

A REVIEW ON GOLD NANOPARTICLES SYNTHESIS AND CHARACTERIZATION

1. ABSTRACT:

Gold nano particles (GNP) have exceptional biocompatibility and possess unique structural, electronic, magnetic, optical, catalytic and molecular recognition properties. Hence GNPs are very attractive for many biological applications. Being noble metal, the GNPs are resistant to oxide formation. Their electronic, magnetic and optical properties are size dependent. The optical properties of gold nano particles depend on nano particles size, shape, aggregation state, and local environment and are tuneable throughout the visible and near-infrared region (Angshuman Pal *et al*, 2007). The current paper gives a brief review of different methods of synthesis of GNP with uniform size and shape and characterization of GNP by UV spectroscopy, Dynamic light scattering, Scanning electron microscopy and Fourier transform Infra Red spectroscopy along with applications of GNPs

Key word: gold nano particles

2. Introduction:

Nanotechnology scale set by National Nanotechnology Initiative, US is 1 to 100 nm. The lower limit is set by size of the atom which is one fourth of an nm diameter, since nanotechnology has to build its devices from atoms and molecules. The upper limit is arbitrary to some extent, but it is around the size that phenomenon is not observed in large structures and can be made use in nano devices. The quantum effects become dominant when the size reaches typically at distance 100 nm or less. Increase in surface to volume ratio imparts different mechanical, catalytic and thermal properties than the bulk material of same element. There are two approaches to synthesize nanostructure:

- Top down method- breaking down bulk to nano scale
- Bottom up method- putting atoms or molecules or unit together to form nanostructure

History of Gold nanoparticle:

The first evidence of gold nano particles prepared by man is the Lycurgus Cup from the Roman times in the 4th century A.D. which is having unique optical property. In reflected light it appears green while red in transmitted light. The reason behind this was found to be presence of gold and silver nano particles. Besides the Lycurgus Cup, bright red colour in ‘ruby glass’ is due to gold nano particles. Ruby glass is used as the red colour in stained glass church windows. Just like bulk gold the beautiful appearance of the gold nano particles has immense benefits and thus making them one of the most widely studied and abundantly used nano particles. Faraday was the first who studied gold nano particles in 1857. Faraday believed that the bright ruby red colour of the colloidal gold was due to the extremely small size of the individual particles, which interacted with light in a different manner compared to metallic gold, but could not prove it. Gustav Mie was the first to provide an explanation on the dependence of colour on the metal particle size.

The philosopher and medical doctor Francisci Antonii published very first book on gold nano particles then known as colloidal gold named Panacea Aurea-Auro Potabile; Bibliopolio Frobeniano in 1618 and gave the ability of colloidal gold to cure diseases, such as heart, venereal problems, dysentery, tumors and epilepsy. In 1676, the German chemist Johann Kunckels wrote a chapter “drinkable gold that contains metallic gold in a neutral, slightly pink solution that exert curative properties for several diseases” giving the explanation that gold has to be present in such a small size that it can’t be seen by the human eye. A colorant in glasses, “Purple of Cassus” was popular in the 17th century which is a colloid of the heterocoagulation of gold particles with tin

dioxide. In 1718, Hans Heinrich published treatise stating that the stability of drinkable gold preparation was noticeably enhanced by the use of boiled starch. French dictionary noted that drinkable gold was containing gold in its elementary form but it was under extremely subdivided and suspended in a liquid, in 1769. In 1794, Mrs. Fuhlame reported in the book “An Essay on Combustion with a View to a New Art of Dying and Painting” that silk was dyed with colloidal gold. In 1818, Jeremias Benjamin Richters stated that the gold in pink and purple solution contains finest subdivision degree while when fine particles aggregate give yellow colour. In 1857, Faraday reduced chloroaurate (AuCl_4) by phosphorus in CS_2 in two phase system to produce deep red colored colloidal gold solutions. He studied the optical properties of the optical properties of thin films obtained from dried colloidal solutions and observed reversible colour changes from bluish-purple to green due to pressurizing (Marie-Christine Daniel et al 2004). Gold colloids have been used for years as contrast agents in electron microscopy as gold is very electron-dense. More recently, gold nano particles are used in biological optical imaging and sensing applications.

Properties of gold nano particles:

GNPs have exceptional biocompatibility and possess unique structural, electronic, magnetic, optical, catalytic properties molecular recognition properties. GNPs are very attractive for many biological applications. Being noble metal, the GNPs are resistant to oxide formation. Their electronic, magnetic and optical properties are size dependent. The optical properties of gold nano particles depend on nano particles size, shape, aggregation state, and local environment and are tuneable throughout the visible and near-infrared region (Angshuman Pal *et al*, 2007). The magnificent range of colours shown by colloidal gold is due to a phenomenon called Surface Plasmon Resonance (SPR). SPR can be considered as the collective oscillation of the conduction band electrons in the metals (Nguyen Ngoe Long *et al* 2009). This is a characteristic surface property of nano sized particle and is not exhibited by individual atoms or bulk materials. Conduction band electrons of noble metals interact strongly with electromagnetic waves of the visible range. When the size of the particle is less than or comparable to one tenth of the wavelength of the incident light, all the electrons in the particle resonates, resulting in strong absorption of the particular wavelength and particles of different sizes resonate at different wavelengths. This gives rise to different colors of the colloidal gold.

One of the useful features of the SPR band is its dependence of the position and width on the particle size. This size effect is visible as red shift for increasing particle sizes. The extinction curve is an important means to determine sizes and morphology of a particle.

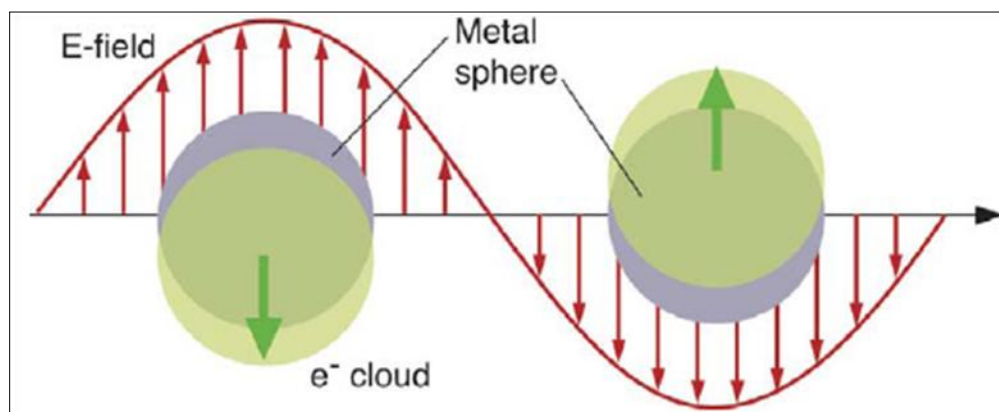


Fig 1: The electromagnetic light induces a collective oscillation of the metal conduction electrons across the nano particles (Sugunan *et al.*, 2004).

The various applications are based on the fact that the color of the colloid depends not only on the particle size, but also on the shape, the refractive index of the surrounding media and the separation between the particles. A variation in any of these parameters will result in a quantifiable shift in the SPR absorption peak. The capping agent used in stabilizing the nano particles of gold, can be chosen such that it attaches to specific molecules and so upon exposure to those molecule, they gets adsorbed on to the surface of the nano particles thereby changing the effective refractive index of the immediate surroundings of the nano particles. If the molecules that we are trying to detect are larger than the nano particles, they will adsorb a few nano particles, making them agglomerate into lumps. This will reduce the particle spacing, resulting in a shift in the SPR changing the color of the colloidal gold (Sugunan *et al.*, 2004) giving potential to be used in sensors, imaging and assay fields. According to Mie theory, Surface Plasmon Band (SPB) is absent for GNPs less than 2nm and greater than 500nm.

Advantages and applications of GNPs:

A high efficiency of excitation of surface plasmon waves (SPWs) on the gold nanoparticles surface gives the opportunity of use of GNPs in the development of biosensors and in medical photo thermal therapy (Krasovskii *et al.*, 2007). Being a Noble metal, GNPs are resistant to oxide formation under ambient conditions. This helps in the controlled attachment of organic molecules from the gold surface by either manipulating the gold-sulphur bond or electrostatic attraction. Gold nano particles have attractive optical properties such as the surface plasmon resonance (SPR) band in the visible region of the absorption spectrum of the colloidal solution. Gold has a high atomic number, a feature which makes it readily detectable in electron microscopes or by X-rays (Pissuwan *et al.*, 2006). Due to their ease of synthesis, uniform distribution of size, rich surface chemistry and a lack of toxicity; GNPs have been used for conjugation with numerous bio molecules for site-specific delivery.

The surface chemistry of gold facilitates the coating, functionalization and integration of GNPs with many bimolecular moieties (Iosin *et al.*, 2009). The GNPs can carry various therapeutic agents and biomolecules, including DNA, proteins, peptides, and low molecular weight compounds and can penetrate across different barriers through small capillaries into individual cells. The surface modification of gold nano particles can be done and can be conjugated with many biomolecules for many bioanalytical and biomedical application (Nishima Wangoo *et al.*, 2008). The conjugation of protein with nano particles not only allows stabilization of the system but it also introduces biocompatible functionalities in the nano particles for further biological interactions or coupling. BSA conjugated nano particles show improved stability against flocculation, increased quantum yield and low toxicity(Housni *et al.*,2008).and these conjugates can be used for the targeting purpose. The Surface modification of GNPs with BSA can improve the nonspecific binding of the protein. Also BSA conjugated gold nano particles can be used as a better drug delivery vehicles compared to the protein alone, as they can be detected in solution due to the optical properties of gold nano particles.

The free electrons present in the conduction band of GNPs make them potential candidates to bind with thiols and amine. Thus, they can be easily tagged with various proteins and biomolecules rich in amino acids leading to important biomedical applications including targeted drug delivery, cellular imaging, biosensing and bioanalysis. With their high free electron densities, GNPs serve as excellent contrast enhancement agents in the detection of tumors. GNPs have exceptional biocompatibility and possess unique structural, electronic, magnetic, optical and catalytic properties which make them to be a very attractive material for biosensor, immunosensor, chemisensor and electrocatalyst (Huang L *et al.*, 2007).Most important

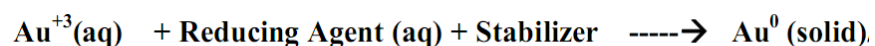
application of GNP bioconjugates is therapeutic delivery. Such bioconjugates can be targeted to specific cells *in vivo* and can be used for drug release.

Synthesis of gold nanoparticles:

GNPs synthesis can be done by number of methods (Marie-Christine Daniel *et al*, 2003)-

- by reductant like Trisodium citrate, NaBH_4
- The Brust-Schiffrin method (two-phase synthesis and stabilization by thiols)
- By sulphur ligands such as xanthates⁶⁶ and disulfides, 67-69 di-70a and trithiols, 70b and resorcinarene tetrathiols
- By other ligands-Phosphine, Phosphine Oxide, Amine, and Carboxylate Ligand, Isocyanides, Acetone, Iodine
- By use of Microemulsion, Reversed Micelles, Surfactants, Membranes, and Polyelectrolytes.
- By seeding growth
- By Physical Methods using Photochemistry (UV,Near-IR), Sonochemistry, Radiolysis, and Thermolysis

The very common approach for controlling the size of nano particles is the reasonably easy solution-phase chemical method. The single phase water based reduction of gold salt by citrate was introduced by (Turkevich *et al*.1973) and refined by Frens which produces spherical particles over a tunable range of sizes. The chemical synthesis of metal particles by reduction of the corresponding metal salts is a simple process, which requires the mixing of the reagents at well defined external conditions. These conditions can affect the final morphology of the particles.



(Kimling *et al.*, 2006) has reported in their study that reactant concentration and other parameters, such as temperature or pH value also have a strong influence on the morphology. Reducing the temperature in the thermal citrate reaction drastically affects the size and quality of the final particles toward larger diameters and irregular shapes. The chemical reduction method depends on variety of parameters that can influence the physical and chemical properties of the resulting nano particles. The choice of a capping agent is a very important factor of controlled growth of nano particles. The selective growth of metal nano particles can be achieved by varying such reaction conditions as type and concentration of the metal reactions, or depending on the different absorbability of capping reagents for the metal nucleus. The particle size and shape in colloidal dispersions are strongly influenced by the interplay between two processes-nucleation and cluster growth, which occur during preparation.

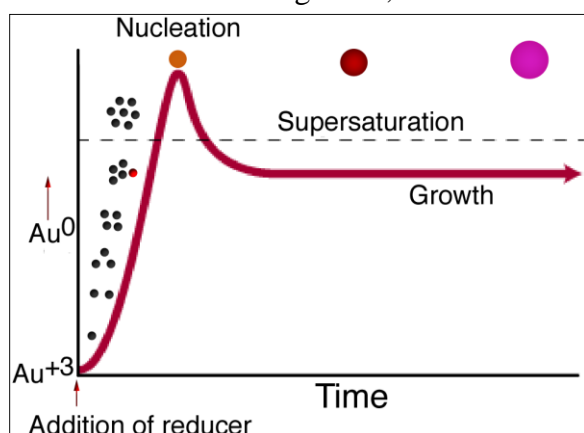


Fig. 2: Schematic representation of mechanism of nanoparticles

After addition of reducing agent the gold ions present in solution form atoms whose concentration reaches beyond supersaturation forming nuclei. Central icosahedral gold cores of 11 atoms are produced at nucleation sites. Once nuclei are formed, consequent depositions of the newly formed nuclei will occur on the already formed nuclei. The growth-rate of dispersed nuclei can be varied by controlling the temperature and concentration during the reaction which results in to production of colloidal particles of different sizes. At a fixed concentration of gold salt in solution, as the concentration of the reducing agent is increased the number of nuclei that form is increased. The more nuclei, the smaller the gold particles are produced. For the preparation of mono disperse colloids, it is essential that large amount of nuclei should be formed in a short period of time at the beginning of the chemical reaction. The nuclei formed will grow rapidly causing the concentration of the dispersed species to fall below the nucleation concentration. As a result, new nuclei are not formed and simultaneous growth of the existing nuclei takes place.

Characterization techniques of GNP:

The characterisation of gold nano particles can be done by different instruments like;

UV-Visible Spectroscopy:

Ultraviolet and visible spectroscopy is used for quantitative analysis of the sample. This instrument works on the principle of Beers –Lambert Law which states that the concentration of a substance in a sample is directly proportional to the absorbance ‘A’.

$$\text{Absorbance (A)} = \text{constant} \times \text{concentration} \times \text{cell length}$$

The law is true for monochromatic light, provided that physical and chemical state of the substance does not change with the concentration. When monochromatic radiation passes through a homogeneous sample, the intensity of the emitted radiation depends upon the thickness (L) and concentration (c). The ratio “ I_0/I ”, known as transmittance is expressed as a percentage and referred to as % transmittance.

Mathematically, absorbance is related to percent transmittance T by the expression:

$$A = \log_{10}(I_0/I) = \log_{10}(100/T) = kcL$$

Where L is the radiation path through the sample, “c” is the concentration of absorbing molecules in the path and “k” is the extinction coefficient that depends upon the nature of the molecule and wavelength of incident radiation. Absorbance is sometimes referred to as “extinction” or “optical density”.

UV-Vis analysis could be performed on gold nano particles dispersed in a solvent or embedded in the insulator matrix. Due to The optical properties i.e. SPR of the synthesized nano particles are evaluated by UV–VIS spectrophotometry in order to estimate the particles size or to monitor the degree of dispersity. Gold nano particles show λ_{max} in the region 500 to 550. Further UV-VIS spectra can give the information about kinetic behavior of the nano particles and the size and shape of gold nano particles formed. SRP band shifts to lower wavelength as the size of GNPs decreases and the intensity of absorption of SRP band indicates the concentration of GNPs formed in the solution and the intense peak, more uniform are the particles (Wolfgang Haiss *et al*, 2007).

Dynamic light scattering (DLS):

It is also known as quasi-elastic light scattering (QELS) or photon correlation spectroscopy (PCS). DLS is mainly used to measure hydrodynamic sizes of nano particles, polydispersities and aggregation effects of a protein sample which are the important parameters for the crystallization of proteins. When a beam of monochromatic laser light passes through onto a colloidal solution the particles that are present in the colloidal solution scatter light in all

directions. Due to the Doppler Shift, it causes changing the wavelength of the incoming light. This change is related to the size of the particle. If the size of the particle is larger, the Brownian motion is slow but in case of smaller particles it is vice-versa. the velocity of Brownian motion is defined by the property of translational diffusion coefficient.

$$d(H) = \frac{kT}{3\pi\eta D}$$

Where as

$d(H)$ = hydrodynamic diameter

D = translational diffusion coefficient

k = Boltzmann's constant

T = absolute temperature

Note: Diameter that is measured in DLS is a value that refers to diffusion behavior of the particle with a fluid so it is referred to as a hydrodynamic diameter. The diameter obtained is the diameter of a sphere that has the same translational diffusion coefficient as the particle.

(Kevin Su-Wei Wu, 2007)

Scanning electron microscopy (SEM):

The scanning electron microscope (SEM) is capable of producing images of sample surface. In SEM, electrons are thermionically emitted from a tungsten or lanthanum hexa-boride (LaB6) cathode filament towards an anode; alternatively electrons can be emitted via field emission (FE). The electron beam which typically has an energy range from a few keV to 50 keV is focused by two successive condenser lenses into a beam of very fine spot size (~ 5 nm). The beam then passes through the objective lens, where pairs of scanning coils deflect the beam either linearly or in a raster over a rectangular area of the sample surface. As the primary electrons strike the surface, they are in elastically scattered by atoms in the sample. Through these scattering events, the primary beam effectively spreads and fills a tear-drop-shaped volume extending about 1 μm to 5 μm into the surface. Interaction in this region leads to the subsequent emission of electrons which are then detected to produce an image.

It gives the information about the topography, morphology, composition and crystallography of the sample.

Fourier transform Infra Red spectroscopy (FTIR):

The Fourier transform infrared spectroscopy is also called as vibrational spectroscopy. It yields the information pertaining to chemical bonds. It allows the measurement of substances in any state (gas, liquid, or solid), with only less amount of sample and minimal interference from coexisting substances. Moreover, the process is swift, involving simple manipulations. IR absorption occurs when bipolar molecular moments in the middle IR region 4000 to 400 cm^{-1} are altered by molecular vibration.

In FTIR spectra both the liquid and solid samples can be analysed. In case of solid samples, these can be milled with potassium bromide (KBr) to form a very fine powder and then this powder is compressed into a thin pellet which can be analyzed. KBr is also transparent in the IR.

The beam containing many different wavelengths of light in infra red region are passed through sample at once. The atoms or group of atoms absorb photon at a frequency matching to their vibration frequency and the remaining beam intensity transmitted is measured by detector. The Fourier transform (a mathematical algorithm) is required to convert raw data into actual graph, hence named. It gives the idea of structure of ligand bound to the surface of nano particles (Hongyu Zhu et al, 2010).

Conclusion: GNPs are prepared by chemical reduction of the metal salts in the presence of a stabilizer which binds to their surface to impart high stability and rich linking chemistry and provides the desired charge and solubility properties. The strength of the reductant and action of

the stabilizer in synthesis of nano particles is critical. Various reagents can be used as reducing agent for the synthesis of GNPs such as sodium/potassium borohydrate, hydrazine and salts of tartarate, or organic ones like, sodium citrate, ascorbic acid and amino acids. Citrate reduction of tetrachloroauric acid (HAuCl₄) is the most preferred method for the preparation of GNPs as particle size of the resultant colloids is well controlled and it gives monodisperse colloids. In the citrate reduction method, tri sodium citrate initially acts as the reducing agent to reduce Au³⁺ ions to Au⁰ and later it causes stabilization by forming a layer of citrate ions on the GNPs surface through electrostatic repulsion between individual particles. Citrate is a common electrostatic stabilizing agent for GNPs (kimling *et al*, 2006). Several factors play important role to determine the final size of GNPs such as Concentration of redutant- The suitable molar ratio of sodium citrate to HAuCl₄ is a crucial factor in determining the morphology of the final gold nano particles (Huang *et al.*, 2006), the speed of addition and Mixing of the redutant with the gold solution may also influence the particle size distribution (Roe *et al.*, 1987) and the concentration of gold stock (Kimling *et al*, 2006).

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