through the capillary or the surface of the nanoparticle can be modified using antibodies or receptor proteins to target tumor cells and enter the cell via receptor mediated endocytosis, i.e. active targeting [49].

Azadirachtaindica acts as a biological sink for fabrication of gold nanoparticles (GNPs) and its applications in efficient delivery of anticancer drug doxorubicin (DOX). Sucrose density gradient centrifugation used to isolate the spherical GNPs of <50 nm from the mixture (containing both spherical and non-spherical) of nanoparticles synthesized using leaves of A. indica at inherent Ph [50]. The stability of GNPs due to the biological capping agents can be scrutinized by measuring the flocculation parameter which was found to be in the range of 0–0.65. On the surface of these capped GNPs, doxorubicin attached along with activated Folic Acid (FA) as navigational molecules for targeted drug delivery. The GNPs-FA-DOX complex was found be non-toxic for normal cells and

considerably toxic for HeLa cells. The drug loading capacity of the GNPs was found to 93%. Doxorubicin release kinetics using GNPs followed 1st order at pH 5.3 which is ideal for solid tumor targeting50.

Breast cancer is a major complication in women and numerous approaches are being developed to over-come this problem. A possible route for green synthesis of GNPs) using leaf extract of Mimosa pudica and its anticancer efficacy in the treatment of breast cancer cell lines is studied. The synthesized nanoparticles were found to be effective in killing cancer cells (MDA-MB-231 & MCF-7) which were studied using various anticancer assays (MTT assay, cell morphology determination, cell cycle analysis, comet assay, Annexin V-FITC/PI staining and DAPI staining). Cell morphological analysis showed the changes occurred in cancer cells during the treatment with GNPs. Cell cycle analysis revealed apoptosis in G0/G1to S phase. Similarly in Comet assay, there was an increase in tail length in treated cells in comparison with the control. Annexin V-FITC/PI staining assay showed prompt fluorescence in treated cells indicating the translocation of phosphatidylserine from the inner membrane. PI and DAPI staining showed the DNA damage in treated cells [51].

In another report, Sharon's group [52] have successfully used biologically synthesized gold nanoparticles to ferry anti-cancer drug Berberine using Folic acid as targeting molecule. Gold nanoparticles were synthesized using Trapabispinosa extract and folic acid and berberine was anchored on the surface via amide linkages. Drug release profiles were studied at different pH using standard statistical models. Cytotoxicity analysis revealed that the gold nanoparticle drug conjugate was showing efficient targeting towards folate receptor positive HeLa cells.

GNPs of various shapes have been evaluated against a variety of human cancer cells as promising agents for cancer therapy. The triangular shaped Au core-Ag shell nanoparticles were obtained by the reduction of gold ions by lemongrass extract by electrostatic complexation of Ag+ ions with negatively charged lemongrass reduced gold nanoparticles followed by reduction of the surface-bound Ag+ ions by ascorbic acid [53].

GNPs are also being tried for Photo-Thermal Therapy (PTT) of Cancer. In PTT GNPs embedded within tumors generate heat in response to exogenously applied laser light. It has been well documented as an independent strategy for highly selective cancer treatment. Gold-based nanoparticles are the main mediators of PTT because they offer: (i) biocompatibility, (ii) small diameters that enable tumor penetration upon systemic delivery, (iii) simple gold-thiolbioconjugation chemistry for the attachment of desired molecules, (iv) efficient light-to-heat conversion, and (v) the ability to be tuned to absorb near-infrared light, which penetrates tissue more deeply than other wavelengths of light. In addition to acting as a standalone therapy, gold nanoparticle-mediated PTT has recently been evaluated in combination with other therapies, such as chemotherapy, gene regulation, and immunotherapy, for enhanced anti-tumor effects. When delivered independently, the therapeutic success of molecular agents is hindered by premature degradation, insufficient tumor delivery, and off-target toxicity. PTT can overcome these limitations by enhancing tumor- or cell-specific delivery of these agents or by sensitizing cancer cells to these additional therapies. All together, these benefits can enhance the therapeutic success of both PTT and the secondary treatment while lowering the required doses of the individual agents, leading to fewer off-target effects. Given the benefits of combining gold nanoparticle-mediated PTT with other treatment strategies, many exciting opportunities for multimodal cancer treatment are emerging that will ultimately lead to improved patient outcomes [54].

Over recent decades, one of the most important and complex problems facing our society is the multi-drug resistance of human cancer cells and pathogens to most clinically approved therapeutics. Recent advances in nanoscience and nanotechnology have expanded our ability to design and construct nanomaterials with targeting, therapeutic, and diagnostic functions. Photo Thermal Therapy (PTT) is a minimally invasive therapy in which photon energy is converted into heat in order to

kill cancer cells. Gold nanoparticles (GNPs) with a high light-to-heat conversion capability are among the most important candidates for PTT. GNP-assisted PTT has shown great success in recent years, suggesting promise for future applications. The efficacy of photo thermal therapy by using gold nanoparticles with laser irradiation on two cancer cell lines [i.e. HeLa (human cervical cancer cell line) and AMN3 (mammary adenocarcinoma) in vitro, in addition, using gold nanoparticles without irradiation , were compared. The results showed that photo thermal therapy was more effective than gold nanoparticles alone o. It also showed that PTT was more effective on AMN3 than HeLa cell line, showing that the effect is related to the type of cell line. The impact of concentration, incubation period and size of particles were also observed. The present study suggests that photo thermal therapy by gold nanoparticles is a good candidate for medical application as anticancer in future [55].

2. GNP based biosensors

The role of incorporation of gold nanoparticles (50-130 nm in diameter) into a series of photocurablemethacrylic-acrylic based biosensor membranes containing tyrosinase on the response for phenol detection can be investigated. A range of gold nanoparticles concentrations from 0.01 to 0.5 % (w/w), incorporated into these membranes during the photocuring process. The addition of gold nanoparticles to the biosensor membrane led to improvement in the response time by a reduction of approximately 5 folds to give response times of 5-10 s. The linear response range of the phenol biosensor was also extended from 24 to 90 mM of phenol. The hydrophilicities of the membrane matrices demonstrated strong influence on the biosensor response and appeared to control the effect of the gold nanoparticles. For less hydrophilic methacrylic-acrylic membranes, the addition of gold nanoparticles led to a poorer sensitivity and detection limit of the biosensor towards phenol. Therefore, for the application of gold nanoparticles in the enhancement of a phenol biosensor response, the nanoparticles should be immobilized in a hydrophilic matrix rather than a hydrophobic material. The results reported here have shown that the incorporation of gold nanoparticles in the methacrylic-acrylic type of polymer membranes containing tyrosinase can have beneficial effects on the response of biosensors for phenol determination. The most obvious benefits are the improvement in response times and the linear response range of the biosensor. However, incorporation of gold nanoparticles in less hydrophilic membranes has resulted in the reduction in biosensor sensitivity and also yielded poorer detection limits towards phenol. Thus, the hydrophilicity of the membrane matrix where the nanoparticles and enzyme are immobilized plays an important role in influencing the response of the biosensor. For the application of gold nanoparticles in the enhancement of a phenol biosensor response, the nanoparticles should be immobilized in a more hydrophilic matrix rather than a hydrophobic material [56].

3. Biogenic GNPs as possible agent in anti-malarial activity

Muruganet. al. reported gold nanoparticles biosynthesized from Cymbopogoncitratus leaf extract were tested against larvae and pupae of the malaria vector Anopheles stephensi and the dengue vector Aedesaegypti 37.

4. Anti-fungal activity of biogenic GNPs

The aqueous seed extract of Abelmoschusesculentus were used to synthesize GNPs and its antifungal activities were tested against Pucciniagraministritci, Aspergillusflavus, Aspergillusniger and Candida albicans. The synthesized GNPs hence, has a great potential in the preparation of drugs used against fungal diseases [57].

5. Use of biogenic GNPs in cosmetics

Glutathione (GSH) is well-known for its anti-oxidant properties such as cosmetic purpose and also to reduce cancer progression. GNP and GSH together demonstrate possibility to use GSH-capped GNPs that take advantages of both antioxidant and high surface ratio properties 35. GSH is a tri-peptide molecule. It is one of important biological antioxidants, preventing damage to important cellular components caused by reactive oxygen species such as free radicals and peroxides [58]. GSH is widely used as a supplement in

numerous diseases such as cancer, AIDS, sepsis, trauma for regulation of nitrogen balance that involve in survival rate and disease progression [59,60]. In addition, antioxidant properties of GSH have been increasingly used for recent cosmetic application. GSH attaching on the surface of GNP results in increase of nanoparticle stability compared with citrate-stabilized GNPs.

6. Biogenic GNPs for enhanced physiological behaviours

Coating GNPs with GSH, a tri-amino acid peptide, also leads to a class of renal clearable GNPs with high resistance to serum protein adsorption that can effectively target tumors [61]. By using GSH coated luminescent GNPs as surface ligands to decorate SPIONs, not only it successfully integrate magnetic and fluorescence properties together, but also significantly enhances the hybrid nanoparticles (HBNPs) physiological stability and minimize serum protein adsorption, opening up a new path to develop multimodality contrast agents with enhanced physiological behaviours.

***7.* Biogenic GNPs as biomarker**

MetalloThioneins (MTs) are a type of low molecular weight proteins with rich cysteine. Metallothioneins are a useful tool to reveal the presence of bioavailable metals, and may be involved in both metal detoxification and antioxidant defense [62].The cysteinylsulfurs of MTs act as bridging and terminal ligands for coordinating divalent metal ions, involving the essential and toxic metals in two metal-thiolate clusters. Hence, MTs play an important role in the trace elements' transport, the detoxification of toxic metals and the scavenging of free radicals. Also, a positive association between MTs and tumors was observed in breast, ovarian, uterine and prostate cancer. Thereby, MTs have been extensively investigated as a biomarker of some diseases and various types of cancer. They were also used as an effective biomarker for evaluating heavy metal poisoning and environmental pollution degree. Therefore, it is significant to develop a sensitive, selective and practical method for detecting MTs in body fluid for biomedical study, clinical diagnosis and environmental monitoring. It is known that citrate capped GNPs coated with traces of mercury possess peroxidase-like properties that can catalyze the oxidation of 2, 2′-azino-bis (3-ethylbenzothiazoline- 6-sulfonate) (ABTS) to form a blue product in acetate buffer of pH 4.5. It is found that if the GNPs are first aggregated by the cysteine-rich metallothioneins, the peroxidase like properties of the resulting aggregates (GNP-Hg-MTs) cause a largely accelerated oxidation of ABTS. The effect of adding MTs to such a solution is used to quantify the MTs by a kinetic assay. The introduction of MTs in the solution of GNPs-Hg (II)-ABTS-H2O2 caused a noticeable enhancement in the absorbance of the system. The absorbance of the system increased gradually with the rise of the concentration of MTs, which is proportional to the MTs concentration. These results demonstrate that MTs can promote the H2O2-mediated oxidation of ABTS in the presence of GNP-Hg peroxidase mimic. The method was successfully applied to the determination of MTs in (spiked) human urine. The strategy may pave the way for related detection platforms [63].

Conclusion

The problem with most of the existing chemical and physical methods of nanomaterial production is that they are extremely expensive and also involve the use of toxic, hazardous chemicals, which may pose potential environmental and biological risks. Further, it is an unavoidable fact that the metal nanoparticles synthesized have to be handled by humans and must be available at cheaper rates for their effective utilization. Thus, there is a need for an environmentally and economically feasible way to synthesize these nanoparticles. This paved a way to use biomimetic approaches for the benign synthesis of these nanoparticles [43]. In biological synthesis, the major advantage is that it is carried out in an aqueous and doesn't require any additional reducing agent. Another feature to be noted is the shelf life of bio-nanoparticles is relatively longer than any other. GNPs are frequently being used in medical field as a theranostic agent. Moreover, role played by GNPs ranges from biomarkers to bio-delivery vehicles in medicine, anti-aging components to biosensors. Such wide real time

applications of GNPs make their synthesis, on a large scale under facile conditions with defined morphology, all the more valuable. Biological synthesized GNPs prove to be non-toxic carriers of drugs for treatment of cancer cells. Inherently surface passivated functional groups aid in direct attachment of drug molecules without the need of any extra linker. Moreover, targeting experiments prove them to kill cancer cells with minimum harm to normal non-infected cells. Thus, due the excellent optical and physical properties, biocompatibility and easy synthesis, GNPs prove to be an excellent drug delivery agent for treatment of diseased cells.

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