Chapter 4

Synthesis of Prussian Blue Nanoparticles-Embedded Heterogeneous Catalyst for Hydrogen Peroxide Detection

4.1 INTRODUCTION

Bi-metallic coordination compound Prussian blue nanoparticles (PBN), a well-known inorganic polymeric material, has been considerably researched and employed for many electrocatalytic applications (Karyakin et al., 2004; Pandey et al., 2018b; Pintado et al., 2013; Qiu et al., 2007). The Prussian blue (PB) formulates as a cubic framework of $Fe^{+3}[Fe^{+2}(CN)_6]$ having metals in different oxidation state viz. Fe^{+3} and Fe^{+2} linked via CN ligand (Karadas et al., 2012). PB is well known as artificial peroxidase due to its advanced mimetic activity (Karyakin et al., 2000; Pandey and Pandey, 2014; Pandey et al., 2018a). One of the potent appliances of such nanomaterials is designing a chemically modified electrode as an electrochemical sensor. In the previous chapter, we have discussed the synthesis of PBN involving EETMSi and cyclohexanone from single precursor potassium ferricyanide. As-synthesized PBN displayed excellent photo-Fenton catalysis during the dye degradation process (Singh and Pandey, 2020). Applicability of such nanomaterials in the development of electrochemical sensors for H_2O_2 detection has been attempted herein. PB synthesis involves the generation of nano-dimensions from single metal ion precursor solutions. However, these nanoscale PB materials exhibit high surface area and catalytic ability but have less practical applicability and challenge, especially for a solution-based reaction. PBN have been developed as fascinating and versatile heterogeneous catalyst for various applications such as electro-oxidation (Aksoy et al., 2016; Pintado et al., 2013), hydrogen peroxide (H_2O_2) reduction (Jin et al., 2012; Uyanik and Pekin, 1970), nitration of organics (Pasnoori et al., 2014), wet chemical oxidation (Doumic et al., 2013; Li et al., 2015a,b; Lin et al., 2016), electro-reduction (Bai et al., 2009), ni- trophenols reduction (Chen et al., 2015), etc. Such kind of catalytic activity directedto investigate the response of fabricated heterogeneous nano-dispersion towards H_2O_2.

Further, the practicability of EETMSi, for modulation of nano-dispersion PBN in a heterogeneous matrix, has been undertaken in this work. However, a few attempts have been developed for substrate-supported PBN, still possessing limitation of consistent and sta- ble growth of particles. So we proposed here silica (SiO_2), which are highly porous nanostructured materials having a high specific area. Besides, there is even an imperative demand to develop catalytically effective, convenient-to-prepare, simple to use, and easy to recoverable substrate-supported PB catalyst. Non-toxicity, low-cast, non-reactivity, hydrophilicity, and remarkable biocompatibility stand out SiO_2 as a suitable matrix for encapsulation of PBN and designing of the novel biosensor. Eventually, SiO_2 indulged with mesopores allows the successful insertion of PBN embedded over and within their accessible pores. Herein, initially PBN has been attempted to examine its electrochemical behaviorby cyclic voltammetry. Besides, differential pulse voltammetry was carried out to understand the intermolecular interaction between the PBN and analyte molecules.

The re-cyclability and easy separation are the crucial parameters for the employment of materials at a large scale during the industrial process. Meanwhile, a novel method has been attempted to fabricate PBN@SiO_2 by sequential chemical reduction process. Noteworthy, the PBN@SiO_2 was found to be specific during H_2O_2 catalytic oxidation via interacting with the iron species present in embedded PBN of the synthesized matrix. The oxidation process was similarly attempted in optimized reaction conditions to understand the re-cyclability and catalytic potentiality of PBN@SiO_2. Constructive re-cyclization of the PBN@SiO_2 was observed as a fascinating insight into the catalytic process in terms of economic scope.

4.2 EXPERIMENTAL

4.2.1 Materials

2-(3,4-epoxycyclohexyl)ethyltrimethoxysilane (EETMSi) and cyclohexanone were obtained from Pubchem. Potassium ferricyanide was purchased from Merck, India. Graphite powder (particle size, 1-2 μm), nujol oil (density = 0.838 g mL^{-1}) and SiO_2 were obtained from Sigma-Aldrich Chemical Co. India. H_2O_2 (assay percentage purity 30-32 % v/v) was purchased from Fischer Scientific (ACS certified). All other chemicals used were of analytical grade and procured from the commercial source. Milli-Q water was used throughout the whole experiment to avoid any interference of metals and for accurate analysis of specific species.

4.2.2 EETMSi mediated synthesis of PBN and PBN@SiO_2

The synthesis of PBN was accomplished using EETMSi and cyclohexanone from single precursor potassium ferricyanide by chemical reduction technique, as discussed in the previous section (chapter 3). PBN preparation involves addition of 20 μl of EEMTSi (0.10 M) into 100 μl of potassium ferricyanide (0.03 M) under stirring condition. Later, 20 μl of cyclohexanone has been added to the reaction mixture and the resultant was kept in an oven at 343 K for 8 hours, which yielded deep blue homogeneous colloidal sol of PBN. The obtained nanosuspension was further purified by solvent extraction using ethyl acetate. Moreover, PBN@SiO_2 was prepared by the chemical reduction technique with the following procedure. Primarily, 10 mg of SiO_2 was suspended in 100 μl of EETMSi (1.2 M) under the stirring condition. After 3 hours, the un-adsorbed EETMSi content was removed with methanol followed by centrifugation. Later on, 200 μl of potassium ferricyanide aqueous solution (0.03 M) was added to the alkoxysilane modified SiO_2 (suspension) under vigorous stirring condition (800 rpm). Further, cyclohexanone was added to the corresponding alkoxysilane modified $K_3Fe(CN)_6$@SiO_2 suspension, and left to stand in an oven at 338 Kfor overnight. Afterward, the unabsorbed PBN were removed by washing (5 times) with methanol/water (2:1) solvent, and the residual was collected via centrifugation.

4.2.3 Instumentation

The morphology of as-synthesized PBN nanoparticle over SiO_2 was analyzed through FE-SEM (Scanning electron microscopy) of FEI Company of USA (SEA) PTE, LTD. The elemental confirmation and mapping analysis were accomplished with ZEISS (Oxford Instrument, USA). The Rigaku X-ray diffractometers (SMART LAB, RIKAGU Corporation, Japan) with Cu Ka radiation (l=l.5406 A°) was used to examine the diffractograms of the sample. The analysis was performed in the scan range of 10-90° for PBN parti- cles. FTIR spectra recorded on an ALFA FTIR Bruker-ATR, Ettington, Germany. The XPS analysis was performed using the ESCA and AES System (Surface Nano Analysis, GmbH, Germany) equipped with Al-ka (1486.6 eV). X-ray source operating at a power of385 W and hemispherical analyzer (PHOBIOS 150 3D Delayline Energy Analyser). The C-1s peak (284.5 eV) was used as an internal reference to calibrate the absolute binding energy. Electrochemical experiments viz; DPV (differential pulse voltammetry) and CV (cyclic voltammetry) were accomplished on an Electrochemical Workstation Model CHI660B, CH Instrument Inc., TX, in a three-electrode configuration with a working volume of 3 ml. The electrode body used for electrochemical measurement has been purchased from Bioanalytical systems. An Ag/AgCl and platinum wire are served as reference and counter electrodes, respectively. All potentials given in the text are relative to the Ag/AgCl. The working electrode was a PBN modified carbon paste electrode (CPE). The active paste of PBN was synthesized by mixing 100 μl of PBN suspension with 60 mg spectroscopic grade graphite powder (particle size 1-2 μm), followed by ultrasonication for 30 minutes and left to dry in a vacuum oven for overnight. The compositions of the typical active paste are i.e., graphite powder = 68% w/w, nujol oil = 28% w/w, and PBN= 4% w/w.

4.3 RESULT AND DISCUSSION

4.3.1 Study of the electrochemical behavior of PBN through cyclic voltammetry

The electrochemical behavior of the PBN modified electrode has been investigated in 0.1 M KNO_3 solution at the scan rate of 10 mV/s vs. Ag/AgCl. The cyclic voltammogram

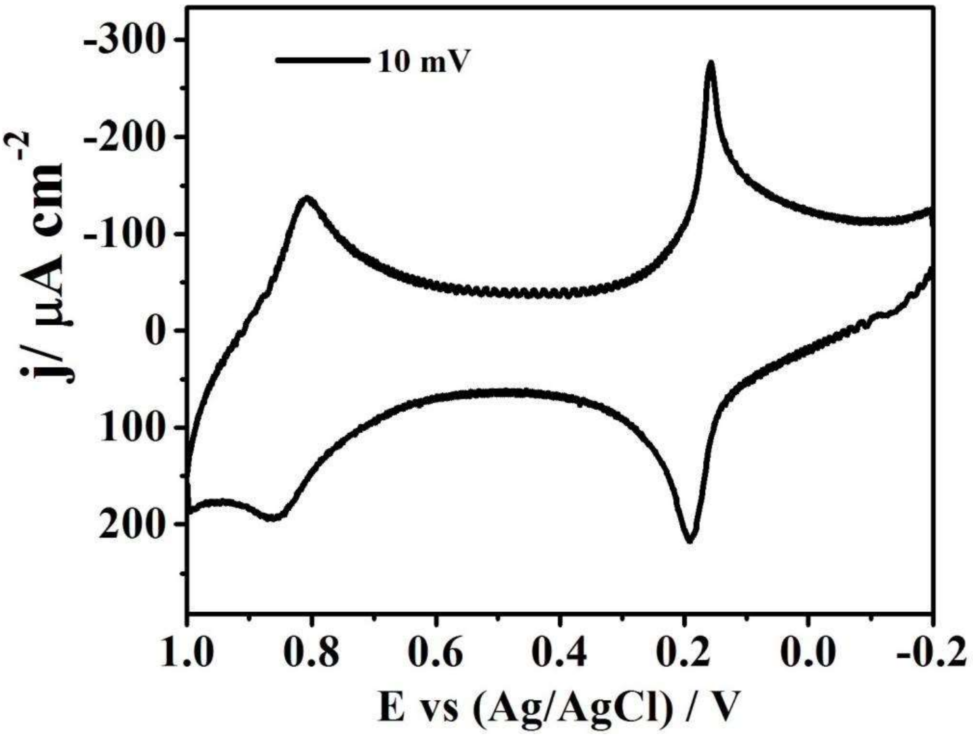

Figure 4.1: Recorded cyclic voltammogram of PBN in 0.1 M KNO_3 solution.

of PBN comprised of two reversible redox couples, located at two different potentials, are revealed in Fig 4.1 (Itaya et al., 1982). The redox peak, centered at lower positive potential (0.2 V) attributed due to the oxidative-reductive response of Prussian white (PW) and PB. Besides, the peak response at higher positive potential (0.9 V) owing to the oxidation of PB to BG (PY) and reduction of BG to PB. While potassium ion performs as the counter ion throughout the electrochemical process (Farah et al., 2013). The low peak separation (Ep = Epa −Epc) of 29 mV (close to 0 mV) unveil an adsorbed electro-active species on the electrode surface. The sharpness of redox peak defines the quality of PB with facile electron transferand indicating the surface dependent redox activity (Ellis et al., 1981).

4.3.2 Electrochemical sensing of H_2O_2 through PBN

The H_2O_2 sensing with high sensitivity and selectivity over low potential is a crucial point (Karyakin and Karyakina, 1999). It was demonstrated that PBN exhibited size-dependent electrocatalytic activity concerning onset potential and current response. Meanwhile,

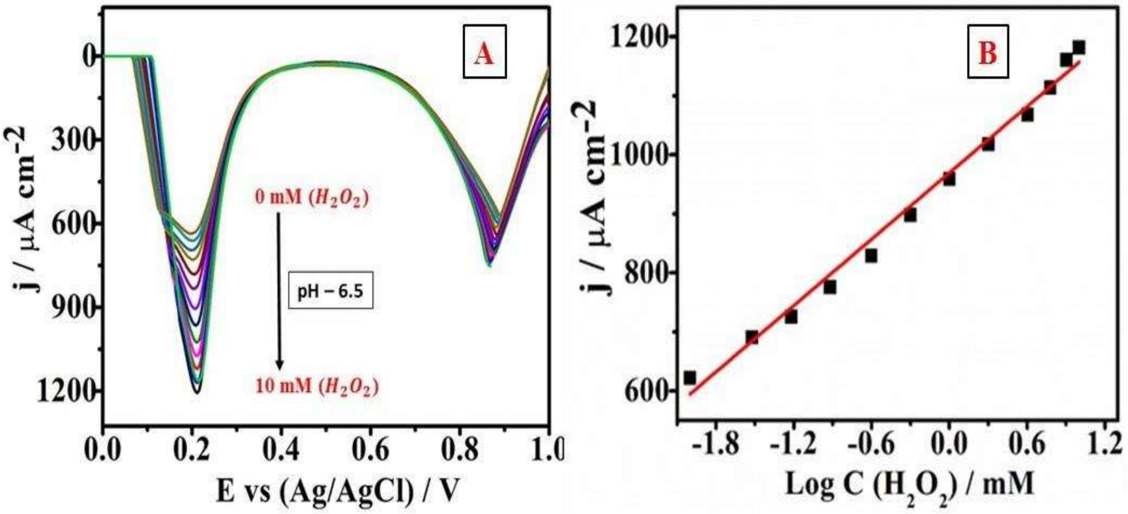

Figure 4.2: Study of electrochemical response of PBN in the presence of H_2O_2 (0-10 mM) by DPV (A). Concentration dependent calibration curve for H_2O_2 (B).

EETMSi and cyclohexanone mediated smaller-sized PBN (PBN_4) has been elected to evaluated their electrochemical sensing ability towards H_2O_2 in PBS (0.1 M, pH-6.5) containing 0.1 M KCl as supporting electrolyte. The differential pulse voltammogram (DPV) of PBN attributes a peak near to 0.2 V justifying the direct-oxidation of PW to PB and other at 0.9 V corresponds to PB oxidation to BG (PY) as shown in Fig. 4.2A (Buleandra et al., 2014). Accordingly, the presence of the same in the bulk reaction system was examined through an electrocatalytic reduction of H_2O_2 at the surface of PBN modified graphite paste electrode. The recorded differential pulse voltammogram depicts the enhancement in the oxidation current of PBN as a function of H_2O_2 concentration (0-10 mM) variation as shown in Fig. 4.2A (Ma et al., 2013). Electrocatalytic reduction of H_2O_2 over the surface of the nanoparticles modified electrode allowed the significant amplification in the electrochemical response ofPBN at their respective peak potential (Fig. 4.2B). The outcomes conclude that H_2O_2 underwent to catalytic reduction and enhancing the electrochemical response of PBN via transferring it discharge electron effectively to the surface of the nanomaterials modified electrode.

To considering its electrochemical response towards H_2O_2, the fabricated heterogeneous catalyst PBN@SiO_2 has been attempted sequentially to evaluate their catalytic response towards the similar analyte. Besides that different techniques (SEM, FTIR, XRD, XPS) has been performed to examine the modulation of PBN over SiO_2 surface.

4.3.3 SEM analysis of fabricated PBN@SiO_2

The shape and size of as-synthesized PBN@SiO_2 was characterized by scanning electron microscopy (SEM). The surface of SiO_2 reported to exhibit an irregular morphology with micro-porous (6.1 Å) and mesoporous (63.54 Å) arrangement (Dutta et al., 2005). The HRSEM analysis indicates the heterogeneously distributed nanocubes and nano-sphere (70-120 nm) of PBN, embedded over SiO_2 as shown in Fig. 4.3(A,B,C).

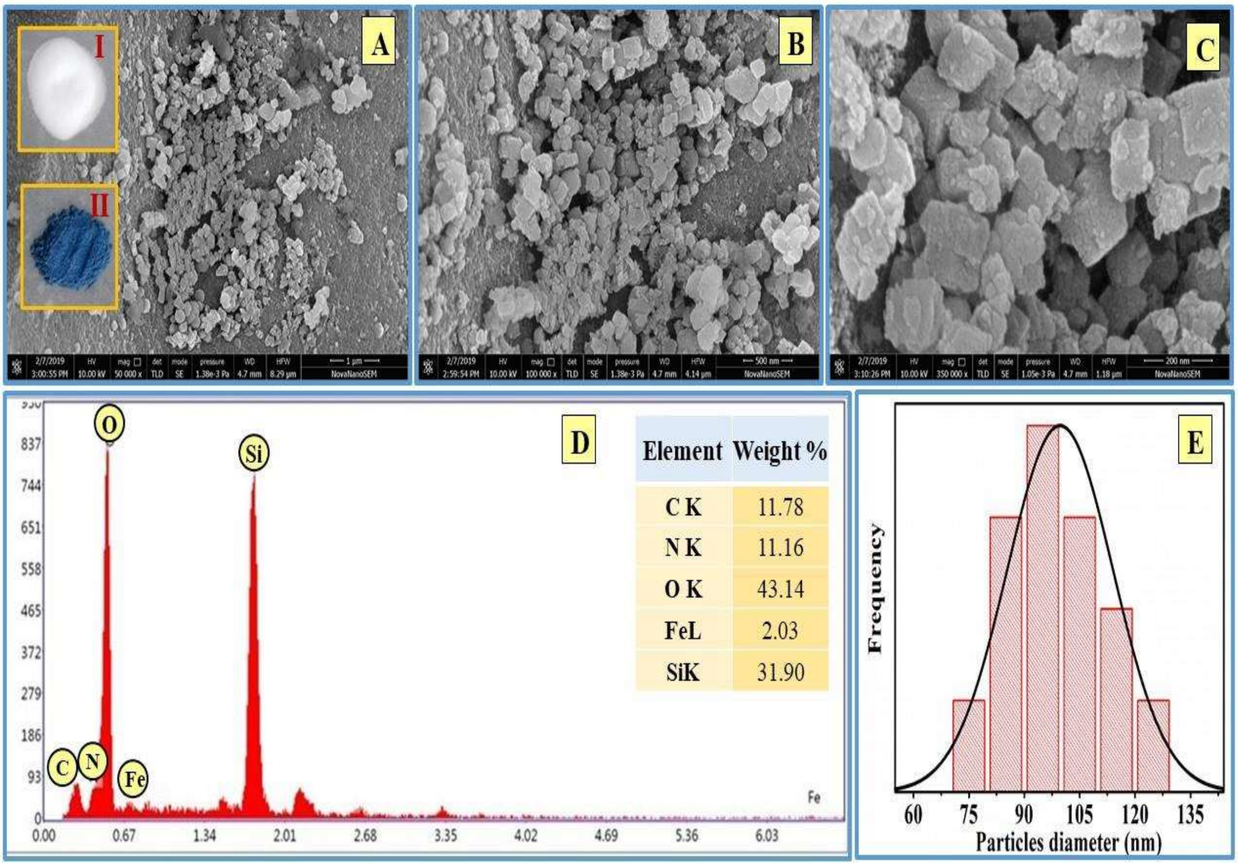

Figure 4.3: HR SEM images of PBN@SiO_2 at different magnification displayed the successful formation of PBN over SiO_2 (A-C), the visual images; (I) and (II) in inset of Fig. (A), corresponds to SiO_2 and PBN@SiO_2 respectively. The EDX spectrum revealed the presence of all mandatory elements (D). Bar histogram displaying the particle size distribution curve of corresponding nanoparticles (E).

The strong peak observed in the EDX spectrum of PBN@SiO_2 confirmed the presence of all mandatory elements such as Fe, C, N, Si, and O (as shown in Fig. 4.3D) and approved similarly their individual PBN and SiO_2 nature. The prepared PBN@SiO_2

system revealed the weight percentage at 11.78 % of C, 11.16 % of N, 43.14 % of O, 31.90 % of Si, and 2.03 % of Fe in the EDX spectrum for the corresponding sample. The corresponding histogram (Fig. 4.3E) revealed the average particle-diameter of 100 nm for the foremost existing (82%) nanocubic shaped PBN.

4.3.4 Mapping analysis

In addition, elemental mapping analysis of selected area shows the presence of about 5 wt %, 6 wt %, 34 wt %, 4 wt %, and 51 wt % of C, N, O, Fe and Si respectively over PBN@SiO_2 surface (Fig. 4.4).

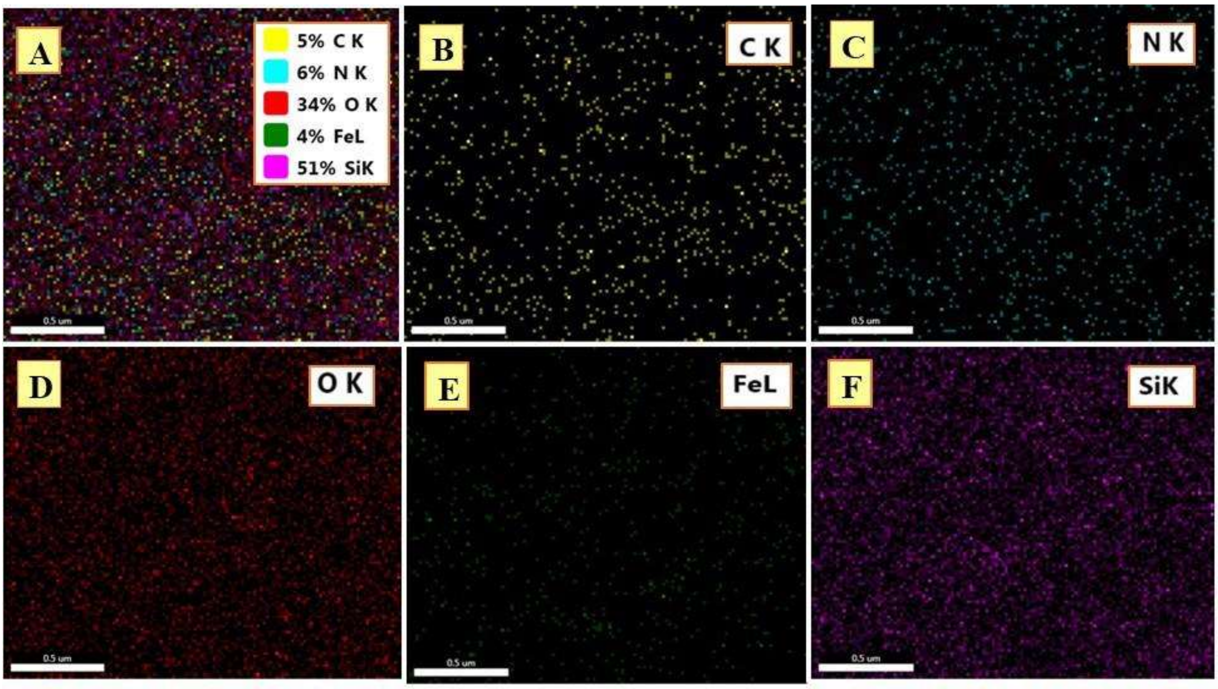

Figure 4.4: Mapping analysis of selected area in PBN@SiO_2 (A) identified to the presence of all elemental species; carbon (B), nitrogen (C), oxygen (D,) iron (E), and silicon (F) with their relative quantity.

4.3.5 FTIR analysis

The broad bands centred at 3548 cm^{-1} and at 1632 cm^{-1} (Fig. 4.5) are consigned to the stretching and bending vibrations of silanol groups (SiOH) respectively in the SiO_2

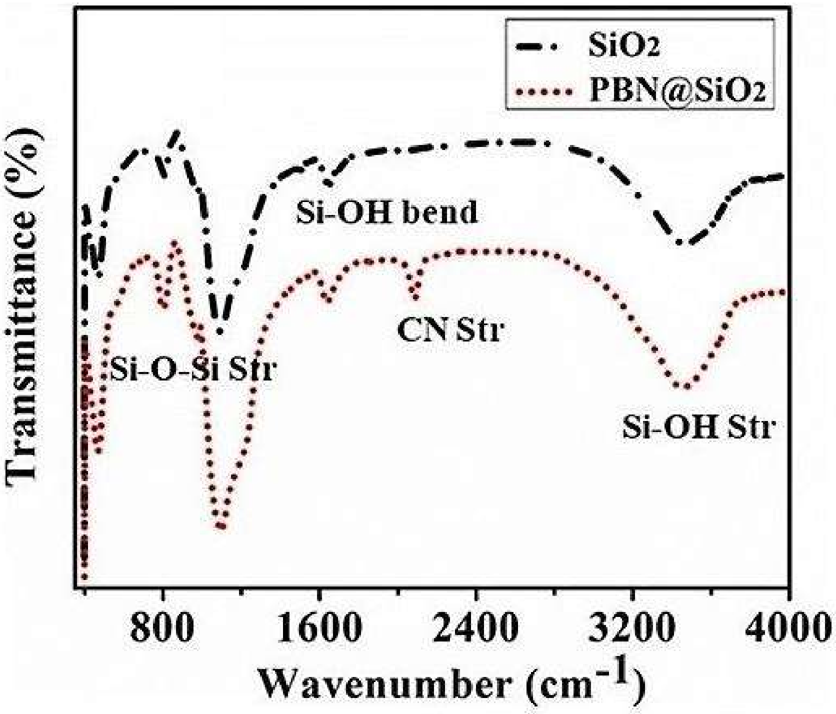

Figure 4.5: FTIR spectrum of as-synthesized PBN@SiO_2 and heterogeneous support SiO_2.

(Antony et al., 2014). The bands located at 1093 cm^{-1} and 801 cm^{-1} in the spectrum correspond to the characteristic anti-symmetric and symmetric stretching modes (Si-O-Si) of SiO_4 units (Antony et al., 2014). The characteristic prominent peak at 2096 cm^{-1} located in the IR spectrum (Fig. 4.5) resemble with the stretching mode of $Fe^{II}-C-N-Fe^{III}$ moiety in PBN (Ayers and Waggoner, 1971) that indicates the successful formation of nanoparticles over SiO_2.

4.3.6 XRD analysis

The crystallinity of as prepared PBN@SiO_2 has been investigated by P-XRD as displayed in Fig. 4.6. The SiO_2 comprised a similar broad peak centred at 2θ = 22 (101) concludes their amorphous nature (Liang et al., 2012). In addition, the peak indexing at 2θ values of 17.6 (200), 24.3 (220), 37.8 (400) indicated the successful modulation of crystalline PBN with face-centered cubic crystal lattice (JCPDS - 73-0687).

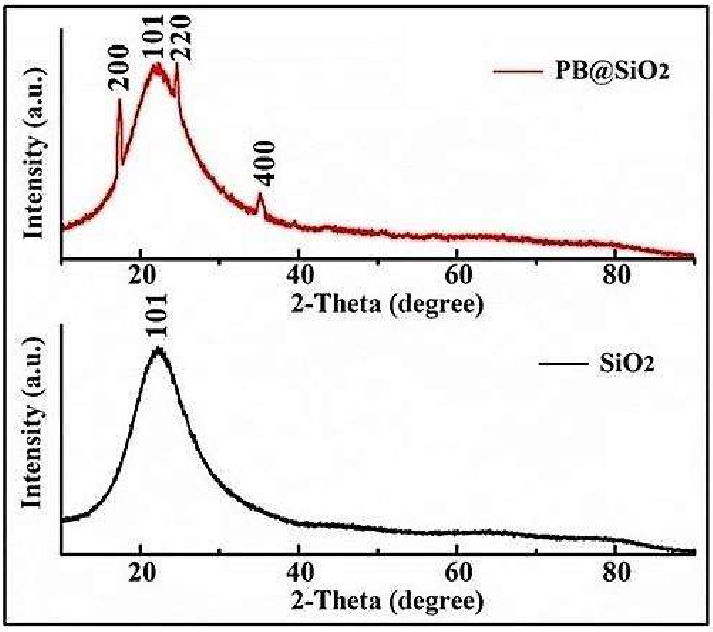

Figure 4.6: P-XRD of SiO_2 and as-synthesized PBN@SiO_2 unveiling the presence of planes.

4.3.7 XPS analysis

The XPS survey scans (Fig. 4.7A) indicated the presence of Si, O, Fe and C in PBN@SiO_2.

Identification of Fe (II) and Fe (III) species in PBN@SiO_2

After refined fitting, the spectrum can be de-convolved into three peaks. Fig. 4.7B displayed the XPS peaks centred at binding energy of 721.27 eV and 708.34 eV for Fe $2p^{1/2}$ and Fe $2p^{3/2}$ respectively, illustrate the characteristic Fe^{+2} moiety of PBN. In addition, spectrum was also enclosed with a peak centered at B.E. of 712.12 eV corresponds to Fe^{+3}species. The position of the following peak is in good agreement with previous literature reports for the characteristics Fe^{+3} and Fe^{+2} components of PB materials (Datta and Datta, 1990).

SiO_2 system

The peak position of Si 2p spectrum corresponding at 103.63 eV of B.E. (Fig. 4.7C) has characteristics of Si (IV) in SiO_2 type compound (Cros et al., 1990).

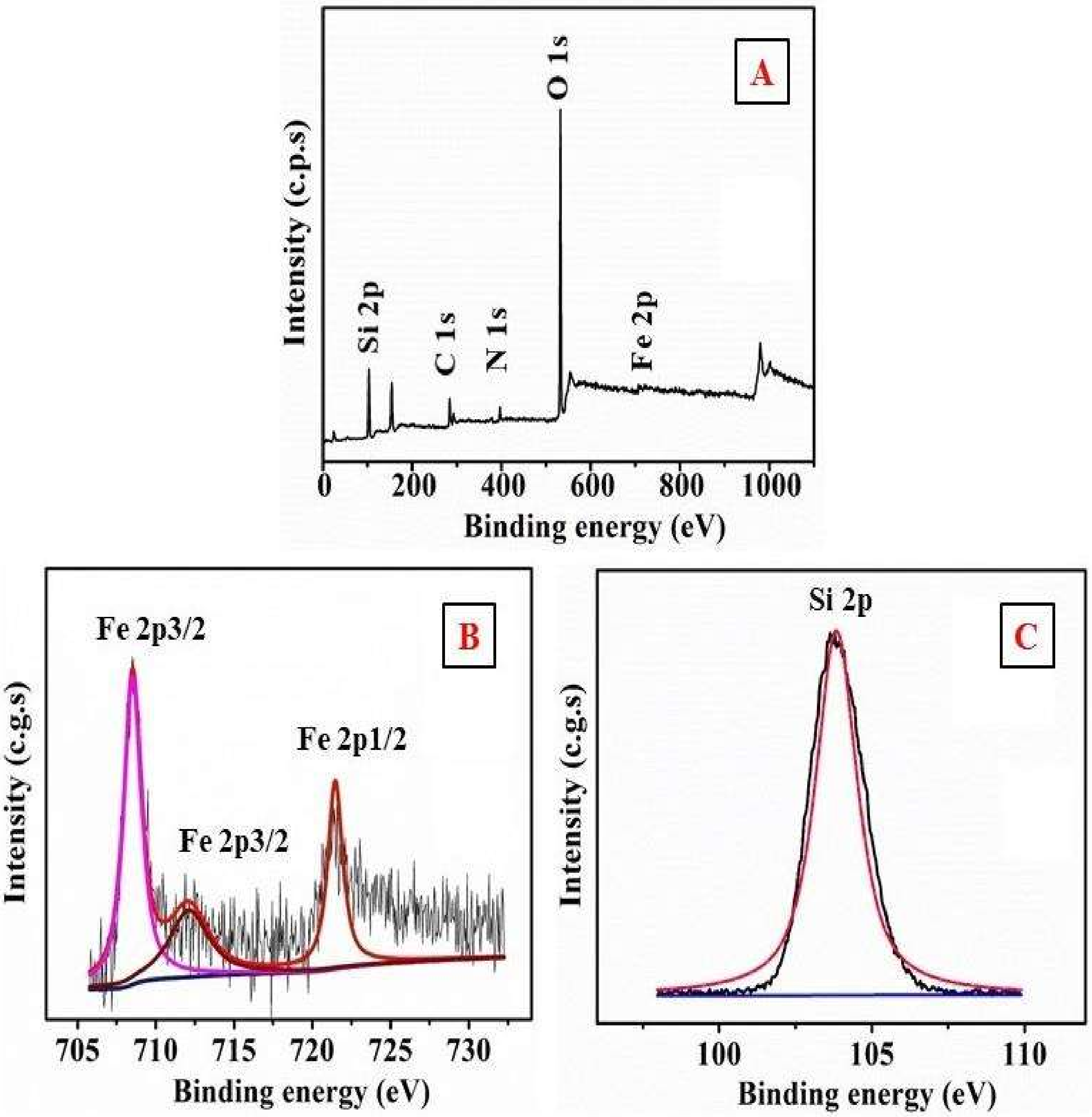

Figure 4.7: XPS analysis of PBN@SiO$_2$ exhibiting complete survey scan with all recognized species (A), Fe^{+2} and Fe^{+3} species in PBN@SiO$_2$ (B), and identified chemical states of Si (IV) in PBN@SiO$_2$ (C).

4.3.8 Peroxidase mimetic activity of PBN@SiO_2

Catalytic performance of PBN@SiO_2 has been examined subsequently for H_2O_2. Since, the PBN@SiO_2 beads are challenging to placed at the surface of the electrode, accordingly the presence of the analytes in the bulk reaction system was determined by the spectrophotometric method. The process involves catalytic oxidation of chromogenic peroxidase substrate o-dianisidine with H_2O_2 in the presence of catalyst. Experiment was performed by adding catalyst (20 mg) and o-dianisidine dye (50 μM) with H_2O_2 (1.0 mM) in phosphate buffer (2 ml).

The whole reactant setup was allowed to stand at room temperature for 10 min to complete the catalytic process. Later on, when the change in colour was observed the catalyst was separated through centrifugation and residue (supernatent) has been underwent for absorbance measurememt. The oxidized product features a brown colour appearance dependent to the H_2O_2 content having the absorption maxima near to 480nm (Fig. 4.8) in the UV-Vis spectrum and eventually justified the peroxidase enzyme-like characteristics of as-prepared PBN@SiO_2 (Kireyko et al., 2006). H_2O_2 interacts with PBN molecules at the surface of SiO_2 and induces a change in colour of the chromogenic substrate, once the catalytic oxidation process has been introduced. Besides, the experiment was also performed to analyze the interaction and reactivity of unsupported SiO_2 towards H_2O_2 in the presence of o-dianisidine and the outcome revealed none of such sort of catalytic oxidation process.

Consequently, the catalytic ability of PBN@SiO_2 towards H_2O_2 have been investigated in the presence of the different concentrations of analytes with constant o-dianisidine and catalyst amount in phosphate buffer (0.1 M, pH 7.0). The oxidized product features a brown colour appearance dependent to the H_2O_2 content having the absorption maxima near to 480 nm (Fig. 4.9) in the UV-Vis spectrum. Absorption maxima of the reaction product are found to enhances as a function of increasing H_2O_2 concentration under the effect of PBN@SiO_2. In addition the recyclability of the catalyst was investigated further via subsequent repetitive cycle of oxidative-catalytic process. It was observed that catalyst start to deploying their peroxidase catalytic activity eventually after 10th repetitive cycle of oxidation process.

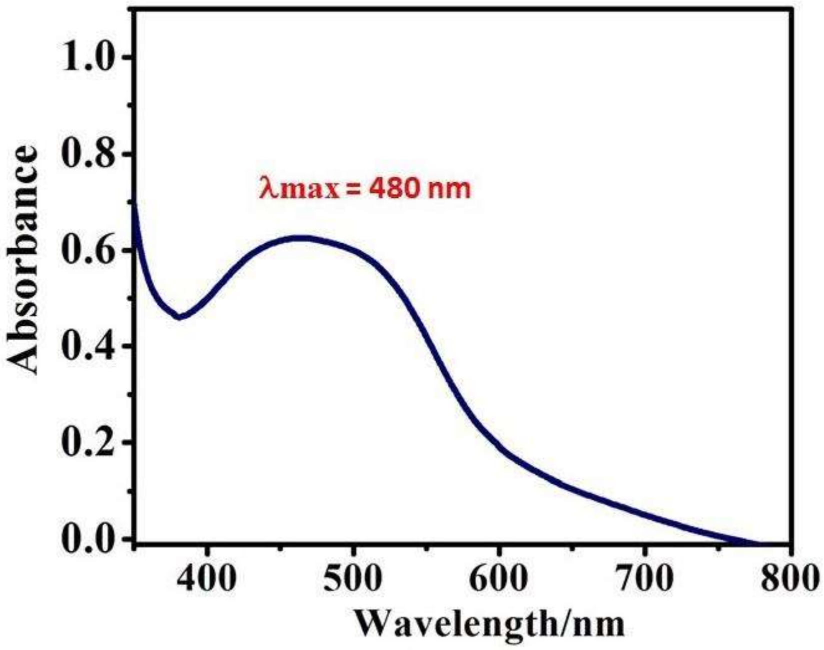

Figure 4.8: UV-Vis spectra exhibiting peroxidase like activity of PBN@SiO_2 towards H_2O_2 by oxidizing chromogenic substrate (o-dianisidine).

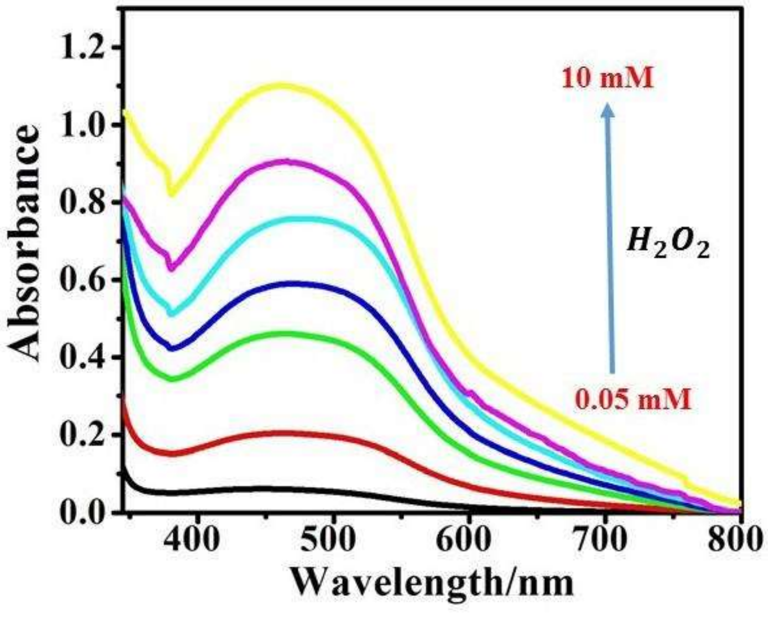

Figure 4.9: Recorded UV-Vis spectra displaying change in the absorption maxima correspond to o-dianisidine oxidation with respect to H_2O_2 concentration variation in the presence of PBN@SiO_2 catalyst.

4.4 CONCLUSION

Novel heterogeneous matrix PBN@SiO_2 has been prepared by a chemical reduction method using single precursor potassium ferricyanide functionalized with alkoxysilane. The SEM analysis confirmed the successful modulation of cubic and spherical shaped particles of PBN over SiO_2. Further XRD and XPS analyse the crystallinity and chemical state of as-synthesized heterogeneous catalyst. Besides the characteristic CN stretching peak of PBN identified through FTIR analysis confirmed their successful modulation over SiO_2. XPS study identify the presence of both Fe^{+2} and Fe^{+3} moiety in PBN@SiO_2. Electrochemical behavior of PBN was characterized by cyclic voltammogram and differential pulse voltammogram. In addition, mimetic activity of heterogeneous catalyst PBN@SiO_2 was investigated through spectrophotometric observation based on H_2O_2 catalytic reduction. The heterogeneous nanomaterial was found to be promising for analyte detection with better recyclability and catalytic ability.