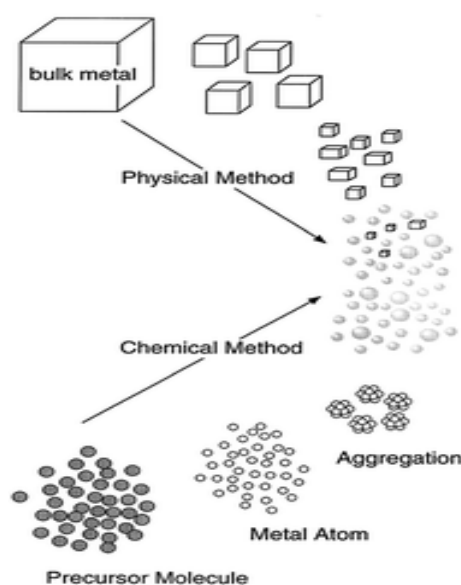


Over the last few years, there is growing interest in the development of noble metal nanoparticles (NMNPs) because of their excellent optical, electronic and catalytic properties, which are different from their bulk counterparts. The diverse and prominent applications of nanoparticles (NPs) in sensors, catalysts, nanoelectronic devices, biochemical tagging reagents and optical switches, reflects its intriguing features in technological development. Several methodologies have been developed for the synthesis of metal nanoparticles (MNPs). Their first natural classification is obviously into “top-down” and “bottom-up” [Wang *et al.*, (2004); Sanchez *et al.*, (2011); Saha *et al.*, (2012); Liu *et al.*, (2013); Kao *et al.*, (2013); Yu *et al.*, (2013); Khan *et al.*, (2013); Gawande *et al.*, (2015)] synthetic strategies, which can also be considered as physical and chemical methods, respectively. In top-down approach, large objects are modified to give smaller features, for example by lithographic techniques, film deposition and growth, laser-beam processing and mechanical techniques. Within the bottom-up category, a wide variety of synthetic methods such as sol-gel process, hydrothermal synthesis, thermal decomposition of organometallic compounds, electrochemical reduction, radiolytic reduction, photochemical reduction and chemical reduction of metal salts. The Schematic illustration of top-down and bottom-up approaches for the preparation of NMNPs by Zahmakiran *et al.*, (2011) as shown in Figure 1.1.



**Figure 1.1.** Schematic illustration of top-down and bottom-up approaches for the preparation of NMNPs. [Zahmakiran *et al.*, 2011]

NMNPs such as silver, palladium and their bimetallic nanoparticles (BMNPs) have shown promising applications for several technological developments of commercial significance [Saha *et al.*, (2012); Liu *et al.*, (2013); Kao *et al.*, (2013); Yu *et al.*, (2013); Khan *et al.*, (2013); Gawande *et al.*, (2015)]. An overview of the NMNPs on the synthesis and applications is described *vide infra*.

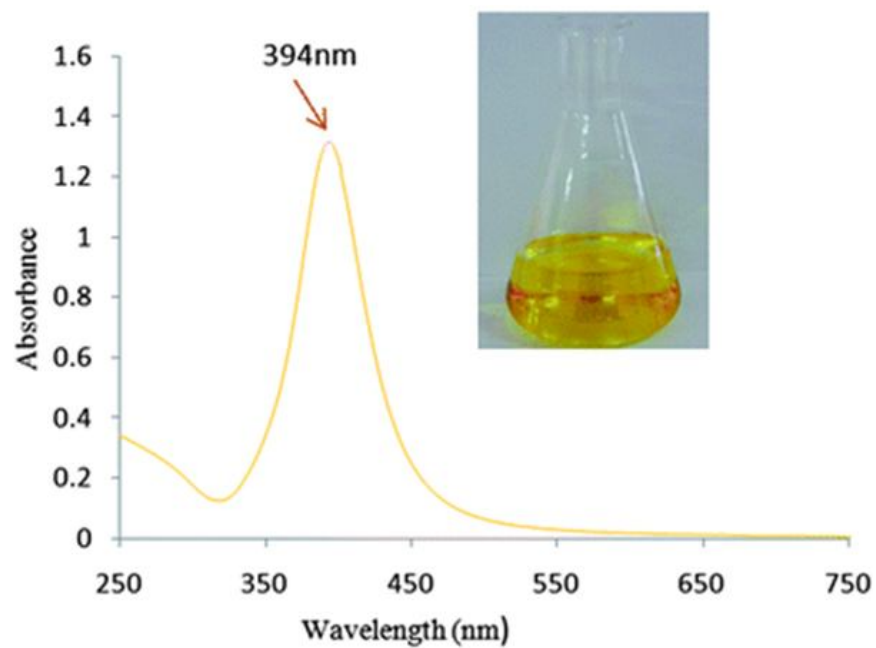
## **1.1. AN OVERVIEW OF NOBLE METAL NANOPARTICLES**

### **1.1.1. Synthesis of Noble Metal Nanoparticles**

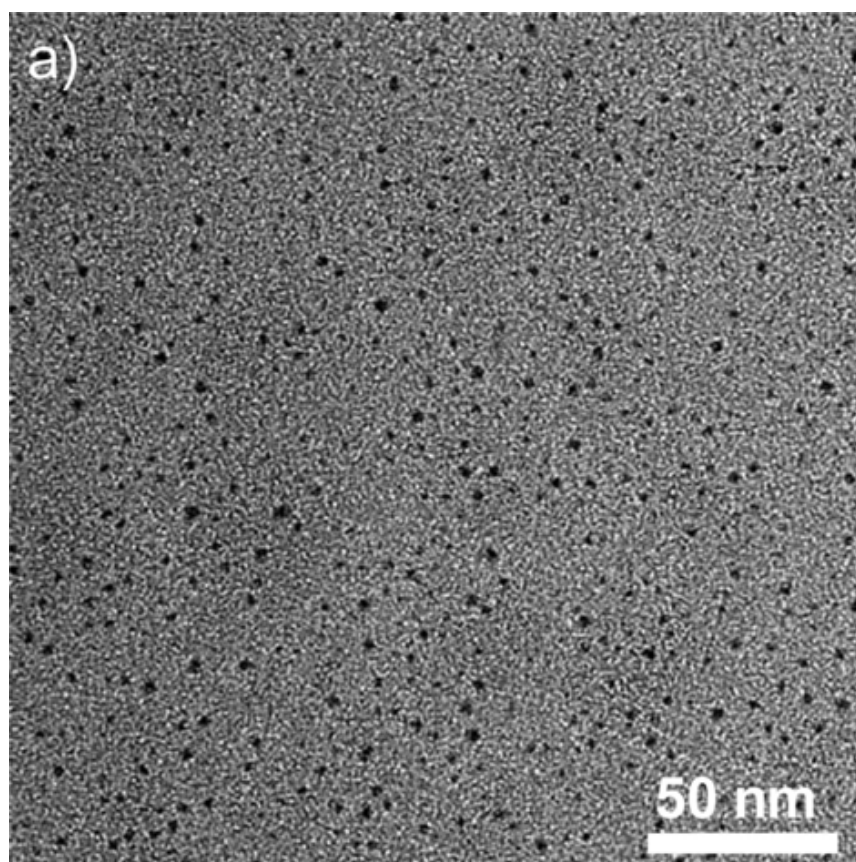
#### ***1.1.1.1. Silver nanoparticles***

Over the last few years, the scientific and practical interest in silver nanoparticles (AgNPs) was exclusively caused by the possibility of their use as highly dispersed supports for enhancing the signals from organic molecules in the Raman spectroscopy [Creighton *et al.*, (1979); Lee and Meisel., (1982)]. Fundamental studies shows that AgNPs exhibit a rare combination of valuable properties namely, unique optical properties associated with the surface plasmon resonance (SPR), well-developed surfaces, catalytic activity and high electrical double layer capacitance [Henglein., (1989)]. So, they serve as a material in the development of electronic, optical and sensor devices. In the last 2 decades, the scarcity of modernization led to core attention of scientific community to synthesize and analyze the AgNPs. Chemical synthesis is the most frequently method for the preparation of AgNPs for their stability and colloidal dispersions in water or organic solvents. Generally, sodium borohydride, tri-sodium citrate, hydrazine, ascorbic acid used as the reducing agents for aqueous solvents, whereas in organic solvents the most popular one is ethanol, which has been used by Toshima and coworkers [Hirai *et al.*, (1979); Toshima and Yonezawa., (1992); Wang and Toshima., (1997)]. Figlarz's polyol method was adapted for the production of silver nanoparticles [Fievet *et al.*, (1989)]. Isopropanol was also used for the preparation of silver colloids in basic conditions [Huang *et al.*, (1993)]. DMF [Santos and Marzan., (2002)], DMSO [Gattarno *et al.*, (2002)] and Formamide [Han *et al.*, (1999)] were also used for the synthesis of

AgNPs. The reduction of silver ions ( $\text{Ag}^+$ ) with various complexes leads to the formation of silver atoms ( $\text{Ag}^0$ ), which is followed by agglomeration into oligomeric clusters. These clusters eventually lead to the formation of colloidal AgNPs [Tao *et al.*, (2006); Wiley *et al.*, (2005); Lee and Meisel, (1982); Shirtcliffe *et al.*, (1999); Nickel *et al.*, (2000); Chou and Ren, (2000); Evanoff and Chumanov, (2004); Sondi *et al.*, (2003); Merga *et al.*, (2007); Ahmadi *et al.*, (1996); Kapoor *et al.*, (1994)]. Previous studies showed that use of a strong reducing agent resulted in small particles that were somewhat monodispersed, but the generation of larger particles was difficult to control. Use of a weaker reducing agent resulted in a slower reduction rate, but the size distribution was far from narrow size [Lee and Meisel, (1982); Shirtcliffe *et al.*, (1999); Schneider *et al.*, (1979)]. So, there is a need to use stabilizing agents to stabilize dispersive NPs during the synthesis process. The most common method to protect the NPs with protective agents that can be absorbed on or bind onto the nanoparticles surface, avoiding their agglomeration [Oliveira *et al.*, (2005); Bai *et al.*, (2007)]. Dodecanethiol and most commonly polymers like, poly (vinylpyrrolidone) (PVP), poly (ethylene glycol) (PEG), poly (methacrylic acid) (PMAA) and polymethylmethacrylate (PMMA) were used as stabilizing agents for the synthesis of AgNPs. Recently, biosynthetic methods utilized as reducing agents like polysaccharides, biological microorganism such as bacteria and fungus or plants extract, emerged as a simple and viable alternative for synthetic procedures to obtain AgNPs. AgNPs are extraordinarily efficient at absorbing and scattering light that depends upon the size and the shape of the particle. The typical image and spectra of AgNPs as reported earlier by Hajizadeh *et al.*, (2011) as shown in Figure 1.2. The particle size of AgNPs as reported earlier by Liang *et al.*, (2014) justified that nanodimension scale as shown in Figure 1.3.



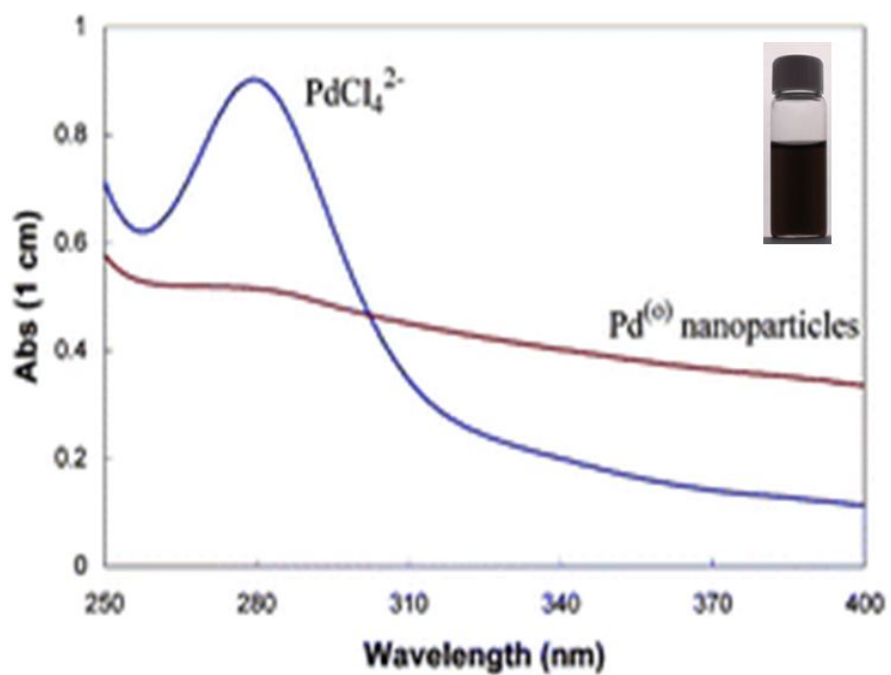
**Figure. 1.2.** Visual images of AgNPs and their corresponding UV-Vis spectra [Hajizadeh *et al.*, (2011)].



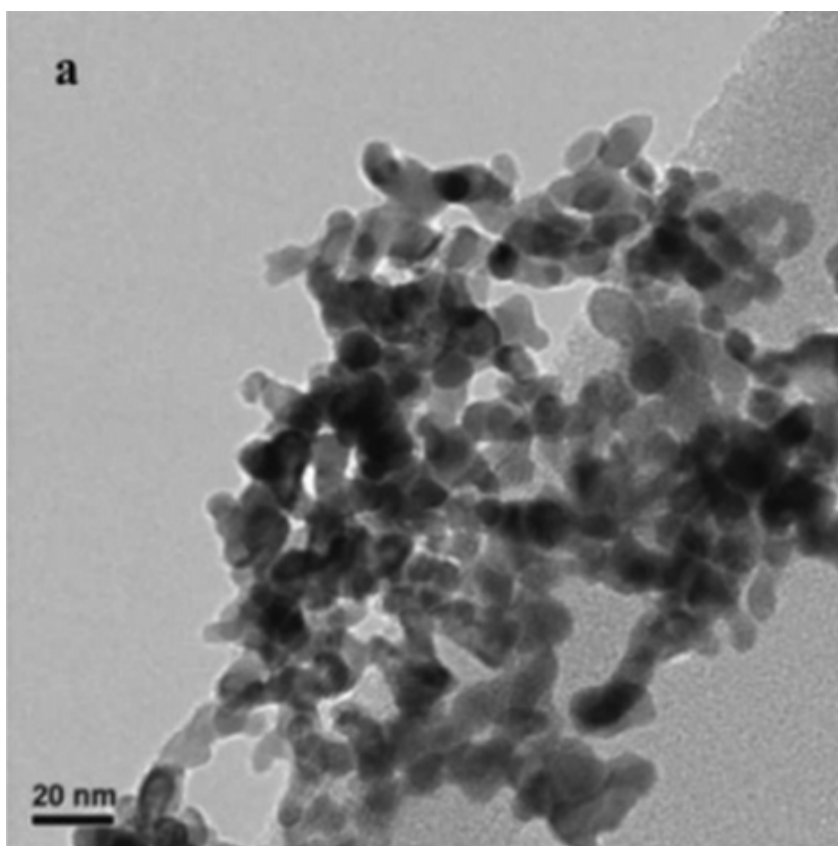
**Figure. 1.3.** TEM image of AgNPs [Liang *et al.*, (2014)].

### **1.1.1.2. Palladium nanoparticles**

Palladium nanoparticles (PdNPs) are of great importance as catalytic materials, as well as for a number of other applications such as hydrogen storage [Rehyani *et al.*, (2011); Adams *et al.*, (2011); Parambath *et al.*, (2012); Kumar *et al.*, (2015); Castillo *et al.*, (2015)] and sensing [Mubeen *et al.*, (2007); Wang *et al.*, (2012); Leng *et al.*, (2015); Shin *et al.*, (2015)]. The synthesis of PdNPs has been widely studied by chemical and electrochemical methods using a variety of stabilizers including organic ligands, salts, surfactants, polymers and dendrimers and also their potential benefits in catalytic applications. The synthesis of PdNPs with controlled particle size and shape [Xiong *et al.*, (2005); Yang *et al.*, (2014); Dumas and Couvreur., (2015)] can play a crucial role in a catalytic process with highest selectivity in a various reaction. Several synthetic approaches and different metal precursors have been demonstrated to generate PdNPs having different shapes and sizes e.g., chemical reduction of PdCl<sub>2</sub> by NaBH<sub>4</sub> [Jana *et al.*, (2000); Kochkar *et al.*, (2011); Li *et al.*, (2015); Holade *et al.*, (2015)] and by arc-discharge [Bera *et al.*, (2004); Sano *et al.*, (2014)], magnetic stirring at 80°C of Pd(OAc)<sub>2</sub> [Luo *et al.*, (2005)], reduction in supercritical carbon dioxide of Pd(OAc)<sub>2</sub> [Kameo *et al.*, (2003); Zhang *et al.*, (2014)], thermally induced reduction of Pd(Fod)<sub>2</sub> [Ho and Chi., (2004)], and sonochemical reduction of PdCl<sub>2</sub> [Okitsu *et al.*, (1996); Chen *et al.*, (2001)]. To prevent the formation of undesired agglomerated PdNPs, the processes have been performed in the presence of various surfactant molecules and stabilizing agents [Drinkel *et al.*, (2014)]. The ultrasonic reduction method of palladium salts (chlorides, acetates) in various media (aqueous, polyols) has been used to synthesized novel PdNPs with a much smaller size, higher surface area, and narrower size distribution than those prepared by several methods [Okitsu *et al.*, (1996); Chen *et al.*, (2001)]. However, few studies have been performed on the preparation and characterization of PdNPs produced by reduction of palladium (II) nitrate in aqueous solution with ethylene glycol and PVP as reducing and stabilizing agents, respectively. The typical image and spectra of PdNPs as reported earlier by Yoon *et al* as shown in Figure 1.4. The particle size of PdNPs as reported earlier by Zhou *et al* justified that nanodimension scale as shown in Figure 1.5.



**Figure. 1.4.** Visual images of PdNPs and their corresponding UV-Vis spectra [Yoon et al., (2003)].



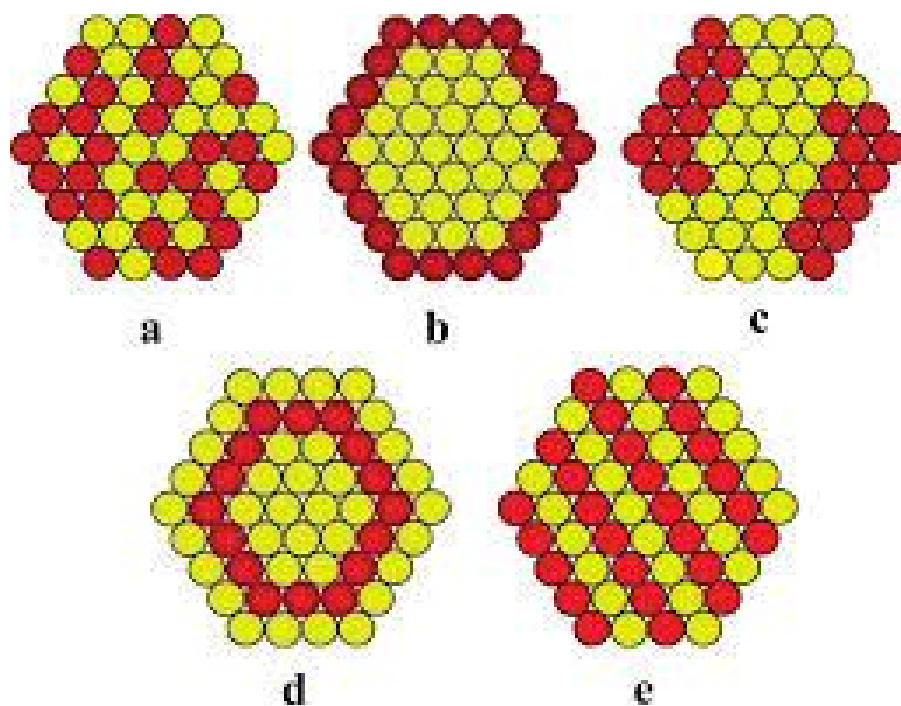
**Figure. 1.5.** TEM image of PdNPs [Zhou *et.al* (2006)].

### **1.1.1.3. Bimetallic nanoparticles**

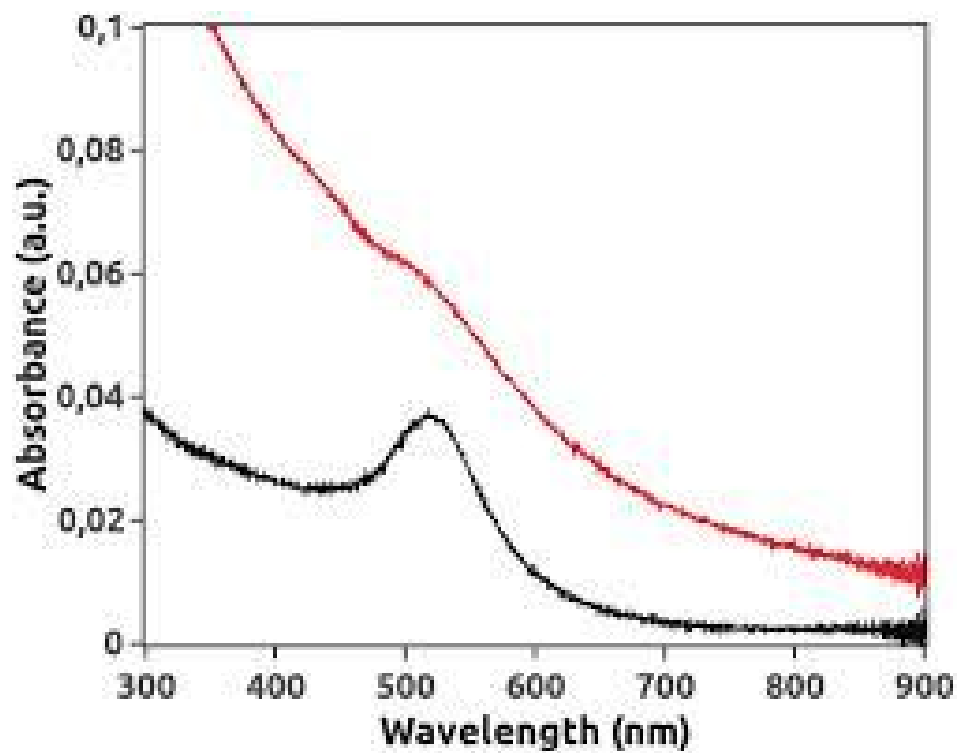
Inspired by the excellent property of NMNPs, considerable attention has been made towards the synthesis of bimetallic nanoparticles (BMNPs). BMNPs, composed by two distinct metal elements, having certain mixing pattern or chemistry sequence or geometry architecture and perform specific functions. Recently, BMNPs have attracted noticeable experimental and theoretical interest because of their optical and catalytic properties [Yoon *et al.*, (2005)]. Their performance often surpasses the enhancement of the properties associated with their single counterparts and exhibits many interesting and useful properties with broad application fields generally explained by synergistic effects of nanomaterials [Zhang *et al.*, (2005)]. By changing components and morphologies of BMNPs, their relevant physical, electrical, or chemical performances can be successfully modified [Linic *et al.*, (2004)]. BMNPs consisting three types of structures, in which the distribution of each metal element is not found as in the bulk: (1) a core-shell structure, in which one metal element forms an inner core and the other element surrounds the core to form a shell, (2) the cluster-in-cluster structures, there is one element forms nanoclusters and the other element surrounds the nanoclusters, acts as a binder and (3) alloy structure, where two kinds of metal elements provide an alloy. Several methods have been used for the synthesis of BMNPs, like, radiation-induced synthesis, electrochemical synthesis and sonochemical synthesis which normally do not require chemical reducing agents [Mallik *et al.*, (2001); Doudna *et al.*, (2003); Abedini *et al.*, (2013); Toshima and Yonezawa., (1998); Cheng *et al.*, (2011); Kim *et al.*, (2014); Mizukoshi *et al.*, (1997); Mizukoshi *et al.*, (2000)]. The use of reducing agents has been extensively investigated that require the participation of reducing agents. For example, Mallin and coworkers [Mallin *et al.* (2002)] used sodium borohydride as a reducing agent and sodium citrate as a capping agent to prepare stabilized Au/Ag bimetallic nanoparticles. Chen and Chen [Chen *et al.* (2002)] used hydrazine as a reducing agent, for the synthesis of Au/Ag BMNPs. BMNPs containing gold as one of the

elements have been synthesized in a variety of ways. Such process involved the participation of gold nanoparticles (AuNPs) as a counterpart with Ag [Lu *et al.*, (2013), Tsao *et al.*, (2014), Park *et al.*, (2011), Gomez *et al.*, (2013) Fu *et al.*, (2013), Zhu (2009), Yu *et al.*, (2011), Zhu *et al.*, (2014)], Pd [Annan *et al.*, (2011); Yang *et al.*, (2011); Zhu *et al.*, (2012); Li *et al.*, (2012); ] Pt [Song *et al.*, (2012); Li *et al.*, (2013)], TiO<sub>2</sub> [Marx *et al.*, (2011)], Fe [Liao *et al.*, (2012)], Zn [Ajai *et al.*, (2013)], Cu [Kim *et al.*, (2014); Sra *et al.*, (2004)], ZrO<sub>2</sub> [Abdelsayed *et al.*, (2006)], and Fe<sub>2</sub>O<sub>3</sub> [Davies *et al.*, (2013)]. Although BMNPs have been known for a long time however, Schmid's group [Schmid., (1992)] was the first to report the synthesis of core-shell bimetallic nanoparticles. AuNPs were covered with a Palladium or Platinum shell when an aqueous solution of these AuNPs was added to a solution of its corresponding salts. Stabilization of the bimetallic nanoparticles could be achieved using the Brust procedure in the presence of thiols [Bethell *et al.*, (1996); Brust and Kiely., (2002)]. Such stable BMNPs were synthesized with group (Pd, Pt) [Song *et al.*, (2012)] and group (Cu, Ag, Au) [Kim *et al.* (2014); Tsao *et al.*, (2014)] metals, all containing Au as one of the two metals. The mole ratios of metals both in and on the surface of the bimetallic cores differed significantly from the metal: salt ratio used in the synthesis of BMNPs. These BMNPs are of great interest and importance because their SPR properties are distinct from those of their constituent components [Lu *et al.*, (2013); Tsao *et al.*, (2014)]. As described earlier by Robert. W. J. Scott showed several representative architectures of BMNPs an Alloy, Core-shell, Cluster-on cluster, Sub-shell and Intermetallic as shown in Figure 1.6. Figure 1.7 shows the UV-Vis spectra of Au-Pd by the group Spitale *et al.* In the spectra black line exhibits the characteristic SPR band at around 510-520 nm of AuNPs and red line after the addition of palladium. It may be inferred that a Pd shell has been formed.





**Figure. 1.6.** Several possible representative architectures of BMNPs including (a) an alloy, (b) core-shell, (c) cluster-on-cluster, (d) sub-shell, and (e) intermetallic [Scott *et al.* (2015)].



**Figure. 1.7.** UV-Vis spectra of bimetallic nanoparticles of Au-Pd [Spitale *et al.* (2015)].

## 1.1.2. Applications of Noble Metal Nanoparticles

### 1.1.2.1. Silver nanoparticles

AgNPs have shown promising applications in various areas, viz, biomedical, material science, and in catalysis as compare to their bulk solid. AgNPs have already proves that it is one of the most important group of nanomaterials for biosensing approaches, as well as in other biomedical applications. AgNPs have many different effects on human health relative to bulk material from which they are produced [Albrecht *et al.*, (2006)]. Increase the biological activity of nanoparticles can be beneficial, detrimental or both. Exposure of metal containing nanoparticles to human lung epithelial cells generated reactive oxygen species, which lead to oxidative stress and damage of the cells [Limbach *et al.*, (2007)]. The biocompatibility and toxicity were exhibited by AgNPs. AgNPs are also used in wastewater treatment utilities and to biological systems [Rani *et al.*, (2009)]. The inhibitory effects of AgNPs on microbial growth were evaluated at a treatment facility using an extent respirometry technique. The nitrifying bacteria were susceptible to inhibition by AgNPs, which could have detrimental effects on the microorganisms in wastewater treatment [Gurunathan *et al.*, (2009)]. It is a non-toxic, safe inorganic antibacterial agent, capable of killing microorganisms that cause diseases [Jeong *et al.*, 2005]. AgNPs has ability to exert a bactericidal effect at minute concentration [Percivala *et al.*, 2005] and has a significant potential for a wide range of biological application such as antibacterial agents for antibiotic resistant bacteria, preventing infections, healing wounds and anti-inflammatory [Taylor *et al.*, 2005]. Apart from that, other important application of AgNPs in biosensing, due to their high selectivity and sensitivity. In addition to that the colorimetric detection of various analytes has been performed through the utilization of AgNPs. The AgNPs can be used for selective and colorimetric detection of cysteine in the presence of other amino acids and glutathione (GSH) through the formation of Ag-S covalent bonds between cysteine and AgNPs as well as the electrostatic interaction between  $\text{COO}^-$  and  $\text{NH}_3^+$  among cysteine molecules [Wang *et al.*, (2008); Huang *et al.*, (2013)]. Although various NPs have been evaluated as enzyme mimetics including ferromagnetic NPs [Gao *et al.*, (2007); Zhang *et al.*, (2010)], NMNPs [Pradeep *et al.* (2009); Jensen *et al.* 1999] and carbon-

based nanomaterials as oxidase or peroxidase mimetic [Lin *et al.* (2014), Xie *et al.*(2012)]. Few reports have also been focused on AgNP-based mimetic reaction. [Jiang *et al.*, (2012); Ma *et al.* (2016)]

### **1.1.2.2. Palladium nanoparticles**

As a consequence, nanoparticles of palladium have been heavily studied in a wide range of catalytic applications including hydrogenations [Hemantha and Sureshababu., (2011); Zhang *et al.*, (2014)] oxidations [Yang *et al.*, (2014); Shinde *et al.*, (2015)] carbon-carbon bond formation [Cheong *et al.*, (2010); Shendage *et al.*, (2013)] and electrochemical reactions in fuel cells [Zhou *et al.*, (2006); Yang *et al.*, (2014)]. However, it should be noted that the applications of palladium go beyond catalysis. For example, palladium adsorb hydrogen has also led to being utilised in hydrogen storage [Rehyani *et al.*, (2011); Adams *et al.*, (2011); Parambath *et al.*, (2012); Kumar *et al.*, (2015); Castillo *et al.*, (2015)] and sensing applications [Mubeen *et al.*, (2007); Wang *et al.*, (2012); Leng *et al.*, (2015); Shin *et al.*, (2015)]. Palladium is often mixed with other platinum group metals like ruthenium, rhodium, osmium, iridium, and platinum, has strong catalytic activity for reactions such as hydrogenation, dehydrogenation, oxidation and hydrogenolysis [Li *et al.*, (2001); Mei *et al.*, (2007)]. PdNPs are used as catalyst for the acetoxylation of ethylene to vinyl acetate and in the manufacturing of sulfuric acid and methanol [Tacke *et al.*, (1998); Chen *et al.*, (2006)]. Due to its lower cost, it is also used in telecommunication and electric equipment as Pd-based contact materials to replace gold [Antler., (1987)]. In addition to that, PdNPs has also been widely used to prepare dental filling materials and produce fine jewellery. For many years, automobiles have been equipped with Pd-based catalysts to minimize the emission of carbon monoxide, nitrogen oxides and hydrocarbons from fuel combustion [Beutel *et al.* (2009) US7576031 B2 ]. In biomedical applications, Pd-103 is widely used in permanent low dose radiation (LDR) brachytherapy as a prostate cancer treatment. Palladium emits higher levels of radiation over a shorter period of time than iodine-125 [Bownes and Flynn., (2005); Mayer *et al.*, (2004)]. Meanwhile, Pd (II) complexes are able to intercalate in the double helix of DNA and subsequently kill cancerous cells [Brabec *et al.*, (2006)]. PdNPs catalyzed Suzuki

cross-coupling reaction of aryl halides with aryl boronic derivatives is a very suitable and extensively studied approach to C–C bond formation [Deshmukh *et al.*, (2011); Uberman *et al.*, (2012)]. One of the predominant applications of PdNPs is selective hydrogenation of unsaturated compounds, e.g., phenol hydrogenation to cyclohexanone and semi-hydrogenation of alkynes [Liu *et al.*, (2009)]. PdNPs of a few nanometers in diameter exhibit size-dependent (electronic, optical, etc) electrocatalysis, and has shown a profound impact on reaction rates and mechanisms [Klejin *et al.*, (2014); Kim *et al.*, (2015)].

### **1.1.2.3. Bimetallic nanoparticles**

Recently, BMNPs have received considerable attention because of their importance for magnetic, optical, and catalytic applications in a variety of fields. Their value arises from their distinctive properties, which are clearly different from those of monometallic nanoparticles [Tao *et al.*, (2008); Ferrando *et al.*, (2008); Burda *et al.*, (2005)]. The field of BMNPs synthesis is currently an area of considerable interest, especially in the area of catalysis, where the BMNPs often possess greater catalytic activity than that of their monometallic counterparts. This is because bimetalization can improve catalytic properties of the original single-metal catalysts and create a new property, which may not be achieved by monometallic catalysts. Due to their unusual chemical and physical properties, they are expected to have potential applications in many technologies, such as opto-electronic nanodevices, catalysts, and chemical sensors [Villa *et al.*, (2015); Zhang *et al.*, (2013)]. In recent years, tremendous progresses have been made on bimetallic nanocrystals because of the newly developed capability in controlling the composition and thus their physical and chemical properties. For example, heterostructures of Pt–M (M =Au, Pd, Ru) BMNPs show much improved catalytic performance toward oxygen reduction [Yeo *et al.*, (2011); Koenigsmann *et al.*, (2011); Mao *et al.*, (2011); Liu *et al.*, (2012)], formic acid oxidation [Lu and Chen., (2011); Habas *et al.*, (2007)], and methanol oxygen [Guo *et al.*, (2010a); Wang *et al.*, (2011); Rivera *et al.*, (2004)], compared with Pt catalysts [You *et al.*, (2013)]. Some of the BMNPs show improved catalytic activity in comparison to pure gold catalyst [Yen *et al.*, (2009); Menezes *et al.*, (2013)]. For instance, Hutchings and coworkers have successfully prepared bimetallic Au–Pd nanoparticles and extensively

investigated their catalytic properties for different reactions [Enache *et al.*, (2006); Hutchings *et al.*, (2008)]. Ag/Au BMNPs have unique catalytic, electronic, and optical properties, which are distinct from those of the corresponding monometallic nanoparticles [Xiong *et al.*, (2006); Zhang *et al.*, (2013)].

## **1.2. NANOCOMPOSITES OF NOBLE METAL NANOPARTICLES**

### ***1.2.1. Synthesis of Nanocomposites***

Nanocomposites are composites in which at least one of the phases shows dimensions in the nanometre range ( $1 \text{ nm} = 10^{-9} \text{ m}$ ) [Camargo *et al.*, (2009)]. In the broadest sense this definition can include porous media, colloids, gels and copolymers, but it is more usually taken as the solid combination of a bulk matrix and nanodimensional phases differing in properties due to dissimilarities in structure and chemistry. The mechanical, electrical, thermal, optical, electrochemical and catalytic properties of the nanocomposite were differing from that of the component materials. Nanocomposite materials have emerged as suitable alternatives to overcome limitations of microcomposites and monolithics, while posing preparation challenges related to the control of elemental composition and stoichiometry in the nanocluster phase. Nature using natural reagents and polymers such as carbohydrates, lipids and proteins, for making strong composites such as bones, shells and wood. In the early 1990s, Toyota Central Research Laboratories in Japan reported work on a Nylon-6 nanocomposite [Braun *et al.*, (1997)], for which a very small amount of nano filler loading resulted in a pronounced improvement of thermal and mechanical properties. ‘The properties of nanocomposite materials depend not only on the properties of their individual parents but also on their morphology and interfacial characteristics’, says Kanartzidis [Gleiter *et al.*(1992); Hussain *et al.* (2006)]. Depending on the phases involved, nanocomposites can be classified as inorganic, organic, or hybrid organic/inorganic materials. A narrower classification uses the chemical nature of the bulk matrix and of the nanodimensional phases: (a) ceramic matrix nanocomposites (CMnC) consist of a bulk ceramic with finely dispersed inorganic or organic nanofiller, to achieve improved mechanical, optical, electrical, and/or magnetic properties, as well as corrosion-resistance and other protective capacities [Pathak *et al.*, (1986)], (b) polymer-matrix nanocomposites (PMnC) have their enhanced

performance due to an organic polymer matrix and to metallic or other inorganic or nanofillers even in extremely low concentrations [Kawasumi *et al.*, (1997); Vaia and Giannelis (1997)] and (c) metal-matrix nanocomposites (MMnC) have a metal matrix reinforced either with a ceramic nanofiller, carbon nanotubes or fibers, silicon carbide, or boron/carbon nitride [Tomasko *et al.*, (2003); Watkins *et al.*, (1995); Ciriminna *et al.*, (2013)] as shown in Table 1.1.

**Table 1.1.** Different types of Nanocomposites

| Class          | Examples   |
|----------------|--|
| <b>Metal</b>   | Fe-Cr/Al <sub>2</sub> O <sub>3</sub> , Ni/Al <sub>2</sub> O <sub>3</sub> , Co/Cr, Fe/MgO, Al/CNT, Mg/CNT   |
| <b>Ceramic</b> | Al <sub>2</sub> O <sub>3</sub> /SiO <sub>2</sub> , SiO <sub>2</sub> /Ni, Al <sub>2</sub> O <sub>3</sub> /TiO <sub>2</sub> , Al <sub>2</sub> O <sub>3</sub> /SiC, Al <sub>2</sub> O <sub>3</sub> /CNT |
| <b>Polymer</b> | Thermoplastic/thermoset polymer/ layered silicates, polyester/TiO <sub>2</sub> , polymer/CNT, polymer/layered double hydroxides.   |

### 1.2.2. Application of Nanocomposites

Nanocomposites offer enhanced chemical and physical characteristics such as high specific area, superior mechanical properties, electrical conductivity, or optical properties, thereby improving their sensitivity and specificity [Mustafa *et al.*, (2014)]. Promising applications of nanocomposite systems are numerous, comprising both the generation of new materials and the performance enhancement of known devices such as fuel cells, sensors and coatings. Nanocomposites containing inorganic spherical and anisotropic nanoparticles, with assemblies of nanoparticles in block copolymers (BCPs) toward functional hybrid materials are reported [Kao *et al.*, (2013)]. There are extensive research which have been devoted towards the development of semiconductor-metal nanocomposites consisting of different classes of materials with coherent interfaces [Hu *et al.*, (2012); Haldar *et al.*, (2012); Ding *et al.*, (2012); Motl *et al.*, (2012); Wang *et al.*, (2013)]. Several studies have reported that graphene-based hybrid composites exhibit superior performance compared with the individual components because of the synergistic effects between each component. The formation of graphene-based composites is greatly advantageous to the design of a new type of versatile material for high-performance, transparent conducting films

(TCFs), field-effect transistors (FETs), sensors, supercapacitors, solar cells, lithium ion batteries, electrodes, drug carriers, gas barriers, photocatalysts, and photoconductors [Lee *et al.*, (2015)]. On the other hand, Prussian blue (PB) has been widely used as an electrocatalytic materials for analytical applications [Yang *et al.*, (2011); Nossol and Zarbin., (2012); Gao *et al.*, (2014)]. Particularly, it was denoted as an “artificial peroxidase” because of its rapid catalytic rate toward the reduction of hydrogen peroxide ( $H_2O_2$ ) at low overpotential [Karyakin *et al.*, (2000); Gao *et al.*, (2014); Sitnikova *et al.*, (2014)]. The fabrication of PB-based nanocomposites is usually focused on developing various conductive materials, such as graphene, carbon nanotubes, titanate nanotubes, polypyrrole and other substrates [Zhang *et al.*, (2012); Ni *et al.*, (2013); Cao *et al.*, (2010); Zou *et al.*, (2015); DeLongchamp *et al.*, (2004); Yamamoto *et al.*, (2009)]. Many noble metals have been commonly used to prepare enzyme-free  $H_2O_2$  sensors due to their large specific surface area, excellent conductivity and extraordinary electrocatalytic properties [Giovanni *et al.*, (2012); Wang *et al.*, (2013); Jiang *et al.*, (2013)]. Apart from many applications of Nanocomposites, the BMNPs and their composites have been extensively used in electrocatalytic material for their use in electrochemical sensor design. In addition to that such materials have also shown peroxidase mimetic activity for developing technology for biomedical applications which are discussed *vide infra*.

#### ***1.2.2.1. Electrochemical Application***

One of the important electrochemical applications is the sensors fabrication which reflect a qualitative and quantitative analysis of multicomponent species in a given sample. The term "chemical sensor" is used for a sensitive element that determines the detectable presence, concentration or quantity of a chemical species of interest in a given sample. Chemical sensors can operate on two main principles: first is a chemical principle, where the analytical signal arises from a chemical reaction between the component being determined and a sensitive layer consisting of a specific chemical substance. And the second is physical principle, where physical parameters are being measured like absorption or reflection of light in different regions of the spectrum, conductivity and mass with a change of concentration of the component being determined. In this connection, biosensors, the

operation of which is based on the biochemical interaction of a sensitive layer (it may consist of living organisms, tissues, cells, membranes, enzymes, antibody preparations) with chemical components being determined, are more appropriately called a variation of chemical sensors. An inherent part of a chemical sensor is a transducer or converter of the energy of chemical, biochemical, or physical processes, usually into an electrical signal, which in turn is transmitted to the appropriate electronic device for further processing.

The interaction of the target analyte with the sensing layer produces a signal when it is interrogated by a suitable transducer. Depending upon the mode of signal transduction, chemical sensors can be classified as thermal sensors, mass sensors, optical sensors and electrochemical sensors. Electrochemical sensors used in electroanalytical determination contain two basic functional units; receptor part, which transforms the chemical information into a form of energy and transducer part which transforms the energy, bearing chemical information, into a useful signal. Various kinds of electrochemical techniques can be used for this purpose and the most common techniques are amperometry, potentiometry and conductometry. Based on these techniques the electrochemical sensors are divided into three main categories:

- **Amperometric Sensors**

The basic principle involved in amperometric devices is the measurement of current at an electrode surface at a fixed electrode potential. The concentration of the target analyte is proportional to the current generated at the electrode surface. The first report of this type of sensor was the Clark oxygen electrode. Amperometric sensors are extensively used in the sensing of analytes in electroanalytical chemistry. NMNPs and their composites provide surface for the direct oxidation or reduction of electroactive ions present in the solution. Different methods have been employed for the fabrication of electrodes in amperometric sensors [Pandey *et al.*, (2001)d; Pandey *et al.*, (1999c); Erden *et al.*, (2013); Kotanen *et al.*, (2013)]. Many reports are available employing chemically modified electrodes as amperometric sensors [Pandey *et al.*, (2005b); Dey *et al.*, (2013); Kul *et al.*, (2013); Qi *et al.*, (2013)]. There have been many articles that discuss the amperometric biosensors based on mediated or



unmediated electron transfer and biosensors for many species such as glucose, fructose and cholesterol have been developed [Pandey *et al.*, (2001)d; Pandey *et al.*, (1999c)].

- **Potentiometric Sensors**

Potentiometric sensors measure the potential difference between the reference electrode and the indicator electrode without polarizing the electrochemical cell, i.e., negligible quantity of current is allowed. A reference electrode is required to provide a constant half-cell potential while indicator electrode develops a variable potential depending on the activity or concentration of a specific analyte in solution. The variation in potential is proportional to the logarithmic of target analyte concentrations. Potentiometric sensors can be broadly classified into three basic types as: ion selective electrodes (ISE), coated wire electrodes (CWE) and ion selective field effect transistors (ISFET) [Minh *et al.*, (1990); Pandey *et al.*, (2000); Pandey and Singh., (2001a); Pandey and Mishra., (2004a); Abramova *et al.*, (2013); Guzinski *et al.*, (2013)].

- **Conductometric Sensors**

Conductometric sensors measure the change in electrical conductivity or resistance of a film or a bulk material due to the presence of a specific analyte. Such materials are termed as chemoresistors [Soldatkin *et al.*, (2013); Nguyen-Boisse *et al.*, (2013); Hossein-Babaei *et al.*, (2012)]. Conductometric methods are fundamentally non-selective. There are some practical considerations that make conductometric methods attractive, such as low cost and simplicity, since no reference electrodes are needed. Improved instrumentation has contributed to rapid and easy determination of analytes, based only on the measurement of conductivity. There are two major classes of chemoresistors: (1) low-temperature (room temperature) chemoresistors with polymeric and organic sensitive coatings and (2) high-temperature (200-600 °C) chemoresistors with semiconductor metal oxide coatings. Chemocapacitors (dielectrometers) are another class of conductometric sensors. They rely on changes in the dielectric properties of a sensing material upon analyte interaction.

Based on these findings our work is directed towards the Electrochemical Sensors. To fabricate an Electrochemical Sensors Mediator- Modified Carbon paste electrodes are taken into consideration because of its several peculiar features. The electrons from the active center of the redox enzyme to the electrode surface at a measurable rate. The introduction of an electron transfer mediator in the construction of an electrochemical biosensor ultimately involves the occurrence of coupled catalytic reactions, the first one being associated with the regeneration of the enzymatic activity and the second one with the regeneration of the mediator electrochemically advantages of using mediator are that mediator permits the enzymatic reaction to proceed independently of oxygen tension and electrochemical detection of the mediator can be performed at a lower over voltage, thereby decreasing the effects of potential interfering species. The major requirements in the development of mediated electrochemical biosensor for a practical application are:

- (i) The capability of the biosensors to probe trace levels of the target analyte,
- (ii) Reproducibility of the sensor response,
- (iii) Wide linearity and first response time of the sensor.

These requirements can be fulfilled by,

- (i) Effective coupling of the immobilized biomolecular together with the mediator on electrode surface;
- (ii) Involving a suitable mediator with fast second order reaction rate associated with reaction between the biomolecular and the mediator;
- (iii) Good electrochemical behavior of the mediator within the immobilized phase;
- (iv) Insolubility of redox couples of the immobilized mediator in aqueous solutions.

Although there are several methods available for the development of mediated electroanalytical biosensor however the present topic is restricted to the designing of such electroanalytical sensor based on application of carbon/graphite paste matrix.

However, the charge transfer within paste depends on its electron relaying properties. Obviously the hopping sites would be more if the graphite paste is homogeneous along with the incorporated mediator and enzyme thereby reducing the distance the two relays.

The mediated mechanism of electron exchange has shown well-defined electrochemistry associated with the designing of enzyme-based biosensor and has been extensively studied involving a variety of mediators. However the practical implementation is restricted to a few mediators mainly because of the problem associated with

1. The immobilization of the mediator at the electrode surface.
2. Stability of the redox couple within the immobilized phase in aqueous solution;
3. Sensitivity of the mediator to species other than the enzymatic redox center;
4. Fast second-order reaction rate between redox center and mediator;
5. Reversible heterogeneous kinetics;
6. Low overpotential and
7. Stability with respect to pH, temperature and oxygen.

Although the Ferrocene and its derivatives have been shown to be an efficient mediator for the regeneration of various oxidoreductase, enzymes through the mediated mechanism.

#### ***1.2.2.2. The Artificial Peroxidase Activity of Nanomaterials***

Natural enzymes attracting great interest in clinical diagnosis, biotechnology, chemistry, environmental science and other fields. Enzymes-catalyzed reactions possess remarkable advantages such as high specificity and high efficiency under mild conditions. However, natural enzymes have some drawbacks such as; (i) they can be easily denatured by environmental changes because their catalytic activity depends on the integrity of their native protein conformation; (ii) they can be digested by proteases and (iii) the preparation and purification are usually difficult, time-

consuming and expensive. Therefore, efforts have been paid to constructing enzyme mimetic with similar functions to natural enzymes. Accordingly, various efforts have been made for an alternative to natural enzymes such as cytochrome P, hemin, hemeatin, porphyrin, cyclodextrin, peroxidase and so on [Wang *et al.*, (2007); Sono *et al.*, (1996); Zhang *et al.*, (2010); Zhang *et al.*, (2013)].

Nowadays, Yan and co-workers reported that inorganic nanoparticles ( $\text{Fe}_3\text{O}_4$ ) possess intrinsic peroxidase-like activity and have revealed their use as peroxidase replacement [Gao *et al.*, (2007)]. This surprising discovery has opened the door for the development of nanoscaled inorganic materials in the biochemical assay. Afterwards, a variety of metal-based and carbon-based nanomaterials including AgNPs, AuNPs, PdNPs, PtNPs, Au@Pt, Au@Pd, Au@Ag, nanorods, carbon nanodots, graphene and its derivatives, single-walled carbon nanotubes, etc., have emerged as enzyme mimics [Jiang *et al.*, (2012); Lien *et al.*, (2012); Ma *et al.*, (2011); Nangia *et al.*, (2012); Guo *et al.*, (2011); Tao *et al.*, (2013); Ju and Kim., (2015)]. The NMNPs that mimic enzymes are very interesting as they have better properties comparing to natural enzymes like more resistance to pH, temperature, large surface-to-volume ratio and protease digestion. In addition to that, they are cheap and easier to prepare and store [Asati *et al.*, (2009); Chen *et al.*, (2012b); Wang *et al.*, (2012)]. Therefore, there is still challenging demand to evaluate the catalytic efficiency of such materials and to compare with that of natural enzyme, thereby allowing the precise control of the mimetic character and ultimately leading to the development of new materials as powerful peroxidase i.e. Horseradish peroxidase (HRP) replacement during enzyme and immune sensors development.

Natural enzyme Peroxidase catalyzes the conversion of different chromogenic substrates such as *o*-dianisidine (ODA), tetramethylbenzidine (TMB), di-azo-aminobenzene (DAB), and *o*-phenylenediamine (OPD) into coloured products using  $\text{H}_2\text{O}_2$  as the oxidizing agent [Josephy *et al.*, (1982); Ma *et al.*, (2011)]. This chromogenic reaction has been used to determine  $\text{H}_2\text{O}_2$ . Similarly, in glucose detection technique, horseradish peroxidase (HRP) is used to catalyze reaction between a chromogenic substrate and  $\text{H}_2\text{O}_2$ , generated from glucose-GOx system, to produce a coloured reaction product. However, this method has a major drawback in

the sense that HRP being a natural enzyme becomes easily denatured and loss its activity by slight environmental changes. Thus, for practical application, HRP have to be removed with artificial enzyme to eliminate a number of obstacles. To highlight the recent progress in the field of nanomaterial-based artificial enzymes, which are designated as “nanozymes” has been reviewed [Wei and Wang., (2013)]. In this regard gold [Peng and Miller., (2011); Song et al., (2011)] silver [Jiang et al., (2012)] and PB based nanomaterials have also been found to exhibit peroxidase-like activity towards the oxidation of a variety of peroxidase substrate [Zhang *et al.*, (2010); Zhang et al., (2013); Zhang et al., (2014); Pandey and Pandey., (2013b)].

### **1.3. CHALLENGES IN NOBLE METAL NANOPARTICLES SYNTHESIS**

The content described in section 1.1 to 1.2 although, provided valuable information on the synthesis and application of MNPs however, many of them restrict their use in practical application due to aggregation, because nanosized metal particles in the solution are active and prone to coalesce due to Van-der Waals forces and high surface energy unless they are protected. Accordingly, various methodologies like chemical, thermal and photochemical processes for the synthesis of AgNPs, PdNPs, AuNPs, BMNPs and Nanocomposites have been attempted. In previous section, described protocols for the synthesis of NMNPs restrict its application in solid state configuration, stringently confined its applicability to only aqueous phase, controlled nucleation, solubilisation in variety of solvents and functionalization. From synthetic point of view, the main challenge is to explore new synthetic protocols that allow the preparation of NMNPs in a controlled manner, obtaining (i) a narrow size distribution, because the properties of nanoparticles are highly size dependent, (ii) protection against aggregation, and (iii) functionality for further conjugation. Beside this the insolubility of these nanoparticles in a variety of organic solvents is one of the major problems that restricted direct manipulation of MNPs for practical application. Therefore, a method is needed to disperse AgNPs, PdNPs and BMNPs into a solvent creating the nanodispersion liable for the development of new nanoparticles-based devices that can be designed with fine patterns in solid-state configuration. Therefore, it is essential to inspect a systematic research on these issues and to provide a deeper

insight for practical applicability which has been the prime attention for present research investigation exploring the possibility of investigating a new synthetic route on nanoparticles synthesis.

#### **1.4. ORIGIN OF THE PRESENT RESEARCH PROGRAMME**

The finding demonstrated in preceding section justified the potential application of nanomaterial in heterogeneous catalysis. Accordingly there has been prime attention on the synthesis of such nanomaterial leading to the formation of nanostructured solid-state matrix for catalytic applications. One of such approach being adopted in our laboratory is the development of organically modified sol-gel (ORMOSIL) film derived from the use of functional alkoxysilanes [Tripathi, (2002); Sharma, (2002)]. Accordingly the role of hydrophilic and hydrophobic organically functionalized alkoxysilanes in the formation of ORMOSIL has been extensively studied in our laboratory [Pandey *et al.*, (1999c); Pandey *et al.*, (1999d); Pandey *et al.*, (2001c); Pandey *et al.*, (2001d); Pandey *et al.*, (2001e)]. Optimum ratio of hydrophilic and hydrophobic alkoxysilanes played central role in deriving the ormosil film for practical design. A novel process on ormosil film formation from functionalized alkoxysilane using two different combinations [(I) and (II)] of sol-gel precursors. Combination (I) involved the role of hydrophilic 3-aminopropyltrimethoxysilane (3-APTMS) and hydrophobic 2-(3,4-epoxycyclohexyl) ethyltrimethoxysilane (ECETMS) precursors whereas Combination (II) reveals the role of hydrophilic Trimethoxysilane (TMS) and hydrophobic 3-Glycidoxypropyltrimethoxysilane (GPTMS) during the formation of ORMOSIL [Pandey *et al.*, (1999a); Pandey *et al.* (2003a); Pandey *et al.* (2003c); Pandey *et al.* (2003d)]. These ormosil films provided excellent matrix for the encapsulation of redox active proteins and encapsulated biological sensing component not only yielded in probing biochemical interaction but the nanostructured matrix itself introduced electrocatalysis during electrochemical sensing. One of the problems encountered during electrochemical sensing while using such nanomaterial was poor diffusion of sensing reagent like oxygen while probing the glucose oxidase catalyzed reaction based on non-mediated bioelectrochemistry. Such problem has been subsequently controlled by incorporating water leachable material and graphite particles within nanostructured matrices [Tripathi., (2002)].

Although non-mediated bioelectrochemistry yielded good electrochemical responses during glucose biosensing, however directed us to design mediated/electrocatalytic sensors for selective and sensitive biosensing. Accordingly, the incorporation of redox active materials like Ferrocene and its derivatives/ Ferricyanide within such matrix has been sought [Tripathi., (2002)]. The ORMOSIL matrix was efficient enough to encapsulate small molecule like ferrocene within nanostructured domains with excellent stability, however the redox active material was not able to communicate with redox active protein as per requirement of mediated/ electrocatalytic biosensors. Accordingly, efforts were made to overcome such limitation by incorporating electrocatalyst like palladium/TCNQ-TTF within nanostructured domains [Pandey *et al.*, (2003b); Pandey *et al.*, (2003e)]. Fortunately, the interaction of palladium chloride ( $\text{PdCl}_2$ ) and GPTMS while making ormosil film with TMS has been recorded [Pandey *et al.*, (2001c); Pandey *et al.*, (2003c)]. The presence of glycidoxy residue in one of the alkoxy silane precursor like GPTMS yielded novel finding on the formation of palladium embedded ormosil for electrocatalytic applications. The presence of palladium within ormosil matrix yielded excellent redox electrochemistry of electron transfer relay present in same nanostructured domains [Pandey *et al.*, (2001c)]. The interaction of  $\text{PdCl}_2$  and GPTMS has been identified based on NMR, Mass and UV-Visible spectroscopy. It was found that palladium chloride opens the epoxide ring of glycidoxy-residue and in turn gets reduced forming Pd-Glymo complex [Sharma (2002)]. The formation of Pd-Si linkage from the interaction of  $\text{PdCl}_2$  with TMS was also recorded [Pandey and Upadhyay., (2005a)]. Such chemical reactivity of functional alkoxy silanes yielded in developing a library of electrocatalytic sites within nanostructured domains [Singh, B., (2007)]. These finding demonstrated the reducing ability of functional alkoxy silanes and directed us to understand whether such functional activity could be exploited in the conversion of other noble metal cations (gold ion and silver ion) into respective nanoparticles.

Another interesting observation during ormosil formation from 3-APTMS and ECETMS is that encapsulating potassium ferricyanide and carrier within ormosil in the presence of cyclohexanone and tetrahydrofuran [Pandey *et al.*, (2004b); Pandey and Upadhyay., (2005b)]. It was found that 3-APTMS treated potassium ferricyanide was converted into white Prussian blue during ormosil formation [Pandey and

Upadhyay., (2005b)]. These finding ultimately leads to the synthesis of water soluble PBNPs involving active role of cyclohexanone and 3-APTMS [Pandey and Pandey., (2013a)]. These reagents not only enabled into the synthesis of water soluble PBNPs but also allowed the synthesis of super-peroxidase mimetic mixed metal hexacyanoferrate [Pandey and Pandey., (2013b)]. Such finding reflected specific interaction of 3-APTMS and metal cations revealing its role in the synthesis of functional nanomaterials. Accordingly, we intended to understand the reducing ability of 3-APTMS and GPTMS during the synthesis of functional noble metal nanoparticles which has been undertaken in current research program. The functional ability of such nanomaterial may also be exploited in the synthesis of nanocomposites of PBNPs and NMNPs that has also been undertaken in the present thesis.

## **1.5. OBJECTIVE OF THE PRESENT INVESTIGATION**

The description yielded in section 1.4 framed the objective of the present thesis work. The role of functional alkoxy silane in the synthesis of functional nanomaterials has been our prime attention. Specifically the objective of the present thesis is the synthesis of silver, palladium, bimetallic nanoparticles and their nanocomposites involving the active role of 3-APTMS and GPTMS, organic reducing agents [Cyclohexanone, Tetrahydrofuran hydroperoxide (THF-HPO) and Formaldehyde] and subsequent applications of these nanomaterials in electrocatalysis and peroxidase mimetic. Our attention for meeting these objectives as given below:

- (1) To synthesized AgNPs using 3-APTMS, GPTMS and silver nitrate under ambient conditions that enables controlled process, solubility, functionality and nanogeometry.
- (2) To investigate the synthesis of AgNPs involving the role of organic reducing reagents i.e. Cyclohexanone or Formaldehyde that enables the synthesis of the same in the presence of 3-APTMS.
- (3) To synthesized the Bimetallic nanoparticles of silver and gold (Ag-Au/Au-Ag) using 3-APTMS, cyclohexanone, silver nitrate and tetrachloroauric acid.



- (4) To synthesized the PdNPs involving the role of other organic reagent i.e. THF-HPO that synthesized the same in the presence of 3-APTMS.
- (5) To investigate the synthesis of Bimetallic nanoparticles of palladium and gold using 3-APTMS, THF-HPO, potassiumtetrachloropalladate and tetrachloroauric acid.
- (6) To investigate the synthesis of PdNPs using Cyclohexanone in the absence and the presence of 3- APTMS.
- (7) To synthesized the Nanocomposite of Prussian blue nanoparticles (PBNPs) with AgNPs or bimetallic Au-Ag using 3-APTMS, cyclohexanone, potassium ferricyanide, silver nitrate and tetrachloroauric acid.
- (8) To investigate the characterization of above synthesized nanomaterials using suitable techniques.
- (9) To analyze the applications of PdNPs and Fc-COOH modified graphite paste electrodes in the development of electrochemical sensor for biologically important analyte Ascorbic Acid and PBNP/AgNP modified graphite paste electrode in the development of electrochemical sensor for biologically important analyte H<sub>2</sub>O<sub>2</sub>.
- (10) To experimentally investigate the intrinsic peroxidase-like activity of above synthesized nanomaterials.

## **1.6. WORKPLAN OF THE PRESENT INVESTIGATION**

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

- [1] Investigation of specific interaction of silver ions with 3-APTMS.
- [2] Specific interaction of GPTMS with 3-APTMS capped silver ions.
- [3] Synthesis of AgNPs based on the interaction of active concentration of 3-APTMS and GPTMS.
- [4] Dispersibility of AgNPs in both aqueous and non-aqueous solvents.

- [5] Synthesis of AgNPs by using 3-APTMS, silver nitrate, cyclohexanone / formaldehyde and varying the concentrations of cyclohexanone / 3-APTMS keeping constant concentration of silver nitrate.
- [6] Characterization of AgNPs through TEM, EDX and FT-IR.
- [7] Synthesis of Bimetallic nanoparticles of gold-silver based on the mixing of 3-APTMS treated desired concentration of tetrachloroauric acid / silver nitrate and fixed amount of cyclohexanone.
- [8] Tetrahydrofuran hydroperoxide mediated conversion of palladium ions into PdNPs in presence of 3-APTMS at ambient condition.
- [9] Characterization of PdNPs through TEM, AFM and UV-VIS spectroscopy.
- [10] Chemical synthesis of Bimetallic nanoparticles of palladium and gold via sequential and simultaneous process using 3-APTMS, THF-HPO, potassiumtetrachloropalladate and tetrachloroauric acid.
- [11] Investigation of specific interaction of PdNPs and silanol groups.
- [12] Further investigation of organic amine which control the nanogeometry and functional ability of the PdNPs.
- [13] Cyclohexanone mediated conversion of single precursor (i.e.,  $K_2PdCl_4$ ) into PdNPs at ambient conditions using cyclohexanone and in the presence or absence of 3-APTMS.
- [14] Chemical synthesis of Nanocomposite of PBNPs with AgNPs or Au-Ag using desired concentrations of metal salts and fixed concentration of 3-APTMS and cyclohexanone.
- [15] Characterization of above nanocomposites via TEM and UV-VIS spectroscopy.
- [16] Development of amperometric sensor for Ascorbic Acid based on PdNPs and FcCOOH modified electrodes. Several electroanalytical techniques like cyclic voltammetry and amperometry were performed to assess the sensor efficiency.

- [17] Fabrication of PBNP-AgNP modified electrode in the development of electrochemical sensor for biological analyte  $H_2O_2$  using cyclic voltammetric technique.
- [18] Investigation about the peroxidase mimetic activity of as synthesized Nanocomposites / PdNPs and to evaluate the variation in their kinetic catalytic efficacy towards the determination of analyte like  $H_2O_2$ .