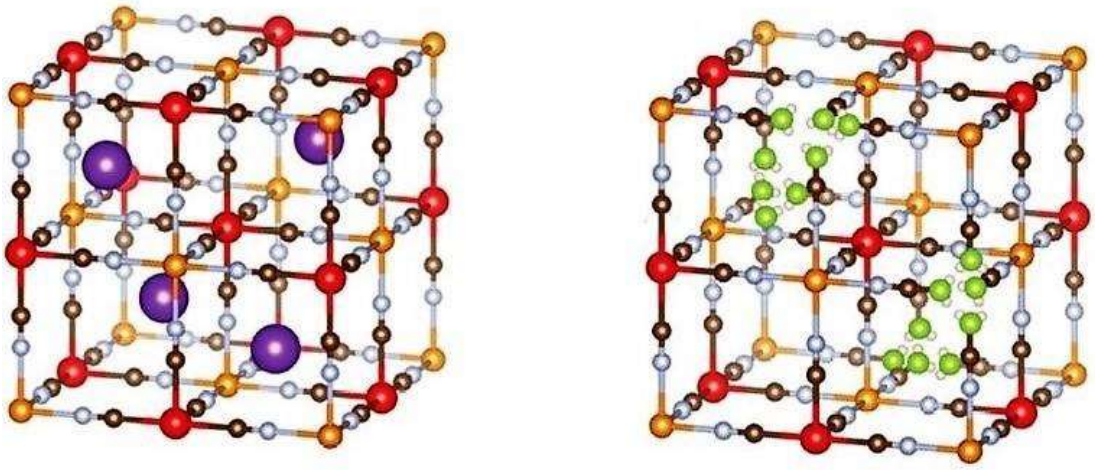
Chapter 1

General Introduction

The materials in their nanoscale exhibit unique chemical and physical properties as compared to their bulk structure (Thanh et al., 2014; Vollath and KGaA., 2008; Mauter et al., 2008; Häkkinen et al., 2003; Roco et al., 1999; Vaseashta et al., 2005; Jeevanandam et al., 2018). Nano-range materials do not only occur in nature but could also be synthesized by humans. Nanoparticles of different metals have achieved significant attention from the scientific community. Since the size of these nanoparticles intrigue physical, chemical and surface electronic properties which have assigned due to their large surface to volume ratio (Astruc et al., 2005). Meanwhile, the appliance of different metal nanoparticles (Ag, Au, Pd, Pt, and Ru) has received enormous attention due to their size-dependent optical, catalytic, and physicochemical properties (Haruta et al., 1989; Hutchings and Haruta, 2005; Qu et al., 2018; Shan et al., 2012; Tang and Cheng, 2015; Wang et al., 2011b; Zhao et al., 2017). Characteristics of these nanomaterials can be easily tuned by controlling its size, shape, and the surrounding chemical environment (solvent, ligand).

In ancient times, the different industries used these nanoscale materials only as the colourants. The blue pigment "Prussian blue" (PB) belongs to a significant family of functional materials, i.e., the transition metal hexacyanoferrates. PB was accidentally discovered (1704) by Berlin painter "Heinrich Diesbach" who tried to create a red colour paint via employing cochineal insect (Ware, 2008). This synthetic coordination compound was considered only a mere dye in the late 1930s until the investigation began exploring its chemical nature and properties. It is the simplest representative of metal hexacyanoferrates (MHCFs) comprised of two metal centers. Among the inorganic materials, this MHCFs attained a wide range of properties and functionality and have received significant attention from the scientific community. Accordingly, different synthetic protocols have been explored to attain MHCFs with enhanced property towards different practical applications. Besides PB is quite interesting because of its two unique characteristics;



(A) (B)

Figure 1.1: 3-D framework of soluble (A) and insoluble PB (B); demonstrated with Fe(II) [red], Fe(III) [yellow], C [golden], N [grey], K [purple], and H_2O molecule [green colour] [Shokouhimehr et al., (2010)].

(i) its "zeolitic" consequences as the significance of the open structure of their crystal lattice, and (ii) its intense blue colour due to charge transfer transition between the iron metals of mixed valance state (Ludi, 1981; Ludi and Güdel, 1973).

1.1 CRYSTAL STRUCTURE OF PRUSSIAN BLUE

The crystal structure of PB was firstly investigated by Keggin and Miles and had suggested that it comprised of a face-centered cubic (fcc) type of unit cell based on the powder diffraction pattern (Keggin and Miles, 1936). Depending on their structural variation, PB has been further categorized into two types. The first case is "soluble" PB; $A^{I}Fe^{III}[Fe^{II}(CN_6)].yH_2O$, where y= 1-5 and A is a monovalent cation such as K^+ and Na^+ and the second type is "insoluble" PB; $Fe_4^{III}[Fe^{II}(CN_6)]_3.xH_2O$, where x= 14-16 (Ludi and Güdel, 1973). Keggin and miles presumed that potassium ion accommodates the interstitial lattice position in order to maintain the crystal's electro-neutrality. Further, Ludi and co-workers precisely studied the crystal of PB and based on their electron, and neutron diffraction investigation (Buser et al., 1972, 1977) states that it contains ab-

out 14 to 15 molecules of water (Herren et al., 1980). The crystal structure of PB possesses the Fm3m space group and designated in such manner so that both iron centers are linked via the same CN ligand. Figure 1.1 displays the two distinct structures of PB, i.e., the soluble and insoluble (Shokouhimehr et al., 2010). Both soluble and insoluble form of PB is highly indissoluble and possess less solvability (Ksp= 10^{-40}). However, the soluble PB easily peptized as blue colloidal sol and forming a clear solution in water, while in the insoluble one, a quarter of [$Fe^{II}(CN)^{4-}$] unit is missing from crystal lattice to compensate for the charge and neutrality. This structural variation creates some vacant positions in the case of insoluble PB. Meanwhile, two different types of water molecules are introduced within its imperfect crystal; (i) co- ordinative water molecules that occupy the vacant position in the crystal lattice and (ii) zeolitic water molecules that lies in the coordination sphere (Boxhoorn et al., 1985; Imanishi et al., 1999). This unique open zeolitic nature of PB has been enormously explored as a "chemical sponge" for adsorbing low molecular weight molecules such as H_2O_2 and O_2 (Boxhoorn et al., 1985).

1.2 COLOUR OF PB

Crystal of PB designated in such a manner that each ferrous is linked directly with a carbon atom, and the nitrogen atom is attached with ferric of the same CN group (Ludi and Güdel, 1973). The ferrous ion (Fe^{+2}) is surrounded with an octahedral environment of carbon having low spin (S=0) configuration, however ferric (Fe^{+3}) lies within the octahedral environment of high spin configured nitrogen (S=5/2). The five electrons are present in the respective orbitals of nitrogen as well as carbon hole, although the sixth one is thought to favor mostly in the t_2g of carbon hole to avoid the inter-electronic repulsion as displayed in Figure 1.2.

The charge transfer that took place from t_2g of carbon hole to t_2g in nitrogen hole is reasonable for the blue colour appearance of this coordination complex and requisite about 14300 cm^{-1} of energy for such electronic transition (Robin, 1962). This amount of energy (14300 cm^{-1}) is more likely to resemble with the coordination complexes, as compared to the electronic energy (10150 cm^{-1}) attributed due to transition from t_2g of

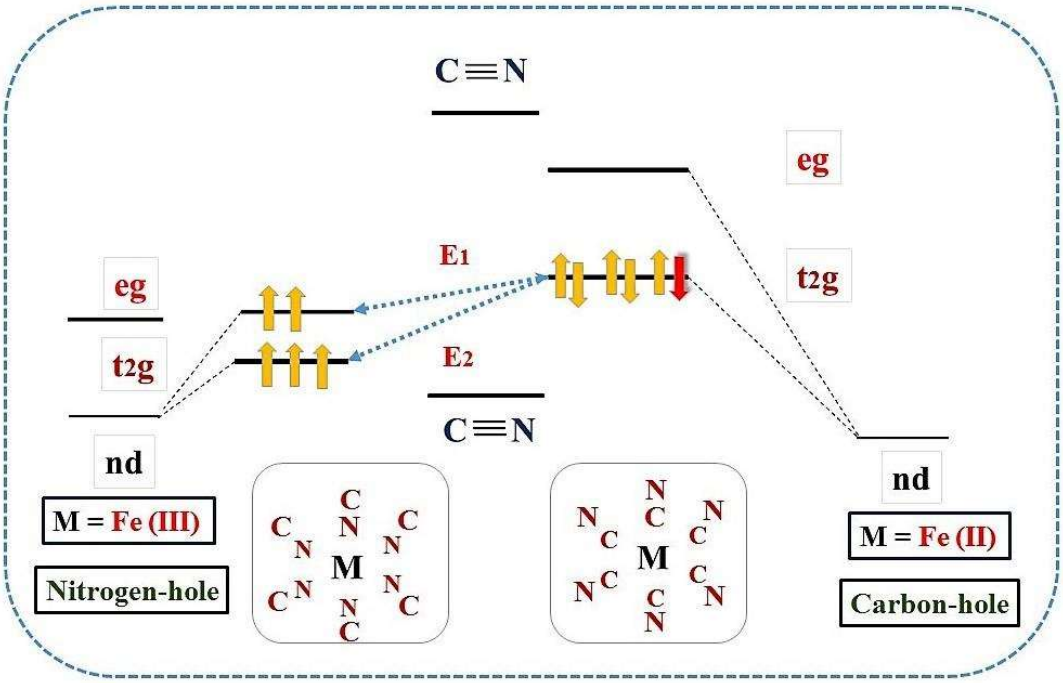


Figure 1.2: Electronic arrangement of valence electrons of Fe(II) and Fe(III) in octahedral environment of carbon and nitrogen hole respectively.

carbon hole to e_g in nitrogen hole.

1.3 PROPERTIES OF PB

1.3.1 Electrochemical

V. D. Neff firstly reported about the electrochemical nature of PB film (Neff, 1978). While Itaya and co-workers thoroughly investigated about the electrochemistry of PB film (Itaya et al., 1982a) and stated that each of the cyclic voltammogram consist of two redox couples vs. Ag/AgCl. The peak centered at lower positive potential (0.2 V) attributed to the oxidation-reduction response of PB to fully reduced Prussian white (PW) and vice versa. In contrast, peak II is due to oxidation of PB to fully oxidized Prussian yellow (PY) or Berlin green (BG) and reduction of PY (BG) species to PB. The response assigned at lower positive potential (0.2 V) is reasonable due to the active participation of high spin system $Fe^{3+/2+}$, while at higher positive potentials (0.9 V) low spin $[Fe(CN)_6^{3-/4-}]$ contributes and interprets to the reversible redox process.

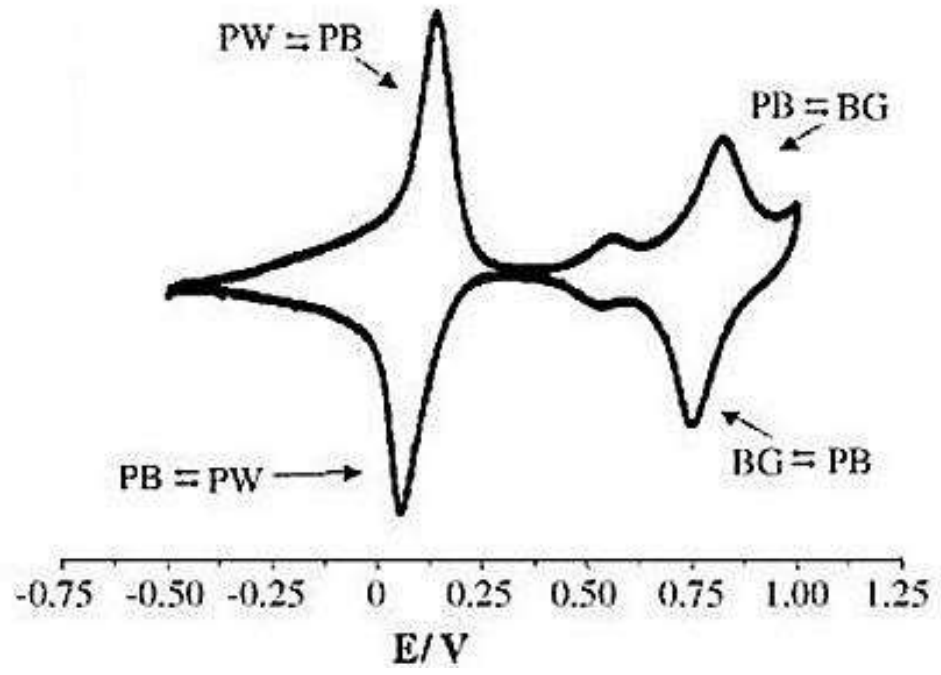


Figure 1.3: Cyclic voltammogram of a PB modified electrode showing oxidation and reduction peak [Ricci and Palleschi, (2050)].

The Karyakin group has thoroughly investigated the active contribution of supporting electrolytes, and they inferred that the large-sized cation could easily penetrate thefilm and facilitate the PB oxidation/reduction process as it possesses a smaller hydrated radii (Karyakin et al., 2001). While the cations with larger hydrated radii could block the reversible redox process after a very few cycles, concluding the retain electrochemical response and optimum behavior of PB in the presence of K^+. Similarly, the electrochemical activity of PB was also observed to sustain even in the presence of others cations like; Rb^+, Cs^+ and NH_4^+. However, the other metals such as; Na^+, Li^+ and H^+ often retards the electrochemical activity of PB after a very few redox cycles. The PB's redox property depends on the channel radius of lattice and the hydrated ionic radii of chosen cations. Since, PB lattice easily accommodates cation with lower hydrated radii such as; K^+ (1.25 Å), Rb^+ (1.28 Å), Cs^+ (1.19 Å), and NH^+ (1.25 Å) through their compatible channel radius (about 1.6 Å) (Ricci and Palleschi, 2005). The electrochemical behavior of mixed metal hexacyanoferrates of nickel, copper, manganese, chromium,

gallium, indium, palla-dium, zinc, vanadium, and silver has also been investigated in detail (de Tacconi et al., 2003; Düssel et al., 1996; Greene et al., 1961; Eftekhari et al., 2004). In addition to that, the electrochemical behavior of rare-earth metals has been studied by many research groups (Liu and Chen, 2002; Sheng et al.,2008; Wu and Cai, 2005). The electrochromic property of PB and Prussian blue analogues (PBAs) was assigned due to an alteration in the charge transfer oscillation energy of metallic centers in the lattice, which leads to simultaneous interchange in its colour.

$$KFe^{III}[Fe^{II}(CN)_6] + e^- + K^+ \rightarrow K_2Fe^{II}[Fe^{II}(CN)_6]$$

Soluble "PB" *Prussian White*

$$KFe^{III}[Fe^{II}(CN)_6] \rightarrow Fe^{III}[Fe^{III}(CN)_6] + e^- + K^+$$

Soluble "PB" *Prussian Yellow*

And the redox process incorporating insoluble PB has also been anticipated via Ricci and Palleschi (2005) as follows:

$$Fe_4^{III}[Fe^{II}(CN)_6]_3 + 4e^- + 4K^+ \rightarrow K_4Fe_4^{II}[Fe^{II}(CN)_6]_3$$

Insoluble "PB" *Prussian White*

$$Fe^{III}[Fe^{II}(CN)_6]_3 + 3X^- \rightarrow Fe^{III}[Fe^{III}(CN)_6]_3X_3 + 3e^-$$

Insoluble "PB" *Prussian Yellow*

Electrochemical behavior of PB concludes the following statements; (i) The reduction process accomplished through the loss of cations in both "soluble" and "insoluble" forms of PB, (ii) while oxidation process involves the cationic loss for the soluble form and intake

of electrolyte anions by the insoluble form of "PB". Throughout the oxidation/reduction process, a flux of cations passes through the holes and the channels of the PB crystal and subsequently maintains the charge compensation throughout the lattice.

1.3.2 Electrochromic

The electrochromism is an inherent property of any material to induce an alteration in the colour in response to the redox process or applied electrochemical potential. Neff firstly reported the electrochromic behavior of PB thin film in 1978. In late 1982, Itaya firstly utilized PB as the sole materials to develop an electrochromic device (Itaya et al., 1982b). So, depending on their electron transfer mechanism, the electrochromic material can be categorized as reducing and oxidizing colouring substances. The PB can be reduced into a colourless form known as PW, and could also be oxidized into pale-green coloured PY, also known as BG. It summarized here that PB film exhibits electrochromic equilibrium between its colourless and coloured state (blue) and displaying fast response and high durable cycles (5×10^6). The reversible behavior of PB established such materials as an ideal candidate to developing an electrochromic device (ECD) which causes a change in colour in the response of the typical oxidation and reduction process as follows:

$$\underset{Blue}{Fe^{III}[Fe^{II}(CN)_6]^- + e^-} \longleftrightarrow \underset{Colourless}{Fe^{II}[Fe^{II}(CN)_6]^{2-}}$$

$$\underset{Blue}{Fe^{III}[Fe^{II}(CN)_6]^-} \longleftrightarrow \underset{Yellow}{Fe^{III}[Fe^{III}(CN)_6] + e^-}$$

The peculiar type of structural arrangement and ease of metal substitution leads to the formation of PBAs of a different colour, dependent on their metal composition, which further exploited in forming electrochemical devices (de Tacconi et al., 2003; Karyakin et al., 2001). A summaryof electrochromism behavior in various MHCFs has been displayed in Table 1.1

Table 1.1: Electrochromic behaviour of different MHCFs

MHCFs	**Colour**	
	Reduced State	**Oxidized state**
CuHCF	Reddish brown	Yellow
PdHCF	Green	Orange
NiHCF	Light grey	Yellow
InHCF	white	Yellow
VHCF	yellow	Blue-green

1.3.3 Photo-physical

PB and PBAs based materials have received enormous attention and emerged as an attractive material for the development of optical sensors and in solar energy conversion. These PB and PBAs modified electrodes have been employed in a photoelectrochemical reaction as materials possess a high molecular mass and polynuclear mixed valent iron cyanide complex structure (Muñoz et al., 2012; Feng et al., 2021; Ghobadi et al., 2021). Photo-current obtained under applied preferable potential was found to retain their stability and reversibility as a influence of irradiation in on and off working conditions. Konchi had investigated and stated that PB and related compounds act as hole transfer agents during the photo irradiation-induced electrochemical process (Koncki, 2002). Meanwhile, PB, in collaboration with Ru(bpy) initiates photolysis of the water molecules into hydrogen and oxygen, via transferring an excited electron from photo-active species (Ru(bpy)) to nanoparticles, under the appliance of irradiation (Zhao et al., 1998).

Static quenching of Photoluminescent Ru(bpy) complexes by PB colloids has been evoked via stepwise complex formation between the positively charged pendant co-polymer complex(Ru(bpy)) and negatively charged (PB) (Zhao et al., 1998).

The photo-response of PB was examined in the absence and the presence of I^{-}/I_2 redox system in the KCl solution (Upadhyay et al., 1991). Study of the photoelectrochemical behavior of PB doped TiO_2 nanocomposites was performed and concluded their photo-current switching behavior de-pendent on the induced electrode potential (Szacilowski et al., 2006).

1.3.4 Magnetic

PB and PBAs have been modulated as a very approaching candidate in designing of molecular-based magnets due to the following interesting facts; (i) They can be easily synthesized from $[M(CN_6)]^{n-}$ building blocks at room temperature. (ii) Metal spin centers are preferably bonded with ligand via a covalent link and structured into a 3D cubic network. (iii) Proposes easy substitution with metallic carriers of different spin and oxidation states and formulates a wide range of structural motifs. PB itself retained their magnetic characteristics eventually at a low curie temperature (Tc = 5.6K) due to their long-range of ferromagnetic arrangement. The PB comprised of CN linked centers; the diamagnetic Fe (II) in low-spin d^6 Fe^{II} (in strong field environment) and paramagnetic Fe (III) in high-spin configuration d^6 Fe^{III} (in weak field sites), through the bond distance of 10 Å, which leads to weak exchange coupling between the metals.

Davidson and Welo firstly reported about the magnetic susceptibility in PB (Davidson and Welo,2002). While, PBAs of different metals such as Mn^{II}, Fe^{II}, Co^{II}, Ni^{II} and Cu^{II} con- tains the paramagnetic metal centers with stronger exchange coupling and shorter bondlength (5 Å) in the ranges of 3 to 50K curie temperature (Tc) (Bozorth et al., 1956).

Recently, Tokoro and Ohkoshi have summarized the magnetic functionalities of all PBAs prospectively (Tokoro and Ohkoshi, 2011).

1.3.5 Charging and Discharging Characteristics

The mixed MHCFs possess a zeolitic type of structure and facilitates the intercalation and de-intercalation of alkali cations in the crystal throughout the electrochemical redox process. Besides, an electrodeposited film of PB over ITO and SnO_2 attained a high range of stability and reversibility in the order of $10^5 - 10^7$ cycles and established as a potent material for battery development. Meanwhile, PB has been used as an anodic and cathodic material in secondary cell evolution by V.D Neff. Accordingly, other MHCFs have also emerged as an exciting candidate for similar development. Some approaches have been introduced to incorporate PB with copper hexacyanoferrate (CuHCF) to de- velop the secondary cell (Grabner and Kalwellis-Mohn, 1987). Significant progress has been driven in order to achieve the solid-state secondary cell based on the charging and discharging properties of; (i) solid-state PB, (ii) CuHCF-PB, and (iii) ZnHCF-PB (Jay- alakshmi and Scholz, 2000a,b). Further, Ali Eftekhari invented solid-state secondary cell via incorporating; (i) two different transition metal hexacyanometallates as anodic and cathodic materials, and (ii) PB-based cathode and potassium as anodic material. The reported secondary cell exhibits superior performance in terms of elated voltage (1.5 V) and cyclability (more than 500) as compared to the previous reports (Eftekhari, 2003, 2004). So, the charge transfer kinetics and cyclic stability were nominated to be essential keys during the optimization of PB and other MHCFs compounds to achieve an excellent product with an enhanced property. Accordingly, Cui and co-workers had proposed that PBAs such as; copper hexacyanoferrate (NiHCF) and CuHCF nanoparticles could be a potent candidate for large scale battery appliance due to its inexpensive synthesis route, long-range cyclability, stability, high rate capability and greater extent of energy efficiency (Wessells et al., 2011a,b).

1.4 APPLICATION OF PB AND ITS ANALOGUES MODIFIED ELECTRODES IN CHEMICAL SENSING

In sensing technology, the electrochemical sensor is an important class which deals with the device that can detect and quantify even the lower concentration of analyte based on their selectivity, and have been shown the prime advances. Based on the transducer mode, electrochemical sensors are classified as conductometric, potentiometric, and amperometric, which deals with the measurement of conductance, potential, and current of the cell, respectively. Plenty of unique characteristics and functionality of these sensors have achieved enormous interest in the electrochemical society and exploited in device modulation. The chemically modified electrodes (CMEs) are fabricated by modifying a chemically unproductive surface into productive ones by selective multifunctional MHCFs (Pournaghi-Azar and Dastangoo, 2002). The high chemical and electrochemical stability of these nano-range inorganic materials have attributed a wide range of practical applicability. A different substrate such as; gold (Au), platinum (Pt), glassy carbon (GC), and graphite paste (GP) have been widely employed for the preparation of chemically modified electrode of MHCFs. These MHCFs have been shown very promising applicability as; energy storage materials, ion sieving materials, electrocatalyst, and sensing devices in several disciplines. Accordingly, the investigation has been widely conducted based on the applicability of MHCFs in chemical sensing.

1.4.1 Electrochemical Sensor for Oxidizable Compounds

PB and PBAs are auspicious material for the development of electrochemical sensors. Various groups had developed electrochemical sensors, based on nanocomposites, and investigated their electrocatalytic response towards cysteine molecule (Abbaspour and Ghaffarinejad, 2008; Majidi et al., 2010; Qu et al., 2011; Sattarahmady and Heli, 2011). An electrochemical sensor, based on nanocomposites of PB with gold nanoparticles (AuNP) and palladium nanoparticles (PdNP), has been developed and unveil the synergistic effect of nano-dimension over their electrocatalytic performance (Pandey and Pandey, 2012c).

Similarly, Chen et al. had employed PB and PBAs for cysteine determination and simultaneously reported a very general kinetic model for consideration of both; the cysteine diffusion and the electrocatalytic response (Chen et al., 2006). The study of electro- chemical and electrocatalytic activity of reduced glutathione (GSH) has been performed over CNFs-PDDA/PB nanocomposites modified TiO_2 electrode (Muthirulan and Velmurugan, 2011). Besides, an amperometry sensor of the cobalt hexacyanoferrate (CoHCF) and CuHCF modified electrodes has also been developed and employed for glutathione (GSH) sensing (Ravi Shankaran and Sriman Narayanan, 2002). These modified electrodes facilitate the glutathione (GSH) catalytic oxidation at a very low over-potentials and inan extensive pH range. Meanwhile, palladium was inserted as a charge transfer bridge between the PB and the Al layer (Pournaghi-Azar and Ahour, 2008). Recently, the developed NiHCF-AuNPs sensor shows functionality towards GSH, and its catalytic ability was observed to be influenced by the size of AuNPs (Pandey and Pandey, 2012a).

Prussian blue nanoparticles (PBN) was observed to retain their electrocatalytic activity towards dopamine (DA) even after the successful modulation over single-walled carbon nanotubes (Adekunle et al., 2012). So, PB and PBAs have been widely explored in designing of electrochemical and chemical sensors towards many oxidizable electroactive species (Karyakin, 2001; Koncki, 2002). Successful determination of dopamine (DA)and ascorbic acid (AA) has been accomplished at the separate oxidation peak poten-tial, on using CTAB (cetyl trimethylammonium bromide) functionalized SnHCF mod- ified graphite paste electrodes (Hosseinzadeh et al., 2009). The nanocomposite of PB doped single-walled carbon nanotube was synthesized and applied through an easy and controlled electrode adsorption process for nitrite sensing (Adekunle et al., 2011). Cui synthesized chitosan (CS) coated PBN with the combination of carbon nanospheres (CNS)and graphene nanosheets (GNS), and justified the high electrochemical activity of these modified nanomaterials in terms of the low detection limit, less response time, and high sensitivity during redox-mediated nitrite oxidation process (Cui et al., 2012).

PB doped multiwalled carbon nanotubes demonstrate the remarkable electrocatalytic activity to- wards hydroxylamine oxidation and possess a linear response, ranges from 1.5 μM to 2.0 mM (Zhang et al., 2010a). Many groups had fabricated MHCFs based sensors and thoroughly assign for hydrazine sensing, which possesses a high catalytic activity (Gholivandand Azadbakht, 2011; Jiang et al., 2011; Kumar et al., 2011; Narayanan and

Scholz, 1999;Yang et al., 2011). Recently Komkova group has reported that boron-doped diamond (BDD) electrodes modified with Prussian Blue (PB), attain utmost sensitivity towards H_2O_2 (Komkova et al., 2020). Electrodeposited Prussian Blue on carbon black modified disposable electrodes has been used for direct enzyme-free H_2O_2 sensing in a Parkinson's disease (Rojas et al., 2018). A paper-based amperometric glucose biosensor, developed with Prussian Blue-modified screen-printed electrodes, has been proven to be a green biocompatible immobilization matrix for glucose oxidase (GOx) (Sekar et al., 2014).

1.4.2 Electrochemical Sensor for Non-electroactive Cations

The open zeolitic type of structure of PB and PBAs had further directed to examine such nanomaterials applicability as potentiometric and voltammetric sensors for size-dependent intercalation/deintercalation process (Ho and Lin, 2001). Since PB possess a channel radius of 0.16 nm, so allows the passes of ions such as Cs^+ (cesium), K^+ (potassium), Rb^+ (rubidium), and NH^+ (ammonium) of smaller hydrated radii of 0.119, 0.125, 0.128, and 0.125 nm, respectively. So, structural investigation concludes that cations possessing larger hydrated radii can not intercalate through the crystal lattice of PB and PBAs. Accordingly, these materials have been developed as ion-selective electrodes by Scholz and co-workers. It was analyzed that iron (III), nickel (II), cadmium (II), copper (II), and silver (I) based MHCFs modified electrodes are most promising for K^+ (potassiumion) determination, while nickel (II) and cadmium (II) based materials are well applicablefor radioactive species (Cs^+) determination (Düssel et al., 1996).

PB and PBAs have also been widely employed in the development of sensors for K^+, Tl^+, and Cs^+ (Coonet al., 1998; Hartmann et al., 1991; Jain et al., 1982; Krishnan et al., 1990; Tani et al.,1998; Thomsen and Baldwin, 1989; Zhiqiang et al., 1991; Estelrich et al., 2021; Mullaliu et al., 2019). The fabricated PB-supported ion-selective electrode was utilised for identification of even low concentration of Tl^+ ($2 \times 10^{-8} M$) (Kahlert et al., 1996) and therefore elevated interest in the drug industry (Labianca, 1990; Ware, 2008). The FDA (Food Drug industry) approved PB as a safeand effective drug in the treatment of contaminated metals like; Tl^+, Cs^+ and similarly,allows the radioactive substance trapping in the gastrointestinal tract.

PB sensor has been formulated in the form of nanotubes for Na^+ ion analysis (Nguyen-

Boisse et al., 2014). CTAB (cetyl trimethylammonium bromide) modified PB electrode was employed in the transport analysis of characteristics species such as; Cs^+, K^+, Rb^+, and NH^+ ions (Vittal et al., 2008). Dual sensing of discriminating ions (K^+ and Na^+) was approached successfully through selective inter-deintercalation of K^+ and comparative inhibition of Na^+ (Ang et al., 2011).

1.4.3. Advanced Transducer for Hydrogen peroxide (H_2O_2)

Hydrogen peroxide (H_2O_2) is an important analyte which abundantly used in different industries such as; chemical, textile, food, and many others. It is also the by-product of many enzymes catalyzed biochemical reactions. H_2O_2 has both an oxidizing as well as reducing property. However, the direct electrochemistry of such materials normally require a higher voltage to accomplish the breakdown process. From the last decades, different electrochemical and biosensors have been specifically developed in order to quantify the electroactive materials (H_2O_2). The subsequent effort has been made initially by Boyer via implementing PB as an electrochemical mediator during H_2O_2 sensing (Boyer et al., 1990). While innovation, in selective detection of H_2O_2 at much lower applied potential over the PB modified electrode, was made initially by the Karyakin group (Karyakin et al., 1994, 1995). They conclude that PB possess multiples times (>100) more electrocatalytic activity towards H_2O_2 ($K = 5 \times 10^2 M^{-1}s^{-1}$) as compared to O_2 moiety.

The peculiar zeolitic structure of PB enabled to the penetration of low weight molecules throughout the framework. They also claim that PB facilitates the H_2O_2 reduction at low potential (0.0 V vs. Ag/AgCl) and displays the high selectivity and sensitivity (in the micromolar range)for the selected analytes (Karyakin et al., 1996). The low operating potential (0.0 V vs.Ag/AgCl) minimized the interference of undesired electrochemical species. In additionto that, the quantified rate constant ($K = 3 \times 10^3 M^{-1}s^{-1}$) was high enough in regardsof PB assisted H_2O_2 catalytic reaction, as compared to the natural peroxidase enzyme assisted process ($K = 2 \times 10^4 M^{-1}s^{-1}$), under the similar optimized condition (Dunford and Hasinoff, 1970). Owing to both; the high selectivity and activity towards H_2O_2, PBhas designated as “artificial peroxidase” (Karyakin and Karyakina, 1999; Karyakin et al., 2000). Consequently, several works has been attempted with PB modified electrode for H_2O_2 determination (Chi and Dong, 1995; Dostal et al., 1995; Moscone et al., 2001; Ricci and Palleschi, 2005).

PB screen printed electrodes has also been developed as transducer during H_2O_2 detection (de Mattos et al., 2003; Ricci et al., 2003). PB and nanocomposite of PB itself, have been extensively employed as a sensor for H_2O_2 analysis by several groups (Du et al., 2010; Gaitán et al., 2010; Haghighi et al., 2010; Komkova et al., 2013; Li et al., 2012; Mokrushina et al., 2013; Salazar et al., 2012a; Sitnikova et al., 2011). Recently, different work has been accomplished by our research group in order to develop AuNPs-PB nanocomposites for H_2O_2 sensing. The electrode displays a significant rise in the sensitivity towards the analyte (Pandey and Chauhan, 2012) as compared to individual PB. Further, work has been extended with the modified electrodes which have shown the importance of palladium nano-dimension over electrocatalytic activity of PB towards H_2O_2 (Pandey and Pandey, 2012b).

1.4.4. Electrochemical Biosensors based on PB and PBAs

A biosensor is a functional device for monitoring living and biological systems. The performance of these analytical devices strongly depends on the electron-transport between the electrodes and the targeted biomolecules. Fabrication of biosensor involves the critical step of immobilizing biological entities on the surface of electrodes. Since H_2O_2 is the major and an essential product of several biological processes, meanwhile PB ac- complished as a redox mediator during the process of analyte detection with selectivity. Accordingly, extensive work has been executed to formulate PB based biosensors, which offers an attractive electrocatalysis. Karyakin was the first who demonstrated the applicability of MHCFs in biosensor development via immobilizing glucose-oxidase on the top of material with the help of the Nafion membrane (Karyakin et al., 1994, 1995). After that, several articles were published by many different groups demonstrating the analytical accomplishment of PB-based biosensors towards glucose detection (Ahmada- linezhad et al., 2009; Chen et al., 2012b; Fu et al., 2011; Jaffari and Pickup, 1996; Wang et al., 2011a; Cinti et al., 2018; Lin et al., 2015; Valiūnienė et al., 2017; Rekertaitė et al., 2019). Similarly, MHCFs constituted biosensors have been developed via immo- bilizing respective enzyme to the selective layer of PB or its composites, to concern a large number of analytes

including cholesterol (Li et al., 2003; Vidal et al., 2004), ethanol (Karyakin et al., 1996), glutamate (Wang et al., 2003), lactate (Salazar et al., 2012b), NADH (Gurban et al., 2008; Radoi et al., 2007), and uric acid (Piermarini et al., 2013). As conferred earlier, that PB shows an interesting electrochromic dimension dependent on their electron-transfer phenomenon. Accordingly, an optical transducer was fabricated, which involves an alteration in PB film absorption (λmax of 720 nm) as a function of analyte (H_2O_2) concentration variation. (Koncki and Wolfbeis, 1998a,b). The glucose biosensor was fabricated by immobilizing glucose-oxidase (GOx) on the top of colourless PW and later was eventually oxidized by the in-situ produced H_2O_2 (Koncki et al., 2001; Lenarczuk et al., 2001a,b). This optical sensing ability bucked up such kind of material to employed in pharmaceutical analysis.

1.4.5 Photo-Fenton Catalysis based on PBN

PB was designated as a photo-Fenton catalyst in degrading organic pollutants of the water. Since, PB contains iron of different oxidation state which undergoes the inter-metallic electron-transfer process, on absorbing maximum wavelength of light of the solar spectrum (Liu et al., 2010, 2011) as follows;

$$Fe^{III}[Fe^{III}(CN)_6] + e^- \rightarrow Fe^{III}[Fe^{II}(CN)_6^-]$$

Active interaction of PB with H_2O_2 has been conceded during water pollutant degra- dation. The materials underwent to its excited state and furnished a highly reactive free radical species after interacting with H_2O_2 (Samain et al., 2013).

$$Fe^{+2} + H_2O_2 \rightarrow Fe^{+3} + OH^{\bullet} + OH^{-}$$

$$Fe^{+3} + H_2O + UV \rightarrow Fe^{+2} + H^{+} + OH^{\bullet}$$

$$FeOH^{2+} + UV \rightarrow Fe^{+2} + OH^{\bullet}$$

The advanced oxidation process (AOPs) is sympathetic as it involves the generation of highly reactive free radical species such as; $OH^{\bullet}$ and $O2^{\bullet}$ and facilitates the degradation of organic contaminants (OShea and Dionysiou, 2012; Pignatello et al., 2006). Iron-based species (Fe^{2+}/Fe^{3+}) underwent to interact with (H_2O_2) and effectively furnished $OH^{\bullet}$ through the photo-Fenton and Fenton process (Enami et al., 2014; Klamerth et al., 2012; Pignatello et al., 1999). It was stated that controlled nanogeometry facilitates the electron-transfer to a greater extent, due to the larger surface area of nanoparticles, and could influence the Fenton cycle compared to the bulk PB (Samain et al., 2013). In-depth investigation carried by Li in 2015, concluded the high catalytic efficiency of nanoparticles towards Rhodamine B (RhB) degradation and gave a deeper mechanistic insight of heterogeneous Fenton reactions (Li et al., 2015b). In 2010, Liu has emphasized the influence of alkali cations on iron hexacyanoferrate's photo-Fenton catalytic ability towards RhB pollutant (Liu et al., 2010). Besides, RhB degradation has also been performed under visible light irradiation (λmax >420 nm) via the appliance of photo-Fenton catalyst and was observed to obey a pseudo-first-order kinetics (Liu et al., 2011).

Nanocomposites of PB/TiO_2 has also been synthesized in order to evaluate its photocatalytic response towards organic pollutants in water (Li et al., 2015b). Recently, PBAs (Fe-Co) was developed as the photo-Fenton catalyst (Li et al., 2015a). Effective removal of sulfamethazine was performed at neutral pH through the appliance of magnetic PBAs, which established such Cu-Fe oxide as the photo-Fenton catalyst. PB/Chitosan-based hybrid hydrogel and aerogel have been employed to treat wastewater using sunlight (Mai et al., 2017). Photo-Fenton catalyst; $BiFe(CN)_6.4H_2O$ is highly efficient for the resolution of environmental pollutant (Gao et al., 2018). Besides, the microcrystal of PB was similarly explored as a catalyst during the photodegradation of organic pollutants (Wang et al., 2018).

1.5 THE ARTIFICIAL PEROXIDASE LIKE ACTIVITY OF NANOMATERIALS: APPLICATION IN H_2O_2 AND GLUCOSE SENSING

The natural enzyme belongs to a class of biologically efficient catalyst seeking significant attention in different fields such as; clinical, chemical, environmental, and biotechnical. Enzyme catalyzed reaction possesses remarkable efficiency and high specificity under mild condition. Besides all these interesting facts, they also feature some of the following drawbacks; (i) They quickly underwent denaturation under the slight change in environmental condition, as their activity entirely depends on the integrity of native protein conformation. (ii) It gets easily digested by proteases enzyme. (iii) They involve an expensive, complicated and time-consuming preparation and purification process. Accordingly, tons of efforts have been attributed during the construction of alternative mimetic enzymes such as hemin, hematin, porphyrin, cytochrome, and so on (Sono et al., 1996; Wang et al., 2007; Zhang et al., 2012; Nguyen et al., 2021; Fan et al., 2020; Liu et al., 2017). Peroxidase is the natural occurring enzyme which catalyze the oxidation of chromogenic substrates such as TMB (tetramethylbenzidine), DAB (di-azo-aminobenzene), OPD (o-phenylenediamine) and ODA (o-dianisidine) into coloured product via H_2O_2 breakdown (Josephy et al., 1982; Ma et al., 2011; Drozd et al., 2016).

Similarly, horseradish peroxidase (HRP) displayed the higher sensitivity and catalytic activity towards in-situ generated H_2O_2 of the glucose-GOx system and, in turn, displayed the analyte concentration-dependent change in absorption spectra after oxidizing the chromogenic dye. However, the drawbacks are associated with the natural enzyme, as it retards the process and in turn its catalytic efficiency, under the reverse condition and causes denaturation. Different works have been initiated towards horseradish peroxidase (HRP) replacement, with other artificial enzymes, to eliminate the drawbacks of this natural process.

Initially, inorganic nanoparticles (Fe_3O_4) have been ascertained to possess intrinsic peroxidase-like activity (Gao et al., 2007). This innovation directed to examine the other nanoscale materials for the biochemical assay. A composite nanozyme of Au nanoparticles, encapsulated by Au nanoclusters (AuNP@AuNCs) was examined for glucose analysis (Chen et al., 2019). A variety of carbon-based nano- materials, including AgNPs, PtNPs, Au@Pt nanorods, graphene, carbon nanodots, and single-walled carbon nanotubes and some derivatives were developed as enzyme mimics (Guo et al., 2011; Jiang et al., 2012; Lien et al., 2012; Ma et al., 2011; Nangia et al.,2012; Tao et al., 2013; Nasir et al., 2017; Jin et al., 2019). All these reported materials involve easy preparation techniques (Asati et al., 2009; Chen et al., 2012a; Wang et al., 2012) and possess more interestingand enhanced properties due to their large surface-to-volume ratio, with better resistivity towards change in pH, temperature and enzyme digestion.

The recent progress in the PB based nanomaterials have found to exhibit the peroxidase-like mimetic activity towards various peroxidase substrates and hence designated as "nanoenzymes" (Wanget al., 2012; Su et al., 2016; Ma et al., 2019; Zhou et al., 2019). PB modified iron oxide nanoparticles (PB@Fe_2O_3) were also fabricated, which shows peroxidase-like mimetic activity (Zhang et al., 2010b; Wang et al., 2011). The obtained kinetics parameters conclude the strong catalytic ability of PB@Fe_2O_3 towards substrates with high affinity (3 fold) as compared to similar-sized magnetic nanoparticles. The high catalytic activity of PB@Fe_2O_3 towards peroxidase substrate "TMB" in the presence of H_2O_2 was also reported by Dutta (Dutta et al., 2012). Besides, Zhang successfully synthesized PB modified ferritin nanoparticles (PB-Ft NPs) and applied it as a peroxidase substrate for glucose detection (Zhang et al., 2013).

1.6 CHALLENGES IN THE SYNTHESIS OF PB AND ITS ANALYTICAL CHEMISTRY

The conventional routes of PB synthesis involves the direct mixing of $[Fe(CN)_6]^{4-}$ with Fe^{3+}, and have been employed exhaustively by the different scientific community and implemented for various applications.

However, such process of PB synthesis has the following drawbacks; (i) The traditional process leads to the formation of solid precipitate due to uncontrolled nucleation and inconsistent growth of the crystal. (ii) The insolubility of these resultant PB in the common solvent restricts their practical applicability as well as confined the electro-deposition of the film in the various frame. (iii) Functionality in PB also restricts such nanomaterials applicability, moreover the practicality of the same was evolved with bio-compatible ligand and displaying their selective binding affinity towards the biomolecules during sensing process. (iv) From an application point of view, the stoichiometric ratio of two metals in 3-D frameworks is critical in order to achieve materials with enhanced polycrystalline nature. (v) Template and reversed micelles techniques rigorously restricts their encompassing application.

Although, in order to control the nanoparticle's dimension, different synthetic methods have been approached via choosing several reagents (Gotoh et al., 2007; Hu et al., 2012; Jia, 2011; Qian et al., 2013; Yamadaet al., 2009; Zhai et al., 2008; Koshiyama et al., 2018; Zhao et al., 2019). Besides controlling the size, the improved processibility, stability, and crystallinity of materials also emerged as one of the prerequisite problems throughout the nanoparticle synthesis. Therefore, a new method is requisite, which not only controls the particle dimension but simultaneously allows the stable nano-dispersion formation into various solvents for the development of PB-based devices. Moreover, it is also crucial to investigate these material's catalytic potential with the following challenges; (a) Whether this new methodology could provide stability to these particles besides influencing their size. (b) Whether the dimension of such nano-ranged materials could affect their catalytic performance. (c) Whether such methods could allow the successful incorporation of such nanomaterials within the heterogeneous matrix. (d) Whether such modified materials can retain their electrocatalytic ability towards selected analytes. (e) Whether nanomaterials could possess the recyclability in order to achieve its large scale implementation. It is also imperative to acquit a systematic approach to these issues. Therefore, an essential attempt has been incorporated in this present study, to control the nano dimension of PB during synthesis and provide more in-depth insight for their catalytic application. These two parameters have been the premier module for this present investigation.

1.7 ORIGIN OF THE PRESENT RESEARCH PROBLEMS

The earlier works were deploying extensive use of functional alkoxysilane in the thin-film fabrication of organically modified silicate (ormosil) for practical applicability (Glezer and Lev, 1993; Pandey et al., 1996, 1999a,b,c; Tatsu et al., 1992). These productive thin films have been utilized previously for successful encapsulation of; noble metals (palladium, ruthenium), enzyme (glucose-oxidase), redox materials (ferrocene, ferricyanide, etc.), and for biosensors modulation in our laboratory (Pandey et al., 2001a,b,c, 2004). The nature of the thin film entirely depends on the optimum concentration and the composition of the designated alkoxysilane.

The appliance of several functional alkoxysilanes demonstrates an active role in designing electrochemical sensors/biosensors in their optimized condition. Moreover, the organic moiety comprising both the ion-exchanger and ion-recognition sites has been successfully incorporated within a matrix and subsequently alters the mediator's electrochemistry. In order to that, several functionalized hydrophilic [trimethoxysilane (TMS), 3-aminopropyltrimethoxysilane (3-APTMS)] and hydrophobic [3-glycidoxypropyltrimethoxysilane (3-GPTMS), 2-(3,4-epoxycyclohexyl)ethyltrimethoxysilane (EETMSi)] alkoxysilanes have been explored in multiple applications (Pandey et al., 2001c, 2003a,b). Functional alkoxysilane like; 3-GPTMS and EETMSi have emerged as reducing agents during PdNP preparation, which similarly allow the introduction of nanostructured within an organically modified network of silicates (Pandey et al., 2001a,b, 2004). Besides, 3-APTMS and cyclohexanone were discovered to play a significant role during the successful conversion of potassium ferricyanide into nanosized PB nanoparticles (Pandey and Pandey, 2013b). Recently, EETMSi played a crucial role during stable PdNP formation and sequentially provided a template for bi-metallic and multi-metallic analogues fabrication (Pandey and Shukla, 2016).

Such finding directed us to examine the role of unexposed "EETMSi" during PB nanoparticles synthesis. The functionalized alkoxysilane, like EETMSi, was further attempted with some other organic reducing agents to expose their influential role during nanoparticle synthesis and its nanostructured heterogeneous modification, which are reported herein. Moreover, such a procedure also excludes the restriction encountered with conventional mode of nanoparticle synthesis and thus can regard as a foremost accomplishment. Further, EETMSi has also been discovered as a stabilizer, despite its reduced ability, and found to orient the growth of crystal during nanostructured formation by specifying its dynamic character.

1.8 OBJECTIVES OF THE PRESENT INVESTIGATION

The active involution of functionalized EETMSi during PdNP synthesis, directed to investigate the consciousness of functionalized epoxy moiety for PBN synthesis and its heterogeneous fabrication, which remained concealed before the current research. Apart from several chemical and electrochemical techniques which are adopted for the PB synthesis often deals with instantaneous nucleation and leads to the formation of an agglomerated and large-sized particles, mostly insoluble in variety of solvents which possess limitation and restricts their practical applicability. Subsequently, assembling such materials with uniform nucleation and controlled-morphological properties is still a challenging task. Besides most of the reported procedures restrict the dispersibility of these nanomaterials in various solvents and accordingly decline their obvious utility for practical means, directed towards systematic search on the control synthetic protocol and appliance of the same to eliminate the problems. Accordingly, a mechanistic approach to the reactivity of functional alkoxysilanes (EETMSi) with THF-HPO has been examined during the controlled synthesis of PBN. The premise of the thesis is the controlled synthesis of PBN and its use as a catalyst for various analytes. Considering that the coherent objectives of the proposed thesis are as follows:

- To examine the functional ability of EETMSi, tetrahydrofuran-hydroperoxide (THF-HPO), and cyclohexanone during the course of PBN chemical synthesis using single precursor potassium ferricyanide under the ambient reaction condition to facilitates the controlled nucleation.

- Optimization of functional alkoxysilane EETMSi, during the process of nanoparticle synthesis, to attain the stable, and well-dispersed nano-sized material for its improved applicability.
- To analyze the functionality, stability, and ease of formation of stable nano-dispersionof PBN in the various solvents for their large scale implementation.
- To understand the specificity of functional alkoxysilane, the chemical conversion of potassium ferricyanide into PBN should also be attempted with potential agent in the absence of EETMSi to figure out the role of EETMSi in stabilization.
- Development of PBN as a catalyst in various forms; as a homogeneous colloidal suspension, in the constitute of the heterogeneous matrix after fabrication over the solid support like silica (SiO_2), and in the appearance of nanosuspension modified graphite should also be attempted to examine their heterogeneous catalytic potential.
- Study of the catalytic potential of these homogeneous and heterogeneous PBN towards biologically significant analytes in relevant application.
- Characterization of the as-synthesized homogeneous and heterogeneous nanomaterials by using suitable techniques.
- Appliance of these PBN in the development of an electrochemical sensor for the analysis of biologically important analytes like., H_2O_2, pyrogallol, and glucose.
- Study of the nanogeometry and nanodiameter consequences over the intrinsic peroxidase mimetic activity, and catalytic ability of PBN towards H_2O_2.
- To investigate the impact of noble metal's influence over the catalytic potential of alkoxysilane functionalized PBN in relevant applications.
- To examine the catalytic ability of PBN as photo-Fenton reagent during pollutant degradation and study of its mechanistic approach.
- Influential active participation of EETMSi with other reducing agents has to attemptto understand its selectivity during PBN synthesis.

1.9 WORK PLAN OF THE THESIS

First part of the work plan includes "functional alkoxysilane mediated controlled synthesis

of prussian Blue Nanoparticles (PBN), Enabling silica-alginate bead development; nanomaterial for selective electrochemical sensing" meeting the partial objectives listed in section 1.8 on the role of 2-(3,4-epoxycyclohexyl)ethyltrimethoxysilane (EETMSi) that has been explored thoroughly to accomplish the PBN synthesis. The consequence of EETMSi with THF-HPO and cyclohexanone has been successfully exhibited throughout the fabrication process. Besides, EETMSi also discovered to influence the nano-dimension of PBN and, in turn, to its catalytic property.

The second part of work plan is on synthesis and application of functional PBN; A photo-Fenton catalyst for organic dye degradation subsequently cover the particle objectives of the thesis.

Next part of the work plan is focused on "synthesis of PBN-embedded heterogeneous catalyst for hydrogen peroxide detection" and meeting partial objectives of the thesis innovating the synthetic insertion of PBN within mesoporous support behaving as potential heterogeneous catalyst for chemical/biochemical sensing.

Finally the last pahse of work plan is focused on "palladium prussian blue nanoparticles; as homogeneous and heterogeneous electrocatalysts" meeting the partial objectives and innovate the formation of known electro-catalysis palladium with prussian blue nanoparticles as both homogeneous and heterogeneous formulations. Accordingly, the entire research plan mainy cover the specific points of objectives proposed in section 1.8 as list below;

- To explore the dual characteristics, i.e., the reducing and stabilizing ability of theEETMSi throughout the synthesis process.
- To synthesize the PBN using EETMSi, cyclohexanone, and THF-HPO from single precursor potassium ferricyanide.
- To attain a stable nano-dispersion, optimization of all the reactants including themetal precursors have to carry out.
- Optimization of the EETMSi to investigate the influential role of the alkoxysilane over the morphology and dimension of nanoparticles.
- To find out the dispersibility limit of these PBN in a different solvent.

- To examine the influence of nano-dimension over the PBN's electrochemical behavior.
- To Investigate the size influence of PBN on its peroxidase-mimetic activity and catalytic activity concerning H_2O_2.
- Development of PBN doped silica-alginate bead to estimates its stability and thecatalytic response towards antioxidant pyrogallol.
- To investigate the potential of the PBN, as photo-Fenton reagent for pollutant degradation.
- Investigation of the nanoparticle dimension significance over photocatalytic abilityof PBN.
- Study of the catalyst re-utility and re-cyclability for large scale implementation.
- Appliance of PBN as the catalyst in diverse forms., colloidal suspension, mixedwith graphite, as the heterogeneous matrix, to evaluate its catalytic ability towards various analytes.
- To modulate the PBN in heterogeneous matrix (SiO_2), in order to fabricate PBN@$SiO_2$and it appliance as catalyst, with good operational stability and recyclability, for the peroxidase mimetic activity investigation.
- Successful Fabrication of Pd-PBN to investigate the impact of the nano dimensionof PdNP on the catalytic ability of PBN towards H_2O_2.
- To investigate the compatibility of hydrophilic amino-functionalized palladium with PBN at the nanoscale to tune the catalytic property of PBN, and the possibility of immobilization of enzymes over these nanoparticles modified graphite electrode.
- Study of the influential parameter of PdNP over PBN's catalytic ability by facilitating the glucose-sensing process after the successful immobilization of enzyme.
- To characterize the nanoparticles using several sophisticated techniques, namely, Scanning Electron Microscopy (SEM), Transmission Electron Microscopy (TEM), Fourier Transform Infrared spectroscopy (FTIR), and X-Ray Diffraction (XRD). Photoelectron Spectroscopy (XPS) analysis was used to investigate the valence stateof metal nanoparticles.