Supplementary file

Fabrication of a Polypyrrole-Functionalized Biogenic $Mg(OH)_2$@MgO Immunosensor, Synthesized Using *Graptopetalum paraguayense* Leaf Extract, for Selective and Efficient Impedimetric Detection of Cholecalciferol (Vit-D_3)

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2.7. Characterization techniques

The characterizations of B-$Mg(OH)_2$@MgO/PPy NCs by ultra-violate (UV) spectroscopy instrument (Agilent Cary 60 UV-vis) confirm through the absorbance, conducting and non-conducting behaviour, Fourier Transform Infrared (FTIR) spectroscopy (modal FTIR 4700 make: JASCO, TOKYO) providing information of available functional groups, X-ray diffraction (XRD) spectroscopy (D8-ADVANCE(eco)/(Bruker)) correct the information of crystalline shape, structure and size was validate by International Centre for Diffraction Data (ICDD). Zeta-DLS instrument Zatasizer Ultra (ZSU5700), Malvern Panalytical (UK) was used to confirm particle size and zeta potential of particles, and a Circular dichroism (CD) spectroscopy instrument JASCO. Modal: CD Polarimeter J-1500 for characterization of PPy coiled at MgO NPs surface. The X-ray photoelectron spectroscopy (XPS) (PHI 5000 Versa Probe (lll) (scanning XPS microprobe)) provides information of a particular element and its corresponding binding energy. The Scanning electron microscope (SEM) and Energy Dispersive X-ray (EDX) (Carl Zeiss company instrument; model EVO-18 Research) sample preparation coating through spattering of Au on B-$Mg(OH)_2$@MgO/PPy NCs and using source laser Lanthanum Hexaboride (LaB_6). Scanning Electron Microscopy (SEM) confirms the morphology, size, and high-quality 3D image, and Energy-dispersive X-ray (EDX) spectroscopy confirms elements presence as well as percentages in the compound. Transmission electron microscopy (TEM) and high resolution (HR-TEM), and selected area electron diffraction (SAED) (Techai $G^2$20 TWIN, FEI company of USA) these are confirming the particle size, shape, d-spacing between lines, crystalline and amorphous nature. The electrochemical analysis of B-$Mg(OH)_2$@MgO/PPy NCs has been done by the instrument electrochemical workstation CorrTest CS studio 350. The electrode functionalization of glass sheets coated with Indium Tin Oxide (ITO) was cut into small pieces (1 cm × 2 cm) and dipped in a mixture of H_2O_2: NH_3: H_2O with a volume ratio of 1:1:5, respectively. After that, the pieces

were baked in an oven for one hour at 80 ℃ for adherence of the OH groups uniformly on the ITO surfaces. The ITO (Resistivity-20 ohm/sq) electrode is used as the working electrode for the fabrication of a biosensor.

Table S1. *Binding Energy data of Mg, O, C and N elements of B-Mg(OH)$_2$@MgO/PPy NCs.*

Elements	***Binding energy range (eV)***	***a plot maximum binding energy (eV)***	***b plot maximum binding energy (eV)***	***c plot maximum binding energy (eV)***
Mg	96 – 104 eV	101.0 eV	99.9 eV	100.3 eV
O	526-537 eV	530.1 eV	529.0 eV	529.2 eV
C	279-295 eV	283.0 eV	282.4 eV	282.6 eV
N	394-410 eV	390.7 – 399.9 eV	397.4-398.7 Ev	397.6 eV

Table S2. interference study of different interference towards the Vit-D$_3$ by the fabricated impedance biosensor was summery.

Interference	***Current density (j mA.cm^{-2})***	***Concentration***	***% RSD***
Vitamin D$_3$	*0.6004*	*70 nM*	*0.00 %*
Vitamin D$_3$ + Vitamin B$_2$	*0.5737*	*70 nM + 5000 nM*	*4.45 %*
Vitamin D$_3$ + Vitamin B$_{12}$	*0.5906*	*70 nM + 100 pM*	*1.64 %*
Vitamin D$_3$ + Vitamin C	*0.6003*	*70 nM + 4000 nM*	*0.03 %*
Vitamin D$_3$ + Urea	*0.5975*	*70 nM + 400000 nM*	*0.49 %*
Vitamin D$_3$ + Glutamic acid	*0.6239*	*70 nM + 100000 nM*	*3.91 %*
Vitamin D$_3$ + Glucose	*0.6305*	*70 nM + 500000 nM*	*5.00 %*
Vitamin D$_3$ + Cholesterol	*0.6139*	*70 nM + 70 mg/dl*	*2.25 %*
Mixture	*0.6209*		*3.40 %*

Table S3. tabulated the real blood serum and spiked serum sample study % recovery, determination of concentration and % RSD toward the standard Vit-D$_3$ concentration was calculated by CV study at SES by fabricated BSA/Ab Vit –D/ B-Mg(OH)$_2$@MgO/PPy NCs /ITO biosensor towards Vit-D$_3$.

Standard Vit-D_3 Current density (j mA.cm^{-2})	*Standard Vit-D_3 + Blood serum Current density (j mA.cm^{-2})*	*Determination of concentration of real sample*	*% Recovery*	*% RSD*	*Standard Vit-D_3 + BSA Current density (j mA.cm^{-2})*	*Determination of concentration of real sample*	*% Recovery*	*% RSD*
1 nM (0.4492)	**1 nM + BS (0.4839)**	1.07 nM	107.7 %	7.7 %	**1 nM + BSA (0.4741)**	1.05 nM	105.5 %	5.5 %
5 nM (0.4547)	**5 nM + BS (0.4804)**	5.28 nM	105.6 %	5.6 %	**5 nM + BSA (0.4804)**	5.28 nM	105.6 %	5.6 %
30 nM (0.4569)	**30 nM + BS (0.4786)**	31.42 nM	104.7 %	4.7 %	**30 nM + BSA (0.4860)**	31.91 nM	106.3 %	6.3 %
60 nM (0.4740)	**60 nM + BS (0.4928)**	62.37 nM	103.9 %	3.9 %	**60 nM + BSA (0.4889)**	61.88 nM	103.1 %	3.1 %
90 nM (0.4813)	**90 nM + BS (0.4986)**	93.23 nM	103.5 %	3.5 %	**90 nM + BSA (0.4932)**	92.22 nM	102.4 %	2.4 %
120 nM (0.4896)	**120 nM + BS (0.4988)**	122.25 nM	101.8 %	1.8 %	**120 nM + BSA (0.4982)**	122.10 nM	101.7 %	1.7 %
150 nM (0.5017)	**150 nM + BS (0.5074)**	151.70 nM	101.1 %	1.1 %	**150 nM + BSA (0.5023)**	150.17 nM	100.1 %	0.1 %
200 nM (0.5095)	**200 nM + BS (0.5092)**	199.88 nM	99.9	0.1 %	**200 nM + BSA (0.5157)**	202.43 nM	101.2 %	1.2 %

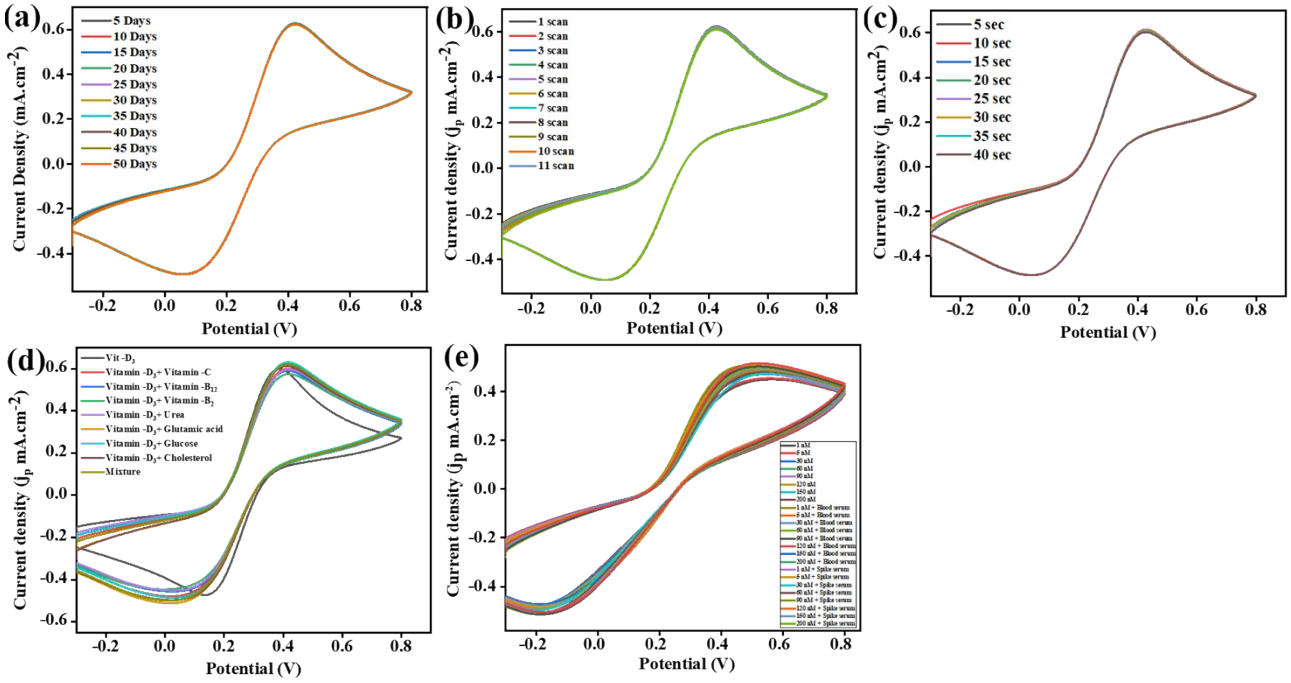
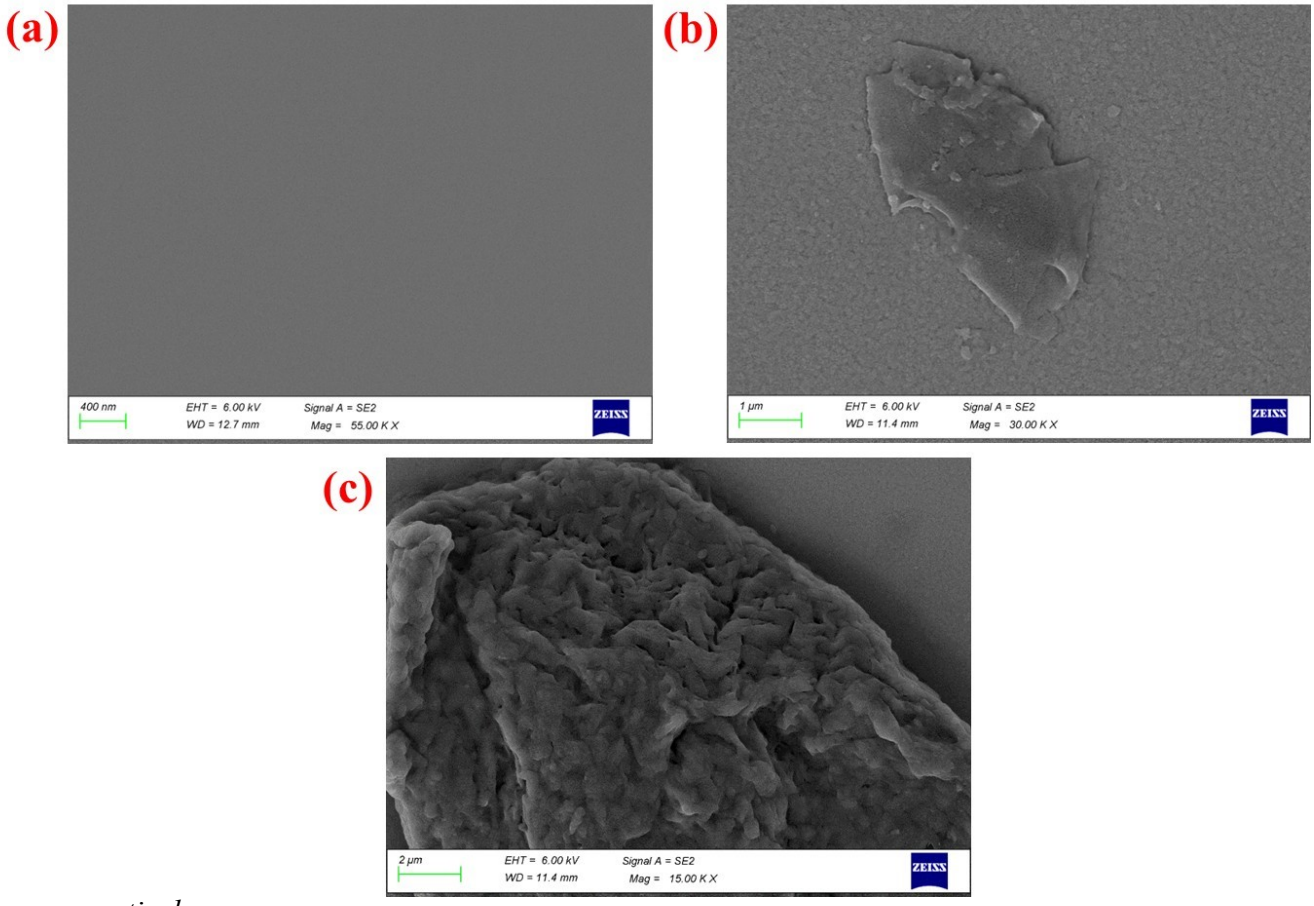


Figure S.1. *CV graph of fabricated BSA/Ab Vit –D/ B-$Mg(OH)_2$@MgO/PPy NCs /ITO biosensor towards Vit-D_3 study at at $[Fe(CN)_6]^{3-/4-}$ containing PBS (5mM, 0.9 % NaCl, pH 5.7) in -0.3 to 0.8 V scan rate 50 mV/sec a) CV graph of stability study, b) CV graph of reusability, c) CV graph of response time, d) CV graph of interference study and e) CV graph of real sample study.*

Figure S.2 *SEM image a) Bare ITO of 400 nm resolution SEM image, b) B-Mg(OH)2@MgO/ppy-NCs/ITO electrode 1 µm resolution SEM image and c) B-Mg(OH)2@MgO/ppy-NCs/Ab-Vit-D/BSA electrode of 2 µm resolution SEM image,*



respectively.

4.6. Competence of the fabricated BSA/Ab Vit –D/ B-Mg(OH)$_2$@MgO/PPy NCs/ITO biosensor towards Vit-D$_3$

A repeatability and reproducibility study was optimized by CV studies at SES. The repeatability of the fabricated biosensor towards Vit-D_3 was done at 1 nM, 70 nM & 200 nM concentrations of Vit-D_3 until the 10 continuous scans of three electrodes. Figure S.3 a), b) & c) of line + symbol plot of three different electrodes on 1 nM, 70 nM & 200 nM concentration of Vit-D_3 until the 10 continuous scans. While in Figure S.3. d) is a line + symbol plot of

concluding that three electrode studies have retained their repeatability until these scans. Reproducibility of fabricated biosensor towards Vit-D_3 was done at 1 nM, 70 nM & 200 nM concentration of Vit-D_3 has been done for 10 continuous scans, respectively. In Figure S.4 shown Line + Symbol plot of each 1 nM, 70 nM & 200 nM concentration of Vit-D_3 at 10 continuous scans, respectively.

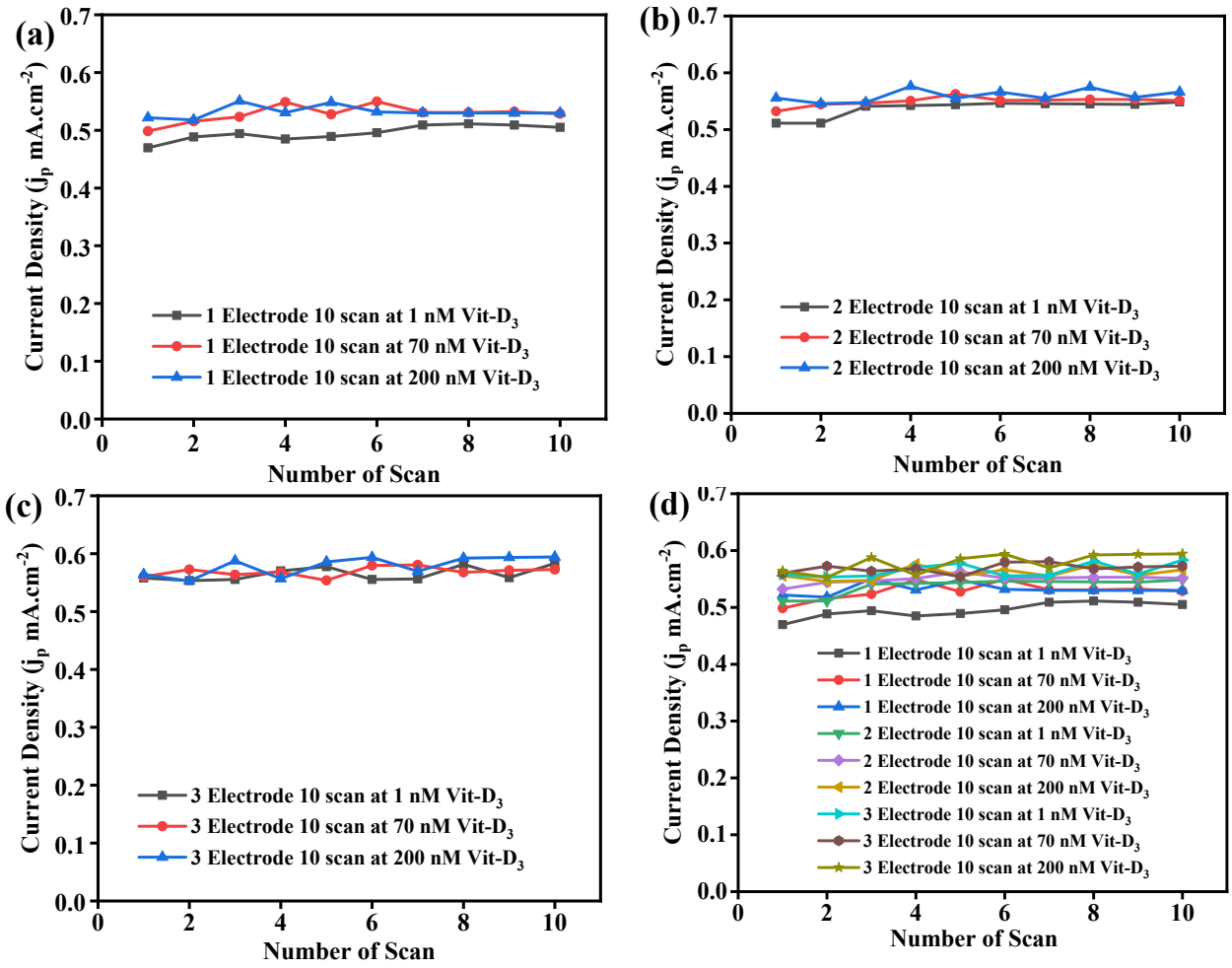


Figure S.3 Plot of reproducibility study in Buffer (pH-5.7) containing $Fe^{+2/+3}$ ion in the potential range (-0.3 to 0.8 V) has been done by cyclic voltammetry (CV). Figure S.3.a), b) & c) line + symbol plot of ITO/B-Mg(OH)2@MgO/ppy NCs/Ab Vit-D/BSA electrode at 1 nM, 70 nM and 200 nM concentration of Vit-D_3 and Figure S.3.d) conclude data of Figure.S.3.a, b & c line + symbol plot of ITO/B-Mg(OH)2@MgO/ppy NCs/Ab Vit-D/BSA electrode at 1 nM, 70 nM and 200 nM concentration of Vit-D_3.

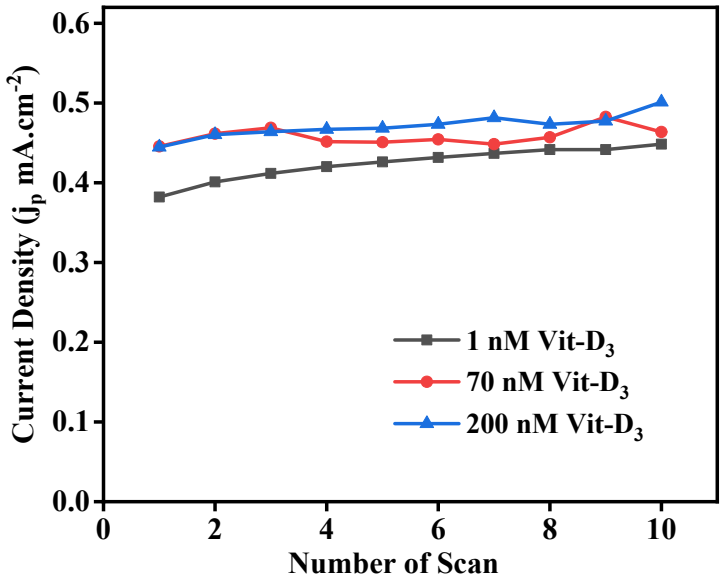
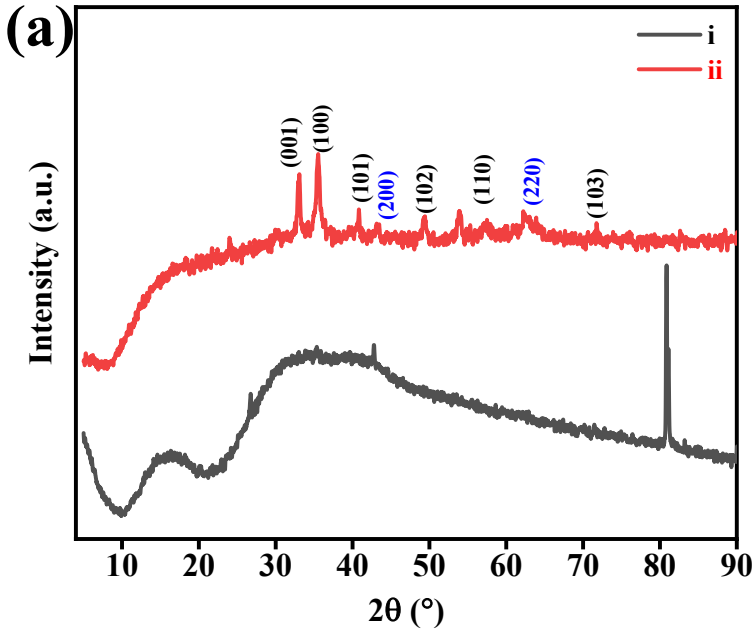
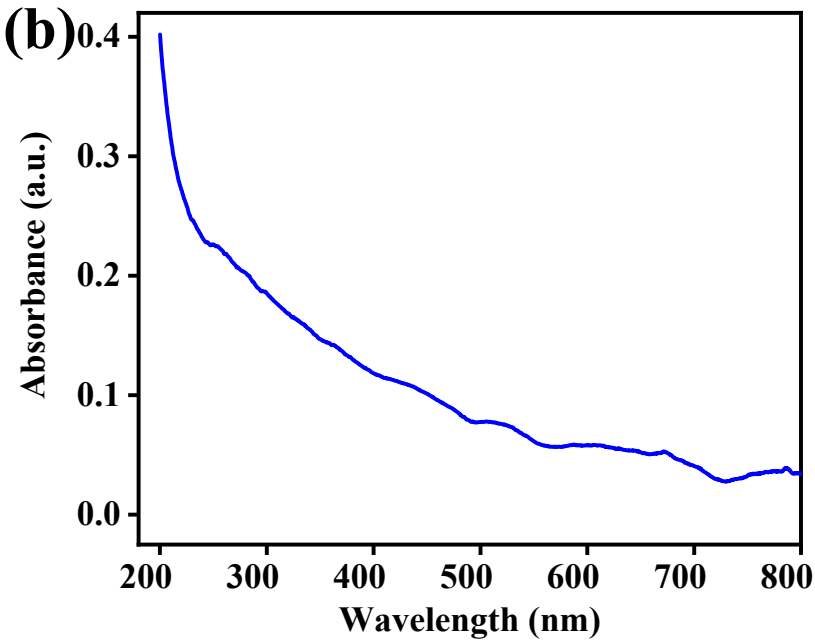


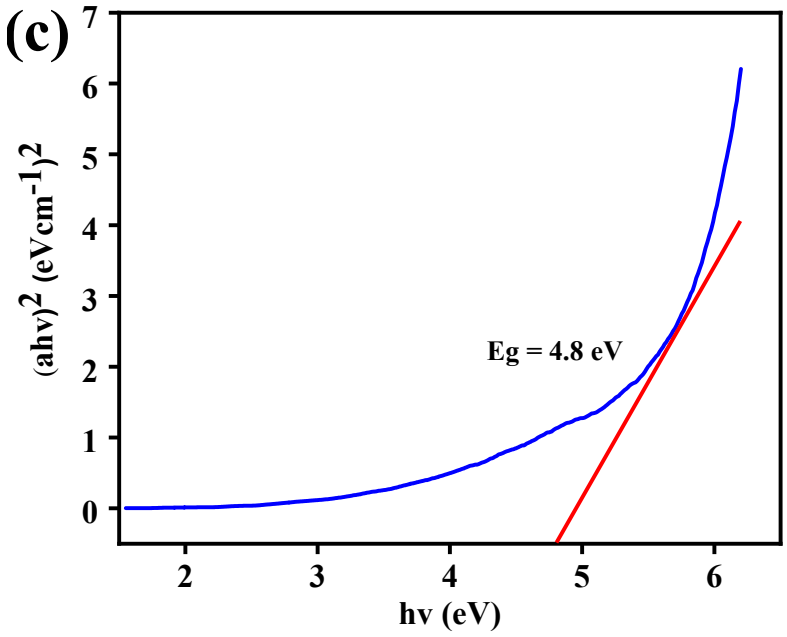
Figure S.4. *Plot of the Repeatability study in Buffer (pH-5.7) containing* $Fe^{+2/+3}$ *ions in the potential range (-0.3 to 0.8 V) has been done by cyclic voltammetry (CV). Here, B-Mg(OH)2@MgO/ppy NCs/Ab Vit-D/BSA electrode performances were done by a minimum at least 30 times (low 10 times (1 nM); medium 10 times (70 nM); high 10 times (200 nM) concentration of Vit-*D_3.

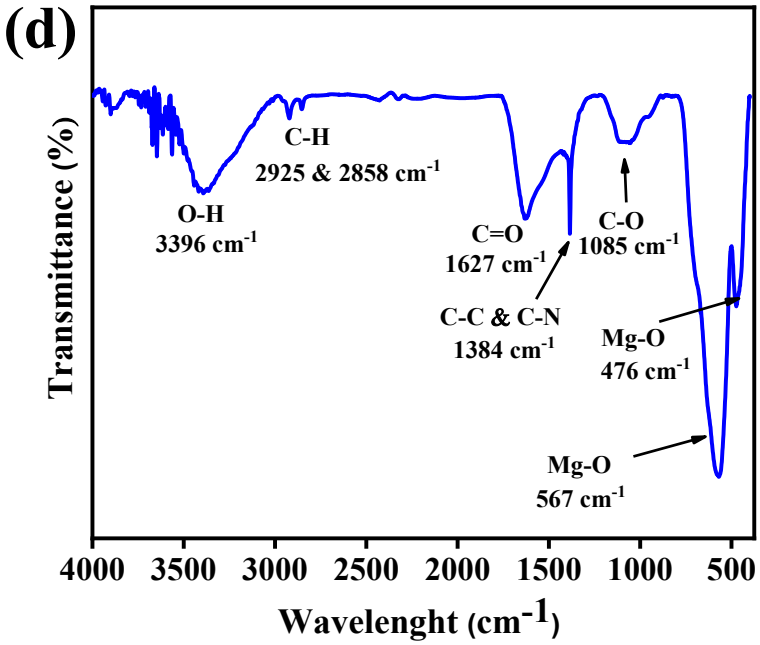
Data Availability Statement

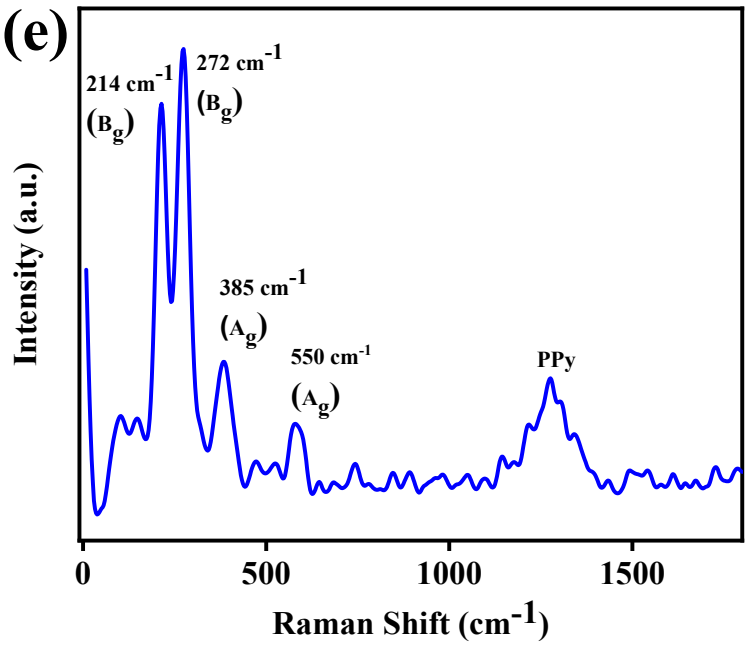
The data supporting this article have been included as part of the Supplementary Information.

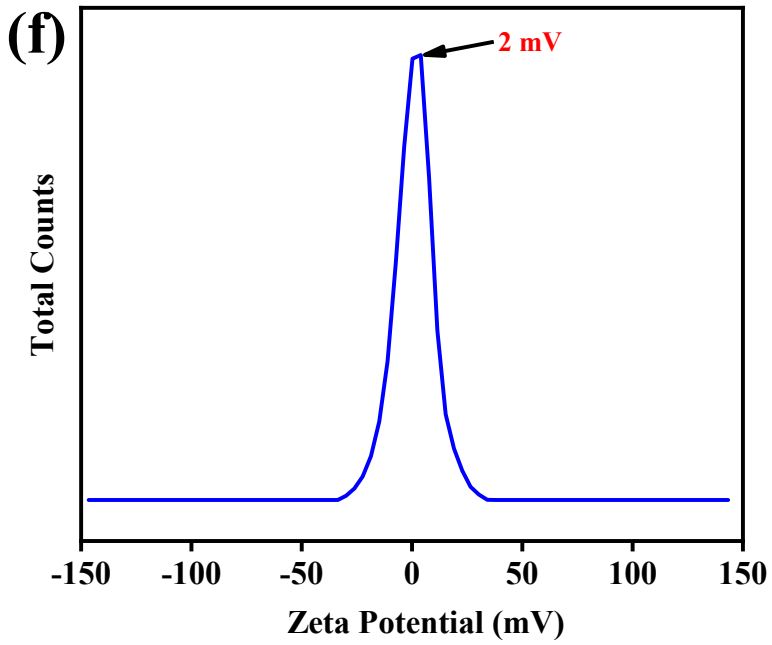





(c)
(ahv)2 (eVcm-1)2
hv (eV)
Eg = 4.8 eV


(d)
Transmittance (%)
Wavelenght (cm-1)
O-H
3396 cm-1
C-H
2925 & 2858 cm-1
C=O
1627 cm-1
C-C & C-N
1384 cm-1
C-O
1085 cm-1
Mg-O
476 cm-1
Mg-O
567 cm-1


(e)
214 cm-1
(Bg)
272 cm-1
(Bg)
385 cm-1
(Ag)
550 cm-1
(Ag)
PPy
Intensity (a.u.)
0
500
1000
1500
Raman Shift (cm-1)


(f)
2 mV
Total Counts
-150
-100
-50
0
50
100
150
Zeta Potential (mV)

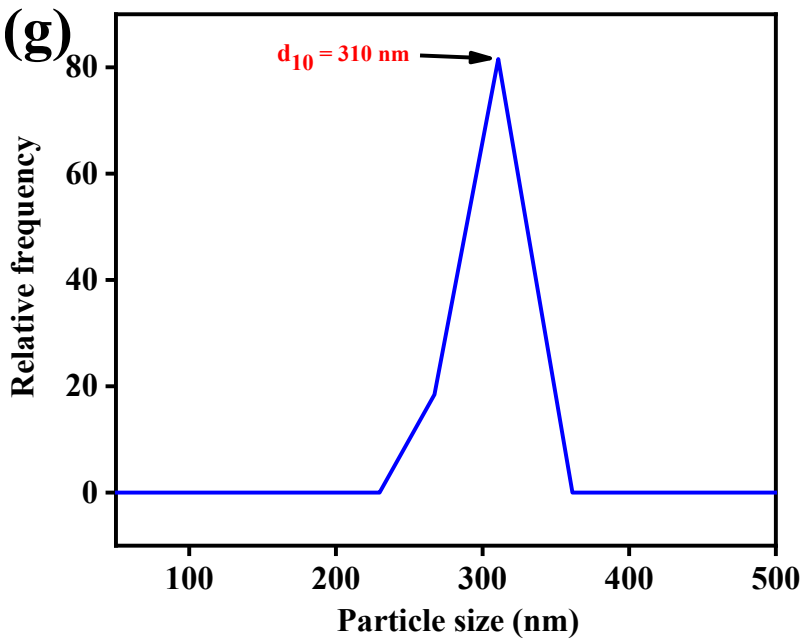
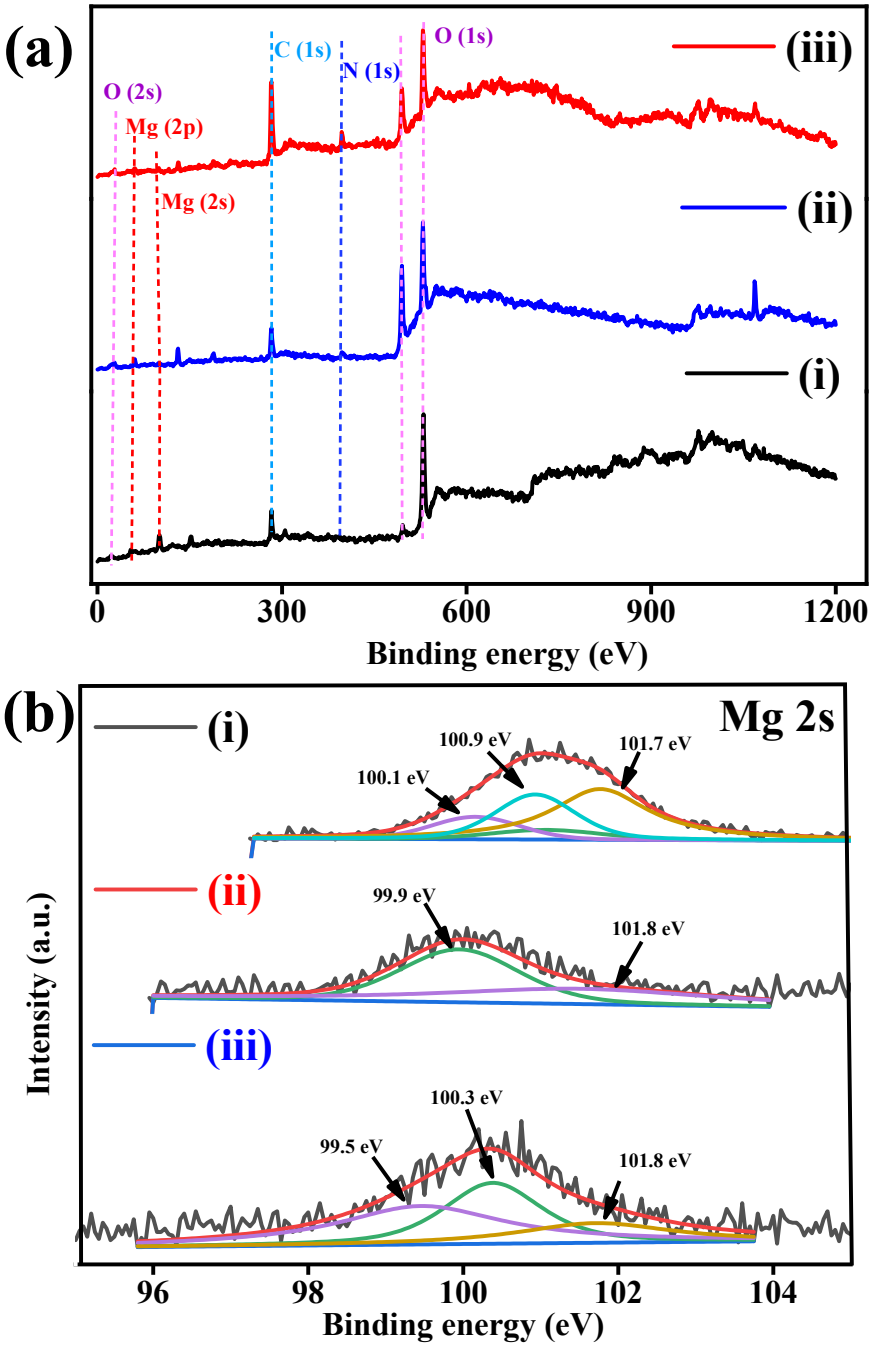
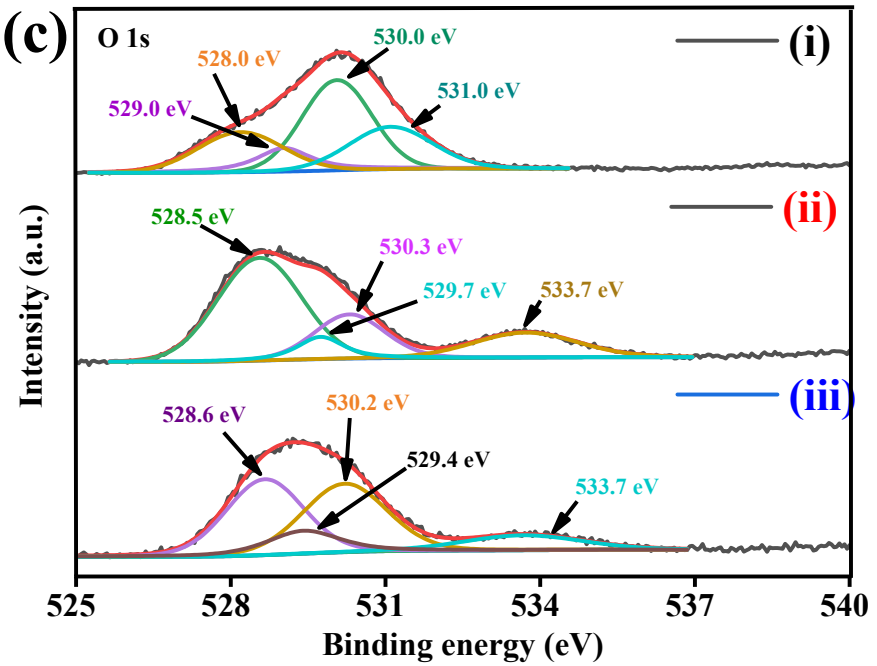
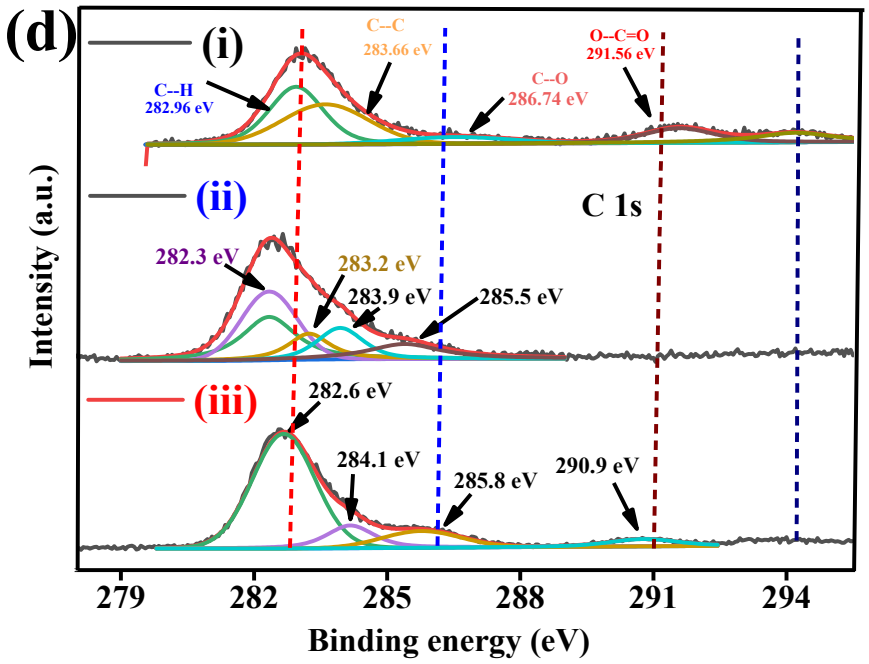


Figure 2: optoelectronic characterization of B-Mg(OH)$_2$@MgO/PPy NCs a) XRD spectra, i) Non-calcination B-Mg(OH)$_2$@MgO/PPy NCs, b) UV visible spectra and insert c) Touc's plot of band gap, d) FTIR spectra, e) Raman spectra, f) Zeta potential spectra and g) DLS spectra of B-Mg(OH)$_2$@MgO/PPy NCs, respectively. (Original origin data file)


(a)
O (2s)
Mg (2p)
Mg (2s)
C (1s)
N (1s)
O (1s)
(iii)
(ii)
(i)
0
300
600
900
1200
Binding energy (eV)
(b)
Mg 2s
(i)
100.9 eV
101.7 eV
100.1 eV
(ii)
99.9 eV
101.8 eV
(iii)
100.3 eV
99.5 eV
101.8 eV
Intensity (a.u.)
96
98
100
102
104
Binding energy (eV)


(c)
O 1s
528.0 eV
530.0 eV
529.0 eV
531.0 eV
(i)
(ii)
528.5 eV
530.3 eV
529.7 eV
533.7 eV
(iii)
528.6 eV
530.2 eV
529.4 eV
533.7 eV
Intensity (a.u.)
525
528
531
534
537
540
Binding energy (eV)


(d)
(i)
C--C
283.66 eV
O--C=O
291.56 eV
C--H
282.96 eV
C--O
286.74 eV
(ii)
C 1s
282.3 eV
283.2 eV
283.9 eV
285.5 eV
(iii)
282.6 eV
284.1 eV
285.8 eV
290.9 eV
Intensity (a.u.)
279
282
285
288
291
294
Binding energy (eV)

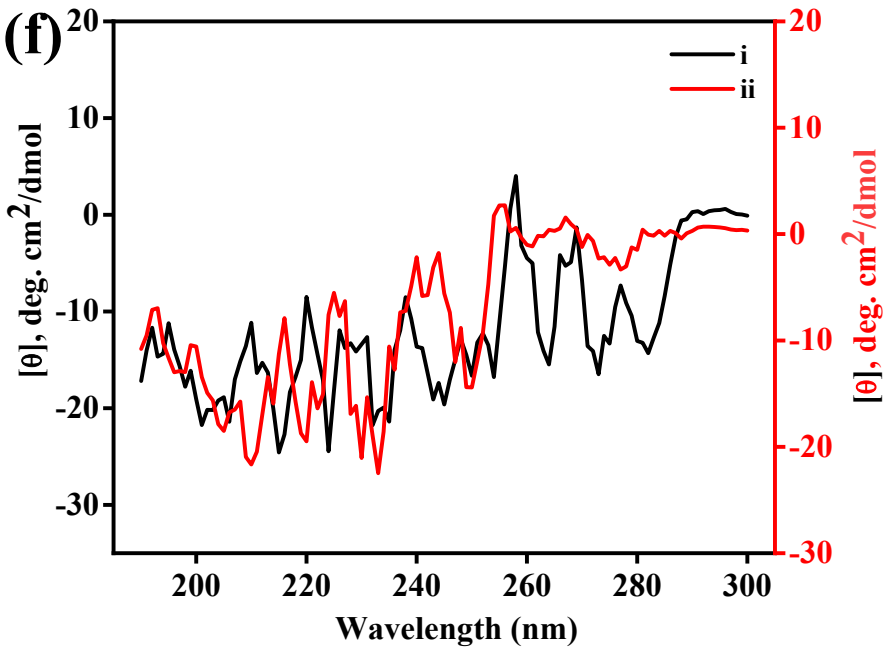
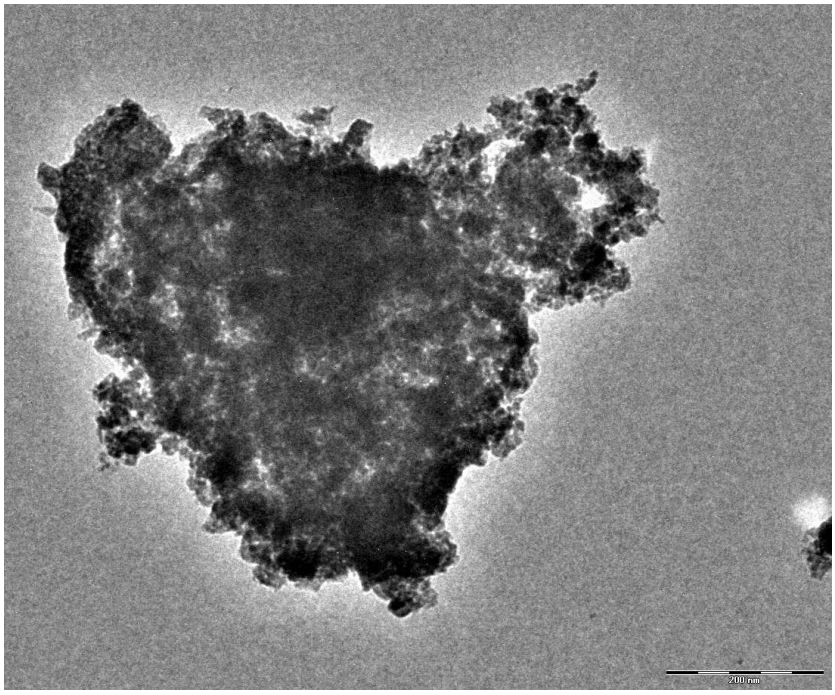
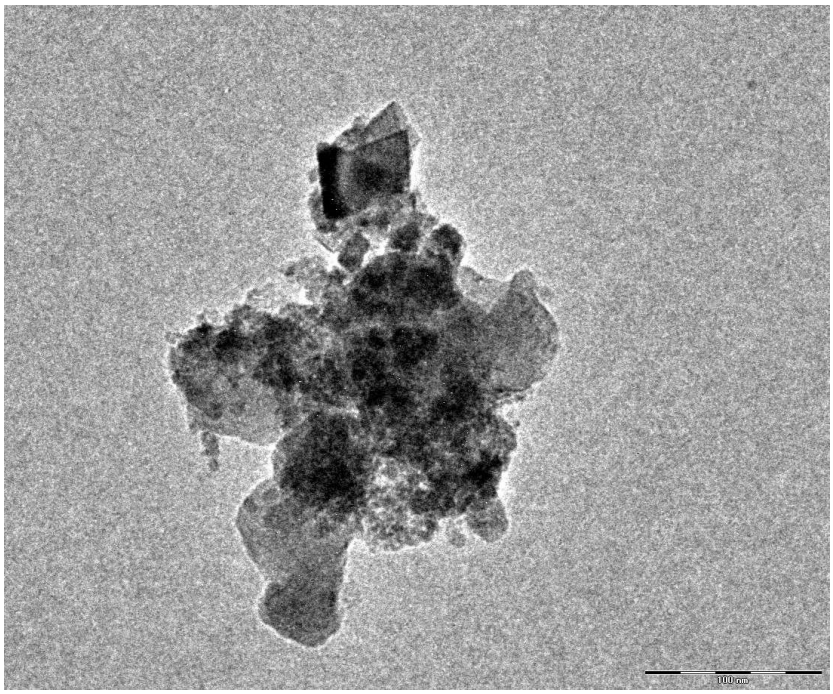


Figure 3: XPS spectra of i) B-Mg(OH)$_2$@MgO/PPy NCs relative binding energy and after immobilization of monoclonal antibody at that surface is ii) Ab Vit-D/Mg(OH)$_2$@MgO/PPy NCs and iii) final prepared impedance biosensor BSA/ Ab Vit-D/Mg(OH)$_2$@MgO/PPy NCs respectively. a) survey plot b) binding energy of Mg 2s, c) binding energy of O 1s, d) binding energy of C 1s, e) binding energy of N 1s, respectively i),ii) and iii) and f) CD spectra of i) B-Mg(OH)$_2$@MgO/PPy NCs and ii) Ab Vit-D/B-Mg(OH)$_2$@MgO/PPy NCs, respectively.

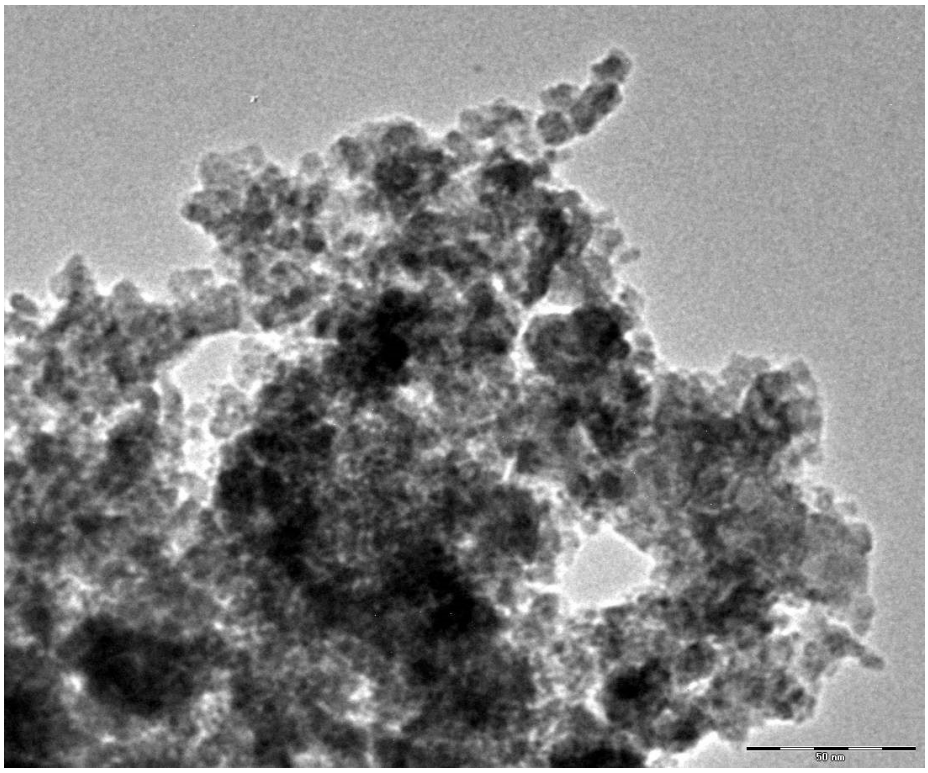
(a)



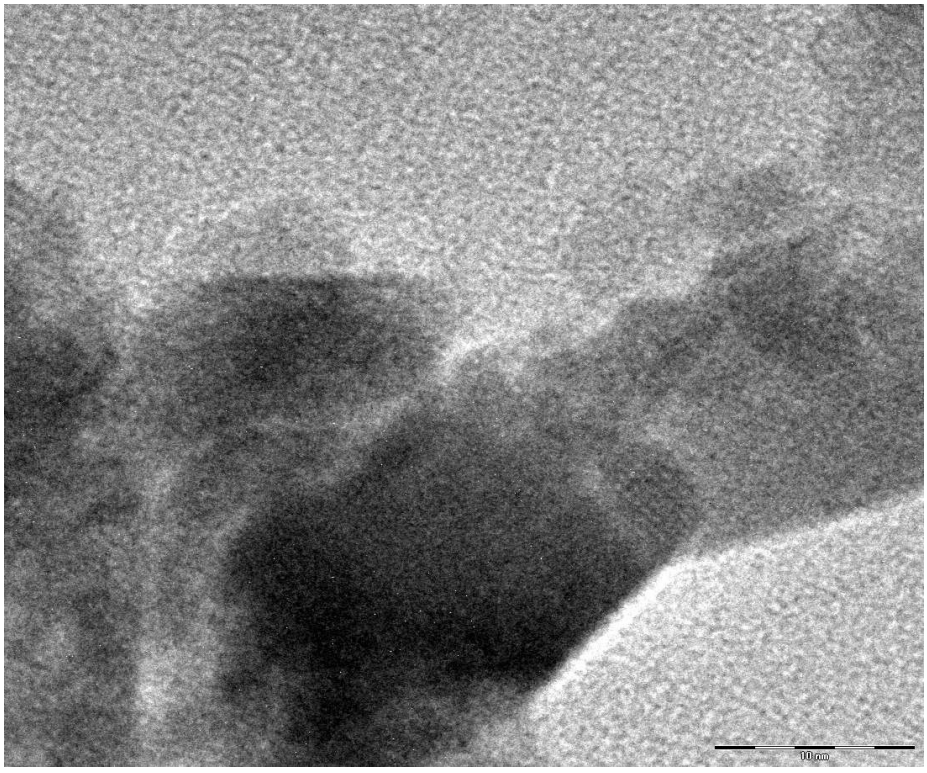
(b)

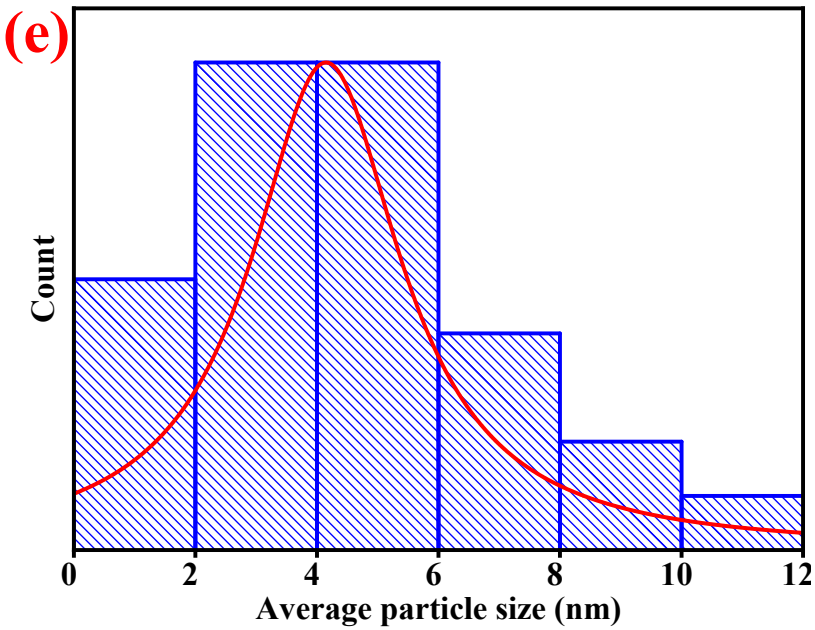


(c)

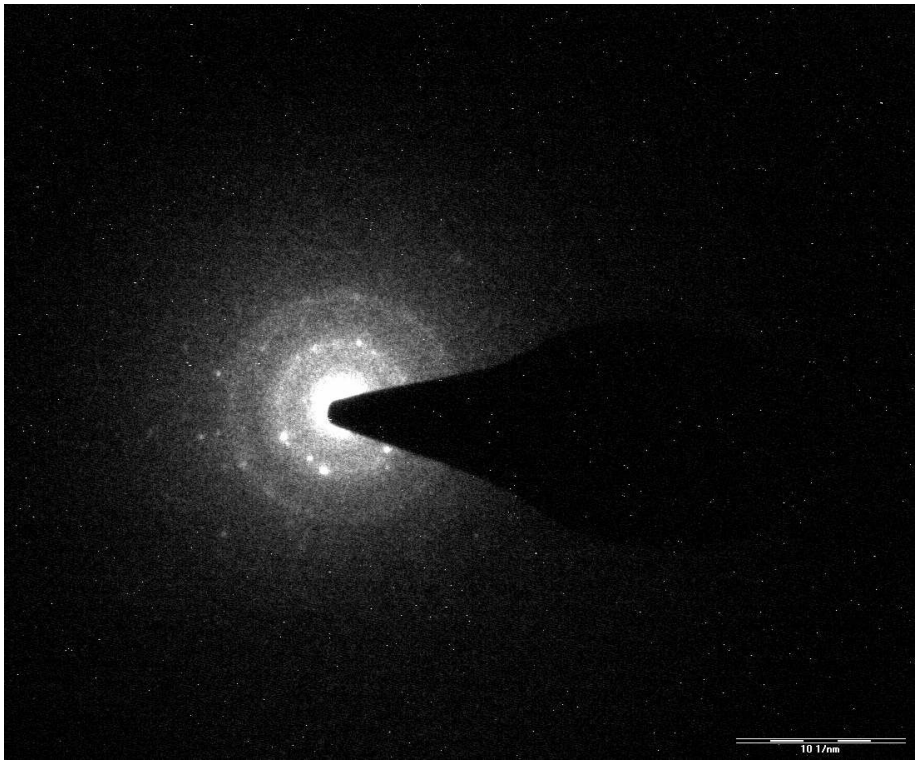


(d)

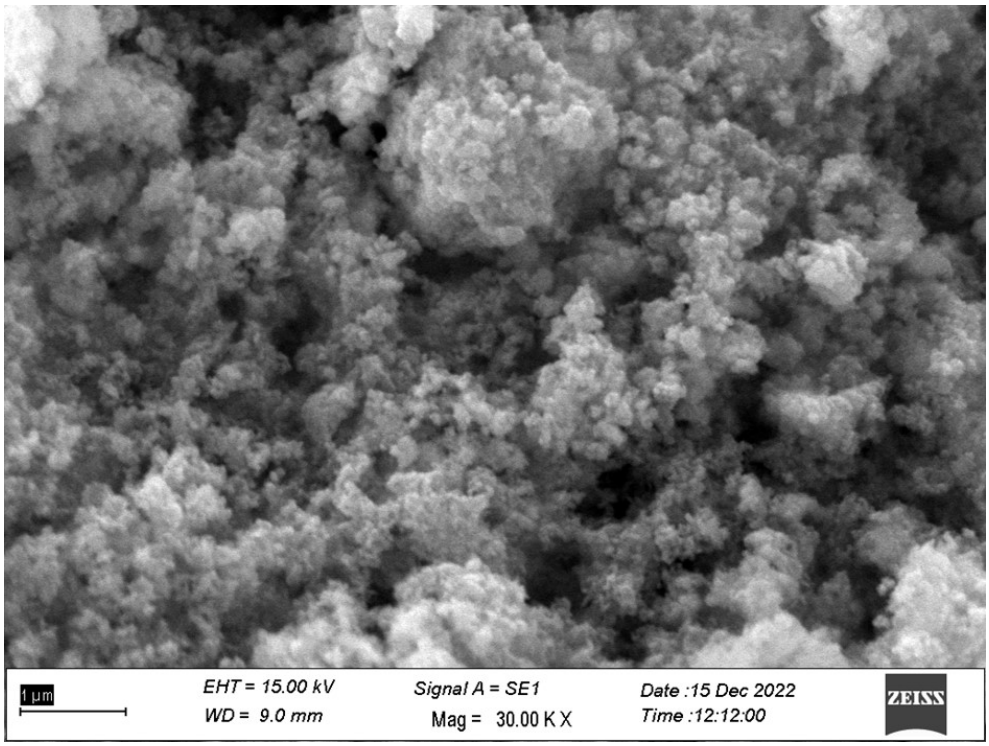




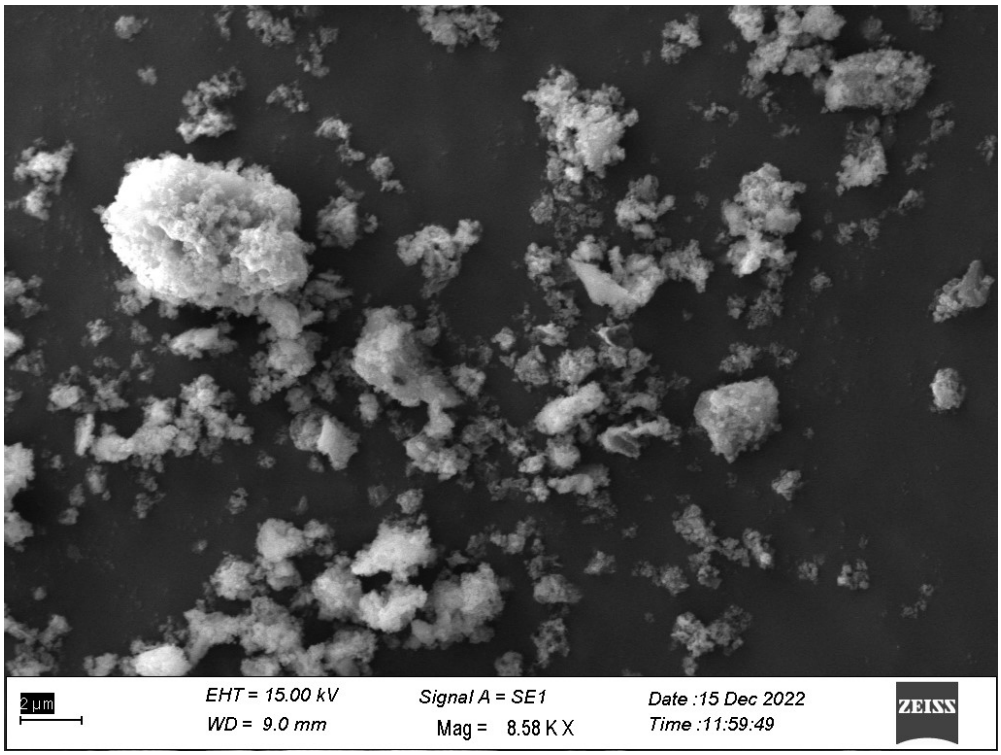
(f)



(g)


1 µm
EHT = 15.00 kV
WD = 9.0 mm
Signal A = SE1
Mag = 30.00 K X
Date :15 Dec 2022
Time :12:12:00
ZEISS

(h)


2 µm
EHT = 15.00 kV
WD = 9.0 mm
Signal A = SE1
Mag = 8.58 K X
Date :15 Dec 2022
Time :11:59:49
ZEISS

(i)

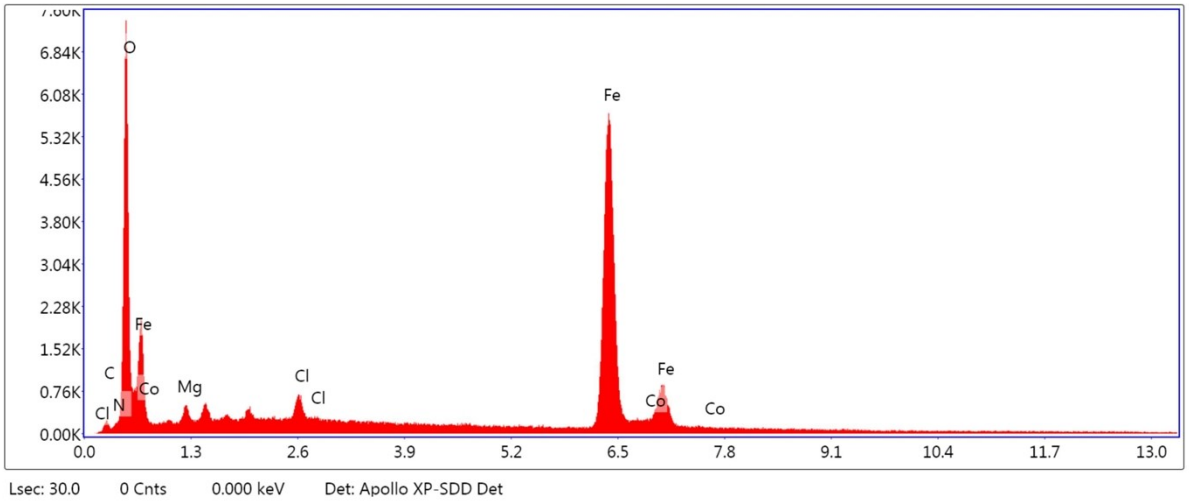


Figure 4: TEM images of B-Mg(OH)$_2$@MgO/PPy NCs a) 200 nm, b) 100 nm, c) 50 nm and d) 10 nm resolution respectively, e) histogram plot for average particle size, f) SEAD pattern, g) SEM image of B-Mg(OH)$_2$@MgO/PPy NCs of 2 µm resolution , h) SEM image of B-Mg(OH)$_2$@MgO/PPy NCs of 1 µm resolution, and i) EXD spectra in inserted table with element weight% and atomic %, respectively.