BIOSYNTHESIS, CHARACTERIZATION AND pH STUDY OF GOLD NANOPARTICLES USING PLANT EXTRACTS

By

Chandan Singh

(061706)

MAY2010

Submitted in partial fulfillment of the Degree of Bachelor of Technology

DEPARTMENT OF
BIOTECHNOLOGY & BIOINFORMATICS
JAYPEE UNIVERSITY OF INFORMATION TECHNOLOGY
WAKNAGHAT, SOLAN, HP, INDIA

CERTIFICATE

This is to certify that the project report entitled **"BIOSYNTHESIS,CHARACTERIZATION AND pH STUDY OF GOLD NANOPARTICLES USING PLANT EXTRACTS"** submitted by Chandan Singh in partial fulfillment of the award of degree of Bachelor of Technology in Biotechnology to Jaypee University of Information Technology, Waknaghat, Solan has been carried out under my supervision.

(Dr. Harvinder Singh)
Dept. of BT and BI
JUIT, Waknaghat

(Dr Vineet Sharma)
Dept.of Physics
JUIT, Waknaghat

Certified that this work has not been submitted partially or fully to any other University or Institute for the award of this or any other degree or diploma.

Chnadan Singh

ACKNOWLEDGEMENT

Apart from my continuous effort, my work could never have been complete without the constant help and support of a number of people, towards whom i would like to extend my sincerest gratitude.

First and foremost, it is my proud privilege to have the opportunity to thank my Project Guides **Dr..Harvindrer Singh and Dr Vineet Sharma** .It was an honor to carry out my project work under his expert guidance and supervision, without which my work would never have been possible. I take this opportunity to acknowledge our deep respect and gratitude to the head of department of Biotechnology **Dr. R.S. Chauhan** for all his support and Valuable inputs ,i thank him for reviving biotechnology as the over all mentor by leading with a vision and teaching us with a knowledge imparting attitude this successful compilation of final year project, depends on the knowledge and attitude inculcated in the total length of the course ,so i want to express my sincere thanks to all the faculty who taught me during the 4 years of B.Tech.

We would also like to express our gratitude for **Dr. Y. Medury,** the Vice Chancellor of our university, and **Brig. (Retd.) Balbir Singh,** the Registrar of our university for allowing us to work in the university's laboratories and providing me with all necessary facilities, equipment and chemicals.

I would indeed be failing in my duties if I did not acknowledge the constant support and immense patience of the staff of the biotechnology lab of our university. Lastly, i would like to thank my parents whose constant encouragement gave me the courage and enthusiasm to complete this work.

Chandan Singh

061706

TABLE OF CONTENTS

INDEX OF FIGURES

INDEX OF TABLES

LIST OF ABBREVIATION

AuNP :	Gold nanoparticle
SERS:	Surface Enhanced Raman Scattering
TEM:	Transmission Electron Microscopy
FR:	Follate Receptor
XRD:	X-ray diffractometer
EGFR :	Epidermal growth factor receptor
FTIR:	Fourier transform infrared spectroscopy
CLL :	Chronic lymphocytic leukemia
RES:	Reticuloendothelial system
NAPT:	Nanoshell-assisted photo-thermal therapy
nm	Nano-meter

ABSTRACT

The synthesis of nanocrystals is in the limelight in modern nanotechnology .Biosynthesis of nanoparticles by plant extract is currently under explotitation .Gold nanoparticles are generated using two different plant extracts first is black peeper and second is Liquorice root,and pH study is performed in both the cases ,in the case of black peeper extract maximoum absorption was found at 530 nm and the pH of four samples were 5,7,8 and 10.In the case of Liquorice root the maximum absorptios was found at 538 nm and the pH 4.3 ,6.2 ,8.4 and 10.2,but the intensity of the colours in case of Liquorice root is better than that of black peeper extract,which reflects the the differences in the shape ,size and surface morphology of gold nanoparticles synthesized by two different methods.We are waiting for TEM(Transmission Electron Microscopy) results which will clearly differentiate all the nanoparticles with respect to their shape,size and surface morphology.

CHAPTER 1

INTRODUCTION

Nanoscience and nanotechnology are steering mankind into new realms of efficient and miniature tools and gadgetry. Every scientific field, be it a fundamental science such as physics, chemistry or biology or applied science such as medicine or engineering is exploding with new discoveries at the nanoscale level. One such discovery lies in the potential to exploit nanoparticles and nanostructures in biomedicine to diagnose and treat diseases in general, and cancer and tumor in particular. This dissertation project highlights the design and development of novel gold nanoparticle conjugates that target cancers and tumors in order to assist with diagnosis and therapy of cancer. According to "National Cancer Institute" it can be defined as follows.

"The field of research that deals with the engineering and creation of things from materials that are less than 100 nanometers (one-billionth of a meter) in size, especially single atoms or molecule"

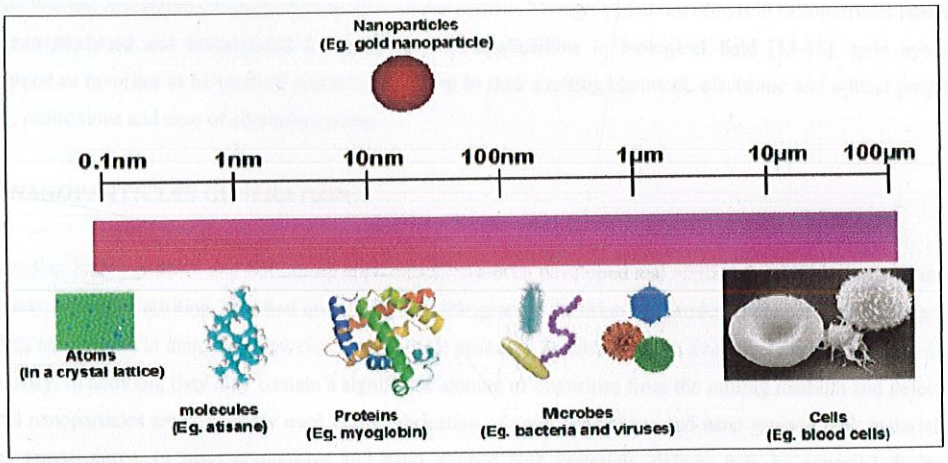

Figure 1.1: Nanoparticles in comparison with other biological entities

1.1 NANOPARTICLES AND THEIR PROPERTIES:

A nanoparticle is by definition a particle where all the three dimensions are in nanometer scale. Nanoparticles are known to exist in diverse shapes such as spherical, triangular, cubical, pentagonal, rod-shaped, shells, ellipsoidal and so forth. Nanoparticles by themselves and when used as building blocks to construct complex nanostructures such as nanochains, nanowires, nanoclusters and nanoaggregates find use in a wide variety of applications in the fields of electronics, chemistry, biotechnology and medicine, just to mention few: For example, gold nanoparticles are being used to enhance electroluminescence and quantum efficiency in organic light emitting diodes [1]; palladium and platinum nanoparticles are used as efficient catalysts [2]; glucose sensors are developed based on silver nanoparticles [3]; and iron oxide nanoparticles are used as contrast agents in diagnosing cancer in Magnetic Resonance Imaging (MRI) [4]. Nanoparticles contain small enough a number of constituent atoms or molecules that they differ from the properties inherent in their bulk counterparts. However, they contain a high enough a number of constituent atoms or molecules that they cannot be treated as an isolated group of atoms or molecules . Therefore, nanoparticles exhibit electronic, optical, magnetic and chemical properties that are very different from both the bulk and the constituent atoms or molecules. For example, the striking colors of metallic nanoparticle solutions (such as gold and silver) are due to the red shift of the plasmon band to visible

frequencies, unlike that for bulk metals where the plasmon absorption is in the UV region (a plasmon is a quantum of collective oscillation of free electrons in the metals). This red shift of the plasmon occurs due to the quantum confinement of electrons in the nanoparticle, since the mean free path of electrons is greater than the nanoparticle size [5, 6]. Additionally, the optical properties of nanoparticles depend significantly on their size and shape as well as on the dielectric constant of the surrounding medium. For example, in spherical gold nanoparticles, the plasmon absorption red shifts with increasing diameter of the nanoparticle [7]. Likewise, quantum dots (semiconductor nanoparticles such as CdSe and CdTe) exhibit red shift in their band gap (emission) as their size increases [8, 9]. Silver nanoparticles of spherical, pentagonal and triangular shape appear blue, green and red respectively under a dark field microscope, suggesting strong correlation between optical property and shape of the nanoparticles [10]. Gold nanorods exhibit different optical properties than their spherical counterparts. Gold nanorods show two plasmon resonances, one a transverse plasmon at 520 nm and the other a longitudinal plasmon at longer wavelengths. Unlike the transverse plasmon mode, the wavelength of the longitudinal plasmon mode increases with increasing aspect ratio of the nanorods [11]. Additionally, gold nanoparticles dispersed in different solvents exhibit plasmon absorption at different wavelengths suggesting the effect of surrounding media [12]. Nanoparticles have large surface to volume ratio, thus surface related phenomena/properties are drastically affected with slight modification of size, shape and surrounding media of nanoparticles. Therefore, the optical properties desired of nanoparticles depending on application can be tuned by generating the nanoparticles of definite size and shape in preferred media and henceforth, develop new effective nanomaterials and nanodevices. Nanoparticles (1-500 nm) are much smaller than human cells which are about 10-20 μm (Figure 1.1). However, nanoparticles have sizes similar to that of the biomolecules encountered at the cellular level. This unique size of nanoparticles facilitates development of nanodevices/nanosensors that can travel into cells to probe proteins (enzymes and receptors) or the DNA inside the cell or outside the cell. Consequently, the first step involved in developing nanodevices/nanosensors is to produce hybrid nanoparticles: nanoparticles labeled with molecules that can investigate or target the specific cellular entities. Though a plethora of hybrid nanoparticles labeled with peptides and proteins have been produced and investigated for their potential applications in biological field [13-15], gold hybrid nanoparticles specifically have emerged as favorites in biomedical applications owing to their exciting chemical, electronic and optical properties along with their biocompatibility, dimensions and ease of characterization.

1.2-METHODS OF NANOPARTICLES GENERATION:

Many techniques, including both top-down and bottom-up approaches,have been developed and applied for the synthesis of nanoparticles. Top down approaches include milling or attrition, repeated quenching and lithography. Attrition can produce nanoparticles ranging from a couple of tens to several hundreds nanometers in diameter. However, nanoparticle produced by attrition have a relatively broad size distribution and varied particle shape or geometry. In addition, they may contain a significant amount of impurities from the milling medium and defects resulting from milling. Such prepared nanoparticles are commonly used in the fabrication of nano composites and nano grained bulk materials, which require much lower sintering temperatures. In nano composites and nano grained bulk materials, defects may be annealed during sintering, size distribution, particle shape, and a small amount of impurities are relatively insensitive for their applications. Repeated thermal cycling may also break a bulk material into small pieces, if the material has very small thermal conductivity but a large volume change as action of temperature. A big volume change associated with phase transition can be effectively utilized in this approach. Although very fine particles can be produced, this process is difficult to design and control so as to produce desired particle size and shape. It is also limited to materials with very poor thermal conductivity but a large volume change. Lithography, which will be discussed inis another method to make small particles. Bottom-up approaches are far more popular in the synthesis of nanoparticles and many methods have been developed. For example, nanoparticles are synthesized by homogeneous nucleation from liquid or vapor, or by heterogeneous nucleation on substrates. Nanoparticles or quantum dots can also be prepared by phase segregation through annealing appropriately designed solid materials at elevated temperatures. Nanoparticles can be synthesized by confining chemical reactions, nucleation and growth processes in a small space such as micelles. Various synthesis methods or techniques can be grouped into two categories: thermodynamic equilibrium approach and kinetic approach. In the thermodynamic approach, synthesis process consists of (i) generation of supersaturation, (ii) nucleation, and (iii) subsequent growth. In the kinetic approach, formation of nanoparticles is achieved by either limiting the amount of precursors available for the growth such as used in molecular beam epitaxy, or confining the process in a limited space such as aerosol synthesis or micelle synthesis. In this chapter, the attention will be focused mainly on the synthesis of nanoparticles through thermodynamically equilibrium approach. However, some typical kinetic approaches such as microemulsion, aerosol pyrolysis and template-based deposition, will be highlighted as well. For the thermodynamic equilibrium approach, this chapter will take the solution synthesis of nanoparticles as an example to illustrate the fundamental requirements and consideration, however the fundamentals

and principles are applicable to other systems without or with minimal modification. For the fabrication of nanoparticles, a small size is not the only requirement. For any practical application, the processing conditions need to be controlled in such a way that resulting nanoparticles have the following characteristics: (i) identical size of all particles (also called monosized or with uniform size distribution), (ii) identical shape or morphology, (iii) identical chemical composition and crystal structure that are desired among different particles and within individual particles, such as core and surface composition must be the same, and (iv) individually dispersed or mono dispersed, i.e. no agglomeration. If agglomeration does occur, nanoparticles should be readily redispersible. Nanoparticles discussed in this chapter include single crystal, polycrystalline and amorphous particles with all possible morphologies, such as spheres, cubes and platelets. In general, the characteristic dimension of the particles is not larger than several hundred nanometers, mostly less than a couple of hundred nanometers. Some other terminologies are commonly used in the literature to describe some specific subgroups of nanoparticles. If the nanoparticles are single crystalline, they are often referred to as nanocrystals. When the characteristic dimension of the nanoparticles is sufficiently small and quantum effects are observed, quantum dots are the common term used to describe such nanoparticles. Now whatever methods are mentioned above are based on the use of harazadous chemical which are quite harmfull for the humans and enviorment,so now the new method for the synthesis of nanoparticles are introduced which are based on the biological entities.

1.3- GOLD NANOPARTICLES(AuNPs)-

Bulk gold is well known for being inert; however, the nanoparticulate sizes of gold display astronomically high chemical reactivity [16, 17]. Consequently, the rich surface chemistry of AuNPs allows surface modification reactions with wide varieties of chemical and biochemical vectors to tailor to the needs of biomedical applications including imaging and therapy of cancer [18-20]. Hybrid AuNPs are produced by the interaction of highly reactive nascent AuNPs with chemical functionalities present on simple chemical molecules or on specific molecules of biological interest (for example biomolecules including peptides and proteins) [13, 21]. These hybrid AuNPs labeled with tumor seeking biomolecules provide efficient vehicles for site specific delivery of nanoparticles carrying imaging and therapeutic probes to target cancer cells [22-25]. In X-ray CT imaging, the contrast appears due to variations in the electron densities (in turn attenuation coefficients) of different tissues. Gold being a metal with ` high electron density, tumor site specific hybrid AuNPs can be used as effective contrast agents in X ray CT imaging [26]. Gold nanoparticle solutions are bright red/purple colored due to Plasmon absorption. Any surface modification of AuNPs results in the shift of plasmon absorption wavelength. This change in optical property of AuNPs when coming in contact with the probe biomolecule is exploited to develop biosensors [13, 27].

1.4-BIOLOGICAL METHODS:

Synthesis of nanoparticles using biological entities has great interest due to their unusual optical [28] chemical [29],photoelectrochemical [30], and electronic [31] properties. A wide variety of physical, chemical and biological processes results in the synthesis of nanoparticles, some of these are novel and others are quite common. Nature has devised various processes for the synthesis of nano- and micro-length scaled inorganic materials which have contributed to the development of relatively new and largely unexplored area of research based on the biosynthesis of nanomaterials [32]. The synthesis and assembly of nanoparticles would benefit from the development of clean, nontoxic and environmentally acceptable "green chemistry" procedures, probably involving organisms ranging from bacteria to fungi and even plants [33]. Hence, both unicellular and multicellular organisms are known to produce inorganic materials either intra-or extracellularly [34],The *Verticillium* sp. fungal biomass when exposed to aqueous $AgN0_3$ solution resulted in the intracellular formation of silver nanoparticles, while *Fusarium oxysporum* biomass resulted in the extracellular silver nanoparticles [35]. The use of microorganisms such as bacteria, yeast, fungi and actinomycetes has been described for the formation of nanoparticles and their applications . The rate of intracellular particle formation and therefore the size of the nanoparticles could, to an extent, be manipulated by controlling parameters such as pH, temperature, substrate concentration and exposure time to substrate . Efforts have also been made to manipulate the shape and size of gold nanoparticles produced extracellularly by microorganisms through altering key growth parameters [36]. Efforts are also being given for the synthesis of nanoparticles of different chemical composition, sizes and controlled monodispersity. For example synthesis of biominerals which are composite materials and consist of an inorganic component and a special organic matrix (proteins, lipids, or polysaccharides) that controls the morphology of the inorganic compound. We reviewed here on the synthesis of nanoparticles using various living organisms.

1.5 –USE OF ORGANISM IN SYNTHESIS OF GOLD NANOPARTICLES:

Here, we summarize some of the organisms used in the biosynthesis of nanomaterials and describe the properties that should be inherent for the production of nanoparticles of desired characteristics. Some organisms which have been used for the production of nanoparticles are summarized in Table I.

1.6-USE OF BACTERIA:

Pseudomonas stutzeri AG259 isolated from silver mines has been shown to produce silver nanoparticles [37]. The synthesis of magnetic nanoparticles has been reported by using magnetotactic bacteria . Magnetotactic bacteria such as *Magnetospirillum magneticum* produce two types of particles; some produce magnetic (Fe304) nanoparticles in chains and some produce greigite (Fe3S4) nanoparticles, while some other produce both types of nanoparticles. Similarly in the presence of exogenous electron donor, sulphate-reducing bacterium *Desulfovibrio desulfuricans* NCIME 8307 has been shown to be synthesizing palladium nanoparticles reported that common *Lactobacillus* strains found in buttermilk assisted the growth of microscopic gold, silver, and gold-silver alloy crystals of well-defined morphology. Recently, bacterial cell supernatant of *Pseudomonas aeruginosa* was used for the reduction of gold ions resulting in extracellular biosynthesis of gold nanoparticles [38]. This would help in understanding the biochemical and molecular mechanism of nanoparticles synthesis. The cell filtrate has helped in achieving better control over size and polydispersity of nanoparticles. Further, this has documented that extracellular synthesis of nanoparticles using cell filtrate could be beneficial over intracellular synthesis. Morphological control over the shape of gold nanoparticles has been achieved by using *Plectonema boryanuln* UTEX 485, a filamentous cyanobacterium. When it was reacted with aqueous Au(S203)~- and AuCli solutions at 25-100 °C for up to I month and at 200°C for 1 day resulted in the precipitation of cubic gold nanoparticles and octahedral gold platelets, respectively . The mechanisms of gold bioaccumulation by cyanobacteria *(Plectonema boryanum* UTEX 485) from gold(II1)chloride solutions have documented that interaction of cyanobacteria with aqueous gold(II1)-chloride initially promoted the precipitation of nanoparticles of amorphous gold(l)-sulfide at the cell walls, and finally deposited metallic gold in the form of octahedral (III) platelets near cell surfaces and in solutions . Adding further to the mechanism, a sulfate-reducing bacterial enrichment was used to destabilize gold(l)-thiosulfate complex to elemental gold and proposed that this could occur by three possible mechanisms involving iron sulfide, localized reducing conditions, and metabolism[39].

TABLE 1-

USE OF VARIOUS BIOLOGICAL ENTITIES IN THE PRODUCTION OF NANOPARTICLES:

Biological entity	Nanoparticles produced	size
Aspergillus fumigatus (Fungus)	Ag	5-25 nm
Colletotrichum sp. (Fungus)	Au	20-40 nm
Candida glabrata (Yeast)	CdS	20 A
Desulfovibrio desulfuricans (Bacterium)	Palladium	20-50 nm
Fusarium oxysporium (Fungus)	Ag	20-40nm

Fusarium oxysporium (Fungus)	Au	3-11 nm
Fusarium oxysporium (Fungus)	Zirconia	15-30 nm
Pseudomonas aeruginosa	Au	15-30 nm
Trichothecium sp. (Fungus)	Au	20 nm
P. jadinii (Yeast)	Au	Few 100 nm

1.7-USE OF PLANTS:

Several plants havebeen successfully used for efficient andrapid extracellular synthesis of silver and gold nanoparticles. Leaf extracts of geranium (*Pelargonium graveolens*)[40], lemongrass (41,*Cymbopogon flexuosus*)[41], *Cinnamommum camphora[*42] neem (*Azadirachta indica*)[43], *Aloe vera[44]*, tamarind (*Tamarindus indica[45]*) and fruit extract of *Emblica officinalis*[[46], have shown potential in reducing Au(III) ions to form gold nanoparticles Au(0) and silver nitrate to form silver nanoparticles Ag(0). Biomasses of wheat (*Triticum aestivum*)[47] and oat (*Avena sativa*)[48],alfalfa (*Medicago sativa*)[49], native and chemically modified hop biomas [50], and remnant water collected from soaked Bengal gram bean (*Cicer arietinum*)[51] have also been used for gold nanoparticles synthesis. However, alfalfa (*Medicago sativa*), *Chilopsis linearis [*52], and *Sesbania* seedlings showed synthesis of gold nanoparticles inside living plant parts. However, alfalfa (*Medicago sativa*) sprouts[53] and *Brassica juncea* germinating seeds[54] have been used for silver and Ag–Au–Cu alloy nanoparticle synthesis. We have tabulated these various plants exploited for the synthesis of gold and silver nanoparticles (Table 2). In characterizing the components of living organisms involved in such nanoparticle synthesis, the role of reductases and reducing equivalents has been discovered. In one such example nitrate reductase from a fungus (*Fusarium oxysporum*) has been documented to catalyze the reduction of silver nitrate to silver nanoparticles utilizing NADPH as reducing agent. Besides these extracellular enzymes, several naphthoquinones and anthraquinones with excellent redox properties have been reported in *F.oxysporum* that could act as an electron shuttle in metal reductions. These studies have demonstrated the involvement of proteins, polyphenols and carbohydrates in the synthesis of metal nanopartilces. All these constituents are present in plants and might be responsible for the synthesis of metal nanoparticles.However, in plants the involvement of such constituents in nanoparticle synthesis needs experimental proof. Isolated quercetin (natural plant pigment) and polysaccharides have been used for silver nanoparticle synthesis. The influence of different factors on the formation of nanoparticles in freshly brewed tea extracts has been investigated. The mean particle size and number of nanoparticles increase with decreasing temperature. In the presence of caffeine more particles were formed, while theophylline catalyzes synthesis of fewer nanoparticles. This suggests that these various compounds could be responsible for various kinds of nanoparticle

synthesis through biogenic routes. Nanoparticle application in catalysis, sensors and medicine depends critically on the size and composition of the nanoparticles. Thus different routes leading to the synthesis of nanoparticles of various shapes and sizes have extended the choice of properties that can be obtained.The optoelectronic and physiochemical properties of nanoscale matter are a strong function of particle size. Nanoparticle shape also contributes significantly to modulating their electronic properties. In the subsequent sections we have described the use of plants or their extracts in the synthesis of gold, silver and gold/silver nanoparticles in a controlled manner for various purposes.

TABLE 2-

PLANTS USED IN THE SYNTHESIS OF SILVER AND GOLD NANOPARTICLES:

Plants	Silver and/or Gold nanoparticles	Size
Medicago sativa	Gold	4–10 nm 15
Chilopsis linearis	Gold	1.1 nm 36
Pelargonium Graveolens	Gold	21–70 nm
Hop biomass	Gold	17 Å,
Avena sativa	Gold	17 Å,
Cicer arietinum	Gold	35 nm
Tamarindus indica	Gold	14 nm
Triticum aestivum	Gold	*9* nm
Sesbania	Gold	10–30 nm
Medicago sativa	Silver	6–20 nm
Aloe vera	Silver and gold	8 nm
Emblica officinalis	Silver and gold	10–20 nm, 15–25 nm

1.8-BIOSYNTHESIS OF NANOPARTICLES:

1.8.1 BOTH SILVER AND GOLD:

In addition to the individual synthesis of either silver or gold nanoparticles, plants have been reported for their potential for both silver and gold nanoparticle synthesis. Sun-dried biomass of *Cinnamomum camphora* leaf when incubated with aqueous silver or gold precursors at ambient temperature produces both silver nanoparticles (55–80 nm) and triangular or spherical gold nanoparticles. The marked difference in shape of

gold And silver nano particles could be attributed to the comparative potential of protective and reductive biomolecules from leaf extracts. The polyol and water-soluble heterocyclic components were mainly responsible for the reduction of silver ions or chloroaurate ions.Neem (*Azadirachta indica*) leaf broth has also been used for the extracellular synthesis of pure metallic silver, gold and bimetallic Au/Ag nanoparticles. Use of neem leaf extract for nanoparticle synthesis has an advantage in terms of the rapid formation of stable silver and gold nanoparticles at higher concentrations. The silver and gold nanoparticles were polydisperse, with a large percentage of gold particles exhibiting an interesting flat, plate like morphology, while silver nanoparticles formed in the mixtures were spherical, polydisperse and of 5 to 35 nm in diameter. This characteristic of competitive reduction of Au3+ and Ag+ ions by neem leaf extract leads to the synthesis of bimetallic Au core–Ag shell nanoparticles ranging in size from 50 to 100 nm.7 However, control over the shape and size of gold and silver nanoparticles has been obtained with the use of *Aloe vera* leaf extract as reducing agent. The extract volume used for the synthesis of nanoparticles and temperature during the reaction had a great impact on the synthesis of characteristic nanoparticles. This biogenic route has been explored for the synthesis of gold nanotriangles and spherical silver nanoparticles. The morphology of such nanoparticles was characterized by transmission electron microscopy TEM, Hence, desired optical properties have been provided to nanoparticles by controlling the reaction conditions. Different constituents of the extract have been reported to be responsible for the synthesis of single crystalline gold nanotriangles with edge lengths of ~350 nm and spherical silver nanoparticles of size 15.2 ± 4.2 nm..In the main, leaf extracts of plants have been used for the synthesis of silver and gold nanoparticles. However, amla (*Emblica officinalis*), an Indian gooseberry fruit extract, showed potential for extracellular synthesis of gold and silver nanoparticles. Reaction of aqueous silver sulfate and chloroauric acid solutions with *Emblica officinalis* fruit extract leads to rapid reduction of the silver and chloroaurate ions to highly stable silver and gold nanoparticles. TEM images confirmed the formation of silver (10–20 nm) and gold (15–25 nm) nanoparticles. Interestingly, these Ag and Au nanoparticles were phase transferred into an organic solution, i.e., cationic surfactant octadecylamine. Transmetallation reaction between hydrophobized silver nanoparticles and hydrophobized chloroaurate ions in chloroform resulted in the formation of gold nanoparticles. Plants have also been used for the synthesis of intracellular alloy nanoparticles. Mixed alloy nanoparticles of Au, Ag and Cu metals showing good specificity and reactivity have been synthesized by living *Brassica juncea* plants. For this, seeds of *Brassica juncea* were sown in soil enriched with gold chloride, silver nitrate and copper chloride solutions. After 14 days' growth on such metal ion-enriched soil, the biomass was harvested and dried at 110 °C,. Their analysis has documented that about half of the gold was in the form of metal and the remaining half in the form of soluble Au+ salt. Further, a STEM-EDX map has shown that all three elements – Au, Ag and Cu – were present in all the nanoparticles and their size ranged from 5 to 50 nm. However, the composition of these three elements varied depending on the size of the nanoparticles. The larger nanoparticles contained 20–55 atom% of Au, 80–44 atom% of Ag and up to 1 atom% of Cu,while smaller nanoparticles contained 43 atom% of Au, 57 atom% of Ag and less than 1% of Cu.

1.8.2 SILVER:

Plants or their extracts have also been explored in the synthesis of silver nanoparticles alone using silver ions as substrate. Aqueous silver nitrate solution, after reacting with geranium leaf extract, led to rapid formation of highly stable, crystalline silver nanoparticles (16–40 nm), which assembled in the reaction medium into quasilinear superstructures. The rate of nanoparticle synthesis was very high,which justifies use of plants over microorganisms in the biosynthesis of metal nanoparticles through greener and safer methods. Control of the size of silver nanoparticles has been shown to be a function of reaction time. In silver nanoparticles synthesized by treating silver ions with *Capsicum annuum* L. extract, the crystalline phase of the nanoparticles changed from polycrystalline to single crystalline and their size increased with increasing reaction time. Five hours reaction time led to spherical and polycrystalline shaped nanoparticles (10±2 nm).With increase in reaction time to 9 h and 13 h, the size of the nanoparticles was increased to 25±3 nm and40±5 nm, respectively. Identifying the responsible molecules involved in the synthesis of nanoparticles, about 3 nm protein moieties have been found to be capping the silver nanoparticles. Furthermore, amine groups containing proteins were found to cause the reduction of silver ions, leading to silver nanoparticle synthesis in the solutions.40 Interestingly, alfalfa plants have been shown to be an efficient in *in vivo* synthesis of silver nanoparticles. Here, roots were capable of absorbing Ag(0) from the agar medium and transferring it to the shoot. There silver atoms arrange themselves by undergoing nucleation to form nanoparticles. The nucleated nanoparticles further join to form larger arrangements, suggesting different organization levels. TEM dark field images of such nanoparticles showed various size silver nanoparticles that were connected by non-crystalline silver atomic wires. Regulation of the size and amount of silver nanoparticles synthesis has been documented by using stems of the rice-paper plant as reducing and stabilizing agents. Such control was possible by varying the reaction conditions. Under optimized conditions, the content of silver particles of size below 100 nm in the matrix can reach as

high as 1.8% by weight. Different-sized silver nanoparticles synthesized through this route have been tested for their antimicrobial activity. The antimicrobial activities intermsofminimum inhibitory concentration (MIC) for these nanoparticle composites were 14.1 mg (Ag) L−1 and 28.1 mg (Ag) L−1 for *Escherichia coli* and *Candida albicans*, respectively, in agar gel. These values are comparable to that for colloidal nanosilver.41 Recently efforts have been made towards identifying the molecules involved in synthesis of silver nanoparticles. It was observed that plant quercetin (3,5,7,3_,4_-pentahydroxyflavon, C15 HgO7O2H2O) was involved in very quick, simple and highly stable nanoparticle synthesis. Quercetin catalyzes such synthesis in thepresence of an air oxygen inwater : AOT:*n*-alkane system. For this, a micellar solution of quercetin was prepared by adding the solid pigment to 0.15 mol L−1 AOT solution in either octane or heptane. After achieving a stationary value of quercetin concentration in micellar solution, an adequate volume of this solution was added to a water solution of AgNO3 or Cu(NO3)2 to the extent necessary to obtain the desired salt concentration and hydration level. The whole system was shaken for 3–5 min and formation ofmetal nanoparticles was indicated by the appearance of an intensive red-brown coloration. It was found that particles were of very small size and with increasing time or quercetin concentration there was no increase in particle size; however, increase in number of particles per unit volume was obtained.

1.8.3 GOLD:

A very simple procedure was followed in the synthesis of gold nanoparticles using geranium (*Pelargonium graveolens*) leaf extract. Leaves were finely cut and boiled with water. A small quantity of leaf broth was inoculated with an aqueous solution of 1 mmol L−1 chloroauric acid (HAuCl4) and allowed to react for 2 h. This short exposure of leaf broth with aqueous chloroaurate ions caused a rapid reduction of the metal ions leading to the formation of stable gold nanoparticles of variable size and shapes such as rods, flat sheets and triangles.Control over shape and size of gold nanoparticles has been achieved using lemongrass (*Cymbopogon flexuosus*). Anisotropic gold nanotriangles have been synthesized by the reaction of lemongrass extract with aqueous gold ions. Size of the gold nanotriangles was controlled by varying the concentration of the lemongrass extract in the reaction medium. With increasing amounts of extract added to the HAuCl4 solution the average size of the triangular and hexagonal particles decreased, while the ratio of the number of spherical nanoparticles to triangular/hexagonal particles increased. In contrast to geranium, synthesis of gold triangles with 1 mmol L−1 aqueous chloroaurate (HAuCl4) solution took longer and completed in 48 h. The gold nanotriangles synthesized by the lemongrass plant seem to grow by a process involving rapid reduction, assembly and room temperature sintering of 'liquid-like' spherical gold nanoparticles. The anisotropy in nanoparticle shape results in large near-infrared absorption by the particles and highly anisotropic electron transport in films of the nanotriangles. Such nanotriangles could be building blocks for the synthesis of electrically conductive thin films (coatings), which can be used effectively in vapor sensing. Similarly, bioreduction of HAuCl4 by tamarind leaf extract led to the formation of flat and thin single crystalline gold nanotriangles with unique and highly anisotropic planar shapes. These may find application in photonics, optoelectronics and optical sensing. Proteins and biomolecules from Bengal gram beans (*Cicer arietinum*) mediate the bioreduction of aqueous Au(III) ions directing the formation of triangular gold prisms. Control of the morphology of gold particles has been achieved by varying compositions of gram bean extract and aqueous Au(III) solution. This represents an environmentally friendly and economic gold nanoparticle biosynthesis. Furthermore, the rate of nanoparticle synthesis with this bean has been found to be comparable to chemical methods. Thoroughly washed and finely cut leaves of *Pelargonium graveolens* have also been used in gold nanoparticle synthesis. Leaves were boiled, leading to rupture of leaf cell walls and thus release of reducing agents along with intracellular material. The filtered broth was incubated with 1 mmol L−1 HAuCl4 aqueous solution. The appearance of a pink-ruby red color indicated gold nanoparticle synthesis. Most probably, the stabilizing/reducing molecules were citronellol and geraniol, which are known to be present in larger concentrations in *Pelargonium graveolens* leaves. TEM images show multiply twinned (MT) decahedral and icosahedral shaped particles ranging in size from 20 to 40 nm, which probably serve as seeds for the growth of nanorods. Truncated triangular (prismatic) gold nanoparticles were frequently observed in higher-magnification TEM images. Rod-like and prismaticmorphology had created some new optical, electronic and catalytic properties in gold nanoparticles.4 Alfalfa (*Medicago sativa*) biomass also efficiently reduce gold(III) ions to gold(0) nanoparticles of different types such as fcc tetrahedral, hexagonal platelet, icosahedral multiple twinned, decahedral multiple twinned, and irregular shaped particles. Among these, icosahedral and irregular particles were abundant or more frequently formed. One possibility for the formation of larger particles could be coalescence of smaller particles Use of ground dry wheat (*Triticum aestivum*) biomass with 0.3 mmol L−1 potassium tetrachloroaurate solution at various pH values (2–6) for 3.5 h caused bioreduction of Au(III), leading to the synthesis of gold nanoparticles (10–30 nm). Various shaped nanoparticles have been observed, such as fcc tetrahedral, fcc hexagonal platelets, irregular shaped, rod shaped, decahedral multiple twinned, and icosahedral multiple twinned shapes. A similar pH-dependent synthesis of various shaped gold nanoparticles

has also been shown with oat (*Avena sativa*) biomass. In this, the biomass and a solution of Au(III) was reacted for a period of 1 h at pH values of 2–6. The Au(III) ions binding to biomass have been found to be pH dependent and the highest adsorption (~80%) occurred at pH 3. Smaller nanoparticles, in fair amounts, were observed at pH 3 and 4, whereas larger nanoparticles were observed at pH 2. Nanoparticles were fcc tetrahedral, decahedral, hexagonal, icosahedral multiple twinned, irregular, and rod shaped. Rapid extracellular formation of gold nanoparticles of short duration has also been shown using *Sargassum wightii*.37 Control over the size of gold nanoparticles has been achieved using native and chemically modified hop biomasses. Au(III) was reduced to Au(0) by approximately 81%, 70%, and 83% by the native, esterified, and hydrolyzed hop biomass, respectively. However, the average particle radii of gold nanoparticles synthesized by native hops biomass was 17.3 Å and size was reduced further to 9.2 Å by using esterified hop biomass, while with hydrolyzed hop biomass the size of gold particles was increased to~25 Å. Carboxyl or other oxygen-containing ligands in the esterified hop biomass have been found to be responsible for the binding and reduction of gold(III) to gold(0).13 In contrast to the above-described plants, sweet desert willow (*Chilopsis linearis*) has been demonstrated the ability for intracellular gold nanoparticle synthesis. This plant has the capability to take up gold (Au) from gold-enriched media (160 mg Au L−1 in agar) and synthesized nanoparticles of average size of 8, 35, and 18 Å in root, stem, and leaves, respectively. The average size of the Au nanoparticles formed by various tissues has been found to be related to the concentration of Au accommodated in tissues and their location in the plant. Intracellular nanoparticle synthesis ability documents its potential use for phytoextraction applications. This ability could further be enhanced by providing thiocyanate. However, using this plant,very small size nanoparticles (0.55 nm) could alsobe synthesized. Similarly, growth of *Sesbania* seedlings in chloroaurate solution resulted in the accumulation of gold with the formation of stable gold nanoparticles in plant tissues. TEM analysis revealed the intracellular distribution of monodisperse nanospheres. This study has proposed that reduction of the metal ions was catalyzed by secondary metabolites present in cells.

1.9-CHARACTERIZATION OF NANOPARTICLES AND ASSOCIATED MOLECULES:

Microscopic techniques such as scanning electron microscopy, transmission electron microscopy and atomic force microscopy are mainly used for morphological studies of nanoparticles. Before morphological studies are carried out, there is need to standardize the synthesis of nanoparticles using plants or their extracts. The formation of various nanoparticles from their different salts gives characteristic peaks at different absorptions that can be monitored using UV-vis spectroscopy. For example, silver nanoparticles formation from silver ions show an absorption peak around 450 nm, while gold nanoparticles show an absorption peak around 550 nm. Similarly, several other metal nanoparticles give characteristic absorption peaks. A progressive increase in the characteristic peak with increase in reaction time and concentration of plant extracts with salt ions is a clear indicator of nanoparticle formation. UV-vis absorption spectra show peaks characteristic of the surface plasmon resonance of nano sized particles.4–19 The X-ray diffraction (XRD) technique is used to establish the metallic nature of particles. X-rays are electromagnetic radiation with typical photon energies in the range of 100 eV–100 keV. For diffraction applications, only short-wavelength X-rays (hard X-rays) in the range of a few angstroms to 0.1 Å (1–120 keV) are used. Because the wavelength of X-rays is comparable to the size of atoms, they are ideally suited for probing the structural arrangement of atoms and molecules in a wide range of materials.The energetic X-rays can penetrate deep into the materials and provide information about the bulk structure. However, Fourier transform infrared (FTIR) spectroscopy is a chemical analytical technique, which measures infrared intensity *versus* wavelength (wave number) of light. It is used to determine the nature of associated molecules of plants or their extracts with nanoparticles. Based upon the wave number, infrared light can be categorized as far infrared (4–400 cm−1), mid infrared (400–4000 cm−1) and near infrared (4000–14 000 cm−1). Infrared spectroscopy detects the vibration characteristics of chemical functional groups ina sample.When an infrared light interacts with matter, chemical bonds will stretch, contract and bend. As a result, a chemical functional group tends to adsorb infrared radiation in a specific wavenumber range regardless of the structure of the rest of the molecule. For example, the CO stretch of a carbonyl group appears at around 1700 cm−1 in a variety of molecules. Hence, the correlation of band wave number position with chemical structure is used to identify a functional group in a nanoparticle associated molecule in a sample. The wave number positions where functional groups adsorb are consistent, despite the effect of temperature, pressure, sampling, or change in molecular structure in other parts of the molecules. A FTIR spectrometer obtains infrared spectra by first collecting an inter ferogram of a sample signal with an interferometer, which measures all of the infrared frequencies simultaneously. A FTIR spectrometer acquires and digitizes the interferogram, performs the FT function, and outputs the spectrum. This technique has been used in the characterization of silver

and gold nanoparticles and their associated molecules from plant extracts in various studies. Raman spectroscopy is a widely used tool to characterize material composition, sample temperature, and strain from analysis of the material-specific phonon mode energies. It requires very little sample preparation and a rapid, non-destructive optical spectrum is easily achieved. Raman spectroscopy is conventionally performed with green, red or near-infrared lasers. These wavelengths are below the first electronic transitions of most molecules, as assumed by scattering theory. The situation changes if the wavelength of the exciting laser is within the electronic spectrum of a molecule. In that case the intensity of some Raman-active vibrations increases by a factor of 102–104. This resonance enhancement or resonance Raman effect can be quite useful. The selection of nanoparticles for achieving efficient contrast for biological and cell imaging applications, as well as for photo thermal therapeutic applications, is based on the optical properties of the nanoparticles. It has been described by the use of Mie theory and the discrete dipole approximation method to calculate absorption and scattering efficiencies and optical resonance wavelengths for three commonly used classes of nanoparticles: gold nanospheres, silica–gold nanoshells, and gold nanorods. The calculated spectra clearly reflect the well known dependence of nanoparticle optical properties, viz. the resonance wavelength, the extinction cross-section, and the ratio of scattering to absorption, on the nanoparticle dimensions.Use of surface-enhanced Raman spectroscopy (SERS) in new material characterization, concept development and in identifying their applications has been thoroughly reviewed.

1.10-APPLICATIONS:

As we know, production of nanoparticles can be achieved through different methods. Chemical approaches are the most popular methods for the production of nanoparticles. However, some chemical methods cannot avoid the use of toxic chemicals in the synthesis protocol. Since noble metal nanoparticles such as gold, silver and platinum nanoparticles are widely applied to human contact areas, there is a growing need to develop environmentally friendly processes of nanoparticle synthesis that do not use toxic chemicals. Therefore, biological methods of nanoparticle synthesis using plant or plant extract in addition to microorganisms have been suggested as possible ecofriendly alternatives to chemical and physical methods. Using plants for nanoparticle synthesis can be advantageous over other biological processes by eliminating the elaborate process of maintaining cell cultures. It can also be suitably scaled up for large-scale synthesis of nanoparticles. These reasons make the biological synthesis of nanoparticles more valuable, though people are thinking that their use is similar to that gained by chemical methods. This article, however, will give a positive message that nanoparticles synthesized through greener routes are much safer for human use. We have further compiled a few applications where such nanoparticles can be used in a safer mode for various human applications. The use of gold nanoparticles dates back to the 16th century, for both medical and staining purposes. Thereafter, gold nanoparticles have found application in analytical methods such as colorimetric techniques for the determination of heavy metal ions in aqueous solutions.Gold nanoparticles possess catalytic activity, and hence are used for reactions such as the water gas shift reaction and selective oxidation of CO.[55,56,57] Gold nanoparticles also used in the field of sensors [58,59] In biology, gold nanoparticles are used for the development of biosensors, DNA labels[60] and in medicine.[61] However, spherical gold nanoparticles have been used to generate functional electrical coatings. In medical applications, gold nanoparticles have been used in treating B-chronic lymphocytic leukemia (CLL). CLL is an incurable disease predominantly characterized by apoptosis resistance. Earlier CLL treatment was with anti-VEGF antibody; however, treatment was found to be more effective when VEGF antibody was attached to the gold nanoparticles. Apoptosis with gold–AbVF was higher than with the CLL cells exposed only to VEGF antibody or gold nanoparticles. Non-coated gold nanoparticles alone were able to induce some level of apoptosis in CLL B-cells. Thus gold nanoparticles could be used for treating CLL. Gold nanoparticles have also been used in the Carter-Wallace home pregnancy test 'First Response'. This uses conventional micrometer-sized latex particles in conjunction with gold nanoparticles (<50 nm diameter),which make them pink. The micro-and nanoparticles are derivatized with antibodies to human chorionic gonadotrophin, a hormone released by pregnant women. When mixed with a urine sample containing this hormone, the micro- and nanoparticles are co-agglutinated and the resulting clumps are colored pink.[62] The plasmon resonance absorption of colloidal gold particles has been exploited in a proposed DNA detection method.[63] .A unique, sensitive, and highly specific immunoassay system for antibodies using gold nanoparticles has been developed.[64] Crumbliss and co-workers have found that adsorption of redox enzymes to colloidal gold causes no loss of enzymatic activity. The enzyme-covered nanoparticles when electrodeposited onto platinum gauze or glassy carbon resulted in synthesis of enzyme electrodes.[65] Owing to the distinct biological activity of silver nanoparticles, they have found application in ecology and medicine. Silver nanoparticles showed good antimicrobial activity and therefore can be used for purification in water-filtering apparatus [66] The interaction of metallic nanoparticles with biomolecules, microorganisms and viruses is another expanding field of research. It was noticed that silver nanoparticles undergo a size-dependent interaction with HIV-1.

Nanoparticles ranging in size from 1 to 10 nm readily interact with the HIV-1 virus via preferential binding to gp120 glycoprotein knobs. This specific interaction of silver nanoparticles inhibits the virus from binding to host cells, demonstrated by *in vitro* study. Hence, silver nanoparticles could find application in preventing as well as controlling HIV infection. Silver nanoparticles also find application in topical ointments and creams used to prevent infection of burns and open wounds.[67] Another widely used application is in medical devices and implants prepared with silver-impregnated polymers. In addition, silver-containing consumer products such as colloidal silver gel and silver-embedded fabrics are now used in sporting equipment.[68] Hydrophobic Ag–Au composite nanoparticles show strong adsorption and good electrical conducting properties, and therefore can be used in enzyme electrode design. These nanoparticles can assist electron transfer between the enzyme and the bulk electrode surface. In these composite particles, current response of electrodes with molar ratios silver (50)–gold (50) and silver (25)–gold (75) was higher than that with silver (75)–gold (25) molar ratio.Gold and silver nanoparticles have also found application in surfaced-enhanced Raman spectroscopy (SERS). Use of SERS surfaces prepared by self-assembly surfaces prepared by self-assembly of gold and silver nanoparticles on glass and other substrates has shown a high degree of reproducibility. A novel, direct, rapid, and label-free electrochemical immunoassay based on a core/shell Ag–Au nanoparticle monolayer as sensing interface has been developed for probing IgG.50 Use of such nanoparticles suggests that they may be explored in many more applications for human benefit.

1.11-GOLD NANOPARTICLES IN CANCER DIAGNOSIS AND THEARPY:

It is important to understand the difference between normal and cancerous tissue to effectively develop hybrid nanoparticles in cancer diagnosis and therapy. Normal tissues have tight, continuous vessel walls interspersed with 9 nm pores frequently and 50 nm pores infrequently. Therefore, small molecules can easily penetrate all types of tissues in contrast to large molecules such as polymers that do so very slowly. However, tumor tissues, inflammatory tissues, and reticuloendothelial system (RES)-rich organs, such as the liver, spleen and bone marrow have discontinuous capillary walls and a large number of ~ 100 nm pores. Additionally, these discontinuous capillary walls have no basal lamina allowing particles less than 100 nm to penetrate easily [69]. Interestingly, tumor tissues lack a lymphatic system for eliminating lipophilic and polymeric materials from them [70]; therefore, once the particles penetrate the tumor tissues, they cannot be eliminated easily. Accordingly, tumors exhibit enhanced penetration and retention effect (EPR effect) for 50-100 nm particles [71]. AuNPs conjugated to appropriate tumor avid biomolecules with mean sizes in 50-100 nm range are ideal for targeting tumors for imaging and therapy purposes. Connor *et al.* have shown that gold nanoparticles are inherently non-toxic to human cells despite being taken up into the cells. However some precursors used to generate nanoparticles might be toxic [72]. This result is significant for the toxicity of gold nanoparticles can be controlled by using non-toxic reagents to produce them. Optical and electronic properties of AuNPs can be utilized to enhance the contrast in molecular imaging for the detection of cancer at early stages. For example, AuNPs labeled with monoclonal antibodies against EGFR (epidermal growth factor receptor) that are over-expressed in skin cancer were shown to localize on the abnormal cervical SiHa cell lines by imaging via endoscope-compatible microscopies, such as optical coherence tomography and reflectance confocal microscopy [73, 74]. The AuNPs appeared as bright spots on the surface of cell lines in a bright field image. Hainfeld *et al.* have demonstrated the use of gold nanoparticles as X-ray contrast agents in imaging breast cancer. 1.9 nm AuNPs were injected via a tail vein into Balb/C mice bearing EMT- 6 subcutaneous mammary tumors and imaged by a clinical mammographic unit. A 5 mm tumor growing in one thigh was clearly evident from its increased vascularity and resultant higher gold content in the X-ray image . Treatment of cancer has different routes such as chemotherapy, photo-thermal therapy and radiotherapy. AuNPs have been investigated for potential candidates to assist in photo-thermal therapy and radiotherapy. O'Neal *et al.* examined the feasibility of nanoshell-assisted photo-thermal therapy (NAPT). Polyethylene glycol (PEG) coated nanoshells, (<130 nm diameter) consisting of silica core with a gold shell and exhibiting an absorption peak in the 805-810 nm region, were injected intravenously into a mice bearing subcutaneously grown colon tumor. The nanoshells accumulate at the tumor site due to enhanced penetration and retention effect (EPR effect) over a 6 hr period. Subsequently, the tumors were exposed to NIR light (808 nm diode laser, 800 mW) resulting in absorption of infra-red light by nanoshells and consequent generation of heat that caused irreversible damage to the tumor. In this study, the nanoshell-assisted photothermal treated tumor displayed complete regression and these mice remained healthy and tumor-free for < 90 days following treatment, unlike the sham group (exposure to NIR alone) and the control group (no injection and exposure) where the tumors kept growing . This outcome provides impetus to develop AuNPs with NIR absorbance for effective treatment of cancer. Hainfeld *et al.* demonstrated that the irradiation of AuNPs accumulated in tumor with 250 kVp X-rays caused shrinkage of tumor in mice with subcutaneously grown mammary carcinoma tumor. It was also found that that treatment with X-rays alone had no therapeutic effect on the tumor]. This study illustrates the possible use of AuNPs in radiotherapy with use of X-rays. These examples of AuNPs in cancer therapy demonstrate the potential of AuNPs in therapy but are yet to comprise target specificity by their conjugation to suitable biomolecules. Recent work by Kannan and Katti *et al.* investigated gum arabic labeled radioactive

AuNPs that localize in liver. This study combines the therapeutic property of radioactive gold 198Au (max = 0.96 MeV, t1/2 = 2.7 days) and target specific biomolecule to form a powerful radiopharmaceutical for targeted drug delivery. This gum Arabic labeled radioactive gold can be used to treat liver cancers with higher radiation dose inherent to radioactive nanoparticles that contain thousands of radioactive atoms .

OBJECTIVE:

The objective of the present study are as follows:

1. Synthesis of gold nanoparticles using black peeper and Liquorice root extract.
2. Characterization of gold nanoparticles using XRD and TEM.

CHAPTER 2

SYNTHESIS OF GOLD NANOPARTICLES USING "BLACK PEEPER" EXTRACT

Scientific classification

Kingdom: Plantae

Family: Piperaceae

Genus: Piper

Species: P. nigrum

Binomial name Piper nigrum

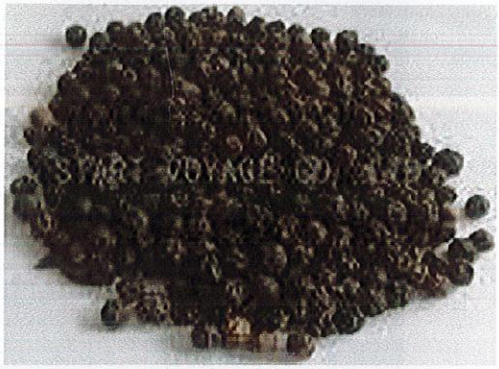

Figure 2.1 Black peeper

Black pepper (Piper nigrum) is a flowering vine in the family Piperaceae, cultivated for its fruit, which is usually dried and used as a spice and seasoning. The fruit, known as a peppercorn when dried, is a small drupe approximately 5 millimetres (0.20 in diameter), dark red when fully mature, containing a single seed. Peppercorns, and the powdered pepper derived from grinding them, may be described simply as pepper, or more precisely as black pepper, white pepper, or green pepper. Black pepper is native to South India and is extensively cultivated there and elsewhere in tropical regions

2.1-Materials- Tetrachloroauric acid purchased from sigma –aldrich ,we got black peeper(***Piper nigrum,*** Family Piperaceae*)* from the local market.Water used throughtout experiment taken from Milipore.

2.2-Preperation of black peeper extract

5 gms of black peeper purchased from the local market was washed properly in running water several times ,then washed in autoclaved water to remove impurites, then added to 10 ml of water and put overnight at room temperature, next morning the liquid was separated using musilin cloth and black peeper seeds were discarded, in the following step the broth was centrifuged at 15000 rpm for 30 minutes to separate the remaing impurities.

2.3 -Biosynthesis of gold nanoparticles using black peeper extarct

1ml of black peeper extract prepared from above method added into 10 ml of 2 milimolar tetrachloro auric acid solution, then the solution was incubated for 6 hours for complete bioreduction.

2.4-Generation of gold nanoparticles at different pH

The pH of 2 mili molar tetrachloro auric acid and the black peeper extract were 2.65 and 6.3 respectively. Now four samples are generated using stock solutions solutuion NaoH and Hcl,the resultant pH of the samples were 5,7 ,8 and 10.

2.5-Result and discussion:

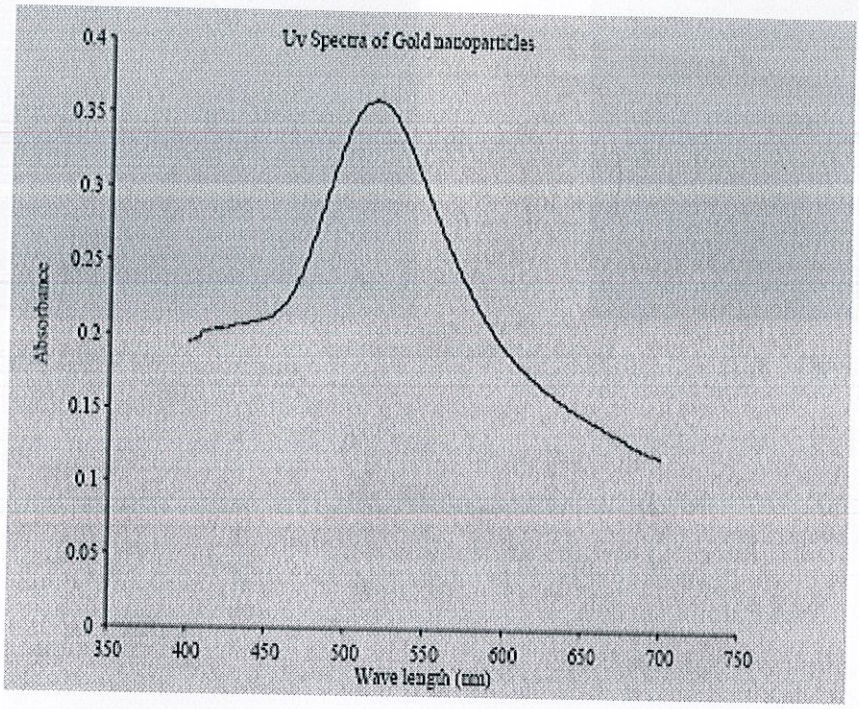

Figure 2.2: UV-Visible spectrum of gold nanopartiles

2.5.1.1 Uv-visible spectrophotometer-The bioreduction of the samples was monitored by periodic sampling of aliquots and measuring the uv-ivisible spectra in 10 mm optical path length quartz cuvettes with Perkin –Elmer UV –visible spectrophotometer .

Here we successfully generated gold nanoparticles using black peeper. Red wine colour appeared after one hour of addition of black peeper extract ,which is visible in the form of vertical red bar ,which goes on increasing with time and after the complete reduction it totally converted into the red.the red colour is due to the surface Plasmon vibrations with goldnanoparticles ,initially solved by Mie in 1908 by solving Maxwell's equation for an electromagnetic light wave interacting with small metallic spheres.The absorbtion peak is 530 nm ,which clearly indicates larger nanoparticles and also supported by peak width,since both absorption and peak width increase with the particle size ,this kind of direct size dependence is termed as extrinsic size effects.

2.5.2-pH study of gold nanoparticle- pH profile experiment were performed plays a very important role in the stability of gold nanoparticles and stability of gold nanoparticles is essentitial for their further use .the initial pH of 2 milimolar gold solution was 2.65 and initial pH of the black peeper extract was 6.3,now with the stock solution of NaoH preapared four samples having pH 5,7,8 and 10 ..Difference in the colour which is clearly visible in the figures(2.3,2.4,2.5 and 2.6) ,shows nanoparticles having different size and shape .one of the most important aspect of our experiment is that the nanoparticles generated at various pH are stable for more than two months ,which was very essential for the next part of our experiment.

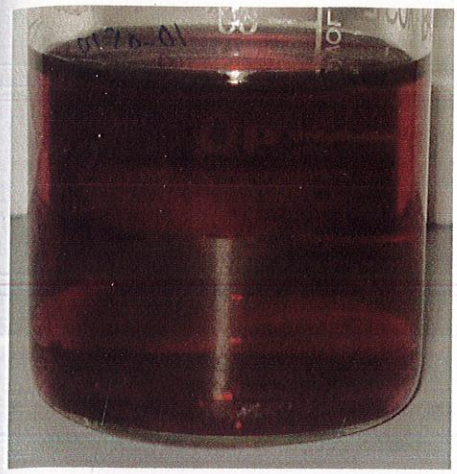

Figure 2.3 (pH 5.0)

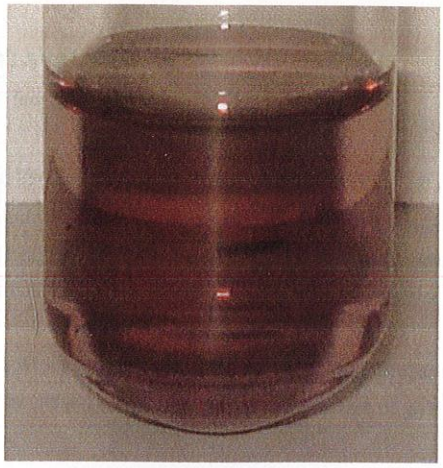

Figure 2.4 (pH 7.0)

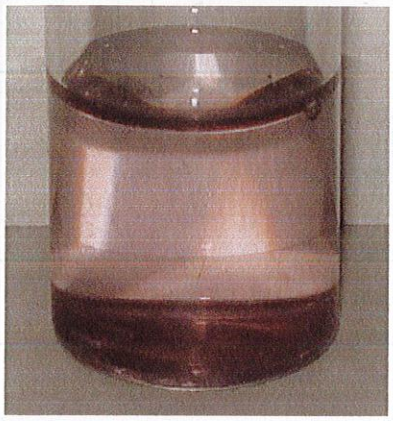

Figure 2.5 (pH 8.0)

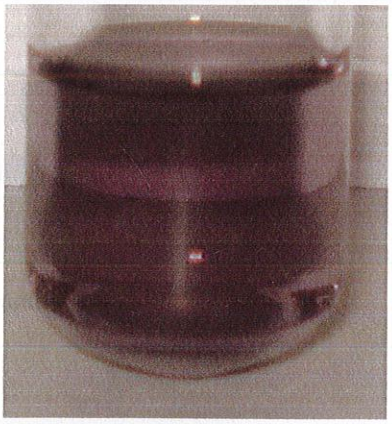

Figure 2.6 (pH 10.0)

2.5.3-TEM(TRANSMISSION ELECTRON MICROSCOPY) ANALYSIS-

Samples are sent for the analysis,and result is expected to come in few days.

2.5.4-XRD(X-RAY DIFFRACTOMETER) ANALYSIS- Performing an XRD analysis consists of measuring the direction and intensities at which crystalline matter diffracts Xrays. Placing an individual crystal in a fixed orientation in a monochromatic X-ray beam can at the best lead to a single diffracted beam, which does not allow identification of the crystal structure. In order to identify the structure, one must present the crystal to the Xray beam under all orientations to record all possible diffracted beams within the angular range covered.With a single crystal, this can be accomplished by rotating the sample about several axes. The most common technique for XRD is called powder diffraction because it uses powdered materials.Here xrd pattern obtained for gold nanoparticles synthesized using black peeper exract.The Bragg reflections corresponding to the various set of planes are observed that may be indxed on the basis of the fcc structure of gold ,the interesting point is that nanocrystals are highly anisotropic in nature.

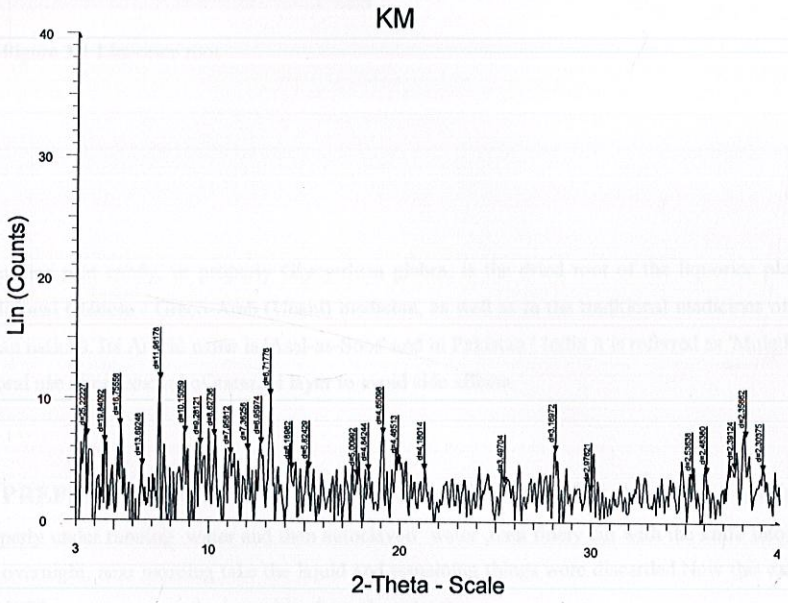

Figure 2.7 Xrd pattern of synthesized gold nanoparticles using black peeper extract

CHAPTER 3

SYNTHESIS OF GOLD NANOPARTICLES USING "LIQUORICE ROOT'EXTRACT

Scientific classification

Kingdom: Plantae

Genus: Glycyrrhiza glabra

Species: G. glab

Figure 3.1 Liquorice root

Liquorice root candy, or properly Glycyrrhiza glabra, is the dried root of the liquorice plant, which is eaten as a candy. It is also used in traditional Chinese / Greco-Arab (Unani) medicine, as well as in the traditional medicines of Japan, Korea, Vietnam, Pakistan, India and other Asian nations. Its Arabic name is 'Asal-as-Soos' and in Pakistan / India it is referred as 'Mulethi'. The Greco-Arab (Unani) Medicine recommend its oral use after removal of external layer to avoid side affects.

3.1PREPERATION OF LIQUORICE ROOT EXTRACT -10 gms of Liquorice root, purchased from local market then washed properly under running water and then autoclaved water ,then finely cut with the knife into small pieces ,then kept into 30 ml distilled water for overnight, next morning take the liquid and remaining things were discarded.Now this extract is taken for the centrifugation for 30 minutes at 12000 rpm ,to remove the impurities from the extract.

3.2-BIOSYNTHESIS OF GOLD NANOPARTICLES USING LIQUORICE ROOT EXTRACT -1 ml of freshly prepared extract is added to 5 ml of 2 mili molar gold solution ,the reduction get started as we add the Liquorice root extract,which is in the form of change of colour from yellow to deep wine red ,which takes 4 hours for the complete reduction.

3. 3-RESULTS AND DISCUSSION-

3.3.1-pH STUDY OF GOLD NANOPARTICLES GENERATED USING LIQUORICE ROOT EXTRACT - Influence of of pH on the synthesized gold nanoparticles is studied,experiment were performed using NaoH and Hcl as stock solutions to make alteration in ph of the original solution.the initial ph of 2 mili molar gold solution was 3.2 and initial pH of the Liquorice root extract was 6.1,four samples having pH 4.3,6.2,8.4 and 10.3 , were generated using the NaoH and Hcl stock solutions.Difference in the colour which is clearly visible in the figures(3.2,3.3,3.4 and 3.5) ,shows nanoparticles having different size and shape ,even in this case gold naoparticles are very much stable .One of the most important point we notice that intensity of colours at different pH is more as compared to the previous case.

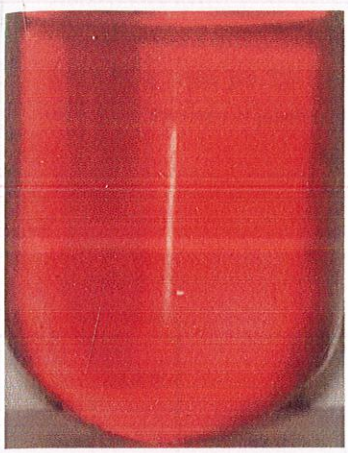

Figure 3.2(pH 4.3)

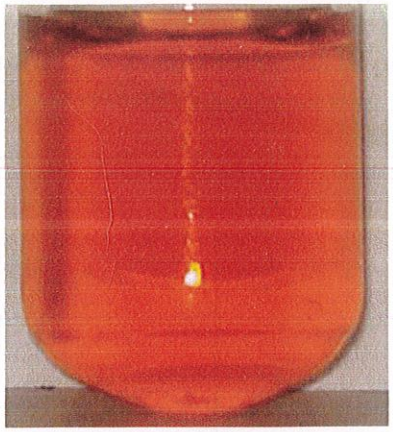

Figure 3.3(pH 6.2)

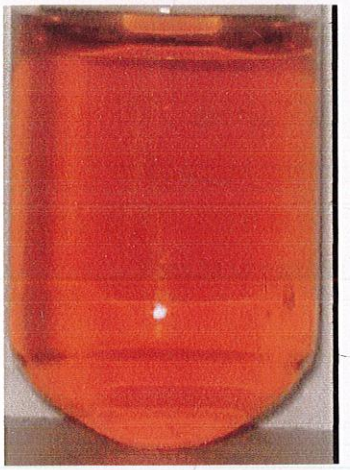

Figure 3.4(pH 8.4)

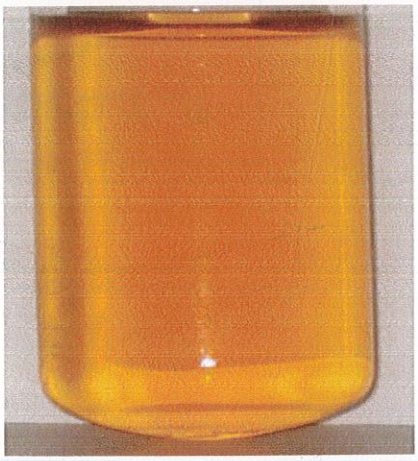

Figure 3.5(pH 10.2)

3.3.2-UV–VISIBLE SPECTRUM ANALYSIS-

Here the uv-visble spectrum of gold nanoparticles is done with ELICO spectrophotometer ,available in our biotechnology lab and the graph were drawn using origin pro 7.5 software .In this case maximum peak is obtained at the 538 nm ,which is 8 nm greater than our previous experiment.the shape of graph is also different from the previous ,which reflects the difference in size and shape of gold nanoparticles.

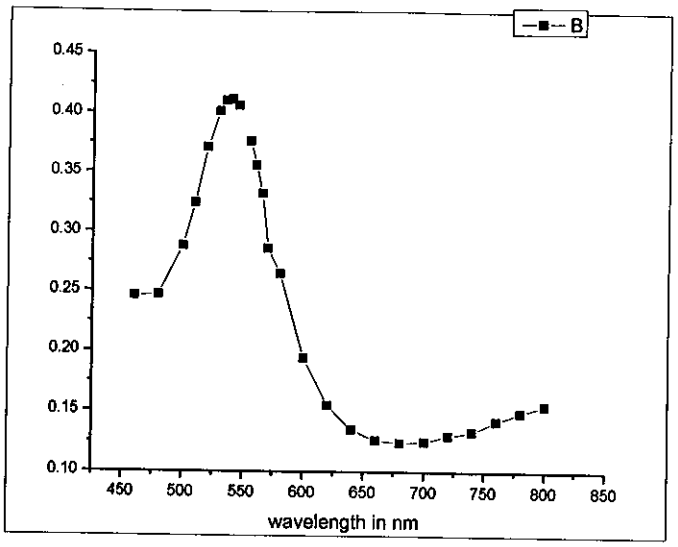

Figure 3.6 Uv-visible of gold nanoparticles

3.3.3-XRD(X-RAY DIFFRACTOMETER) ANALYSIS -XRD is very Important technique for the characterization of gold nanoparticles.in xrd a collimated beam of x-rays ,with a wavelength typically ranging from 0.7 to 2 A,is incident on a specimen and is diffracted by the crystalline phase in specimen according to Bragg's law.XRD pattern obtained for films of gold nanoparticles synthesized using Liquorice root extract on the glass substrate . Number of prominent Bragg reflection can be seen,which indicates fcc structure of gold nanopartiles.

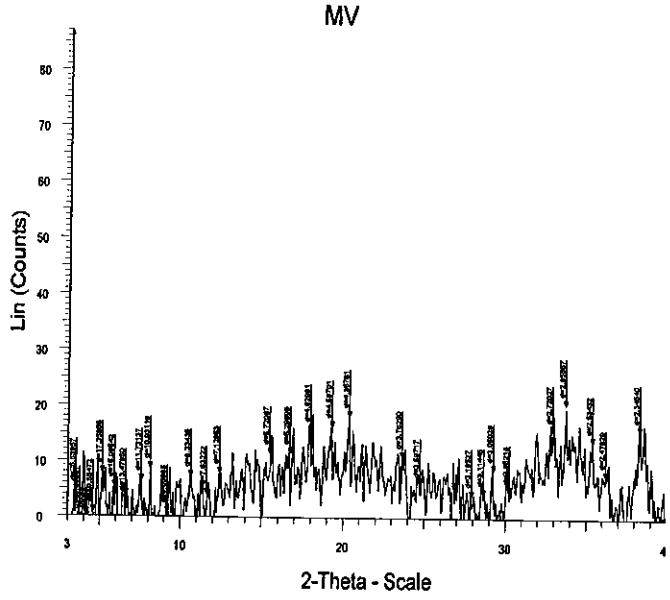

Figure 3.7 Xrd pattern of synthesized gold nanoparticles using

Liquorice root extract

CHAPTER 4

FUTURE DIRECTIONS

Despite the fact that there have been significant developments in anti-cancer technology, such as radiotherapy, chemotherapy and hormone therapy, cancer still remains as the second leading cause of death following heart disease in the United States. Most often, the main cancer treatment is chemotherapy utilizing highly potent drugs, which include mitomycin, paclitaxel and camptothecin. In many cases, these chemotherapeutic agents show a dose responsive effect, and cell kill is proportional to drug exposure .Highly aggressive style of dosing is thus necessary to eradicate neoplasms; however, high-dose chemotherapy is hindered by poor selectivity for cancer cells and severe toxicity to normal cells. Clearly, this lack of tumor-specific treatment is one of the many hurdles that needs to be overcome by current chemotherapy. An ideal solution to current chemotherapy limitations would be to deliver a biologically effective concentration of anti-cancer agents to the tumor tissues with very high specificity. In order to reach this ultimate goal, tremendous amount of efforts were undertaken to develop tumor-selective drugs by conjugating anti-cancer drugs to hormones, antibodies and vitamin derivatives. Among them, one low molecular weight vitamin compound, folic acid, shows a great deal of promise as a tumor-homing agent. Folate is a member of vitamin B family and plays an essential role in cell survival by participating in the biosynthesis of nucleic and amino acids. This essential vitamin is also a high affinity ligand that enhances the differential specificity of conjugated anti-cancer drugs by targeting folate receptor (FR)-positive cancer cells. The FR, a tumor associated glycosylphosphatidylinositol anchored protein, can actively internalize bound folates and folate conjugated compounds via receptor-mediated endocytosis. It has been found that FR is up-regulated in more than 90% of non-mucinous ovarian carcinomas. It is also found at high to moderate levels in kidney, brain, lung, and breast carcinomas while it occurs at very low levels in most normal tissues. The FR density also appears to increase as the stage of the cancer increases Exploiting the above-mentioned facts, it is hypothesized that folate conjugation to anti-cancer drugs will improve drug selectivity and decrease negative side effects. Based on the previous research that folate conjugation allows a drug molecule to target and become endocytosed into FR-positive cancer cells, numerous types of anti-cancer drugs were conjugated and evaluated for their biological activity .Particularly, folate-mitomycin C conjugates, EC72 and EC118, were found to be highly cytotoxic and outstandingly selective for FR-positive M109 cells. In addition, EC72 and EC118 significantly extended lifespan of nu/nu mice with human KB xenografts without evidence of toxicity to major organs or delayed cumulative myelo suppression, the most common negative side effect of mitomycin C. Furthermore, combination therapy with paclitaxel produced a synergistic anti-tumor effect without any apparent adverse effects, suggesting a possibility of adjuvant use of folate conjugated drugs. Overall, performance of EC72 and EC118, both *in vitro* and *in vivo*, proves that folate conjugation enhances drug specificity thereby reducing lethal toxicity.

CONCLUSION

Here we report the successful generation of gold nanoparticles using black peeper extract and Liquorice root extract.Both the experiments are very important ,since we are first who used black peeper and Liquorice root as reducing agents for the synthesis of gold nanoparticles.Nanoparticles generated in both the methods are stable for more than two months.These simple procedures of gold nanoparticles synthesis has several advantages such as cost effectiveness ,compatability for biomedical and pharmaceutical applications as well as large scale commercial production of gold nanoparticles.

BIBLIOGRAPHY

1. Park, J.H., Y.T. Lim, O.O. Park, J.K. Kim, J.W. Yu, and Y.C. Kim, *Polymer/Gold nanoparticle nanocomposite light-emitting diodes: Enhancement of electroluminescence stability and quantum efficiency of blue-light-emitting polymers.* Chemistry of Materials, 2004. 16(4): p. 688.

2. Narayanan, R. and M.A. El-Sayed, *Catalysis with transition metal nanoparticles in colloidal solution: Nanoparticle shape dependence and stability.* Journal of Physical Chemistry B, 2005. 109(26): p. 126

3. Aslan, K., Z. Jian, J.R. Lakowicz, and C.D. Geddes, *Saccharide sensing using gold and silver nanoparticles - a review.* Journal of Fluorescence, 2004. 14(4): 391.

4. Riviere, C., F.P. Boudghene, F. Gazeau, J. Roger, J.N. Pons, et al., *Iron oxide nanoparticle-labeled rat smooth muscle cells: Cardiac MR imaging for cell graft monitoring and quantitation.* Radiology, 2005. 235(3): 959.

5. Eustis, S. and M.A. El-Sayed, *Why gold nanoparticles are more precious than pretty gold: Noble metal surface plasmon resonance and its enhancement of the radiative and nonradiative properties of nanocrystals of different shapes.* Chemical Society Reviews, 2006. 35(3): p. 209.

6. Daniel, M.-C. and D. Astruc, *Gold Nanoparticles: Assembly, Supramolecular Chemistry, Quantum-Size-Related Properties, and Applications toward Biology, Catalysis, and Nanotechnology.* Chemical Reviews (Washington, DC, United States), 2004. 104(1): p. 293.

7. Berciaud, S., L. Cognet, P. Tamarat, and B. Lounis, *Observation of intrinsic size effects in the optical response of individual gold nanoparticles.* Nano Letters,2005. 5(3): p. 515.

8. Neeleshwar, S., C.L. Chen, C.B. Tsai, Y.Y. Chen, C.C. Chen, S.G. Shyu, and M.S. Seehra, *Size-dependent properties of CdSe quantum dots.* Physical Review B, 2005. 71(20).

9. Masumoto, Y. and K. Sonobe, *Size-dependent energy levels of CdTe quantum dots.* Physical Review B, 1997. 56(15): p. 9734.

10. Mock, J.J., M. Barbic, D.R. Smith, D.A. Schultz, and S. Schultz, *Shape effects in plasmon resonance of individual colloidal silver nanoparticles.* Journal of Chemical Physics, 2002. 116(15): p. 6755.

11. Eustis, S. and M. El-Sayed, *Aspect ratio dependence of the enhanced fluorescence intensity of gold nanorods: Experimental and simulation study.* Journal of Physical Chemistry B, 2005. 109(34): p. 16350.

12. Huang, S.H., K. Minami, H. Sakaue, S. Shingubara, and T. Takahagi, *Optical spectroscopic studies of the dispersibility of gold nanoparticle solutions.* Journal of Applied Physics, 2002. 92(12): p. 7486.

13. Katz, E. and I. Willner, *Integrated nanoparticle-biomolecule hybrid systems: Synthesis, properties, and applications.* Angewandte Chemie-International Edition, 2004. 43(45): p. 6042.

14. Niemeyer, C.M., *Nanoparticles, proteins, and nucleic acids: Biotechnology meets materials science.* Angewandte Chemie-International Edition, 2001. 40(22): p. 4128.

15. Pellegrino, T., S. Kudera, T. Liedl, A.M. Javier, L. Manna, and W.J. Parak, *On the development of colloidal nanoparticles towards multifunctional structures and their possible use for biological applications.* Small, 2005. 1(1): p. 48

16. Kell, A.J., R.L. Donkers, and M.S. Workentin, *Core Size Effects on the Reactivity of Organic Substrates as Monolayers on Gold Nanoparticles.* Langmuir, 2005. 21(2): p. 735.

17. Xue, C., G. Arumugam, K. Palaniappan, S.A. Hackney, H. Liu, and J. Liu, *Construction of conjugated molecular structures on gold nanoparticles via the Sonogashira coupling reactions.* Chemical Communications (Cambridge, United Kingdom), 2005(8): p. 1055.

18. Astruc, D., M.-C. Daniel, and J. Ruiz, *Dendrimers and gold nanoparticles as exoreceptors sensing biologically important anions.* Chemical Communications (Cambridge, United Kingdo20. Letsinger, R.L., C.A. Mirkin, R. Elghanian, R.C. Mucic, and J.J. Storhoff, *Chemistry of oligonucleotide-gold nanoparticle conjugates.* Phosphorus, Sulfur and Silicon and the Related Elements, 1999. 144-146: p. 359.

21. Nam, J.-M., S.I. Stoeva, and C.A. Mirkin, *Bio-Bar-Code-Based DNA Detection with PCR-like Sensitivity.* Journal of the American Chemical Society, 2004. 126(19): p. 5932.

22. Balogh, L.P., S.S. Nigavekar, A.C. Cook, L. Minc, and M.K. Khan, *Development of dendrimer-gold radioactive nanocomposites to treat cancer microvasculature.* PharmaChem, 2003. 2(4): p. 94m), 2004(23): p. 2637

25. Chen, J., F. Saeki, B.J. Wiley, H. Cang, M.J. Cobb, et al., *Gold Nanocages: Bioconjugation and Their Potential Use as Optical Imaging Contrast Agents.* Nano Letters, 2005. 5(3): p. 473.

26. Hainfeld, J.F., D.N. Slatkin, T.M. Focella, and H.M. Smilowitz, *Gold nanoparticles: a new X-ray contrast agent.* British Journal of Radiology, 2006. 79(939): p. 248.

27. Storhoff, J.J., S.S. Marla, P. Bao, S. Hagenow, H. Mehta, et al., *Gold nanoparticle-based detection of genomic DNA targets on microarrays using a novel optical detection system.* Biosensors & Bioelectronics, 2004. 19(8): p. 875.

28- Krolikowska A, Kudelski A, Michota A, Bukowska J (2003) SERS studies on the structure of thioglycolic acid monolayers on silver and gold. Surf Sci 532:227-232

29- Kumar A, Mandal S, Selvakannan PR, Parischa R, Mandale AB, Sastry M (2003) Investigation into the interaction between surface-bound alkylamines and gold nanoparticles. Langmuir 19:6277-6282

30-Chandrasekharan N, Kamat PV (2000) Improving the photoelectrochemical performance of nanostructured TiO_2 films by adsorption of gold nanoparticles. J Phys Chern B 104: 10851-1 0857

31- Peto G, Molnar GL, Paszti Z, Geszti 0, Beck A, Guczi L (2002) Electronic structure of gold nanoparticles deposited on SiOx/Si. Mater Sci Eng C 19:95-99

32-Sastry M, Ahmad A, Khan MI, Kumar R (2004) Microbial nanoparticle production. In: Niemeyer CM, Mirkin CA (eds) Nanobiotechnology. Wiley-VCH, Weinheim, Germany, pp 126-135

33- Bhattacharya D, Rajinder G (2005) Nanotechnology and p0tential of microorganisms. Crit Rev Biotechnol 25:199204

34- Mann S (ed) (1996) Biomimetic materials chemistry. VCH Publishers, New York

35- Senapati S, Mandal 0, Ahmad A, Khan MI, Sastry M, Kumar R (2004) Fungus mediated synthesis of silver nanoparticles: a novel biological approach. Indian J Phys 78A:I0I-105 Senapati S, Ahmad A, Khan MI, Sastry M, Kumar R (2005) Extracellular biosynthesis of bimetallic Au-Ag alloy nanoparticles. Small 1:517-520

36-Gericke M, Pinches A (2006b) Microbial production of gold • nanoparticles. Gold Bull 39:22-28

37- Joerger R, Klaus T, Olsson E, Granqvist CG (1999) Spectrally selective solar absorber coatings prepared by a biomimetic technique. Proc Soc Photo-Opt Instrum Eng 3789:2-7\

38- Husseiny MI, EI-Aziz MA, Badr Y, Mahmoud MA (2007) Biosynthesis of gold nanoparticles using *Pseudomonas aeruginosa.* Spectrochim Acta A: Mol Biomol Spectrosc 67:1003-1006

39- Lengke M, Ravel B, Fleet ME, Wanger G, Gordon RA, Southam G (2006b) Mechanisms of gold bioaccumulation by filamentous cyanobacteria from gold(III)-chloride complex. Environ Sci Technol 40:6304-6309

40-- Shankar SS, Rai A, Ahmad A and Sastry M, Controlling the optical properties of lemongrass extract synthesized gold nanotriangles and potential application in infrared-absorbing optical coatings. *Chem Mater* 17:566–572 (2005)

41- Huang J, LiQ, Sun D, Lu Y, Su Y, Yang X, *et al*, Biosynthesis of silver and gold nanoparticles by novel sundried *Cinnamomum camphora* leaf. *Nanotechnology* 18:105104–105114 (2007).

42-Shankar SS, Rai A, Ahmad A and Sastry M, Rapid synthesis of Au, Ag, and bimetallic Au core–Ag shell nanoparticles using neem (*Azadirachta indica*) leaf broth. *J Colloid Interf Sci* **275**:496–502 (2004).

43- Chandran SP, Chaudhary M, Pasricha R, Ahmad A and Sastry M, Synthesis of gold nanotriangles and silver nanoparticles using *Aloe vera* plant extract. *Biotechnol Prog* 22:577–583 (2006).

44- Ankamwar B, Chaudhary M and Sastry M, Gold nanotriangles biologically synthesized using tamarin leaf extract and potential application in vapor sensing. *Synth React Inorg Metal-Org Nano- Metal Chem* 35:19–26 (2005)

45- Ankamwar B, Damle C, Ahmad A and Sastry M, Biosynthesis of gold and silver nanoparticles using *Emblica officinalis* fruit extract, their phase transfer and transmetallation in an organic solution. *JNanosci Nanotechnol* 5:1665–1671 (2005).

46- Armendariz V, Gardea-Torresdey JL, Jose-YacamanM, Gonzalez J, Herrera I and Parsons JG, Gold nanoparticles formation by oat and wheat biomasses, in *Proceedings –Waste Research Technology* Conference at the Kansas City, Mariott-Country Club Plaza July30–Aug1 (2002).

47- Gardea-Torresdey JL, Gomez E, Peralta-Videa JR, Parsons JG, Troiani H and Jose-YacamanM, Alfalfa sprouts: a natural source for the synthesis of silver nanoparticles. *Langmuir* **19**:1357–1361 (2003).

48- L ′ opezaML, Parsonsb JG, Peralta Videab JR and Gardea-Torresdey TL, AnXASstudy of thebinding and reduction of Au(III) byhopbiomass. *Microchem J* 81:50–56 (2005).

49-Ghule K, Ghule AV, Liu JY and Ling YC, Microscale size triangular gold prisms synthesized using Bengal gram beans (*Cicer arietinum* L.) extract and HAuCl4 × 3H2O: a green biogenic approach. *J Nanosci Nanotechnol* 6:3746–3751 (2006).

50- Gardea-Torresdey JL, Parsons JG, Gomez E, Peralta-Videa J, Troiani HE, Santiago P, *et al*, Formation and growth of Au nanoparticles inside live alfalfa plants. *Am Chem Soc* **2**:397–401 (2002).

51- Rodriguez E, Parsons JG, Peralta-Videa JR, Cruz-Jimenez G, Romero- Gonzalez J, Sanchez-Salcido BE, *et al*, Potential of *Chilopsis linearis* for gold phytomining: using XAS to determine gold reduction
and nanoparticle formation within plant tissues. *Int J Phytoremed* 9:133–147 (2007).

52- Sharma NC, Sahi SV, Nath S, Parsons JG, Gardea-Torresdey JL and Pal T, Synthesis of plant-mediated gold nanoparticles and catalytic role of biomatrix-embedded nanomaterials. *Environ Sci Technol* 41:5137–5142 (2007).

53-Haverkamp RG, Marshall AT and van Agterveld D, Pick your carats: nanoparticles of gold–silver–copper alloy produced *in-vivo*. *J Nanopart Res* 9:697–700 (2007).

54- Andreeva D, Low temperaturewater gas shift over gold catalysts. *Gold Bull* 35:82–88 (2002). 66 -Grisel R, Weststrate KJ, Gluhoi A and Nieuwenhuys BE, Catalysis by gold nanoparticles. *Gold Bull* **35**:39–45 (2002).

55- Hutchings GJ and Haruta M, A golden age of catalysis: a perspective. *Appl Catal A* 291:2–5 (2005)

56- Yanez-Sedeno P and Pingarron JM, Gold nanoparticle-based electrochemical biosensors. *Anal Bioanal Chem* 382:884–886 (2005).

57-Liu J and Lu Y, Colorimetric biosensors based on DNAzyme-assembled gold nanoparticles. *J Fluoresc* 14:343–354 (2004).

58- Tang D, Yuan R and Chai Y, Ligand-functionalized core/shell Ag–Au nanoparticles label-free amperometric immun-biosensor. *Biotechnol Bioeng* 94:996–1004 (2006).

59- Paciotti GF,Myer L,Weinreich D, Goia D, Pavel N,McLaughlin RE, *et al*, Colloidal gold: a novel nanoparticle vector for tumor directed drug delivery. *Drug Deliv* **11**:169–183 (2004).

60- Bangs LB, New developments in particle-based immunoassays: introduction. *Pure Appl Chem* 68:1873–1879 (1996).

61- Elghanian R, Storhoff JJ, Mucic RC, Letsinger RL and Mirkin CA, Selective colorimetric detection of polynucleotides based on the distance-dependent optical properties of gold nanoparticles. *Science* **277**:1078–1081 (1997).

62- Thanh NTK and Rosenzweig Z, Development of an aggregation-based immunoassay for anti-protein A using gold nanoparticles. *Anal Chem* 74:1624–1628 (2002).

63- Crumbliss AL, Perine SC, Stonehuerner J, Tubergen KR, Zhao J, Henkens RW, *et al*,Colloidal gold as abiocompatible immobilization matrix suitable for the fabrication of enzyme electrodes by
electrodeposition. *Biotechnol Bioeng* **40**:483–490 (1992).

64- Revina AA and Egorova EM, Radiation-chemical technology for the synthesis of stable metallic and bimetallic clusters, in *International Conference on Advances in Technology in the 21st Century (ICAT '98)*, Moscow, 5–9 October, pp. 411–413 (1998).

65- Becker RO, Silver ions in the treatment of local infections. *Met Based Drugs* 6:297–300 (1999).

66- Silver S, Bacterial silver resistance: molecular biology and uses and misuses of silver compounds. *FEMS Microbiol Rev* **27**:341–353 (2003).

70. Fukumori, Y. and H. Ichikawa, *Nanoparticles for cancer therapy and diagnosis.* Advanced Powder Technology, 2006. 17(1): p. 1.

71. Maeda, H., L.W. Seymour, and Y. Miyamoto, *Conjugates of Anticancer Agents and Polymers - Advantages of Macromolecular Therapeutics Invivo.* Bioconjugate Chemistry, 1992. **3**(5): p. 351.

72. Qi, X.R., Y. Maitani, T. Nagai, and S.L. Wei, *Comparative pharmacokinetics and antitumor efficacy of doxorubicin encapsulated in soybean-derived sterols and poly(ethylene glycol) liposomes in mice.* International Journal of Pharmaceutics,1997. 146(1): p. 31.

72. Connor, E.E., J. Mwamuka, A. Gole, C.J. Murphy, and M.D. Wyatt, *Gold nanoparticles are taken up by human cells but do not cause acute cytotoxicity.* Small, 2005. **1**(3): p. 325.

73. Sokolov, K., M. Follen, J. Aaron, I. Pavlova, A. Malpica, R. Lotan, and R. Richards-Kortum, *Real-time vital optical imaging of precancer using antiepidermal growth factor receptor antibodies conjugated to gold nanoparticles.* Cancer Research, 2003. 63(9): p. 1999.

74. Sokolov, K., C. Robinson, T. Collier, R. Richards-Kortum, M. Follen, and R. Lotan, *Metal nanoparticles as biospecific contrast agents for cancer imaging.* Trends in Optics and Photonics, 2002. **71**(OSA Biomedical Topical Meetings,2002): p. 376.

PUBLICATIONS

1- Singh,C., Singh. H. Abstract Id: Nanomedicine: From nano-oncology to nanosensing. *Nanotech India-2009,* 14-16th August, 2009, Kochi, Kerala, India,

INTERNATIONAL AWARD/NOMINATION

1-Nominated for "***Dr Y S R Bio Asia Innovation award 2010***",for desigining "A Kit for Bioethanol Production using domestic waste".