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General Introduction

The materials in nanoscale exhibit unique physical and chemical properties compared to bulk materials (Gross et al., 2006; Klabunde and Richards, 2009; Vollath, 2008) and such materials are not only man-made but also occur naturally. Naturally occurring nanoscale material can be organic (viruses) as well as inorganic (aluminosilicates) in nature, produced by natural processes like volcano or weathering (Lead and Wilkinson, 2006; Hough et al., 2011).

In ancient times the most spectacular effect of nanoscale material (nanoparticles, NPs) was seen as color pigment in glass and luster industry (Colomban, 2009; Sciau and Hashim, 2012). Metal nanoparticles (MNPs) have been used to color glasses. Lycurgus cup, as shown in Fig 1.1, kept in British museum is one very famous example of nanoparticles use in coloring glasses. In this case gold nanoparticles (AuNPs) have been used to give ruby red color to the cup. Romans manufactured this cup in fourth century, it appears green in daylight and red when illuminated from inside (Leonhardt, 2007; Freestone et al., 2007). The attention received by MNPs from scientific community can be ascribed to the large surface to volume ratio, intriguing physical and chemical characteristics and surface electronic properties (Astruc et al., 2012). The use of metal nanoparticles for applications

like molecular recognition, biomedicine, energy transfer, sensors and as catalysts is well established (Otsuka et al., 2013; Shan et al., 2012; Williams et al., 2014; Hu and Liu, 2014; Cho et al., 2014; Wen et al., 2013; Wen et al., 2013; Wang et al., 2013; Mikami et al., 2013). Among MNPs (Au, Ag, Pd, Pt and Ru) AuNPs have received tremendous attention because of many important features. First, they exhibit unique size dependent physico-chemical and optical properties. Second, these properties can be tuned by controlling the size, shape and surrounding environment (solvent, ligand etc.). Third, these AuNPs are sub-cellular, non cytotoxic and biocompatible. Fourth, Stable AuNPs of desired shape can be synthesized easily. One very important property of AuNPs is multifunctionalization that has been exploited for detection of small molecules and biological targets.



Figure1.1. The Lycurgus Cup in reflected (left) and in transmitted (right) light. © Trustees of the British Museum

1.1. CONVENTIONAL METHODS FOR THE SYNTHESIS OF GOLD NANOPARTICLES

Considerable efforts have been devoted since last two decades for the synthesis of AuNPs with a focus on control over its size, shape, dispersibility and functionality. The approaches

for the preparation of AuNPs include both “top-down” and “bottom-up” approaches (Daniel and Astruc, 2004). The first scientific study in the field of AuNPs was done by Michael Faraday (Faraday and Philos, 1857). He devised a biphasic synthesis strategy in which aqueous solution of gold salt, sodium tetrachloroaurate, $[\text{Na}(\text{AuCl}_4)]$ was treated with phosphorus dissolved in Carbon disulfide. After a short reaction yellow colored solution of $[\text{Na}(\text{AuCl}_4)]$ turned into ruby red indicating the synthesis of AuNPs. Since Faraday, several methods have been given for AuNPs synthesis. Following are the important methods:

1.1.1. Turkevisch Method

In 1951, Turkevisch developed this method which remains the most popular method for the synthesis of AuNPs till date due to the ease of synthesis, cheap and non-toxic nature of stabilizer. In this method sodium citrate is used both as stabilizer and reducer. It initially acts as reducer to Au^{3+} producing Au^+ and Au^0 and then as stabilizer due to the electrostatic repulsion between the negatively charged oxygen on citrate. The AuNPs thus produced are spherical with size around 20nm (Turkevisch et al., 1951). Later, another scientist Frens(Frens, 1973) extended the work of Turkevisch and successfully synthesized variable sizes of AuNPs by varying the ratio of satabilizer and gold salt. Further, he also noticed that the size of AuNPs were inversely proportional to the stabilizer concentration i.e. to produce larger particles, less sodium citrate should be added while smaller NPs can be obtained by increasing the concentration of citrate. This method utilizes aqueous medium for the synthesis of AuNPs hence can be used in aqueous phase only.

1.1.2. Brust Method

The method was given by Brust and Schiffrin (1994) to produce AuNPs in organic solvents that normally form separate layer with aqueous solvent. This method for the synthesis of thiol-protected AuNPs was a breakthrough in the field of AuNPs synthesis. It is a two-phase

synthesis strategy in which thiol-gold interactions are used to protect gold nanoparticles from agglomeration using thiol ligands. Synthesis protocol involves the transfer of AuCl_4^- from aqueous to toluene media by the use of surfactant tetraoctylammonium bromide and reduced by sodiumborohydride in the presence of octane thiol. A change in color from yellow to deep brown takes place in organic media on addition of sodium borohydride, NaBH_4 (Brust et al., 1994). The AuNPs obtained in this case have a size range 1.5-5nm. These NPs exhibit higher stability due to superior Au-thiol interaction. The AuNPs can be further dried and redispersed in organic solvent of choice. The AuNPs obtained in this case show dispersibility in organic phase.

1.1.3. Martin method

This method was developed by Martin and Etah (2010) for the production of “naked AuNPs”. The trick is to stabilize AuNPs, produced by the action of NaBH_4 on aqueous HAuCl_4 solution, without using stabilizer like citrate or alkanethiols. The NPs produced through this method are monodispersed with diameter ranging from 3.2nm-5.2nm. Martin method is further modified to give octanethiol stabilized AuNPs. The naked NPs obtained through this method can be coated with dodecanethiol and might be phase-transferred to hexane by shaking with mixture of acetone, water and hexane for 30 minutes.

1.1.4. Amine as capping Ligands

AuNPs can be stabilized by using sodium citrate or by exploiting the strong Au-thiol interactions. While mostly thiol ligands have been used for AuNPs functionalization a variety of other ligands have also been used. Amines are also important as stabilizer because they are omnipresent i.e. present everywhere in biological and environmental system. The

first report on the use of amine for AuNPs synthesis was given by Leff et al. (1996). These workers produced hydrophobic AuNPs by using dodecylamine or oleylamine in place of dodecanethiol of Brust-Schiffrin method. Thus, this method is also regarded as a modified type of Brust-Schiffrin method. The precursor made by the interaction between Au^{3+} and long chain containing primary amine $[\text{AuCl}(\text{NH}_2\text{R})]$ result in AuNPs upon decomposition in air or in tetrahydrofuran (Gomez et al., 1945). Further, phase transfer technique has been used to generate organically soluble monodispersed AuNPs using amines like Laurylamine and octadecylamine (Kumar et al., 2003). Oleyl amine (Aslam et al., 2004), aromatic amines (Newman and Blanchard, 2006), amino acids (Bhargava et al., 2005; Selvakanan et al., 2003; Selvakanan et al., 2004; Leontowich et al., 2011); Mandal et al., 2002), diamines (Selvakanan et al., 2004), tetraoctylammonium (Isaacs et al., 2005), porphyrins (Kotiah et al., 2010), and hyperbranched polyethylenimine (Duan and Nie, 2007) have been used as reducing/capping agents in synthesis of AuNPs, while a direct one-pot synthesis of amine-stabilized AuNPs using 3-(trimethoxysilylpropyl)-diethylenetriamine has been reported (Zhu et al., 2005). The controlled synthesis of AuNPs by simple heating in ionic liquid has been developed recently, for which quaternary ammonium-based room-temperature ionic liquids was employed as a solvent, a reducing agent, and as a stabilizer (Huang et al., 2011). Piperazine derivatives have also been used as reducing/capping agents during gold nanoparticles synthesis (Das and Raj, 2011).

1.2. TYPES OF GOLD NANOPARTICLES

AuNPs can be characterized based on their shape, size and properties. The first obtained AuNPs were spherical in shape. Later various other shapes or forms like nanocages,

nanoshells and nanorods etc. were obtained. Fig. 1.2 shows the pictorial representation for various forms of AuNPs.

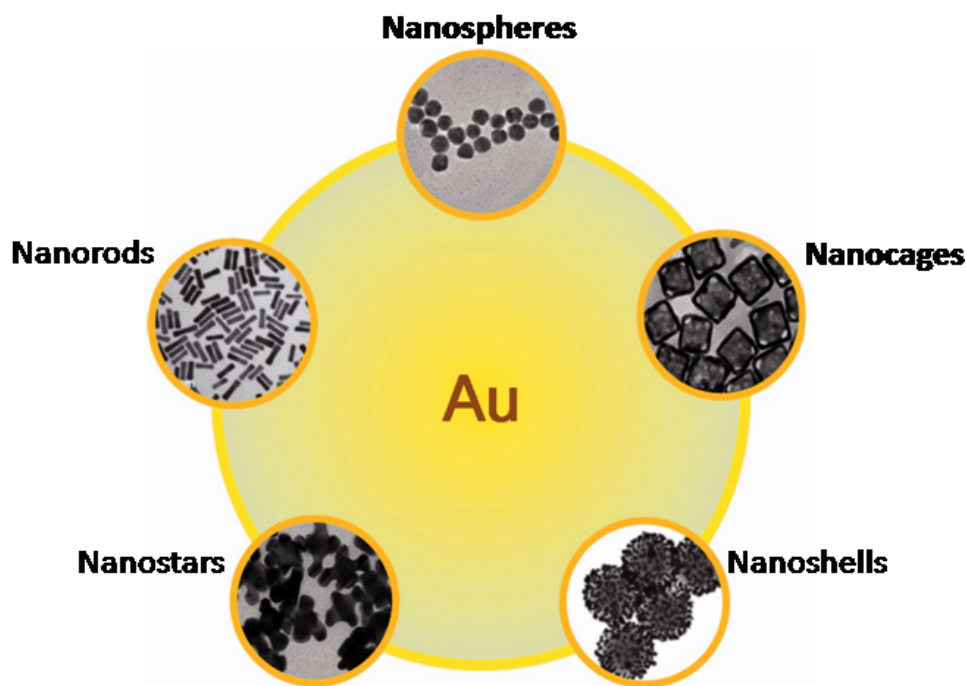


Figure1.2. Different shapes acquired by gold at nanoscale.

1.2.1. Goldnanospheres

Au nanospheres were obtained by reducing aqueous HAuCl_4 with different reducing agents under different conditions. Most commonly used reducing agent was citric acid (Frens, 1973; Turkevich et al., 1951). The diameter of the spheres varies from 1-100nm. The size of the nanosphere depends on the reaction conditions like concentration of the reactants. The UV-Vis spectra of Au nanospheres give peak between 510nm-560nm. The absorption spectra shows red shift i.e. shifts towards longer wavelength with increased size of the spheres. The sharpness of the peak indicates the size distribution.

1.2.2. Goldnanorods

The strategy that worked for the synthesis of Au nanorods was template method based on the electrochemical deposition of Au within the pores of nanoporous polycarbonate or alumina template membranes (Martin, 1994; van der Zande et al., 1997). The pores of the template membrane decide the diameter of nanorods, this way the diameter of nanorods is predetermined. The length of Au nanorod is controlled by the amount of Au deposited within membrane pores. The most common way for the synthesis of Au nanorod would be the “Seed-mediated synthesis”, as it gave higher aspect ratios in comparison to other methods (Jana et al., 2001; Busbee et al., 2003).

1.2.3. Goldnanoshells

These are more precisely called as a nanoshell plasmon and their diameter range from 10nm to 200nm. The thickness of the shell is controlled by the amount of Au added to the core. These are type of nanoparticles with dielectric core covered with a shell of metal, usually gold (Au) (Loo et al., 2004). Due to nanoscale resonance phenomenon, they have very large optical absorption and scattering cross-sections. They possess remarkable set of physicochemical properties making them ideal candidate for cancer detection.

1.2.4. Goldnanocages

Goldnanocages were synthesized in 2006, by the replacement reaction of silver nanocages with aqueous solution of HAuCl_4 . Here, silver nanocages serve as sacrificial template, metamorphosed into hollow space internally within Au nanostructures through galvanic replacement (Chen et al., 2006; Chen et al., 2005). AuNPs differ from Au nanocages in light absorption as former absorbs light in visible region while later absorbs light in near-infra red

region. The dimensions and wall thickness of the nanocages is a function of molar ratio of silver to HAuCl_4 .

1.3. PROPERTIES OF GOLD NANOPARTICLES

1.3.1. Redox activity

Discrete electron transition energy levels are present in AuNPs due to the quantum size effect that AuNPs possess. Molecule-like redox behavior of AuNPs has been demonstrated by taking hexanethiol functionalized AuNPs having 1.62nm size (Quinn et al., 2003; Antonello et al., 2007). It has been shown through experiments that these possess 15 redox states at room temperature. The application of AuNPs in electrochemical devices is due to the quantized capacitance charging behavior of AuNPs that can be tuned by varying the local environment of NPs (Schmid and Simon, 2005; Subramaniam et al., 2005).

1.3.2. Surface Plasmon Resonance (SPR)

SPR in AuNPs is produced due to the collective oscillations of conduction electrons across the NPs caused by resonant excitation by incident photons. The UV-Vis spectra display a peak corresponding to this excitation which is thus called “Surface Plasmon Band”. The origin of this phenomenon was elucidated by Mie (1908). He solved the maxwell’s electromagnetic equation for small spherical particles interacting with electromagnetic field. The resonant condition for AuNPs is fulfilled by visible light justifying the intense coloration in AuNPs (Halas et al., 2011; Jain et al., 2008; Link and El-sayed, 2003). The surface plasmon band is not given by very small NPs or by bulk material and also it depends not only on size but also on the solvent, reducing agent, stabilizer and temperature etc. With increase in size of the AuNPs the band shows red-shifting. Aggregation of small AuNPs also results in red shifting due to interparticle plasmon coupling changing the color of

AuNPs suspension from red to blue, which has been utilized in colorimetric sensing (Srivastava et al., 2005; Su et al., 2003).

1.3.3. Fluorescence quenching

The Fluorescence intensity of a sample is reduced as a result of fluorescence quenching. In case of AuNPs, fluorescence quenching takes place when surface Plasmon band of AuNPs overlaps with emission spectrum of fluorophores (Sapsford et al., 2006; Gersten and Nitzan, 1981; Oh et al., 2005). To examine this phenomenon fluorophores must be appended onto the surface of AuNPs. The resonant energy transfer has led to packages in biophotonics and materials science (Imahori and Fukuzumi, 2001; Imahori et al., 2001; Lakowitka, 1999; Wilcoxon et al., 1998). Both radiative and nonradiative paths show particle dependence, with higher efficiencies of quenching occurring with small nanoparticles (Bigioni et al., 2000; Link and El-Sayed 2000; Mohamed et al., 2000; Dulkeith et al., 2005). AuNPs can also act as an electron acceptor to quench the fluorophores in a photo-induced transfer of electrons.

1.4. APPLICATION OF GOLD NANOPARTICLES

1.4.1. Colorimetric Sensing

Aggregation of small AuNPs results in interparticle surface coupling causing a change in color of suspension from red to blue which is visible to the naked eye. The color change provides a platform for sensing of the target analyte having potential to cause AuNPs aggregation or redispersion.

1.4.1.1. Detection of Alkali and Alkaline Earth Metal Ions

Potassium ion detection has been done by exploiting AuNPs with surface modified with 15-crown-5 ethers. Addition of K^+ analyte causes aggregation due to the formation of

multidentate interparticle complexes with the chelating ligand (Lin et al., 2002). The detection of metal ions with AuNPs generally requires the surface modification with appropriate chelating ligands.

1.4.1.2. Detection of Heavy Metal Ions

Heavy metal ions are hazardous to human health and hence their detection is very important. Heavy metal ion detection is also based on the technique of heavy metal ion chelation with the ligands appended onto the AuNPs surface. Kim et al (2001) developed a very simple colorimetric technique for the detection of heavy metal ions. Mercaptoundecanoic acid functionalized-AuNPs were used to cause the aggregation of AuNPs on addition of heavy metal ions. Carboxylate group present on the ligand served as metal ion receptor for Pb^{2+} , Cd^{2+} and Hg^{2+} but not for Zn^{2+} .

1.4.1.3. Detection of Anions

It is well known that detection of anions is difficult and challenging as compared to cations. It happens perhaps because of several reasons, such as, lower charge to radius ratio, pH sensitivity, wide range of geometries and solvent dependent binding affinity and selectivity (Beer and Gale, 2001). However, along with cations, AuNPs have also showed promising results in detecting anions. For example, AuNPs coated with ethylene glycol appended isothiuronium units were successfully used to detect F^- in water using 3-nitrophenylboronic acid as a mediator at pH 5.5 (Minami et al., (2008). Likewise, Itoh et al. (2004) have applied ionic liquid functionalized AuNPs for the sensing of anion like I^- and PF_6^- . These later authors have used ionic liquids based on the imidazolium cation for colorimetric sensing (Garcia-Etxarri et al., 2010).

1.4.1.4. Detection of Small Organic Molecules

A competitive colorimetric glucose assay has been demonstrated by using assemblies of concanavalin A (Con A) and dextran-functionalized AuNPs (Aslan et al., 2004; Aslan et al., 2005). Multivalent binding with Con A cross-links dextran-coated nanoparticles and glucose, when added into the system, competitively binds Con A releasing the dextran-coated AuNPs that can be readily monitored by UV-Vis spectrophotometry (Aslan et al., (2004) with a glucose dynamic sensing range of mM. This system can potentially be useful to diagnose the blood glucose level in healthy people (3–8mM) and in diabetics (2–40 mM), because of the wide detection range (Aslan et al., 2005). Matsui et al. (2004) have used molecularly imprinted polymers (MIP) with embedded AuNPs (Au-MIP) as a colorimetric sensor for adrenaline (Matsui et al., 2004).

1.4.2. Fluorescence-based sensing

Huang and Murray (2002) have demonstrated a “Fluorescence Resonance Energy Transfer(FRET)” based assay for the detection of metal ions. For this they took tiopronin-coated AuNPs and a very common cationic fluorophore $[\text{Ru}(\text{bPy})_3]^{3+}$. Electrostatic complexation between negatively charged AuNPs and positively charged $[\text{Ru}(\text{bPy})_3]^{3+}$ resulted in fluorescence quenching of the $[\text{Ru}(\text{bPy})_3]^{3+}$. Addition of analyte (metal ions) breaks the electrostatic complexation restoring the fluorescence for $[\text{Ru}(\text{bPy})_3]^{3+}$ (Huang and Chang, 2006). In another interesting piece of work hairpin-FRET based system has been utilized for sensing DNA by labeling molecular beacons with AuNPs (Dubertret et al., 2001). The DNA probe conjugates with dye in such a fashion that it forms a hairpin-like structure with effective fluorescence quenching occurring between dye and AuNPs. With the addition of DNA, the hairpin structure is straightened making available again the

fluorescence signals from the dye. Semiconductor quantum dots (QDs) have also been utilized for the development of FRET-based AuNPs assay for the detection of proteins (Medintz et al., 2003). QDs and AuNPs have been utilized by Melvin and co-workers (2005) and they have reported a fluorescent competitive assay for the detection of DNA. In this work, AuNPs were assembled with Cadmium Selenide (CdSe) QDs through cDNA strands causing fluorescence quenching of the CdSeQDs. Addition of complementary oligonucleotides then displaces the AuNP-DNA from the QD-DNA, resulting in QD fluorescence restoration.

1.4.3. Electrical and Electrochemical Sensing

AuNPs properties like conductivity, high surface area and catalytic properties (Katz et al., 2004) make them perfect material for electrochemical detection of analytes (Shipway et al., 2000; Guo and Wang, 2007; Pumera et al., 2007; Willner and Willner, 2002). The electronic properties of monolayer protected AuNPs depends on number of factors and can be tuned by varying the size, distance between particles, functionality and chemical environment (Zamborini et al., 2002). Chemiresistors, rely on this sensitivity through changes in electrical conductance upon interaction with chemical species. A number of chemiresistor vapor sensors have been reported since last decade by utilizing thiol functionalized AuNPs (Ahn et al., 2004; Im et al., 2009; Joseph et al., 2007). One such vapor sensor was devised by Wohltjen and coworkers (1998) in which vapor sensor was fabricated by depositing a very fine film of octane-thiol coated AuNPs onto an interdigitated microelectrode. The conductance of the film decreased rapidly in the presence of toluene and tetrachloroethylene due to the swelling caused by it (Wohltzen and Snow, 1998). Lahav et al. (1999 a,b; 2000) have produced nanostructured assemblies via synergistic combination of electroactive

AuNPs (negatively charged citrate AuNPs) and macrocyclic compounds (oligocationic cyclophanes) to provide useful sensor systems. The assembly process was further repeated in stepwise manner to attain an assembly of anionic AuNPs and oligocationic cyclophanes. For sensing, the bipyridiniumcyclophanes serve as π -acceptors (Odell et al., 1988) for the association of π -donor substrates such as hydroquinone in their cavities, generating an electrochemical response (Blonder et al., 1998).

1.4.4. Therapeutics

AuNPs are widely used for biomedical applications, as they are non-cytotoxic and biocompatible in nature (Shukla et al., 2005). The AuNPs on excitation by light at wavelengths from 700 to 800 nm produce heat, given their tendency to absorb near-IR radiation. This property of AuNPs is utilized to eradicate targeted tumors (Boisselier and Didier., 2009). AuNPs have natural tendency to accumulate near tumour cells and when light is applied to a tumor containing gold nanoparticles, the particles rapidly heat up, killing tumor cells in a treatment also known as hyperthermia therapy. Therapeutic agents can also be appended onto the surface of AuNPs that will ensure the accumulation of drug near tumour cells and avoid side effects on other healthy cells (Dreaden et al., 2012; Francois et al., 2011; Kodiha et al., 2014; Wang et al., 2010). The large surface area-to-volume ratio of gold nanoparticles enables their surface to be coated with hundreds of molecules (including therapeutics, targeting agents, and anti-fouling polymers).

1.4.5. Catalyst in organic reactions

For very long gold has been considered as non reactive metal. However, later it was discovered by bond and coworkers that AuNPs can be utilized to catalyze organic synthesis for selective reduction of 1,3 butadiene to butane (Bond and Sermon, 1973; Bond et al.,

1973; Sermon et al., 1979). Gold-based catalysts have been used extensively in organic transformation since last few years and are a topic of much current interest. Colloidal suspension of AuNPs when used as catalyst cannot be recovered from the system and also adds metal impurities into the system. However, AuNPs in powder form have no such limitation and is preferred over suspension form. In order to use AuNPs in powder form it has to be adsorbed on some solid support like polymer fibers (He et al., 2014; Huang et al., 2008), porous solids (Dotzauer et al., 2006) and clays (Wu et al., 2012) etc. Coalescence and agglomeration are two main problems encountered while causing the AuNPs adsorption onto the support because catalytic activity of the NPs diminishes as the AuNPs size grows bigger than 10nm. AuNPs have been used to catalyze organic reactions like Hydrogenation reaction, Nitro reduction reaction etc.

1.4.5.1. Hydrogenation reaction

The most studied reduction reaction in organic is the selective reduction of carbonyl (C=O) bond in the presence of other unsaturated bonds. Hydrogenation of a C=C bond is favoured over a C=O bond by nearly 35 kJ mol^{-1} , hence there have been various modifications in catalysts to achieve maximum selectivity for C=O reduction. Shibata et al. (1988) described the very first application of gold for the reduction of unsaturated carbonyls. They used Au–Zr alloy to reduce crotonaldehyde to 2-butenal with 60% chemo-selectivity (Shibata et al., 1988). Later, Hutchings and Bailie (1999) also carried out crotonaldehyde reduction using supported Au catalysts over ZnO and ZrO₂.

1.4.5.2. Nitro reduction

Aromatic amines are industrially very important amines, especially in dyes and pigments. Many methods are available to cause the reduction with the involvement of intermediates

like azoxy, nitroso, nitro etc. which makes the reduction process complicated as several other products are formed. Attempts have been made to cause the selective reduction of nitro to amine (Blaser et al., 2009). In this context, Chen et al. (2006) and Corma et al. (2006) independently developed gold catalyzed hydrogenation of nitroarenes. In these works, high conversion (~100%) and high selectivity (>90%) were achieved.

1.5. BIMETALLIC NANOPARTICLES

Properties of binary catalysts are very different from their individual components and it has been observed that binary catalysts exhibit better stability and activity than their monometallic analogues (Zhang et al., 2007; Suo et al., 2007). PdAu bimetallic systems are important among various bimetallic systems as they display high activity towards useful reactions both organic and electrochemical. Among organic reactions are hydrogenation reaction, acetoxylation reactions and oxidation reactions, etc. (Mizukoshi et al., 2000; Chen et al., 2005). Electrochemical reactions with enhanced activity in the presence of bimetallics are methanol oxidation, 2-propanol oxidation and formic acid oxidations, etc. (Zu et al., 2008; Zhou et al., 2007). The bimetallics that are formed can either form alloy structure or attain core-shell morphology and the catalytic activity largely depends on the design of the bimetallic formed (Alayoglu et al., 2008). The catalytic performance of the bimetallic PdAu NPs also depend on the composition of the catalyst i.e. the feed ratio of Pd/Au salts used for the fabrication of catalyst. Efforts have been made to synthesize bimetallics having control over size, shape, geometry and properties. The synthesis can be carried out sequentially or simultaneously. In concurrent methods of bimetallic synthesis, salts of Pd and Au are mixed first followed by reduction and stabilization. In sequential method of bimetallic synthesis,

either PdNPs are grown over preformed AuNPs or AuNPs are grown over preformed PdNPs.

In one report, radiolytic reduction method has been adapted, through which poly acrylic acid stabilized Pd-Au alloy NPs are produced (Ksar et al., 2009). In another report, sequential reduction protocol is followed to synthesize Pd-Au bimetallic NPs using Poly(vinylpyrrolidone) as stabilizer (Rosales et al., (2007). PdAu nanodendrites have been made in the presence of strong reductant hydrazine using Poly(vinylpyrrolidone) as stabilizer (Lee et al., 2010). Further, some workers (Yun et al., 2010), involving co-reduction method, have also developed the use of ethylene glycol and sodium citrate as reducing and stabilizing agent, respectively, for the formation of PdAu bimetallic nanoparticles.

1.6. TRIMETALLIC NANOPARTICLES

Multimetallic NPs exhibit higher selectivity, sensitivity and stability compared to the monometallic components (Kang et al., 2011; Wang et al., 2011; Xia et al., 2008). Multimetallic nanoparticles either contain 3 or more than three components ($n \geq 3$). In the past decades, many studies have been made on bimetallic nanoparticle concerning their size, shape and stability. Bimetallic NPs with well-defined morphologies include flower-like, wire-like, dendritic structures etc. (Hong et al., 2011; Xu et al., 2011; Lim et al., 2009). Despite the advantages of multimetallic NPs discussed above, a very few reports are available concerning trimetallic nanoparticles in comparison to monometallic and bimetallic NPs. For example, Gonziaz et al. (2011) reported the formation of Pd-Au-Ag nanoboxes from Ag nanocubes by sequential or simultaneous galvanic exchange. Synthesis of triple layered Au@Pd@Pt with core-shell morphologies has been reported by Wang and co-

workers (2010) having better catalytic activity than bimetallic core-shell nanoparticles. Use of ZnO nanowires as sacrificial template for the production of Pt-Pd-Ag ternary alloy nanotubes with nanoporous framework is reported by Choi et al. (2011). In another report, Kang et al. (2013) have also reported one pot synthesis of Au@PdPt core-shell NPs with a well defined octahedral Au core and dendritic Pd-Pt alloy shell without any assistance of presynthesized template by the use of dual reducing agents (ascorbic acid and hydrazine). In another approach, Karthikeyan and Loganathan (2013) have used microwave radiation for the synthesis of trimetallic nanocomposites (Au-Pt-Ag) and nanotubes from aqueous solution of metal precursor using citrate as reductant and PVP as stabilizer. Venkatesan and Santhanalakshmi, (2012) have also reported the synthesis of Au-Ag-Pd trimetallic NPs by simultaneously causing the reduction of precursor salts in the presence of cetyltrimethylammoniumbromide (CTAB) as capping agent.

1.7. PEROXIDASE LIKE ACTIVITY: APPLICATION IN H₂O₂, GLUCOSE AND GLUTATHIONE DETECTION

Peroxidase enzyme has always remained main target of biomimetic chemist as peroxidase enzyme activates H₂O₂ to perform reactions. Hydrogen peroxide (H₂O₂) ranks among important biological compound as it performs a myriad of oxidation reactions in nature. Chromogenic substrates such as o-dianisidine (ODA) and 3,3',5,5'-tetramethylbenzidine (TMB) are converted into colored products using H₂O₂ as the oxidizing agent and horseradish peroxidase (HRP) enzyme as catalyst. This chromogenic reaction has been used for the determination of H₂O₂.

The ability of nanomaterial to behave as peroxidase mimick catalyst has attracted great interest due to the difficulty involved with natural enzymes. The nanomaterials could

also catalyze (like peroxidase enzyme) the oxidation of chromogenic substrates to develop colorful aqueous solutions using H_2O_2 as oxidizing agent (Long et al., 2011; Tao et al., 2013; Zhang et al., 2013; Jv et al., 2010). Gao et al. (2007) discovered intrinsic peroxidase-like activity of ferromagnetic particles, similar to the catalytic activity of natural enzymes such as HRP. The drawbacks with the use of natural enzymes are that their preparation, purification, storage are time-consuming and costly; also their catalytic activity is unstable i.e. inactivates with time. In comparison to natural enzymes, nanomaterials are advantageous in following respects: (i) high stability and favorable catalytic activity, (ii) low-cost and easy availability. Specially, the properties of nanomaterials, behaving as artificial enzyme mimics are retained well. For example, magnetic properties of Fe_3O_4 nanoparticles are retained when functioning as a peroxidase mimic (Gao et al., 2007). So far, a large number of nanomaterials have been discovered with peroxidase-like activity, such as gold nanoparticles (AuNPs) (Wang et al., 2012; Deng et al., 2014), gold nanoclusters (Wang et al., 2011), metal organic frameworks (Hou et al., 2015), carbon nanotubes (Song et al., 2010), carbon dots (Shi et al., 2011) or graphene oxide (Song et al., (2011)). The peroxidase-mimicking nanomaterials could overcome many serious disadvantages of natural enzymes (Long et al., 2011; Liu et al., 2013) and also be helpful in the field of biochemical sensing, mainly confined to H_2O_2 (Jv et al., 2010), H_2O_2 -generating substances, including glucose (Jv et al., 2010), cholesterol (Kim et al., 2011) or H_2O_2 -consuming biochemicals, such as glutathione, melamine (Ding et al., 2010). Therefore, it is highly imperative to develop simple and colorimetric strategies to widen the applications of nanomaterials' peroxidase-like activity in the field of biochemical sensing.

Liu et al. (2014) have reported the role of negatively charged AuNPs as peroxidase mimic and its application in causing dopamine oxidation. In another report, Jv et al. (2010) used positively charged as a peroxidase mimic and the activity has been utilized for the detection of H_2O_2 and glucose. Recently, kiwi juice prepared AuNPs exhibited an excellent peroxidase mimetic activity and thus utilized for the detection of cysteine [Li et al., (2016)].

1.8. PARANITRO PHENOL REDUCTION

Para nitrophenol (PNP) is a phenolic compound with nitro group at its para position. PNP is a water-soluble pollutant, largely produced during the manufacturing of fungicides, insecticides, drugs, etc. The reduction product of PNP i.e paraaminophenol (PAP) is utilized for many organic synthesis reactions.

In order to evaluate the catalytic ability of metal nanoparticles, the reduction of PNP in the presence of excess NaBH_4 using MNPs as catalyst, has become a model reaction (Jin et al., 2012; Sun et al., 2009; Santos et al., 2012; Li et al., 2012). In the absence of catalyst the reduction doesn't take place because of the kinetic barrier set up by two mutually repelling negative ions PNP^- and BH_4^- (Huang et al., 2010). Metallic nanoparticles provide surface for the adsorption of reactants, where, the transfer of electrons takes place from BH_4^- to PNP. The adsorption process of reactant onto the nanoparticles surface helps overcoming the kinetic barrier of the reaction. In the presence of excess NaBH_4 , the reaction follows pseudo-first order with respect to the concentration of PNP as has been proved by various workers (Zhang et al., 2009; Kuroda et al., 2009; Wu et al., 2012). The reduction of yellow colored PNP to colorless PAP can be detected spectrophotometrically either by

monitoring the decrease in absorbance value of PNP (~400nm) or increase in the absorbance value of PAP (~315nm).

1.9 CHALLENGES IN THE SYNTHESIS OF AuNPs AND ITS MULTIMETALLIC ANALOGUE

The conventional routes of AuNPs synthesis described above have been used exhaustively by scientific community for its application in different fields. These methods have been further modified by the addition of several reagents like PVP, PAP etc. to make the AuNPs conducive for the concerned applications. However, such techniques for AuNPs synthesis have following limitations: (i) Generally the AuNPs are produced as aqueous suspension (Turkevisch method). The use of such NPs in organic solvent causes agglomeration. Similarly the AuNPs having compatibility with organic solvent (Brust- Schiffrin method) are not compatible in aqueous system. (ii) The AuNPs fabricated through conventional routes are generally produced as dilute solution. The initial concentration of AuNPs precursor (i.e. Au^{3+}) is very low. (iii) The AuNPs made through conventional routes are not very stable to changes in pH- and Salt- concentration which limits there use for several applications and the AuNPs have to be modified to serve the purpose. The AuNPs made through Turkevisch method agglomerate on addition of single drop of salt. The AuNPs should be able to adapt to different conditions (change of solvent, change of pH and change of salt concentration) without undergoing any change in its size or shape. (iv) Attempt to convert the homogenous suspension of AuNPs to heterogeneous matrix by adsorbing over some solid support (TiO_2 , Al_2O_3 etc.) causes an increase in size of the AuNPs i.e. undergo agglomeration.

An attempt has been made in the present thesis to synthesize AuNPs utilizing the amphiphilic and bifunctional nature of 3-APTMS to overcome the limitations mentioned above. Also, the advantage of multimetallic NPs ($n \geq 3$) over monometallic NPs and limited studies on multimetallic nanoparticles encouraged the use of 3-APTMS for generating multimetallic NPs. The motivation behind use of functional alkoxysilanes has been discussed in the topic “Objectives of the research programme”.

1.10. ORIGIN OF THE PRESENT RESEARCH PROGRAMME

Synthesis of sol-gel glasses has received widespread attention because of its applications in various directions (Glezer and Lev., 1993; Sampath and Lev., 1996; Tatsuet al., 1992; Pandey et al., 1996; Pandey et al., 1999). Accordingly, the use of several functionalized alkoxysilanes in optimum ratio of hydrophilic character [trimethoxysilane, 3-APTMS] and hydrophobic character [epoxycyclohexyltrimethoxysilane, 3-glycidoxypropyltrimethoxysilane (3-GPTMS)] have been demonstrated for yielding organically modified thin films for multiple applications. (Pandey et al., 2001a; Pandey et al., 2003a, 2003b, 2003c) Functional alkoxysilanes have been used for casting thin films (through sol-gel processing) that can be used for encapsulating enzymes (glucose oxidase), redox material (ferrocene, ferricyanideetc) and noble metal ions (palladium, ruthenium) and for its use as biosensor (Pandey et al., 2001b, 2001c; Pandey et al., 2004; Pandey et al., 2001a). The property of the thin film depends on the concentration and composition of the alkoxysilanes used. Extensive report has been documented on these lines in our laboratory. Functional alkoxysilanes have been used in our laboratory for fabricating nanostructured thin films of organically modified silicates. Some of the functional alkoxysilanes like 3-GPTMS and trimethoxysilane have potential to act as reducing agent for Palladium chloride

(PdCl₂) that allowed the introduction of Pd within nanostructured network of organically modified silicates (Pandey et al., 2004; Pandey et al., 2001b, 2001c). Accordingly, such findings directed to examine the role of functional alkoxy silane during the synthesis of noble metal nanoparticles. The role of such functional alkoxy silanes during the synthesis of metal nanoparticles has been attempted and the finding on the synthesis of AuNPs and multimetallic analogue are reported herein, eliminating the limitations encountered in conventional route of nanoparticle synthesis that can be regarded as a major achievement. In addition to that 3-APTMS has been explored as stabilizer for nanostructured matrices directing its active role in NPs synthesis.

1.11. OBJECTIVES OF THE PRESENT INVESTIGATION

The main objective of the present work was to carry out the synthesis of AuNPs utilizing 3-APTMS and variety of other reducing agents (3-GPTMS, THF-HPO, cyclohexanone, formaldehyde, acetaldehyde, acetone and t-butylmethylketone) for its use as catalyst. The AuNPs synthesized have been used as catalyst in various forms; as colloidal suspension, mixed with graphite, in thin film form or as a hybrid with siloxane polymer both in suspension and solid state. The use of functional alkoxy silanes specifically 3-APTMS, have following properties



- (i) 3-APTMS is amphiphilic in nature due to the presence of hydrophilic amine group and hydrophobic or lipophilic three carbon containing propyl chain, the AuNPs that has been made using 3-APTMS can also be dispersed or fabricated in various solvents.
- (ii) The amine group of 3-APTMS moiety has the ability to form imine (-C=N-) linkage during the

synthesis of AuNPs when used with organic reducing agent like formaldehyde (-C=O). As this imine linkage is reversible in nature, it has a great propensity to play attractive role in the synthesis of AuNPs. (iii) The 'trimethoxysilyl' [-Si(OMe)₃] of 3-APTMS can be utilized for the conversion of homogenous nanoparticle suspension to heterogeneous solid matrix without addition of any external support. Generally, to use AuNPs as heterogeneous catalyst it is to be adsorbed on some solid support like TiO₂. The Presence of trimethoxysilyl group in the stabilizer has an advantage over conventional NPs as there is no need to add any support externally. The trimethoxysilyl group can also be utilized for casting film of the nanomaterial through sol-gel processing.

1.12. WORK PLAN FOR THE PRESENT INVESTIGATION

The work plan employed in the present thesis is as follows:

- To investigate the mechanism operating during functional alkoxysilanes (3-APTMS and 3-GPTMS) mediated synthesis of AuNPs and assess the dispersibility of NPs in different solvents in relation to alkoxysilanes molar ratio.
- To carry out the synthesis of AuNPs, using 3-APTMS and THF-HPO in aqueous medium, and study the effect of catalytic imine linkage on the peroxidase mimetic ability of AuNPs.
- To test the potential of a variety of reducing agents, such as, cyclohexanone, formaldehyde, acetaldehyde, acetone and t-butyl dimethyl ketone, in order to

provide a better amphiphilicity to AuNPs synthesized using amphiphilic 3-APTMS.

- To investigate the pH- and salt- tolerance abilities of AuNPs as a function of different reducing agents and varying 3-APTMS concentrations and test the pH- and salt-tolerance of AuNPs for homogenous catalysis of potassium ferricyanide mediated oxidation of ascorbic acid.
- To exploit the reagents, 3-APTMS and formaldehyde, for the synthesis of trimetallic NPs involving Au^{3+} , Ag^+ and Pd^{2+} . Multimetallic nanoparticles exhibit better performances than their monometallic counterparts.
- To investigate the catalytic potential of trimetallic (Au/Pd/Ag) NPs in facilitating the reduction of p-Nitrophenol into p-Aminophenol and compare the catalyzing ability with monometallic (Au) and bimetallic (Au/Pd) NPs.
- To decipher the reason/s for the formation of “siloxane-AuNPs” hybrid in organic solvents but not in water utilizing amphiphilic reagents “3-APTMS and acetone”.
- To fabricate the “siloxane-AuNPs” hybrid in solid state and use it as catalyst, with good operational stability, for the reduction of PNP to PAP in the presence of excess NaBH_4 .
- To characterize the nanoparticles using several sophisticated techniques, namely, Scanning Electron Microscopy (SEM), Transmission Electron Microscopy (TEM) and Atomic Force Microscopy (AFM). X-ray

Photoelectron Spectroscopy (XPS) characterization tool was used to investigate the oxidation states of trimetallic NPs.