

### **Supplementary Information**

## **Impedimetric Sensing of C-Reactive Protein Using Novel Molecularly Imprinted Polymer Coupled with Bismuth-Enhanced Cobalt Ferrite Nanocomposites for Cardiovascular Risk Assessment**

Siddhima Singh<sup>1</sup>, Astha Singh<sup>1</sup>, Neelottma Singh<sup>1\*</sup> and Jay Singh<sup>2\*</sup>

*<sup>1</sup>Sam Higginbottom University of Agriculture, Technology and Sciences, Prayagraj-211007  
Uttar Pradesh, India*

*<sup>2</sup>Department of Chemistry, Institute of Sciences, Banaras Hindu University, Varanasi -221005,  
Uttar Pradesh, India*

\*Corresponding Author: Email: [neelottma.singh@shiats.edu.in](mailto:neelottma.singh@shiats.edu.in) (N.S.), (ORCID ID: 0000-0002-7124-2702; Phone: +91-8299585369) and [jaysingh.chem@bhu.ac.in](mailto:jaysingh.chem@bhu.ac.in) (JS), (ORCID ID: 0000-0002-3793-0450; Phone: +91-9871766453)

## Characterization Technique

To assess the properties of synthesised  $\text{Bi}_{(0.15)}\text{CoFe}_2\text{O}_4$  NPs, various characterization techniques were used to evaluate their structural, morphological, electrochemical, and compositional properties. Powder XRD (X-ray diffraction) spectroscopy [PAN Analytical EMPYREAN RAYONS-X] diffractometer, with  $\text{Cu K}\alpha$  X-ray radiation at a scanning step size of  $0.013^\circ$ , had a wavelength of 0.154056 nm. The  $2\theta$  values for spectra collection ranged from  $10$  to  $80^\circ$ , as XRD identifies the crystalline phase and lattice parameters. Fourier-transform infrared spectroscopy (FTIR) (model FTIR 4700, JASCO, TOKYO) confirmed the binding energies of potential functional groups in the range of  $400\text{--}4000\text{ cm}^{-1}$  at  $25^\circ\text{C}$ . Samples were prepared using KBr pellets, which measure light transmission through solid samples. UV analysis by SHIMADZU, model no. A125361 UV-1900I determined the optical properties of  $\text{Bi}_{(0.15)}\text{CoFe}_2\text{O}_4$  NPs, indicating how the material absorbs light in the ultraviolet and visible regions. Brunauer-Emmett-Teller (BET) analysis, conducted using Quantachrome USA, determined the specific surface area of the synthesised material and provided insights into porosity. An atomic force microscope (AFM), model 5500 from Agilent, was used to confirm the surface morphology of  $\text{Bi}_{(0.15)}\text{CoFe}_2\text{O}_4$  NPs. X-ray photoelectron spectroscopy (XPS) was performed with a PHI 500 VersaProbe-III microprobe (ULVAC-PHI, Tokyo, Japan) to analyze the elemental composition of the synthesized NPs. A JEOL 500 FT-NMR measured  $^1\text{H}$ -NMR spectra at 500 MHz, while a SCIEX X500R (QTOF-MS) mass spectrometer recorded the mass spectra. Field Emission Scanning Electron Microscopy (FE-SEM) (Carl Zeiss EVO-18 Research, model) utilized focused sources from laser Lanthanum Hexaboride ( $\text{LaB}_6$ ). The samples were coated with Au and Pd on adhesive carbon tape. FE-SEM provided high-resolution images showing the structural morphology and topography, including agglomeration, pores, and cracks in  $\text{Bi}_{(0.15)}\text{CoFe}_2\text{O}_4$ . HR-TEM and SEAD were performed using a Technai 200 Novatouch LX4 (FEI, USA) to calculate interplanar spacing ( $d$ -spacing)

and particle size, as well as to reveal the shape and morphological features of the synthesized material. After confirming the structural and morphological characteristics, electrochemical analysis was conducted using an electrochemical workstation, Corrtest CS studio 350, to study the electrochemical behavior and conductivity of the fabricated ITO electrode. Techniques included CV (Cyclic Voltammetry), Differential Pulse Voltammetry, and EIS (Electrochemical Impedance Spectroscopy).

**Table S1: Shows the pore size of the prepared MIP and NIP.**

| Material | Surface Area (m <sup>2</sup> /g) | Pore Volume | Pore size (nm) |
|----------|----------------------------------|-------------|----------------|
| MIP      | 25.813 m <sup>2</sup> /g         | 0.085 cc/g  | 3.533 nm       |
| NIP      | 22.800 m <sup>2</sup> /g         | 0.093 cc/g  | 3.524 nm       |

**Table S2: Scan rate analysis Bi<sub>(0.15)</sub>CoFe<sub>2</sub>O<sub>4</sub>/MIP/ITO electrode**

| S.no | Scan rate(mV/s) | Square root of scan rate (mV/s) | E <sub>pa</sub> | E <sub>pc</sub> |
|------|-----------------|---------------------------------|-----------------|-----------------|
| 1    | 10              | 3.1622                          | 0.320428        | 0.0987232       |
| 2    | 20              | 4.4723                          | 0.350068        | 0.0747150       |
| 3    | 30              | 5.4772                          | 0.367558        | 0.0539671       |
| 4    | 40              | 6.3245                          | 0.381782        | 0.0412220       |
| 5    | 50              | 7.0710                          | 0.393638        | 0.0272913       |
| 6    | 60              | 7.7459                          | 0.404901        | 0.0195850       |
| 7    | 70              | 8.3666                          | 0.415572        | 0.0059507       |
| 8    | 80              | 8.9442                          | 0.4173506       | 0.0011627       |
| 9    | 90              | 9.4868                          | 0.4303921       | -0.0091655      |
| 10   | 100             | 10                              | 0.4389876       | -0.0165754      |

**Table S3: Response timing analysis of Bi<sub>(0.15)</sub>CoFe<sub>2</sub>O<sub>4</sub>/MIP/ITO electrode**

| S.No. | No. of days | Current (mA) | Recovery %  | RSD%     |
|-------|-------------|--------------|-------------|----------|
| 1     | 5           | 0.8291203    | 100         | 0        |
| 2     | 10          | 0.8456119    | 101.1989047 | 1.989047 |

|    |    |           |             |            |
|----|----|-----------|-------------|------------|
| 3  | 15 | 0.8386347 | 101.1475294 | 1.147529   |
| 4  | 20 | 0.8107258 | 97.78144377 | 2.218556   |
| 5  | 25 | 0.8075543 | 97.398929   | 2.6010700  |
| 6  | 30 | 0.7961370 | 96.021892   | 3.9781078  |
| 7  | 35 | 0.7948684 | 95.868886   | 4.1311134  |
| 8  | 40 | 0.7942341 | 95.792383   | 4.2076161  |
| 9  | 45 | 0.7891598 | 95.180373   | 4.81962629 |
| 10 | 50 | 0.7815483 | 94.262352   | 5.7376474  |
| 11 | 55 | 0.7745710 | 93.420822   | 6.579177   |
| 12 | 60 | 0.7663252 | 92.426298   | 7.573701   |

**Table S4: stability analysis of Bi<sub>(0.15)</sub>CoFe<sub>2</sub>O<sub>4</sub>/MIP/ITO electrode**

| S.No. | No. of days | Current (mA) | Recovery % | RSD %     |
|-------|-------------|--------------|------------|-----------|
| 1     | 1           | 0.7392756    | 100        | 0         |
| 2     | 2           | 0.729315     | 98.6526540 | 1.3473459 |
| 3     | 3           | 0.731578     | 98.9587644 | 1.0412355 |
| 4     | 4           | 0.731578     | 98.958764  | 1.0412355 |
| 5     | 5           | 0.729315     | 98.652654  | 1.3473459 |
| 6     | 10          | 0.722071     | 97.672775  | 2.327224  |
| 7     | 15          | 0.729315     | 98.652654  | 1.3473459 |
| 8     | 20          | 0.732937     | 99.1425931 | 0.857406  |
| 9     | 25          | 0.730220     | 98.775071  | 1.224928  |
| 10    | 30          | 0.727051     | 98.346408  | 1.6535917 |
| 11    | 35          | 0.722071     | 97.672775  | 2.3272241 |
| 12    | 40          | 0.719354     | 97.305253  | 2.694746  |
| 13    | 45          | 0.722976     | 97.795193  | 2.2048069 |
| 14    | 50          | 0.714827     | 96.69289   | 3.3071022 |
| 15    | 55          | 0.717091     | 96.999143  | 3.0008565 |
| 16    | 60          | 0.707583     | 95.713019  | 4.2869803 |

**Table S5: Reusability of Bi<sub>(0.15)</sub>CoFe<sub>2</sub>O<sub>4</sub>/MIP/ITO electrode**

| S.No. | No. of scan | Current (mA) | Recovery % | RSD%     |
|-------|-------------|--------------|------------|----------|
| 1     | 1           | 0.690938     | 100        | 0        |
| 2     | 2           | 0.690736     | 99.970836  | 0.029163 |

|    |    |          |            |            |
|----|----|----------|------------|------------|
| 3  | 3  | 0.690553 | 99.944278  | 0.055721   |
| 4  | 4  | 0.690296 | 99.907082  | 0.092917   |
| 5  | 5  | 0.690135 | 99.883781  | 0.1162188  |
| 6  | 6  | 0.689329 | 99.7671281 | 0.2328718  |
| 7  | 7  | 0.688805 | 99.691289  | 0.3087107  |
| 8  | 8  | 0.687515 | 99.504586  | 0.4954134  |
| 9  | 9  | 0.686870 | 99.4112351 | 0.5887648  |
| 10 | 10 | 0.686749 | 99.393722  | 0.6062772  |
| 11 | 11 | 0.685943 | 99.2770697 | 0.722930   |
| 12 | 12 | 0.684452 | 99.0612617 | 0.93873821 |
| 13 | 13 | 0.683848 | 98.973858  | 1.02614127 |
| 14 | 14 | 0.683646 | 98.94462   | 1.055376   |
| 15 | 15 | 0.680906 | 98.5480607 | 1.4519392  |

**Table S6: Standard (Analyte) for interference of Bi<sub>(0.15)</sub>CoFe<sub>2</sub>O<sub>4</sub>/MIP/ITO electrode**

|   |          |                |
|---|----------|----------------|
| 1 | 80 ng/ml | 0.819466508 mA |
|---|----------|----------------|

**(a)- Ascorbic acid interference of Bi<sub>(0.15)</sub>CoFe<sub>2</sub>O<sub>4</sub>/MIP/ITO electrode**

| S.No. | Concentration (ng/ml) | Current (mA) | Recovery % | RSD%       |
|-------|-----------------------|--------------|------------|------------|
| 1     | 60                    | 0.737359788  | 89.980466  | 10.0195333 |
| 2     | 70                    | 0.745022311  | 90.9155290 | 9.0844709  |
| 3     | 80                    | 0.746415497  | 91.0855403 | 8.91445962 |
| 4     | 90                    | 0.749550165  | 91.4680658 | 8.5319341  |
| 5     | 100                   | 0.750595054  | 91.595574  | 8.4044257  |

**(b)- cholesterol interference of Bi<sub>(0.15)</sub>CoFe<sub>2</sub>O<sub>4</sub>/MIP/ITO electrode**

| S.No. | Concentration (ng/ml) | Current (mA) | Recovery % | RSD%       |
|-------|-----------------------|--------------|------------|------------|
| 1     | 60                    | 0.833466839  | 101.708468 | 1.7084689  |
| 2     | 70                    | 0.834129935  | 101.789386 | 1.7893869  |
| 3     | 80                    | 0.83459102   | 101.845653 | 1.84565346 |
| 4     | 90                    | 0.835522436  | 101.959314 | 1.9593147  |
| 5     | 100                   | 0.832936362  | 101.643734 | 1.6437345  |

**(c)- Glucose interference of Bi<sub>(0.15)</sub>CoFe<sub>2</sub>O<sub>4</sub>/MIP/ITO electrode**

| S.No. | Concentration (ng/ml) | Current (mA) | Recovery % | RSD%       |
|-------|-----------------------|--------------|------------|------------|
| 1     | 60                    | 0.775071615  | 94.5824640 | 5.4175359  |
| 2     | 70                    | 0.762927929  | 93.100562  | 6.899437   |
| 3     | 80                    | 0.776492688  | 94.755878  | 5.2441215  |
| 4     | 90                    | 0.753368004  | 91.933959  | 8.066040   |
| 5     | 100                   | 0.75298044   | 91.886664  | 8.11333560 |

**(d)- Histamin interference of the Bi<sub>(0.15)</sub>CoFe<sub>2</sub>O<sub>4</sub>/MIP/ITO electrode**

| S.No. | Concentration (ng/ml) | Current (mA) | Recovery % | RSD%      |
|-------|-----------------------|--------------|------------|-----------|
| 1     | 60                    | 0.702917311  | 85.7774300 | 14.222569 |
| 2     | 70                    | 0.7050099    | 86.0327900 | 13.967209 |
| 3     | 80                    | 0.706597382  | 86.2265114 | 13.773488 |
| 4     | 90                    | 0.708617813  | 86.473065  | 13.526934 |
| 5     | 100                   | 0.7097000187 | 86.605128  | 13.394871 |

**(e)-Uric acid interference of the Bi<sub>(0.15)</sub>CoFe<sub>2</sub>O<sub>4</sub>/MIP/ITO electrode**

| S.No. | Concentration (ng/ml) | Current (mA) | Recovery % | RSD%      |
|-------|-----------------------|--------------|------------|-----------|
| 1     | 60                    | 0.773778184  | 94.424625  | 5.5753741 |
| 2     | 70                    | 0.775071576  | 94.5824592 | 5.4175407 |
| 3     | 80                    | 0.77617096   | 94.7166177 | 5.283382  |
| 4     | 90                    | 0.776882327  | 94.8034263 | 5.1965736 |

|   |     |             |           |            |
|---|-----|-------------|-----------|------------|
| 5 | 100 | 0.777593693 | 94.890234 | 5.10976526 |
|---|-----|-------------|-----------|------------|

**(f)-Hemoglobin interference of the  $\text{Bi}_{(0.15)}\text{CoFe}_2\text{O}_4/\text{MIP}/\text{ITO}$  electrode**

| S.No. | Concentration (ng/ml) | Current (mA) | Recovery %    | RSD%       |
|-------|-----------------------|--------------|---------------|------------|
| 1     | 60                    | 0.793010753  | 96.7715880097 | 3.22841199 |
| 2     | 70                    | 0.85950764   | 104.88624386  | 4.8862438  |
| 3     | 80                    | 0.918971626  | 112.14267050  | 12.142670  |
| 4     | 90                    | 0.922333743  | 112.552951    | 12.552951  |
| 5     | 100                   | 0.92950036   | 113.427498    | 13.427498  |

**(g)-Trypsin interference of the  $\text{Bi}_{(0.15)}\text{CoFe}_2\text{O}_4/\text{MIP}/\text{ITO}$  electrode**

| S.No. | Concentration (ng/ml) | Current (mA) | Recovery %  | RSD%       |
|-------|-----------------------|--------------|-------------|------------|
| 1     | 60                    | 0.844910289  | 103.1049201 | 3.10492018 |
| 2     | 70                    | 0.892153066  | 108.869985  | 8.8699852  |
| 3     | 80                    | 0.793672719  | 96.852368   | 3.1476318  |
| 4     | 90                    | 0.832752221  | 101.621263  | 1.62126369 |
| 5     | 100                   | 0.903963759  | 110.311251  | 10.3112513 |

**Table S7: Real Sample analysis of the CRP on the  $\text{Bi}_{(0.15)}\text{CoFe}_2\text{O}_4/\text{MIP}/\text{ITO}$  belectrode**

| S.No. | Concentration (ng/mL) | Current (mA) |
|-------|-----------------------|--------------|
| 1     | 40                    | 0.814914     |
| 2     | 50                    | 0.840125     |

|   |    |           |
|---|----|-----------|
| 3 | 60 | 0.84228   |
| 4 | 70 | 0.84532   |
| 5 | 80 | 0.8512477 |

**(a) Real Sample analysis of the serum-1 on the  $\text{Bi}_{(0.15)}\text{CoFe}_2\text{O}_4/\text{MIP}/\text{ITO}$  electrode**

| S.No. | Concentration (ng/mL) | Current (mA) | Recovery %   | RSD%       |
|-------|-----------------------|--------------|--------------|------------|
| 1     | 40                    | 0.852265128  | 104.58339139 | 4.58339139 |
| 2     | 50                    | 0.864724864  | 102.92807907 | 2.9280790  |
| 3     | 60                    | 0.866671698  | 102.89591323 | 2.89591323 |
| 4     | 70                    | 0.869007899  | 102.80224045 | 2.80224045 |
| 5     | 80                    | 0.872512199  | 102.49802761 | 2.4980276  |

**(b) Real Sample analysis of the serum 2 on the  $\text{Bi}_{(0.15)}\text{CoFe}_2\text{O}_4/\text{MIP}/\text{ITO}$  electrode**

| S.No. | Concentration (ng/mL) | Current (mA) | Recovery %    | RSD%       |
|-------|-----------------------|--------------|---------------|------------|
| 1     | 40                    | 0.886618005  | 108.79891103  | 8.79891103 |
| 2     | 50                    | 0.890301227  | 105.97242997  | 5.97242997 |
| 3     | 60                    | 0.89251161   | 105.96376620  | 5.96376620 |
| 4     | 70                    | 0.89416733   | 105.77856078  | 5.77856078 |
| 5     | 80                    | 0.900982573  | 105.842573613 | 5.84257361 |

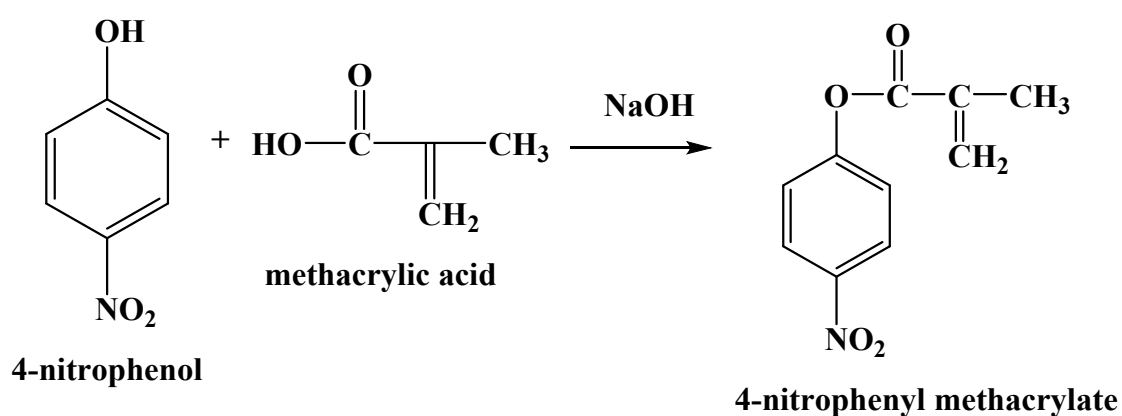


**(c) Real Sample analysis of the serum 3 on the Bi<sub>(0.15)</sub>CoFe<sub>2</sub>O<sub>4</sub>/MIP/ITO electrode**

| S.No. | Concentration (ng/mL) | Current (mA) | Recovery % | RSD% |
|-------|-----------------------|--------------|------------|------|
| 1     | 40                    | 0.81341109   | 99.81536   | 0.18 |
| 2     | 50                    | 0.82021859   | 97.630531  | 2.36 |
| 3     | 60                    | 0.83042985   | 98.59298   | 1.40 |
| 4     | 70                    | 0.84064113   | 94.44636   | 0.5  |
| 5     | 80                    | 0.86058728   | 101.097    | 1.09 |

**(d) Real Sample analysis of the serum 4 on the Bi<sub>(0.15)</sub>CoFe<sub>2</sub>O<sub>4</sub>/MIP/ITO electrode**

| S.No. | Concentration (ng/mL) | Current (mA) | Recovery %  | RSD%   |
|-------|-----------------------|--------------|-------------|--------|
| 1     | 40                    | 0.817845322  | 100.37970   | 0.35   |
| 2     | 50                    | 0.821491563  | 97.782056   | 2.21   |
| 3     | 60                    | 0.83295117   | 98.89241    | 1.10   |
| 4     | 70                    | 0.842848116  | 99.70757    | 0.2924 |
| 5     | 80                    | 0.846873466  | 99.48610307 | 0.5    |



**Figure S1: Reaction of 4-nitrophenyl methacrylate**

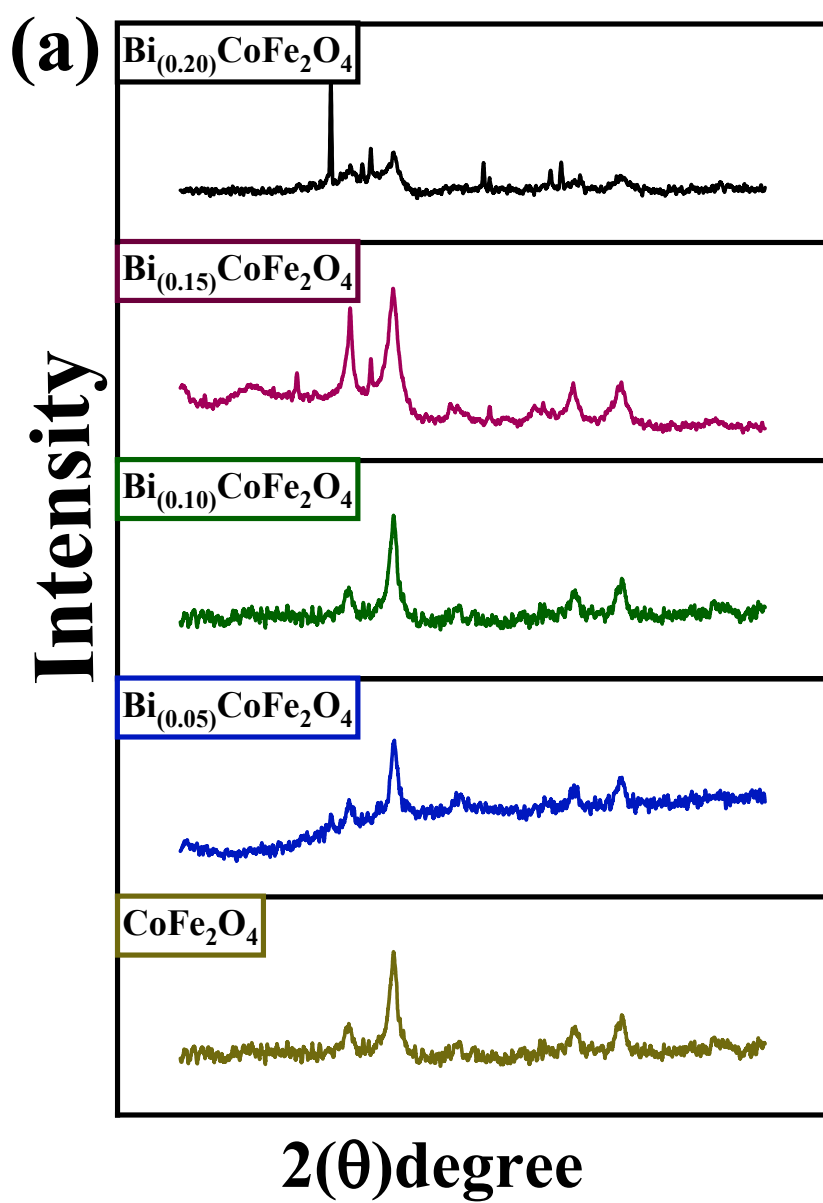
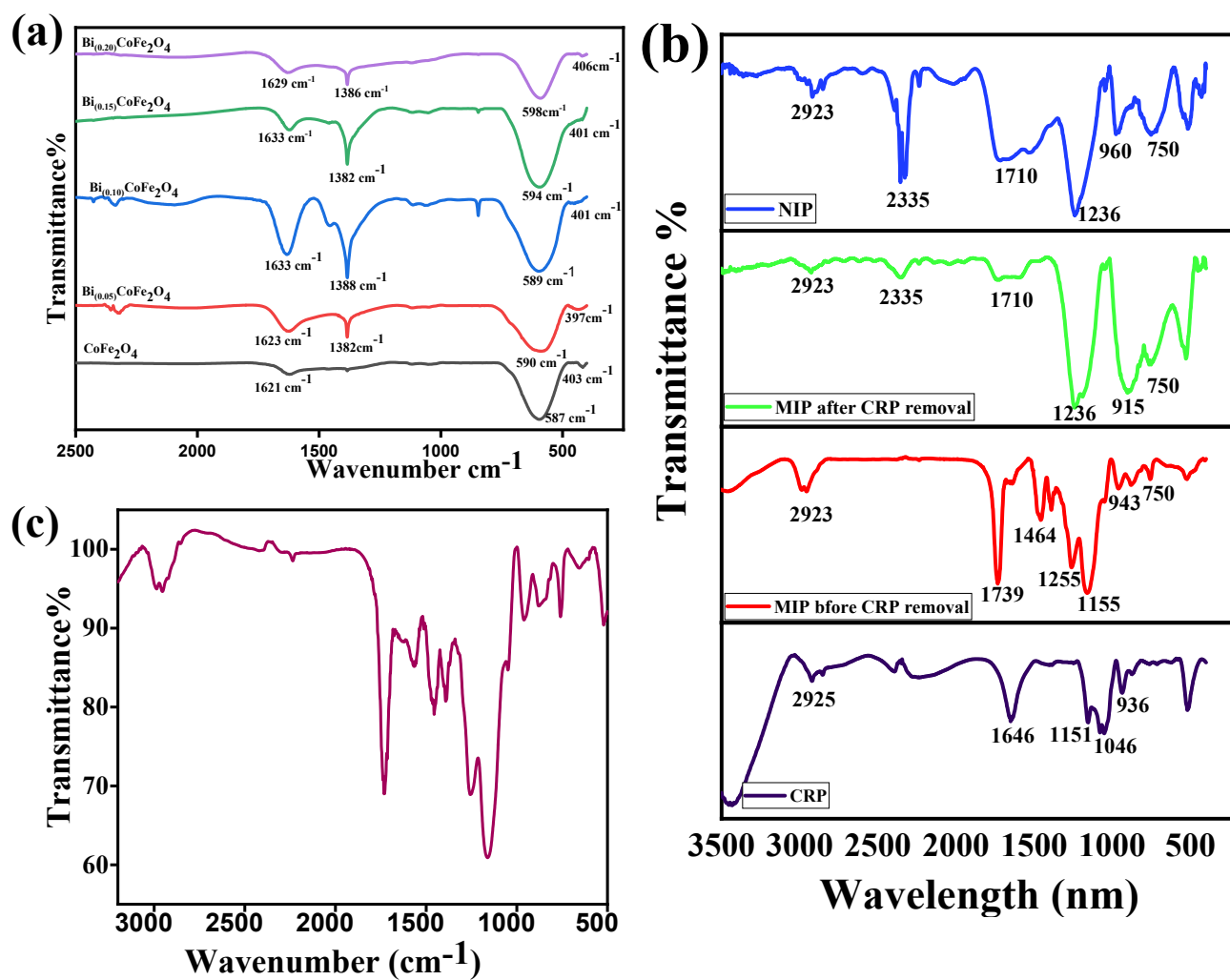
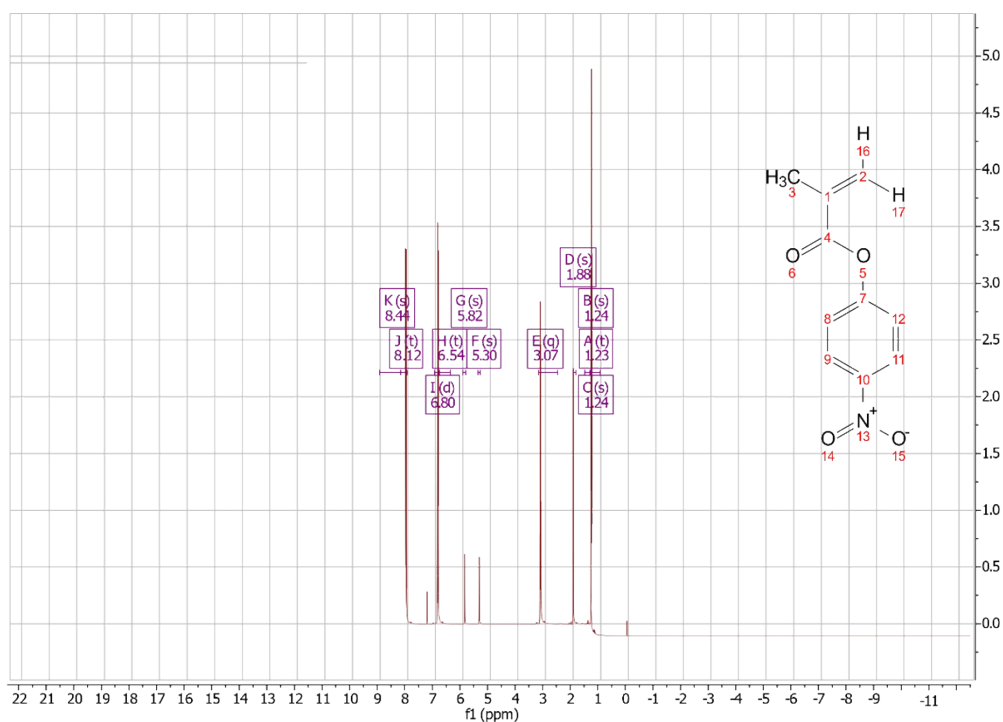


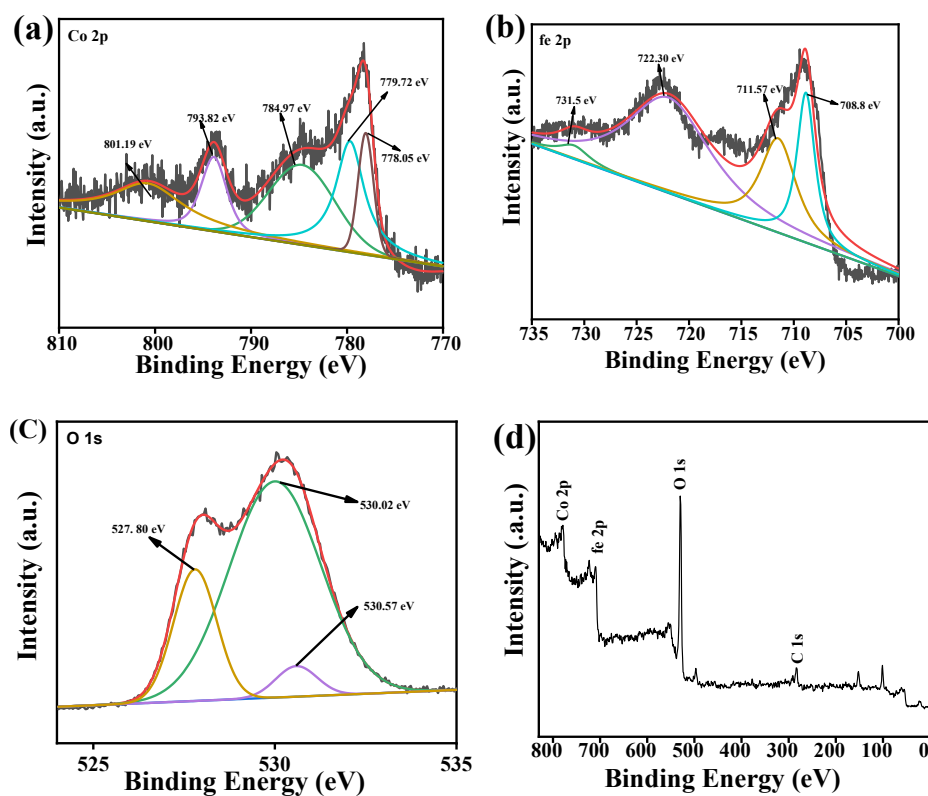
Figure S2: (a) shows XRD data of  $\text{Bi}_x\text{CoFe}_2\text{O}_4$ ,



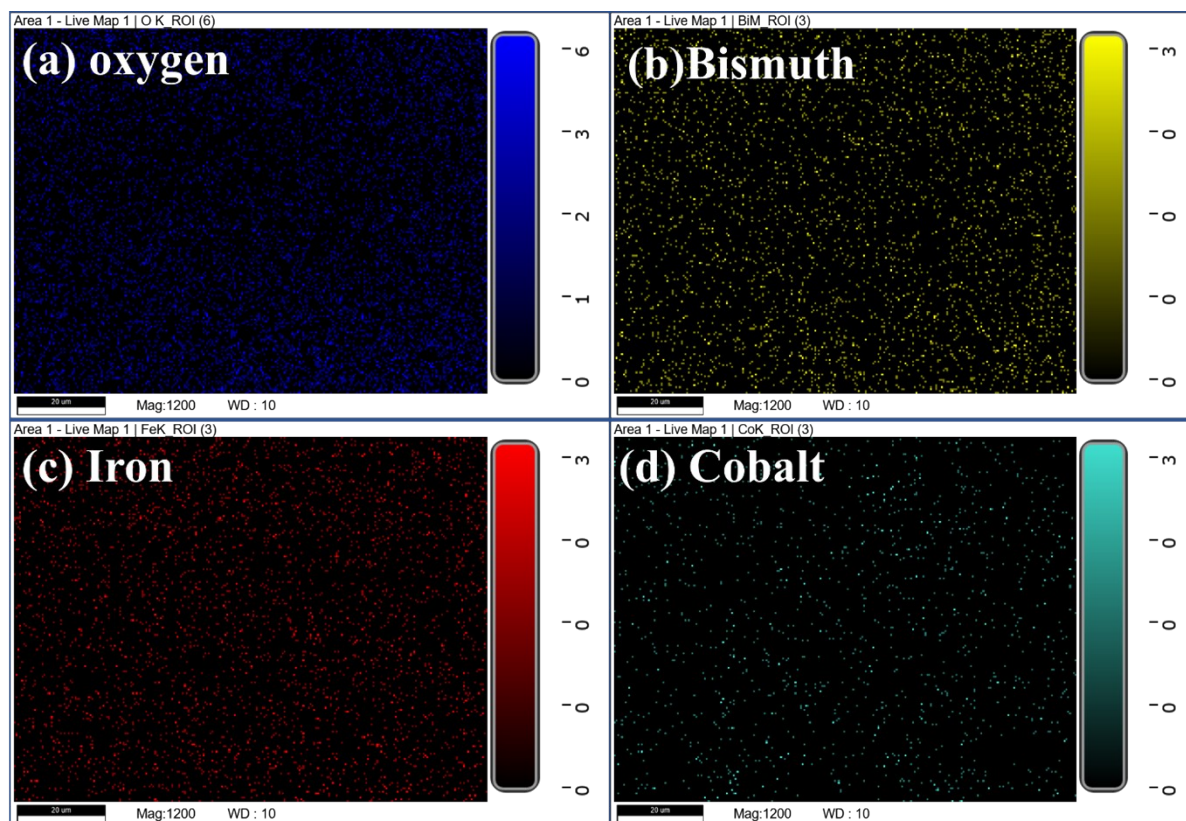
**Figure S3:** (a) Shows FTIR plot of  $\text{Bi}_x\text{CoFe}_2\text{O}_4$ , and (b) shows plot of NIP, MIP<sub>before</sub>, MIP<sub>after</sub> and CRP, and NIP (c) shows Monomer (4-nitrophenyl methacrylate)



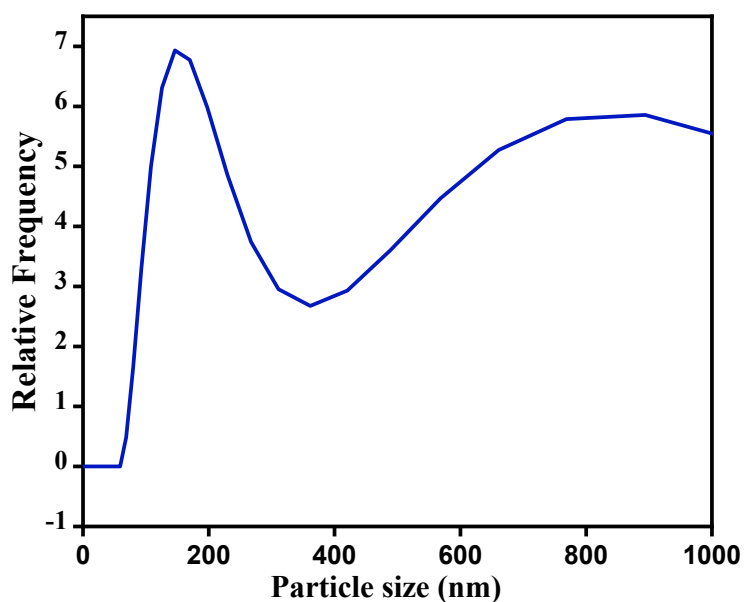
**Figure S4: NMR of Monomer (*4-nitrophenyl methacrylate*)**



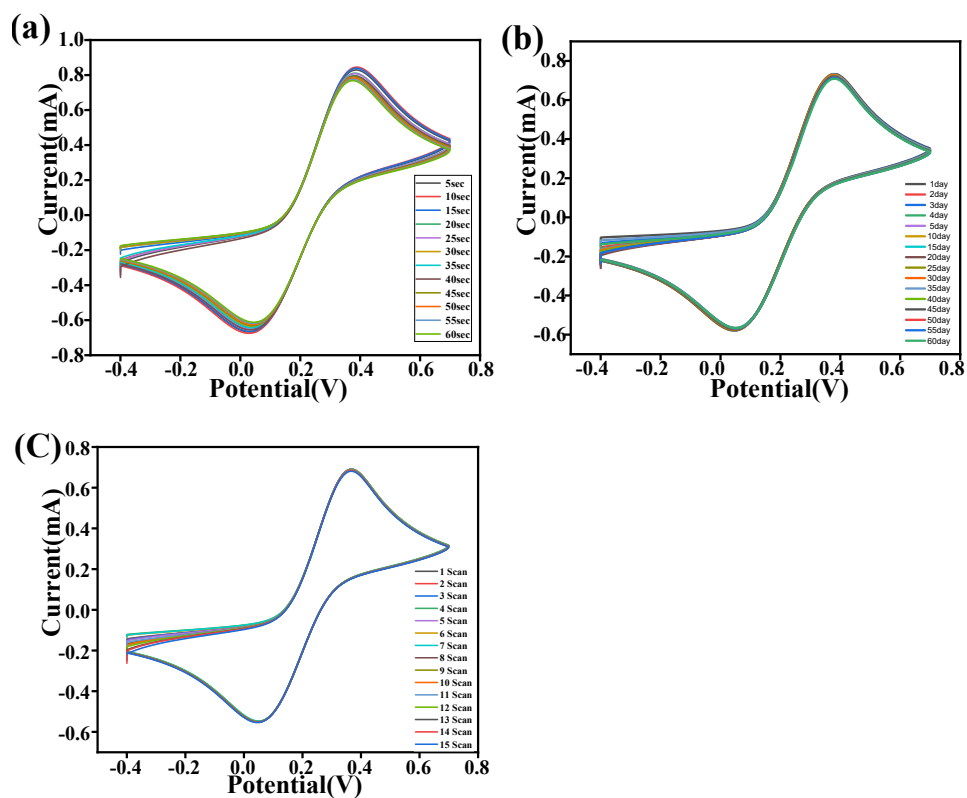
**Figure S5: XPS spectrum (a) Co 2p (b) Fe 2p (c) O1s (d) survey scan**



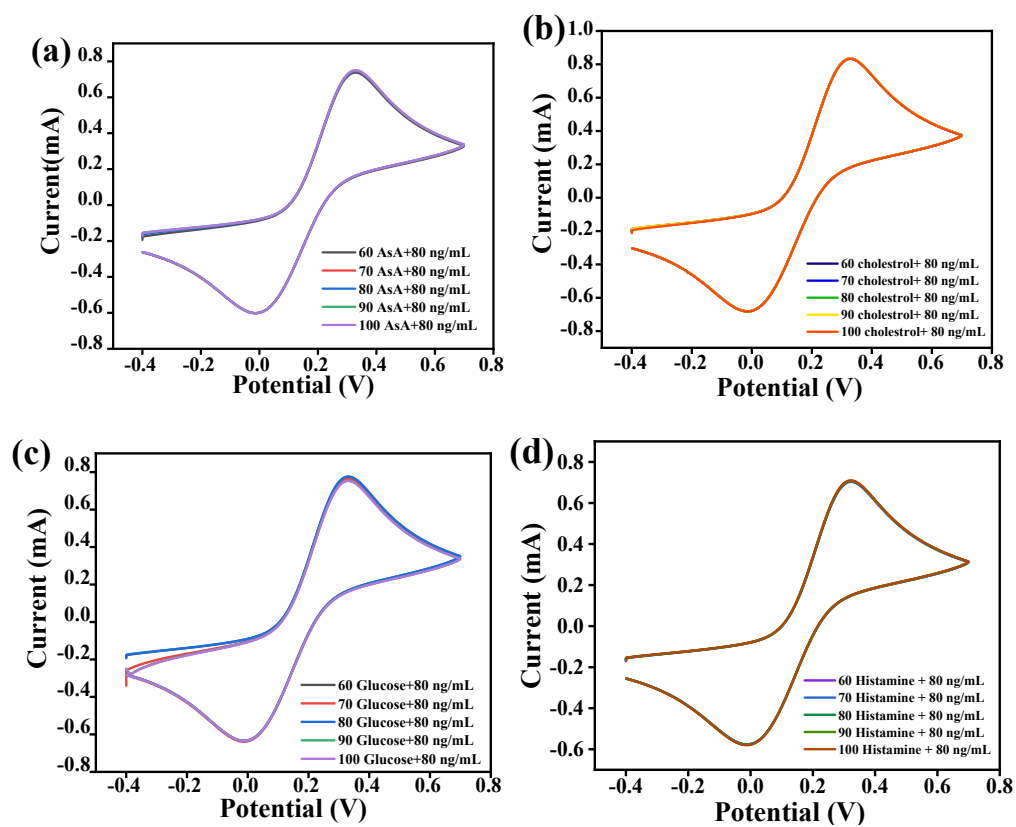
**Figure S6: Elemental mapping demonstrating the (a) presence of oxygen, (b) presence of Bismuth, (c) presence of iron, and (d) presence of cobalt**

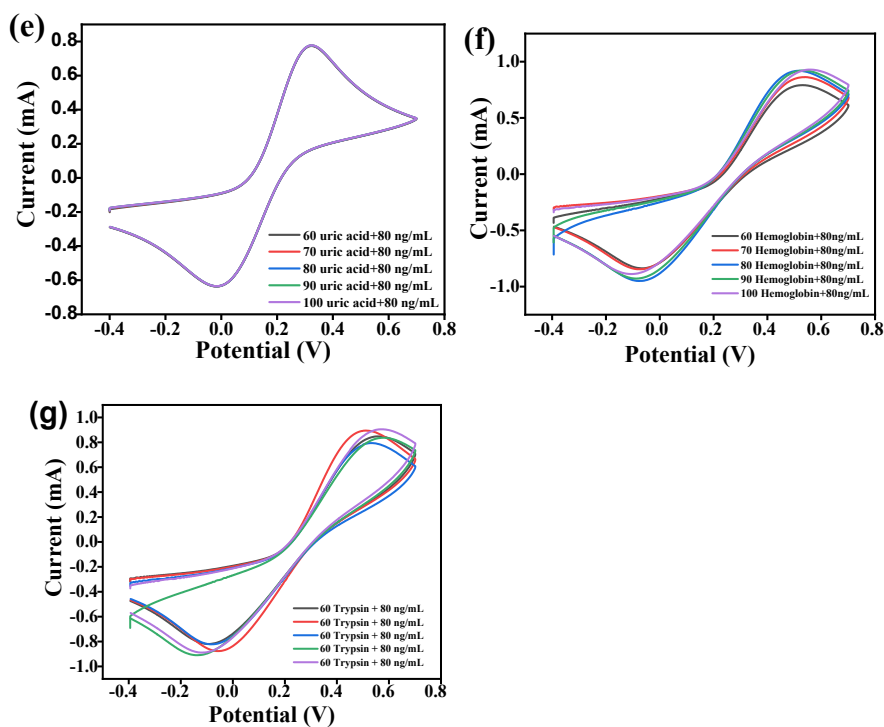


**Figure S7: shows the DLS plot of  $\text{Bi}_{0.15}\text{CoFe}_2\text{O}_4$**

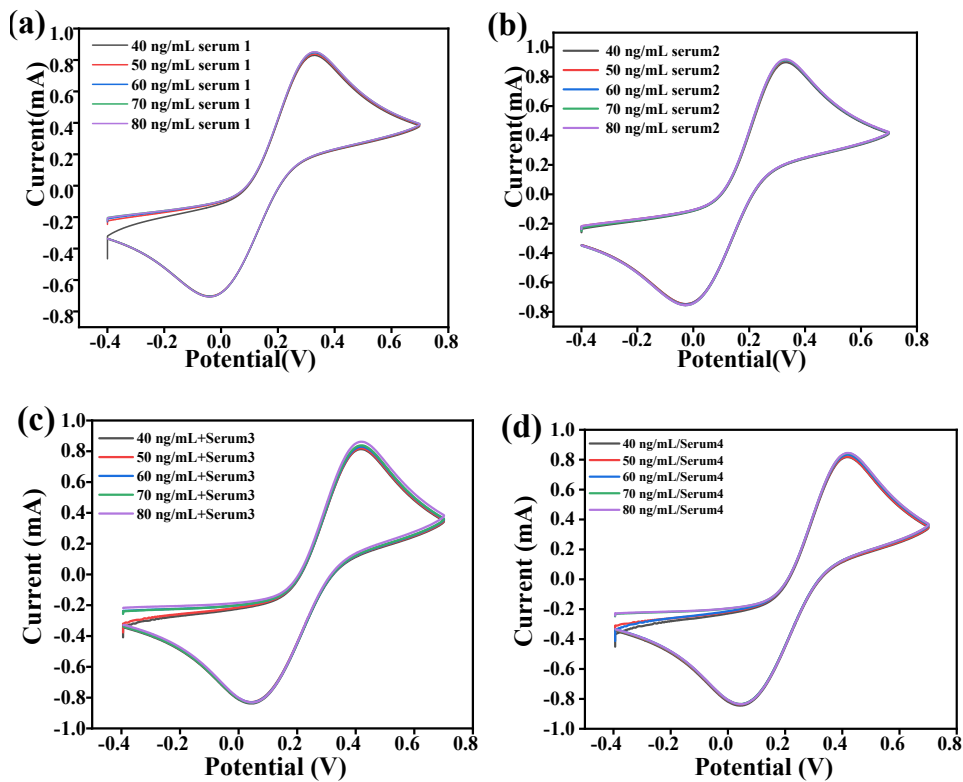


**Figure S8: CV plot (a) response timing, (b) stability, (c) reusability**



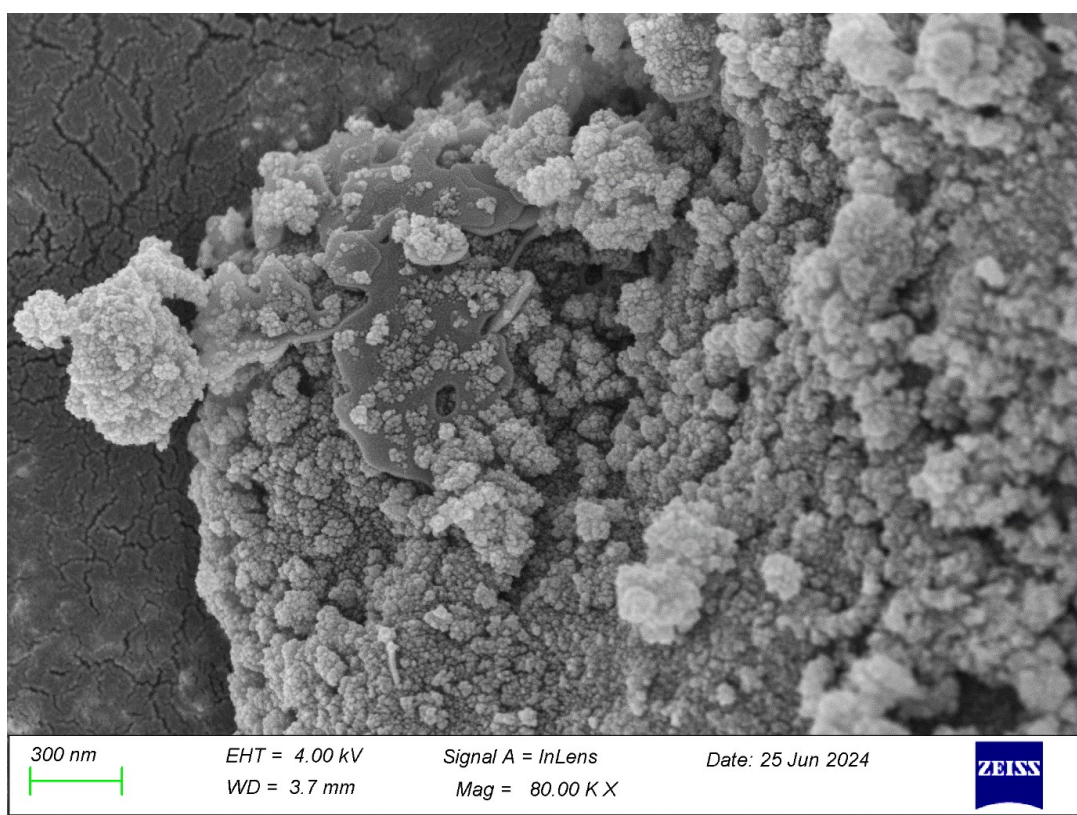
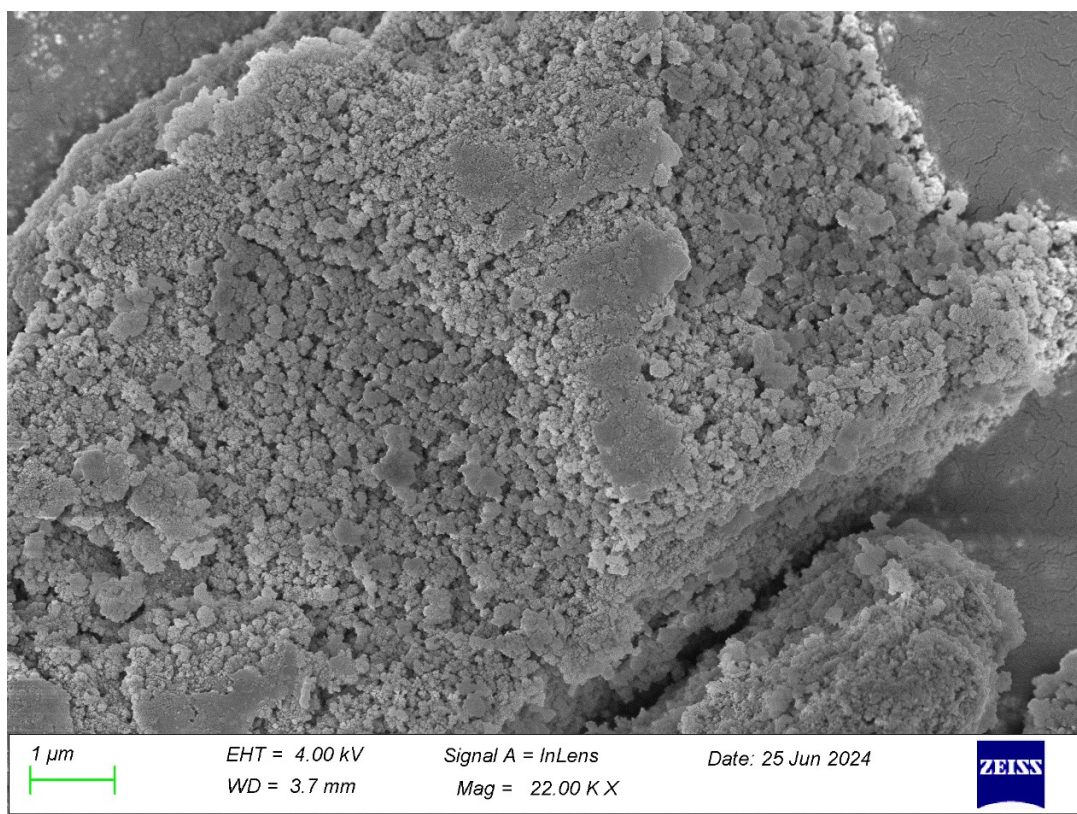


**Figure S9: Interference CV plot (a) Ascorbic acid (AsA), (b) cholesterol, (c) Glucose, (d) Histamine, (e) uric acid, (f) Hemoglobin, (g) Trypsin**



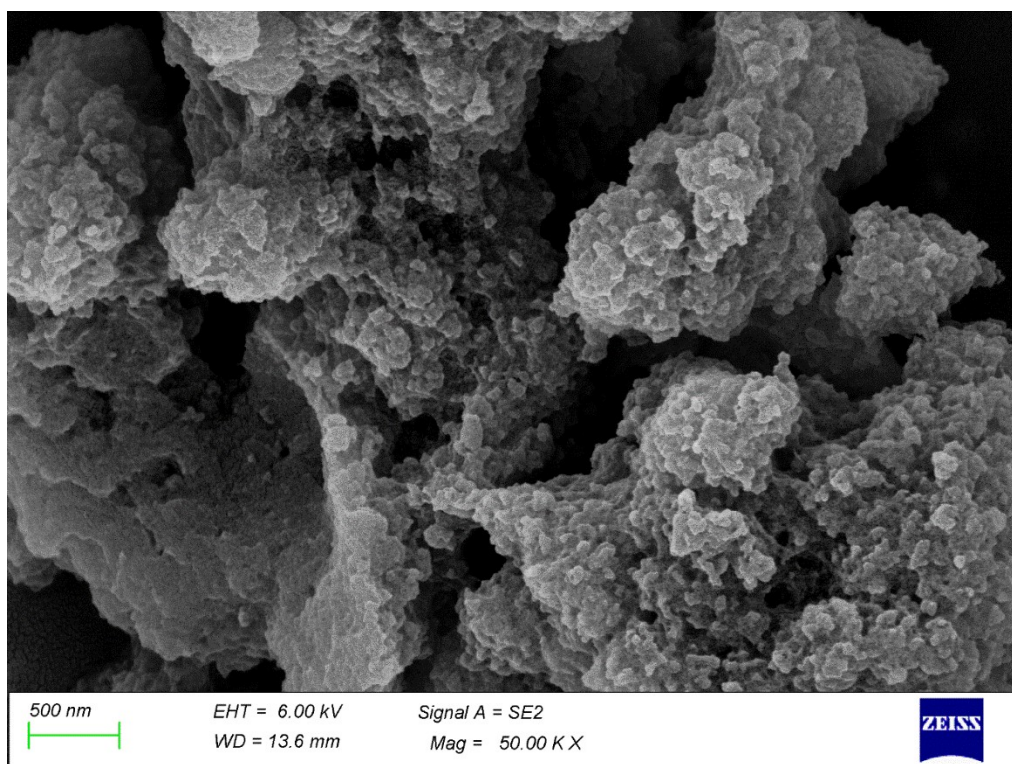
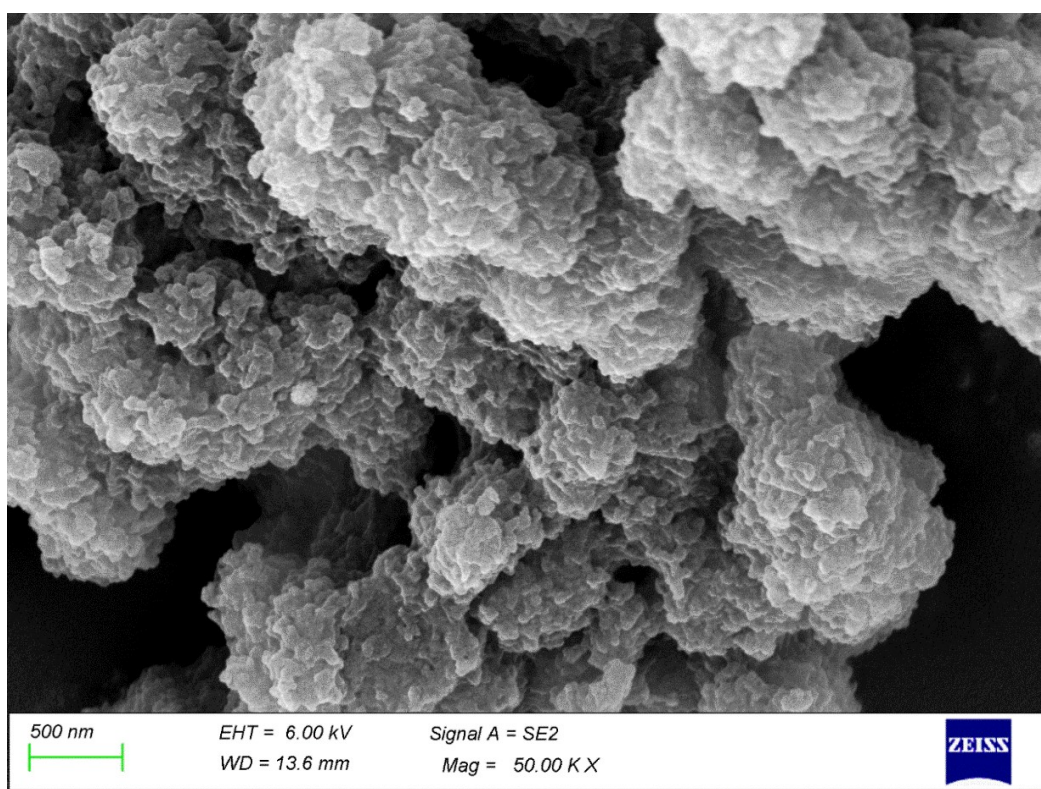
**Figure S10: Real sample CV plot (a) serum 1 (b) serum 2 (c) serum 3 (d) serum 4**



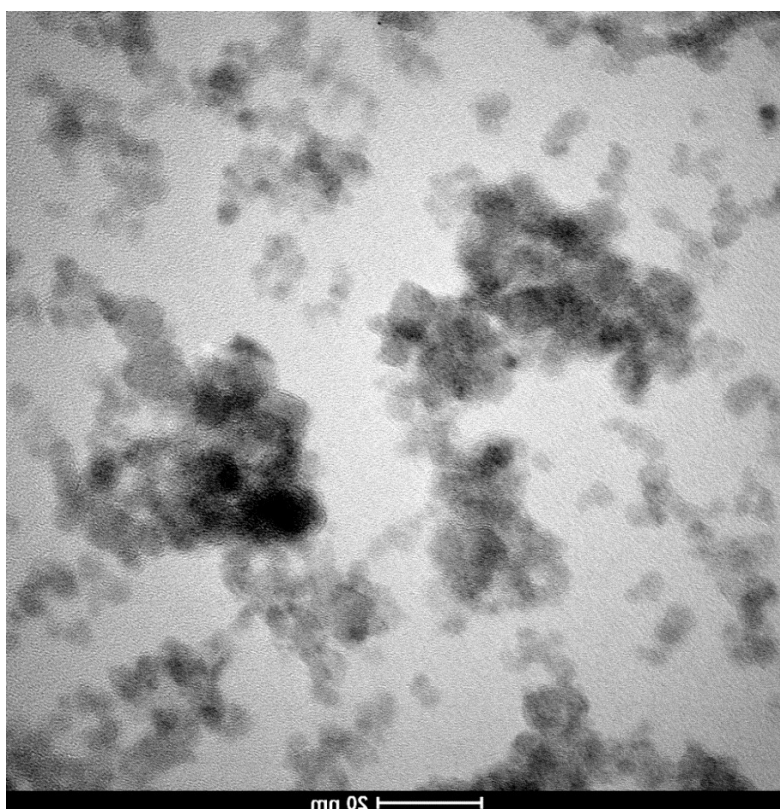
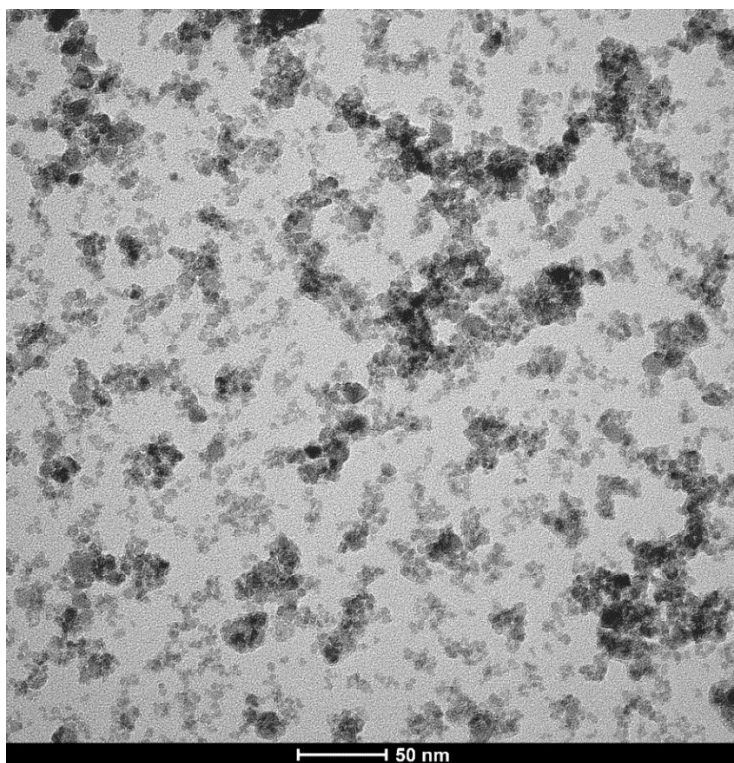


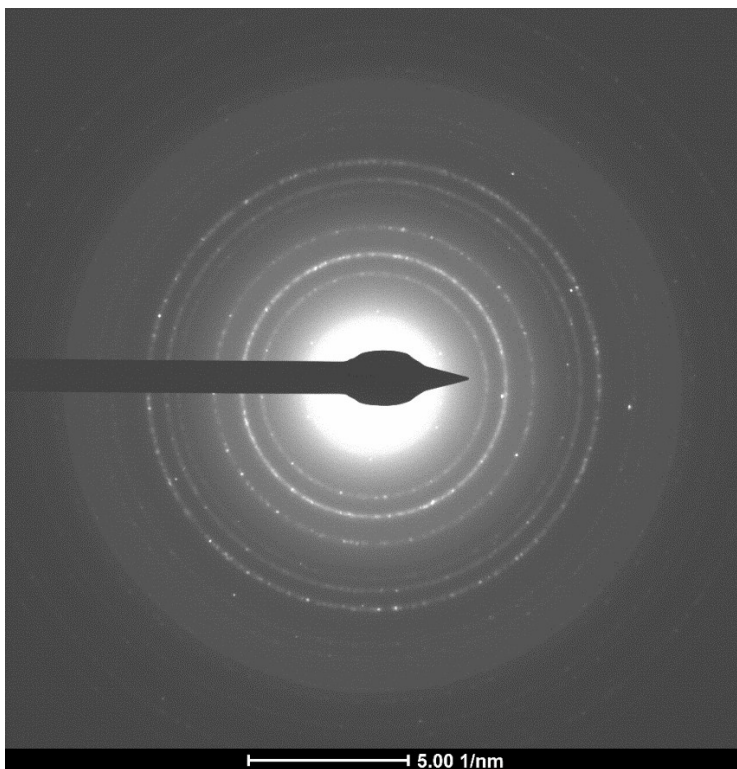
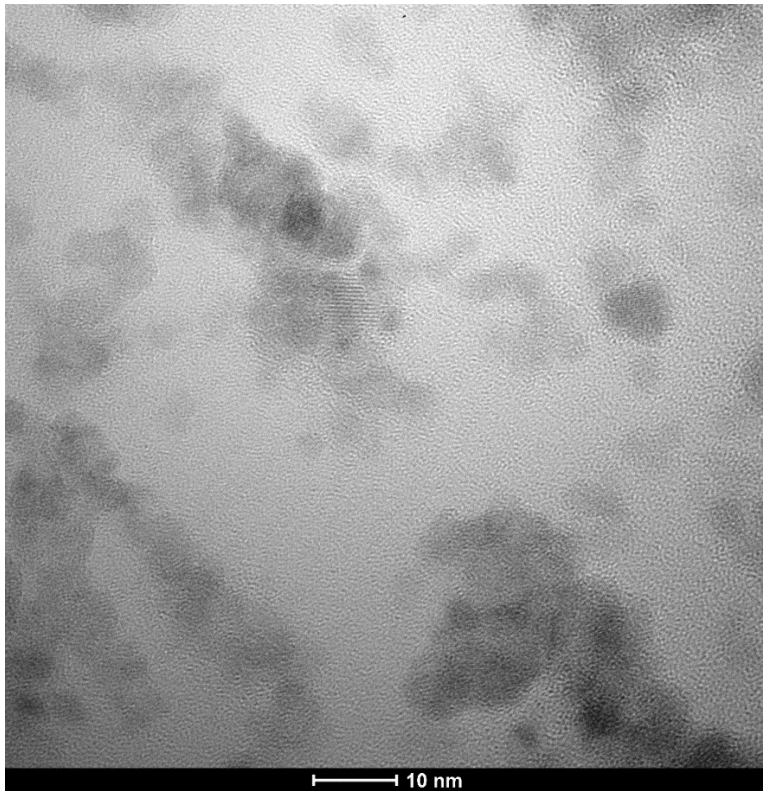
**Figure S11: FE-SEM original image of  $\text{Bi}_{0.15}\text{CoFe}_2\text{O}_4$  is used in main manuscript**



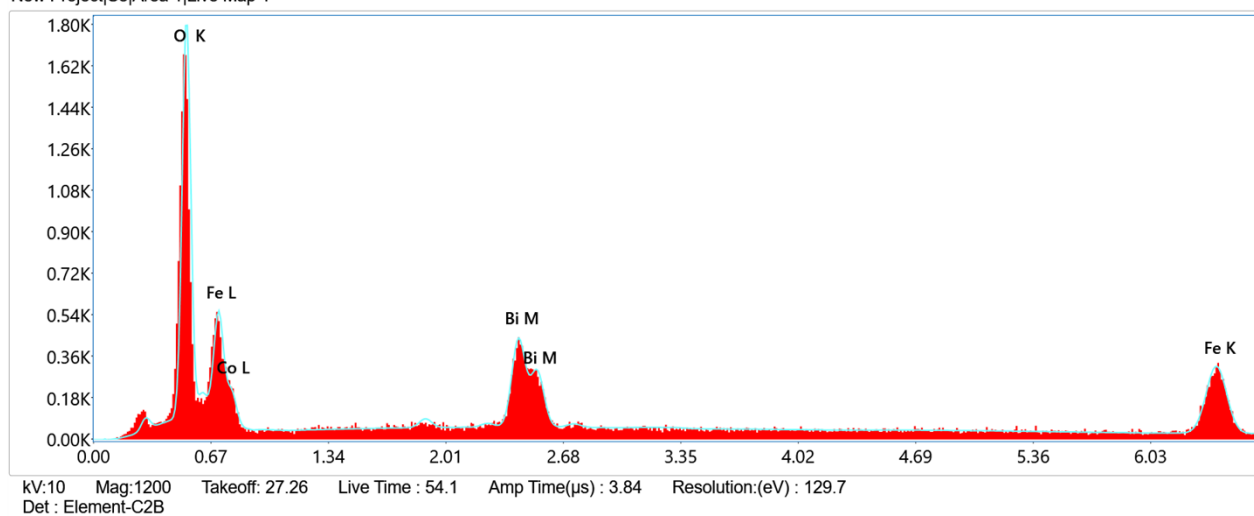


**Figure S12: FE-SEM original image (a) NIP (b) MIP is used in the main manuscript.**





New Project[S8]Area 1[Live Map 1



**Figure S13: HR-TEM original image of  $\text{Bi}_{(0.15)}\text{CoFe}_2\text{O}_4$  is used in the main manuscript**