

Design and Deployment of Electrochemical Sensors using Conductive Metal Organic Frameworks and their Nanohybrids



Thesis submitted in partial fulfilment

for the Award of Degree

Doctor of Philosophy

by

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Dedicated to my family

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It is certified that the work contained in the thesis titled “**Design and Deployment of Electrochemical Sensors using Conductive Metal Organic Frameworks and their Nanohybrids**” by **Shubhangi** has been carried out under my supervision and that this work has not been submitted elsewhere for a degree.

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Preface

Biosensors are crucial bioanalytical tools capable of detecting any analyte of interest within a sample solution. While the conventional diagnostic methods require certain demands such as trained personnel and advanced infrastructure, with the processes being time-consuming, they also have the biggest drawback of not being able to be accessed at remote locations. Electrochemical biosensors are emerging as newer alternatives to such conventional tools with the ability to function in remote settings, as point-of-care diagnostics as well. They are being continuously improved to detect clinical and environmental analytes with higher sensitivity and selectivity through incorporation of advanced materials and novel signal enhancement strategies. The surfaces of the sensors are functionalized with suitable biorecognition elements or the matrix components themselves are catalytic enough to detect the target analyte at suitable potentials. Functionalization with advanced nanomaterials often impart excellent catalytic properties to these sensing platforms along with increment in their surface area to many folds.

In this work, we have attempted to develop multiple sensing platforms with metal organic frameworks as essential matrix components, to detect a range of clinical and environmental analytes, both in ideal as well as real samples. They also offer potential for deployment for on-site detection of these analytes to ensure better health. In this thesis project, four different biosensors have been designed using metal organic frameworks and its composites to detect hydrogen peroxide, folic acid, nitrite, and uric acid. All materials and composites reported in the work have been thoroughly characterized through physical and electrochemical routes before their application in sensor development.

In the first work, creation of a nanohybrid platform has been attempted for rapid detection of hydrogen peroxide (H_2O_2), which has tremendous role in area of medical diagnostics. Conventionally, the peroxidase (POD) enzyme catalyses H_2O_2 however, it is

prone to inherent chemical and thermal instabilities reducing the overall stability and shelf life of sensor probe. A possible solution for this problem has been attempted in this work where a non-enzymatic peroxidase mimic nanohybrid probe comprising of gold nanodendrites (AuND), nickel metal organic framework (Ni-MOF), and hydrazine has been synergistically deployed for rapid detection of H_2O_2 . The developed sensor probe has been rigorously characterized through various physical as well as electrochemical characterization techniques. It demonstrated impressive analytical performance, with a linear dynamic range (LDR) between $1 \times 10^{-8} \text{ M}$ to $1 \times 10^{-15} \text{ M}$, with the limit of detection (LOD) being $0.34 (\pm 0.05) \times 10^{-15} \text{ M}$, making it suitable for trace analysis as well. The probe's average response time with changing H_2O_2 concentrations was 5.02 ± 0.42 seconds, making it an agile sensing platform for H_2O_2 detection. The nanohybrid probe displayed minimal response towards interferants such as superoxide radicals, ascorbic acid, cysteine, glucose, alanine, and citric acid, which usually co-exist in real sample matrix. In order to investigate the real-life applicability of the developed sensor probe, a real sample analysis involving synthetic serum was adopted which yielded a current recovery between 90.20 - 94.14%. The probe fabrication time and on-chip synthesis procedure are facile, making it a robust and efficient sensing platform for H_2O_2 free radical in clinical settings.

In the second work, an electrochemical sensor has been developed for folic acid (FA), an essential water-soluble vitamin and a precursor for enzymes which requires timely and precise monitoring in serum of individuals with varying clinical diagnosis. An attempt has been made in this direction through our work where, rapid detection of FA through its oxidation via. metal centres from hybrid nanomaterials, is deployed for signal generation. A non-enzymatic, non-immunometric approach involving a sandwich model comprising of NiMOF layered between gold nanoparticles (AuNPs) and gold nanodendrites (AuNDs) incorporated within a sensor matrix has been deployed for the purpose. The probe displayed

great analytical performance with a LDR between 1×10^{-3} M to 1×10^{-11} M, with the LOD being 0.48×10^{-11} M. The probe's average response time with respect to changes in FA concentration was recorded as less than 2.1 seconds, making it a rapid sensing platform for FA detection. The real-life applicability of the developed sensor was tested in serum, followed by a breast cancer cellular microenvironment-based analysis which yielded a current recovery between 95.11-98.17%. The cancer cell FA uptake was also validated through a live cell imaging based on the standard method of fluorescence. The less fabrication time of the developed sensor when compared to existing ones makes it a facile and efficient sensing platform for FA in clinical settings. This current study is the first report on conjunction of 1D, 2D and 3D materials as sensing matrix for molecular detection application.

In the next chapter, we have attempted the stacking of two MOFs, a Ni-MOF, followed by Co-MOF, which was further functionalized with gold nanoparticles to detect nitrite ions (NO_2^-) in real/environmental water samples. Nitrite is a naturally occurring plant nutrient present in the soil and is also used as a preservative in industries however, can generate carcinogenic precursors at higher concentrations. The catalytic sensing probe was developed to detect NO_2^- through its electrochemical oxidation to NO_3^- at a specific potential. The GCE/Ni-MOF/Co-MOF surface was deposited through a facile and faster approach of electrodeposition, with Co-MOF being developed through a novel electrodeposition route. The final sensing probe was thoroughly characterized by various physical and electrochemical techniques. It displayed worthy analytical performance in terms of its wide linear dynamic range between 1×10^{-3} M and 1×10^{-8} M and a limit of detection of 4.2 nM. It exhibited a rapid average response time of about <1.5 second, making it an ultrasensitive detection platform for NO_2^- in environmental water samples. A systematic integration with a

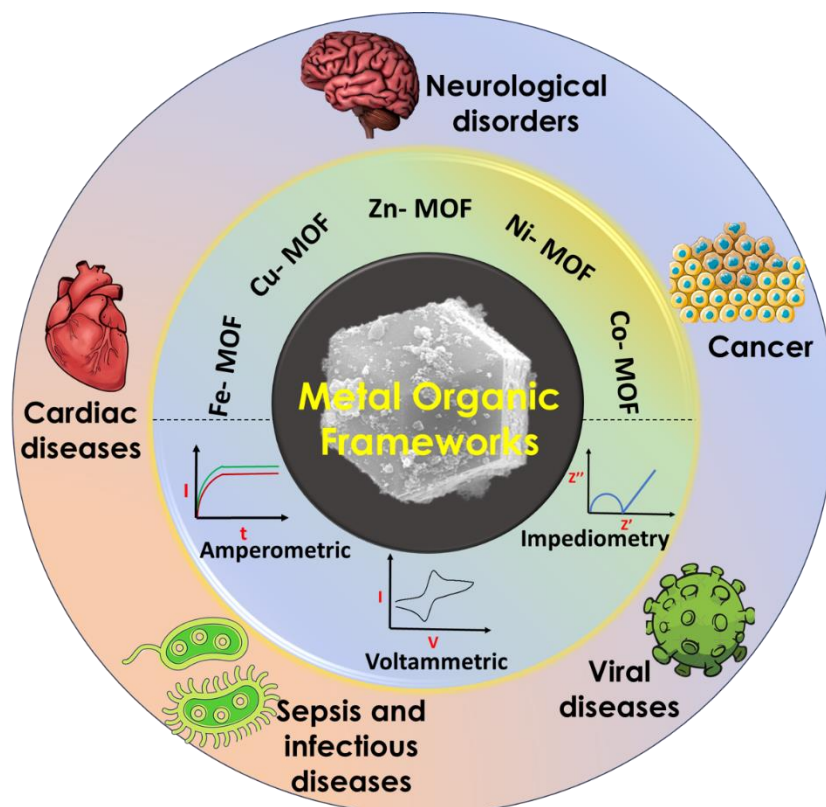
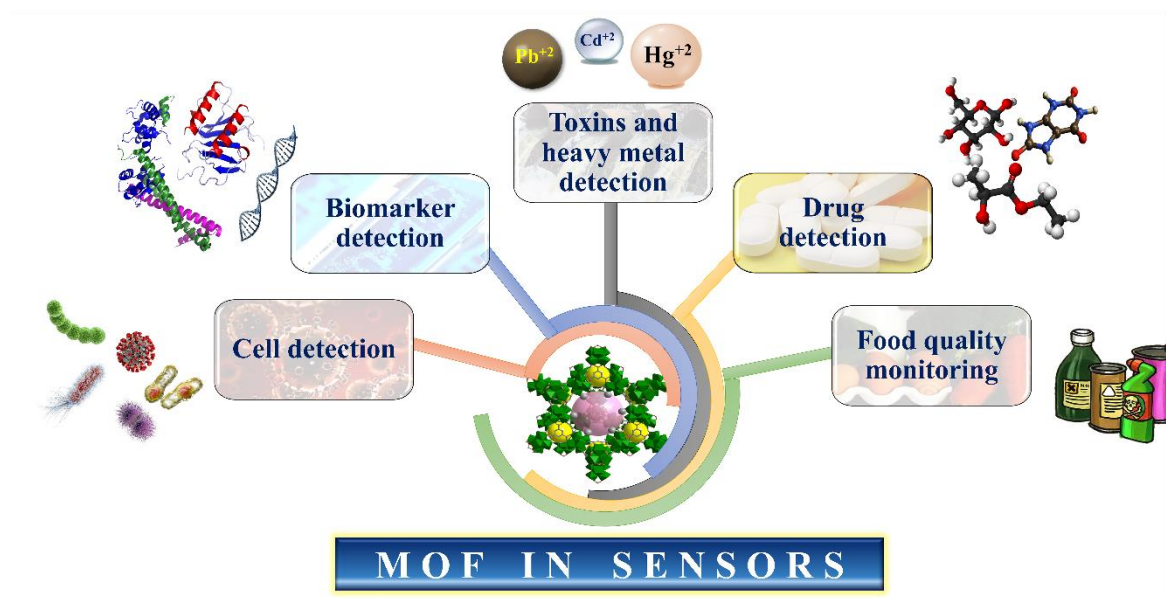
smartphone application has also been proposed for on-site application of the sensor where a single-fed input value determines the real-time NO_2^- concentration in test water samples.

In the next and final work, novel sensing platform comprising of cobalt/molybdenum (Co/Mo) bimetallic MOF has been attempted for the sensitive detection of uric acid (UA) in clinical ranges. Conventionally, UA is detected through an enzyme called uricase which converts UA to allantoin. However, the enzyme-based diagnostic solutions are prone to chemical and thermal instabilities making the process cumbersome and tedious. To eliminate such challenges, the developed non-enzymatic detection system uses a highly catalytic bimetallic sensing module which electrochemically facilitates conversion of UA to allantoin, and yields electrochemical signal outcomes. The developed sensing probe was characterized through physical and electrochemical techniques. UA has been used as the sample target analyte to validate the efficacy of the bimetallic system using differential pulse voltammetry (DPV) and chronoamperometry (CA). The preliminary results hold immense promise for the developed system in application for sensing a wide range of analytes through electrochemical methods in clinical settings.

In the sixth and final chapter of the thesis, we have identified the possible challenges in development of such MOF-based sensing platforms to project better research opportunities for the upcoming researchers. The work is aimed towards the fabrication sensitive platforms exploring the potential of novel synthesis methods for MOF synthesis along with its deployment in the sensor matrix development. The work can be further extended to development of hand-held point-of care or miniaturized systems which can be used for on-site diagnostics with little or no technical training requirements.

Chapter I

Introduction



1. General Introduction

Despite all of the medical advancements and sophisticated methods, universal healthcare is becoming a significant issue among all of the problems we face today. As a result, a healthy life for all people, regardless of age, is emphasized as a crucial component of sustainable development in the United Nations General Assembly's developmental plan, "Transforming our world: the 2030 agenda for sustainable development." On the other hand, disease outbreaks are getting more frequent in our globe. Because of a shift in lifestyle, a significant number of new cases of metabolic illnesses, such as obesity, diabetes, and even cancer, are now being found in younger populations. Despite the rapid development of improved drugs and cures, the number of new cases of cancer and the deaths associated with it are increasing annually. In the modern world, drug overdose and drug resistance-related illnesses are becoming serious issues. By identifying these cases as soon as feasible, the significant impact of such problems might be lessened. The diagnostic tools that identify these illnesses can also be used to document health problems for future monitoring, which is necessary for medication regimens.

A number of tools have been created to address these health concerns and are frequently employed in clinical sample analysis. Hospitals perform these biochemical tests using devices such as spectrophotometers, chromatography, and colorimetry. All of these tools search for biomarkers, which are molecules of biological or chemical origin linked to a specific condition. The concentration of these compounds changes beyond the clinical range in sick conditions. Therefore, the creation of POC devices can make advantage of these biomarkers. Despite their great strength, these devices have several drawbacks. They require a costly setup with skilled staff, a lengthy procedure, and a high sample volume. These restrictions impede the devices' portability, on-site detection, and speedy diagnosis.

To avoid such limitations in diagnosis, miniaturized devices have been developed to detect the biomarkers. Miniaturization of the devices would make them robust, reliable under emergency cases, and can be carried to remote locations easily.

1.1. Introduction to Biosensors

According to the International Union of Pure and Applied Chemistry, biosensor can be defined as “a device that uses specific biochemical reactions mediated by isolated enzymes, immune-systems, tissues, organelles or whole cells to detect chemical compounds usually by electrical, thermal, or optical signals”.

A biosensor is a bio-analytical instrument that uses a variety of chemical or biological molecules with certain characteristics found in the sensing matrix to identify an analyte. The three main components of a biosensor are a processor, a transduction system, and biorecognition. Enzymes, antibodies, aptamers, entire cells, and other chemical or biological components that can interact with the analyte selectively are examples of biorecognition elements. Together with the biorecognition element, the transducer transforms this biorecognition event into a quantifiable signal. The transducer transforms the signal into various quantifiable forms after detecting changes in pH, temperature, charge, mass, etc.

An amplifier in the biosensor's following section reduces signal background noise and boosts weak signals so the analyte may be measured. After analyzing the data, the processor shows it on the digital display so that it can be further processed. New signal amplification techniques are integrated into the biosensor to attain better analytical results as a result of technological advancements.

Since its invention in 1956, biosensors have experienced remarkable growth and improvement. Clark created the first oxygen electrode sensor, which served as the basis for further studies in this field. This work served as the foundation for Yellow Spring Instruments' 1975 development of the YSI Model 23 Analyzer, the first commercially available glucose biosensor. In the

ensuing decades, a plethora of novel commercial sensors, including as subcutaneous implantable in vivo glucose monitoring electrodes and self-testing blood glucose strips, inundated the market based on the biosensor's premise in order to detect glucose and other clinically significant molecules.

1.2. Classification of Biosensors

1.2.1. Biosensors can be classified based on the **application of external labels**, i.e. agents to facilitate the analytical performances of the sensors.

A. Label-free biosensors

Label-free biosensors are those that can detect the analyte without the use of an external label. No external label is required for sensing activity if the analyte is a redox-active molecule or if the biosensing matrix is made especially to interact with or catalyze the analyte. The majority of label-free biosensors are derived from potentiometry or impedimetric biosensors. Based on the shift in the resonant frequency of the matrix components, Maramatshu et al. created an unlabeled biosensor for the detection of immunoglobulin IgG. The sensor could distinguish between IgA and IgG. Using a label-free biosensor has the benefit of directly sensing the analyte, without the need for an immobilization step using a secondary antibody or tagged molecule.

B. Labeled biosensors

Biosensors are referred to as labeled biosensors when they use external labels to detect the analyte. The twofold confirmation of an analyte's existence is a benefit of utilizing a labeled tag. Additionally, a labeled tag (such as a fluorescence tag) can identify the presence of a single molecule in a sample, something that unlabeled biosensors find difficult to accomplish. The most widely used labels are fluorescently tagged DNA probes and different enzymes, such as horseradish peroxidase and alkaline phosphatase (ALP). Pallela et al. employed AuNP-polymer-antibody to capture the cell in order to create a labeled biosensor for the detection of

metastatic cancer cells. For dual confirmation of the presence of cells, a secondary hydrazine-AuNP-Ab2 tag was employed, in which the analytical signal was produced by the catalysis of H_2O_2 by hydrazine. The disadvantages of using a tagged molecule lies in their sensitivity towards various environmental conditions (e.g. fluorescent tags are sensitive to incident light and interference from commonly coexisting molecules).

1.2.2. Sensors can also be classified on the basis of the **type of transducer used**, as discussed further:

A. Optical Biosensors

An optical biosensor is a type of biosensor that detects changes in the target analyte's optical characteristics. UV-Vis. adsorption and reflectance, fluorescence, phosphorescence, luminescence, surface plasmon resonance, internal reflection, and other photometric characteristics are frequently employed in optical biosensors. When physical or chemical changes occur in the analyte sample, an optical biosensor detects the change in the altered phase, polarization, or frequency of the input light. There are two primary types of optical biosensors: one that detects variations in the biomolecules' inherent optical characteristics, frequently as a result of interaction with a particular target molecule. This class is inexpensive and follows easy steps because it doesn't require the usage of any labels or optical probes.

SPR-based biosensors, interferometer-based biosensors (such as Mach-Zehnder, Young's, Hartman, and backscattering interferometers), optical waveguide-based biosensors, optical-resonator-based biosensors, optical-fiber-based biosensors, photonic crystal biosensors, etc. comprise the majority of non-labeled optical biosensors. In this, a fluorescent tag is affixed to the molecule of interest in the second class of labeled biosensors. The additional process of identifying the molecules in the class is the major drawback, though. However, a single analyte molecule in the sample, such as the circulating tumor cells, may be detectable by label-based biosensors due to their extremely high sensitivity. On the basis of optical measurements of

analytes, new affordable wearable technology for ongoing health monitoring are being developed in recent times.

B. Mechanical/ Piezoelectric biosensors

Another class of biosensors are mechanical/piezoelectric biosensors, which can sense the analyte by measuring the change in its mechano-physical properties like pressure, acceleration, temperature, strain, or force applied. Piezoelectric materials like bernalite, quartz, Rochelle salt, etc. vibrate under the influence of an electric field depending on the thickness and nature of the material. Each piezoelectric material vibrates with a certain resonating frequency, which is measured by the biosensor. The change in the resonating frequency is proportional to the mass added/removed from the sensing surface, which can be calculated using the Sauerbrey equation. The piezoelectric sensors can reach upto a resolution of 1 μg . Quartz crystal microbalance (QCM) is the most widely used mechanical biosensor capable of sensing an analyte in solid, liquid and gaseous phases. The biggest advantage that they offer is that the analyte can be sensed directly, without using any label. However, environmental factors like humidity can affect the sensing activity of these biosensors. Unlike others, the biggest advantage that they offer is excellent mass resolutions (nanoelectromechanical system have achieved a zeptogram resolution), label-free detection, ease to study the interaction between a biological entity and small molecules, and capability to fabricate small microfluidics devices.

C. Electrochemical biosensors

The ‘Physical Chemistry and Analytical Chemistry’ division of the International Union of Pure and Applied Chemistry (IUPAC), defined the electrochemical biosensors as follows: “an electrochemical biosensor is a self-contained integrated device, which is capable of providing specific quantitative or semi-quantitative analytical information using a biological recognition element (biochemical receptor) which is retained in direct spatial contact with an electrochemical transduction element.

The biorecognition event in electrochemical biosensors was transformed into a detectable signal in the form of a measurable current, potential, or change in conductivity. Because of their affordability, ease of manufacture, and potential for downsizing, electrochemical biosensors are the most widely utilized biosensors among the aforementioned categories. Electrochemical biosensors can function in a very tiny sample volume, in contrast to optical and mechanical biosensors. Very LOD values are the main benefit of employing them, though. Additionally, they are not affected by fluorophores, chromophores, or other particles, which is typically the case with optical ones. They may therefore be more effective in identifying bacteria, cancerous cells, entire cells, etc.

Electrochemical biosensors are classified into three major categories: (i) Amperometry, that measures variations in current; (ii) Potentiometry, that measures a change in charge accumulation and potential; and (iii) Conductometry or impedimetric that measures the change in conducting behaviors of sensing sample.

- (I) **Amperometry:** This kind of electrochemical biosensors function by measuring the current when a voltage is applied across the electrodes. The electrodes are held at a constant potential, and the changes in the current are measured as a function of the analyte concentration present in it. When a constant potential is applied, the current in the three-electrode system can be explained using the Butler-Volmer equation.
- (II) **Potentiometry:** In such biosensors, the accumulated charge potential at the working electrode is measured in comparison to the reference electrode, when no current flows between them. This kind of system gives information about the charged molecules present in the solution, where the concentration of the analyte and the potential is governed by the Nernst equation.
- (III) **Conductometry/ Impedimetric:** Unlike the above-mentioned techniques, the impedimetric biosensors do not measure the charged ion concentration, but measure the

impedance of the cell across the working and counter electrode. Impedimetric biosensor does not require any detection tag, hence it is generally used for label-free detection of an analyte. This is also used to study protein or another biomolecule immobilization over the electrode surface. This can also be used for miniaturization of detection devices and can be integrated for chip development.

1.2. Role of nanomaterials in the development of biosensors

Because of their distinct geometric and electrical characteristics, nanomaterials improve a biosensor's analytical capabilities. They behave differently than the individual atoms that make them up, and they also alter their properties when they are present in close proximity to another complementary substance to form a composite. These materials are very promising for developing biosensors with increased efficiency because of their altered geometric and electrical properties. A bigger catalytic surface is produced by a higher surface to volume ratio, and this is used to reduce the size of the detection equipment without sacrificing sensitivity. The creation of point-of-care detection modules can greatly benefit from this trait, which does away with the necessity for large devices and the drawbacks that come with them. The following outcomes are the consequence of functionalizing the sensing surface with nanomaterials: Larger active surface area resulting into better sensitivity.

- a) An increase in the conductivity results in a fast transfer of electrons between the electrode and the reaction center.
- b) An increase in the catalytic activity results in the oxidation/reduction of analyte at a lower potential, which helps distinguish structurally similar molecules in sensing.
- c) Surface modification results in limited interaction of interfering molecules with the electrode and increases the selectivity.
- d) A large surface area also leads to fast diffusion and pre-concentration of target molecule onto the electrode surface.

Different class of nanomaterials can be explored for biosensor development based on their surface, chemical, and electronic properties. In our work, we have focused primarily on a specific class of materials, which are MOFs (Metal organic framework).

2. Introduction to MOFs

MOFs are a diverse class of multifunctional, crystalline and porous materials with their evolution dating back to late 1990s¹. These porous materials often referred to as ‘coordination polymers’, are formed through coordination of organic linker molecules with metal ions by their self-assembly^{2,3}. They find their applications in a plethora of fields ranging from gas storage⁴⁻⁷, gas separation⁸, catalysis⁹⁻¹² to drug delivery, theranostics and sensing¹³⁻¹⁷. For example, Cu-based MOFs are often used as nanozymes like proteases, peroxidases and laccases¹⁸⁻²⁰. The applications of these MOFs are supported by their commendable properties such as uniform and tunable pore sizes, large accessible surface area, high thermal and chemical stabilities¹. Some MOFs have intrinsic properties like low toxicity, inherent stability and good biocompatibility such as Zr-based MOFs²¹. However, for expansion and enrichment of their properties, further, these MOFs are often decorated with functional species to form composite materials with synergistic or improved features²²⁻²⁴. For example, Wen et al. in their recent work improved the electron-transfer efficiency of ZIF-8 by conjugating it with graphene for fabrication of a pesticide biosensor matrix²⁵. Similarly, in a work by Lai et al.²⁶, a porphyrin-based MOF was doped with Mn^{2+} ions to selectively detect glutathione. In another study by Kulandaivel et al.²⁷, copper-based MOF nanozymes after being doped with Cu^{2+} ions, were found to be catalytic with oxidoreductase activity which helped in enhanced colorimetric paper-based detection of Cr^{6+} . Apart from doping, the key to MOF’s reticular design lies with binding of metal nodes to different linker molecules yielding MOF structures of varying pore sizes and morphology. There is liberty for precise control of preferred topology by change in the molecular building blocks²⁸. The guest accessible voids can be exploited as per needs for the

development of optical, electromechanical, electrochemical, chemicapacitor sensors, and sensor arrays. For sensing applications, the target moiety's counter molecule can be either encapsulated or adsorbed on the MOF surface making its physical characteristics of significant interest. In addition to these intrinsic properties of MOFs, they are also engineered for conjugation of various receptors to develop a sensing interface for molecular analysis^{29–31}.

With all its applications gaining eminence widely, its biosensing based-applications are currently trending in the research arena^{32,33}. Biosensors of various types depending upon the type of inducers, ranging from optical^{34,35}, electrochemical^{36,37}, photo-electrochemical^{38,39} and mechanical⁴⁰, have found their utility in this field. MOFs can either be directly used as biosensing platforms⁴¹ or can be modified/functionalized with different ions⁴², nanoparticles^{43,44}, polymers⁴⁵, composites⁴⁶, or as combinations of these⁴⁷ to be used for effective fabrication of ultrasensitive detection systems. The use of MOFs involving multiple metal ions is on rise due to the synergistic effect of such systems leading to better analytical abilities^{47,48}. For instance, Du et al.⁴⁹ synthesized bimetallic CuCo-MOF (ingrown on Ni-foam), with adjustment of metal ions in a specific ratio to develop a non-enzymatic electrochemical glucose biosensor. The sensor was capable of detecting glucose within a limit of detection (LOD) of about 0.023 mM without the use of any enzymes along with an improved electron transfer capability due to metal ion synergies. These types of work would pave path towards the development of MOF-based biosensors having diverse functionalities in coming years.

Based on a scientific survey conducted by us on the online database “Scopus”, we discovered that there were no recent review articles highlighting MOF-based detection of different categories of molecules of clinical importance, as shown in **Figure 1.1**. There are some recently published articles which are really informative but are stringent in their content with a limited scope of study^{50–54}. There still remains a lacuna in holistic understanding of these

coordination compounds from their synthesis methods to their applications in electrochemical and optical biosensors which we have tried to address through this article. The first segment of the article discusses about properties and various synthetic approaches that are usually adopted in MOF fabrication. Then, the further segments highlight about free radicals, ions, small molecules, macromolecules and *in vitro* detection assays that have been explored in the recent times using these materials as sensor probe. Illustrative schemes and tables have been incorporated in order to give maximal overview of the sensing mechanism, fabrication parameters, dynamic range, LOD and real sample considered in those studies. Future prospects and limitations have also been discussed to promote further research in this field.

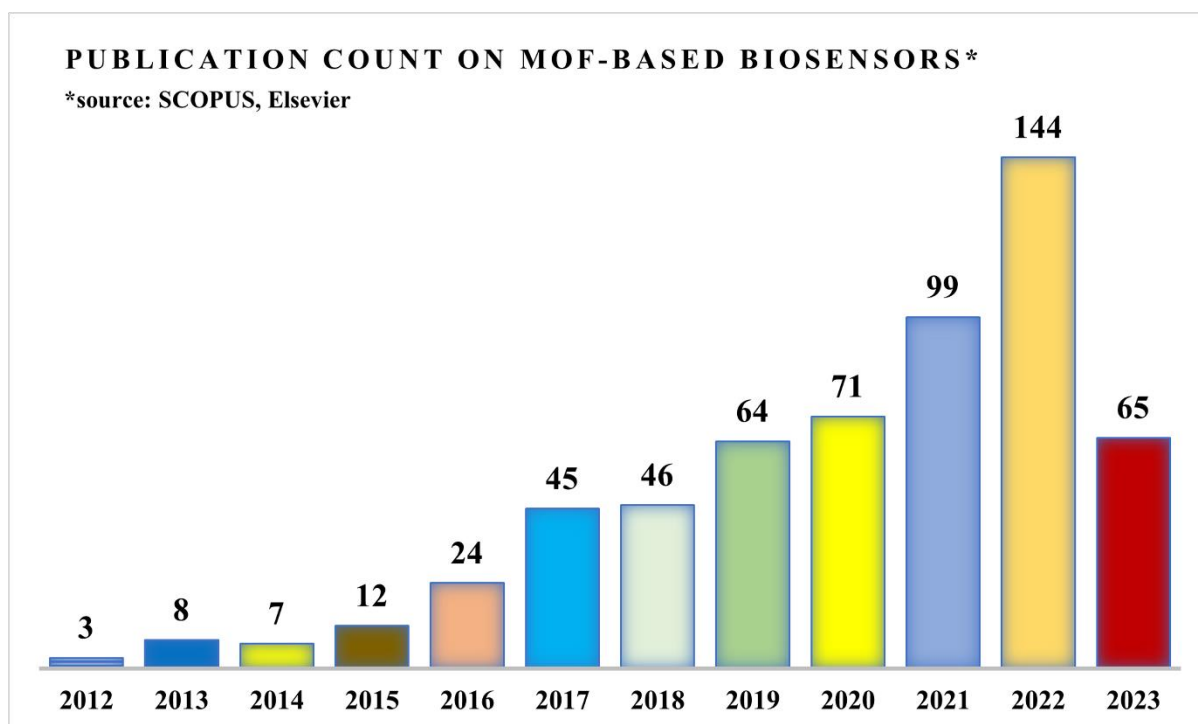


Figure 1.1. A graphical demonstration giving an outlook of recently published research articles (in the last decade) on MOF-Based Biosensors, as per the online database “Scopus” using keywords “metal organic frameworks biosensors”, till June 2023.

2.1. Properties of MOFs

MOFs possess a plethora of advantageous properties (mechanical, catalytic, optical and electronic) owing to their porous structure and crystalline nature. Amongst the most common

MOFs, zeolitic imidazolate frameworks (ZIFs) possess a distinct resemblance with zeolites with M-Im-M instead of the Si-O-Si angles present in the latter⁵⁵. Some of the properties supporting role of MOFs in biosensing matrices have been discussed in the section below.

2.1.1. Mechanical properties

While discussing about the mechanical properties of ZIFs, the initial Diamond Anvil Cell diffraction experiments to understand the behaviour of ZIF-8 at high temperature and pressure was done by Cheetam et al.⁵⁶ They found that with an increase in solvent accessible volume, the hardness and elastic modulus of ZIF-4 increases. Coudert et al.⁵⁷ in their computational study highlighted the amorphous nature of ZIF-4 and ZIF-8 on compression can be explained by a “shear-mode softening mechanism”. In another study by Ryder and Tan⁵⁸, the role of topology of MOFs with similar chemical components was investigated where it was found that the stability of MOFs is dependent over spatial orientation of its nodes and linker moieties. For example, ZIF-3 possesses an exceptionally low Young’s and shear modulus. Modification of the properties of linker molecules like electronic and steric bulk can also act as an enhancer or diminisher of the MOFs mechanical properties as a whole⁵⁹. The mechanical properties of MOFs have been reported to impart structural stability to biomaterials⁶⁰, however, such direct studies are still scarce in the field of biosensors but highlight their possible potential in the development of robust biosensors in forthcoming future.

Studies on phase diagrams of ZIFs (ZIF-62, ZIF-4) at high temperature and pressure revealed that liquid and glass MOF phases can be achieved at elevated hydrostatic pressure where the melting point of these MOFs can be lowered to a great extent. However, for conducting such studies, a slight change in test conditions like the choice of hydrostatic fluid can alter the results drastically. Other MOFs like MOF-5 and HKUST-1 have also been studied extensively. Both of these MOFs being cubic in nature, with the former being a prototypical carboxylate MOF, a rise in temperature from 25-100 °C can result into a steady drop in its hardness and Young’s

modulus⁶¹. For MOF-5, a lot of studies have been done for calculating the change in its amorphization with application of pressure treatment, showing a pore collapse at pressure nearing 10.3 MPa⁵⁶.

2.1.2. Optical properties

The hybrid composition of MOFs encompasses it with a variety of optical properties being it fluorescence, phosphorescence or scintillation. They have shown to exhibit extraordinary emission characteristics ranging from metal-based emissions in lanthanides (more commonly known as ‘Ligand-to-metal charge transfer’) to exciplex-excimer emissions and ligand-based luminescence⁶². MOFs containing lanthanides are also known to exhibit “antenna effect” wherein they undergo indirect activation from adjoining ligands emitting distinctive luminescence. Eu^{3+} and Tb^{3+} ions are amongst the most reported MOFs in optical sensing applications due to their distinct visible luminescence properties due to evident Stokes shifts^{63,64}. Apart from above discussed factors, it has also been found in studies that MOFs containing d^{10} transition elements devoid of any unpaired electrons exhibit linker-based luminescence with linker moiety instead of metals being the focal point of the luminescence^{62,65}. MOFs tailored with materials with better properties and stability tend to minimize diffusion barriers and solve mass transfer issues leading to enhanced ion and energy transport^{66,67}. In a recent article by Jamali et al.⁶⁸, MOF-5, with enhanced fluorescence properties and direct band gap, was subjected to metal-semiconductor-metal photodetectors to determine its optical properties when conjugated with zinc oxide (ZnO). The MOF acted as an electron acceptor molecule with an enhancement in its charge transfer characteristics by almost 6 folds.

2.1.3. Electronic Properties

Significant electrical conductivity is quite an uncommon trait in MOFs due to the presence of insulating organic linker moieties and a relatively poor π - π conjugation⁶⁹. Previous reports

have cited them as less conductive (conductivity between 10^{-8} and 10^{-10} S cm⁻¹) or insulating, limiting their applications in electrochemistry and fuel cells⁷⁰. Hitherto they were being looked upon as highly non-conductive materials. However in recent times, use of MOFs has also been explored by tuning their electron density and bandgap through incorporation of semi-conductive materials such as BiVO₄⁷¹. The conductivity originates from ion or electron transport phenomenon occurring between host and guest species⁷². With advancements in material science in the last decade, newer conductive MOFs have been devised giving MOFs the role in sensory platforms⁷³. Electrical conductivity in MOFs can either follow band-like or charge-transport mechanism involving redox-hopping. Along with it, the charge transfer characteristics of MOFs can be levied on the functional groups present on their surfaces. Narrowing of bandgap is possible in case of increase in the sp²-hybridized carbon atoms. Also, in case of linker molecules like amine and nitro groups, the p-orbitals might interact with the aromatic carbon atoms facilitating band gap modulation⁷⁴. For lesser discussed redox-hopping mechanisms in MOFs, a recent article by Li et al., discusses thoroughly on the redox conductivity of Zn-based MOFs grown on fluorine-doped tin oxide surface exhibiting exceptional conductivity and minimal electrical resistance⁷². Such approaches can be fascinating for future development of microporous conductors and reconfigurable electronics with applications in sensors.

2.1.4. Catalytic properties

Studies on MOFs have also highlighted their catalytic behaviour. In an article where Co-based MOF executed synthesis of multicomponent reactions had a Co metal centre linked with 4, 6-diamino-2-thiopyrimidine that imparted it with lewis acid properties⁷⁵. Not only MOFs, but also their derivatives such as Ni/NiO/C composite have found to be excellent electrocatalysts for hydrogen evolution reactions⁷⁶. MOFs with Cu⁺ sites have found their applicability in CO₂ hydrogenation to alcohols due to their catalytic behaviour and unique coordination assembly

at the room temperature⁷⁷. Catalytic properties of MOFs, both photocatalysis or electrocatalysis, can be attributed to their enriched electron transfer capabilities from their metal centers to their respective ligand molecules^{78,79}.

2.2. General Methods of MOF Synthesis

MOFs have garnered tremendous interest recently, with efforts put up in modulation in its synthesis and post synthesis modifications. They have been synthesized through innumerable ways since its popularization by Yaghi et al. around 1995^{80,81}. The primary concept of MOF synthesis revolves around linking of the organic linker molecule with the metal center to develop a porous framework with the objective of not disrupting the integrity of the linker molecule during the synthesis procedure. Variation in the surface area and pore size of MOFs is highly subjective to the temperature changes and reaction conditions. A general schematic representation of the different synthetic routes for MOFs has been shown in **Figure 1.2**.

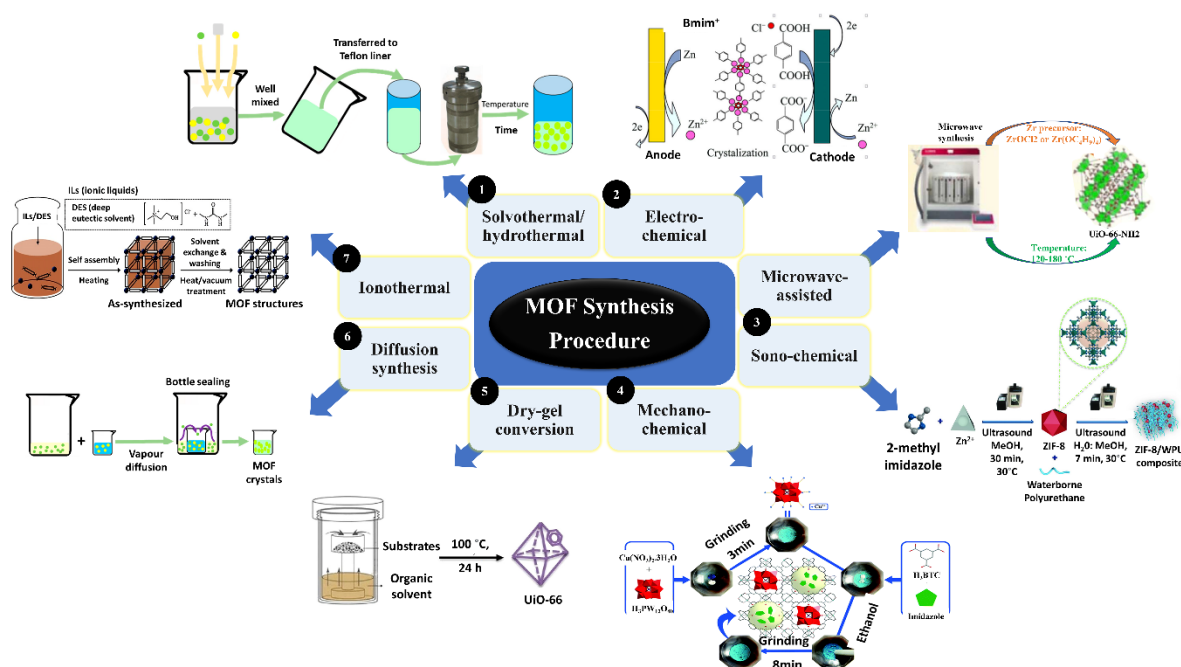


Figure 1.2. A general schematic representation of the prime MOF synthesis routes. Reprinted with permission from Ref.^{82–88}. **1)** Solvothermal/ hydrothermal synthesis, Copyright 2018, Bian et al.; **2)** Electrochemical synthesis, Copyright 2015, Yang et al.; **3)** Microwave-assisted synthesis and Sono-chemical synthesis, Copyright 2022, Solis et al.; Copyright 2022, Borse et al.; **4)** Mechano-chemical synthesis, Copyright 2021, Wang et al.; **5)** Dry-gel conversion,

Copyright 2017, Lu et al.;⁶) Diffusion synthesis, Copyright 2018, Bian et al.;⁷) Ionothermal synthesis, Copyright 2016, Zhao et al.

These synthetic routes used have been briefly discussed with recently published examples for better clarity in section below.

2.1.1. Solvothermal MOF synthesis

Reaction temperature is a key parameter for synthesis of MOFs. So, the method of MOF synthesis can be broadly categorized as solvothermal and non-solvothermal synthesis. According to Albrecht Rabenau⁸⁹, reactions taking place under autogenous pressure (above 1 bar) in closed vessel above the boiling point of the solvent are termed as solvothermal synthesis. Such synthesis can also be termed as hydrothermal synthesis in aqueous solvent system when temperature exceeds 100°C. A generalized scheme for solvothermal MOF synthesis has been shown in **Figure 1.2.1**. However, this process can be easily understood through examples discussed in the section ahead. For instance, in a recent article on S-doped CAU-17 based tetracycline removing system, different proportions of CH₄N₂S were added to a mixture of solution containing bismuth nitrate [Bi(NO₃)₃·5H₂O] and benzene-1,3,5-tricarboxylic acid [H₃BTC]. They had been dissolved in methanol and dimethyl formamide (DMF) solvent systems respectively and the reaction mixture was stirred and heated at 120 °C for 2 h in a Teflon lined reactor, leading to MOF synthesis⁹⁰. Similar case has also been reported for MIL-100(Fe) MOF which has been synthesized at 150 °C for 12 hours in an autoclave⁹¹. The major advantage of this method includes chemical and structural stability along with morphological symmetry⁹² whereas the disadvantage lies in optimizing the temperature conditions for desirable morphological characteristics.

2.1.2. Electrochemical MOF synthesis

Electrochemical synthesis is a fast, clean and cost-effective method for nanoparticle fabrication including MOFs⁹³. A general representation of electrochemical MOF synthesis has been shown

in **Figure 1.2.2**. This method has been widely favoured due to its potential in growing MOFs in specific patterned thin films and versatile coatings. The pioneers of electrochemical MOF synthesis are believed to be researchers at Baden Aniline and Soda Factory (BASF) who proposed salt free continuous synthesis, advantageous for an industrial setup. The process typically involves supplying a metal building block (placed at the anode) to a synthetic solution containing organic linker and a conducting salt⁹⁴. Other reports have similar claims for metal salt-free synthesis of MOFs too^{95,96}. The reaction conditions are carefully controlled for production of bulk MOF material. A well-known MOF structure $[\text{Cu}_3(\text{BTC})_2]$ was electrochemically formed on pure copper electrodes where Cu^{2+} ions were linked to a tridentate ligand H_3BTC ⁹⁷. On application of anodic voltage at the copper electrodes, the Cu^{2+} ions dissolve into the synthesis solution which contained a mixture of H_3BTC and methyltributylammoniummethyl sulfate (MTBS). It is relatively easier to obtain crystals of varying sizes (2-50 μm) with simple alterations in synthesis conditions. For example, a ten-fold rise in the applied anodic voltage increases the metal ion concentration near the surface facilitating thin films formation with smaller crystals. On contrary to this, larger crystals are obtained on addition of more water to the synthesis solution resulting from slowed nucleation. In a study on electrochemical synthesis of MOF-5, polished zinc was used as an anode and titanium sheet was taken as the cathode⁸⁴. Terephthalic acid was dissolved in DMF in which zinc nitrate was added to serve as the conductive medium. Afterwards, an ionic liquid (1-butyl-3-methylimidazolium (Bmim) chloride) was added into this solution and agitated with a magnetic agitator. With passage of current, a white flocculent substance was obtained which is the desired MOF. The study stated that the MOF-5 synthesized by this method possessed higher thermal stability and better crystallinity which can be accredited to π - π stacking interactions, coordination bond and ionic bond prevalence. This method could be a choice for fabrication of films and layers of MOFs since the thickness can be controlled by alteration in applied voltage

and current in mild synthesis conditions^{98,99}.

2.1.3. Microwave-assisted (MW) and ultra-sonochemical (US) MOF synthesis

MW involves usage of microwave irradiation as energy source for conducting solid-solid as well as solution-based syntheses. Both MW and US syntheses are considered inexpensive and unconventional irradiation systems on the basis of various parameters of concern like phase-selective crystallization, degree of acceleration and crystal size reduction. A general perspective of these processes can be gained through **Figure 1.2.3** but some recent examples would make things more lucid. For example, in MOFs like ZIF-8, the conventional methods rely on nitrate and acetates of zinc as precursors which are expensive. However, for ZnO precursors, the problem lies on poor conversion rate and relatively high reaction time. Yet, the ZnO-based synthesis possesses multiple benefits among which one is being sustainable leading to water as a by-product. In a latest study by Bazzi et al.¹⁰⁰, both the US and MW methods of ZIF-8 synthesis have been discussed where the former led to a better conversion rate while more particle dispersion and better material regeneration was seen in the latter. The group had tried synthesizing ZIF-8 in different types of solvents and demonstrated the product usage in phosphate adsorption study. MW-assisted MOF synthesis is often adopted for its shorter reaction time, superior phase purity, uniform particle morphology and simplicity of procedure¹⁰¹. However, a study on MW-assisted MOF synthesis stated its shortcomings highlighting about generation of hotspots in MW reactor due to complex distribution of MW field which results into localized overheating. This is unfavorable for growth and nucleation of MOFs' crystals. These hotspots are regulated by various operating parameters such as dielectric property of reactants, input power, stirring rate and shape/dimension of the cavities that are needed to be taken care of¹⁰².

Alternatively, ultrasounds have been used for MOF synthesis mostly for its energy efficiency

benefits accompanied with a reduced reaction time¹⁰³. The frequency of ultrasonic waves can alter its application causing innumerable physical and chemical variations in a non-equilibrium state of reaction. In a zinc-based MOF synthesis, zinc nitrate hexahydrate along with adipic acid was added to DMF and the solution was transferred to a round bottom flask (RBF). Then, an ultrasonic probe (dia. 13 mm) was dipped into it and the synthesis was carried out at 65% power. The synthesized particles were found to be equally thermal stable with smaller crystallite size when compared to those synthesized by other methods¹⁰⁴. In another study by Bui et al.¹⁰⁵ for Fe₃O₄ encapsulated ZIF-8 synthesis, sonochemical method was chosen over all other methods to increase the microporous loading efficiency and enhancement of reaction rate caused by confined high temperature and pressure-mediated cavitation of the MOF.

2.1.4. Mechanochemical MOF synthesis

This efficient yet simple method involves greener approach of solvent-free or extremely low-solvent synthesis using grinding of only powdered raw material. This solid-solid reaction is a convenient method over the conventional solvent intensive methods, which poses potential advantage for large scale production and eases handling by many folds. **Figure 1.2.4** shows a diagrammatic general representation of this process. Recently, ZIF-8 was mechanochemically synthesized by Imaoka et al.¹⁰⁶, wherein, they compared the relative crystallinity and convenience of synthesis with conventional methods involving usage of solvent systems. Their method used 50 yttria-stabilized zirconia (YTZ) ball-based milling of powdered ZnO and 2-methylimidazole in a ceramic pot. The synthesis was carried out at 150 rpm for one hour and was catalyzed by cobalt acetate or zinc acetate dihydrate in two different reaction conditions. This acetate-assisted mechanosynthesis procedure can be explained as reaction between cobalt acetate with 2-methyl imidazole yielding acetic acid which thereby dissolves ZnO. The mass transfer by *in situ* generated acetic acid and hydrated water encouraged formation of the complex between the ionized metal to the linker molecule. Both, the complex formation and

dissolution of the complex by continuously forming acetic acid, led to agglomeration and growth of the macroporous structure. An alternative to this ball-milling process could be the single screw (SSE) and double screw (DSE) extrusion methods where a screw is employed with a gradually increasing root diameter covering large portion of a conveying section, and a kneading element in the final zone. However, in case of SSE, the work is applied through compressive forces which are generated by a reduction in screw channel depth upon movement from feeding to metering section. The barrel of the extruder is heated to a temperature which exceeds the melting point of the ligand. The extrusion process possesses better control on temperature in the barrel which is missing in the case of ball-milling where temperature cannot be standardized and is inevitable due to friction¹⁰⁷.

2.1.5. Dry-gel conversion (DGC) MOF synthesis

DGC is a rapid process which involves treating amorphous gel powder in minimal solvent conditions without the use of modulators to form crystalline compounds in small reactor volumes⁸⁷, as represented in **Figure 1.2.5**. It was described by Ploskonka et al.⁹⁹ for synthesis of UiO-66 and UiO-66-NH₂, where heating under a vacuum environment promoted the nucleation without any need for solvent exchange by virtue of acetone's low boiling point. Lu et al.⁸⁷ synthesized UiO-66 MOF through the DGC route without the use of any acid or salt. Earlier reports too claim for such benign synthesis route for zeolites and zeolitic membranes aided by vapors of water or volatile amines^{108,109}. This method eliminates the usage of toxic solvents and release of hazardous by-products⁸⁷. It is a relatively safer, economical and environment-friendly technique that avoids use of acid modulators which sometimes limits the yield of the reaction. This method is often used in conjugation with other techniques like ball-milling as seen in case of Cr-MIL-101 synthesis where the properties of both methods were envisaged to obtain MOF with a greater surface area and higher yield¹¹⁰.

2.1.6. Diffusion-based MOF synthesis

The principle of diffusion-based MOF synthesis is absolutely contrary to the conventional solvothermal method. In this method, the reagents are separately placed within a sealed container in small glass vials. The method can be understood through the general schematic diagram in **Figure 1.2.6**. The container's atmosphere gradually fills with the surface evaporation of chemicals thereby diffusing into the reagent to complete the reaction and synthesize the target compound. In a typical MOF-70 synthesis method, lead nitrate $[\text{Pb}(\text{NO}_3)_2]$ and 1,4-benzenedicarboxylic acid (BDC) were supplemented to the DMF solvent under stirring¹¹¹. The solution was diluted with ethanol and hydrogen peroxide, later filtered and kept in glass vials within amber glass container along with a solution of trimethylamine and DMF. The lid was sealed with silicone oil with the standing solutions undisturbed for a period of 7 days to obtain the desired product. The method is feasible at room temperature and pressure making it highly sustainable yet time consuming. In another Cd-based MOF synthesis, similar method was employed where the aqueous solution of reagents was layered over metal solution in a crystal tube to obtain the desired MOF in 30 days¹¹².

2.1.7. Ionothermal MOF synthesis

This benchtop and time-efficient synthesis method is often regarded as an alternative to mechanochemical process. It tries to overcome the shortcomings of mechanochemical technique like use of specialized instruments (ball-mills and extruders) to evolve as a solvent-free, cost-effective and green synthesis approach. It also poses a control over MOF crystallization kinetics and induces chirality via inclusion of ionic liquids within the framework. However, the condition lies in usage of low-melting point salt which can serve the dual purpose of reaction solvent as well as that of a metal precursor.

The general schematic representation of ionothermal MOF synthesis can be seen in **Figure 1.2.7**. To understand it further, as per a recent study on synthesis of Ca-based MOFs, use of deep eutectic solvent was explored for creation of ionic environment¹¹³. This environment was favorable over other synthetic approaches for prevention of competing coordination by water. This is because the alkali metal's ability to bind with water molecules is higher over any other ligand due to its oxophilicity. The reaction involved terephthalic acid and calcium nitrate $[\text{Ca}(\text{NO}_3)_2]$ addition to a vial containing choline chloride and eutectic urea deep eutectic solvent (e-urea DES). The mixture was heated to 140 °C for 10 days to obtain the desired MOF. In another study by Azbell et al.¹¹⁴, a similar approach without the use of eutectic mixture was chalked out where low melting metal precursor salts were added to an autoclave containing equivalent molar concentration of linker and the temperature was maintained just above the melting point of the salt to facilitate MOF synthesis without the use of solvent. On extraction of solid product, washing was done via different approaches, Soxhlet extraction also being one of them. This method is largely untapped and is restricted to certain specific categories of MOFs like ZIFs and carboxylates. Stable azolate and salicylate MOFs are now being explored too but the area remains greatly unexplored.

For all above methods, each one has certain restricted usage e.g., methods like sonochemical, microwave-based and mechanosynthesis might involve high energy consumption within complex processes with an additional requirement for advanced machinery, lengthy reactions, introduction of undesirable anions and tough process regulations. Hence, the synthesis scheme can be modified and adopted as per one's choice of material and its implementation in various fields.

3. Application of Biosensors in Molecular Sensing and Disease Diagnosis

3.1. Detection of Clinically Significant Molecules Using MOF-based Sensors

3.1.1. Free Radicals and Metal Ions

3.1.1.1. MOF-based Electrochemical sensors for free radical and metal ion detection

Hydrogen peroxide (H_2O_2) is one of the crucial markers for clinical and physical conditions aiding to their early diagnosis^{115,116}. It has often been linked with several other disorders too such as Parkinson's disease, Alzheimer's disease and myocardial infarction^{117,118}. According to Huang et al.¹¹⁹, ultrathin 2D MOF nanosheets can be used as nanozymes for electrochemical biosensing of H_2O_2 . The 2D Cu-HHTP-NSs being ultrathin can facilitate better charge mobility along with exposed active sites for anchorage of functional molecules. The sheet surface was decorated with in-grown AuNPs for structural and electrical property enhancement. Peroxidase-like activity (POD) was explored for the composite in the presence of H_2O_2 to oxidise 3,3',5,5'-tetramethylbenzidine (TMB) in the pH range of 3.2-4.8, exhibiting blue colour. Interfacial behaviour and electrocatalytic performance of the modified electrode was also confirmed using electrochemical impedance spectroscopy (EIS) and cyclic voltammetry (CV) measurements, respectively. Electrode sensitivity was measured through amperometry which showed a fast signal response in 50 nM to 2 mM H_2O_2 concentration within 3s. Electrochemical sensing was seen in wide linear dynamic range (LDR) of 50 nM-16.4 mM H_2O_2 with LOD of 5.6 nM. The group used colon cancer cells for analysis which when stimulated with N-formyl-methionyl-leucyl-phenylalanine initiate H_2O_2 generation[95]. Similarly, Xie et al.¹²⁰ in their study on electrochemical H_2O_2 detection in HeLa cells, electrodeposited gold-platinum NPs on Co-MOF modified glass carbon electrode (GCE). The 2D Co-MOF nanosheets exhibited electrocatalytic activity for the reduction of H_2O_2 in the meantime also acting as a supporting material. The catalytic current exhibited a linear trend with rise in concentration of H_2O_2 from 0.1 μM to 5 mM and 5mM to 60 mM, as measured by amperometry measurements, with an LOD of 0.02 μM (**Figure 1.3A**). In another study on H_2O_2 detection by Xiong et al.¹²¹, a lower LOD of 2.32 nM was achieved through ZIF-67 MOF-

derived Co_3O_4 NPs functioning as H_2O_2 nanozyme (**Figure 1.3B**). The study focussed on live detection of H_2O_2 in A549 (human lung cancer cell), 4T1 (mouse breast cancer cell) and HUVEC (human epithelial cells) cells by $\text{Co}_3\text{O}_4@\text{CNBs}$ modified GCE at -0.22 V. A linear response was recorded, through CV and amperometry analysis, with H_2O_2 concentrations from 0.01 to 359 μM . Similar MOF/MOF-based composite's peroxidase-like activity have been exploited in past few years for sensitive H_2O_2 detection^{122–128}.

Apart from H_2O_2 , efforts are underway on the development of highly sensitive detection probes for highly reactive oxygen species (hROS) like superoxide ($\text{O}_2^{\bullet-}$), peroxynitrite (ONOO^-), hydroxyl radical ($\bullet\text{OH}$), singlet oxygen ($^1\text{O}_2$) and hypochlorite (ClO^-) which are modulators of redox homeostasis and cellular signalling in living systems^{129,130}. Their overaccumulation can lead of oxidative stress which can thereby result into human pathologies like inflammation, carcinogenesis, neurological disorders and ischemia-reperfusion injury etc.^{131–133}. Another recent study proposed a superoxide anion sensor ($\text{O}_2^{\bullet-}$) where Ag and 2-aminoterephthalate based MOF was used to derive a carbon-based nanomaterial for probe fabrication¹³⁴. A GCE electrode surface was coated with the AgNPs-MC to fabricate the electrochemical sensor which exhibited excellent $\text{O}_2^{\bullet-}$ detection in living human glioma cells. Chronoamperometry analysis confirmed a LDR of the constructed sensor between 3.69×10^{-11} to 5.26×10^{-7} M and 1.68×10^{-6} to 3.06×10^{-4} M with an LOD of 12.3 pM.

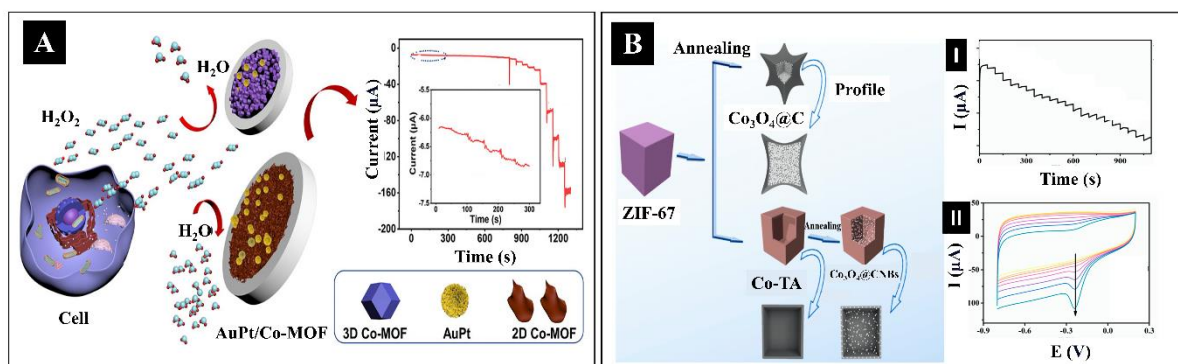


Figure 1.3. (A) Schematic illustration showing H_2O_2 detection in HeLa cells, electrochemically. Adopted from Xie et al.[120], copyright 2022. (B) Illustration depicting ZIF-67 based nanozyme sensor for H_2O_2 detection. I) Amperometric response of the carbonized

MOF fabricated on GCE surface against different concentrations of H_2O_2 , when scanned at -0.22V in PBS solution. II) CV response of the MOF-based sensor in the presence of different H_2O_2 concentrations. Reprinted with permission from Xiong et al.[121], copyright 2022.

3.1.1.2. MOF-based Optical sensors for free radical and metal ion detection

MOF-based optical sensors have also been devised in recent times for free radical and metal ion detection. In a latest work by Huang et al.¹³⁵, a redox-active luminescent MOF, Mg(DHT), was synthesized for encapsulation of hydroquinone moiety for sensitive detection of $\bullet OH$ and ClO^- . The MOF comprised of 2,5-dihydroxyterephthalic acid (H_2DHT), a bidentate ligand, which endowed it with large Stokes shift and an ultrafast response due to enol transfer aided by enol-keto tautomerism. On the other hand, presence of Mg^{2+} as the metal centre enhanced the luminescence efficiency due to reduced non-radiative decay. The hydroquinone underwent a reversible transformation by $\bullet OH$ and ClO^- to become quinone because of its reducing capability. The MOF displayed a strong fluorescence at 556 nm which got quenched upto 76% and 90% in the presence of $\bullet OH$ and ClO^- species, respectively (**Figure 1.4A**). The same results were conferred with UV-vis data where oxidation of hydroquinone led to appearance of a new absorption peak at 285 nm. The probe was also used for endogenous detection of the ROS species in MCF-7 cells, which were pre-treated with lipopolysaccharide (LPS) and phorbol 12-myristate 13-acetate (PMA) to simulate oxidative stress induction. The results proved applicability of the biosensor for imaging and monitoring of ROS in live cells within a detection range of 2.5-50 μM and an LOD of 0.22 μM and 0.18 μM for $\bullet OH$ and ClO^- , respectively. Similar sensors for superoxide anions have also been fabricated with a different principle where MOFs have been used as electrocatalysts for *in situ* real time detection of cellular $O_2\bullet^-$. Zhang and group¹³⁶ used Mn-MOF in three different forms (NPs, nanorods and nanolollipops) to detect the target ROS species in MCF-7, H9c2 and HepG2 cells. The concentration of $O_2\bullet^-$ was initially determined through spectrophotometry where the reduction of ferricytochrome c was

investigated. For electrochemical measurements, Mn-MOF was then coated on the GCE surface to record the oxidation of $O_2^{\bullet-}$ at 0.69 V in an LSV curve. For comparison of all three forms of MOF, half-wave potential and peak current values were considered for evaluation of individual electrochemical performance. Amperometric technique was employed for sensitive detection of $O_2^{\bullet-}$ which revealed the biosensor to be highly sensitive in an LDR of 3.0-1350 nM with an LOD of 1.0 nM. The highest sensing performance was observed for Mn-MOF nanolollipops modified electrode due to heavily exposed active sites and faster charge transfer kinetics benefitted from its unique spatial structure aiding to ultrasensitive sensing.

Mercury ions (Hg^{2+}) are one of the heavy metal pollutants which are environmental contaminants posing global health hazard by contributing to biomagnification. They cause neurological and cardiovascular disorders along with impairment at molecular level including genetic mutations and mitosis defects^{137,138}. In an article by Uttam et al.¹³⁹, calixarene was conjugated with ZIF-8 MOF to enable sensing as well as adsorption capabilities for the metal ions (**Figure 1.4B**). The adsorption of metal ions on DanC4A (dansyl appended calix[4]arene) was investigated in THF solvent system. DanC4A showed an excitation peak at 345 nm and a strong emission at 510 nm. When subjected to Hg^{2+} , it showed a reduction in its absorbance band at 345 nm and simultaneously increment at 285 nm. The limit of detection was found to be 0.56 μ M. Confocal microscopy was also done for sensing of the target metal ion by DanC4A in HeLa cells. The green fluorescence which was seen upon treatment of cells with DanC4A, gradually faded with its intensity decreasing to ~60% indicative of Hg^{2+} -receptor interaction.

Precious metals like Pd^{2+} and Au^{3+} are known for their unique physical and chemical properties like catalytic activity, high electrical conductivity and corrosion resistance. They find their enormous industrial applications in the form of computer hard drives, wires and switches, etc.¹⁴⁰ with a global surge in their demands each year. With their growing demands but limited supply, there is a dire need for recovery of these precious metals from e-wastes and for

environmental remediation purposes along with minimization occupational health hazards linked to recycling^{141,142}. In a study by Alharbi et al.¹⁴³, an azo-HNTA-MIL-101(Al) sensor was fabricated for fluorescent, ultrasensitive detection of Pd^{2+} and Au^{3+} ions. The group initially synthesized an azo dye and then used it as a linker for preparing the Al-MOF using solvothermal method. This approach enabled coupling of all amino groups of 2-aminoterephthalic acid with 2-naphthol even before the MOF synthesis. An increase in chemosensor sensitivity was found to be proportional to the increased functionalized amino groups on the MOF surface thereby increased adsorbed target metal ions. The absorbance of the sensor increased with an increase in Pd^{2+} and Au^{3+} ions whereas its fluorescence emission decreases significantly. This could be possibly because of the selective reactivity of Pd^{2+} with the carboxylic group and Au^{3+} with the phenolic group of 2-naphthol leading to the change in sensor spectra. The LOD for Pd^{2+} was reported as 6.79 ppb and that for Au^{3+} was 3.57 ppb with an LDR of 0 ppm- 0.3 ppm and 0 ppm- 0.1 ppm, respectively. The applicability of developed biosensor was tested against extraction of these metal ions from phone-grounded motherboards and was found to be efficient and cost-effective.

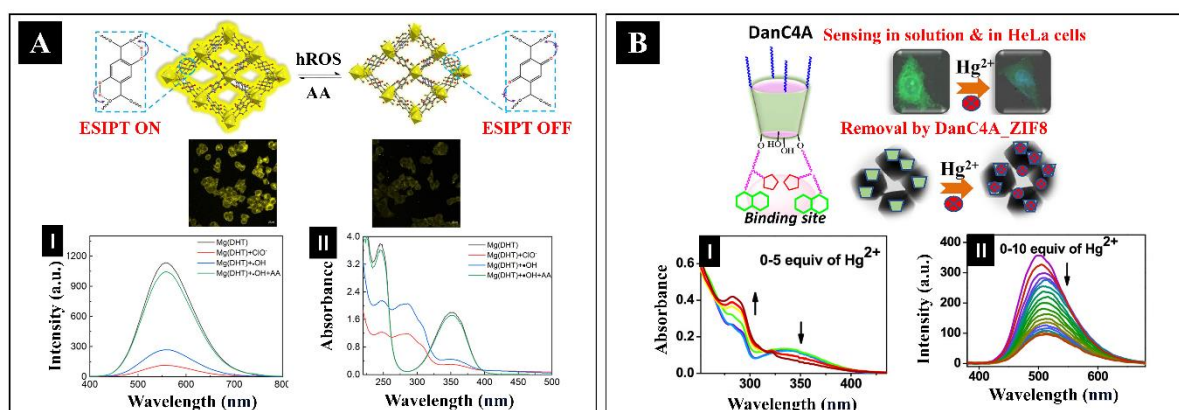


Figure 1.4. (A) Schematic representation of MOF incorporating hydroquinone moiety for detection of highly reactive oxygen species (hROS) through ESIPT by linker. (I) Fluorescence and (II) UV-vis absorption spectra of MOF with different ROS species. Reused with permission from Huang et al.[135], copyright 2022. (B) Diagrammatic illustration of DanC4A MOF-based sensor for sensitive detection of Hg^{2+} ions. (I) Absorption and (II) Fluorescence spectrum of different concentrations of Hg^{2+} ions in Tetra hydrofuran (THF) solution, when titrated with DanC4A. Reprinted with permission from Uttam et al.[139], copyright 2022.

Recently reported MOF-based biosensors for detection of free radicals and metal ions have been comprehensively discussed in **Table 1.1** below with their respective target analytes, detection techniques, fabrication strategies, real samples considerations and detection efficiency parameters (LDR and LOD).

Table 1.1. Detailed list of latest MOF-based biosensors for sensing of free radicals and metal ions (N.R.: Not Reported)

S.No.	Target Analyte	Detection Mechanism	Sensor Composite	Fabrication strategy	Mechanistic Role of MOF	Real Sample	LDR	LOD	Reference
1.	H ₂ O ₂	CV, EIS, CA	SWNT-MOF(Cu)	Gold wire was dip-coated with catalytic ink which facilitated nanotube growth. Then it was modified with SWNTs deposited through CVD. Finally, the surface was electrodeposited with <i>in situ</i> synthesized Cu MOF.	Increase in electroactive surface area	Live cells (RAW 264.7), Raw milk	1 to 2000 μ M	0.21 μ M	Nandhini et al. ¹⁴⁴
	H ₂ O ₂	DPV	BSA-AuNPs-MFOF	BSA-capped AuNPs were synthesized through sonication which were further embedded in MFOF.	Increase in electroactive surface area	Human whole blood, Egg yolk, Butter, Milk, Goat meat, Cheese, Yogurt and ice cream, Beer and apple juice, live cells (RAW 264.7 cells)	100 nM to 10 mM	18.4 nM	Arul et al. ¹⁴⁵
	H ₂ O ₂	CV, CA	Ce-UiO-66-BPDC	Ce-UiO-66-BPDC flakes were covered entirely with Au-Pt NPs through electrodeposition.	Increase in electroactive surface area	A549 cancer cells	0.15 to 64.8 and 114.8 to 1164.8 μ M	89 nM	Yang et al. ¹⁴⁶
	H ₂ O ₂	Colorimetry	IrNCs@Ti-MOF	IrNCs@Ti-MOF, TMB and various concentrations of H ₂ O ₂ were added into sodium acetate–acetic acid buffer to record chromogenic reaction.	Ideal support matrix with high porosity, robustness	Rabbit serum	0 to 900 μ M	14.12 μ M	Mu et al. ¹⁴⁷
	H ₂ O ₂	Colorimetry	M-PCN-222 where, M = Mn, Fe, Co, and Ni	Microwave-assisted hydrothermal synthesis of MOFs was done that exhibited improved peroxidase-mimicking activity. Presence of Fe-PCN-222, H ₂ O ₂ ,	Contains porphyrin active sites exhibiting	Milk, Orange juice, Grape juice, human tears	Fe-PCN-222:	Fe-PCN-222: 0.3 μ M	Shu et al. ¹⁴⁸

				and TMB, resulted into oxidation of TMB into blue ox-TMB.	potential biomimetic		1 to 100 mM	Mn-PCN-222: 0.9 μ M	
					catalytic activities			Co-PCN-222: 1.0mM	
								Ni-PCN-222: 9.6 μ M	
	H ₂ O ₂	Fluorescence, Colorimetry	Cyclodextrin MOF (CD-MOF)	The CD MOF exhibited peroxidase (POD) and oxidase (OXD)-like property. POD-like activity using TMB as POD substrate with 'OH release readout using visible colour change. OXD-like activity was determined using L-ascorbic acid assay and TMB colorimetry.	Peroxidase and oxidase-like activity, hydrolytic stability	HepG2 cells	0.11 to 0.95 mM	0.06 mM	Tan et al. ¹²⁷
2.	Hg ²⁺	ECL, CV, EIS	Ag-MOF	Capture strand DNA (c-DNA) with a terminal modification was attached to Au-electrode. Then, mercury-ion aptamer was adhered to electrode by being paired with c-DNA. ECL signal reduction was done using streptavidin aided by biotin as linker molecule.	High specific surface area and large pore diameter	Tap water, Lake water	300 fM to 1 μ M	66 fM	Liu et al. ¹⁴⁹
3.	Cr ³⁺	PEC, EIS	Cu-MOF	Tetra-ammine cobalt phthalocyanine-based COF was grown over a Cu-MOF substrate via covalent bonds. This acted as an efficient platform for immobilization of DNA strand which binds to Cr ³⁺ ions, thereby detecting it.	High porosity and rich functionality tightly anchored the DNA strands	River water, Tap water	0.1 pM to 100 nM	PEC: 14.5 fM EC: 22.9 fM	Zhang et al. ¹⁵⁰
4.	OH ⁻ , Al ³⁺ , Fe ³⁺	Fluorescence	Zn-DHNDC MOF	Zn ²⁺ and 4,8-dihydroxynaphthalene based MOF was functionalized with OH ⁻ . When MOF was subjected to OH ⁻ ions, ESIPT suppression turned on bluish fluorescence. When MOF was subjected to Al ³⁺ ions, a CHEF-mediated green luminescence was observed. When MOF was subjected to Fe ³⁺ ions, a CHEQ-mediated fluorescence quenching was observed.	Participates in excited state intramolecular proton transfer	N.R.	N.R.	OH ⁻ : 48 nM Al ³⁺ :95 nM Fe ³⁺ :33 nM	Bhowal and Ghosh ¹⁵¹

5.	Al ³⁺ and Ga ³⁺	Photoluminescence	Eu-MOFs (JXUST-11)	Eu-based MOF acted as a photoluminescent sensor for detection of Al ³⁺ and Ga ³⁺ by fluorescence enrichment visualized through naked eye. The 'N' and 'S' atoms of H ₂ BTDB ligand's thiadiazole ring acted as the site for metal coordination.	Exhibits excellent luminescence	MCF-7 cells	N.R.	Al ³⁺ : 2.9 ppm Ga ³⁺ : 10.2 ppm	Li et al. ¹⁵²
6.	F ⁻ and Cr ₂ O ₇ ²⁻	Fluorescence	UiO-66-NH ₂ @EY	Ratiometric fluorescent probe was constructed by utilizing Zr-MOF cavity to encapsulate eosin Y molecule. F ⁻ detection was through effect of hydrogen bond whereas, for Cr ₂ O ₇ ²⁻ , it was based upon energy competition transfer mechanism.	Provides cavities for eosin Y encapsulation	Sweat latent fingerprint, human serum, HepG2 cells	N.R.	F ⁻ 34.7 nM Cr ₂ O ₇ ²⁻ 22.3 nM	Zhang et al. ¹⁵³
7.	Cu ²⁺	Fluorescence	Fe-MIL-88NH ₂ @RhB	Ratiometric fluorescent probe was fabricated with the aid of encapsulated RhB into porous framework of Fe-MIL-88NH ₂ . The MOF composite was mixed with different concentrations of target ion solutions to get optical measurements due to binding of ion with -NH ₂ group of the MOF.	Provides cavities for eosin Y encapsulation	A549 cells, human serum, river water, reed leaves	N.R.	4.23×10 ⁻⁶ M	Zhang et al. ¹⁵³
8.	Cu ²⁺ and Fe ²⁺	Fluorescence	Tb-TATB MOF	MOF was synthesized through solvothermal route. When Tb ³⁺ was excited at 349 nm, it gave characteristic transitions at 490, 544, 582 and 620 nm. The fluorescent intensity gradually decreased with an increase in ion concentration at 544 nm	Abundant luminescence sites, visible color, and sharp characteristic emission peak	N.R.	N.R.	Cu ²⁺ : 2.92 μM Fe ²⁺ : 4.84 μM	Zhang et al. ¹⁵⁴
9.	ClO ⁻	Fluorescence	UiO-66-NH ₂	The MOF was developed which on excitation at 400 nm exhibited new emission at 533 nm on reaction with ClO ⁻ , which otherwise emits at 432 nm.	Excellent thermal stability, water tolerance, and permanent porosity	Water, L02, Hela and RAW 264.7 cells	0.1 to 150 μM	63.8 nM	Yu et al. ¹⁵⁵

Abbreviations:

CV: Cyclic Voltammetry, EIS: Electrochemical Impedance Spectroscopy, CA: Chrono Amperometry; SWNTS: Single Walled Nanotubes; CVD: Chemical Vapour Deposition; DPV: Differential Pulse Voltammetry; BSA: Bovine Serum Albumin, AuNPs: Gold Nanoparticles; MFOF: Metal

free organic framework; Pt: Platinum; TMB: 3,3',5,5'-Tetramethylbenzidine; RhB: Rhodamine B dye; ECL: Electro-chemi Luminescence; DNA: Deoxy-ribonucleic Acid; PEC: Photoelectrochemical Luminescence; COF: Covalent Organic Framework; ESIPT: Excited State Intramolecular Proton Transfer; CHEF: Chelation Enhanced Fluorescence; CHEQ: Chelation Enhanced Quenching

3.1.2. Small Molecules

Small molecules' detection gains its importance due to their easy penetration and involvement in human metabolic pathways^{156,157}.

3.1.2.1. MOF-based Electrochemical sensors for small molecule detection

Pesticides and herbicides are current environmental menace due to severe toxicity even at mild concentrations. Some of the organophosphorus pesticides like methylparathions, chlorpyrifos are known to cause acetylcholinesterase (AChE) inhibition leading to serious nervous disorders, human poisoning and even death^{158,159}. These potent threats have urged for development of sensitive detection tools against these hazardous chemicals. In a work by Wen et al.²⁵, ZIF-8 has been used as a substratum for immobilization of AChE enzyme on its periphery for the sensitive detection of isocarbophos (ICP) (**Figure 1.5A**). GCE electrode surface was drop-casted with AChE-CS/Gr/ZIF-8 where graphene (Gr) was used to enhance conductivity of the system. CV, EIS and DPV tests were done at -0.2 to 0.6 V, 0.23 V and 0.3 to 0.9 V, respectively. AChE catalysed acetylthiocholine to generate acetate and thiocholine (TCI) on hydrolysis. This TCI got oxidized further at a specific voltage to generate oxidative current corresponding to its concentration. Cabbage leachate and tap water samples were used for analysis by spiking it with different concentrations of ICP. The LDR for the sensor was found to be in the range 0.5 to 100 ng/mL with an LOD of 0.18 ng/mL.

Another study by Niu et al.¹⁶⁰ on electrochemical detection of paraquat herbicide, a fast sterilant and lethal herbicide to humans, uses the same ZIF-8 skeleton (**Figure 1.5B**). ZIF-8 and para-sulfonylcalix[4]arene (pSC4) coated with AuNPs on GCE electrode were found to be excellent sensing platform for the herbicide with an LDR of 0 pM to 10 nM and a detection limit of 0.49 pM. EIS measurements were done within a frequency range of 0.1 Hz to 100 kHz with $[\text{Fe}(\text{CN})_6]^{3-/4-}$ as the electronic mediator. The activity of the sensor was confirmed against

real tap water samples spiked with the target herbicide. Paraoxon, another organophosphorus pesticide, has been detected through 2D cMOFs with Cu and Ni metal centers coupled with organic ligands namely hexahydroxytriphenylene (HHTP), and tetrahydroxy-1,4-quinone (THQ)¹⁶¹. The Cu₃(THQ)₂-based biosensor used AChE for electrocatalysis of acetylthiocholine chloride (ATCh) and thereby its oxidative conversion to oxTCH was aided by the MOF substrate. This conversion was recorded at a remarkable lower potential of 0.3 V due to electroactivity of thiocholine (a product of the reaction cascade) in presence of the MOF substrate (Cu₃(THQ)₂) when compared to Cu₃(HHTP)₂ and Ni₃(HHTP)₂. The biosensor worked efficiently with an LDR of 1 to 1000 ng mL⁻¹ and LOD of 0.37 ng mL⁻¹. The sensor's applicability was also checked by testing on real samples such as soil, water, vegetables and fruits.

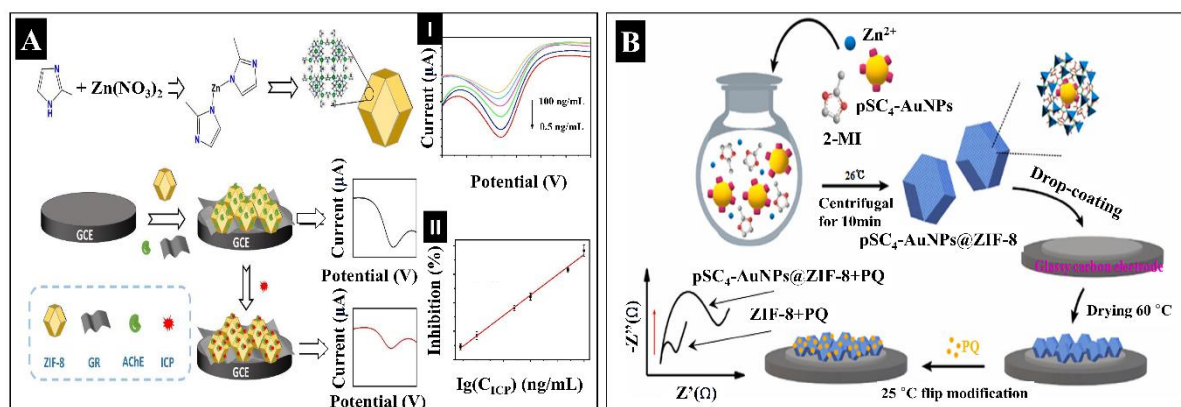


Figure 1.5. **A)** Schematic representation depicting applicability of acetylcholinesterase enzyme immobilized-MOF for electrochemical detection of organophosphorus pesticides. **I)** DPV response curve, and **II)** Calibration curve showing enzymatic inhibition rate vs logarithmic pesticide (ICP) concentration. Adopted with permission from Wen et al. [25], copyright 2022. **B)** A graphical illustration depicting electrochemical sensing of paraquat herbicide using ZIF-8 nanocomposite. Reused with permission from Niu et al. [160], copyright 2022.

3.1.2.2. MOF-based Optical sensors for small molecule detection

Apart from electrochemical biosensors, MOFs have found their application in sensing small molecules through optical means too. Another potent carcinogen and human health threat, 2,4-Dichlorophenoxyacetic acid (2,4-D) is a prominent herbicide with auxin-like properties that is

widely used in agriculture¹⁶². Its high-water solubility and non-biodegradability^{163,164} enables it to easily enter human food chain and act as an endocrine disruptor. According to the International Agency for Research on Cancer (IARC), it has been recognized as a potent human carcinogen which makes its environmental surveillance important for public health concerns^{165,166}. In a recent article on its colorimetric detection, use of Co-hemin MOF was demonstrated where Co-hemin oxidized TMB into ox-TMB. The detection of 2,4-D did not involve addition of H₂O₂, depicting the composite's oxidase-like activity, with a wide LDR from 0.1 $\mu\text{g mL}^{-1}$ to 240 $\mu\text{g mL}^{-1}$ and LOD of 33 ng mL^{-1} ¹⁶⁷. The biosensor had its applicability tested for real samples like rice and apple too.

Glucose is a crucial energy provider in living organisms for cellular activity and homeostasis^{168,169}. A poorly balanced blood glucose level may lead to diabetes and related diseases such as renal failure, blindness and cardiovascular diseases^{170,171}. With a rise in diabetes cases worldwide, the demand for ultrasensitive glucose-based biosensors has become crucial. With conventional methods like high performance liquid chromatography¹⁷², gas chromatography¹⁷³, capillary electrophoresis¹⁷⁴, spectrometry¹⁷⁵, electrochemistry^{176,177}, electroluminescence¹⁷⁸, etc. still prevalent in field of glucose diagnostics, the urge for its rapid, accurate and cost-effective quantification still remains a field of research. According to the guidelines by the American Diabetes Association (ADA), a person is termed diabetic if its blood glucose level exceeds 6.1 mM¹⁷⁹. In a recent article by Wu et al.¹⁸⁰, Ag conjugated MIL-101 (Fe) was used for colorimetric sensing of blood glucose. The conjugate behaved as a peroxidase mimic nanozyme which in presence of H₂O₂ (released from glucose oxidation by glucose-oxidase (GOx)) caused oxidation of TMB into ox-TMB to yield colorimetric response at 652 nm (**Figure 1.6A**). The LDR of the detection platform to glucose levels was found to be 0.5 to 50 μM with an LOD of 0.23 μM . The response was verified and reliability was checked using actual human serum samples. Apart from MOF composites acting as enzyme, MOFs have

also been used as enzyme immobilization units for sensitive glucose detection. In a study, defected ZIF-8 based biohybrid hydrogel containing GOx was used to release H_2O_2 from glucose oxidation. This release further cascaded into another process where peroxidase enzyme labelled with rhodamine-B fluorophore converted ABTS into ABTS^+ which was analysed using a mobile application. This diagnosis met the quantitative range of 0.05-4 mM glucose level¹⁸¹. Label-free colorimetric sensing of glucose by exploring peroxidase-like behaviour of MOFs like Cu-MOF@Pt and IrNCs@Ti-MOF has also been done recently where the LODs were 0.42 mM and 20.0 μM , respectively^{147,182}. Adenosine diphosphate (ADP) and Adenosine triphosphate (ATP) are two cellular metabolites whose ratio indicates cellular energy status and regulates various cell signalling activities. Their concentration determination can be quite informative for early diagnosis of diseases including cancers¹⁸³. Recently, Wang et al.¹¹⁸ proposed an ultrasensitive quantification system for simultaneous sensing of ADP and ATP (**Figure 1.6B**). They fabricated a system comprising of clustered regularly interspaced short palindromic repeats (CRISPR) and associated proteins (Cas)12a integrated to ZIF-90 MOF nanocomposite encapsulating oleic acid capped $\text{Ag}_3\text{AuS}_2@\text{Fe}_3\text{O}_4$. The ADP aptamer binding with the MOF composite was facilitated by amidation between amino group of the former with the latter's carboxyl group. In presence of ADP nucleotide target, it bound to the aptamer forming an ADP/aptamer complex, releasing and activating the Cas12a. It was followed by binding of the Cas12a/crRNA and thereafter cleavage of the ssDNA reporter, exhibiting a strong fluorescence emission signal at 520 nm within an LDR of 0.03 to 5 nM with an LOD of 0.022 nM. On the other hand, the ATP competed with Zn^{2+} to cause ZIF-90 splitting and release of the encapsulated composite from its cavity. These composite-constituents led to quenching of fluorescence intensity which was caused by polyethyleneimine-modified carbon dots. Linear relationship was established between the fluorescence intensity at 450 nm and ATP concentration with an LDR of 0.1 to 5 nM with an LOD of 0.079 nM. The biosensor

applicability was tested in diluted serum samples spiked with different concentrations of ADP and ATP to compare with commercially available detection kits. This shows the fruitfulness of these robust materials in the sensitive recognition of small molecules from different domains.

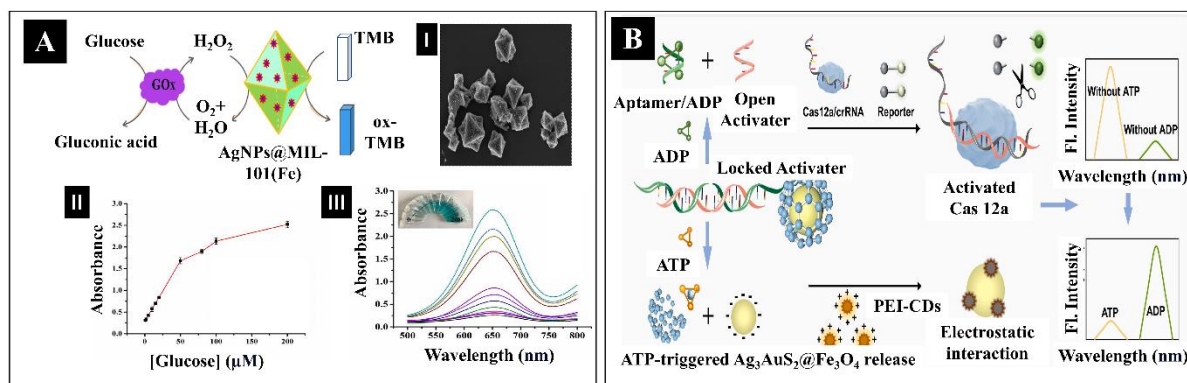


Figure 1.6. *A)* Diagrammatic representation of MOF encapsulated with silver nanoparticles for glucose sensing through colorimetry *I)* SEM image of AgNPs@MOF hybrid. *II)* Calibration curve of absorbance vs glucose concentrations. *III)* Absorption spectra of ox-TMB (at 652 nm) for different glucose concentrations. Reprinted with permission from Wu et al. [180], copyright 2022. *B)* Pictorial illustration for simultaneous quantification of ATP and ADP using a ZIF-90 composite. Reprinted with permission from Wang et al. [118], copyright 2022.

Recently reported MOF-based biosensors for detection of small molecules have been comprehensively discussed in **Table 1.2** below with their respective target analytes, detection techniques, fabrication strategies, real samples considerations and detection efficiency parameters (LDR and LOD).

Table 1.2. Detailed list of latest MOF-based biosensors for sensing of small molecules. (N.R.: Not Reported)

S.No.	Target Analyte	Detection Mechanism	Sensor Composite	Fabrication strategy	Mechanistic Role of MOF	Real Sample	LDR	LOD	Reference
1.	Glucose	CA, EIS	SWNT-MOF(Cu)	Nanotube catalytic ink was prepared to further dip coat a gold wire. It was followed by nanotube synthesis using CVD and electrodeposition.	Increase in electroactive surface area	Whole blood and milk	1 to 3000 μ M	0.16 μ M	C. Nandhini et al. ¹⁸⁴
	Glucose	CV, EIS, CA	NiCo-MOFs	NiCo-BTC nanosheets were synthesized through solvothermal method. The sensor system was synthesized through magnetron aided sputtering of concentric circular pattern on a PET matrix.	Excellent electrical conductivity and faster ion mobility	Sweat	10 to 200 μ M	10 μ M	Zha et al. ¹⁸⁵
	Glucose	EIS, CV	ZIF-8	PET substrate was modified by bonding it to hydrogel. The PET surface was functionalized with Aam, BIS, APS, TEMED, SA, GS NPs as monomers, cross-linker, initiator, accelerator, crosslinking agent and crosslinking metal ions source, respectively.	Encapsulate protein into the matrix	Honey	0.75 to 4 mg/mL	N.R.	Wang et al. ¹⁸⁶
	Glucose	CA	Cu-MOF (HKUST-1-MWCNTs)	Au electrode was pre-treated with H ₂ O ₂ and H ₂ SO ₄ mixture. Then, it was electrodeposited with Pt and DA+HKUST-1-MWCNTs+GOx the oxidation of DA was triggered by H ₂ O ₂ . EP tube was used to avoid water evaporation.	Offers high porosity to improve the active specific surface area of enzyme	Human serum	0.005 to 7.0 mM	0.12 μ M	Chen et al. ³¹
	Glucose	CA	ZIF-8@ZIF-67	Polyimide film was used as substrate for flexible electrode fabrication. PI film was transformed into graphene using universal CO ₂ pulsed laser (LIG) followed by spray coating H-NPC solution. Temperature/pH, glucose and lactate sensors were fabricated by different functionalization.	Source of nanoporous carbon (NPC) materials	Human sweat	0.3 μ M to 1.5 mM	0.025 μ M	Asaduzza man et al. ¹⁸⁷

	Glucose	Colorimetry	M-PCN-222 where, M = Mn, Fe, Co, and Ni	Microwave-assisted hydrothermal synthesis of MOFs was done which then exhibited improved peroxidase-mimicking activity. Presence of Fe-PCN-222, H ₂ O ₂ , and TMB, resulted into oxidation of TMB into blue colored ox-TMB. GOx was introduced in the system for glucose sensing.	Contains porphyrin active sites exhibiting potential biomimetic catalytic activities	Milk, Orange juice, Grape juice, human tears	Fe-PCN-222:1 to 80 μ M	Fe-PCN-222: 0.4 μ M	Shu et al. ²⁹
	Glucose	Colorimetry	IrNCs@Ti-MOF	IrNCs@Ti-MOF, TMB and varying concentrations of H ₂ O ₂ were added into sodium acetate-acetic acid buffer to record chromogenic reaction.	Provides ideal support with high porosity, robustness	Rabbit serum	0 to 600 μ M	20.0 μ M	Mu et al. ¹⁴⁷
	Glucose	SPR	M-BDC (where, M = Cu, Mn, Ni, and Zr)	The synthesized M-BDC MOFs were hybridized with the Au surface embedded on the SPR chip sensor.	Capturing and co-localizing enzymes and receptors with the target biomolecule	Human serum	Bare Au:5 to 20 mmol/L Cu-BDC:1 to 20 mmol/L Mn-BDC:1 to 20 mmol/L Ni-BDC:1 to 20 mmol/L Zr-BDC: 0.1 to 20 mmol/L	Bare Au: 14.763 mmol/L Cu-BDC: 10.383 mmol/L Mn-BDC: 4.790 mmol/L Ni-BDC: 4.945 mmol/L Zr-BDC: 0.97702 mmol/L	Gumilar et al. ¹⁸⁸
2.	Cholesterol	DPV	BSA-AuNPs-MFOF	BSA-capped AuNPs were synthesized through sonication which were further embedded in MFOF	High S.area, Support matrix	Human whole blood, Egg yolk, Butter, Milk, Goat meat, Cheese,	250 nM to 3.0 mM	53.2 nM	Arul et al. ¹⁸⁹

						Yogurt and icecream, Beer and apple juice, live cells (RAW 264.7 cells)				
3.	L-cysteine	Colorimetry	IrNCs@Ti-MOF	IrNCs@Ti-MOF, TMB and varying concentrations of H ₂ O ₂ were added to a sodium acetate–acetic acid buffer solution to record chromogenic reaction.	Peroxidase-mimic activity	Rabbit serum	0 to 50 μ M	3.41 μ M	Mu et al. ¹⁴⁷	
4.	Glutathione	Colorimetry	Pi@Ce-doped Zr-based MOF	MOF composite was synthesized by the hydrothermal route and then combined with phosphate to enhance its oxidase-like activity.	Possesses strongly positive surface which combine with phosphate to enhances the composite's oxidase-like activity	Human serum	0.8 to 15 μ M	0.24 μ M	Wang et al. ¹⁹⁰	
5.	Dopamine	CA, CV,	MIL-53 (Fe) on carbonized seaweed	Dried seaweed was carbonized through pyrolysis onto which a ZnO nanomembrane was deposited through ALD through multiple cycles. <i>In situ</i> synthesis of MIL(Fe) was done on these substrates through solvothermal synthesis.	Outstanding electrochemical performance	Urine, Juice	1.0 to 200 μ M	0.73 μ M	Ke et al. ¹⁹¹	
	Dopamine	Co-reactant ECL, CV	Zn-tpMOF	Zn-tpMOF was synthesized through one-pot synthesis. The Zn-tpMOF acted as a luminophore in ECL reactions. It exhibited a strong blue-green luminescence with AIE characteristics. ECL emissions were enhanced using DABCO as the co-reactant.	ECL emitter with AIE property	Serum samples	10 nM to 1 mM	8.2 nM	Fu et al. ¹⁹²	

6.	Sialic Acid	CA	Co/Fe MOF	The sensor was constructed on an Au-SPE through the immobilization of Co/Fe MOF followed by functionalization with N-acetylneuraminic acid aldolase enzyme. The MOF was synthesized through solvothermal route.	Biorecognition element	GD3 ganglioside, HeLa cell lines	0.20 to 1.00 mM	0.026 mM	Kıyıkçı et al. ¹⁹³
	Sialic acid	PEC, Colorimetry, EIS	Au@PCN-224	Au@PCN-224 was synthesized and used along with Bi ₂ S ₃ (Au NPs@Bi ₂ S ₃) to form a sandwich like-arrangement on the electrode surface to detect SA aided by two pairs of cis-diol groups.	Inert electroconductivity and nanozyme activity	Human serum samples	5 to 1000 μ mol/L	1.44 μ mol/L	Cheng et al. ¹⁹⁴
7.	5-fluorouracil	ECL- RET, CV, EIS	Zn-MOF	Ultrasonication based Zn-MOF (energy donor) synthesis was followed with decorated by 5-FU on its surface. CoOOH nanosheets (energy acceptor) were decorated with 5-FU antibody.	Organic luminophore with low trigger potential	Human serum	0.001 to 1000 ng/mL	0.52 pg/mL	Peng et al. ¹⁹⁵
8.	Uric acid	CV, DPV, EIS	Co(II) MOF	GCE electrode surface was modified with Co(II)MOF along with an ink made from carbon black and ethanol.	Monolithic peroxidase mimic	Serum	20 to 350 μ mol/L	0.2 μ mol/L	Wang et al. ¹⁹⁶
9.	Bisphenol A	ECL, CV, EIS	CD-COF	CD-COF and Pt NPs were mixed then coated on the GCE surface mediated by chitosan followed by dropwise addition Fc-ssDNA onto surface of modified electrode.	Improving the ECL response	Water	1.0×10^{-14} mol/L to 1.0×10^{-5} mol/L	2.21 fM	Ma, et al. ¹⁹⁷
	Bisphenol A	CV, DPV	2D Cu-TCPP	2D Cu-TCPP was fabricated with Tyr assembled within it layer by layer. GCE was polished and drop-casted with the above Tyr@Cu-TCPP composite for electrochemical measurement.	Preserve biological functions of the assembled biomacromolecules	Milk, Plastic mineral water bottle samples	3.5 nM to 18.9 μ M	1.2 nM	Ma et al. ¹⁹⁸
10.	Trenbolone	ECL, CV	Eu-BDPO MOF	Au deposition on GCE electrode surface was followed by drop casting TB antibody solution. Then, TB standard substances followed by antigen@Eu-BDPO were dropped on electrode to complete the ECL biosensor assembly.	ECL luminophore	River water	10 fg/mL to 100 ng/mL in all three pH conditions.	pH 5.0: 3.95 fg/mL pH 7.4: 2.36 fg/mL	Zhao et al. ¹⁹⁹

								pH 8.5: 5.48 fg/mL	
11.	Hypoxanthine	LSV, CA	Al-TCPP(Zn) MOF	Al-TCPP(Zn) was spread over ITO surface followed by casting xanthine oxidase on its surface.	Photoactive material	N.R.	1 to 100 μ M	0.1 μ M	Wang et al. ²⁰⁰
12.	Ochratoxin A (OTA) Mycotoxin	EIS, CV, DPV	Co-MOFs	AuNPs were conjugated with Co-MOFs. Streptavidin was modified on composite surface followed labelling with DNA2. The GCE electrode was modified with Au NPs@Ti ₃ C ₂ MXene. Then, the above synthesized complex was dropped over the surface to complete electrode fabrication.	Electrochemical signal tag and internal reference probe	Red wine	1 to 50 ng/mL	0.31 fg/mL	Guan et al. ²⁰¹
	OTA	Chemiluminescence	UiO-66-NH ₂	Fe ₃ O ₄ @SiO ₂ @UiO-66-NH ₂ was used as the working solution. The sensor was a kind of an optical fibre-mediated homogeneous chemiluminescent immunosensor which could estimate the adsorption kinetics and isotherms.	Pre-enrichment of OTA in food matrices by OTA adsorption	Crushed wheat, corn, bread mixture	0.1 to 100 ng/mL	0.28 ng/mL	Wei et al. ²⁰²
13.	Kanamycin	EIS, ECL	Dy-MOF	Dy-MOF was used as a reaction accelerator, with Gd(III) complex GdPc(acac) as an ECL emitter, all assembled on a GCE electrode.	Co-reaction accelerator	Milk	0.001 pg/mL to 1000 ng/mL	0.3 fg/mL	Duan et al. ²⁰³
	Kanamycin	PEC	Cu-MOF	ITO electrode was coated with Cu-MOF through drop-casting. Then, HP1 DNA was coated over it later being blocked with mercaptohexanol. Different concentrations of cDNA was added dropwise finally functionalizing with SP DNA@ZIS/ZnS QDs@Au-Ag NPs.	Good PEC material showing cathodic photocurrent	Human serum, Milk	1 pM to 1 μ M	0.34 pM	Du et al. ²⁰⁴
14.	Lactate	CA	ZIF-8@ZIF-67	Polyimide film was used as substrate for flexible electrode fabrication. PI film was transformed into graphene using universal CO ₂ pulsed laser (LIG) followed by spray coating H-NPC solution. Temperature/pH, glucose and lactate sensors were fabricated by different functionalizations.	Source of nanoporous carbon materials	Human sweat	0 to 56 mM	4 μ M	Asaduzzaman et al. ¹⁸⁷

15.	H ₂ S	Fluorescence	RhB/UiO-66-N ₃ MOF	RhB/UiO-66-N ₃ MOF acted as a reaction-based ratiometric fluorescent nanosensor. The MOF acts as a platform for accommodation of guest molecule (H ₂ S) triggering reduction of -N ₃ to -NH ₂ emitting blue fluorescence which enhances with increasing concentration of H ₂ S.	Inherent photophysical property and porosity with respect to the guest-encapsulating ability	HepG2 cells	0.05 to 5 mM	29.9 μM	Gao et al. ²⁰⁵

Abbreviations: PET: Polyethylene Terephthalate; AAm: Acrylamide; APS: Ammonium persulphate; TEMED: N,N,N',N'-tetramethylethylenediamine; SA: Sodium Alginate; SPE: Screen Printed Electrode; GS NPs: GOx@ZIF-8 NPs; ALD: Atomic Layer Deposition; AIE: Aggregation-induced Emission; MWCNTs: Multiwalled Carbon Nanotubes; DA: Dopamine; SPR: Surface Plasmon Resonance; 5-FU: 5-Fluoro Uracil; ITO: Indium Tin Oxide; LSV: Linear Sweep Voltammetry

3.1.3. Macromolecules

Macromolecules are high molecular mass compounds comprising of small repetitive monomeric units. The most common biological molecules of this class are proteins, RNA or DNA. However, being a polymer is not a mandatory criterion for these to be called ‘macro’ molecules since many heterocyclic compounds such as lipids also fall under them. They play a crucial role as biomarkers, hormones or enzymes in the living system²⁰⁶. The below segment describes a few of such macromolecules which have been detected using MOF-based electrochemical and optical transducing systems in recent times.

3.1.3.1. MOF-based Electrochemical sensors for macromolecules detection

p53 gene, a transcription factor which plays crucial role in activation and expression of numerous genes and microRNA, is also a tumour suppressor²⁰⁷. Its mutation causes a loss in its physiological function leading to promotion of tumour metabolism. Therefore, it can be found in almost half of cancerous tumours in tissues of breast, lung and colon²⁰⁸. Various biosensing approaches have already been adopted for its detection in recent times^{209,210} but either have complicated operation or suffer with a narrow LDR. In a recent article by Lv et al.²¹¹ on MOF based electrochemical biosensor development for p53 detection, Cr-doped MIL-101-NH₂ MOF was fabricated to act as a hybrid sensor with target responsive releasing and self-catalytic properties (**Figure 1.7A**). The MOF encapsulated methylene blue (MB), a signal probe, which was sealed within MOF cavity by a hairpin (HP) DNA acting as a gatekeeper, covalently immobilized on it. On encountering the aptamer, it hybridized to form dsDNA which had a site for cleavage of HP by the nicking endonuclease, to open the pore thereby releasing MB. The rich G-sequences in the cleaved HP formed a Hemin/G-quadruplex DNAzymes which would combine with MOF to catalyse MB in a redox reaction to observe a signal amplification. The electrochemical detection used DPV in a voltage range from -0.5V to 0V. The current

signals generated from released MB corresponded to p53 concentration in a range of 1×10^{-14} to 1×10^{-7} M with an LOD of 1.4×10^{-15} M. This technique can also be adopted for sensitive detection of diverse DNA sequences with specific recognition sites as well.

Insulin, being one of the most important peptide hormones of the body, caters the need for glucose metabolism which thereby regulates normal blood glucose level^{212,213}. Detection of insulin levels can be a holy grail for timely detection of diabetes. However, the prominent detection methods rely on expensive machinery and suffer from poor sensitivity and LOD^{214,215}. Therefore, electrochemical detection methods using aptasensors have now been into major limelight for its ultrasensitive and cost-effective detection. In a recent article by Sakthivel et al.²¹⁶, two MOFs (ZIF-L and Cu-BTC) were synthesized and made into composites to modify Au electrodes for insulin detection upto 0.027 pM within LDR of 0.1 pM to 5 μ M (**Figure 1.7B**). Briefly, the electrode was fabricated by dropcast method where the MOF composite was dropped on to disposable Au electrode surface followed by further surface modification using insulin aptamer. 6-mercaptop-1-hexanol solution was utilized for blockage of remaining active sites. Insulin solution with varying concentrations was prepared and dropped over modified electrode surface to record the corresponding aptamer mediated reaction using DPV.

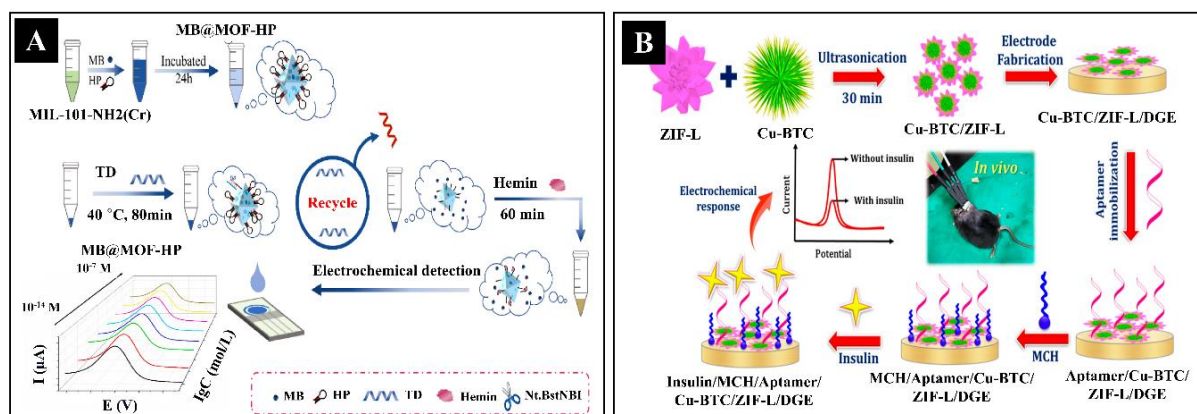


Figure 1.7. **A)** Diagrammatic representation of MIL-101-NH₂ synthesis followed by electrochemical sensing of p53 of varying concentrations in PBS buffer solution. Adopted with from Lv et al. [211], copyright 2022. **B)** Schematic representation of 2D leaf-like ZIF-aptamer sensor for detection of insulin. The DPV response was recorded at several insulin

concentrations followed by real time insulin monitoring. Reprinted with permission from Sakthivel et al. [216], copyright 2022.

3.1.3.2. MOF-based Optical sensors for macromolecules detection

microRNAs are small, non-coding RNAs with a dimension of about 22 nucleotides, that act as gene expression mediators, modulating cellular signalling pathways through mRNA degradation and translational inhibitions^{217,218}. The expression levels of these miRNAs play a pivotal role in prognosis and diagnosis of different diseases with its mis-regulation also relating to cancers^{219,220}. In a latest study on detection of microRNA in prostate cancer cell lines, an assembly of carbofluorescein FAM-Probe21 with NMOF-FA was made to facilitate fluorescence sensing²²¹. Briefly, UiO-66-NH₂ MOF was conjugated to folic acid, followed by mixing it with FAM-Probe21. The expression level of miRNA was determined for different prostate cancer cells like DU145, PC3 and LNCaP of which PC3 is well known for its high expression of folic acid receptors. Fluorescence studies revealed that the target miRNA released the dye-labelled oligonucleotide (Probe21) from the NMOF causing an increase in the fluorescent intensity which was quenched previously due to MOF encapsulation. Therefore, an increase in the miRNA concentration was found proportionate to the fluorescence intensity at 520 nm. The sensor probe was found to be sensitive towards miRNA detection within an LDR of 0 to 1000nM and an LOD of 10 pM.

Apart from RNA molecules, (circulating tumor DNA) ctDNA is also of clinical importance which is a primary triple-negative breast cancer biomarker at an early stage. Its quantitative detection is of significant prognostic and postoperative importance. In a recent work by Zha et al., an ultrasensitive sensing platform for ctDNA was developed using Nd-MOFs (**Figure 1.8A**)²²². The MOF nanosheet was functionalized with ionic liquid along with AuNPs. This MOF-AuNP combination facilitated higher photocurrent response along with a rise in overall surface area thereby increasing the probe active sites. The GCE/NdMOF@AuNP was functionalized with CP which resulted into generation of an “off signal” for ctDNA in visible

light conditions. Post encountering the ctDNA, ferrocene-labelled signalling probes were allowed to attach on the sensing probe which thereby generated a rise in current signals, observable through square-wave voltammetry, termed as a “signal-on”. The model system was found capable of detecting ctDNA in a concentration range of 1.0 fmol L^{-1} to 10 nmol L^{-1} as well as 1.0 fmol L^{-1} to 1.0 nmol L^{-1} for PEC and EC model systems, respectively. Two LODs were estimated for the two systems that were 0.63 fmol L^{-1} and 0.3 fmol L^{-1} .

Ulinastatin (UTI) is a glycoprotein-based protease inhibitor found in human urine²²³. Its role is in the enhancement of microcirculation by prevention of multiple protease generation¹²⁸. Its imbalance can lead to serious health risks which makes its ultrasensitive detection a clinical necessity. Electrochemiluminescence (ECL) can be an important technique for the purpose, using lanthanide-based MOFs (L-MOFs) as the ECL emitter. In another study by Li et al.¹²⁴, europium-based L-MOF has been synthesized via one-pot synthesis method and then, Eu-TATAB has been used as a chromophore for UTI detection and $\text{K}_2\text{S}_2\text{O}_8$ was taken as a co-reactant. The electrode fabrication process involved drop-casting of Eu-TATAB on a polished GCE surface followed by modification with 1-methylimidazole solution for activation of carboxyl group. Then, the surface was modified with antibody Ab_1 attachment followed by immersion of the modified electrode into UTI solutions of varying concentrations. The electrode was finally wrapped with $\text{Ab}_2/\text{CCB}/\text{MNPs}$ subjecting it to ECL detection in $\text{K}_2\text{S}_2\text{O}_8$. Characterization of the ECL sensor was done through EIS which showed a sharp decline in transfer resistance of the modified electrode when compared to bare GCE. The sensor detected UTI within LDR of 0.1 ng mL^{-1} to 10^5 ng mL^{-1} with an LOD of 9.7 pg mL^{-1} . The feasibility of the sensor was demonstrated in spiked real serum samples.

Amongst the clinically importance proteins, one of them is the C-reactive proteins (CRP) which is produced in the liver as a response to inflammation^{224–226}. Its synthesis is regulated by the action of IL-6 on gene accountable for its transcription at the site of wound or affliction²²⁷. This

pentameric protein rises in different pathological conditions ranging from edema, cardiac ailment, autoimmune disorders to even the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)^{228,229}. Therefore, its detection method development is on rise in recent times due to surging cases of CRP increase worldwide. In an article by Ali and Omer²³⁰, they fabricated an ultrasensitive optical biosensor comprising of an aptamer-functionalized Cu-MOF fluorescent nanozyme which detected CRP (**Figure 1.8B**). The biosensor can be operated on dual mode and could detect CRP with an LOD of 40 pg mL⁻¹ in fluorescence mode and 240 pg mL⁻¹ in the colorimetric mode. The POD activity of the Cu-MOF was exploited to oxidize TMB into ox-TMB generating blue color whereas, on addition of RNA to the MOF restricted its POD like-activity thereby diminishing the signal. An addition of CRP to the MOF/RNA composite caused a release of RNA from the MOF surface leading to consequential restoration of POD-like activity. The fluorescence-based method was found to be more sensitive towards CRP levels. COVID-19 patient serum samples spiked with the CRP protein with varying concentrations was used as the real sample for CRP detection.

Human Epidermal Growth Factor, a 185 kDa transmembrane glycoprotein complex, is overexpressed in most of the primary invasive breast cancers (BC) causing uncontrolled tumor growth and metastasis^{231,232}. A rise in its concentration in cancer patients to 15-75 ng mL⁻¹ is observed, which is highly elevated to its normal concentration of about 2-15 ng mL⁻¹²³³. So, development of detection methods for its (or its receptor's human epidermal growth factor receptor 2 (HER-2)) ultrasensitive quantification is of great clinical significance. Recently, a group of scientists have worked on fabrication of an ECL biosensor towards its detection using a novel ECL system comprising of ZMB/S₂O₈²⁻²³⁴. They have used AuNPs decorated graphite-like carbon nitride (g-C₃N₄@Au) as the sensing matrix that could function in a potential range of -1.2 V to 0 V to generate an ECL signal in presence of peroxydisulfate (S₂O₈²⁻) co-reactant. Amino-modified capture probes (CP) were immobilized on this complex via the Au-N bonds.

Additionally, a Zr-based MOF modified with boron carbon oxynitride (ZMB) was also synthesized which exhibited ECL intensity in the potential range of -2.0 V to 0 V with 0.8 mM $S_2O_8^{2-}$. For the fabrication of the ECL biosensor, a pre-treated GCE electrode surface was coated with g- $C_3N_4@Au$. Then on drying, it was incubated with CP solution followed by treatment with BSA to block the redundant non-specific binding sites. The surface was topped with dropping the tracer label to form the modified electrode for HER-2 detection. ECL experiments were performed in 0.1 M PBS (pH 7.0) containing 0.8 mM $K_2S_2O_8$. An ECL intensity which was initially hindered on addition of CP and BSA was greatly enhanced, in form of cathode ECL emission, on introduction of the tracer label. On addition of HER-2 solutions of varying concentration, the tracer label detached from the electrode surface resulting a decline in ECL emissions again. The biosensor worked well in an LDR of 0 fg mL $^{-1}$ to 100 ng mL $^{-1}$ with an LOD of 0.2 fg mL $^{-1}$. The applicability of the sensor was verified on real diluted human serum samples spiked with HER-2 solutions, exhibiting a good recovery of almost 95% . Hence, the discussion proves utility of MOFs in sensing matrices for ultrasensitive detection of macromolecules ranging from biomarkers to hormones and many more.

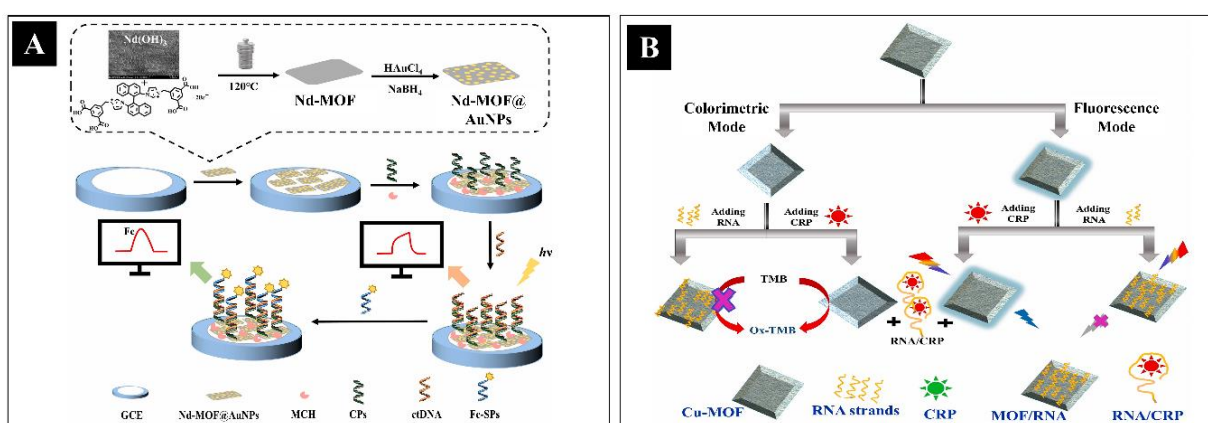


Figure 1.8. **A)** A schematic representation of Nd-MOF-based nanosheets capable of detecting ctDNA facilitated by gold nanoparticles and ionic liquid. Reprinted with permission from Zha et al.²²², copyright 2023. **B)** Graphical illustration of Cu-MOF functionalized with aptamer for ultrasensitive detection of C-reactive protein. Reused with permission from Ali and Omer [230], copyright 2022.

Recently reported MOF-based biosensors for detection of macromolecules have been comprehensively discussed in **Table 1.3** below with their respective target analytes, detection

techniques, fabrication strategies, real samples considerations and detection efficiency parameters (LDR and LOD).

Table 1.3. Detailed list of latest MOF-based biosensors for sensing of macromolecules. (N.R.: Not Reported)

S.No.	Target Analyte	Detection Mechanism	Sensor Composite	Fabrication strategy	Mechanistic Role of MOF	Real Sample	LDR	LOD	Reference
1.	Lincomycin	PEC	Heterostructured NiS@Ni ₃ S ₂ /CdS photoactive composite derived from a Ni-ZIF MOF	Sulfofication of Ni-Zr MOF was done to synthesize a photoactive composite which was modified further to form an aptamer-based (APT) sensing electrode based on a coupling reaction.	High S.Area, Superior photocurrent response	Milk	0.0001 to 1.25 nM	0.067 pM	Zhang et al. ²³⁵
2.	G-triplex mediated DNA of Mycoplasma bovine pneumonia	CV, EIS, DPV	NH ₂ -MIL-53(Al)	GCE surface was electrodeposited with AuNPs which were further immersed into PEG and MOF composite solution. The electrode surface was then drop casted with G3 DNA probe. Methylene blue and phenothiazine were used as electrochemical indicator	Adsorption matrix	10% goat serum	10 ⁻¹⁰ to 10 ⁻⁶ M	1.00 pM	You et al. ²³⁶
3.	Glutathione S-transferase	Colorimetry	Pi@Ce-doped Zr-based MOF	The MOF composite was synthesized by the hydrothermal method and then was combined with phosphate to enhance its oxidase-like activity.	Oxidase-like activity	Human serum	0.0002 to 0.006 U/L	0.19 mU/L	Wang et al. ¹⁹⁰
4.	Alpha-fetoprotein (AFP)	ECL, CV, EIS	NiZn MOF	NiZn as catalysts were synthesized through partial pyrolysis and then the surface was decorated with AuNPs followed by functionalization with anti-AFP proteins on its surface for selective detection of AFP.	Energy-sorption for catalysis	Human serum	5×10 ⁻⁵ to 10 ² ng/mL	0.98 fg/mL	Zhao et al. ²³⁷
5.	I27L gene	ECL	Eu-MOFs	A one-step room temperature synthesis approach was adopted for the Eu-MOF growth. It was then immobilized on a GCE electrode surface successively with AuNPs. Finally, H1(target DNA strand) was drop-casted on it for selective cleavage and detection of the target protein.	ECL emitter	Human serum	1 fM to 10 nM	447 aM	Dai et al. ²³⁸
6.	Exosomal piR-823	Ratiometric Fluorescence	AuNCs/Uio-66-NH ₂	Uio-66-NH ₂ MOF composite was synthesized by hydrothermal route and was subsequently conjugated with AuNCs through Au-N bond.	Support matrix	Human serum	0.04 to 4 pM	10.2 fM	Tong et al. ²³⁹

7.	Thrombin	Photoluminescence, ECL, CV, EIS	$\text{Ru}@\text{Ni}_3(\text{HITP})_2$	$\text{Ru}@\text{Ni}_3(\text{HITP})_2$ was adorned on the GCE, followed by dripping the surface with AuNPs. It was then followed by immobilization of TB aptamer on it. Thereafter, the ferrocene-labelled single-stranded DNA (Fc-S1) was added to conjugate with the TB aptamer to cause ECL signal quenching.	ECL-conductivity enhancer	Human serum	1 fM to 1 nM	0.62 fM	Zhang et al. ²⁴⁰
8.	Neuron-specific enolase (NSE)	ECL-RET, EIS	FeMOFs-sCuO	FeMOFs were prepared through hydrothermal synthesis. They were applied to load sCuO to obtain FeMOFs-sCuO nanocomposite which was used as a quenching probe for P-C ₃ N ₄ -CoPdNPs towards sensitive analysis of NSE.	ECL-RET dual quencher	Human serum	0.00005 to 100 ng/mL	20.4 fg/mL	Hu et al. ²⁴¹
9.	CRISPR/Cas1 4a-based <i>Burkholderia pseudomallei</i> specific DNA sequences detection	DPV, EIS, CV	PtPd@PCN-224	Solvothermal synthesis of PCN-224 was followed by <i>in situ</i> generation of PtPdNPs to facilitate its binding with P-ssDNA	Signal-amplification tag	Human serum	100 aM to 10 nM	12.8 aM	Li et al. ²⁴²
10.	Nanoscale exosomes	ECL, CV	TCPP/UiO-66-NH ₂	TCPP/UiO-66-NH ₂ synthesis was carried out through a one-pot solvothermal reaction followed by post-synthetic metalation with Zn ²⁺ ions. Fluorine-doped tin oxide (FTO) electrodes were coated with positively charged chitosan followed dipping the electrode in negatively charged AuNP solution. Thiol-modified CD63-binding peptide was drop casted on electrode surface.	ECL emitter	HepG2 cells derived exosomes added in exosome depleted fetal bovine serum (FBS)	1.00×10 ⁴ to 3.16×10 ⁶ particles/μL	9.08×10 ³ particles/μL	Wang et al. ²⁴³
11.	Glial Fibrillary Acidic Protein	CV, EIS	Au@ZIF-8@rGO	One-pot solvothermal synthesis of ZIF-8 was done. <i>In situ</i> AuNPs were synthesized in GO, ZIF-8 solution mixture to form the composite.	High S. area	Urine	50.0 to 10 000.0 fg/mL	50.0 fg/mL	Mehmandoust et al. ²⁴⁴
12.	MicroRNA	DPV	Co-MOFs	Co-MOFs were synthesized by solvothermal route and then functionalized with Streptavidin. In another set of experiments, the walker DNA (WD)/blocker DNA (BD) complex, with which miRNA-141 would react,	Signal probe	Human serum	1 fM to 10 nM	0.46 fM	Guan et al. ²⁴⁵

				were decorated on the HP1-functionalized Magnetic Beads' surface to complete probe fabrication.					
13.	α -Glucosidase, Alkaline phosphatase, and AAO	Colorimetry	AuNPs/UiO-66	UiO-66 MOF was prepared through solvothermal synthesis followed by composite formation involving MOF and AuNPs for the colorimetric analysis.	Enhanced peroxidase-like activity	Human serum	α -Glucosidase: 0.005 to 1.9 U/mL Alkaline phosphatase : 1.25 to 37.5 mU/mL AAO: 0.5 to 10 mU/mL	α -Glucosidase: 0.002 U/mL Alkaline phosphatase : 0.14 mU/mL AAO: 0.34 mU/mL	Liu et al. ²⁴⁶
14.	Acetylcholinesterase (AChE)	Colorimetry	Cu-CAT NSs (Copper chatecholate Nanosheets)	2D MOF was synthesized based upon Cu-CAT using dextran-assisted ultrasound exfoliation of the bulk Cu-CAT. The synthesized Cu-CAT nanosheets exhibited peroxidase like activity leading to AChE sensing.	Peroxidase-like activity	HA tablets	0.01 to 4.0 mU/mL	0.01 mU/mL	Wang et al. ²⁴⁷
15.	miRNA-10b	CV, EIS	NMOF@Cu ²⁺	NMOF coordinated with Cu ²⁺ was coupled with a 3D DNA walker. The carboxyl group in the organic ligand of NMOF assembled complementary DNA strand for biorecognition reaction.	DNA strand assembly, Catalysis	Human serum	0.8 pM to 250 pM	0.5 pM	Tang et al. ²⁴⁸
16.	miRNA-21	CV, LSV, DPV	ZIF8	Fluorine-doped tin oxide electrode was primarily electrodeposited with AuNPs with subsequent modification by GDH/NAD ⁺ followed by controlled etching and TA addition.	Encapsulation of moieties	Spiked healthy human serum, human normal liver cells (L-02 cells), human liver cancer cells (HepG2 cells), and human BC cells (MCF-7 cells)	0.01 to 1000 fM	2 aM	Yan et al. ²⁴⁹

17.	Amyloid β 42	Aggregation-induced ECL resonance energy transfer (AIECL-RET), CV	Au@UiO-66-NH ₂	GCE electrode was drop-casted with Ag@ZnPTC/BSA/Ab ₁ . A β 42 antigen solution of varying concentrations was added to the modified electrode surface. Then, finally Au@UiO-66-NH ₂ /BSA/Ab ₂ was dropped on its surface to complete the sandwich biosensor.	ECL emission acceptor and donor	Spiked human serum	10 fg/mL to 100 ng/mL	2.4 fg/mL	Yang et al. ²⁵⁰
18.	HBV DNA	ECL, CV	Al-MOF	Polished GCE surface was drop-casted successively with Al-MOF (synthesized through solvothermal method) and AuNPs. Then, it was dripped with S1 followed by DA-S2. Afterwards, various concentrations of HBV and Exo III were added to its surface successively. Post washing with PBS, the fabricated electrode was tested in HEPES buffer.	ECL activity	Human serum	100 aM to 10 pM	62.1 aM	Chen et al. ²⁵¹
19.	Exosomal miRNA-155	Surface Plasmon Resonance	Zr-MOF	Aptamer-engineered RBCs were labelled with PKH26 and exosomes with PKH67. The fluorescence-labelled engineered RBCs and exosome suspensions were visualized through microscope.	Complexation and strengthening of SPR response	Human plasma	1 fM to 1 $\times$ 10 ⁶ fM	2.03 fM	Fan et al. ²⁵²
20.	SARS-CoV-2 spike antigen	Fluorescence	MOF-5/CoNi ₂ S ₄	MOF-5 was adorned with CoNi ₂ S ₄ , followed with H ₂ TMP for the antigen detection.		Recombinant SARS-CoV-2 spike antigen	NR	5 nM	Rabiee et al. ²⁵³
21.	VEGF165	PEC, EIS	Nd-MOF	Nd-MOF/GCE electrode surface was introduced with dsDNA and ALP. Aptamer-antigen reaction led to aptamer shedding from electrode surface leading to decline in the charge-transfer resistance. Thereafter, ascorbic acid was yielded by the catalysis of l-ascorbic acid 2-phosphate which subsequently reduced HAuCl ₄ to produce AuNPs leading to localized SPR and VEGF165 sensing.	High S.Area	Human serum	0.01 to 100 ng/mL	3.51 pg/mL	Zhong et al. ²⁵⁴
22.	CEA	PEC	Cu-MOF	ITO electrode was coated with Cu-MOF through drop-casting. Then, HP1 DNA was coated over it later being blocked with mercaptohexanol. Varying concentrations of cDNA were added dropwise finally functionalizing with SP DNA@ZIS/ZnS QDs@Au-Ag NPs.	PEC material for cathodic photocurrent generation	Human serum, Milk	0.1 fg/mL to 0.1 ng/mL	0.045 fg/mL	Du et al. ²⁰⁴



Abbreviations:

S.Area: Surface Area; AuNcs: Gold Nanoclusters; TB:Thrombin; GO: Graphene Oxide; HA: huperzine A; GDH: Glucose Dehydrogenase, NADH: nicotinamide adenine dinucleotide; BC: Breast Cancer; TA:Tannic acid; RBCs: Red Blood Cells, CEA: Carcinoembryonic Antigen

3.1.4. In vitro cell detection

3.1.4.1. Prokaryotic cell detection

Bacillus anthracis is a dangerous pathogen known to cause severe pulmonary anthrax in mammals with a fatality of about 90%. Electrochemical and luminescence-based approaches have been readily adopted for recognition of bacterial markers such as 2,6-pyridine dicarboxylic acid (DPA) over other methods due to simpler operation and low cost. In a recent research by Liu et al.²⁵⁵, NH₂ functionalized Eu-based MOF was used for fluorescent-sensing of DPA molecules (**Figure 1.9A**). The MOF exhibited no luminescence in ethanol (MOF dispersive medium) but with addition of DPA, luminescence of both Eu³⁺ and amino ligand NH₂-BDC got recovered due to stable complexation of Eu³⁺ with DPA. Fluorescence intensity of amino ligand at 460 nm was found proportionate to DPA concentrations whereas that of Eu³⁺ at 705 nm shifted to 624 nm, with an LDR of 0-100 µM. Two LODs as 0.36 µM and 0.8 µM were reported for two different solvent systems water and ethanol, respectively. The values were much lower than infectious bacterial spore load for humans.

Another pathogenic bacterium, *Burkholderia pseudomallei* known for its high fatality infection in humans, is nowadays being detected sensitively using MOFs. In a recent article by Wang et al.²⁵⁶, this pathogenic species was detected using Co-doped MOF adorned with AuNPs and N-(4-aminobutyl)-N-(ethylisoluminol) exhibiting improved ECL activity as compared to previously reported biosensors. This was aided by its zero-distance catalytic properties. The LOD was found to be 60.3 aM with a detection capability as low as 9.0 CFU mL⁻¹. Briefly, the biosensor was prepared on GCE electrode with immobilization of Au@Co-MOF@ABEI on its surface followed by aptamer DNA strands which bound to dopamine and thereby quenched the ECL. Then another DNA strand attachment turned the signal “on”. The characterization of electrode was done using techniques such as EIS and CV at every step of electrode modification. LDR was found in the range of 10² to 10⁸ aM. On verification of biosensing

platform in serum samples containing the pathogen, it had a good applicability for detecting *B. pseudomallei* within a range of 1.5×10^1 to 1.5×10^5 CFU mL⁻¹.

Various microbial species are currently of public health concern including *Staphylococcus aureus* which is known to cause life-threatening illness including sepsis, acute renal failure, meningitis and gastrointestinal reaction²⁵⁷. It is often detected through techniques like qPCR and ELISA which are rapid but have high cost and low stability. Therefore, these shortcomings have been resolved through latest works by Chen et al.²⁵⁸. According to the study group, development of MOF-based aptasensor with colorimetry and electrochemical modes of detection was proposed. Cu-MOF conjugated with gold nanoparticles served the dual purpose of catalysis (in chromogenic reaction) and electrochemical response genesis by virtue of its Cu²⁺ dopant (**Figure 1.9B**). Screen-printed carbon electrode was modified with MB aptamer/ssDNA-Au/CuMOF and scanned in the potential range of 0.2-0.6 V with pulse amplitude of 50mV and pulse width of 0.05s for DPV response measurement. The peroxidase-mimicking activity was also explored taking TMB-H₂O₂ reaction system as a typical model system which has a distinct oxidation peak of 450 nm in presence of Au/Cu-MOF. The aptasensor indicated a good electrochemical response in a range of 10 to 10⁸ CFU mL⁻¹ with an LOD of 5 CFU mL⁻¹²⁵⁸.

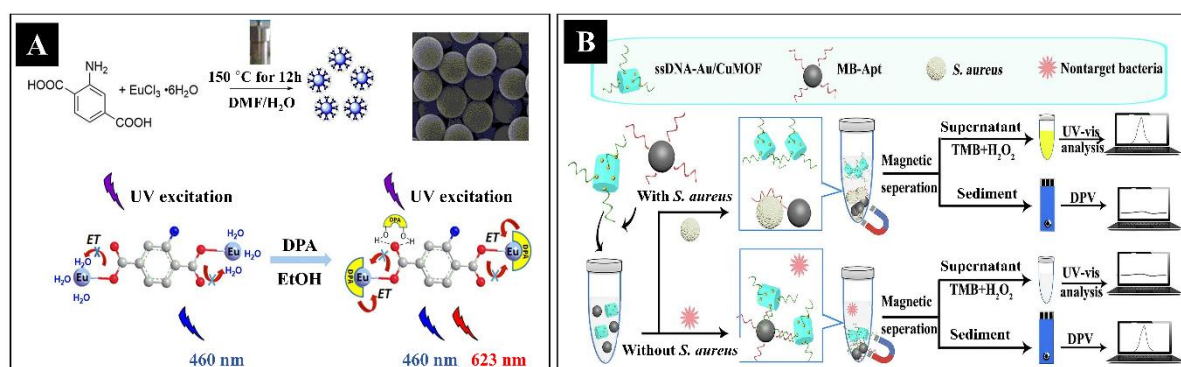


Figure 1.9. **A)** Graphical representation of Eu-MOF for detection of anthrax biomarker (DPA). Inset image corresponds to SEM result for NH₂-BDC-Eu-MOF composite. Reused with permission from Liu et al. [255], copyright 2022. **B)** Pictorial representation of multifunctional MOF-based dual mode (colorimetric and electrochemical) aptasensor for *S. aureus* detection. Reported with permission from Chen et al. [258], copyright 2023.

3.1.4.2. Eukaryotic cell detection

Breast cancer (BC) being one of the most common cancers affecting women globally, if diagnosed at an early stage is usually non-aggressive in nature. However, the aggressiveness of BC is attributed to over expressivity of multiple cell markers including HER-2^{259–262}. Therefore, identification of aggressive BC cells' phenotype can help in therapy and its timely prognosis. Addressing the problem, Wang et al.²⁶³ in their recent study have developed an electrochemical biosensor using a MOF-based probe AuNPs@HRP@ZIF-8 (**Figure 1.10A**). They synthesized HRP-encapsulated ZIF-8 which was further modified using AuNPs. HER-2 protein is a transmembrane tyrosine kinase receptor belonging to the family of epidermal growth factor receptors (EGFR or ErbB). It is overexpressed in different cancer types such as ovarian, gastric, breast and oral^{264,265}. The ZIF-8 surface used in the study catered attachment of peptides specific to target protein HER-2 and MMP-2 due to presence of numerous active sites. Then this recognition sequence was immobilized on indium tin oxide (ITO) electrode as well. On encountering BC cells, the AuNPs@HRP@ZIF-8 was cleaved off leading to target protein detection. The sensing performance was studied using CV and EIS measurements. DPV was also performed from 0.5 V to -0.1 V and i-t curve was recorded at -0.1 V. The results revealed detection range for HER-2 and MMP-2 from 50 fg mL⁻¹ to 50 ng mL⁻¹ and 10 fg mL⁻¹ to 10 ng mL⁻¹ with an LOD of 16.8 fg mL⁻¹ and 5.33 fg mL⁻¹, respectively. The results were verified on real samples to detect the phenotype of four different types of BC cells to quantify these markers. SK-BR-3 and BT-474 cells were found to be more aggressive on the level of HER-2 expression when compared to MCF-7 and MDA-MB-231 triple-negative BC cells. In another study on electrochemical fluorescence-based CDs@ZrHf-MOF, CDs were embedded in the MOF cavity²⁶⁶. The sensor possessed a very low detection limit of 19 fg mL⁻¹ for HER-2 protein and 23 cell mL⁻¹ for MCF-7 cells, with their respective detection ranges of 0.001-10 ng mL⁻¹ and 1×10² to 1×10⁵ cell mL⁻¹. The aptasensor fabrication involved drop-casting of

CDs@ZrHf-MOF suspension on pre-treated Au surface succeeded by incubation with HER-2 aptamer solution to embed the aptamer on its surface. EIS, CV and DPV measurements were carried out to validate sensor assembly at each step. On interaction of HER-2 with the aptamer, complex formation took place which resulted to an increase in the R_{ct} value from 0.261 k Ω to 0.480 k Ω . Apart from BC, cancer cases in general are on rise with colorectal cancers (CRC) contributing a major part of gastrointestinal malignancies globally^{267,268}. Electrochemical method when compared to other diagnosis approaches is highly time efficient, low cost and fluorescent-label free with aptamer-based ones gaining more attention^{269,270}. In a study by Duan and group²⁷¹, CT26 cells present in CRC were selectively detected using Cr-MOF/CoPc hybrid immobilizing cell-targeted aptamer. Au electrode was drop-casted with Cr-MOF@CoPc-2 followed by immersion into aptamer solution. Finally, the modified electrode was dipped into CT26 cell suspension. EIS and DPV studies showed LOD values for the cells to be 36 and 8 cells mL⁻¹ which was further verified through real sample analysis using human serum samples spiked with CT26 cells of varying concentrations.

Circulating tumor cells (CTCs) are easy and less painful source of cancer detection when compared to conventional tissue biopsy²⁷². However, the challenge lies in their rare abundance in blood circulation with only 1-10 circulating cells mL⁻¹ on an average amid a lot of significant interferences surrounding them²⁷³. Recently, Zhang et al.²⁷⁴ have proposed a dual-mode aptamer based electrochemical and fluorescent sensor detecting MCF-7, HepG2, MB-468, HeLa, and MCF-10A cell types (**Figure 1.10B**). The Mapt-EF sensor, fabricated on ITO electrode used Co-Fe bimetallic MOF which was capable of catalysing the disintegration of H₂O₂ to release oxygen. An aptamer chain was designed with a dual purpose of capturing three cancer biomarkers namely, PTK7, EpCAM and MUC1 along with inhibiting the catalase like-activity of the MOF. The ITO electrode was negatively biased to adsorb positively charged compound, MB and TMB molecules imparting high fluorescence and electrochemical signal intensity. CV-

based sensor characterization was done followed with checking its applicability in real blood sample. The sensor was found capable of detecting upto a single cell in the medium with an LOD of 11, 9, 10, 10, 11 cells mL⁻¹ for HepG2, HeLa, MCF7, MDA-MB-468 and MCF-10 BC cells, respectively. Colorimetry-based CTCs detection has also been demonstrated by another group using CuFe-NH₂-MIL-101 MOFs²⁷⁵. The MOF possessing peroxidase-mimicking activity derived its fluorescence ability in the excitation wavelength range of 280-410 nm from Cu present in the MOF composite. EpCAM receptors on the HCT116 cells were used as the transmembrane cell surface glycoprotein which were targeted using specific antibodies to capture the cells. The fluorescence intensity was governed by binding of CuFe-NH₂-MIL-101 with EpCAM antibody. Moreover, the colorimetric response increased with increase in the cell concentrations contrary to the fluorescence readout which decreased over time. The sensor was capable enough to detect cells in the range of 10² to 2×10⁶ cells mL⁻¹ with the LOD being 12 cells mL⁻¹.

Therefore, as per the detailed discussion in the above section it can be emphasized that these MOFs have been a material of choice in recent times for biosensor probe fabrication for detection of all kinds of cells. They can be an optimal sensing matrix when conjugated with cell specific antibodies for ultrasensitive cell detection.

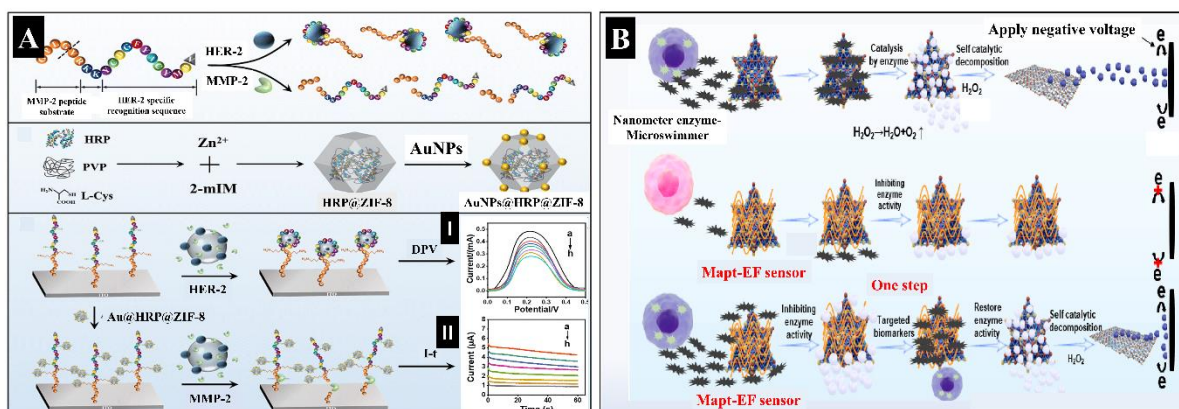


Figure 1.10. A) Diagrammatic illustration depicting MOF-based biosensor for identification of aggressive BC cells using overexpressed HER-2. I) DPV curve for analysis of HER-2 at

varying concentrations. II) Amperometric response for varying concentrations of MMP-2. Adopted from Wang et al. [263], copyright 2023. B) Diagrammatic representation of Co-Fe-MOF for simultaneous detection of multiple cancer markers for CTC capturing, identification and counting. Reprinted with permission from Zhang et al. [274], copyright 2023.

Recently reported MOF-based biosensors for detection of prokaryotic as well as eukaryotic cells have been comprehensively discussed in **Table 1.4** below with their respective target analytes, detection techniques, fabrication strategies, real samples considerations and detection efficiency parameters (LDR and LOD).

Table 1.4. Detailed list of latest MOF-based biosensors for sensing of cells (prokaryotic and eukaryotic). (N.R.: Not Reported)

S.No.	Target Analyte	Detection Mechanism	Sensor Composite	Fabrication strategy	Mechanistic Role of MOF	Real Sample	LDR	LOD	Reference
1.	<i>Salmonella typhimurium</i>	Colorimetry	MIL-88@Pd/Pt	MIL-88@Pd/Pt composite was synthesized by <i>in situ</i> growth of Pd, Pt NPs over the surface of MIL-88 MOF. The complex acted as a peroxidase mimic which along with MNBs caused specific separation and labelling of target pathogen.	Peroxidase mimic and high S.Area	Chicken meat supernatant	4×10^1 to 4×10^5 CFU/mL	32 CFU/mL	Yuan et al. ²⁷⁶
	<i>Salmonella</i>	Colorimetry	NH ₂ -MIL-101(Fe)	Modified magnetic nanobeads MNB- <i>Salmonella</i> -MOF sandwich complexes were made which were used to catalyse o-phenylenediamine to generate colorimetric response.	High S.Area	Spiked chicken meats	1.5×10^1 to 1.5×10^7 CFU/mL	14 CFU/mL	Qi et al. ²⁷⁷
	<i>Salmonella</i>	Colorimetry	ZrPr-MOF	Zirconium praseodymium MOF acted as nanozyme to detect the pathogen by converting TMB to ox-TMB, liberating colorimetric response.	Peroxidase-mimic activity	Water, Milk	10^2 to 10^8 CFU/mL	37 CFU/mL	Mirsadoughi et al. ²⁷⁸
2.	<i>Escherichia coli</i> (<i>E. coli</i>)	PEC, CV, EIS	In ₂ O ₃ /CeO ₂ @mNC	PolyMOF(Ce) were hydrothermally synthesized to adsorb trace indium ions (In ³⁺). Further the complex was calcined at high temperature forming In ₂ O ₃ /CeO ₂ @mNC hybrids.	High S. area, large pore size, multiple functionalities for PEC	Raw milk, Bread	10^{-1} to 10^7 CFU/mL	1.12 CFU/mL	Wang et al. ²⁷⁹
	<i>E. coli</i>	Photoluminescence	NH ₂ -MIL-53(Fe) MOF	NH ₂ -MIL-53(Fe) MOF-metal composite was synthesized and conjugated with galactose and mannose sugars.	Luminescent property and high S. area	Bacteria culture cells	19 to 10×10^6 CFU/mL	8 CFU/mL	Bhatt et al. ²⁸⁰
	<i>E. coli</i>	Fluorescence	Zr-UiO-66-B(OH) ₂	The MOF was functionalized with boric acid to adhere bacterial cells. Ab ₁ -MNP-target bacteria-Zr-UiO-66-B(OH) ₂ complex was created and dispersed in H ₂ O ₂ solution for quantitative estimation of bacteria.	Photocatalytic inactivation of bacteria, fluorescence properties	Drinking water, Milk	5.0 to 2.5×10^4 CFU/mL	1.0 CFU/mL	Zuo et al. ²⁸¹

3.	<i>Staphylococcus aureus</i> (<i>S. aureus</i>)	EIS, DPV	ML-Cu ₂ O@Cu-MOF	A suspension of ML-Cu ₂ O@Cu-MOF was dropcasted on a pre-treated Au electrode surface which was later incubated with a targeting aptamer.	Anchoring ability to aptamer, conductivity	Milk, honey, and biscuit	0 to 1×10 ⁸ CFU/mL	1.6 CFU/mL	Tian et al. ²⁸²
4.	<i>P. aeruginosa</i>	Photoluminescence	NH ₂ -MIL-53(Fe) MOF	NH ₂ -MIL-53(Fe) MOF was synthesized and conjugated with galactose and mannose sugars	Luminescent property and high S. area	Bacteria culture cells	170 to 33 × 10 ⁷ CFU/mL	202 CFU/mL	Bhatt et al. ²⁸⁰
5.	SARS-CoV-2	Colorimetry	MIL-101(CuFe)	CD147-modified MIL-101(CuFe) was prepared which oxidized TMB, yielding ox-TMB in the presence of H ₂ O ₂ , to generate a colorimetric response.	Peroxidase-like activity	SARS-CoV-2 pseudovirus in human serum and real virus sample	SARS-CoV-2 pseudovirus in human serum: 0.003 to 48.9 µg/mL; real virus sample: 10 to 10 ⁴ PFU/mL	SARS-CoV-2 pseudovirus in human serum: 0.09 ng/mL; real virus sample: 3 PFU/mL	Zhao et al. ²⁸³
6.	Cauliflower mosaic virus 35S promoter (CaMV 35S)	PET, FRET	Fe-MIL-88	The FAM-ssDNA probe was fabricated complementary to the targeted CAMV 35S. In its absence, the FAM ssDNA got adsorbed on surface of the Fe-MIL-88 MOF causing a fluorescence quenching event.	High S. area, greater quenching and DNA binding abilities	Transgenic plant genomic DNA	5 pM to 50 nM	0.184 pM	Liu et al. ²⁸⁴
7.	Breast cancer cells (HER-2, MMP-2)	EIS, CV	AuNPs@HRP@ZIF-8	ZIF-8 based probe was modified with AuNPs for binding with the target peptide (Au-S, Au-N bonding) and HRP.	High S. area	BC cells	HER-2: 50 fg to 50 ng/mL MMP-2: 10 fg to 10 ng/mL	HER-2: 16.8 fg/mL MMP-2: 5.33 fg/mL	Wang et al. ²⁶³



Abbreviations:

S.Area: Surface Area; MNBs: Magnetic Nanobeads; PET: Photoinduced electron transfer; FRET: Fluorescence resonance energy transfer; HRP: Horse Radish Peroxidase; BC: Breast Cancer

3.2. Electrochemical MOF-based Biosensing matrices for Disease Diagnosis

In recent times, the convergence of nanotechnology, materials science, and bioengineering has led to revolutionary progress in the advancement of biosensors, heralding a new age of accurate diagnosis and personalized healthcare ^{285,286}. The increasing worldwide prevalence of infectious and fatal diseases, including those that can be transmitted and others that cannot, highlights the dire need for diagnostic methods that are simple, fast, accurate, and affordable ²⁸⁷. Conventional laboratory diagnostic methods do not meet these requirements, thereby requiring novel technologies which are capable of tackling the difficulties such as early diagnosis, continuous monitoring, and point-of-care testing ^{288,289}. Electrochemical biosensors have emerged as plausible solutions overcoming previous methodological setbacks incorporating a plethora of nanomaterials ²⁹⁰.

Among the several materials investigated for the development of sensors, MOFs have emerged as an extremely promising category of materials with distinct features that make them well-suited for use in electrochemical sensing applications ²⁹¹. MOFs, distinguished by their elevated surface area, flexible porosity, and effortless functionalization have improved performance and specificity of sensors to many folds ²⁹². One of the key characteristics of MOFs is their modular structure, consisting of metal nodes and organic linkers, which enables the customization of their properties for the development of sensors that can detect a wide array of proteins and other molecules linked to different diseases ^{293,294}. The combination of the diverse attributes of MOFs and the responsiveness of electrochemical methods has facilitated the advancement of sensors that have improved analytical capabilities, establishing them as preferred materials in the field of healthcare ²⁹⁵. One fundamental aspect of electrochemical sensors based on MOFs is their application in different sensing methods, such as amperometry ²⁹⁶, voltammetry ²⁹⁷, and impedance spectroscopy ²⁹⁸. Such integration enables the identification of biomarkers at extremely low levels, hence allowing for the timely detection

of diseases such as diabetes, cancer, cardiovascular, pulmonary, neurological disorders and infectious conditions ²⁹⁹.

Due to their ease in production and ability to work well with small electronic devices, MOF-based electrochemical sensors have great potential in the advancement of portable and user-friendly diagnostic systems. Commercial availability of MOFs such as ZIF-8, MIL-53(Al), HKUST-1, Fe-BTC and others is an imperative of MOF-enabled product demands in real world ³⁰⁰. Point-of-care testing (POCT) devices have recently been engineered with MOFs to simplify testing and improve analytical performance supplementing devices to overcome the challenges associated with antibody-associated POCTs ³⁰¹. The possibility of incorporating MOF-based sensors with smartphones and wearable technology enhances the versatility of these sensors for the detection of multiple biomarkers by virtue of organic functioning, extended shelf-life and thermal stability ³⁰².

The global burden due to infectious and fatal diseases is increasing and millions of mortalities are occurring from diabetes, viral infections, cardiovascular, pulmonary, and many others ³⁰³. Various studies on MOF-based electrochemical sensors for the detection of different disease-specific biomarkers have recently highlighted MOF-mediated POCT research progression. Li et. al described the MOF based electrochemical sensors solely for the detection of glucose ³⁰⁴, similarly Wang et. al in their study discussed about MOFs used for electrochemical sensing of viruses ³⁰⁵, hence limiting their study to a particular disease. Another study by Li et. al discussed about MOF based sensors for small, macromolecules and cells but their study mainly revolved around the synthesis and fabrication of MOF with a limited discussion on targeting biomolecules ³⁰⁶. Hitherto such studies evidently suggest the rising interest of researchers towards these molecules, our Scopus based analysis also backs the same. The studies that were limited to only a handful of reports on MOF-based electrochemical sensors in 2013 have risen to >200 annual articles. All such reports have emphasized on the role of MOFs in sensor

matrices through molecular perspective while a study encompassing their crucial role through perspective of a range of diseases and their relevant biomarkers is still lacking. This review focusses on the recent advances in different kinds of MOFs, their functionalization strategies, and applications in the realm of electrochemical sensors for disease diagnosis. We have thoroughly studied the role of MOFs as a matrix component in conjugation with different nanomaterials for their application in healthcare and human wellbeing. Additionally, we have discussed various globally developed MOF-based sensor matrices for disease diagnosis in tabulated form describing their target analyte, detection technique, sensor composite, fabrication strategy, mechanistic role of MOF, real sample, linear dynamic range (LDR) and limit of detection (LOD). Also, the challenges and future prospects of MOF-based electrochemical sensors have been discussed which allows the readers to ponder and eventually work towards innovative technological translations from the laboratory to the clinical settings. In addition to improvement of analytical performance, the incorporation of MOFs into electrochemical sensing platforms brings in a new era in point-of-care diagnostics. We hope that our critical analysis will add to the expanding amount of information highlighting the revolutionary potential of MOF-based electrochemical sensors for more accurate diagnosis and enhanced medical outcomes.

Selective and sensitive detection of disease biomarker in a complex biological sample is of utmost importance for early disease diagnosis and prognosis. Electrochemical signal sampling plays a key role in POCT devices where the use of miniaturized electrochemical sensors to detect a range of electrolytes is done by observing a change in current, potential or impedance caused by the redox properties of the analyte species ³⁰¹. Such changes in signals within the MOF-based POCT devices are brought up by MOFs' electronic and catalytic properties levied by the functional groups such as amine and nitro which are often associated with their linker components ⁷⁵. Not only this, Co, Ni and Cu centred MOFs have been found to be highly

electrocatalytic in nature which is attributed to their enriched electron transfer capabilities from their metal centers to their respective ligands ^{76,77}. With advancement in technology, MOFs have gained importance in biomarker detection due to enormous surface tailoring possibilities they offer, bestowing them with catalytic potential, functionalization sites, porosity and huge surface area facilitating molecular adhesions (Shubhangi et al., 2024).

Researchers globally have understood the importance of these porous structures in the field of fluorescence and electrochemical sensing matrices for disease diagnosis. Some of the major disease detection sensing probes with electrochemical MOF-based nanocomposites' constituents have been elaborated in the upcoming sections.

3.2.1. MOF-based electrochemical sensors for Diabetes

Diabetes accounts to almost 4.2 million deaths worldwide with a whopping number of patients aged between 20-79 years, contributing to 11.3% of global deaths ³⁰⁸. The World Health Organization in 2014 projected about >422 million world adult population to be suffering from diabetes mellitus with its prevalence on rise continuously ³⁰⁹. The current impact of this disease is maximal in low and middle-income nations which necessitates its prevention and early diagnosis on an urgent basis. Its consequential impact can be seen on different vital organs; however, its major impact can be governed through its primary marker being elevated blood glucose levels ³¹⁰. MOF-based glucose detection systems can be an enticing approach serving two major roles, either as signal transduction systems or, as a substrate for embedding enzymes and nanoparticles ³¹¹. MOF-based glucose sensors can be pristine or modified MOFs which can be used as a part of electrochemical sensing matrices.

In a recent study by Xu et al., catalytic potential of MOF in basic medium was illuminated ³¹². A MOF/ α -cyclodextrin-reduced graphene oxide (MOF/ α -CD-rGO) hybrid was synthesized on a titanium mesh where the Ni-based MOF acted as an electrocatalyst whereas, the rGO enriched

the composite's electrochemical performance (**Figure 1.11A**). The Ni(II)/Ni(III) redox couple assisted by the OH⁻ in the electrolytic solution oxidized glucose to form gluconolactone, thereby generating amperometric oxidation peak current responses at +0.55 V. Two LDRs from 0.65 μ M-4.828 mM and 4.828-9.178 mM with a LOD of 0.3 μ M was obtained with a rapid response time of 1.9s. Fetal bovine serum sample was used for evaluation of sensor practicability exhibiting high recoveries. Similar enzymatic potential was seen for a non-toxic Fe(II)-MOF in acidic epidermal sweat environment ³¹³. The 3D printed device had a MOF-based Fe-centered nanozyme immobilized on its working electrode (WE) surface which oxidized glucose to gluconolactone generating a defined anodic peak response at -1.2 V. An interesting observation in this case would be a shift in the analytical peak potential from +0.55 V to -1.2 V, from the former discussed sensor, with change in the pH of the electrolyte solution from basic to acidic. The carbon black-poly(lactic acid) (PLA) filament used as a WE which was drop-casted with a solvothermally synthesized Fe(II)-MOF, entrapped using a Nafion film (**Figure 1.11B**). The Fe(II) component of the MOF underwent a cascading reduction [Fe(0)] and thereafter oxidation [Fe(III)] by sweeping the potential between -1.4V to +2.0V. At the oxidized state, the Fe(III) enabled an oxidative conversion of glucose, thereby bringing back the oxidation state of Fe to its original. The sensor had an optimum LDR of 0-600 μ mol L⁻¹ and a LOD of 17.6 μ mol L⁻¹. It is interesting to note that such MOF-based sweat glucose sensors have been focussed upon recently for wearable electrochemical glucose monitoring devices as well. In a study by Xia et al., multiwalled carbon nanotubes/Polydimethylsiloxane (PDMS) based film electrode surface, stamped with CNTs, was functionalized with enzyme mimicking Ni-Co bimetallic MOF to fabricate a wearable glucose sensor ³¹⁴. In this case, the redox reaction occurred between the bimetallic centres Ni and Co in an alkaline medium where Ni(III) and Co(IV) species were converted to Ni(II) and Co(III) states respectively, thereby aiding the oxidation of glucose to gluconolactone at +0.5 V (**Figure 1.11C**). The highly

electrocatalytic coating endowed the sensor with a LDR of 20 μM -1.1 mM and a LOD of 6.78 μM . The non-invasive MOF-based sensors not only have been explored to estimate sweat glucose but also salivary glucose levels. In a recent work on conductive carbon paper layered with Ni-MOF, the probe was configured with extended gate field effect transistor for sensing glucose in a LDR of 20 μM -47 mM, working in physiological pH range at a potential of +0.7 V ³¹⁵. Such MOF-mediated analytical systems can be integrated with smart phone-enabled portable devices too to simplify and introduce the sensor application into public domain and better reachability ³¹⁶.

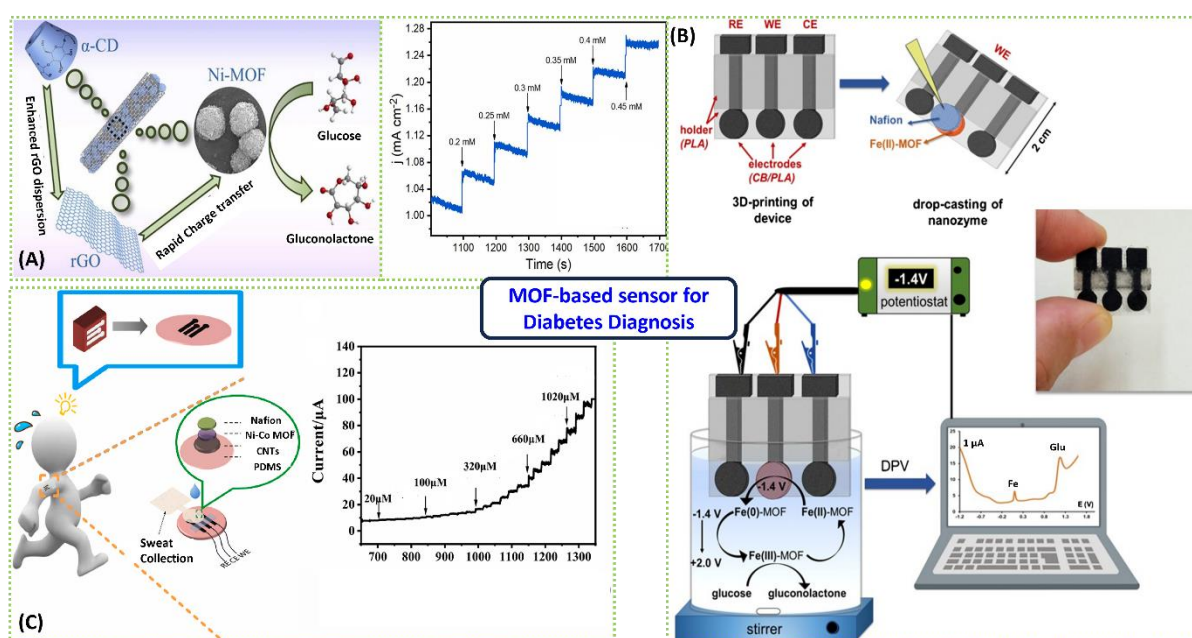


Figure 1.11. A) Schematic representation of Ni-MOF α -CD-rGO hybrid based electrochemical sensor for catalytic conversion of glucose to gluconolactone generating amperometric response (adopted from Xu et al., copyright 2022). B) Fe(II)-MOF based biocompatible nanozyme sweat glucose sensor fabrication strategy, reprinted with permission from Koukouviti et al., copyright 2023. C) Incorporation of Ni-Co bimetallic MOFs, as enzyme mimics, in glucose sweat sensor (adopted from Xia et al., copyright 2023).

In addition to these few representative examples there are several reports MOF-based biosensors for diagnosis of diabetes which have been comprehensively discussed in **Table 1.5** with their respective target analytes, detection techniques, sensor composite, fabrication strategies, mechanistic role of MOF, real samples considerations and detection efficiency parameters (LDR and LOD).

Table 1.5. Detailed list of latest MOF-based electrochemical biosensors for diagnosis of diabetes

S.No.	Target Analyte	Detection Technique	Sensor Composite	Fabrication Strategy	Mechanistic Role of MOF	Real Sample	LDR	LOD	Reference
1.	Glucose	Amperometry, CV	NF/NiCo ₂ O ₄ NWs@Co ₃ O ₄ NPs	NF was topped with NiCo ₂ O ₄ NWs which acted as a substrate for growth of NPs derived from Co ₃ O ₄ MOF to complete the electrode fabrication.	Good structural stability and high conductivity	Mouse serum	0.001-1.7 mmol/L	0.5 μ M	³¹⁷
2.	Glucose	Amperometry	Ni/Mn-LDH-MOF	GCE electrode was used as WE by simply drop-casting the Ni/Mn MOF-74 suspension solution dispersed in Nafion.	Electrocatalytic activity	Human serum	4.9 μ M-2.2 mM	0.87 μ M	³¹⁸
3.	Glucose	Amperometry, CV, DPV	Ni/Co MOF	Nitric acid treated CC was used as a substrate for growing Ni/Co(HHTP)MOF through the process of electrodeposition.	Large surface area, effective active sites, electrocatalytic activity	Human serum, tea, orange juice	0.3 μ M-2.312 mM	100 nM	³¹⁹
4.	Glucose	CV, Amperometry, EIS	Ni/Co-BTC MOF	Polished GCE was potential cycled in the presence of 0.1 M NaOH for 10 consecutive cycles. Then, the electrode was immersed in a solution containing Co(NO ₃) ₂ ·6H ₂ O, Ni(NO ₃) ₂ ·6H ₂ O, and KNO ₃ for a period of 200s followed by dipping into a solution of ethanolic H ₃ BTC to synthesize Ni/Co-BTC MOF on its surface.	Electrocatalytic activity	Human serum	0.001-1.78mM; 1.78-5.03mM	0.187 μ M	³²⁰
5.	Glucose	CV	Ni/Co MOF	The electrode fabrication started with gold layer deposition over a hydrophilic PDMS surface followed by drop-casting Ni/Co MOF nanosheets.	Electrocatalytic activity	Human sweat	20-790 μ M	4.25 μ M	³²¹
6.	Glucose	Amperometry	Ni/Co-BTC MOF	Screen printed PET substrate was modified with CC and Ni/Co-BTC MOF by pasting it to the electrode surface with the aid of a conducting resin followed by covering with KOH-PVA gel.	High conductivity and metal synergy leading to improved catalytic activity	Human sweat	5-205 μ M	0.09 μ M	³²²

7.	Glucose	CV, Amperometry	Ni/Co MOF	rGO/PU fiber was coated with Ag glue on the electrode surface. Then, Ni/Co MOF with 1% Nafion was drop-casted on the modified electrode to obtain the final Ni/Co MOF/Ag/rGO/PU surface.	Large specific surface area and high catalytic activity	Human sweat	10 μ M-0.66mM	3.28 μ M	³²³
8.	Glucose	CV, Amperometry	2D M-BDC MOFs where M= Cu, Mn, Ni, Zr	The MOF powder was mixed with isopropanol and Nafion, and drop-casted on to the surface of polished GCE to obtain the desired probe.	Electrocatalytic potential	N.R.	Ni-BDC MOF: 0.01-0.8 mM	6.68 μ M	³²⁴
9.	Glucose	CV, Amperometry	Ni-MOF	Polished GCE electrode was modified by drop-casting Ni-MOF on its surface to obtain the desired electrode.	High specific surface area and abundant active catalytic sites	Human serum	0.001-2.0 mM, and 2.0-3.4 mM	0.7 μ M	³²⁵
10.	Glucose	CV, Amperometry	Cu-MOF	Cu-MOF/C composite dispersed in DMF was drop-casted onto the GCE. Then, the surface was electrodeposited with NiNPs to obtain NiNP/Cu-MOF-C/GCE.	Electrocatalytic synergy between Cu and Ni metals.	Human saliva	0.3-1150 μ M	0.090 μ M	³²⁶
11.	Glucose	Amperometry	Ni/Co MOF	CCFs were cleaned and pretreated to be used as substrate for MOF growth. Co and Ni salts along with the ligand were dissolved in DMF and the CCF was soaked into this solution followed by hydrothermal synthesis in autoclave. The obtained MOF decorated on the CCF surface was used as the sensor probe.	Electrocatalytic potential	Human blood serum, Saliva	0.04-3.13 mM and 3.63-8.28 mM	0.348 μ M	³²⁷

Abbreviations:

NF: Nickel Foam; NW: Nanowires; NPs: Nanoparticles; GCE: Glassy Carbon Electrode; WE: Working Electrode; CV: Cyclic Voltammetry; DPV: Differential Pulse Voltammetry; EIS: Electrochemical Impedance Spectroscopy; CC: Carbon cloth; HHTP: Triphenylene-2,3,6,7,10,11-hexaol; PDMS: Polydimethylsiloxane; PET: Polyethylene terephthalate; BTC: Trimesic acid; PVA: Polyvinyl Alcohol; rGO: Reduced Graphene oxide; PU: Polyurethane; NiNPs: Nickel Nanoparticles; CCF: Commercial Cotton Fabrics; DMF: Dimethyl Formamide; NR: Not Reported

3.2.2. MOF-based electrochemical sensors for Virus and bacteria-borne infectious Diseases

3.2.2.1. Viral diseases

Infectious diseases originating from viral pathogens can aggravate and result into pandemics as evident from our recent past ³²⁸. These can overturn economies and can lead to human survival challenges. The world has encountered various viruses in recent times ranging from single stranded RNA viruses like Corona virus (COVID-19) ^{329,330}, Middle east respiratory syndrome (MERS), severe acute respiratory syndrome (SARS), and influenza viruses ³³¹. Where these viruses usually affect the pulmonary pathway of humans, viruses like Zika ³³², Hepatitis ^{333,334} and Human immuno-deficiency virus (HIV) ³³⁵ can affect specific organs and thereby lead to multiple organ failures, due to compromised immune system and opportunistic infections by other pathogens. Some vector-transmitted viruses like Chikangunya ³³⁶ and dengue ³³⁷ can cause febrile illness leading to an annual mortality of more than 20,000 in tropical and sub-tropical regions of the world. Recently, some viruses like human papillomavirus, herpes simplexvirus ³³⁸ and Epstein-barr viruses have been known to cause cancers with their unique route of transmission which in cases can even transfer from mother to child ³³⁹.

With the advent of these viral-borne diseases worldwide, there have been tremendous diagnostic advancements in the field ^{331,340,341}. Viruses are mostly diagnosed using detection techniques like real-time PCR, quantitative PCR or high-throughput sequencing techniques which are usually high cost and sophisticated ones ^{342,343}. MOF-based virus biosensors have recently gained limelight due to their positively charged metal centres flanked with abundant functional groups within linker entities which endow them with good charge transfer characteristics aided with hydrogen bonding, electrostatic and, π - π stacking interactions ³⁴². In a study by Lin et al., a Cu-MOF based biosensor was projected to be efficient in detecting

hepatitis B (HBV) viral DNA ³⁴⁴. The Cu-MOF was adopted as a signal probe and was coupled with electrochemically reduced graphene oxide (ErGO), through π - π stacking interactions, as a signal amplification matrix. The MOF possessed a 3D honey-comb like structure with Cu(II) metal centres linked with H₂btca (5-carboxybenzotriazole) as the bridging ligand, giving a penta-clustered arrangement facilitating electron transfer along the b-axis of the MOF (**Figure 1.12A**). The Cu component was also postulated to interact with amino functional groups of the probe DNA molecule leading to their strong binding affinity. With attachment of the target DNA to the sensor probe the redox signal intensity was weakened at -0.050 V signifying the conversion of $\text{Cu}^{2+} \rightarrow \text{Cu}^0$ and further at 0.268 V corresponding to $\text{Cu}^0 \rightarrow \text{Cu}^{2+}$. The sensor worked within an LDR of 50.0 fM-10.0 nM and LOD of 5.2 fM with a DNA hybridization efficiency of 89.9%.

There are only a handful of reports of MOFs as electrochemical sensing matrix component for macromolecular detection including viral proteins and its nucleotide. A noticeable hurdle could be imparting selectivity to these molecules through suitable functionalization, which can be addressed through application of supramolecular chemistry principles. A recent study highlighting one such approach explains the fabrication of sandwich type-DNA sensor for hepatitis B virus (HBV) determination ³⁴⁵. In the probe fabrication strategy, WE was functionalized with rGO sheet adorned with pillar[5]arene (WP-5) stabilized Pd NPs. This modified surface was immobilized with methylene labelled DNA (MB-DNA) by virtue of interaction between MB-DNA with WP5. In a separate setup, ZIF-67 MOF was modified to functionalize hydroxylatopillar[5]arene-AuNPs conjugate possessing superior electrocatalytic properties assisting in signal amplification. The proposed model used the former setup to attach the target antigen DNA sandwiched by the later setup at the top, as per depicted in **Figure 1.12B**. The developed probe worked in an LDR of 1×10^{-15} mol/L- 1×10^{-9} mol/L with a LOD

of 0.32 fmol/L. Such probes can be promising prospects for DNA sensor development using MOFs as matrix components.

3.2.2.2. Bacterial Sepsis

With a rise in global viral diseases like COVID-19, the world experienced an advent rise in opportunistic bacterial infections like those caused by *Staphylococcus aureus* (*S. aureus*). There has also been a dramatic rise in multidrug resistant gram-negative bacterial infections such as rifampin-resistant tuberculosis, caused due to the abrupt and disproportionate usage of antibiotics⁷⁴. Drug-resistant bacterial infections in bloodstream have led to worldwide disease burden with priority concerns against Enterobacteriaceae and Staphylococcaceae bacterial families, as per WHO³⁴⁶, contributing to almost 2,50,000 annual deaths in America and Europe alone³⁴⁷. Neonatal sepsis within the first 3 days of child birth still remains the leading cause for morbidity and mortality, particularly in Asian countries primarily caused by staphylococcus species^{348,349}. So, this global scenario brings us to the urgency of detecting bacterial pathogens from diverse domains through simpler and rapid detection techniques like electrochemistry.

In a recent study on pathogenic strain *S. aureus*, a ratiometric electrochemical sensor was developed based on a ZIF/AuNP composite³⁵⁰. The electrode fabrication comprised of a polished GCE functionalized with a layer of ZIF-MOF, sealed with a film of Nafion. The use of Nafion film was strategically done to adsorb the positively charged AuNPs, followed by incubation in 2-mercaptothylamine for an hour. The electrode was further treated with H1 DNA probe, followed by immersion in trigger DNA solution and thereafter in H2-methylene blue (H2-MB), to facilitate the recycling of the latter. To understand the generation of trigger DNA, **Figure 1.12C** sums up the steps involved in the process which has been briefed as follows. The aptameric probe corresponding to the *S. aureus* DNA was initially bound to helper DNA (HP)

which dissociated on attachment of the target sequence. This detached HP then, got involved in a different set of process where a padlock DNA was ligated to it through a T4 DNA ligase. This ligation triggered a DNA polymerization reaction resulting into generation of two DNA fragments, viz., trigger DNA and ssDNA, aided by BbvCI nicking endonuclease. This trigger DNA, subsequently combined with H1 probe followed by H2-MB probe, generating an electrochemical response. The ratiometric response was a function of ferrocene (Fc) and MB current ratio, with respect to the trigger DNA. With an increase in *S. aureus* concentration, the Fc signal response at 0.43 V was reduced whereas, the oxidation of MB was elevated at the same time at -0.087 V, attributing to a reversal in current change tendency, as depicted through DPV curves in **Figure 1.12C**. The probe exhibited a sensing ability for *S. aureus* within a LDR of $5\text{-}10^8$ CFU/mL, with a LOD of 1 CFU/mL. The sensor applicability was also tested in real samples such as milk and orange juice. With all the discussions revolving around the probe performance so far, the vital role of MOF grabbed the attention of readers towards the multi-fold enhancement of probe surface area due to its incorporation in the matrix. Similar role of MOF-borne surface area enhancement was attempted in a recent study by Ye et al.³⁵¹ where they constructed a dual-channel biosensor which could simultaneously detect two deadly foodborne pathogens *Salmonella typhimurium* (*S. typhimurium*) and *Listeria monocytogenes* (*L. monocytogenes*). The sensor comprised of MOF-based nanotags functionalized with gold nanoparticles (Au@CuMOF and Au@PbMOF) with each containing specific target DNA sequences corresponding to these bacterial species. The nanotags were hybridized onto the surface of electrode and achieved sensitive detection through DPV. The dual channel sensor detected the bacterial genes *invA* and *invlA* efficiently within a LDR of 1×10^{-14} - 1×10^{-8} M and 1×10^{-13} - 1×10^{-8} M, respectively. As far as the detection of the bacteria directly was concerned, *S. typhimurium* was detected within a LDR of $5\text{-}1.0 \times 10^4$ CFU mL⁻¹ (LOD of 2.33 CFU mL⁻¹), and *L. monocytogenes* within LDR of $10\text{-}1.0 \times 10^4$ CFU mL⁻¹ (LOD of

6.61 CFU mL⁻¹). Such studies are suggestive for guest molecule holding capabilities of MOFs, thereby easing electrochemical probe assembly fabrication.

Apart from their role as support matrices, MOFs have also been used as nanozymes in sensors for detection of pathogenic bacterial strains. Peroxidase mimicking activity of MOFs have been explored in recent times (Shubhangi, et al., 2023). One such was explored in a case where bacteria captured through GCE functionalized with vancomycin was detected through a separate AuNP-MOF decorated with Rabbit anti-*S. aureus* rosenbach tropina antibody (Ab₂)³⁵³. This whole system was eventually generating cathodic current response by virtue of MOF exhibiting peroxidase activity and catalysing o-phenylenediamine in presence of hydrogen peroxide. The generated signal was proportional to the concentrations of *S. aureus*. The 2D Fe-based MOF was found capable of detecting *S. aureus* in a concentration range of 10-7.5 x 10⁷ CFU/mL with a LOD of 6 CFU/mL.

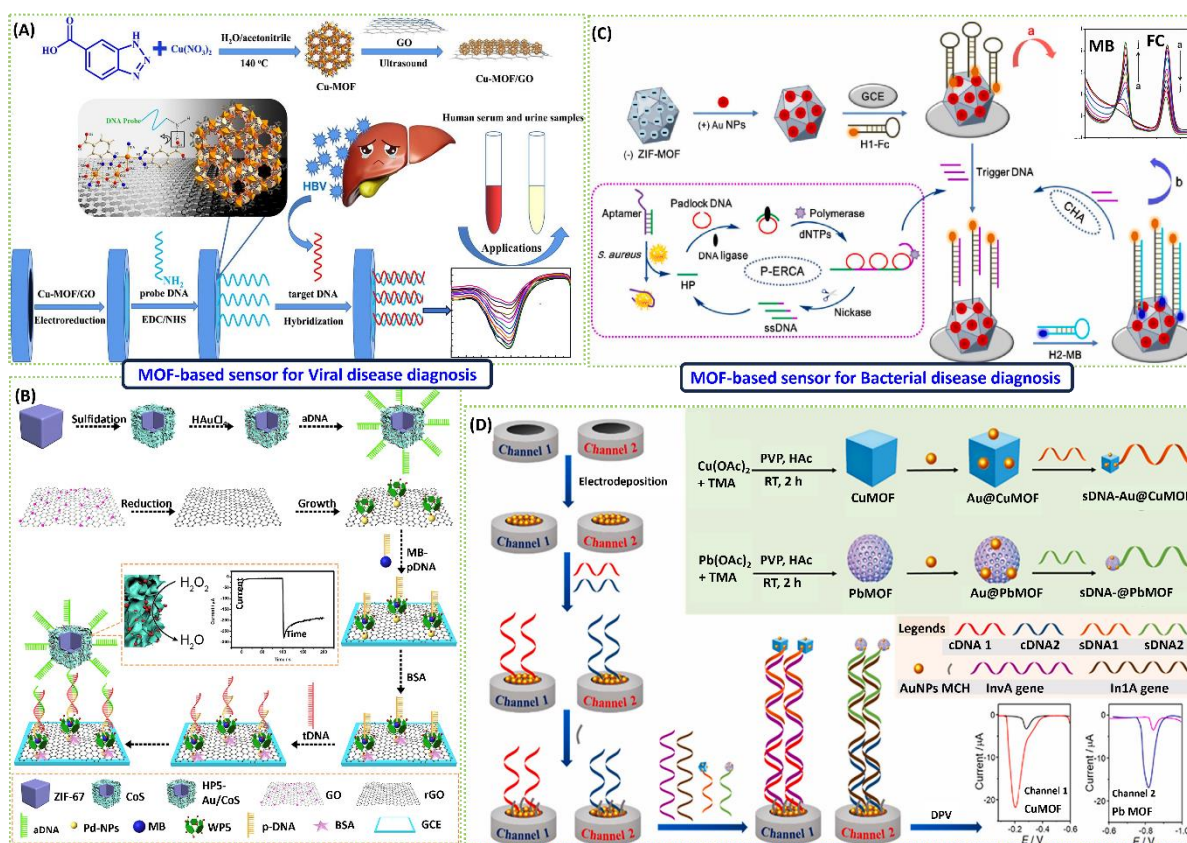


Figure 1.12. *A) and B) schematically demonstrate strategies for fabrication of MOF-based viral disease biomarker, viz., HBV viral DNA (adopted and reprinted with permission from Lin et al. 2020; Qian et al., 2020). C) and D) correspond to studies on bacterial disease diagnosis through electrochemical tools deploying ZIF, Cu and Pb MOFs targeting specific bacterial DNA (reprinted with permission from Shan et al., 2023; Ye et al., 2023).*

The above discussions can be more vividly established through elaborately stated recent reports on MOF-based biosensors for diagnosis of viral and bacterial infectious diseases, as per **Table 1.6** with their respective target analytes, detection techniques, sensor composite, fabrication strategies, mechanistic role of MOF, real samples considerations and detection efficiency parameters (LDR and LOD).

Table 1.6. Detailed list of latest MOF-based electrochemical biosensors for viral and bacterial disease diagnosis

S.No.	Target Analyte	Detection Technique	Sensor Composite	Fabrication Strategy	Mechanistic Role of MOF	Real Sample	LDR	LOD	Reference
Viral Infection diagnosis									
1.	a) Recombinant SARS-CoV-2 spike glycoprotein b) SARS-CoV-2 pseudovirus	EIS	CoFeBDCNH ₂ -CoFe ₂ O ₄ MOF-nanohybrids	The MOF-nanohybrid was coated on a gold chip was used as a substrate. Further, the electrode surface was modified by conjugating it with the virus specific antibody using 11-MUA as the linker entity through SAM.	Conductive synergy between CoFeBDCNH ₂ -MOF and CoFe ₂ O ₄ nano materials.	Serum	a) 10 ng/mL-100 fg/mL b) 10 ng/mL-10 fg/mL	a) 6.68 fg/mL b) 6.20 fg/mL	³⁵⁴
2.	HIV-1 DNA	EIS	Ni/Co-MOF	The Ni/Co-MOF was transformed into NiCo ₂ O ₄ /CoO@CNTs. This composite was then immobilized with HIV-1 probe DNA for its detection.	Carrier for probe DNA, biocompatibility, electrochemical conductivity	Human serum	0.1 pM-20 nM	16.7 fM	³⁵⁵
3.	SARS-CoV-2	EIS	Ni ₃ (BTC) ₂ MOF	Ni ₃ (BTC) ₂ MOF was coated on SPCE surface which was further modified with SARS-CoV-2 S protein specific aptamer followed by dopamine deposition through electro polymerization.	High surface area substrate for covalent immobilization of aptamer	Serum from sick and healthy individuals with SARS-CoV-2 virus	10-10 ⁸ PFU/mL	3.3 ± 0.04 PFU/mL	³⁵⁶
4.	HCV-RNA	CV, EIS	Ni-MOF	The working electrode was fabricated using a GCE electrode coated with the Ni-MOF. The MOF surface was then functionalized with polyaniline followed by a DNA probe and BSA.	Highly porous matrix for loading target DNA probe	Serum	1 fM-100 nM	0.75 fM	³⁵⁷
5.	Hantavirus	DPV	Cu-MOF	GCE surface was electrodeposited with AuNPs-rGO composite and further immobilized with cDNA aided by Au-S bonding. Subsequent attachment of t-DNA was	As a signal tag	Real virus samples	10 ⁻¹⁵ -10 ⁻⁹ M	0.74 fM	³⁵⁸

				followed by its coupling with Cu-MOF-sDNA composite completing the sensing platform.					
6.	HCV-RNA	DPV	Ag/Zn MOF	GCE electrode surface was modified with the Ag/Zn bimetallic MOF and was further modified with the capture probe (nucleic acid-based) for HCV detection.	Immobilization matrix for DNA probe	Human serum	1 fM-100 nM	0.64 fM	³⁵⁹
7.	Foot and mouth disease virus (FMDV)	SWV	TB@MOFs	GCE surface was drop-casted with TB@MOF which was followed by coating with AuNPs. The electrode was then coated with Ab and then treated with BSA for redundant site blockage.	Large surface area for TB encapsulation	Pig serum	0.1 pg/mL-10 ng/mL	0.008pg/mL	³⁶⁰
8.	FMDV	DPV	CdS@Zn-MOF	Pretreated GCE surface was coated with solution of MWCNTs. The modified electrode was then functionalized with Ab ₁ followed by incubation and BSA treatment. The electrode was then treated with different concentrations of antigen followed by Ab ₂ to complete sandwich probe fabrication and initiate immunoreaction. Zn-BTC MOF was used as a label which was stained with CdS for FMDV detection.	As encapsulation matrix for CdS	Human serum	0.5 fg/mL- 5 ng/mL	0.05 fg/mL	³⁶¹
9.	HBV DNA	DPV	Cu-MOF	Non-enzymatic sensor was developed by functionalizing GCE surface with Cu-MOF as electroactive sensor probe. It was further coated with GO, which was electrochemically reduced to ErGO, for signal amplification. Cu-MOF due to its high affinity with DNA, acts a functional platform for its immobilization which can thereby determine concentrations of the target DNA.	As a signal probe and multisite matrix	Human serum, urine	50.0 fM-10.0 nM	5.2 fM	³⁴⁴
10.	HBV DNA	Amperometry	ZIF-67	Polished GCE surface was drop-casted with WP5-Pd/rGO and dried at room temperature. Then, MB-DNA was functionalized onto the modified electrode surface followed by treatment with BSA to block redundant sites. It was followed by addition of different concentrations of target DNA at RT to develop the sandwich type DNA sensor.	As nanobox anchor for adhering probe components	Serum	1 fM-1nM	0.32 fM	³⁴⁵

Bacterial Infection diagnosis									
11.	<i>Pseudomonas aeruginosa</i> (<i>P. aeruginosa</i>)	DPV	Zr-MOF	Cu doped Zr-MOF was used for conjugating the aptamer/DNA/Super P signal probe on the surface of a gold electrode.	Large surface area for Cu-doping	Urine	10-10 ⁶ CFU/mL	2 CFU/mL	³⁶²
12.	<i>Escherichia coli</i> (<i>E. coli</i>) <i>O157:H7</i>	DPV	CdS@ZIF-8	ZIF-8 MOFs were encapsulated with CdS QDs which was further functionalized with polyethyleneimine followed by attachment of bacteria specific antibody. The CdS particles act as signal tags on releasing through HCl leaching event, releasing Cd ²⁺ from MOF cavity and consequently detecting <i>E. coli</i> .	CdS encapsulation matrix	Milk	10 ⁻⁸ CFU/mL	3 CFU/mL	³⁶³
13.	a) <i>E. coli</i> <i>O157:H7</i> b) <i>Staphylococcus aureus</i> (<i>S. aureus</i>) c) <i>Salmonella enterica</i>	DPV	Poly Mn-MOF	The poly Mn-MOF was synthesized using polyH ₂ BDC units linked to 4,4'-bipyridine, MnCl ₂ and polyvinyl pyrrolidone. The developed MOF was conjugated with the bacterium specific aptamer for their respective sensing.	Aptamer adherence, backbone stability and release of metal ions in aqueous system	Milk, Frozen shrimp (Samples were spiked with pathogen)	10-10 ⁸ CFU/mL	a) 1.5 CFU/mL b) 3.5 CFU/mL c) 2.6, CFU/mL	³⁶⁴
14.	<i>Mycobacterium bovis</i> (<i>MB</i>)	SWV	TB@MOFs	GCE surface was drop-casted with TB@MOF which was followed by coating with AuNPs. The electrode was then coated with Ab and then treated with BSA for redundant site blockage.	Large surface area for TB encapsulation	Pig serum	0.1pg/mL-10µg/mL	0.002 pg/mL	³⁶⁰
15.	<i>Mycobacterium tuberculosis</i> antigen (MPT64)	DPV	Fe-MOF	Fe-MOF was functionalized with PEI to form P-MOF which was later drop-casted on electrode surface. A thin film comprising of Au@Pt was deposited on the surface of this MOF-coated electrode. On another setup, C ₆₀ NPs-N-CNTs/GO was doped AuNPs which was further functionalized with MAA II to form a tracer label. This tracer label and the former modified electrode bonded to the MPT64 antigen selectively leading to its detection.	Electrochemical conductivity, absorption of Au/Pt nanoparticles	Human serum of healthy and infected individuals	1 fg/mL-1 ng/mL	0.33 fg/mL	³⁶⁵

16.	<i>S. aureus</i>	DPV	Cu-MOF	GCE surface treated with BSA was attached to vancomycin via EDC/NHS chemistry and then incubated with varying concentrations of bacteria. Following it, Ab ₂ /AuNPs/MOFs was added to the modified electrode surface sealed with Nafion.	Peroxidase-mimic nanozyme	N.R.	10-7.5 × 10 ⁷ CFU/mL	6 CFU/mL	³⁶⁶
17.	<i>S. aureus</i>	DPV	ZIF	GCE surface was modified by coating its surface with ZIF-MOF. It was then functionalized with AuNPs aided by ethanolic Nafion. Further treatment with mercaptoethylamine was done for the adhesion of H1(helper DNA) probe. Then, the target DNA was attached to it surface followed by attachment of H2-MB probe.	Electron transport promoter, provides surface area for absorption	Orange juice, Milk	5-10 ⁸ CFU/mL	1 CFU/mL	³⁵⁰
18.	<i>E. coli</i>	EIS	Cu-MOF	Initially PANI was mixed with the linker molecule H ₃ BTC followed by mixing with the MOF precursor salt to form Cu ₃ (BTC) ₂ -PANI. The prepared MOF composite was drop-casted on the surface of ITO followed by annealing the electrode surface at 100 °C. Then, Ab was incubated using EDC-NHS on modified surface for an hour and un-specific binding sites were blocked through treatment with Tween-20.	Bio-interface for attachment of anti- <i>E. coli</i> antibodies	Water	2.0-2.0 × 10 ⁸ CFU/mL	2 CFU/mL	³⁶⁷
19.	Methicillin-resistant <i>S. aureus</i>	DPV	UiO-66-NH ₂	GCE surface was drop-casted with a dispersed alcoholic mixture of N-doped porous carbon material, dried in oven. Two electroactive dyes, methylene blue and epirubicin were encapsulated within the MOF and treated with different concentrations of target DNA to obtain results.	Nano-carrier	Tap water, Milk samples	5 × 10 ⁻¹⁵ -1 × 10 ⁻¹⁰ M	Bacterial genes: mecA gene: 3.7 fM nuc gene: 1.6 fM	³⁶⁸
20.	<i>S. aureus</i>	EIS, DPV	Zn-Glu@PTBD-COF	The aptameric sensor was fabricated through functionalization with L-glutamic acid, 1,10-phenanthroline-2,9-dicarbaldehyde and benzene-1,4-diamine linker molecules. Aptamer adhesion on composite surface was aided by abundant active sites on the MOF-	MOF defects provide anchoring sites for aptamers	Milk, Honey	10-10 ⁸ CFU/mL	EIS: 2.0 CFU/mL DPV: 1.0 CFU/mL	³⁶⁹

COF surfaces facilitating specific recognition of SA.

Abbreviations:

EIS: Electrochemical Impedance Spectroscopy.; DPV: Differential Pulse Voltammetry; SWV: Square Wave Voltammetry; SAM: Self assembled monolayers; HIV: human immunodeficiency virus; HCV: Hepatitis C virus; HBV: Hepatitis B virus; CNT: Carbon nanotubes; BTC: Trimesic acid; TB: Toluidine blue; BSA: Bovine Serum Albumin; rGO: Reduced graphene oxide ;MB: Methylene Blue

3.2.3. MOF-based electrochemical sensors for Neurological disorders

Neurological disorders are referred to diseases which target the nervous system and include nerves, spine and the brain. They are the leading cause of severe-long term disabilities and second largest cause of deaths in the world ³⁷⁰. More than 600 diseases have been reported in this category and their symptoms largely aggravate with age and are modulated by risk factors. Some of them include dementia, stroke, parkinson's disease, alzheimer's, epilepsy etc. ³⁷¹. The etiologic aspects of such diseases still remain poorly understood therefore their timely detection remains crucial. Various neurological diseases including some of the neurodegenerative disorders have shown involvement of certain neurotransmitters and peptides which can be target analytes for their timely diagnosis ³⁷². MOFs-based electrochemical biosensing platforms can serve as point-of-care diagnostic tools for the timely, sensitive and low-cost detection of these disorders.

Dopamine (DA), a catecholamine neurotransmitter is essentially utilized by cells for conversion of chemical signals into impulses ³⁷³. It is responsible for modulation of the central nervous system and cognitive functioning of organisms ³⁷⁴. Its abnormal levels may affect the human nervous system triggering neurological dysfunctions ³⁷⁵. There have been recent developments on the sensitive detection of DA however, have faced challenges like complicated operations, complex sample handling and overlapping signals. Electrochemical DA sensing could be a plausible solution to such challenges. If the recent developments of MOF-based sensors are looked upon, two different approaches could be visualised. The first one focusses on detection of DA directly through exploitation of its redox behaviour while the second approach relies on catalysing it to further constituents. In a recent study by Zhou et al., CoNi MOF was ultrasonically mixed with GO to develop a sensor probe where the components were coated electrochemically on polished electrode surface to form the final sensor probe, as shown in **Figure 1.13A** ³⁷⁶. The MOF structure exhibits an enhanced conductivity while also

offers abundant sites for DA absorption exhibiting sensitivity towards it in a wide LDR of 0.1–400 μM with a LOD of 0.086 μM , when tested in DPV premises. Bimetallic MOF like this tends to impart synergistic effect to the sensing matrices. In another study on DA detection using a Co-based ZIF-67 MOF discussed about calcination of the ZIF followed by its hydrothermal treatment in a solution of cerium nitrate hexahydrate³⁷⁷. This different strategy not only exposed the pre-existing active sites on the MOF but also prevented the agglomeration of the constituted dopant CeO_2 (**Figure 1.13B**). Again, in this case the electrocatalytic response was a result of Co and Ce metal centre synergies. The GCE/ CeO_2 / Co_3O_4 exhibited an LOD of 0.13 μM with a LDR of 0.13 μM –60 μM using the DPV method. This catalytic sensor was capable of converting DA into DA-Q even in human urine samples but dealt with a small dynamic range of detection. For clinically relevant sensors a wide detection range is favoured which most of the MOF-based sensors lack for³⁷⁴. To overcome this issue, researchers have tried microenvironment modulation in ZIFs where MOFs have been incorporated with composite materials to improve their electrocatalytic behaviour (Shubhangi et al., 2023; Sun et al., 2024). Apart from the examples discussed in the text, there have been many recent advancements on MOF-based DA sensors which have been comprehensively discussed in **Table 1.7**. Apart from DA, there lies a deep lacuna in the field of development of MOF-based electrochemical sensors for this category of disease due to limitations in accessible biomarkers. 5-hydroxytryptamine(5-HT), commonly known as serotonin, is also one of the neurotransmitters which is less explored but plays role in regulating mood and related functions³⁷⁸. Its specific concentration in serum, urine and CSF is important and if altered can cause depression, Alzheimer's disease and irritable bowel syndrome^{379,380}. In a recent study on its electrochemical MOF-based detection, a group utilized Nickel bis (dithiophen-dibenzoic acid) ligand to construct a nickel zinc bis(dithiolene) MOF with good catalytic potential³⁷⁹. The MOF was ultrasonically dispersed with acidified MWCNTs (Ni Zn-MOF/MWCNTs) and

drilled onto the electrode surface to develop the final probe (**Figure 1.13C**). DPV-based oxidative detection of 5-HT was carried out in Tris-HCl solution with a LDR of 0.5-115 μM and LOD of 0.03 μM . Human urine was considered as the real sample matrix to understand the sensor's utility which yielded commendable recovery of 99.94 % to 102.02 %. The current studies though being quite limited but hold tremendous scope for MOF based electrochemical sensing solutions for amyloid disease biomarkers such as amyloid β peptides³⁸¹. Therefore, MOFs could be more emerging sensor matrix components in coming times for neurological disorders.

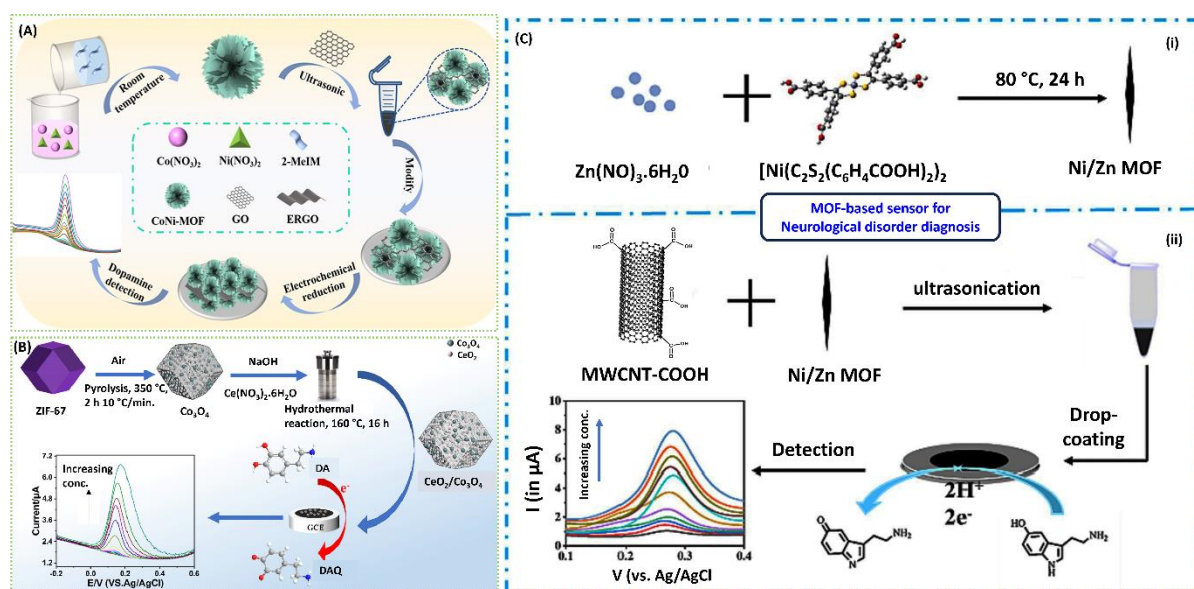


Figure 1.13. A) Illustration on step-wise fabrication of CoNi bimetallic MOF as an efficient platform for dopamine detection, adopted with permission from Zhou et al., copyright 2023. B) Synthesis strategy for electrocatalytic conversion of DA to DAQ through modified ZIF-67 and thereby generation of DPV signal response, reprinted with permission from Ding et al., copyright 2023. C) **Upper panel:** Hydrothermal synthesis of bimetallic Ni/Zn MOF; **Lower panel:** Fabrication of MWCNT functionalized Ni/Zn MOF for oxidative detection of 5-HT, adopted with permission from Chen et al., copyright 2023.

Table 1.7. Detailed list of latest MOF-based electrochemical biosensors for neurodegenerative disease diagnosis

S.No.	Target Analyte	Detection Technique	Sensor Composite	Fabrication Strategy	Mechanistic Role of MOF	Real Sample	LDR	LOD	Reference
1.	Dopamine	CV, DPV	Polyoxometalate- γ -cyclodextrin MOF	POM- γ CD MOF was drop-casted on the laser-scribed graphene electrode (LSGE) and dried at ambient conditions to get POM- γ CD MOF/LSGE.	It acts as an electrocatalyst and provide selective detection	Serum samples and <i>SH-SY5Y neuroblastoma</i> cells	50 nM-1.0 mM	10 nM	³⁸²
2.	Dopamine	DPV	Ni-MOF	A polished GCE electrode surface was coated with Ni-MOF dispersed within ethanol. On drying, the electrode surface was layered with 1% Nafion solution to complete the fabrication process.	Enhances electrocatalytic activity of sensor	Commercial DA hydrochloride injection	0.2-100 μ M	60 nM	³⁸³
3.	Dopamine	DPV	Fe-MOF	2D Fe-MOF was synthesized at room temperature through a wet chemistry approach followed by its pyrolysis at 900 °C forming Fe/CNS. It was then soaked into conc. 1.0 M H ₂ SO ₄ solution forming 2D CNS. This solution was then dispersed in ultrapure water and dropped on clean GCE to obtain the final probe.	Increases surface area and electrical conductivity	Human serum	0.05-80 μ M	0.01 μ M	³⁸⁴
4.	Dopamine	DPV	Ce (III, IV)-MOF	A polished GCE surface was coated with a layer of CB/Ce (MV)-MOFs dispersed in 0.5 % alcoholic Nafion solution. The modified electrode was then dried at 60 °C, 5 minutes to obtain the final probe.	Provides high surface area, chemical stability	DA injections, Urine	0.3-252.0 μ M	0.0955 μ M	³⁸⁵
5.	Dopamine	CV, DPV	β -CD/Ni-MOF	A clean polished GCE surface was coated with β -CD/Ni-MOF suspension and dried under red lamp.	Increases catalytic sites which enhances redox reaction	DA hydrochloride injection, human serum	0.7-310.2 μ M	0.227 μ M	³⁸⁶
6.	Serotonin	DPV	ZnO-Cu MOF/NF	Uniform-sized Ni foam strip was cut and rinsed with conc. HCl to remove any surface oxides. Then, it was pretreated with ethanol, acetone and DI water and later dried to obtain in the desired form. ZnOCu MOF was ultrasonically prepared and dispersed in water using sonication. 100 μ l of this suspension was drop casted onto the Ni-foam	Higher surface area and improved electrocatalytic activity	Simulated blood serum	1ng/mL-1 mg/mL	0.49 nM	³⁸⁷

				and dried at 70 °C for 1 h to obtain the final sensing probe.						
7.	Beta-site secretase (BACE1)	LSSV	ph-AgNPs@Zr-MOF	Cytosine (C) rich sequence-templated AgNPs/Zr-based metal–organic framework (MOF) composite is modified by phenol groups, and then the prepared tag (ph-AgNPs@MOF) is captured in microplate surface by the conjugation reaction of phenolic groups between tyrosine and tag.	Large surface area and water stability	Serum	1-200 pM	0.8 pM	388	
8.	β-amyloid (Aβ) oligomer	DPV	AuNPs/Cu-MOF	By using an aptamer-tethered gold nanoflower (AuNF) modified electrode for target capture and aptamer-tagged gold nanoparticles/Cu-MOF (AuNPs/Cu-MOF) conjugates, an effective sandwich sensor was obtained.	Provides both increased surface area and conductivity	Human Aβ oligomer (82E1-specific) ELISA kit	1 nM-2 μM	0.45 nM	389	
9.	Dopamine	CV, DPV	Cu-Co(2-Meim)MOF @CNF	A CNF mat was fabricated using electrospinning method. The mat was then hybridized with Cu-Co(2-Meim)MOFs through solvothermal method. Post hybridization the composite was washed with ethanol/water mixture and dried at 70 °C.	Enhances catalytic activity and sensitivity of sensor towards analyte	DA: cheese Tyramine: banana	5-800 μM	DA: 44.2 nM Tyramine: 52.7 nM	390	
10.	Dopamine	CV, DPV	DUT-67/ZnCo2O4-MWCNTs	A polished GCE surface was dried under N2 flow. DUT-67/ZnCo2O4 and activated MWCNTs were dispersed in DMF solution and dropcoated onto the surface followed by drying under IR lamp.	Composite provide specificity towards dopamine	DA injection	1.0-180 μM	12 nM	391	

Abbreviations:

EIS: Electrochemical Impedance Spectroscopy; DPV: Differential Pulse Voltammetry; CV: Cyclic Voltammetry; LSSV: linear sweep stripping voltammetry; DMF: Dimethyl Formamide; IR: Infra red ; MeIM: 2-methyl imidazole.

3.2.4. MOF-based electrochemical sensors for Cardio-vascular diseases

Claimed to be one of the most common non-communicable diseases worldwide, cardiovascular diseases are the leading cause deaths which are estimated to rise to a figure of 22.2 million deaths by 2030. As per WHO, 31% of global deaths were cardio-vascular disease-related with heart attacks and strokes being the highest contributors in the statistics ³⁹². Acute myocardial infarction (AMI) or heart attack is usually diagnosed through various approaches commonly being electrocardiography, radioimmunoassay and enzyme-linked immunosorbent assay ³⁹³. However, biosensors involving MOFs could be better choice for point-of-care rapid diagnostic solutions for better management of this disease ³⁹⁴.

A crucial cardiac biomarker is Antigen galectin-3 (GL-3) which belongs to the family of β -galactoside proteins which poses its significance in detection of cardiac fibrosis ³⁹⁵. A study aiming its detection fabricated an amperometric sensor based upon AuNPs functionalized on graphitic carbon nitride nanosheet (g-C₃N₄@AuNPs) surface with Ti-MOF@COF covalent composite as the signal amplification component. A GCE electrode surface was initially modified with g-C₃N₄@Au NPs through a dispersive coating followed by immobilization with anti-GL-3-Ab₁ assisted by amino-gold binding affinity. Thereafter, a parallel preparation of Ti-MOF@COF composite was functionalized with anti-GL-3-Ab₂ which was sandwiched upon the former setup to develop the final immunosensor probe. The amperometric measurements were performed in H₂O₂ solution which acted as a mediator for GL-3 detection (**Figure 1.14A**). The sensor was found to be capable of detecting GL-3 in a wide LDR of 0.0001-20.0 ng mL⁻¹ with a LOD of 25.0 fg mL⁻¹. In another case on detection of a blood-based AMI biomarker detection strategy by Gupta et al., screen-printed electrodes were drop casted and annealed twice at 90 °C for 4 h with ethanolic solutions of Cu₃(BTC)₂/PANI MOF composite to obtain uniform sensor matrix ³⁹³. The electrodes were then incubated in carbodiimide (EDC) and N-hydroxysuccinimide (NHS) solution to facilitate attachment of cardiac troponin I antibody

(anti-cTnI) followed by blocking non-specific binding sites through 0.01% Tween 20. The impedimetric immunosensor was found to be detecting the cardiac troponin I (cTnI) antigen in a clinically significant LDR of 1- 400 ng mL⁻¹ with a LOD of 0.8 ng mL⁻¹. The significance of the sensor was also deduced by testing its practicability in mice serum samples with satisfactory recoveries. The unique properties of MOFs have created their impact on development of such label-free electrochemical sensing approaches. A similar approach involving Cu-MOF for cTnI was attempted by Ling et al. where they fabricated Cu-MOF-74 as a bifunctional material which not only acted as antibody immobilization matrix but also attributed to signal generation³⁹⁶. Briefly, the immunosensor was developed on a GCE surface which was initially electrochemically pretreated with 2.5% K₂Cr₂O₇ and 10% HNO₃ solutions to obtain -COOH groups on its surface. Subsequently, the electrode was activated with EDC/NHS and NH₂-rGO pitching a ground for electrodeposition of Cu-MOF. The MOF was electrodeposited in two step process with an initial Cu-salt deposition at -0.4V followed by electrochemical scanning in PBS solution containing H₄dhtp to produce Cu-MOF-74/NH₂-rGO/GCE. Finally, the surface was adorned with anti-cTnI antibodies aided by -NH₂ condensation reaction between the antibody and EDC-NHS moieties (as shown in **Figure 1.14B**). The DPV-based immunosensor could efficiently detect the target analyte with a LDR of 0.01 pg mL⁻¹-1.0 ng mL⁻¹ and LOD of 4.5 fg mL⁻¹. The standard-addition method-based serum sample analysis projected a commendable recovery of the spiked samples vouching the reliability of the sensor. Similar label-free approaches using MOFs against cTnI have also been adopted by others in recent times^{397,398}. It is worthy to mention that MOF-based sensors against the exploration of several

other cardiac biomarkers like myoglobin, creatine kinase isoenzyme remains the need of the hour with current global health scenario.

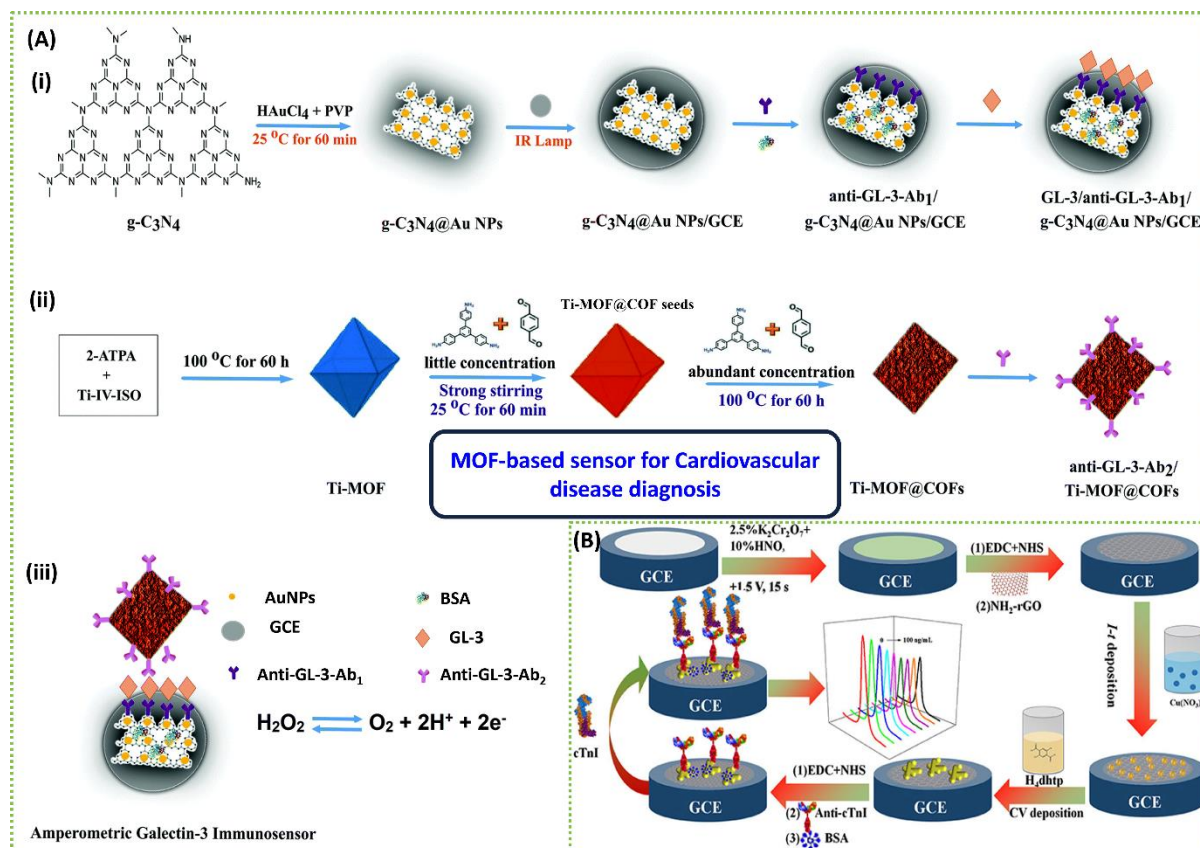


Figure 1.14. A) An elaborated overview on the fabrication of sandwich-type MOF-based sensing and detection principle for GL-3 biomarker. i) The panel describes about functionalization of graphitic carbon nitride sheet with AuNPs followed by attachment of anti-GL3 Ab₁. ii) demonstrates the synthesis of Ti-MOF followed by hydrothermal synthesis of Ti-MOF@COF composite with attachment of anti-GL3 Ab₂. iii) Assembly of setup i) and ii) to form sandwich type sensor generating signal by virtue of H₂O₂ as redox mediator (reprinted with permission from Yola & Atar, copyright 2020). B) Depicts the schematic step-wise probe fabrication involving functionalization of GCE with Cu-MOF-74/NH₂-rGO/GCE anti-body based electrochemical detection of cTnI (reprinted with permission from Ling et al., copyright 2022).

Apart from the examples elaborated in the section above, there have been some noteworthy contributions recently in the field of MOF-based biosensors for diagnosis of cardiovascular diseases using their specific biomarkers which have been highlighted in **Table 1.8** stating their respective target analytes, detection techniques, sensor composite, fabrication strategies, mechanistic role of MOF, real samples considerations and detection efficiency parameters (LDR and LOD).

Table 1.8. Detailed list of latest MOF-based electrochemical biosensors for cardiac and pulmonary disease diagnosis

S.No.	Target Analyte	Detection Technique	Sensor Composite	Fabrication Strategy	Mechanistic Role of MOF	Real Sample	LDR	LOD	Reference
1	C-reactive protein (CRP)	DPV	Zn-MOF	Carbon paste electrode was used and further modified with ZnO/MPC and Ionic liquid composite.	Increase surface area and conductivity and helps in antibody immobilization	Human serum	0.01-1000 ng/mL	5 pg/mL	³⁹⁹
2	CRP	DPV	Cu-MOF	Toluidine blue loaded Cu-MOF was used with AuNPs and drop-casted on GCE surface. Then anti-CRP was deposited after that BSA was used for blocking non-specific sites. Further Au-MOFs-Ab2-CRP was used for specific detection of CRP.	The 3-D structure provide higher surface area which carries antibody	Serum	0.5-200 ng/mL	0.167 ng/mL	⁴⁰⁰
3.	CRP	DPV	Pt-COF	Pt-COF (PtNPs Modified Covalent Organic Frameworks) deposited on electrode surface and then anti-CRP was used. After blocking the nonspecific surface with BSA, MOFs anti-CRP was used for detection.	Increases electrical conductivity	Serum	1-400 ng/mL	0.2 ng/mL	⁴⁰¹
4.	cTnI	EIS	Cu-MOF	Cu ₃ (BTC) ₂ /PANI composite were synthesized using solvothermal method. Then, the composite was drop-casted on the surface of SPEs followed by immobilization of anti-cTnI antibodies.	Composite MOF provide higher conductivity and sensitivity	Mice serum	1-400 ng/mL	0.8 ng/mL	⁴⁰²
5.	cTnI	DPV	Zn-MOF	Fe ₃ O ₄ -COOH was used as a electrocatalyst and Thi used as redox specie and were separately introduced into Zn-MOF. The developed Zn-MOF/Fe ₃ O ₄ -COOH/Thi nanocomposite was used further and anti-cTnI monoclonal antibody was immobilized.	It helps in carrying large number of electrocatalyst and redox specie	N.A.	0.04-50 ng/mL	0.9 pg/mL	⁴⁰³
6.	cTnI	DPV	Cu-MOF-74	The pretreated electrode was covalently modified with NH ₂ -rGO. Further Cu-MOF-74 was electrodeposited over modified surface and then anti-cTnI was immobilized to form final sensing surface.	It provides both surface for immobilization of antibodies	Serum	0.01 pg/mL- 1.0 ng/mL	4.5 fg/mL	³⁹⁶

					and signal enhancement				
7.	cTnI	EIS	FeCo-MOF	FeCo bimetallic MOF was grown on Ni foam forming NH ₂ -MIL-88B(Fe ₂ Co)-MOF/NF surface. Further anti-cTnI antibody was immobilized for specific detection of cTnI.	Modified MOF provide ample active sites for carrying antibody	Serum	10 ng/mL-0.1 fg/mL	13 fg/mL	⁴⁰⁴

Abbreviations:

[BMIM]BF₄ - 1-butyl-3-methylimidazolium tetrafluoroborate ; PAMAM- poly(amidoamine)

3.2.5. MOF-based electrochemical sensors for Cancer

Cancer is a global health burden with its whopping cases standing tall at 18.1 million with 9.6 million mortalities reported in 2018, as per a recent report by WHO (WHO report, 2020). These figures are expected to double by 2040 however, its global response is believed to be uneven and inequitable with almost two-third cases in middle income countries. The spectrum of cancer control interventions comprises of prevention followed by timely and accurate screening and diagnosis. The challenge of cancer diagnosis lies on specificity of sensor towards cancer cells through lesser invasive testing and better detection index ²²⁶. Its early detection is only possible through a powerful sensing of relevant biomarkers such as micro-RNA 21, carcinoembryonic antigen, human telomeric DNA and many others⁴⁰⁶.

Of all diagnostic tools currently available, electrochemical methods are one of the most reliable ones due to their rational design, ease of fabrication, low cost and high-end sensitivity towards biomolecules. Cancer biomarkers not just comprise of macromolecules but also ROS like H₂O₂ ⁴⁰⁷ and superoxide which are now being explored for its early diagnosis. Huang et al. in their recent study have highlighted the significance of detecting H₂O₂ in a range of colon cancer cells (SW-48, NCM-460, and HCT-116) ⁴⁰⁸. For the purpose of microelectrode fabrication, reduced graphene fiber was activated (AGF) through 30% H₂O₂ solution which was further placed in polytetrafluoroethylene (PTFE) autoclaves along with ZnO precursor solution at 90°C for 12 hours. The obtained product was further treated at 400°C to obtain ZnO/AGF and then incubated into 2-methylimidazole to obtain ZIF-8@ZnO/AGF. It was soaked in 0.5 M Cu(NO₃)₂ solution to finally obtain Cu₂(OH)₃NO₃@ZnO/AGF (**Figure 1.15A**). The probe was electrochemically reducing H₂O₂ at -0.8 V which was further calibrated using chronoamperometry. The sensor was capable of detecting the radical in the range of 1 µM–17.4 mM with a LOD of 1 µM. MOFs not only find their purpose as sensor matrix support but also as catalytic metal center providers like in case of a recent study on sensing superoxide

anions (Weizhou, Fan, 2021). In this case, an Ag-based MOF was pyrolyzed to form an AgNPs@C nanocomposite which was deposited onto the surface of a pre-treated GCE electrode to detect $\text{O}_2^{\cdot-}$ released from HeLa cells with an LDR of 3.032×10^{-13} - 5.719×10^{-5} M and LOD of 1.011×10^{-13} M.

Apart from their role in single analyte detection, they have also been explored for detection of multiple cancer biomarkers through a multiplexing approach ^{274,410}. A recent article on detection of multiple liver cancer biomarkers, human tissue transglutaminase 2 (TGM2) and heterogeneous nuclear ribonucleoprotein A1 (hnRNP A1), focusses on development of a $\text{Fe}_3\text{O}_4/\text{MIL-101}(\text{Fe})-(\text{NH})(\text{CO})-\text{COOH}$ (FO/MOF) adorned with alkaline phosphatase (ALP)-labelled detection antibodies ⁴¹⁰. In a single chip, a spatially separated array of eight distinct electrodes was screen-printed in parallel fashion where each electrode comprised of two carbon-ink WE and CE electrodes, and an Ag/AgCl ink RE. Sandwich assays comprising of anti-TGM2 (JU30-02)-FO/MOF/TGM2/ALP-anti-TGM2 (2F4) and hnRNP A1 aptamer-FO/MOF/hnRNP A1/ALP-anti-hnRNP A1 were magnetically assembled onto each WE (**Figure 1.15A-ii**). The ALP present on them could enzymatically convert 4-aminophenyl phosphate (APP) producing electroactive species thereby releasing 2e^- and 2H^+ which facilitated the quantitative analysis of both the biomarkers. The developed probe was designed to detect two biomarkers at a time but the model could be well deployed for detection of up to eight biomarkers simultaneously. The DPV based assay also demonstrated results in clinical undiluted serum samples with promising results for future liver cancer diagnostics however, lacked an upscaling device fabrication approach. An attempt towards solving this was made by Zhang et al. where ZIF-90-ZnO-MoS₂ nanohybrid-based integrated electrochemical liquid biopsy (ELB) platform was developed for direct profiling cancer exosomes from blood ⁴¹¹. The multiplexed point-of-care ELB platform comprised of three WE, one CE and one RE printed on a polyethylene terephthalate substrate sealed with PDMS. Solid-phase redox chemical

species (silver/carbon band) were placed underneath the WE to facilitate Ag^0/Ag^+ redox reaction to eliminate external redox chemicals and matrix effects. ZIF-90-ZnO-MoS₂ was hierarchically grown over the substrate which were immobilized with exosome recognition antibodies through the MOFs -CHO groups. BSA was interlaced for its antifouling property to detect exosomes from culture supernatant of human non-small cell lung cancer cell line (A549) through DPV (**Figure 1.15B**). The developed sensors could efficiently sense trace amounts of CD9, CD63 and CD81 with an LOD of 10fg/mL, 1fg/ mL and, 1fg/mL, respectively. Practical application of the microchip was evaluated by also considering healthy and diseased blood samples generating higher current

values for normal samples. Such rational engineering approaches could pioneer clinically significant MOF-based multiplexing sensing platforms in coming years.

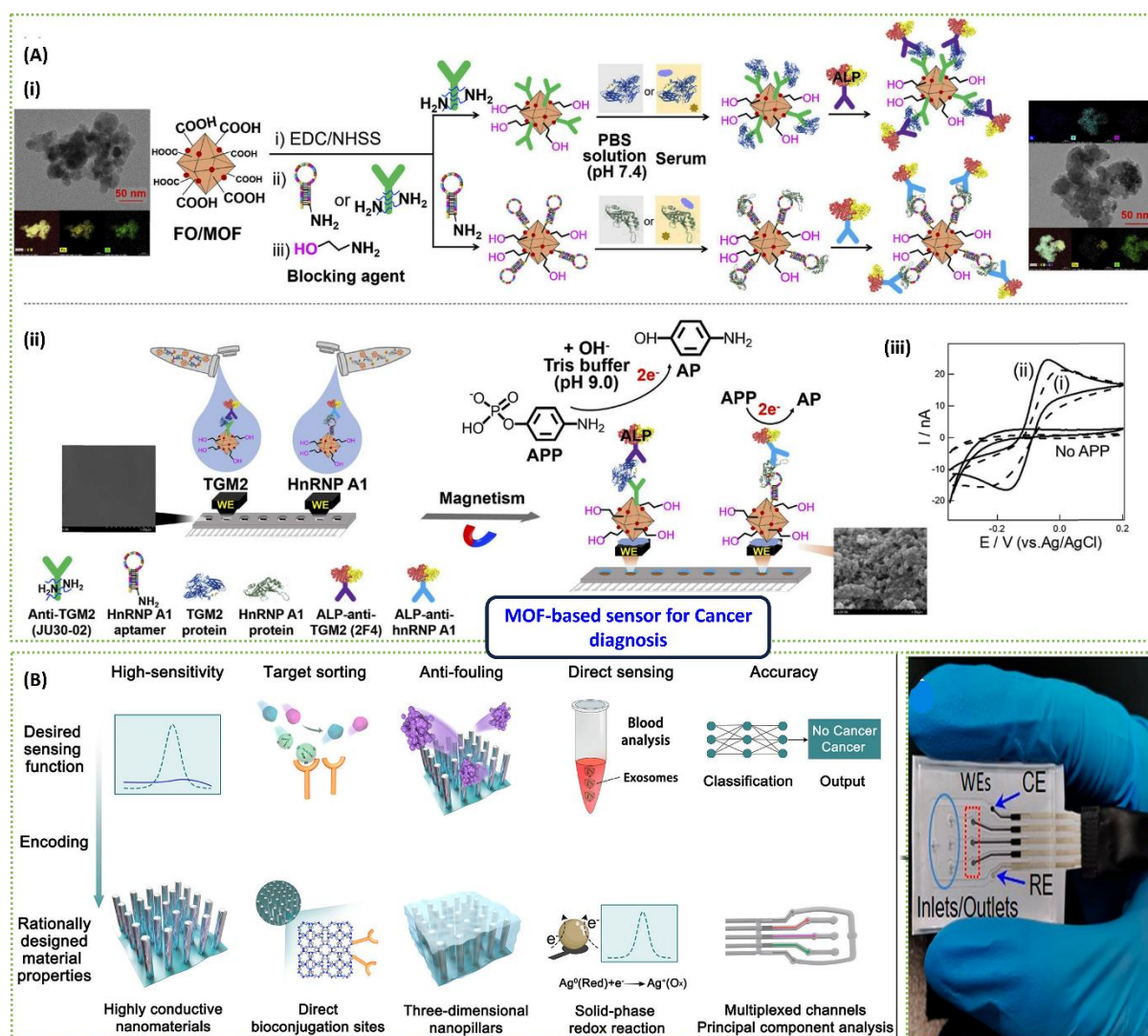


Figure 1.15. A)-i) Representative scheme illustrating the entire process for fabrication of two types of signalling systems, where the first one comprises of FO/MOF conjugated with capturing bioreceptors and the second comprises of ALP-labeled specific antibodies. TEM and EDS mapping of FO/MOF (left inset box) and FO/MOF/hnRNP A1/ALP-anti-hnRNP A1 (right inset box). ii) Representative mechanism of simultaneous detection of TGM2 and hnRNP A1 on fabricated electrode surface. SEM images of bare (left inset box) and final probe (right inset box). iii) CV data corresponding to target protein detection in alkaline medium (pH 9.0), reproduced with permission from J. Li et al., copyright 2023. B) Schematic representation of strategy to impart sensing properties to ZIF-90-ZnO-MoS₂ based integrated electrochemical liquid biopsy platform for effective screening of cancer exosomes (adopted from Y. Zhang et al., copyright 2022).

These recently reported MOF-based biosensors for diagnosis of cancer using its specific biomarkers can be further complimented with other studies elaborately discussed in **Table 1.9** with their respective target analytes, detection techniques, sensor composite, fabrication

strategies, mechanistic role of MOF, real samples considerations and detection efficiency parameters (LDR and LOD).

Table 1.9. Detailed list of latest MOF-based electrochemical biosensors for cancer detection

S.No.	Target Analyte	Detection Technique	Sensor Composite	Fabrication Strategy	Mechanistic Role of MOF	Real Sample	LDR	LOD	Reference
1.	i) Human Epidermal Growth Factor-2 (HER-2) ii) Estrogen receptor	SWV	ZIF-8/Co-MOF	Co-MOF nanosheets were synthesized hydrothermally. ITO was chosen as the WE and was immersed in a solution of ZIF-8 precursors to coat its surface with a thin film. It was further coated with a drop-casted layer of Co-MOF followed by coating it with labeled ssDNA aptamers. The fabricated electrode was incubated with PBS solution containing the target proteins.	High surface area with increased conductivity and also provide adsorption of aptamer	Human serum spiked with HER2 and ER	0-15 pg mL ⁻¹	i) 3.8 fg/mL ii) 6.8 fg/mL	⁴¹²
2.	Circulating tumor DNA (ctDNA)	SWV	Nd-MOF	Template-assisted reagent substitution reaction was used for the synthesis of nanosheets of Nd-MOF which was then integrated with AuNPs to coat onto the GCE electrode surface. The surface was then further functionalized with thiol functionalized capture probes (CPs). On encountering the Fc-labeled signaling probes, the ctDNA-probe hybridization leads to signal generation.	Large surface area providing site to adsorb AuNPs	Healthy human serum	1.0 fM-1.0 nM	0.30 fM	⁴¹³
3.	HER-2	DPV	La-MOF-PbO ₂	GCE electrode was drop-casted with Polyethyleneimine functionalized MoS ₂ NFs (PEI-MoS ₂ NFs) then electrocoated with Au nanocrystals. It was followed by electrode incubation with Ab ₁ and thereby treatment with HER2 samples of varying concentrations.	High specific surface area providing large number of active sites	Human serum	100 fg mL ⁻¹ -10 µg mL ⁻¹	15.64 fM	⁴¹⁴
4.	Matrix metalloproteinase-9	CV, DPV	Fe-MOF	Fe-MOF was synthesized and functionalized with AuNPs and then with peptide2 through sulphhydryl bonding. In another process Au electrode was immobilized with peptide 1. Then the two peptides could integrate with cucurbit[8]uril to complete the probe fabrication.	Increase in conductivity	Serum from metastatic liver cancer patients	0.5 pg-mL ⁻¹ -500 ng-mL ⁻¹	1.30 pg-mL ⁻¹	⁴¹⁵

5.	Alpha-fetoprotein (AFP) Cancer embryo antigen (CEA)	DPV	Cu-UMOFNs@Au NPs and Co-UMOFNs@Au NPs	Cleaned GCE electrode surface was casted with Au@Fe ₃ O ₄ NPs dispersed in 0.5% Nafion solution. It was followed by immersion of the electrode in the mixture of 0.1 mg mL ⁻¹ anti-AFP and 0.1 mg mL ⁻¹ anti-CEA, incubation and later immobilization with Ab ₁ at RT. The last step was blocking of non-specific binding sites using BSA.	Both MOFs are providing high surface area, chemical stability and are carrier for antibodies	Human serum	AFP: 0.001-100 ng mL ⁻¹ CEA: 0.001-80 ng mL ⁻¹	AFP: 0.28 pg mL ⁻¹ CEA: 0.31 pg mL ⁻¹	⁴¹⁶
6.	Programmed cell death Ligand 1 (PD-L1)	EIS	Cu-F/Hs (Copper MOF)	Gold was chosen as the working electrode which was polished and treated with piranha solution followed by washing and drying under N ₂ atmosphere. To fabricate the electrode, it was cycled in 0.5 M H ₂ SO ₄ to remove any oxide layer. Then, PD-L1 aptamer was mixed with TCEP followed by modification with MCH to avoid any unspecific binding. Then, PD-L1 and Ab-Cu-F/Hs were subsequently added to the electrode surface to form sandwich-like arrangement. Finally, the probe was completed post addition and incubation by DA and ODA.	Cu-F/Hs shows oxidase like activity and enhance catalytic activity	Serum	0.5 ng/mL-200 ng/mL	0.11 ng/mL	⁴¹⁷
7.	HER-2	DPV	Zn-Co-MOF@ferrocene (Fc) and Fe-Co-MOF@methylene blue (MB)	Zn-Co-MOF film was electrodeposited onto the electrode surface as a thin film. The formed bimetallic MOF film was used for immobilization of aptamer doped with Fc which acts as a capture probe. In a parallel setup, Fe-Co MOF was synthesized through the hydrothermal route which encapsulated the MB signal molecule. The two MOFs jointly acted as ratiometric sensor probe against target analyte.	Provide active surface for aptamer attachment	Human serum	0.75-250 pg/mL	0.37 pg/mL	⁴¹⁸
8.	Interleukin-1 receptor antagonist (IL-1RA)	EIS	FeCo-MOF/NF	Sliced commercial NF was treated and dried to use as FeCo MOF growth substrate in hydrothermal setup. Functionalization of the formed material was done by initial incubation in EDC-NHS followed by IL-1RA antibody at 4 °C. It was followed by washing in Tween 20-PBS solution and blocking of unwanted sites through BSA. It was then refrigerated for further usage.	Large surface area providing site to adsorb AuNPs	Human serum	10 fg/mL-10 ng/mL	PBS Buffer: 7.30 fg/mL Serum: 7.22 fg/mL	⁴¹⁹

9.	Mucin1	DPV CA	Mn-MOF-74	The aptasensor was constructed with GCE as the WE being electrodeposited with AgNPs followed by coating it with aptamer solution aided by thiol bonding. Later, MCH solution was used to block the non-specific sites followed by functionalizing with varying concentrations of MUC1. Then, Apt/MnO@C@AuNPs were dropped onto the modified electrode surface and was washed with PBS. The probe was stored at 4 °C to finish the final probe.	High specific surface area providing large number of active sites	Human serum	DPV: 0.001 nM- 100 nM CA: 0.001 nM- 10 nM	DPV:0.31 pM CA:0.25 pM	⁴²⁰
10.	microRN A 155	DPV	Cu-MOF	At first, magnetic rod carbon paste electrodes (MCPE) were prepared. In a separate setup, CNF was dispersed in 0.5% Nafion solution to obtain a homogeneous mixture. This mixture was dropped onto the surface of the MCPE followed by dropping CuBTC-AIA solution over it. It was followed by dispersion of Fe@rGO and PANHS solution and further saturation with DMF vapour. The modified probe was immobilized with miR-155 capture probe to obtain the final sensing probe.	Platform for microRNA binding	Human serum	0.2 fM– 500 pM	0.08 fM	⁴²¹

Abbreviations:

GCE: Glassy Carbon Electrode; CV: Cyclic Voltammetry; DPV: Differential Pulse Voltammetry; CA: Chronoamperometry; CC: Carbon cloth; CNF: Carbon nanofibers; PANHS: 1-pyrenebutyric acid-N-hydroxysuccinimide ester; AIA: 5-aminoisophthalic acid; NR: Not Reported

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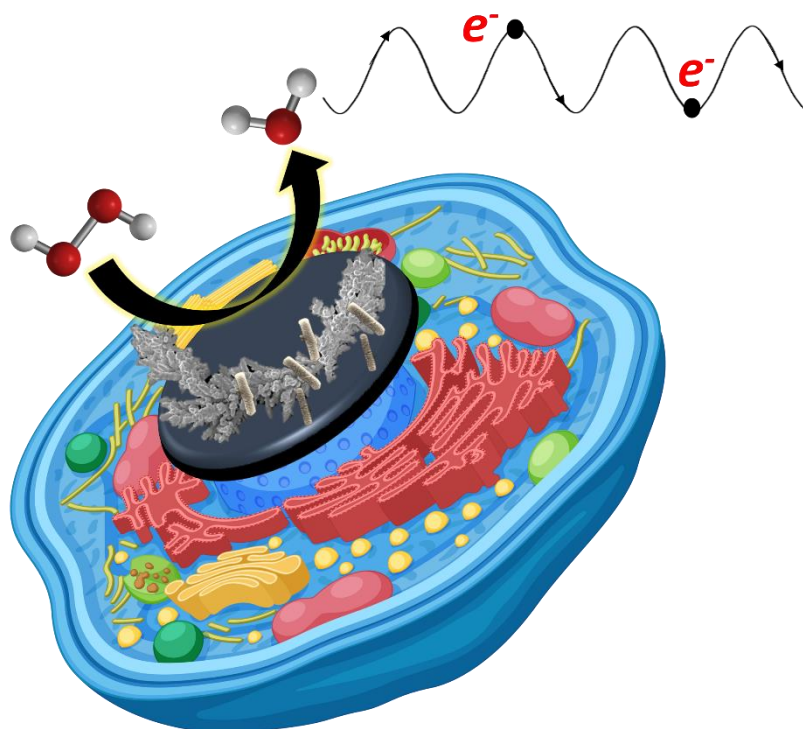
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Chapter II

Electrochemical Assembly of Nickel MOF Decorated Au Dendrites as Peroxidase Mimic for High Performance Hydrogen Peroxide Sensing



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1. Introduction

H₂O₂, an intermediate in various biological reactions and signalling pathways, is difficult to detect in natural premises due to its instability. However, its excessive concentration can pose threat to cellular health by damaging vital proteins and DNA due to the generation of reactive oxygen species. In view of the importance of H₂O₂ a number of analytical techniques such as colorimetry, fluorescence¹, gas chromatography, high performance liquid chromatography (HPLC)², HPLC-UV³ have been used. Techniques like chromatography and colorimetry are highly skill-dependent ones with necessary preprocessing requirements incurring higher costs and labour for the target specimen detection⁴. Whereas, techniques such as fluorescence can have hindered outcomes due to involvement of interferants. Therefore, to avoid such limitations, electrochemical sensing approach could be a plausible solution due to its rapid response, ease of procedure, sensitivity and low cost^{5,6}.

Enzymatic sensors are widely being used now-a-days for sensitive and selective sensing of H₂O₂. POD is a naturally occurring enzyme found in many plants as well as few animal cells. This enzyme is known to be highly selective towards combining with H₂O₂ and methyl or ethyl peroxides⁷. Horseradish peroxidase, a heme-containing enzyme of this class, is known to utilize H₂O₂ in order to oxidise vivid variety of organic and inorganic compounds naturally. However, there are significant disadvantages to this century-old enzyme-based sensing technology, including complicated enzyme orientation and controlled immobilization processes, low stability, a compromised shelf life incurring high costs and high response time⁸. To enhance the economic aspect and facilitate a longer lifetime, peroxide sensors in recent years have focussed on the usage of non-enzymatic sensing platforms which have grasped research limelight lately⁹⁻¹¹.

Newer and versatile non-enzymatic sensing platforms with nanohybrid matrices possessing astounding selectivity and stability have been developed recently for H₂O₂ detection¹². These

non-enzymatic systems often aim to overcome the intrusive chemical and thermal instability of enzymatic sensors making their applicability more diverse. However, most of the non-enzymatic sensors require extensive multistep chemical synthesis approaches as the synthesized materials are majorly drop-casted onto the electrode surface^{13–15}. Nevertheless, these sensor systems also possess low detection limits and require relatively longer detection time¹⁶. Thus, a new nanoengineered system can be developed which incorporates the direct deposition of the matrix components in a highly controlled manner onto the electrode surface as a plausible solution.

Metallic dendrites, fern-like 3-dimensional (3D) branched nanomaterials, are known to facilitate electro-oxidation and electro-reduction of analytes by virtue of the metallic species present in them, such as palladium (Pd), platinum (Pt), silver (Ag), gold (Au) etc.^{17,18}. Furthermore, the branching structure offers a significant number of electroactive sites facilitating electron transfer and thereby conductivity. Another class of materials gaining prominence lately is Metal Organic Framework (MOF). These microporous structures are made up of coordinated metal clusters and organic ligands which when incorporated in electrochemical systems impart them with unique properties such as high surface to volume ratio, large pore sizes, surface tunability, and functional potential¹². However, their properties are often compromised by low conductivity and catalytic properties when compared to classic metallic structures due to the presence of organic linker moieties in their framework resulting into electrode fouling^{19,20}. Much reports on their application in sensor matrices' component have discussed their synthesis through the solvothermal route^{21,22}. There have been recent reports on MOF-based non-enzymatic H₂O₂ sensors which have highlighted MOFs to be suitable sensor matrix component, however, such sensors are dramatically compromised due to micromolar range of detection which does not comply with clinical range of the analyte²³. It is very well established that in heterogeneous biological systems the H₂O₂ concentration

window lies in sub-nanomolar ranges²⁴. Since the outflux of H_2O_2 from cellular microenvironment is dynamic in most of the biological fluids, the detection time should be as low as possible in such complex cellular matrices. Therefore, the sensor should be crafted with desirable traits encompassing all critical features including (i) low detection time, (ii) less contact time with biological fluid to avoid fouling effect, (iii) sub-nanomolar analysis with wide dynamic detection range, and (iv) ease of fabrication. Furthermore, the limiting feature of MOFs in terms of the conductivity can be improved through its systematic fabrication via the electrochemical route²⁵, as an attractive alternative to cope up with its limitations and facilitate lower detection ranges. Considering the uniqueness of metallic dendrites and MOF-based matrices, we hypothesize that a nanohybrid material comprising these can be an interesting combination that can show remarkable performance as a sensor matrix for rapid detection of clinically relevant molecules such as H_2O_2 .

In view of this, an innovative nanohybrid sensor matrix has been designed. A controlled electrodeposition process was adopted for nanoimprinting the AuND over the glassy carbon electrode (GCE) surface. Then, electrochemical deposition of Ni-MOF has been done to improve surface area to many folds. The surface has been further modified by adding a thin coat of hydrazine, which is considered as a powerful electrocatalyst for electrochemical reduction of H_2O_2 ²⁶. The nanohybrid sensing matrix, GCE/AuND/Ni-MOF/Hyd exhibited commendable electrical and catalytic properties and was finally redirected towards the non-enzymatic detection of H_2O_2 . Each layer was characterized using various physical and electrochemical techniques to establish its complete morphology and physico-chemical characteristics. The sensor surface was vigorously studied for its conductive and catalytic behaviour through a series of electrochemical analyses such as, CV, EIS, linear sweep voltammetry (LSV), and chronoamperometry (CA). The analytical strength of the developed sensing probe to detect H_2O_2 was assessed using the LOD and LDR studies. The applicability

of the nanohybrid sensing probe for H_2O_2 detection was validated in synthetic serum samples. The efficacy and selectivity of the sensor towards H_2O_2 through study involving co-existing molecules was also investigated. Reproducibility of results on different electrodes' surfaces over a period of time was checked as well. To the best of our knowledge, this fabricated nanohybrid system is the first sensing module comprising of AuNDs intercalated with MOFs, thereby generating enormous surface area, has been developed for high performance H_2O_2 sensing. The stages of sensor fabrication and the H_2O_2 detection strategy has been illustrated in **Figure 2.1**.

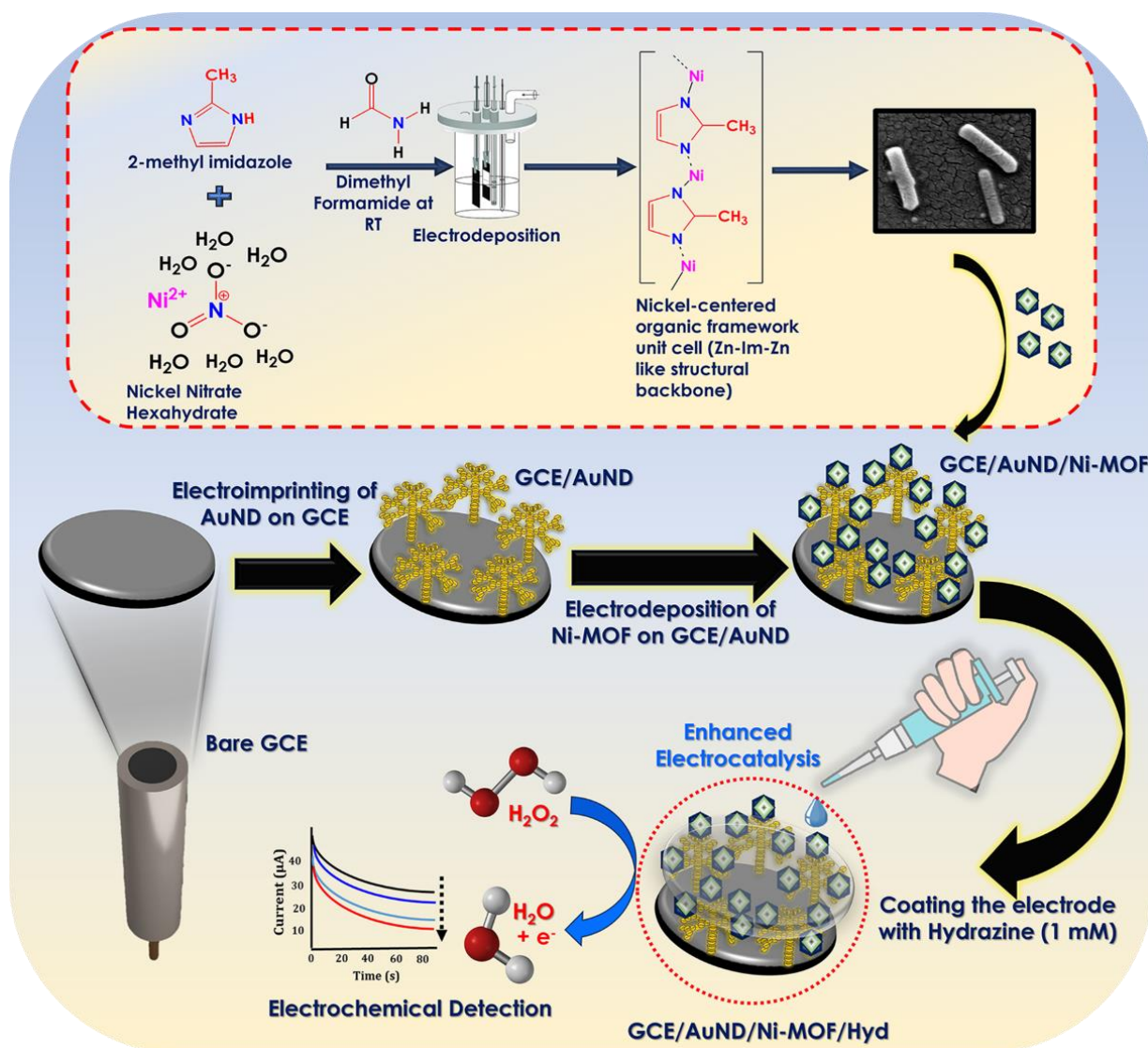


Figure 2.1. The upper panel discusses the structural assembly of Ni-MOF components whereas the lower panel gives an overall detailed diagrammatic representation of the development of nanohybrid sensing probe for electrocatalytic detection of H_2O_2 .

2. Materials and Methods

2.1. Chemicals and Instruments

All reagents and chemicals used in the current experimental setup were of analytical grade. Potassium hexacyanoferrate (II) ($\text{K}_4\text{Fe}(\text{CN})_6$) and potassium hexacyanoferrate (III) ($\text{K}_3\text{Fe}(\text{CN})_6$) were procured from Himedia Pvt. Ltd, Mumbai, India. Nickel nitrate hexahydrate ($\text{Ni}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$), 2-methyl imidazole (2-MeIm), dimethyl formamide (DMF), sodium monophosphate (NaH_2PO_4), sodium bisphosphate (Na_2HPO_4), potassium chloride (KCl),

chloroauric acid ($\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$) were bought by Sisco Research Pvt. Ltd, Delhi, India. Hydrogen Peroxide (H_2O_2) was obtained from Sigma-Aldrich Chemical Co. (India). Double-distilled water from a Milli-Q water purifier (water resistance $18.2 \text{ M}\Omega$) was used for the preparation of standard solutions. Physical characterizations were carried out on a set of instruments facilitated by the Central Instrument Facility Centre (CIFIC) IIT (BHU) such as the XPS [Thermo Fisher K-Alpha instrument], SPM, SEM [FEI Quanta 200 FEG instrument], FTIR [Nicolet iS5, Thermo Electron Scientific Instruments LLC], XRD [Rigaku Miniflex 600 Desktop X-Ray Diffraction System]. An electrochemical analyser (Palm Sense 4.0) comprising of a three-electrode system with GCE as the working electrode and platinum wire, and silver/silver chloride (Ag/AgCl) as auxiliary, and reference electrodes, respectively. The system was used to evaluate the electrochemical characteristics of the nanohybrid sensing probe and thereafter, its analytical efficiency.

2.2. Preparation of GCE/AuND/Ni-MOF/Hyd sensing probe

Initially, the bare GCE electrode surface was polished with the alumina slurry (0.5 micron) and then, was thoroughly rinsed with double-distilled water to wash away all impurities. The first step involved electro-imprinting the AuND over the GCE surface for which 10 mM $\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$ was dissolved in 0.1 M KCl solution and thereafter optimally electrodeposited at -0.30 V vs. Ag/AgCl for 1500 seconds. Further, Ni-MOF was deposited onto the surface of the dendrite by dissolution of 50 mM $\text{Ni}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ and 75 mM of 2-MeIm in 10 ml DMF solvent system at -1.4 V vs. Ag/AgCl for 300 seconds in optimized conditions systematically. The GCE/AuND/Ni-MOF probe was further modified by coating it with 1mM hydrazine prepared in phosphate buffer saline (PBS) to form the final layer as GCE/AuND/Ni-MOF/Hyd post incubation for 20 minutes. Different sets of electrochemistry-based control experiments were conducted to validate the presence of metal ions and hydrazine in a 0.5M sulphuric acid (H_2SO_4), 0.1M NaOH, and 0.1M PBS in vivid experimental settings. In this, each layer of the

nanoengineered probe surface was separately developed and thoroughly characterized for comparative evaluation critically. The final probe was washed with double-distilled water and ethanol sequentially and dried and kept in desiccator for further usage.

Testing process of H_2O_2 involved an initial electrode scanning in 0.1 M PBS buffer solution (blank) with no H_2O_2 present in it. In the next step, H_2O_2 was added in the solution with increasing concentrations (0.1 to 0.5 M H_2O_2), in a three-electrode system setup. This control study was followed by testing the developed probe further at low concentrations of H_2O_2 (10^{-8} to 10^{-15} M H_2O_2) to obtain the calibration plot. The overall reaction of electroreduction of H_2O_2 , aided by hydrazine, can be understood through **Figure 2.1**.

2.3. Electrochemical characterizations of the GCE/AuND/Ni-MOF/Hyd nanohybrid sensing probe

The Zobbles electrolytic solution (ZS) ($\text{K}_3[\text{Fe}(\text{CN})_6]/\text{K}_4[\text{Fe}(\text{CN})_6]$), 5mM; pH-7) was used to carry out the electrochemical characterizations of the GCE/AuND/Ni-MOF/Hyd nanohybrid surface through techniques such as CV and EIS. The EIS data was recorded at a rate of ten points per decade sampling, with an open circuit voltage in the analysis range of 10 to 10^4 Hz. Further, the amperometric responses were logged upto 80 seconds at potential and equilibration time of -0.4 V vs. Ag/AgCl and 10 seconds, respectively, to acquire the standard calibration plot of H_2O_2 .

2.4. Real Sample Analysis

The real-life application of the developed nanohybrid sensing probe GCE/AuND/Ni-MOF/Hyd was validated on synthetic serum samples. For the effective validation study on detection of H_2O_2 , a conventional spike and recovery model was used. The synthetic serum was equilibrated with PBS by 10 times dilution and then spiked with varying concentrations of H_2O_2 . This is a vital step to maintain the molar strength of the real matrix when compared with the standards to prove the sensor's performance. The recorded output current was plotted and

compared with the calibration curve obtained from an initial concentration-dependent study on H_2O_2 in standard buffer solutions.

3. Results and Discussion

3.1. Electrochemical nanoimprinting and characterization of 3D AuND/Ni-MOF/Hyd nanohybrid sensing probe

An optimized synthesis of AuND was done through a simple yet robust electroimprinting technique was followed by Ni-MOF electrodeposition followed by functionalization with a thin layer of Hyd. The electrochemically nanoimprinted **AuND/Ni-MOF/Hyd** probe was thoroughly characterized physically as well as electrochemically to validate its formation. Various physical techniques were adopted at first stage to confirm the probe deposition of MOF layer.

3.1.1. SPM studies

At the first stage, SPM was conducted for each layer of the sensor probe. The electrode was thoroughly cleaned and imprinted with AuND and utilized for the study. In further samples, the Ni-MOF and hydrazine were layered and grown onto the electrode surface and were scanned respectively in tapping mode. The probe and cantilever specifications have been detailed under heading ‘**Additional information**’ in the adjoining box.

The bare surface shows an evenly distributed pattern throughout the pitch of analysis (**Figure 2.2A and 2.2a**). Upon deposition of nanodendrite, enlarged crests of relatively bigger size with significant changes in the surface topology can be seen. A uniform distribution of crests was seen throughout the analysis window suggesting even surface fabrication (**Figure 2.2B and 2.2b**). After this step, on electrodeposition of Ni-MOF, the surface topological characteristics change drastically with additional bigger crests visible which could be due to the successful formation of the MOF, embedded on dendritic surface (**Figure 2.2C and 2.2c**). In the last step, when the hydrazine was coated onto the final probe, a highly patterned surface topology was

observed which was completely different from the previous ones, as seen in **Figure 2.2D and**

2.2d, thereby concluding the steps to final probe fabrication. The SPM analysis was also numerically evaluated through scrutiny of SPM parameter such as z-deflection values, where the incremental shift in values was observed with increase in successive layers on the sensor probe. The z-deflection values obtained at various magnifications establish the uniformity of materials formed through the electrodeposition of layers on the electrode surface. The SPM results can be better understood through values stated in **Figure 2.2**, where a gradual increase in the z-deflection on successive layer deposition is an indicative of successful uniform probe surface fabrication. Since in this study there are defined patterned structures, whose morphology remain uncertain, SEM, EDX and FTIR analyses were taken into consideration for affirmation of structural morphology and chemical insights.

Additional Information: Details on the SPM Cantilever Specifications

Model: NTEGRA Prima; **Company:** NT-MDT Service & Logistics Ltd.

Characterization was performed through a Noncontact Polysilicone probe, tip material comprised of silicon with cantilever chip size being 1.6 x 3.6 mm and thickness of 0.45 mm. The curvature radius of the tip was around 10 mm. The cantilever specifications were as follows:

- Cantilever length (in μm): 94 with a typical dispersion of about ± 2
- Cantilever width (in μm): 34 with a typical dispersion of about ± 3
- Cantilever thickness (in μm): 1,85 with a typical dispersion of about ± 0.15
- Force Constant (N/m) = 12 with a typical dispersion of about $\pm 20\%$
- Resonant Frequency (kHz) = 235 with a typical dispersion of $\pm 10\%$

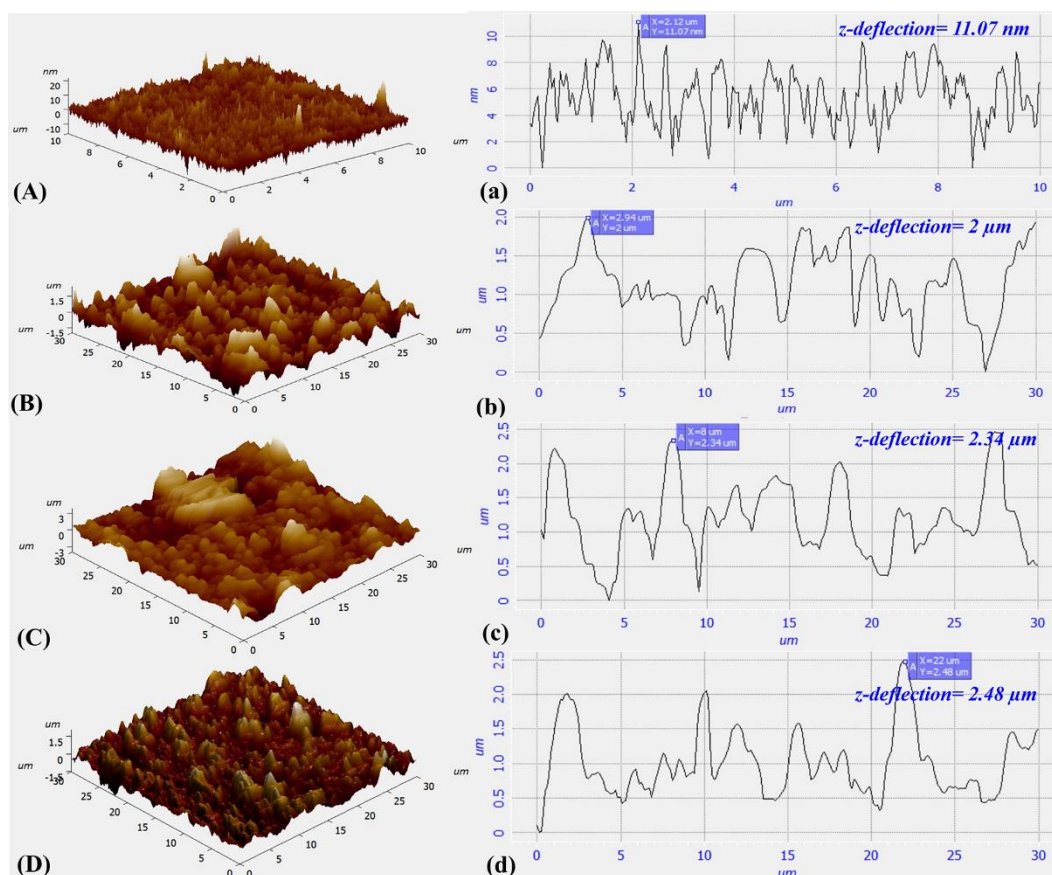


Figure 2.2. (A) SPM 3D and its respective 2D graph (a) illustrating surface of the bare electrode; (B) SPM 3D and SPM 2D (b) graphs corresponding to the second layer of electrode/AuND; (C) SPM 3D and its corresponding SPM 2D graph(c) for the third layer, i.e., electrode/AuND/Ni-MOF; and (D) SPM 3D and SPM 2D graph(d) demonstrating the distinctly developed crests and uniform surface distribution of Hyd.

3.1.2. Physical characterization of sensing probe/ probe materials

SEM analysis was conducted to thoroughly investigate the nanostructural morphology. A clean electrode surface was taken to nanoimprint AuND onto it followed by subsequent modification with Ni-MOF to obtain AuND/Ni-MOF probe surface. **Figure 2.3A** depicts the nanodendritic imprint intercalated with Ni-MOF. A uniformly distributed fern-like forest of Au dendrites flanked with similar structures possessing distinct primary (1°), secondary (2°) and tertiary (3°) branches (specified through orange and red dotted lines) were observed, as shown in **Figure 2.3B and 2.3C** (zoomed image of GCE/AuND/Ni-MOF). The 1° branches were seen bifurcating into smaller 2° branches and then into thinner 3° branches, as evident from the images. Ni-MOF nanostructures as a component of AuND/Ni-MOF hybrid was seen clearly

intercalated with the dendritic structures (as encircled in blue color in the **Figure 2.3B and 2.3C**). To establish the firm belief on the presence of AuND and Ni-MOFs, both the components were exclusively electrodeposited on a bare electrode surface too. This separate study confirmed the leaf-like morphology of AuNDs as well as the rod-like morphology of Ni-MOFs, as seen in the imaged area (**Figure 2.3D and 2.3E**). The zoomed-in view of the Ni-MOF can be clearly seen in **Figure 2.3F**.

Further, to validate the elemental composition of the AuND/Ni-MOF nanohybrid, elemental mapping coupled with EDX analysis was carried out. The mapped results shown in **Figure 2.4A** demonstrates the merged micrograph of all elements viz. Au (**Figure 2.4A-I**), Ni (**Figure 2.4A-II**), N (**Figure 2.4A-III**) and C (**Figure 2.4A-IV**) illustrated through **orange, cyan, dark green, and olive green-colored** patterns, respectively. For better clarity the merged micrograph segregated into constituent element micrographs has been depicted in **Figure 2.4A (I-IV)**. EDX analysis confirms the presence of Au, Ni, N, and C elements in the atomic percentage of about 31.3%, 5.4%, 20.0 %, and 25.2 %, respectively (**Figure 2.4B**). With these atomic proportions, we postulated a structure of the Ni-MOF (as illustrated in **Figure 2.1**) which as a framework should look morphologically identical to Zn-Im-Zn like-arrangement seen in ZIFs. The metal center 'Ni' is postulated to be attached to two imidazole linker moieties through the pyrrolic 'N' lone pairs which makes the ratio look like $[C_4H_6N_2]_2$ connected to one 'Ni' metal center. This makes the ratio of N:Ni as 4:1 which exactly matches with the EDX atomic percentage ratio for N:Ni, forming a 2D framework. Hence, it is possible to infer that Ni-MOF is being formed. Also, the C 1s percentage of the elucidated characterization shows greater atomic percentage of C 1s than the N 1s which is as obvious by their presence in the imidazolate structure where C 1s is in excess. After the confirmatory tests for deducing the elemental composition of the constituents present in the sensor matrix, XRD and FTIR spectra was also obtained to reconfirm the crystalline nature, phase formation and functional groups of the Ni-

MOF (**Figure 2.5**). The FTIR spectrum for Ni-MOF was obtained at a wavenumber scope of 4000-450 cm^{-1} to understand the functional groups present in the structure. The spectrum exhibits various peaks corresponding to the bending and stretching vibrations of the imidazole linker ring in the region of 450-1500 cm^{-1} and are comparable to those reported in previous literature²⁷. The FTIR spectrum presented with peaks corresponding to desirable functional groups confirming the successful synthesis of Ni-MOF through the novel route (**Figure 2.5A**). The -OH stretching vibration at 3450 cm^{-1} corresponds to the moisture as well as hydroxyl moieties present in the MOF structure. Two peaks located at 2929 and 3135 cm^{-1} correspond to =C-H bond vibrations from the aromatic and aliphatic rings in the imidazole structure. In addition to this, two doublet peaks were also observed at 679 and 740 cm^{-1} belonging to the C-H stretching bonds present in the methyl group of the linker. A classic peak at around 472 cm^{-1} corresponds to Ni-N bond which establishes the notion for successful Ni-MOF formation²⁷. Apart from this, the alkene C=C group in the linker contributes to a peak at around 1650 cm^{-1} . Furthermore, in the XRD analysis, the strong diffraction peak at around 14° distinctly confirmed the crystalline structure of the electrodeposited Ni-MOF (**Figure 2.5B**) which is consistent with the previously reported literature²⁸.

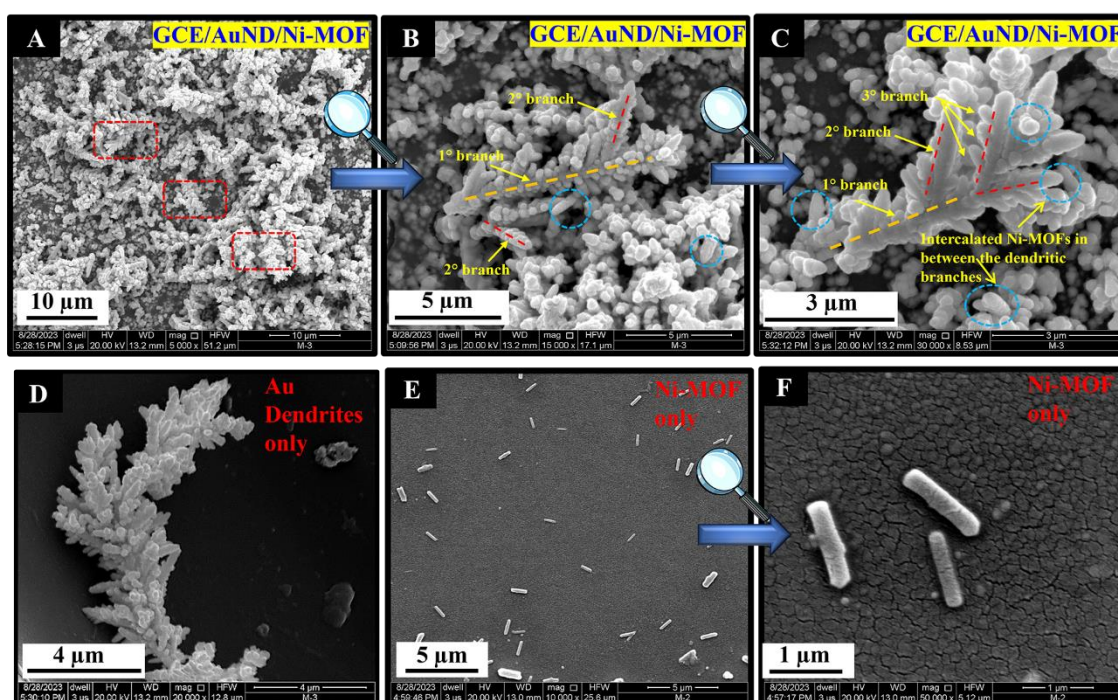


Figure 2.3. (A) depicts the SEM image of AuNDs (red square) intercalated with Ni-MOF at a scale of 10 μm ; (B) and (C) shows zoomed-in SEM micrographs of Ni-MOFs grown over the surface of metallic dendrites (blue circle) at 5 and 3 μm scale, respectively, a distinct fern like architecture of AuNDs with 1°, 2° and 3° branching was seen; (D) shows zoomed-in Au dendrites (E) illustrates uniform distribution of rod-like Ni-MOFs at 5 μm and (F) zoomed-in image of the Ni-MOFs at 1 μm scales, respectively.

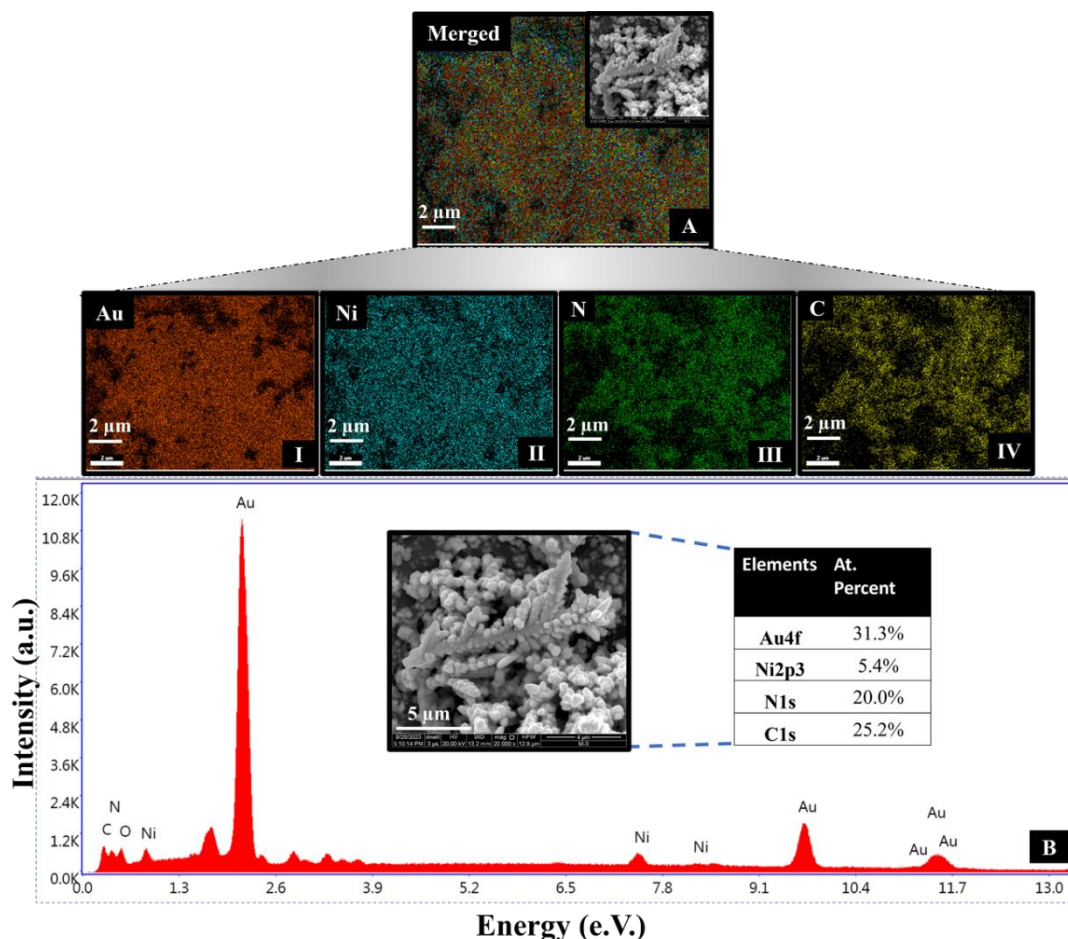


Figure 2.4. (A) depicts the merged elemental mapping of AuND/Ni-MOF probe, showing the distribution of all the elements, viz. Au (orange), Ni (cyan), N (dark green), and C (olive green color) over its surface. The micrograph of individual elements is also depicted separately in 2.4A (I-IV); (B) EDX analysis of AuND/Ni-MOF probe confirming the presence of constituent elements, Au (orange), Ni (cyan), N (dark green), and C (olive green color) with their respective atomic percentage in the nanostructure, as depicted in the adjoining table.

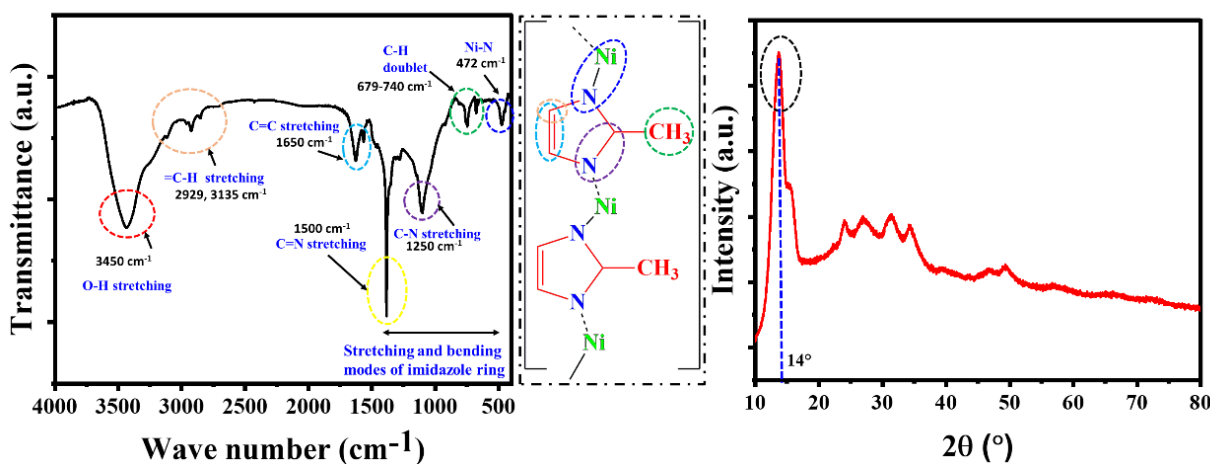


Figure 2.5. (A) FTIR and (B) XRD spectra corresponding to the fabricated Ni-MOF.

These studies are suggestive of the successful coating of different layers at the electrode surface, however, to further dig into the chemical functionalities and oxidation states of all constitutive elements in the developed probe, XPS analysis was performed.

3.1.3. XPS analysis

To validate the elemental composition and the metal oxidation states on the fabricated surface, the sensor probe underwent XPS examination. XPS spectra of Au 4f, Ni 2p, C1s, and N1s elements in the dendrites, MOF and hydrazine components presence on the electrode surface, GCE/AuND/Ni-MOF/Hyd are depicted in **Figure 2.6A, 2.6B, 2.6C, and 2.6D**. The highly resolved XPS spectrum of Au 4f reveals two doublet peaks at 83.66 and 87.48 e.V. (**Figure 2.6A (i) a,b**) which on deconvolution corresponds to characteristic peaks of metallic Au⁰ on the GCE/AuND surface²⁹. These peaks are present in case of GCE/AuND/Ni-MOF/Hyd surface as well (**Figure 2.6A (ii) a,b**) however, their intensity slightly diminishes. This was most likely due to the fabrication of successive layers, confirming their successful coating. The deconvoluted XPS spectra of Ni shows four distinct peaks (**Figure 2.6B (ii) a-d**), for the GCE/AuND/Ni-MOF/Hyd surface, which were not observed earlier in the case of GCE/AuND surface, (**Figure 2.6B (i)**) indicating that the peaks in the former correspond to Ni-MOF only. Of all visible peaks of the spectrum, peaks ‘a’ and ‘c’ correspond to Ni(2p_{3/2}) and Ni(2p_{1/2}) core levels while ‘b’ and ‘d’ represent their corresponding shakeup satellite peaks (sat.), respectively. The peaks located at binding energy values of 855.08, 860.61, 872.58, and 879.09 e.V. can be designated to Ni²⁺(2p_{3/2}), Ni²⁺(2p_{3/2}) shakeup satellite, Ni²⁺(2p_{1/2}), and Ni²⁺(2p_{1/2}) shakeup satellite peaks, respectively^{30,28}. The intense peaks corresponding to Ni²⁺(2p_{3/2}) and Ni²⁺(2p_{1/2}) establish the presence of Ni core levels in its Ni⁺² state within the MOF when bonded to imidazole linker moiety. The hypothesized structure in **Scheme 1** also indicates the Ni metal center to exist as Ni⁺². The difference between the core level Ni peaks (17.5 e.V.) and

the satellite peaks (18.48 e.V.) remained comparable^{30,31}. Further, the sample was examined for the presence of C 1s (**Figure 2.6C (a,b)**) and N 1s (**Figure 5D**) peaks. The peaks at 284.18 e.V. and 287.69 e.V. correspond to C=C and C-N bonds, respectively, which are testament for the presence of these elements in the imidazole backbone of the Ni-MOF, as shown in (**Figure 2.6C (ii) a,b**). These representative peaks, however, were absent when GCE/AuND was examined verifying that the peaks are merely due to the successful coating of Ni-MOF onto the electrode surface. Further, on consideration of the N 1s spectrum, a distinct peak was observed at binding energy value of 398.38 e.V. for GCE/AuND/Ni-MOF and GCE/AuND/Ni-MOF/Hyd as shown in (**Figure 2.6D (ii and iii)**), respectively, confirming the presence of C-N bond which complements with the results of the C 1s spectrum^{28,32}. These peaks signify the imidazole linker moieties of the framework which were absent in case of GCE/AuND (**Figure 2.6D (i)**), suggesting that these peaks are due to the elements of the imidazole framework, exclusively. The N 1s peak intensifies on the addition of hydrazine layer onto the electrode surface in the final step of the GCE/AuND/Ni-MOF/Hyd probe fabrication. The detailed XPS spectra and thereby increment in atomic N1s percentage has been provided in **Figure 2.7** and **Figure 2.8**. XPS analyses, thus, evidenced the presence of both metals as well as non-metals in their desired states on the nanoimprinted surface.

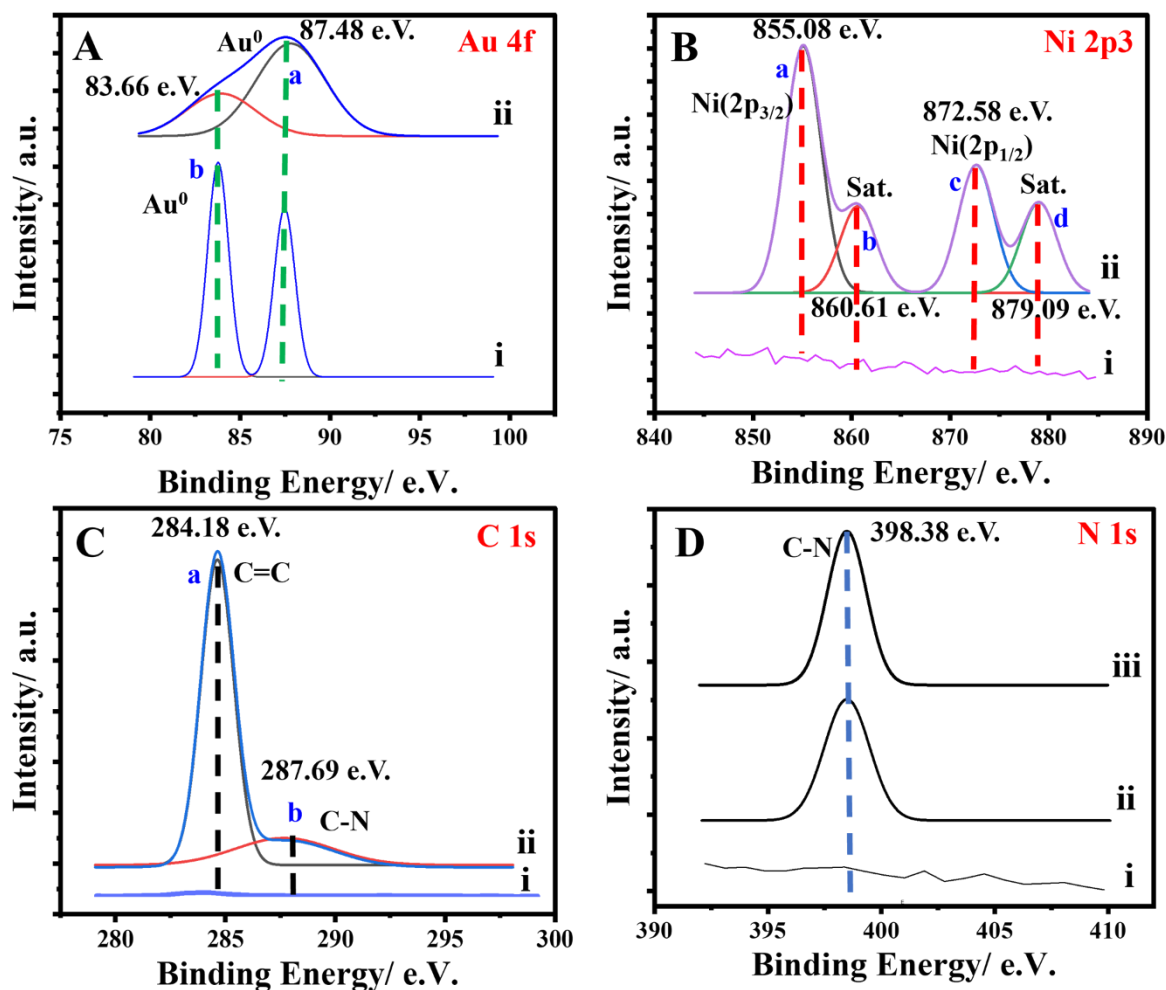


Figure 2.6. XPS spectra stating the oxidation states of elements present in the sensor matrix. **(A)** XPS spectrum corresponding to Au^0 (i) GCE/AuND and (ii) GCE/AuND/Ni-MOF/Hyd probe surfaces; **(B)** XPS spectrum of Ni element (i) for GCE/AuND and (ii) GCE/AuND/Ni-MOF/Hyd surfaces can be deduced into 4 peaks. Absence of these peaks in (i) represent the absence of Ni in GCE/AuND surface. **(C)** represents the XPS spectrum of C 1s (i) in GCE/AuND surface and (ii) GCE/AuND/Ni-MOF/Hyd final probe surface. **(D)** XPS spectra for (i) GCE/AuND (ii) GCE/AuND/Ni-MOF and (iii) GCE/AuND/Ni-MOF/Hyd surfaces.

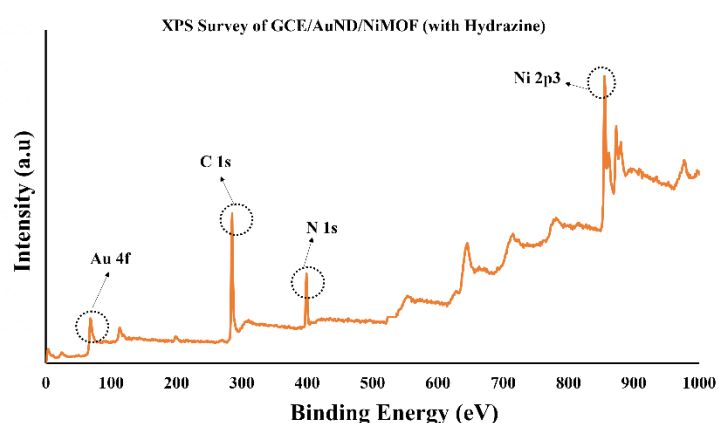
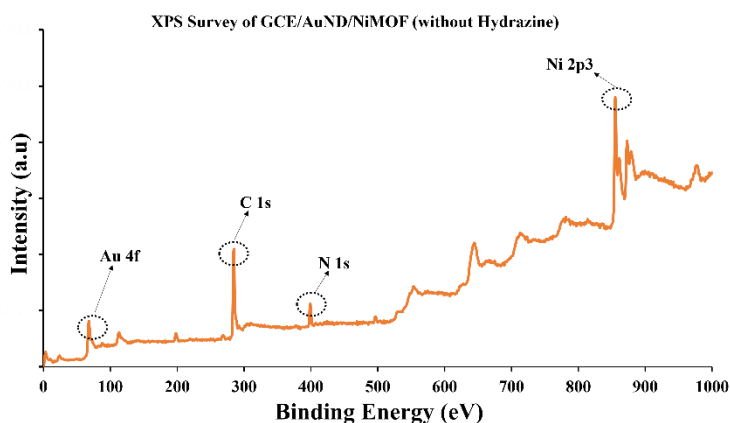


Figure 2.7. A comparison for the XPS spectra of GCE/AuND/Ni-MOF surface without and with the presence of hydrazine. An enhancement in the peak corresponding to N 1s was seen by upto 5 atomic % clearly stating the successful functionalization of probe surface with hydrazine in the final step.

Elements	Atomic Percentage (GCE/AuND/Ni-MOF)	Atomic Percentage (GCE/AuND/Ni-MOF/Hyd)
Au4f	0.05%	0.01%
Ni2p3	12.02%	11.04%
N1s	7.33%	12.46%
C1s	46.5%	50.22%

Figure 2.8. A comparative atomic percentage of GCE/AuND/Ni-MOF and GCE/AuND/Ni-MOF/Hyd. With an introduction of Hyd, there appears an enhancement in N 1s peak implying successful coating of Hyd on the probe surface.

The physical characterisation results were verified through a series of electrochemical experiments as discussed in further sections below.

3.1.4. Validation of Au, Ni, and hydrazine presence at the electrode surface

The presence of Au, Ni, and Hyd in the sensing matrix was systematically examined through a series of experiments with various controls.

The fabricated GCE/AuND/Ni-MOF/Hyd probe was scanned in the potential window of +0.5 V to +1.5 V vs. Ag/AgCl in 0.5 M H₂SO₄ solution. In this case, the representative Au redox peaks were observed at +1.20 (a) and +0.98 V (b) vs. Ag/AgCl (as depicted in **Figure 2.9, black curve**) which are testimony of the presence of Au in the final fabricated probe surface^{33,34}. This was further verified by a negative control experiment where bare GCE was tested in the similar experimental conditions where no redox peaks were observed (**Figure 2.9, green curve**). The elemental validation was done for Ni, where, the GCE/AuND/Ni-MOF/Hyd probe was tested in a solution of 0.1 M NaOH by scanning the potential between -0.5 V to +0.3 V vs. Ag/AgCl. Sharp anodic and cathodic peaks corresponding to Ni were observed at +0.20 V (c) and -0.20 V (d) vs. Ag/AgCl (**Figure 2.9, blue curve**). Similar scan results in bare GCE yielded no peaks at these corresponding potential values signifying the peaks to be exclusively

belonging to elemental Ni³⁵ (**Figure 2.9, brown curve**). In another set of control studies, the GCE/AuND/Ni-MOF/Hyd probe was subjected to 0.1 M PBS and scanned within a potential range of -1.0 V to +0.1 V vs. Ag/AgCl. A sharp anodic peak was observed at -0.20 V (**e**) vs. Ag/AgCl which confirmed the presence of adsorbed hydrazine on probe surface (**Figure 2.9, red curve**). In its negative control counterpart, no peak was seen in case of bare GCE (**Figure 2.9, pink curve**) stating the peak in the former study to be corresponding to hydrazine only²⁶. It was followed by validation of electron transfer mechanism and stability of Au, Ni, and Hyd through a scan rate dependent analysis of final nanohybrid sensing probe in 0.5 M H₂SO₄, 0.1 M NaOH, and 0.1 M PBS solutions, respectively. Cathodic (I_{pc}) and anodic (I_{pa}) peak currents were found to be directly proportional to the scan rates with correlation coefficient values corresponding to 0.99 and 0.99 for Au (**Figure 2.9A**), 0.99 and 0.95(**Figure 2.9B**) for Ni, and 0.88 for hydrazine (**Figure 2.9C**), respectively. These set of experiments successfully confirm the presence of Au, Ni metals and hyd on the developed sensor probe. Along with it, these findings evidently demonstrate that electron transport was taking place primarily aided by surface adsorption phenomenon occurring at the sensor surface. It also evidents the stability of the probe surface even at higher scan rates³⁶, making it an ideal engineered surface for real sample examination.

These outcomes clearly show that the sensor surface underwent adsorption processes and is extremely stable, even at higher scan rates.

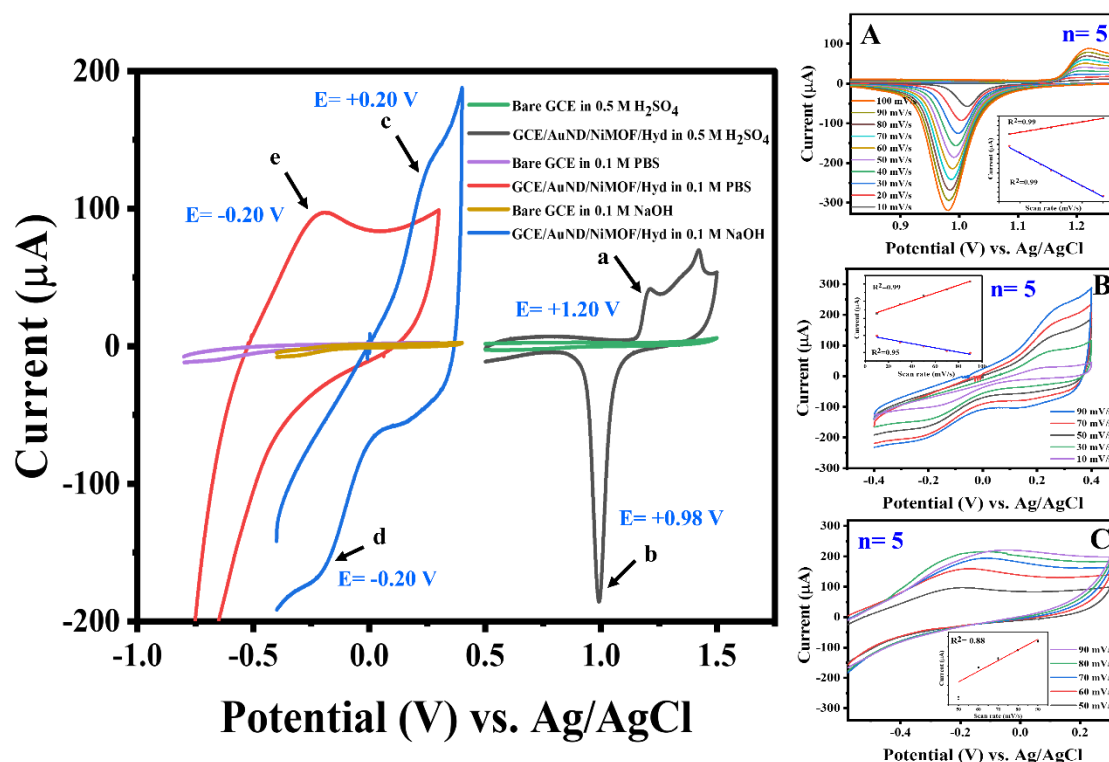


Figure 2.9. CV curve of bare GCE and GCE/AuND/Ni-MOF/Hyd surfaces in 0.5 M H₂SO₄ (black curve), 0.1 M NaOH (blue curve), and 0.1 M PBS (red curve). (A) Scan rate analysis of GCE/AuND/Ni-MOF/Hyd probe in 0.5 M H₂SO₄ with a scan rate varying between 10-100 mV/s; inset representing the linearity between the current and scan rate, (B) Scan rate analysis of GCE/AuND/Ni-MOF/Hyd probe in 0.1 M NaOH with the scan rate varying from 10 to 100 mV/s; inset representing the linearity between the current and scan rate, (C) Scan rate analysis of GCE/AuND/Ni-MOF/Hyd probe in 0.1 M PBS; inset shows corresponding peak values showed linearity. Here, $n=5$ denotes the number of iterations performed.

3.1.5. Electrochemical characterization of GCE/AuND/Ni-MOF/Hyd sensing probe.

Post verifying the presence of desirable sensing probe elements such as Au, Ni metals and Hyd, the fabricated GCE/AuND/Ni-MOF/Hyd probe was proceeded for electrochemical examination to examine its conductive and electrocatalytic properties. CV was conducted in 5 mM ZS at a scan rate of 50 mV/s in an analysis window of 0 to +1.0 V vs. Ag/AgCl ($n=5$) (Figure 2.10A). Peaks within a potential window of +0.2 to +0.8 V vs. Ag/AgCl were observed due to the inherent redox behavior of ZS at GCE (red curve).

Peaks referring to GCE/Ni-MOF (blue curve), GCE/AuND (pink curve), GCE/AuND/Ni-MOF (green curve) and GCE/AuND/Ni-MOF/Hyd electrode (black curve), have been

illustrated in **Figure 2.10**. MOFs have always been known to hamper the conductivity of the sensor probe but in our case, interestingly, when the GCE surface was electrochemically deposited with Ni-MOF, a rise in conductivity was noted. Such electrodeposited MOF-dependent conductivity enhancement has been also observed earlier for different MOFs³⁷. Apart from that, bare GCE when electrodeposited with AuND also exhibited a rise in conductivity. On hybridizing the surface of the GCE with AuND and Ni-MOF sequentially, led to a humongous rise in conductivity (**green curve**). This unexpected rise is an interesting result where we were successfully able to develop a hybrid module comprising of a novel combination of AuND and Ni-MOF for the very first time. A comparative histogram was plotted to understand the redox current response of each layer of the electrode (**Figure 2.10B**). The current density was elevated with addition of Ni-MOF and AuND with the maximum current density observed for GCE/AuND/Ni-MOF, attributed to the synergistic impact of highly conductive surface embedding the conductive MOF components. It was statistically analyzed through a t-test calculation and the p-value was found to be < 0.03.

The numerical quantification of each surface's electrocatalytic activities and charge transfer characteristics was done through the estimation of the effective surface area (A) using the Randles-Sevcik's equation^{38,39}

$$I_p = (2.69 \times 10^5) n^{3/2} ACD^{1/2} v^{1/2} \dots \text{Equation i}$$

where, I_p stands for the highest current output (in amperes), n is the no. of electrons involved in redox reaction (here $n = 1$), A implies the electrodes' effective surface area (in cm^2), C represents the electroactive species' concentration (in mole cm^{-3}), D denotes the diffusion coefficient (in $\text{cm}^2 \text{s}^{-1}$) and its value is $7.6 \times 10^{-6} \text{ cm}^2 \text{s}^{-1}$ for aqueous ferrocyanide, and v is the scan rate (in Vs^{-1}).

The active surface area of GCE, GCE/Ni-MOF, GCE/AuND, GCE/AuND/Ni-MOF, and GCE/AuND/Ni-MOF/Hyd was calculated as $70.11 \times 10^{-3} \text{ cm}^2$, $97.17 \times 10^{-3} \text{ cm}^2$, $150.06 \times 10^{-3} \text{ cm}^2$, $432.96 \times 10^{-3} \text{ cm}^2$, $291.57 \times 10^{-3} \text{ cm}^2$. As lucid these area results are, they clearly emphasize our established notion so far where the conductivity rise is consistent with addition

of subsequent layers on the GCE surface. The area statistics for the GCE/AuND/Ni-MOF nanohybrid sensing probe reveal a rise in about 6.18, 4.46, 2.88 and 1.48 times, when compared to bare GCE, GCE/Ni-MOF, GCE/AuND, and GCE/AuND/Ni-MOF/Hyd, respectively. This backs the higher conductivity and electron transfer capabilities of the designed nanohybrid sensing probe. The conductivity on addition of hydrazine decreases slightly due to presence of organic moieties in it (**black curve**). However, its presence is quintessential for the effective sensing of our target analyte H_2O_2 through reduction.

The validation of CV output was complimented through EIS experiment (**Figure 2.10C**). The values obtained by circuit fitting for GCE (**red curve**), GCE/Ni-MOF (**blue curve**), GCE/AuND (**pink curve**), GCE/AuND/Ni-MOF (**green curve**) and GCE/AuND/Ni-MOF/Hyd electrode surfaces (**black curve**) were plotted as their respective Nyquist plots. The corresponding R_{ct} values were determined through the respective Nyquist plots pertaining to each probe surface viz., 6542 (± 102.12) Ω , 4585 (± 101.49) Ω , 2375 (± 103.55) Ω , 251 (± 103.55) Ω , and 409 (± 108.51) Ω , respectively. There was a gradual decline in the charge transfer resistance with each fabrication step upto GCE/AuND/Ni-MOF layer. A slight increase the resistance of the GCE/AuND/Ni-MOF/hyd was observed complimenting the CV results. The overall EIS findings are in good agreement to the CV analysis (**Figure 2.10D**). This makes it a suitable matrix for the electrochemical sensing of our target analyte.

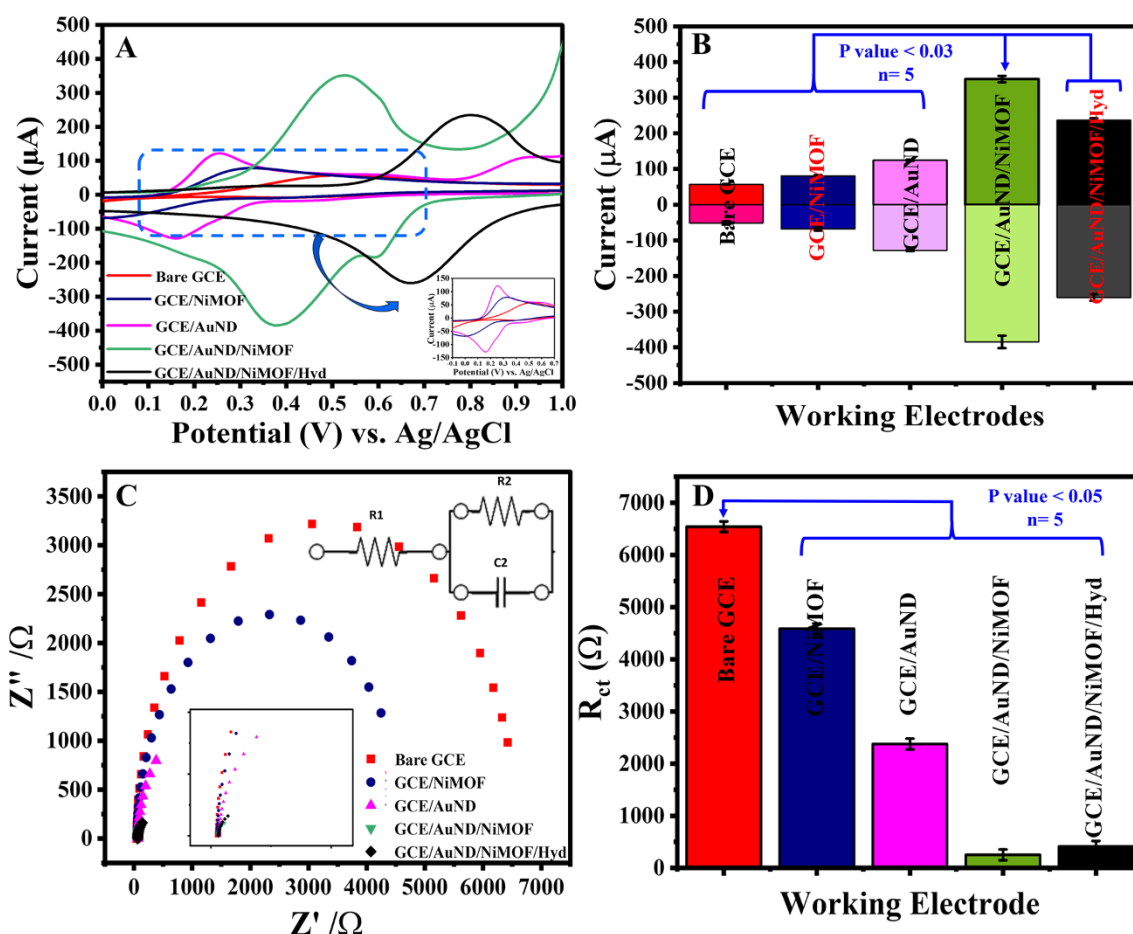


Figure 2.10. (A) CV response of bare GCE (red curve), GCE/Ni-MOF (blue curve), GCE/AuND (pink curve), GCE/Ni-MOF/AuND (green curve), and GCE/Ni-MOF/AuND/Hyd (black curve) surfaces in ZS (5mM; pH-7, scan rate- 50 mV/s); (B) A comparative histogram displays the anodic and cathodic current responses corresponding to each surface, with an amplified response for GCE/AuND/Ni-MOF surface. (C) EIS response in form of Nyquist plots for bare GCE (red curve), GCE/Ni-MOF (blue curve), GCE/AuND (pink curve), GCE/Ni-MOF/AuND (green curve), and GCE/Ni-MOF/AuND/Hyd (black curve) surfaces in ZS (5mM; pH-7, scan rate- 50 mV/s) with its equivalent circuit model; (D) A comparative histogram displays the R_{ct} values of each surface, with the least resistance for the final surface.

To further investigate the electrode behavior, stability and migration of ions at the surface of GCE/AuND/Ni-MOF and GCE/AuND/Ni-MOF/Hyd nanohybrid sensing probe, CV experiments were conducted in 5 mM ZS solution by varying scan rates between 10-100 mV s^{-1} [Figure 2.11 (A and B)]. The developed interface draws attention towards its high stability and diffusion-controlled charge transfer behaviour at elevated potentials. The square root of the scan rates was found to be linearly proportionate to the cathodic (I_{pc}) and anodic (I_{pa}) peak currents having a correlation coefficient of 0.99 (insets of figures). The great charge

conducting capabilities of the nanoimprinted probe illuminates its enormous possibility in futures sensing applications.

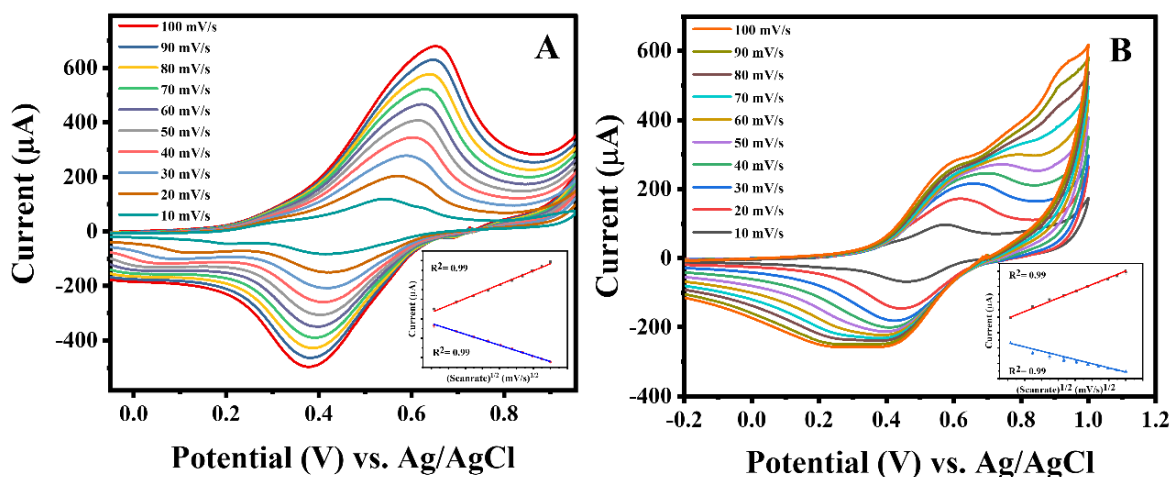


Figure 2.11. (A) Scan rate analysis of the GCE/Ni-MOF/AuND probe in ZS (5mM; pH-7) with scan rate varying between 10 to 100 mV/s to evaluate the stability and charge transfer behaviour at the probe surface; inset corresponding to current at different scan rate showed linearity with a correlation coefficient of 0.99, indicating a diffusion-controlled charge transfer behaviour of final interface; (B) Scan rate analysis of the GCE/Ni-MOF/AuND/Hyd probe in ZS (5mM; pH-7) with scan rate varying between 10 to 100 mV/s to evaluate the stability and charge transfer behaviour at the probe surface; inset shows corresponding current at various scan rates demonstrated linearity with a correlation coefficient of 0.99, indicating a diffusion-controlled charge transfer behaviour of the final probe.

3.2. Analytical performance of GCE/AuND/Ni-MOF/Hyd nanohybrid sensing probe

A set of comparative and control experiments were conducted to undermine the role of each layer viz. bare GCE, GCE/AuND, GCE/AuND/Ni-MOF and GCE/AuND/Ni-MOF/Hyd. The obtained results can be seen through the generated data below. The data was generated for all layers against 0.1 M H_2O_2 concentration (**Figure 2.12 A-B**) to deduce the importance of the constituting matrix material and avoid any misleading interpretations. The bare GCE and GCE/Ni-MOF do not produce any signal response when tested against H_2O_2 . However, there was an increment in current magnitude in the case of GCE/AuND. On combining both the layers, which was the case for GCE/AuND/Ni-MOF, a multi-fold increase in the conductivity as well as surface area was observed (as discussed in **Section 3.1.5**). An increment in the

current response than that in GCE/AuND scenario is also observed. This could be due to activation of the Ni catalytic center when it comes in contact with the Au center of the AuND causing a signal amplification⁴⁰. Then, when the electrocatalytic layer of hydrazine was introduced on this highly conductive surface the enhancement in current response was observed (**purple curve**).

Therefore, this multilayered engineered nanohybrid sensor probe brings out the inherent characteristics of each metal center for deployment in H₂O₂ sensing application which may lead to ultrasensitive detection.

The efficacy of the electrochemically developed nanohybrid sensing probe, GCE/AuND/Ni-MOF/Hyd was analysed for its application by utilizing H₂O₂ as a test molecule. The GCE/AuND/Ni-MOF/Hyd nanohybrid sensing probe was subjected to a series of control experiments to establish that the increment in current magnitude with increase in potential was a resultant of direct and precise electron transfer with H₂O₂. In the first control experiment, a study was conducted at relatively higher concentrations of H₂O₂ i.e., in 0.1 M to 0.5 M H₂O₂. In case of the blank reading, no peaks were seen, however, subsequently, current rose with increasing H₂O₂ concentrations between 0.1 to 0.5 M (**Figure 2.12 C**). The curve shows a linear trend signifying concentration dependent signal, with a correlation coefficient of 0.99 (**Figure 2.12 D**). The regression equation obtained from curve is as follows: $\Delta I (\mu A) = 47.131 (\pm 62.626) + 4471.105480 (\pm 147.5500) \times \text{Conc. [H}_2\text{O}_2 \text{ (M)]}$.

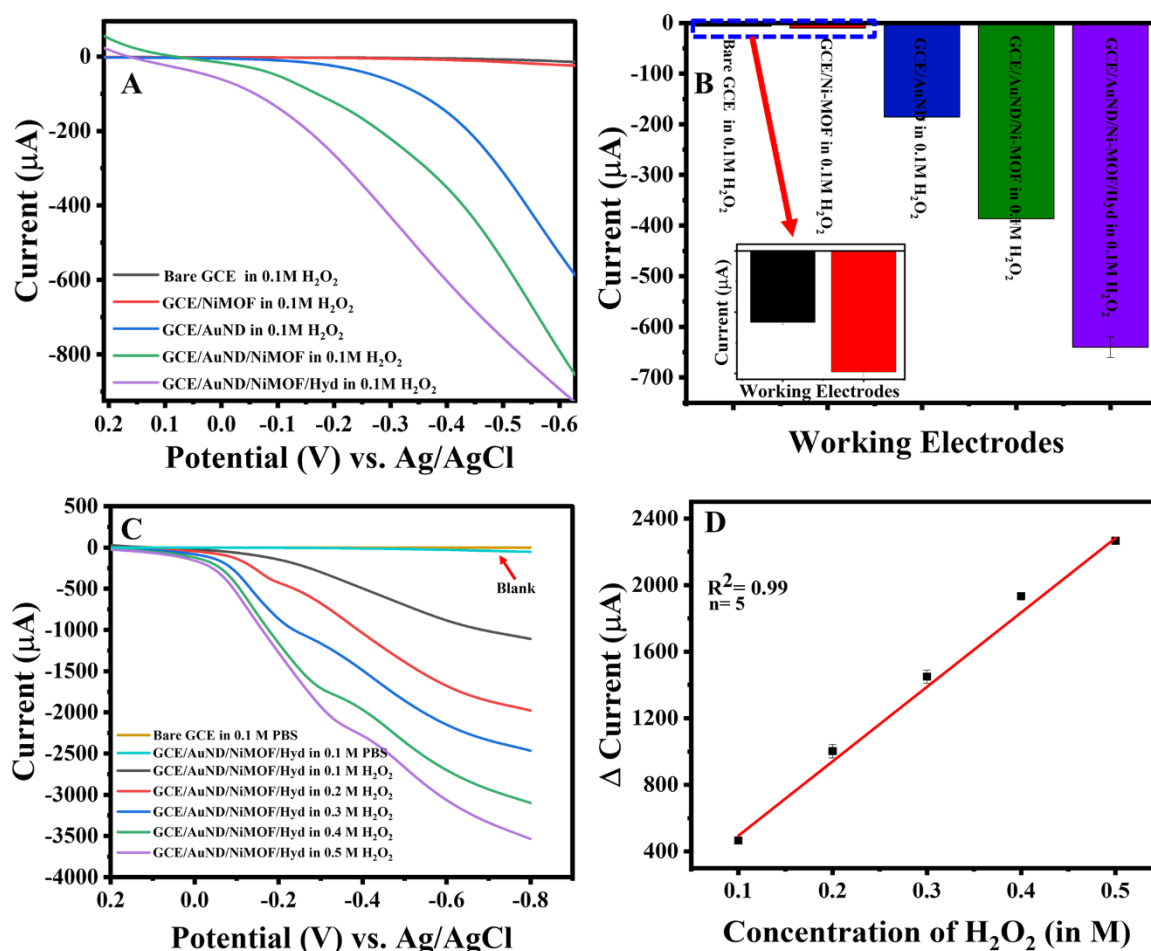


Figure 2.12. (A) A comparative LSV curve corresponding to each layer of GCE/AuND/Ni-MOF/Hyd fabrication in PBS and 0.1 M H₂O₂ dissolved in PBS. (B) Corresponding histogram generated from the layer-by-layer study, plotting the cathodic current response generated by the LSV curve measurements. (C) Concentration-dependent control analysis of GCE/AuND/Ni-MOF/Hyd probe in PBS (cyan curve), and in 0.1 M (black curve), 0.2 M (red curve) and 0.3 M (blue curve), 0.4 M (green curve) and 0.5 M (purple curve) H₂O₂ to validate that peak is exclusively due to H₂O₂; (D) Corresponding calibration curve at varying concentrations ranging from 0.1 M to 0.5 M H₂O₂ in PBS with correlation coefficients of 0.99.

In the second control test, the charge transfer behaviour and stability of the analyte towards the GCE/AuND/Ni-MOF/Hyd probe was analysed. It comprised of scan rate dependent study through an alteration in the scan rates between 10 and 100 mV/s in the presence of 0.1M H₂O₂ (Figure 2.13A). With an increment in scan rates, the cathodic peak currents also increased linearly. The engineered nanohybrid probe demonstrates diffusion-control charge transfer behaviour and excellent surface stability as the cathodic peak current was found to be proportional to the square root of scan rate possessing a correlation coefficient of 0.99 (Figure

2.13B). Therefore, both the control studies inferred that the designed GCE/AuND/Ni-MOF/Hyd nanohybrid sensing probe is highly stable and can effectually sense H₂O₂.

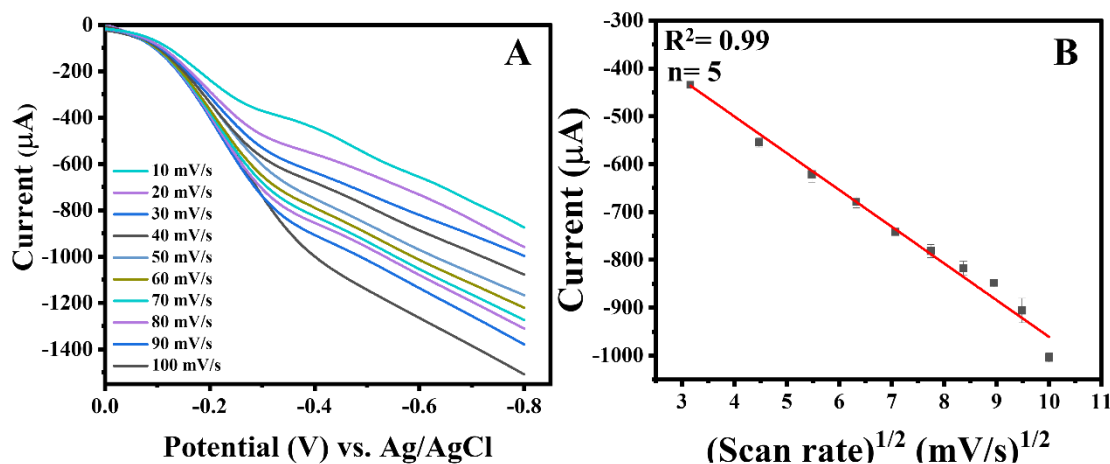


Figure 2.13. (A) Scan rate dependent analysis of GCE/AuND/Ni-MOF/Hyd nanohybrid probe in 0.1M H₂O₂ at a scan rate between 10 and 100 mV/s; As scan speeds increased, peak current rose with a small shift to the negative side; (B) corresponding current at different scan rates showed linearity with a correlation coefficient of 0.99.

The sensing capabilities of the GCE/AuND/Ni-MOF/Hyd nanohybrid probe were further investigated using amperometry to find the minimal concentration since amperometry is considered as a highly sensitive technique³⁷. First of all, the signal output for GCE/AuND/Ni-MOF/Hyd (**blank**) was recorded. In this case, a nominal peak at -0.4 V vs. Ag/AgCl was observed (**brown curve**). Thereafter, the GCE/AuND/Ni-MOF/Hyd probe was evaluated for varying concentrations of H₂O₂ and the signal output was found to be increasing with an increase in H₂O₂ levels (**Figure 2.14A**). On the basis of the amperometry data, the calibration curve showcased a linearity within the concentration range of 1 × 10⁻⁸ to 1 × 10⁻¹⁵ M (**Figure 2.14B**). The LOD of H₂O₂ was calculated as 0.34 (±0.05) × 10⁻¹⁵ M using **Equation ii**

$$LOD = \frac{3SD_b}{\text{slope}} = \frac{3SD_b}{\frac{dy}{dx}} = \frac{3SD_b}{\frac{dy}{d\ln x} \times \frac{d\ln x}{dx}} = \frac{3SD_b}{\frac{dy}{2.303(d\log x)} \times \frac{1}{x}} = \frac{3 \times 2.303 \times SD_b \times x}{\frac{dy}{d\log x}} = \frac{3 \times 2.303 \times SD_b \times x}{\text{Slope of semilog plot}}$$

.....Equation ii

where σ_b represents the blank sample's standard deviation, and m is the calibration plot's slope

The linear increment in amperometry current responses with rise in H_2O_2 concentrations in between 1×10^{-8} to 1×10^{-15} M can be summed up through the regression equation shown below: $I (\mu\text{A}) = 7.64 (\pm 0.42) + 0.48 (\pm 0.03) \log \text{Conc. } [\text{H}_2\text{O}_2 (\text{M})]$.

Response time, which implies to the time essential for the surface to generate a signal response post the injection of the analyte, has a considerable influence on the sensor's efficacy. For examining the same, CA was executed by injecting varying concentrations of H_2O_2 in blank PBS solution. The current peak was measured at a constant potential of -0.4 V vs. Ag/AgCl and response time values were obtained at varying concentrations. **Figure 2.15** depicts the chronoamperometric response on addition of 10^{-9} M H_2O_2 to a stable current **(a)**. After injecting H_2O_2 at point **(b)**, a sharp spike in current due to diffusion **(c)** was noted between 262.63 seconds and 263.86 seconds; it was followed by saturation of current **(d)**. Similar experiments were conducted for different H_2O_2 concentrations, viz. 10^{-10} M, 10^{-12} M, 10^{-13} M, as mentioned in **Figure 2.16**. With an increase in the concentrations, a slight increase in the response time of the GCE/AuND/Ni-MOF/Hyd sensor probe was observed, however, was restricted to <8 seconds. The average response time for the probe was found to be 5.02 ± 0.42 seconds indicating the rapid sensing ability of the system.

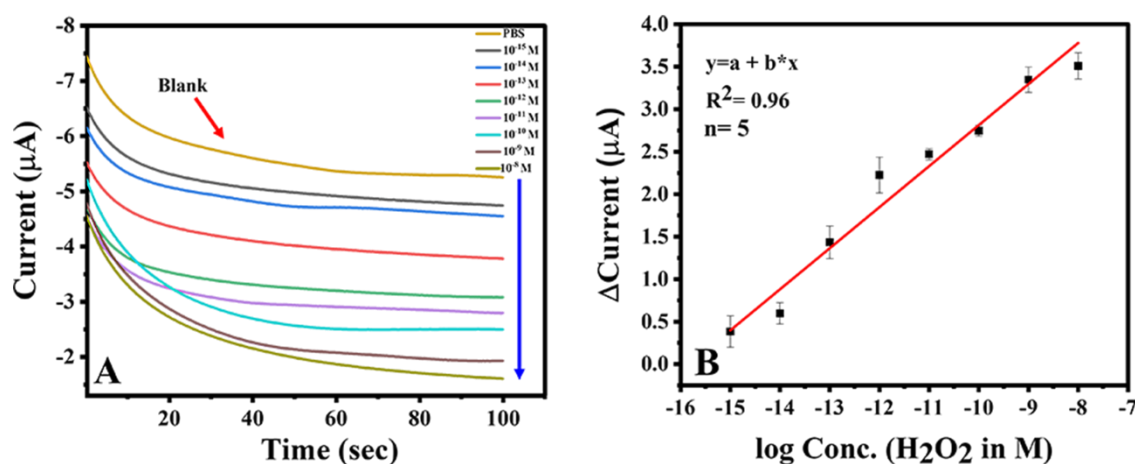


Figure 2.14. (A) The chronoamperometric response of GCE/AuND/Ni-MOF/Hyd sensor probe in the range of 1×10^{-8} to 1×10^{-15} M H_2O_2 in PBS to find the minimal concentration of H_2O_2 ;

(B) Corresponding calibration curve of varying concentrations of H_2O_2 in PBS with a correlation coefficient of 0.96.

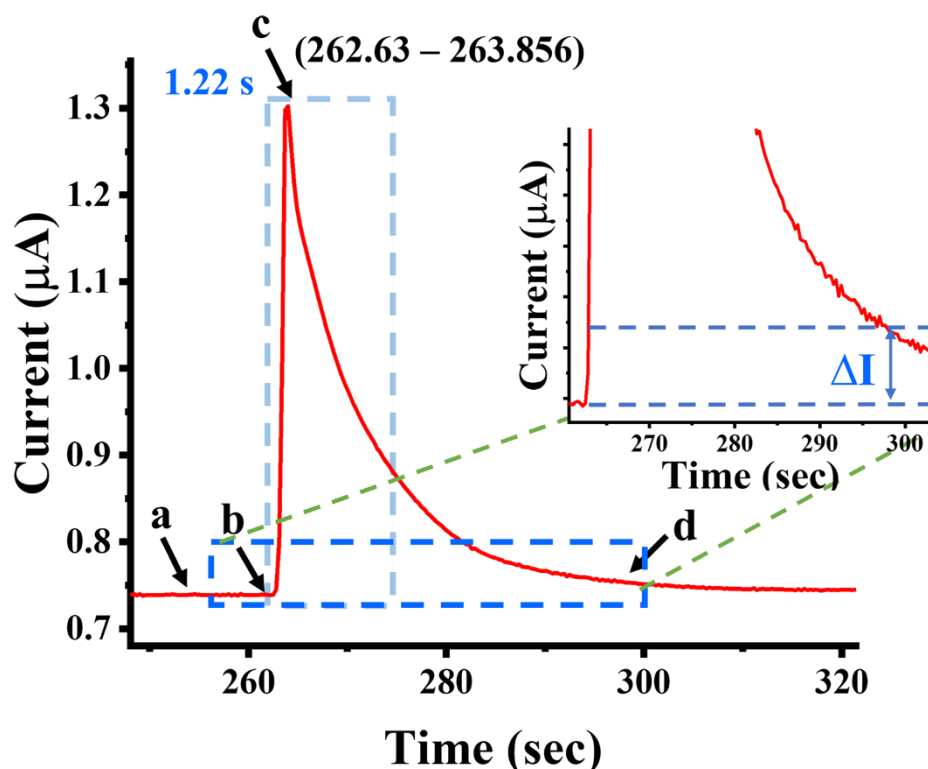


Figure 2.15. Chronoamperometric evaluation of the GCE/AuND/Ni-MOF/Hyd nanohybrid sensing probe in PBS buffer following injection of 10^{-9}M H_2O_2 concentration showing a response time of 1.22 seconds. (a) stabilized current, (b) injection point, (c) spike in current, and (d) differential current magnitude post saturation.

Concentration (in M)	Mean Response Time
10^{-9}	1.5 ± 0.3 sec
10^{-10}	3.3 sec
10^{-12}	7.5 sec
10^{-13}	7.8 sec
Average response time	5.02 ± 0.42 sec

Figure 2.16. Response time of the developed nanosensor probe corresponding to different concentrations of H_2O_2 . The mean response time was found to be 5.02 ± 0.42 seconds

The produced 3D nanohybrid sensitive platform is fascinating because of its short fabrication duration devoid of bulky and conventional synthetic chemical processes, a lower LOD, and faster response time. Additionally, the thorough evaluation of the sensor's analytical performance has been compared to recently published articles on MOF-based H₂O₂ sensors in **Table 2.1**. It is interesting to note that our developed sensor possesses almost 9 times better LOD than few of the recently reported MOF-based sensing setups^{41,42}. Not only this, our developed probe is working optimally in the prescribed clinical ranges²⁴.

Table 2.1. *A comparative analysis of recently published reports on MOF-based sensor.*

S.No.	Probe Composite	Fabrication strategy	Technique used	Reaction time	LOD	LDR	Real Sample	Reference
1.	PA-Tb-Cu MOF	The single material nanoparticle was synthesized by exploring the luminescent behaviour of Tb ³⁺ , catalytic potential of Cu ²⁺ and using m-phthalic acid(PA) as the ligand moiety.	Fluorescence	20 minutes	0.2 µM	0 to 500 µM	Milk samples	Qi et al. ⁴³
2.	Fe-MOF	GCE surface was used the working electrode for the one-step electrodeposition of the MOF film using a precursor solution comprising of metal salt and linker molecule dispersed in a probase in DMF.	LSV	N.R.	N.R.	N.R.	N.R.	Zhang et al. ⁴⁴
3.	Co-MOF	The MOF/titanium mesh hybrid was synthesized hydrothermally in DMF solvent system at 125 °C for 12 hours by dipping Ti mesh into the reaction chamber. The synthesized entrapped MOF in Ti mesh was used as the WE.	CV	3 seconds	0.25 µM	1 to 13,000 µM	A549 cells	Xia et al. ⁴⁵
4.	SWNT-MOF(Cu)	A catalytic ink was dip-coated onto the surface of a gold wire which enabled the growth of the nanotube. It was further modified with SWNTs deposited through the process of CVD. Then, <i>in situ</i> synthesized Cu MOF was electrodeposited to obtain the probe surface.	CV, EIS, CA	50 seconds	0.21 µM	1 to 2000 µM	Live cells (RAW 264.7), Raw milk	Nandhini et al. ⁴¹
5.	Ce-UiO-66-BPDC	The probe was prepared using Ce-UiO-66-BPDC functionalized with Au-Pt NPs through the process of electrodeposition.	CV, CA	8 seconds	89 nM	0.15 to 64.8 and 114.8 to 1164.8 µM	A549 cancer cells	Yang et al. ⁴⁶
6.	IrNCs@Ti-MOF	Chromogenic reaction was recorded for IrNCs@Ti-MOF, TMB sequentially tested against varying concentrations of H ₂ O ₂ in a sodium acetate-acetic acid buffer.	Colorimetry	2-10 seconds	14.12 µM	0 to 900 µM	Rabbit serum	Mu et al. ⁴⁷

7.	M-PCN-222 where, M = Mn, Fe, Co, and Ni	MOFs were synthesized through a microwave-assisted hydrothermal route which displayed a peroxidase-mimic module. Presence of Fe-PCN-222, H ₂ O ₂ , and TMB, caused the conversion of TMB into blue-colored ox-TMB.	Colorimetry	10 minutes	Fe-PCN-222: 0.3 μ M Mn-PCN-222: 0.9 μ M Co-PCN-222: 1.0mM Ni-PCN-222: 9.6 μ M	Fe-PCN-222: 1 to 100 mM	Milk, Orange juice, Grape juice, human tears	Shu et al. ⁴⁸
8.	Cyclodextrin MOF (CD-MOF)	Peroxidase (POD) and oxidase (OXD)-like mimicking properties were seen in the synthesized MOF. The POD-like activity was seen through TMB as POD substrate which exhibited visible colour change readout. The OXD-like activity was determined using the TMB colorimetry as well as a L-ascorbic acid assay.	Fluorescence, Colorimetry	30 minutes	0.06 mM	0.11 to 0.95 mM	HepG2 cells	Tan et al. ⁴⁹
9.	Cu MOF	Polished GCE surface was dipcoated with a mixture of MOF and MWCNTs topped with nafion to obtain the desired electrode.	LSV	N.R.	0.46 μ M	3 to 70 μ M and 70 to 30000 μ M	River water, tap water samples	Zhou et al. ⁵⁰
10.	Cu-porphyrin MOF	Polished GCE surface was dropcasted with AgNPs/ Cu-TCPP nanocomposite to obtain the final sensing probe	CV, Amperometry	N.R.	1.2 μ M	3.7 μ M to 5.8 mM	Disinfectant sample	Ma et al. ⁵¹
11.	Cu MOF	Polished GCE surface was dropped with carboxylated graphene. It was then dipped into the crystalline solution of Cu-MOF precursors to coat the surface with the MOF over 7 hours at RT.	CV, Amperometry	N.R.	6.7×10^{-8} M	2.00×10^{-7} to 1.85×10^{-4} M	Disinfectant sample	Zhou et al. ⁵²
12.	AuNPs-NH ₂ /Cu-MOF	Polished GCE surface was dropcasted with AuNPs-NH ₂ /Cu-MOF suspension to obtain the desired probe.	Amperometry	2 seconds	1.2 μ M	5 to 850 μ M	HeLa cells	Dang et al. ⁵³
13.	Cr-MOF	Polished GCE surface was dropcasted with an aqueous suspension of MIL-53-Cr MOF. Nafion and ethanol.	CV	N.R.	3.52 μ M	25 to 500 μ M	Human serum	Lopa et al. ⁵⁴

14.	Ni-MOF	NiO microspheres on titanium carbide was obtained by calcination of Ni-MOF. It was made into solution of ethanol and was dropcasted on GCE surface.	DPV	N.R.	0.34 μM	10 μM to 4.5 mM	N.R.	Ramachandran et al. ²³
15.	Cu-BTC MOF	ITO was chosen as the WE and was dropcasted with Cu-MOF/GO dispersed in aqueous gelatin. Then, the GO was electrochemically reduced to obtain Cu-MOF/ERGO _n /ITO	Amperometry	N.R.	0.44 μM	4 μM to 17.334 mM	Milk	Golsheikh et al. ²⁰
16.	Ni-MOF	Adipic acid based Ni-MOF was mixed with graphite powder. This was packed into a syringe to form the AP-Ni-MOF/CP working electrode.	Amperometry	15 seconds	0.0009 mM	0.004 mM to 60 mM	Lens cleaning solution	Sherino et al. ⁵⁵
17.	ZIF-8	Carbon sheet based nanozyme was synthesized using ZIF as template through thermal and sonochemical methods.	Amperometry	N.R.	0.76 μM	1 μM to 20 m	HeLa cells	Wang et al. ⁴²
18.	Ni-MOF	Imidazole based Ni-MOF was electrodeposited on GCE surface electrocoated with Au nanodendrites. Hydrazine was drop-casted on the final nanohybrid surface to enhance electrocatalysis.	Amperometry	5.02 $\pm$ 0.42 seconds	0.34 (± 0.13) $\times 10^{-15}$ M	1 $\times 10^{-8}$ to 1 $\times 10^{-15}$ M	Serum	Current Work

3.3. Selectivity assay

The commercial applications of the developed GCE/AuND/Ni-MOF/Hyd nanohybrid probe requires proving its selectivity towards the specific target analyte present amongst many co-existing interferant molecules within a complex matrix. Therefore, we chose various co-existing molecules such as superoxide free radicals, ascorbic acid, alanine, citric acid, cysteine, glucose along with our target analyte H₂O₂, to examine nanohybrid sensing probe's selectivity.

Figure 2.17A highlights the signal response of the co-existing molecules which displayed minimal signals at around -0.4 V vs. Ag/AgCl. **Equation iii** was used to determine the coefficient of selectivity (K_{sel}) of the co-existing molecules, with their respective values stated in **Table 2.2**. The values were found to be significantly low ($K_{sel} \ll 1$), with p-value <0.02 emphasizing good selectivity of the developed GCE/AuND/Ni-MOF/Hyd probe for H₂O₂ analysis.

$$K_{sel} = (\text{Signal})_{\text{Interfering molecules}} / (\text{Signal})_{\text{H}_2\text{O}_2} \dots \text{Equation iii}$$

where K_{sel} denotes the coefficient of selectivity, $(\text{Signal})_{\text{interfering molecules}}$ are the current corresponding to interferants, and $(\text{Signal})_{\text{H}_2\text{O}_2}$ is the response generated by the nanoprobe after being treated with H₂O₂.

Co-existing molecules	Current Signal (μA)	K_{sel}
<i>Ascorbic Acid</i>	0.125	0.19
<i>Alanine</i>	0.010	0.015
<i>Cysteine</i>	0.012	0.018
<i>Citric Acid</i>	0.084	0.132
<i>Glucose</i>	0.009	0.014
<i>Superoxide free radical</i>	0.016	0.025
<i>H₂O₂</i>	0.633	1

Table 2.2. Coefficient of selectivity (K_{sel}) of the co-existing interferant molecules

No significant response was recorded for the co-existing interferants in our experiments due to the electrochemical passivity of developed GCE/AuND/Ni-MOF/Hyd nanohybrid probe towards these molecules. Another reason could be the analytical potential range, which forbidden other electroactive species from experiencing the redox reaction. Further H_2O_2 was measured using the GCE/AuND/Ni-MOF/Hyd probe in synthetic serum samples in order to evaluate the practicability of our developed sensing matrix.

3.4. Real sample analysis

H_2O_2 , an important intermediate in various biological pathways rises in various pathophysiological conditions including cancers. It's excessive concentration in bodily fluids can be a threat to the cellular system which can lead to DNA damage²⁴. The GCE/AuND/Ni-MOF/Hyd sensing matrix was evaluated against H_2O_2 in synthetic serum samples to understand its ability to detect the analyte. Spike and recovery test method was adopted to estimate H_2O_2 in synthetic serum samples for evaluation of the potential of constructed nanohybrid sensing probe. Firstly, synthetic serum was equilibrated in PBS followed by spiking it with various concentrations of H_2O_2 . The current response was then documented under the optimized experimental settings. The recovery percentage of H_2O_2 at different concentrations was calculated using **equation iv**, as follows,

$$\% \text{ Recovery} = | [S]_{H_2O_2} - [B]_{H_2O_2} | / [SS]_{H_2O_2} * 100 \dots \text{Equation iv}$$

where, $[S]_{H_2O_2}$ and $[B]_{H_2O_2}$ are the analytical response of the GCE/AuND/Ni-MOF/Hyd nanohybrid probe in synthetic serum samples with H_2O_2 spiked and blank samples, respectively; $[SS]_{H_2O_2}$ corresponds to the response in the standard buffer with same concentration of H_2O_2 .

The current output generated for varying concentrations of H_2O_2 in the synthetic serum samples (n=5) has been represented using histograms, as shown in **Figure 2.17B (olive green)**. There is a linear increment in the current response with rise in H_2O_2 concentrations, where the current recovery for different H_2O_2 concentrations ranges between 90.20 and 94.14% (RSD <3.8 %), when compared with the standard assay results (**blue histogram**). The mathematical depiction

of the linear increment in signal output with increasing H_2O_2 concentrations can be explained through the regression equation as follows: $\Delta I (\mu\text{A}) = 7.03 (\pm 0.43) + 0.45 (\pm 0.03) \log \text{Conc.} [\text{H}_2\text{O}_2 (\text{M})]$. The sensor efficacy was phenomenal in synthetic serum samples, with a correlation co-efficient of 0.95. During the analysis, the peak current values in real samples were found to be comparable to the corresponding values in the standard calibration plot. These variations could be a resultant of restrictions in ion mobility and minor handling errors in real sample matrices. Serum also comprises of different constituting components however, they did not hinder with our experimental results, thereby, incurring no contrary effects on the sensing matrix. It is also important to mention that the developed nanohybrid sensing probe can be used to accurately monitor H_2O_2 in a variety of biological matrices as it can detect H_2O_2 at various order of magnitudes. These outcomes comprehensibly stipulate that the proposed peroxidase mimic nanohybrid probe is a robust as well as precise sensing platform for H_2O_2 , reminiscing its wide commercial prospects.

3.5. Reproducibility and stability assay

Five different GCE/AuND/Ni-MOF/Hyd electrodes were discretely tested to analyse the electrode-to-electrode reproducibility. Various batches of the sensing electrodes were constructed to measure the current response. Only a slight disparity in the electrode-to-electrode current responses was recorded, with RSD value of about <4.5%. Other test conditions and handling errors could be the suggestive reasons for these minor deviations in the output current. The examination of a single nanohybrid nanoprobe against H_2O_2 was also done for multiple iterations ($n=5$) in succession without any pre-treatment, and the RSD was found to be <3.7%. The stability of the nanohybrid architecture without involving any biorecognition elements (antibodies, enzymes, aptamers, etc.) during its fabrication imparted the GCE/AuND/Ni-MOF/Hyd sensing probe with good stability and reproducibility (**Figure 2.18**). Periodical assessment of the sensor for five weeks was done to conclude its long-term

stability using CA. The current response was recorded at an interval of two-weeks, and the signal was retained up to 95-98% till four weeks. Hence, these results demonstrate nanohybrid sensor probe's reliability and high reproducibility, which are crucial factors governing its shelf life, preservation, and transportation.

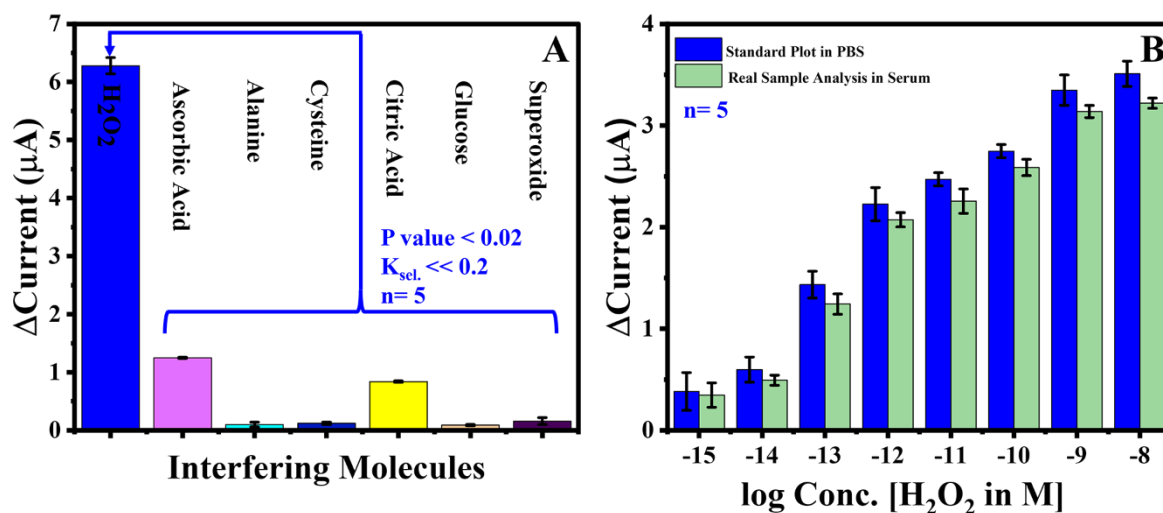


Figure 2.17. (A) Interference analysis of GCE/AuND/Ni-MOF/Hyd nanohybrid sensing probes towards various co-existing molecules and H_2O_2 ; (B) Real sample testing in serum at varying concentrations of H_2O_2 with recovery percentage varied between 90.20 to 94.14%.

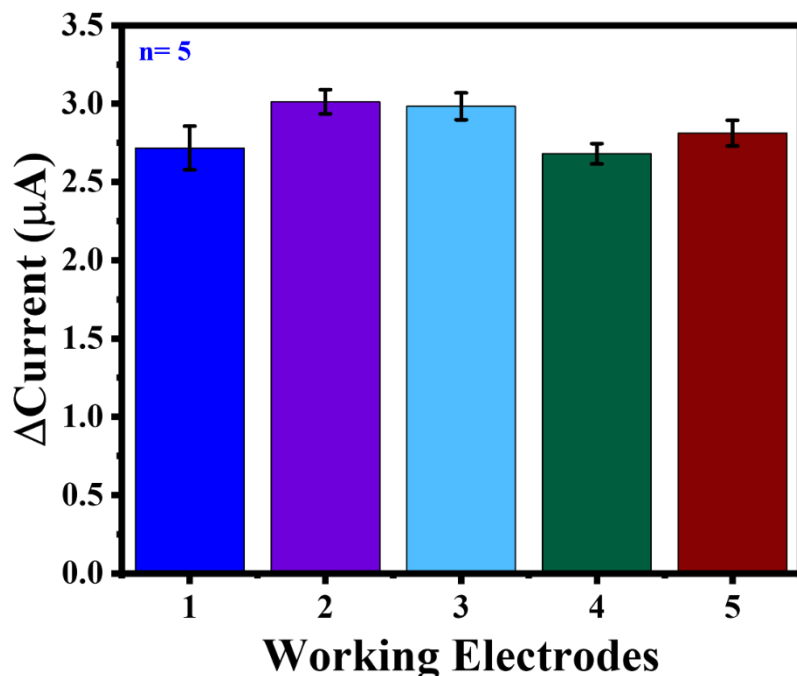


Figure 2.18. Histogram displaying the electrode-to-electrode reproducibility of GCE/AuND/Ni-MOF/Hyd sensing matrix.

4. Conclusion

In this work, a novel 3D AuND/Ni-MOF/Hyd nanohybrid sensor surface was developed which was precisely directed towards the non-enzymatic sensing of H_2O_2 . The electrodeposition variables were keenly optimized for construction of the matrix with superior analytical performance. The addition of successive layers developed into an interesting hybrid module with enormous surface area and charge transfer characteristics favoring sensing applications. The GCE/AuND/Ni-MOF/Hyd nanohybrid engineered sensing probe was characterized through various physical characterization techniques such as SPM, SEM, EDX, XPS along with a number of electrochemical techniques like, CV, EIS, LSV, and CA. A linear trend in the calibration studies within a H_2O_2 concentration range of 1×10^{-8} and 1×10^{-15} M and a LOD of $0.34 (\pm 0.05) \times 10^{-15}$ M was achieved. The nanohybrid sensing probe displayed a commendable response time of 5.02 ± 0.42 seconds, attributing to its excellent sensitivity towards H_2O_2 determination. When subjected to significant concentrations of interfering species prevalent in the biological microenvironment, it exhibited a high H_2O_2 selectivity with $K_{\text{sel}} \ll 1$. The developed nanohybrid probe could accurately sense H_2O_2 in real sample matrices wherein the outcomes were found to be consistent with the standard conditions. The proposed sensor displays good current recovery percentages and therefore, could be an optimal promising platform for sensitive detection of H_2O_2 in serum samples. To the best of our knowledge, this is the first report of AuND and Ni-MOF nanohybrid structure systematically created by electrodeposition process with remarkable conductivity and catalytic properties. This system could be explored for its future integration with the multiplexing systems for a simultaneous rapid detection of diverse analytes. Such an integrated approach, in our opinion, would be integral for pharmacological analysis prospects and an advantageous module for molecular sensing.

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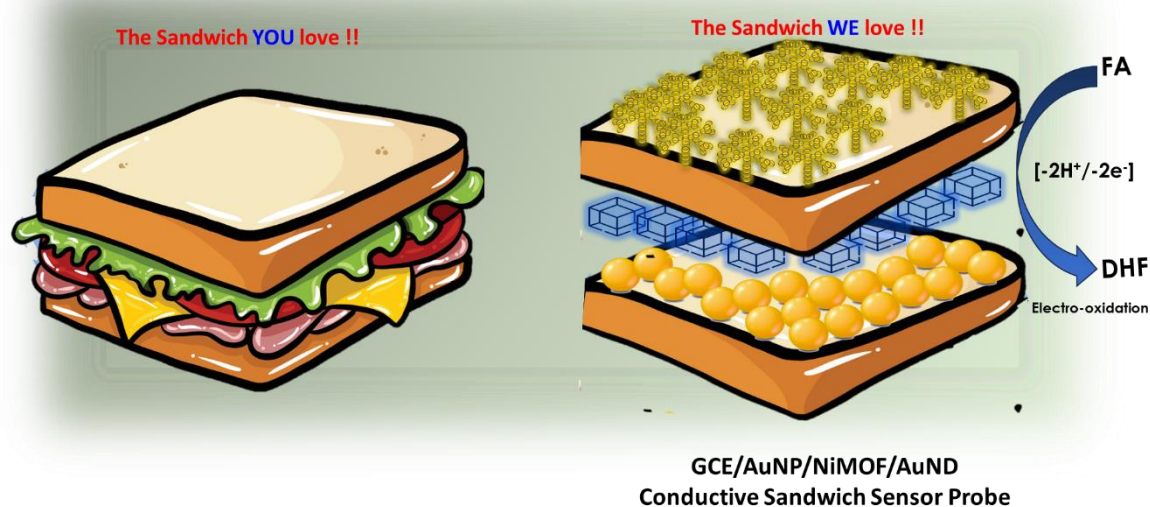
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Chapter III

Pristine NiMOF sandwiched between 1D and 3D engineered Au-particles and dendritic platform for ultra-swift folic acid sensing in cellular microenvironment



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1. Introduction

Folic acid (FA), a common name for pteroylglutamic acid, is an essential water-soluble vitamin (Vit. B9) of the B-complex category. Its primary essentiality revolves around important bodily functions like being precursor for various coenzymes such as ferroheme, synthesis of purines and pyrimidines and thereby, vital role in the fixing, methylation and synthesis of DNA^{1,2}. It is a synthetic substitute of Vit. B9 or folacin which is chemically (2S)-2-[[4-[(2-amino-4-oxo-1Hpteridin- 6-yl)methylamino]benzoyl]amino] pentanedioic acid. It gained its prominence in the beginning of the century as the understanding on its deficiency grew^{3,4}. A decrease in its body fluid concentration ($34.4 \pm 10.4 \times 10^{-9}$ M) has found its linkage to various diseases such as mental deconvolution, leucopenia, gigantocytic anaemia and increased possibilities for heart attacks and cancer⁵. While its supplementation reduces the occurrence of neural tube defects such as spinal bifida in women as well as carcinogenesis due to its involvement in nucleotide synthesis, the over-expression of its receptors on cancerous cells makes its receptors as classic tumour markers due to the cells' risen metabolic FA uptake⁶⁻⁸. There have been conflicting reports on its role in suppressing as well as increasing carcinogenesis in clinical subjects with varying clinical diagnosis⁹⁻¹¹. This makes its judicious supplementation and quantitative detection through selective and sensitive sensing modules the need of the hour. Whilst it is naturally synthesized in green leafy plants, algae and microbes such as yeast and bacteria, its deficiency is coped up in humans through dietary or pharmaceutical supplementation¹².

FA detection has been practiced through various methods including the traditional spectrophotometry¹³, colorimetry¹⁴, mass spectroscopy¹⁵, electrophoretic¹⁶ methods along with sophisticated methods like high performance liquid chromatography¹⁷, flow injection chemiluminescence¹⁸, ELISA¹⁹ and many others. The major drawback of these methods is that they require expensive setups, expertise in operations and are time-consuming. To overcome

these challenges, newer and more sensitive tools such as electrochemical methods have been introduced into the domain of FA sensing which has not only revolutionized the sensing arena by reducing costs and time but also improved the sensor efficacy in terms of FA sensitivity and LOD². Nanostructured materials with varying compositions and functionalities have garnered immense interest in recent times for electrochemical sensing module development²⁰. Nanomaterials of different sizes, electronic properties and surface characteristics can be clubbed together through probe surface nano-engineering to develop novel sensing matrices of desirable functionalities²¹.

MOFs are an emerging class of crystalline porous materials which have transcended their potential in electrochemical sensing roles²². Their catalytic metal centres self-assembled with organic ligands offer opportunities for surface functionalization, chemical and non-chemical molecular adhesions and large surface area paving their functional potential in sensing²³. In regards to their explored roles as FA sensing modules, they have mostly been adopted in optical sensor matrices with rare earth metal centres having their own set of limitations such as high fabrication time and low selectivity²⁴. Conversely, MOF synthesized through the solvothermal synthesis route exhibit poor conductivity in electrochemical sensing due to organic solvent interventions. Switching towards electrochemical MOF synthesis for development of electrochemical sensors could be a pivotal step to surpass the hurdles dealt in this route so far.

3D nanomaterials such as metallic nanodendrites are architecturally remarkable structures comprising of branched stems, possessing commendable conductivity²⁵. Since being from metallic origin, AuND outperform several other 3D materials in their catalytic potential due to presence of enormous active sites²⁶. 1D AuNPs are also widely employed in sensing due to their observable electrical, and catalytic capabilities, in addition to their possible functionalization on surfaces²⁷. Thus, we hypothesize that a composite system comprising

AuND and AuNP with MOF can accentuate the surface charge transfer characteristics by many folds due to the amalgamation of two highly catalytic and conductive surfaces.

In view of this, we have attempted fabrication of a sandwich-like unique GCE/AuNP/NiMOF/AuND sensing platform for the sensitive detection of FA in serum and *in vitro* cellular microenvironment. The probe matrix surface has been initially characterized through scanning probe microscopy (SPM), scanning electron microscopy (SEM), energy dispersive X-ray analysis (EDX-mapping) and Fourier transform infrared (FTIR) analysis followed by electrochemical studies. The electrochemical studies have initially been deployed for the characterization of the metal centres of the sensor probe, followed by understanding each fabricated layer and their roles in sensing. All experiments have been cross-referenced through a set of control experiments, followed by deducing tangible outcomes in terms of LOD, LDR, response time, selectivity, and sensitivity of the probe. The results have been complemented with tabular entries for comparing the developed probe with existing ones. This study is one of its kind, where a simple and highly functional catalytic system has been developed to detect FA in clinical ranges and much beyond. It is worth mentioning that the developed sensing assembly is an amalgamation of materials of all three dimensions (1D, 2D and 3D), leveraging property from each one of them for cumulative outcome.

The **Figure 3.1** illustrates the fabrication of a sandwich model comprising NiMOF layered between AuNPs and AuNDs, its possible role in FA oxidation, and its deployment in real-time FA sensing in MCF-7 cell lines.

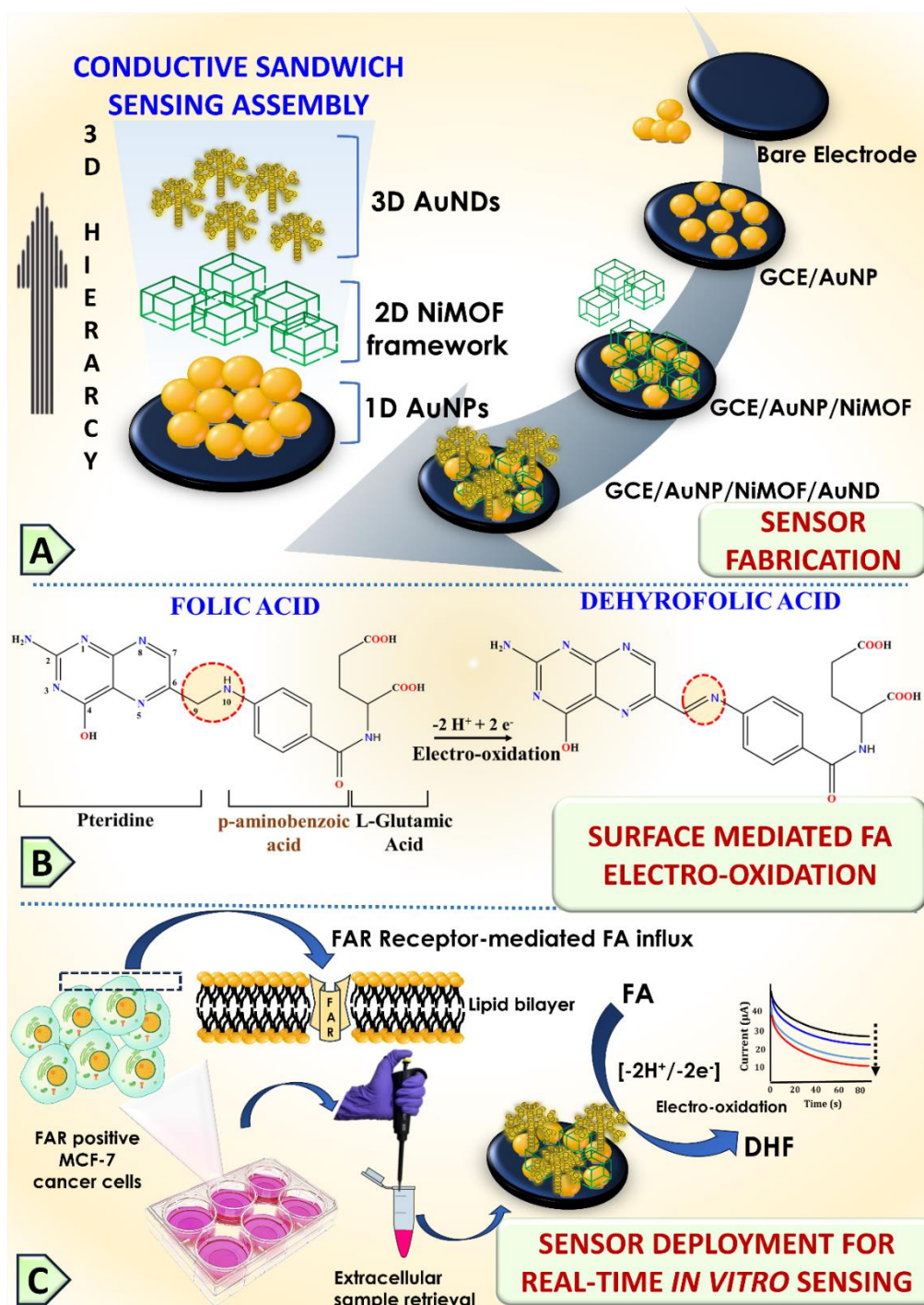


Figure 3.1. The panel (A) showcases the fabrication steps involved for the development of GCE/AuNP/NiMOF/AuND; (B) the proposed mechanism behind electro-oxidative conversion of FA; and (C) overall strategy for deployment of the developed sensor involving the cancer cellular microenvironment.

2. Materials and Methods

2.1. Chemicals and Instruments

All chemicals and reagents used in different sets of experiments in this work were of the analytical grade. ZoBell's electrolytic solution (ZS) ($K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$), 5mM; pH-7) was prepared using potassium hexacyanoferrate (II) ($K_4Fe(CN)_6$) and potassium hexacyanoferrate (III) ($K_3Fe(CN)_6$), procured from Himedia Pvt. Ltd., Mumbai, India. Phosphate-buffered saline (PBS, pH-7) was made in-house using sodium monophosphate (NaH_2PO_4), sodium bisphosphate (Na_2HPO_4), and sodium chloride (NaCl), bought from Sisco Research Pvt. Ltd. (SRL), India. Potassium chloride (KCl), chloroauric acid ($HAuCl_4.3H_2O$), and sulphuric acid (H_2SO_4) were utilized for synthesizing AuNP and AuND precursor solutions, which were procured from SRL, India. Dimethyl formamide (DMF), nickel nitrate hexahydrate ($Ni(NO_3)_2.6H_2O$), and 2-methyl-imidazole (Me-Im) were used for the fabrication of the NiMOF, which were all procured from SRL, India. FA was obtained from Sigma-Aldrich Chemical Co. (India). Double-distilled water from a Milli-Q water purifier (water resistance 18.2 M Ω) was used for the preparation of standard solutions. MCF-7 cell line (breast cancer cells) was procured from NCCS, Pune. Dulbecco's modified Eagle's medium (DMEM) was purchased from Himedia (AL007F), fetal bovine serum (FBS) was bought from Gibco (10270106), PBS was purchased for cell culture experiments from Sigma-Aldrich (D5652-10L) while the Pen-Strep and 6-well plates were procured from Edna BioLabs (PENSTRP-100) and SPL life sciences (30006), respectively.

All physical characterizations were carried out at the Central Instrument Facility Centre (CIFC), IIT (BHU). SPM was performed using NTEGRA Prima by NT-MDT Service & Logistics Ltd., HR-SEM using Nova Nano SEM 450 by FEI, EDS by EDAX Inc. and FTIR using Nicolet iS5 by the Thermo Electron Scientific Instruments LLC. For all the electrochemical measurements, an electrochemical workstation (Palm Sens 4.0, Houten, Netherlands) was deployed. It comprised of a three-electrode system with Ag/AgCl as the

reference, Pt wire as the counter and glassy carbon electrode (GCE) as the working electrode in the current set of experiments.

2.2. GCE/AuNP/NiMOF/AuND sensor probe fabrication

Bare GCE surface was polished and pre-treated as per the standard procedure to clean all surface impurities²⁶. The foremost layer comprising of AuNPs was electrodeposited onto the GCE surface through linear sweep voltammetry (LSV). The pretreatment parameters were optimized as -0.6 V vs. Ag/AgCl, 60 s, where cleaned GCE was dipped into a 0.5 M H₂SO₄ AuNP precursor solution containing 0.001% HAuCl₄.3H₂O. Further, the consecutive layer comprising of NiMOF was fabricated over GCE/AuNP through an electrochemical approach using chronoamperometry (CA) at a potential of -1.4 V vs. Ag/AgCl for 300 s. The MOF precursor solution contained 50 mM Ni(NO₃)₂.6H₂O and 75 mM of 2-MeIm in 10 ml DMF solvent system, which was stirred for 10 minutes before the electrodeposition step. The obtained GCE/AuNP/NiMOF probe was rinsed with Milli-Q water and then further electrocoated with the topmost layer of AuNDs. The synthesis of AuNDs was optimized to devise the final deposition conditions as -0.3 V vs. Ag/AgCl, 1500 s, using a 10 mM HAuCl₄.3H₂O solution, that was dissolved in 0.1 M KCl solution. The final sensing probe, GCE/AuNP/NiMOF/AuND, was then subjected to a set of physical and electrochemical characterizations before use for the purpose of FA sensing. A series of control experiments were performed to confirm the presence and importance of each matrix component in the final sensing assembly. The validation of Au was done through dipping the GCE/AuNP/NiMOF/AuND sensor probe in 0.5 M H₂SO₄, while the estimation of Ni metal was done by dipping the same into 0.1 M NaOH in a separate experimental setting. For all physical characterizations, each layer was individually developed and tested for comparative and critical examination. On completion of the final GCE/AuNP/NiMOF/AuND probe

fabrication, it was double rinsed with Milli-Q water each time, dried at room temperature (RT), and stored in a desiccator for further usage.

2.3. Electrochemical characterization of GCE/AuNP/NiMOF/AuND sensor probe

The first step towards verification of the fabricated GCE/AuNP/NiMOF/AuND sensor probe was through its electrochemical characterization in ZS (pH=7). CV and EIS techniques were adopted for the characterization purposes using the three-electrode setup. EIS data recording was done at the rate of ten points per decade sampling, keeping the open circuit voltage within 10 to 104 Hz. The control studies were performed through DPV followed by recording the calibration responses through CA at a potential of +0.8 V vs. Ag/AgCl for 100 s.

2.4. Real Sample Analysis

2.4.1. Sensor Validation in Serum samples

The applicability of the developed GCE/AuNP/NiMOF/AuND sensing probe was initially validated on serum samples through the standard method of spike and recovery. In this method, the obtained serum was spiked with different concentrations of FA after initial 10x dilution with 0.1 M PBS. The rationale behind dilution of serum samples is to maintain the molar strength of the real matrix for the better understanding of the sensor performance. The recorded output current was plotted and compared to the standard calibration curve to study the FA concentration-dependent studies in a real matrix like serum.

2.4.2. In vitro sensing and real-time FA uptake in cellular microenvironment

MCF-7 cells were cultured in folate-free DMEM supplemented with 10% FBS and 1% penicillin/streptomycin in 6-well plates. Each well was filled with 1.5 ml of folate-free media. Cultures were maintained under standard conditions at 37°C with 5% CO₂ purging and 95% humidity. Cell growth was regularly observed using microscopic examination. Upon reaching 90% confluency, 150 µl of FA dissolved in PBS was added to each well, resulting in a final

concentration of 10^{-4} M FA. Cell growth medium was collected at every 3-hour intervals starting from $t=0$ until $t=24$, encompassing a total of 7 time points. Duplicate wells were prepared for each time point, and corresponding media control was set up, where only media and FA were added without the cells. The collected samples were analyzed, post 1/10 dilution in the PBS buffer, using the FA sensor and compared with the control wells, where no FA was added.

3. Results and Discussion

3.1. Electrochemical surface engineering and characterization of GCE/AuNP/Ni

MOF/AuND sensor probe

Several sets of probe optimization-driven approach were adopted to fabricate the sensor probe. The GCE working electrode was fabricated by electrocoating it with three consecutive layers of AuNPs, NiMOF, and AuNDs to obtain the final GCE/AuNP/NiMOF/AuND sensor probe surface. Various physical and electrochemical characterization experiments were performed to confirm the presence of constituting elements and conductive layers, as discussed in the upcoming sections.

Physical Characterizations

3.1.1. SPM studies

SPM can be a powerful tool to understand the overall surface topology and individual layer thickness. A tapping mode study was conducted on each electrodeposited surface fabricated on a clean electrode. The probe and cantilever specifications can be referred to in detail within **‘Additional Information’** section within a box on the page. The bare electrode surface (referred as WE in **Figure 3.2**) exhibits an evenly distributed pitch of analysis, with the adjoining 2D micrograph stating an average z-deflection of 5.42 nm (**Figure 3.2A**). Upon deposition of the AuNPs as the first layer onto the bare WE, a rise in the crest thickness

throughout the surface was seen with an average z-deflection of 12.53 nm, as evident from **Figure 3.2B**. An even deposition of AuNPs can be inferred from the graph. On further surface modification using NiMOF, the sharpness in peaks is drastically reduced, with enlargement in crests and a significant change in surface topology, and they possess an average z-deflection of 28.40 nm. This could be due to successful fabrication of electrodeposited NiMOF embedded over the AuNPs (**Figure 3.2C**). This was followed by the deposition of the final dendritic layer onto the WE/AuNP/NiMOF surface. The uniformity of the probe was restored with a further increment in the z-deflection to 219.43 nm (**Figure 3.2D**). The SPM study was critically evaluated on the basis of the relative rise in the z-deflection values on successive depositions, as also stated in the respective 2D micrographs. The micrographs were obtained at different magnifications, depicting similarity in outcomes and establishing the notion of uniform layer fabrication at each step. The certainty of individual nanostructures and the chemical insights of those nanostructures were established by further studies like SEM, EDX, and FTIR analyses.

Additional Information: Details on the SPM Cantilever Specifications

Model: NTEGRA Prima; **Company:** NT-MDT Service & Logistics Ltd.

Characterization was performed through a Noncontact Polysilicone probe, tip material comprised of silicon with cantilever chip size being 1.6 x 3.6 mm and thickness of 0.45 mm. The curvature radius of the tip was around 10 mm. The cantilever specifications were as follows:

- Cantilever length (in μm): 94 with a typical dispersion of about ± 2
- Cantilever width (in μm): 34 with a typical dispersion of about ± 3
- Cantilever thickness (in μm): 1,85 with a typical dispersion of about ± 0.15
- Force Constant (N/m) = 12 with a typical dispersion of about $\pm 20\%$
- Resonant Frequency (kHz) = 235 with a typical dispersion of $\pm 10\%$

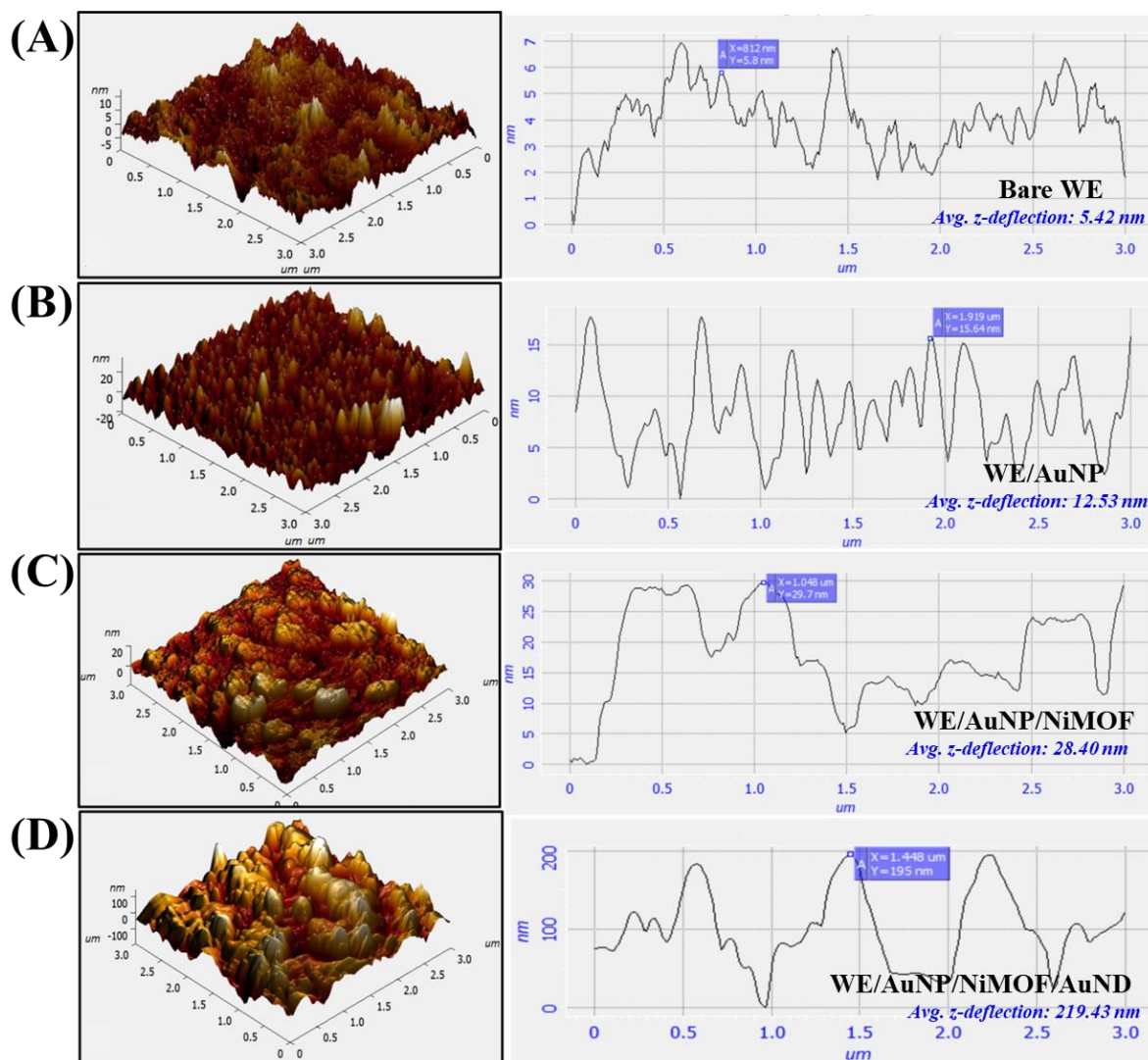


Figure 3.2. SPM 3D and the corresponding 2D micrographs for **A)** bare WE; **B)** first thin film deposited, viz., WE/AuNPs; **C)** the second electrodeposited film of WE/AuNPs/NiMOF; **D)** the final film of WE/AuNPs/NiMOF/AuND.

3.1.2. SEM and EDX analysis

The immaculate nanostructural morphology of the sensing probe was analysed through SEM. A clean electrode surface was taken to nanoimprint AuNP onto it, followed with subsequent modification by electrodeposited NiMOF to obtain WE/AuNP/NiMOF probe surface. **Figure 3.3A** depicts the dense clusters of NiMOF grown over the surface of AuNPs²³. The cabbage-like morphology of the composite is a classical outcome seen in electrodeposited MOFs²⁸. The encircled regions in yellow have been further zoomed-in within the image as ‘insets’ to

understand the morphology on subsequent degree of magnifications. On further modifying the WE/AuNP/NiMOF with AuNDs, the imprinted nanodendritic structures can be seen intercalated between NiMOF clusters. A uniform distribution of AuNDs throughout the analysis pitch can be seen, which has been more clearly demarcated in red dotted circles (Figure 3.3B). Yellow circles still visible in the area refer to the WE/AuNP/NiMOF clusters uncovered with AuNDs. The inset of Figure 3.3B shows an enlarged view of one such AuND with a primary (1°) branch/central rib (denoted in light yellow dotted line), secondary (2°) branch (denoted in pink dotted lines), and, in some cases, tertiary (3°) branches (specified through orange dotted lines). The 1° branch splits into the subordinating branches to mimic an established fern-like morphology of the AuNDs. To understand the morphology of each layer more lucidly, an individual layer of the probe was fabricated on the bare WE surface and analysed under SEM (as depicted in Figure 3.3 ‘C’, ‘D’, and ‘E’ corresponding to AuNP, NiMOF, and AuND, respectively).

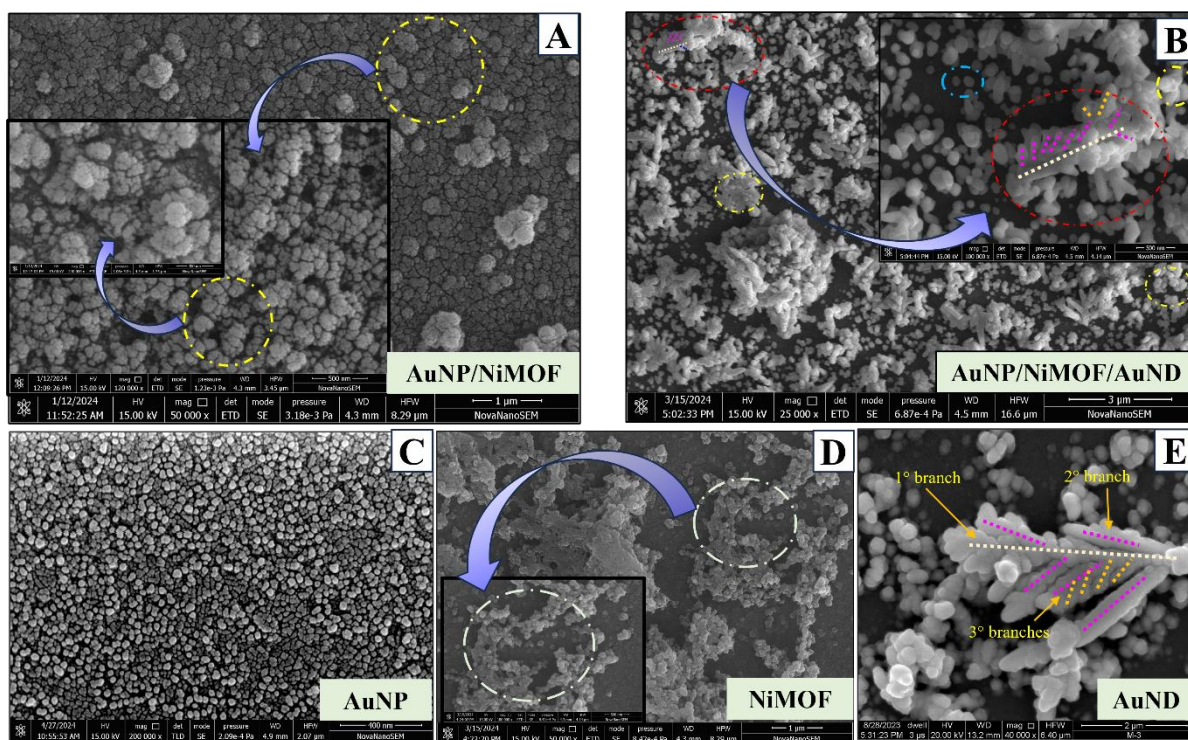


Figure 3.3. SEM micrographs of (A) WE/AuNP/NiMOF; (B) WE/AuNP/NiMOF/AuND; (C) electrodeposited AuNP; (D) electrodeposited NiMOF; and (E) electrodeposited AuND on electrode surface.

For the further validation of the elemental composition of constituents, the WE/AuNP/NiMOF/AuND sensor probe was subjected to elemental mapping coupled with EDX analysis. The obtained results have been represented in **Figure 3.4**, where the **Figure 3.4A(a)** is the actual area under scan. It is to note that the mapped results have been discussed with an assumption that similar and equal distribution of electrodeposited structures are present throughout the pitch of the electrode. **Figure 3.4A(b)** demonstrates the cumulative micrograph of all elements, viz., C (**Figure 3.4B-i**), N (**Figure 3.4B-ii**), Ni (**Figure 3.4B-iii**), and Au (**Figure 3.4B-iv**), illustrated through **green, yellow, orange, and brown-colored** patterns, respectively. The segregation into constituent elemental micrographs has been depicted for lucid understanding of the reader. The EDX analysis of the demarcated area in red (**Figure 3.4C**) confirms the presence of C, N, Ni, and Au elements in the atomic percentage of about 14.77%, 20.34%, 14.68%, and 32.24 %, respectively. We have reconfirmed these elements from the electrochemical methods in further section ahead. To reconfirm the presence of NiMOF onto the electrode surface, FTIR analysis of NiMOF coated electrode surface was done, which confirmed peaks corresponding to desirable functional groups, re-affirming our notion on MOF synthesis.

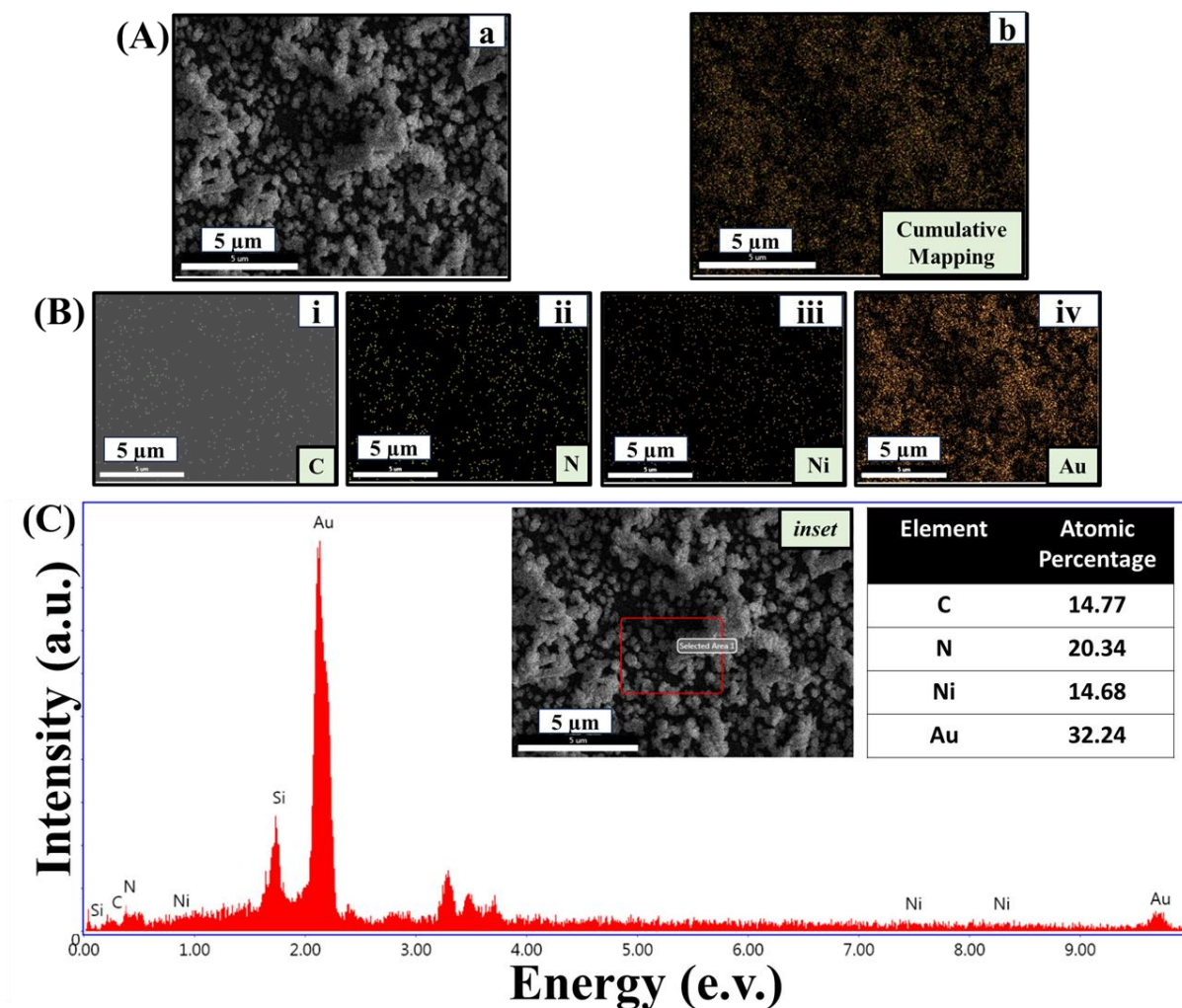


Figure 3.4. The EDX mapping results for the wide area under investigation stated in A(a), with a cumulative mapping result in A(b), which has further been dissociated into individual elemental compositions micrographs of the surface where, (B-i) corresponds to 'C'; (B-ii) corresponds to 'N'; (B-iii) corresponds to 'Ni', and (B-iv) corresponds to Au. The mapped results have been further complemented with elemental table of constituting elements, as shown in tabulated form in (C) part.

3.1.3. FTIR analysis for NiMOF

The FTIR spectrum was obtained to analyse present functional groups in a wide wavelength range of 4000-450 cm^{-1} . The spectrum presented with corresponding peaks confirm the desirable functional groups and thereby successful electro-synthesis of NiMOF through the novel route²³. The bending and stretching vibrations of the imidazole ring, part of the linker moiety, were prominent in the region of 450-1640 cm^{-1} ²⁹. Two doublet peaks were observed in the range of 692-717 cm^{-1} belonging to the C-H stretching bonds present in the methyl group

of the linker. A classic peak at around 474 cm^{-1} corresponds to Ni-N bond, which establishes the notion for successful NiMOF formation²⁹. Apart from this, the C-N, C=N, and alkene C=C stretching vibrations in the linker contributes to a peak at around 1200, 1445, 1640 cm^{-1} . Apart from this, the -OH stretching vibration at 3439 cm^{-1} corresponds to the moisture as well as hydroxyl moieties present in the MOF structure. Two peaks located at 2856 and 2932 cm^{-1} correspond to =C-H bond vibrations from the aromatic and aliphatic rings in the imidazole structure. The FTIR, SEM and EDX-mapping results cumulatively confirm the formation, morphology, as well as composition of the final sensing matrix's constituents.

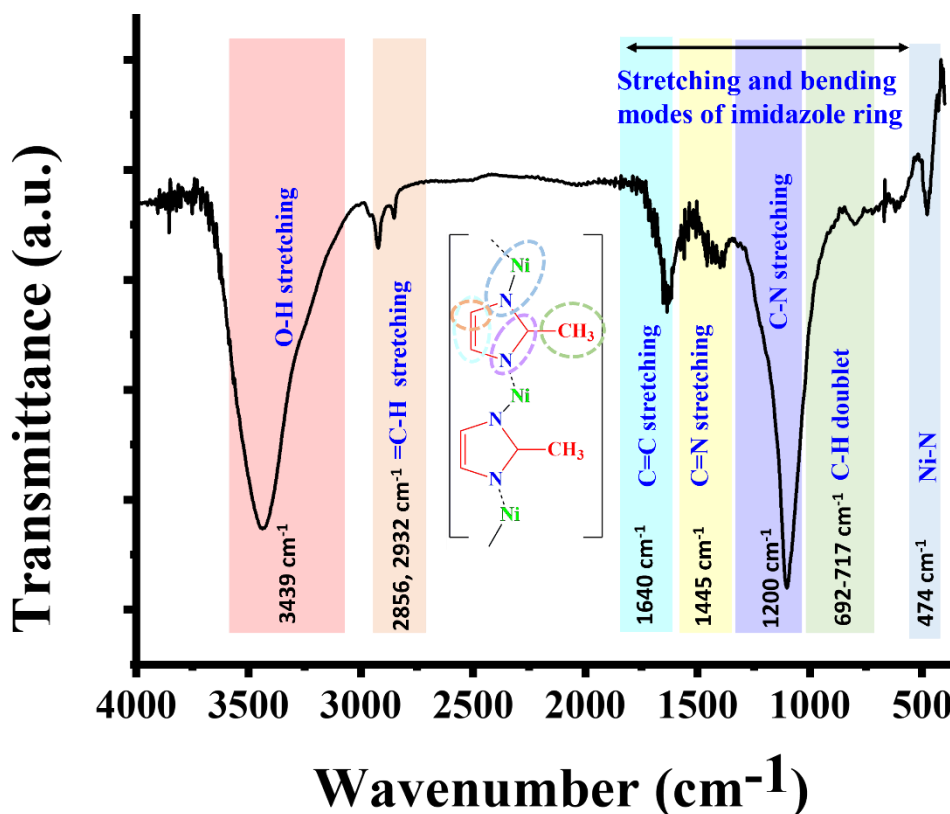


Figure 3.5. FTIR analysis for NiMOF formation

The physical characterisation results were verified through a series of electrochemical experiments as discussed in further sections below.

3.1.4. Catalytic metal elemental validation on the GCE/AuNP/NiMOF/AuND probe surface

The presence of Au in the electrodeposited AuNPs as well as AuNDs, along with the Ni metal centre validation in the NiMOF, on the developed sensor surface were verified through a set of separate experiments and controls on the same electrode in different electrolytes. At the first stage, the initial layer comprising of GCE/AuNP was fabricated and then, scanned within a potential range of +0.4 V to +1.6 V vs. Ag/AgCl in a 0.5 M H₂SO₄ solution to estimate the presence of Au in the fabricated nanostructures. A characteristic ‘Au’ peak was noted in lieu of, in accordance to the electrochemical behavior of the deposited gold in its reduced form as AuNPs on the probe surface, at +0.9 V vs. Ag/AgCl (**Figure 3.6A-i**). A feeble peak around +1.3 V vs. Ag/AgCl was also seen however was lesser prominent in the case. Then, the same electrode was subjected to further modification through the electrochemical synthesis of the NiMOF. It was then, dipped into a solution containing 0.1 M NaOH and scanned within a potential window of -0.5 V to +0.5 V vs. Ag/AgCl. A characteristic Ni oxidation peak was observed at +0.3 V vs. Ag/AgCl (**Figure 3.6B-i**), which was not seen earlier while scanning a bare GCE probe in the same potential window. This affirmed the Ni⁺² presence exclusively post electrodeposition of the NiMOF onto the probe. The final step involving the electrodeposition of the AuNDs was carried out on the surface post Ni estimation. The probe was then, re-immersed into the 0.5 M H₂SO₄ solution, and scanned within the potential window of +0.6 V to +1.2 V vs. Ag/AgCl. There was a significant enhancement in the characteristic peak intensities at +0.9 V vs. Ag/AgCl as well as at +1.2 V vs. Ag/AgCl (which was earlier not that prominent), suggestive of presence of more Au-based deposition on the probe surface (**Figure 3.6C-i**). From this significant rise in Au redox signals, it can be inferred that clusters of AuND are forming on the sensor surface leading to this rise. To eliminate even a modicum of doubt, a negative control study was done, where an unmodified GCE was tested in similar settings, yielding no results in that case. The stability of the individual probe layers and electron transfer mechanisms were validated through a scan rate study as well. The cathodic (I_{pc}) and

anodic (I_{pa}) peak currents were found directly proportional to the scan rates, with the correlation coefficient values corresponding to 0.99 and 0.99 for Au in AuNPs, 0.97 for Ni, and 0.99 and 0.99 for Au in AuNDs, respectively. This demonstrates the stable surface adsorption/adhesion phenomena with stability even at higher scan rates, making the engineered probe ideal for real sample sensing modules as well. Thus, the set of physical and electrochemical characterization studies conclusively validated the presence, composition and overall morphology of the constituting metallic nanostructures in the final GCE/AuNP/NiMOF/AuND sensing probe.

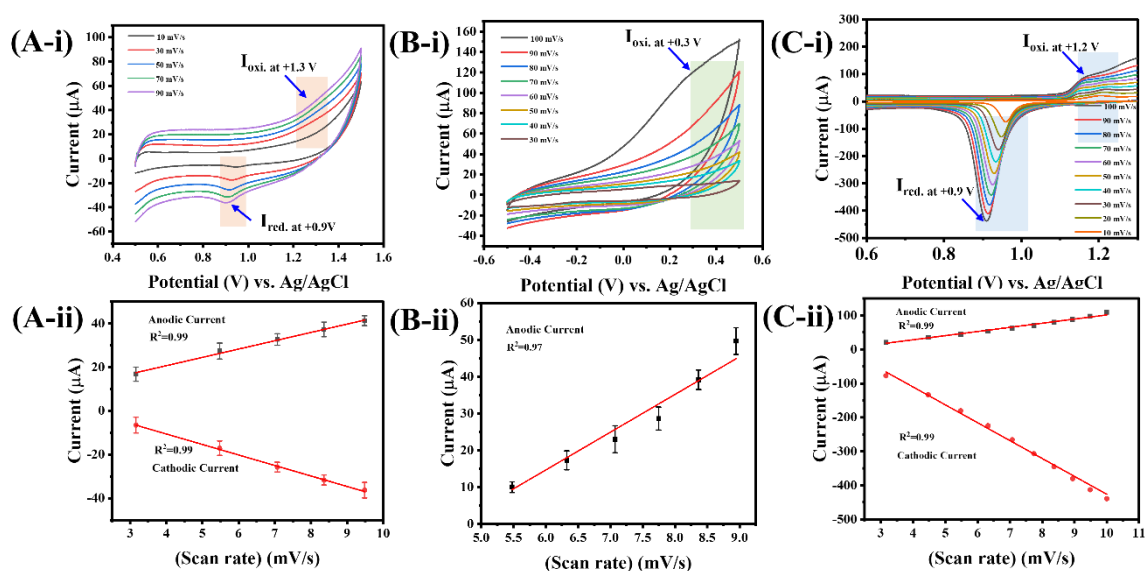


Figure 3.6. Electrochemical estimation of probe elements where A-i and A-ii correspond to the redox and fitting curves for AuNPs, B-i and B-ii refer to the oxidation and fitting curves for Ni metal of the NiMOF, and similarly, C-i and C-ii correspond to redox peak and fitting curves for the Au metal of the AuND component within the final sensing matrix.

3.2. Electrochemical performance of GCE/AuNP/NiMOF/AuND sensor probe

After verifying the presence of desirable metals in the sensor, it was time to proceed with the electrochemical examination to check its electron transfer and electrocatalytic potential. For this purpose, CV was conducted in ZS solution at RT within an analysis window of -0.1 V to +0.5 V vs. Ag/AgCl at a scan rate of 50 mV/s ($n=5$). The peaks observed within the set

window were observed in all cases (including the bare GCE surface, **red curve**), due to the inherent known redox behavior of ZS. Peaks corresponding to GCE/AuNPs (**black curve**), GCE/AuNP/NiMOF (**green curve**), and GCE/AuNP/NiMOF/AuND (**blue curve**) can be referred to in **Figure 3.7 (A, B)**.

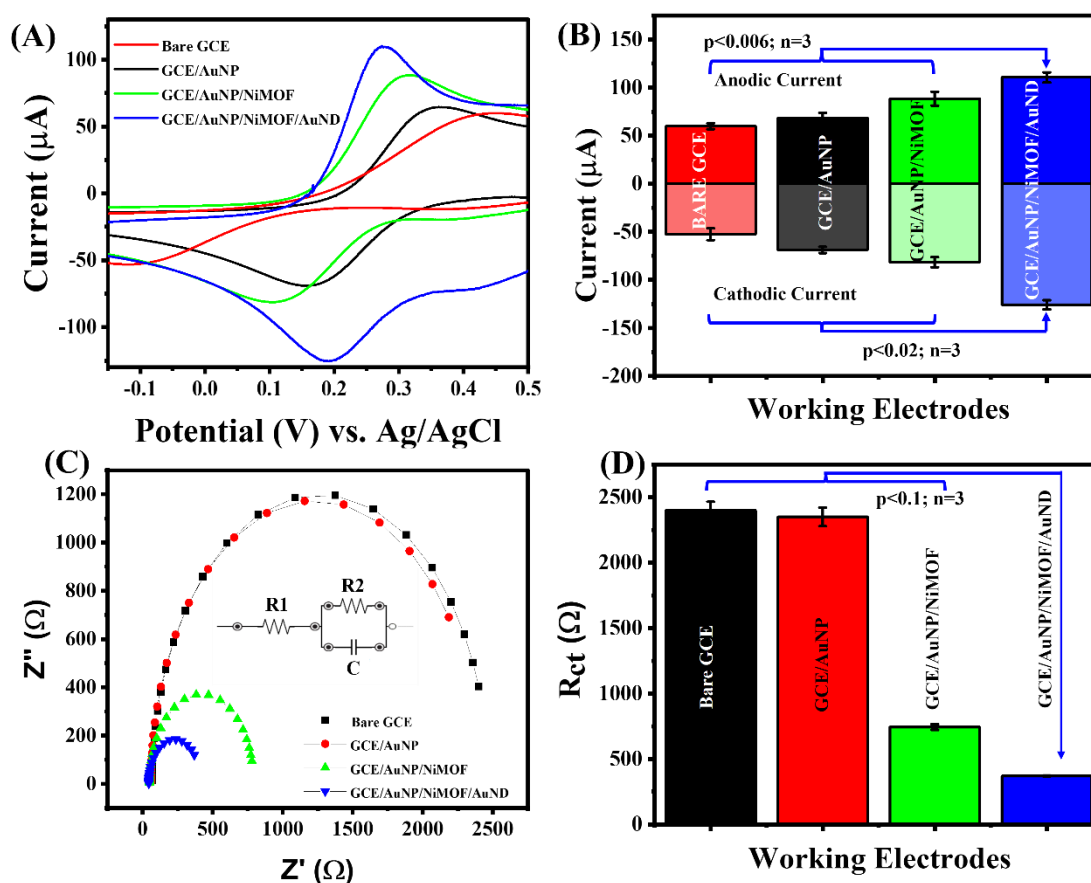


Figure 3.7. (A) CV response of bare GCE (**red curve**), GCE/AuNP (**black curve**), GCE/AuNP/NiMOF (**green curve**), and GCE/AuNP/NiMOF/AuND (**blue curve**) in ZS (5 mM; pH-7, scan rate- 50 mV/s); (B) A comparative histogram displays the anodic and cathodic current responses corresponding to each surface, with an amplified response for GCE/AuNP/NiMOF/AuND surface. (C) EIS response in the form of Nyquist plots for bare GCE (**red curve**), GCE/AuNP (**black curve**), GCE/AuNP/NiMOF (**green curve**), and GCE/AuNP/NiMOF/AuND (**blue curve**) in ZS (5 mM; pH-7) with its equivalent circuit model; (D) A comparative histogram displays the R_{ct} values of each surface, with the least resistance for the final surface.

MOFs have always been concerning on their usage in sensor matrices due to their compromised conductivity. However, we have been able to cope up with this issue through the development

of conductive NiMOF. The MOF has demonstrated enhanced conductivity when synthesized through the electrochemical route. Apart from this, sandwiching the conductive 2D MOF between the 1D AuNPs and 3D AuNDs through a novel arrangement has resulted in an enhancement in overall surface conductivity considerably (**blue curve**). A comparative histogram has also been added to understand the redox behavior of the electrode with the addition of each layer (**Figure 3.7B**). An enhancement in the current was noted on addition of NiMOF, while the highest peak current was seen in the case of GCE/AuNP/NiMOF/AuND, which was adopted as the final sensing probe. The rise in probe conductivity can be attributed to the metal atom synergies within the sensor matrix. The results were analysed through a t-test, where the p-value was found to be <0.006.

The numerical quantification of individual surface's charge transfer characteristics and deduction of their electrocatalytic activities was done through the estimation of the effective surface area (A) using the Randles-Sevcik's equation as follows,

$$I_p = (2.69 \times 10^5) n^{3/2} A C D^{1/2} v^{1/2} \quad \dots \text{Equation i}$$

where, I_p is the highest current output (in amperes), n is the no. of electrons involved in redox reaction (here $n = 1$), A denotes effective surface area of the electrodes (in cm^2), C is the electroactive species' concentration (in mole cm^{-3}), D stands for the diffusion coefficient (in $\text{cm}^2 \text{s}^{-1}$) whose value is $7.6 \times 10^{-6} \text{ cm}^2 \text{s}^{-1}$ for aqueous ferrocyanide, and v is the scan rate (in Vs^{-1}).

On substituting values in the above equation, the active surface area was found to be $73.2 \times 10^{-3} \text{ cm}^2$ for bare GCE, $80.20 \times 10^{-3} \text{ cm}^2$ for GCE/AuNP, $108.47 \times 10^{-3} \text{ cm}^2$ for GCE/AuNP/NiMOF and $135.55 \times 10^{-3} \text{ cm}^2$ for GCE/AuNP/NiMOF/AuND. The results lucidly establish the notion behind addition of subsequent layers and thereby enhancement of charge transfer ability. The effective surface area in the final sensing probe rises to 1.85 times when compared to bare GCE, 1.69 times when compared to GCE/AuNP and 1.25 times when compared to GCE/AuNP/NiMOF. The validation of these CV outcomes was complimented with the EIS experiments (**Figure 3.7C**). The values obtained by circuit fitting for GCE (**black**

curve), GCE/AuNP (red curve), GCE/AuNP/NiMOF (green curve), GCE/AuNP/NiMOF/AuND (blue curve) were plotted in form of their respective Nyquist plots. The corresponding charge transfer resistance (R_{ct}) values were determined for the respective plots as $2401 (\pm 64) \Omega$ for bare GCE, $2350 (\pm 71) \Omega$ for GCE/AuNP, $743.6 (\pm 21.6) \Omega$ for GCE/AuNP/NiMOF and $369.3 (\pm 3.9) \Omega$ for the GCE/AuNP/NiMOF/AuND surface, respectively (**Figure 3.7D**). There was a sharp decline in the R_{ct} on addition of the NiMOF and AuND layers to the surface; thus, the overall EIS findings are in good agreement with the CV results validating the matrix as being conductive.

The charge transfer behaviour and stability of the final probe GCE/AuNP/NiMOF/AuND was elucidated through a scan rate-based study, where the scan rate was varied between 10-100 mV/s in ZS solution. Even at elevated potentials as high as 100 mV/s, the migration of ions on the sensing probe was uninterrupted vouching for the stability of the interface. The square root of scan rates was found to be linearly proportionate to the I_{pa} and I_{pc} peak currents, with a correlation coefficient of 0.99, depicting the diffusion-controlled charge transfer behaviour (**Figure 3.8**). The charge transfer capabilities of the GCE/AuNP/NiMOF/AuND matrix paves path for future sensing applications of the developed probe.

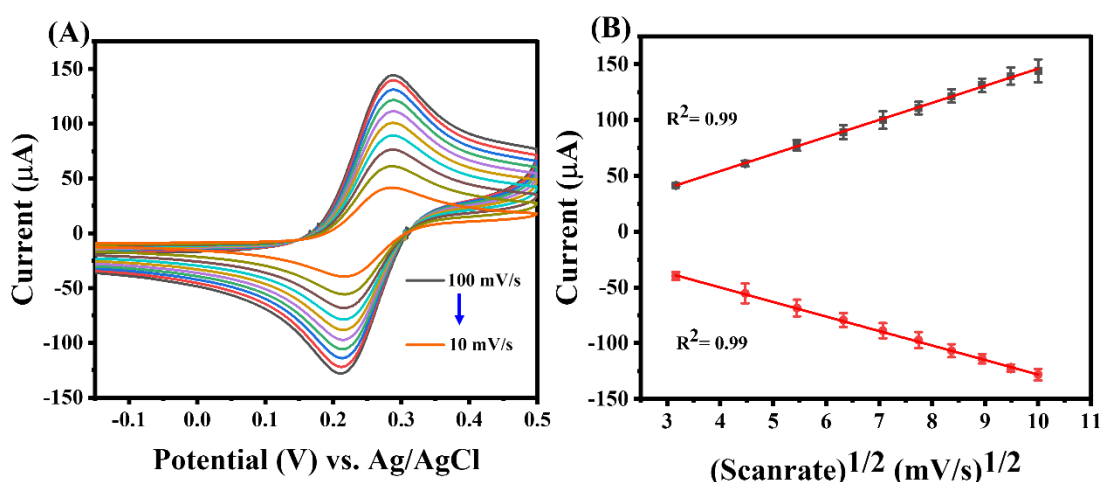


Figure 3.8. (A) Scan rate study of the final sandwich sensing probe GCE/AuNP/NiMOF/AuND in ZS when scanned between 100-10 mV/s; (B) Fitting curves corresponding to anodic and

cathodic peak currents of the (scan rate)^{1/2} study. The linearity of the plot was deduced with a R^2 value of 0.99 for both fitted curves.

3.3. Analytical performance of GCE/AuNP/NiMOF/AuND sensor probe

3.3.1. Control studies

A set of control experiments are mandatory to understand the role and stability of the sensing matrix in presence of standard buffers against the target analyte.

Before proceeding towards sensor analytics, a preliminary study must elucidate the contribution of individual layers in the sensing of FA. For this purpose, GCE, GCE/AuNP, GCE/AuNP/NiMOF, and GCE/AuNP/NiMOF/AuND were separately tested against 10^{-7} M FA. A small oxidative current was seen in case of bare GCE, indicating it not being an active electrode for FA sensing. The signal response was also less significant in case of GCE/AuNP which drastically rose in case of GCE/AuNP/NiMOF. The peaks generated in the probe were however slightly shifted towards the negative potential. A distinct and sharper peak with an enormous I_{pa} at the same potential (i.e., +0.8 V vs. Ag/AgCl), was observed for the GCE/AuNP/NiMOF/AuND sensing probe. Electro-oxidation for FA has been reported at the similar potential in earlier reports on its electrochemical sensing³⁰. This could be because of the catalytic response by NiMOF and stable 3D AuND synergy that generated the optimum signal output (**Figure 3.9A**). The deduced results have been also reported in terms of a simpler

histogram comparing their respective I_{pa} values (**Figure 3.9B**). Therefore, the engineered nanohybrid sandwich model brings out the essence of each component in the detection of FA.

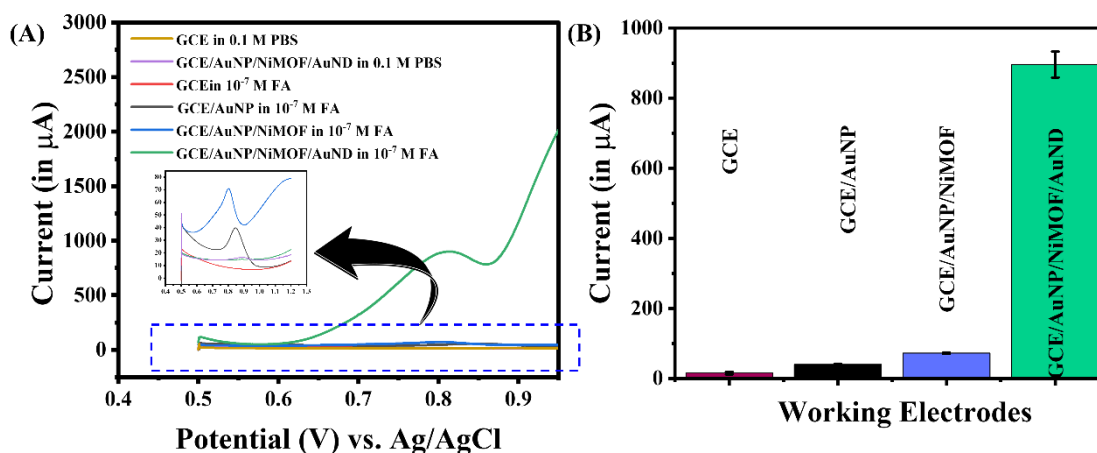


Figure 3.9. (A) Layer by layer study against 10^{-7} M concentration of FA (B) Relative histogram for each layer against the tested concentration of FA.

In order to prove that the signal generated in the first control study was due to FA, we performed the second control experiment. It was important in the perspective to establish the notion that an increase in the current magnitude occurs with an increase in FA concentration due to a precise electron transfer with FA. A voltammetry-based study was conducted considering five concentrations of FA i.e., 10^{-8} M to 10^{-4} M. The current rose gradually with a rise in FA concentration from 10^{-8} M to 10^{-4} M (**Figure 3.10A**). A shift in the peaks on increasing concentration of FA is a common phenomenon, also reported in previous works²⁰. The curve exhibited a linear concentration dependent signal output with a correlation coefficient of 0.99 (**Figure 3.10B**). The regression equation obtained from the curve is as follows:

$$\Delta I (\mu A) = 4811.037 (\pm 236.46) - 435.19 (\pm 25.450) \times \log \text{Conc. [FA (M)]}.$$

In a further addition to this, another control test determining the stability of the GCE/AuNP/NiMOF/AuND probe and charge transfer behavior was analysed in the target analyte. It comprised of a scan rate study between 16 mV/s to 24 mV/s in the presence of 10^{-4} M FA (**Figure 3.10C**). With increase in the scan rate there was observed a linear increment in

the Ipa demonstrating an excellent surface stability and diffusion-controlled charge transfer behavior. The anodic current was found to be proportional to the square root of the scan rate with a correlation coefficient of 0.94 (**Figure 3.10D**). Therefore, both the above studies evidently suggested that the designed hybrid sandwich probe is quite stable and efficiently sense FA.

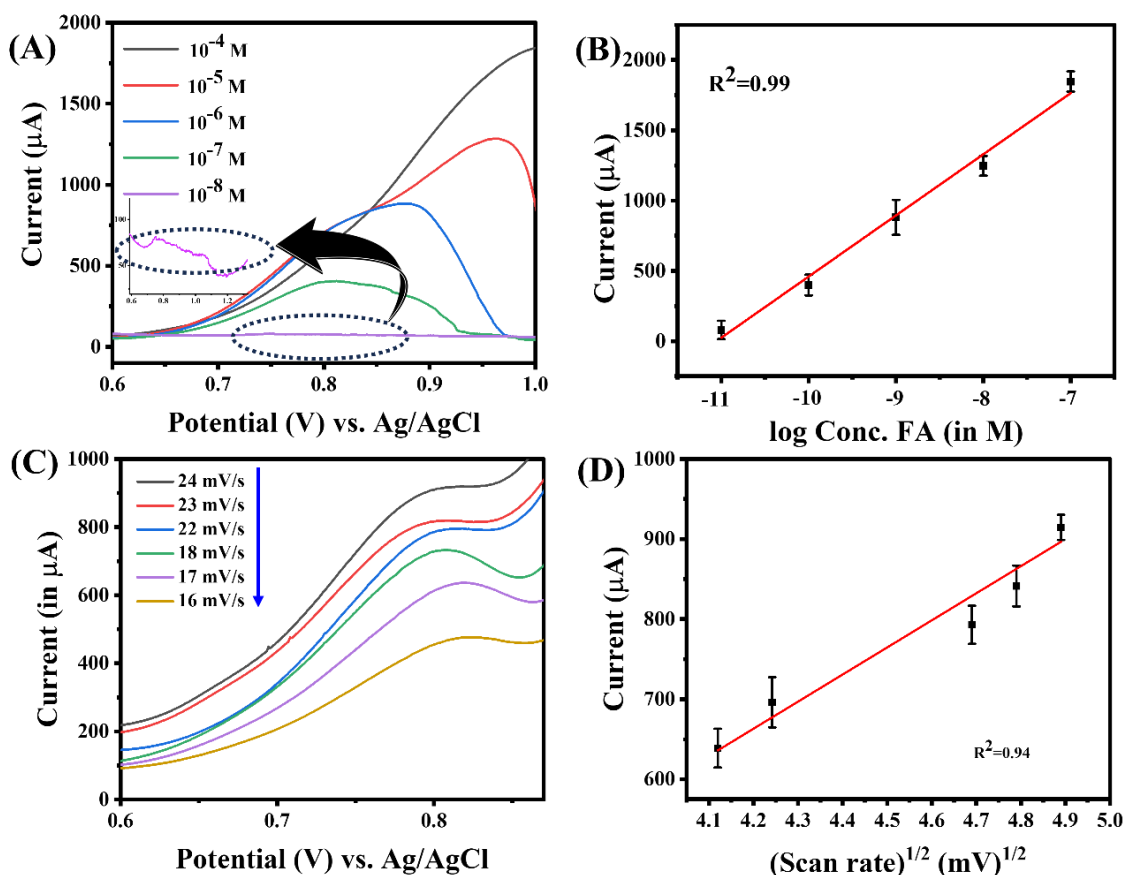


Figure 3.10. (A) Concentration dependent study for FA and its corresponding (B) fitting curve, stating a $R^2=0.99$. (C) Scan rate dependent study in the presence of 10^{-4} M FA and its corresponding (D) fitting curve for $(\text{Scan rate})^{1/2}$ vs. Current (μA).

There were many reports on the property of FA to adsorb and strongly adhere to sensor surfaces which made us ponder if the same was happening in our case too³⁰. Therefore, out of curiosity, we ran a set of experiment where different concentrations of FA treated GCE/AuNP/NiMOF/AuND probe was subjected to FTIR analysis to observe any characteristic peak of FA. To our surprise, the FTIR results were in coherence with our assumptions. The spectra exhibited characteristic peak of FA in the region (**Figure 3.11, A-D**). The aromatic

peaks of FA fall in the region of 1000, 1400 and 1600 cm^{-1} where, the characteristic peak around 1650 cm^{-1} (**region B and C**) corresponds to the -CONH bond while that around 1023 cm^{-1} (**region D**) corresponds to the -NH bond in the FA heterocycles³¹. The broad peaks between 2750-3800 cm^{-1} (**region A**) also signify the presence of -NH and -NH₂ stretching vibrations found in primary and secondary amines of FA^{32,33}. The intensity of these peaks proportionately increases with increase in FA concentration (from 10^{-9} M to 10^{-3} M) and is thereby adsorbed on the final sensor.

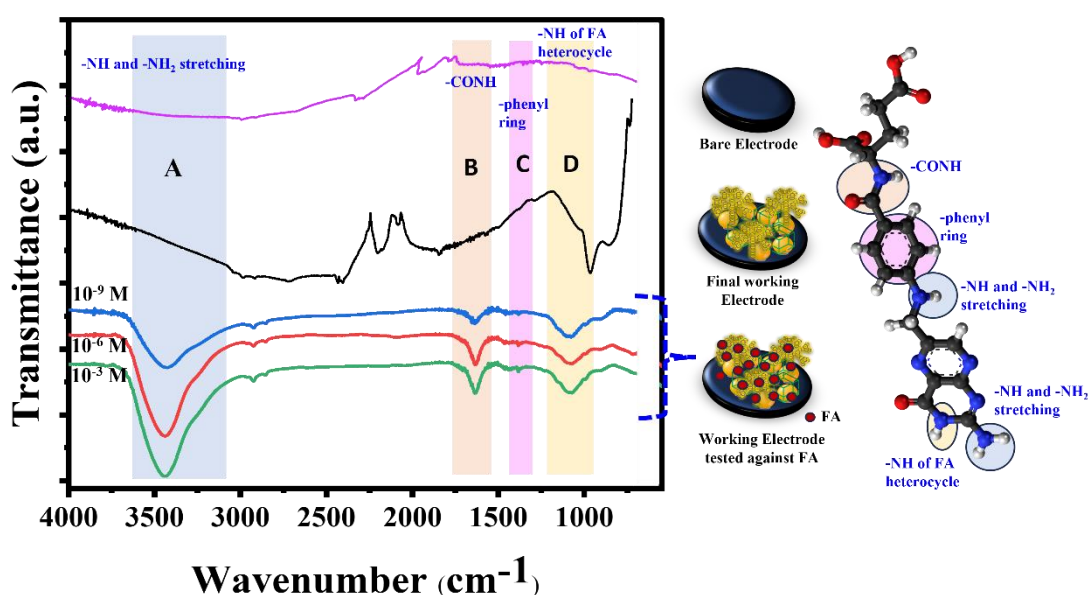


Figure 3.11. Concentration dependent adsorption study of FA on the GCE/AuNP/NiMOF/AuND probe.

Also, the wide sensing potential window and broader peaks in FA oxidation are attributed to the strong adsorption behaviour of the phenyl ring of p-aminobenzoic acid moiety of FA³⁰. The reason for such adsorption could be most likely the diffusion of FA into the assembly and probably an electrostatic as well as π - π stacking interaction between the aromatic ring of FA and MOF's imidazole in our case. The overall sensing phenomena of FA involves the electro-oxidation of FA at the C₍₉₎-N₍₁₀₎ site, where the bicyclic pterin moiety is connected to the p-aminobenzoic acid with the methylene bridge (as depicted in **Figure 3.1**, **panel B**). For a non-enzymatic detection method, tracking the oxidative cleavage of C₍₉₎-N₍₁₀₎ in FA

could be a plausible solution. The anodic oxidation of FA yields a single oxidation peak in the potential range of +0.7 V to +1.0 V ^{30,34} vs. Ag/AgCl in standard buffer solutions due to the conversion of aniline structure in the FA backbone to p-benzoquinone³⁵. The further sensing capabilities of the engineered sensor probe was estimated through the method of CA, as discussed in the upcoming section.

3.3.2. Sensor Analytics

CA is a highly sensitive technique which can be a useful tool to understand even a slightest change in the analyte concentration. Hence, the sensing capabilities of the engineered GCE/AuNP/NiMOF/AuND sandwich probe was investigated through it. Initially, the signal output was recorded for the sensor probe in PBS, it being a supporting electrolyte for FA (**blank**). The response was recorded at +0.8 V vs. Ag/AgCl (**blue curve**), followed by evaluation of the probe against varying concentrations of FA. There was a rise in the current magnitude with increasing concentrations of FA from 1×10^{-11} M to 1×10^{-3} M, with the minimum 'ΔI' between blank and 1×10^{-11} M (**Figure 3.12A**). On the basis of the chronoamperometric data, a calibration curve was plotted between the 'ΔI' vs. log [Conc. (FA in M)], refer **Figure 3.12B**. The curve exhibited a linear trend within the evaluated concentration range of FA from 1×10^{-11} M to 1×10^{-3} with a correlation coefficient of 0.98. The LOD for FA was calculated as $0.48 \pm 0.02 \times 10^{-11}$ M using the **equation i** stated below,

$$LOD = \frac{3SD_b}{Slope} = \frac{3SD_b}{\frac{dy}{dx}} = \frac{3SD_b}{\frac{dy}{d\ln x} \times \frac{d\ln x}{dx}} = \frac{3SD_b}{\frac{dy}{2.303(d\log x)} \times \frac{1}{x}} = \frac{3 \times 2.303 \times SD_b \times x}{\frac{dy}{d\log x}} = \frac{3 \times 2.303 \times SD_b \times x}{Slope \text{ of semilog plot}}$$

...Equation i

where SD_b represents the SD of the blank, and x is the lowest concentration measured.

The linear increment in amperometry current responses with rise in FA concentrations from 1×10^{-11} M to 1×10^{-3} M can be represented through the regression equation as,

$$I (\mu\text{A}) = 0.865 (\pm 0.035) + 0.072 (\pm 0.0042) \log \text{Conc. [FA (M)]}.$$

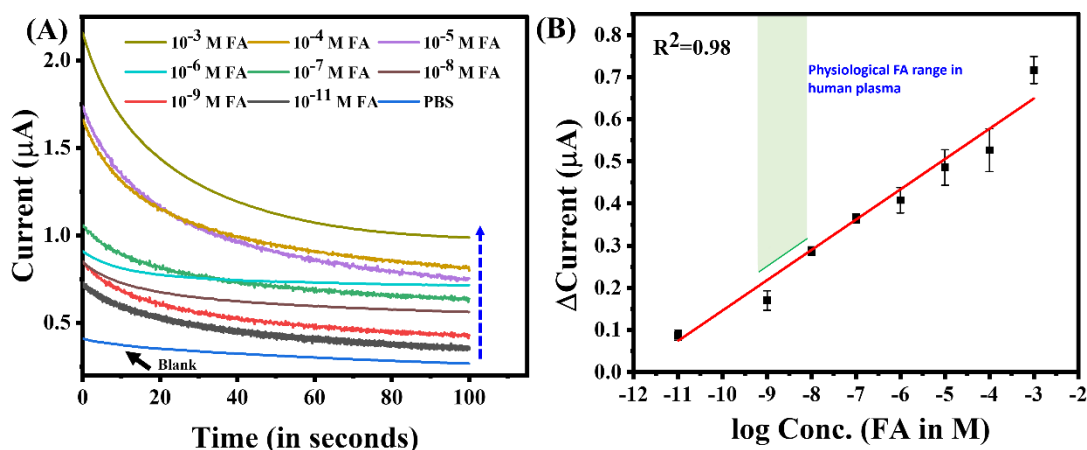


Figure 3.12. (A) Chronoamperometric response curve of the final sensing probe against varying concentrations of FA, ranging from 1×10^{-3} M to 1×10^{-11} M. (B) Calibration plot for the same range of FA with an R^2 of 0.98.

3.3.3. Response time study

Response time refers to the time lapsed for a sensor to respond towards a change in the concentration of buffer on addition of a known concentration of analyte into it. The shorter the response time of a sensor, the more it is believed to be sensitive towards the target analyte³⁶. For examining the same for our engineered GCE/AuNP/NiMOF/AuND surface, CA was used as the suitable method, where at +0.8 V vs. Ag/AgCl, different concentrations of FA were injected in blank PBS solution to evaluate the response of the sensor. **Figure 3.13** shows a typical chronoamperometric response of the sensor towards injection of 200 μl of 10⁻⁸ M FA. In the curve, the sample is injected to stable current **(a)** at a given point of time **(b)**, where a sharp rise in current is noted due to diffusion which goes up to **(c)** within a time span of 1232.01 seconds and 1233.94 seconds. Then, the current gradually decreases with time to reach a plateau, termed as ‘current saturation’ at **(d)**. The saturation current (d) and injection point current (b) possesses a significant difference (ΔI) by virtue of the added FA to PBS electrolytic solution that oxidises contributing electrons. For FA concentration as low as 10⁻⁸ M, the sensor

responded within 1.93 seconds. We had recorded responses for different concentrations and the time lapsed was low for higher concentrations and vice-versa. A table showing response time pertaining to different concentrations of FA viz. 10^{-4} M, 10^{-6} M, 10^{-9} M, has been mentioned in **Table 3.1**. A slight change in the response time was noted with change in concentrations, however remained constrained to <2.1 seconds, indicating the rapid sensing ability of the system. The fast response of the probe is by virtue of three diversified layers, that are individually contributing towards analyte sensing.

Concentration of FA	Response Time
10^{-4} M	1.42 sec
10^{-6} M	1.62 sec
10^{-8} M	1.93 sec.
10^{-9} M	1.9 sec.
10^{-9} M	2 sec.
Average Response Time	<2.1 seconds

Table 3.1. Response time values for different concentrations of FA.

The engineered 3D sensing probe is an interesting surface because of its short and simple fabrication strategy without the involvement of hefty chemical synthesis-based processes, a wide LDR and low LOD

against FA with a lightning-fast response time. A relative evaluation of the developed probe and its parameters with other recently reported FA sensor probe has been summarized in **Table 3.2**.

It is interesting to note that where most of the reported MOF-based FA detection systems have a LOD in the mM ranges, our developed sensor has almost 7 orders lower LOD than the recently reported setups with much simpler fabrication steps and better response time, intersecting the clinical ranges.

Table 3.2. Comparative analysis of recently developed FA sensors comprising MOF as transduction system component with our developed system.

Probe Composite	Fabrication strategy	Technique used	Probe/matrix fabrication time	Reaction time	LOD	LDR	Real Sample	Reference
RE-MOF (Rare earth MOFs, where RE= Eu, Gd, Tb and Dy)	A series of water stable 3D RE-MOFs were synthesized solvothermally in a mixture of DMF and water solvent to yield the fluorescent MOFs.	Fluorescence	Nearly 120 hours	N.R.	0.02 mM	0-120 μ M	N.R.	³⁷
Ni-MOF	Ni-BTC MOF was synthesized through the solvothermal route. A 1 mg/ml solution of the MOF in deionized water was ultrasonicated and dropcasted onto a unmodified SPE surface to form the WE.	CA, DPV	36 hours	N.R.	0.03 $\pm$ 0.001 μ M	0.08 to 635.0 μ M	FA tablets, urine	³⁸
La⁺³/Co₃O₄ nano-cubes	Carbon SPE working electrodes were coated with an aliquot of La ⁺³ /Co ₃ O ₄ nano-cubes dispersed in aqueous system. The modified electrode was allowed to dry at RT before use in sensing.	CA, DPV	>16 hours	N.R.	0.3 μ M	1.0-600.0 μ M	FA tablets, urine	³⁹
Zn-Eu MOF	A heterometallic MOF was prepared through the solvothermal route. It was suctioned with filter paper to obtain MOF-based test strips of the size 1 cm x 2 cm.	Fluorescence	>147 hours	N.R.	1.84 $\times$ 10 ⁻⁸ M	5.90 $\times$ 10 ⁻⁴ to 2.22 $\times$ 10 ⁻⁷ M	N.R.	⁴⁰
ZIF-90	7-amino-4-methylcoumarin was encapsulated within ZIF-90 to fabricate a fluorescent sensor at RT.	Fluorescence	>9 hours	N.R.	3.5 $\times$ 10 ⁻⁶ M	1 $\times$ 10 ⁻⁵ to 1 $\times$ 10 ⁻³ M	Milk, Goat serum	⁴¹
Eu/Tb/Gd MOF (Ln MOF)	Three isostructural MOFs were synthesized solvothermally at 160 °C which was thereafter used for optical sensing of FA.	Fluorescence	>72 hours	1.5 minutes	0.0883 μ M	0–18 μ M	N.R.	²⁴
Ag MOF	The Ag-based MOF was synthesized through the solvothermal route at 160 °C to obtain needle-like crystals.	Fluorescence	>84 hours	5 min.	49 nM	0.1–30 μ M	Human serum and plasma	⁴²
ZIF-67/AgNWs	Screen printed carbon electrodes were modified through dropping	SWV	>24 hours	N.R.	30 nM.	0.1 μ M to 10 μ M	Real serum samples	³⁵

	them with a layer of ZIF-67/AgNWs and airdried at RT to form the final working sensor probe.							
Probe Composite	Fabrication strategy	Technique used	Probe/matrix fabrication time	Response time	LOD	LDR	Real Sample	Reference
MB/ERGO/GCE	On a polished GCE surface, GO was deposited electrochemically forming ERGO/GCE which was then modified by further deposition of MB on its surface through CV.	DPV	>25 hours for GO preparation followed by EC based depositions	N.R.	0.5 mM	4.0 mM to 167 mM	Commercial FA tablets	²⁰
CuONPs/poly MO	A clean and polished GCE surface was drop-coated with a film of CuONPs dispersed in EtOH. This was followed by modification of the surface using MO poly-electrodeposition onto the GCE using CV.	DPV, CA	> 3 hours	N.R.	0.002 μM	0.01 to 1.5 μM	Fresh citrus juices (orange, tomato, and strawberry)	⁴³
NC/CPE (Niacin-modified carbon paste electrode)	Initially, CPE was fabricated from scratch using ground GO and binder. A niacin monomeric solution was electrodeposited onto the CPE through 15 cycles of CV.	CV	N.R.	N.R.	0.09 μM	5.0 μM to 45.0 μM	Fruit juice (orange, lemon and tomato, pharmaceutical sample)	⁴⁴
C/PDA/AuNPs	PDA encapsulated carbon-based material adorned with AuNPs was synthesized through a microwave-assisted approach to be used as a WE.	CA	N.R.	5 minutes	25 nM	0.1–905 μM	Human serum	⁴⁵
PtNPs-GNPs-MWCNTs-β-CD	Initially, a homogeneous dispersed solution of GNPs, MWCNTs and PtNPs was prepared in water followed by addition of β -CD to it and thereby sonication. The reaction yielded PtNPs-GNPs-MWCNT- β -CD composite which was drop-coated on a polished GCE surface to form the WE.	CV	>10 minutes	N.R.	0.48 $\mu\text{mol L}^{-1}$	0.02–0.50 mmol L^{-1}	FA tablets	⁴⁶
AuNP/NiMOF/AuND	A layer of conductive NiMOF	DPV, CA	<40 minutes	1.5 seconds		10^{-3} – 10^{-11} M	Serum and MCF-7 cells	<i>This work</i>

**was sandwiched
between layers of
AuNPs and AuNDs.**

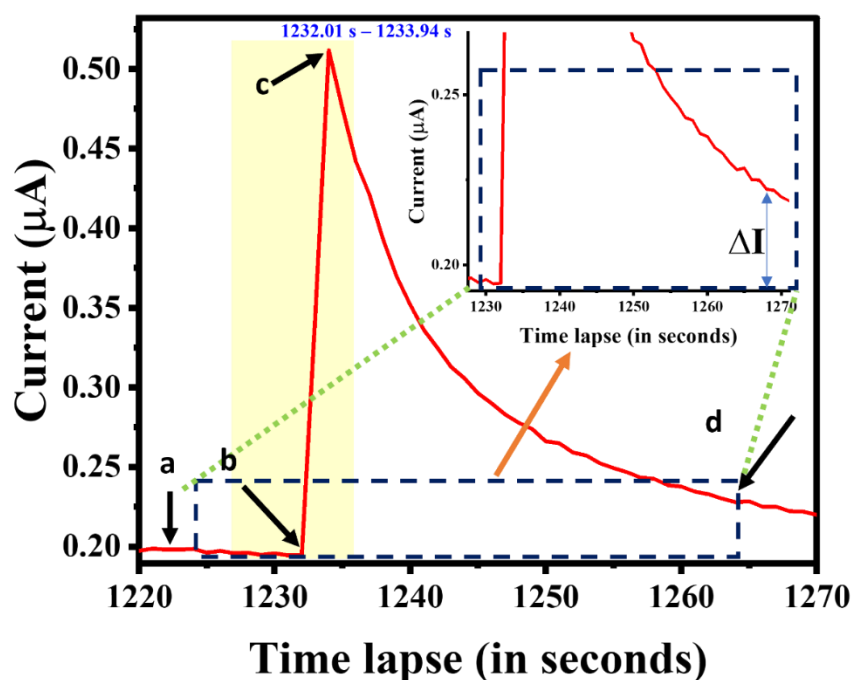


Figure 3.13. The graph denotes the response time curve of the developed sensor probe against spiking 10^{-8} M FA concentration in standard buffer, where (a) is stable current (b) injection point (c) maximum current spike on FA inoculation, and (d) saturation current. (inset) shows the current plateau observed on the recovery stage post FA injection, denoted by ΔI .

3.3.4. Selectivity and reproducibility assay

The translational significance of the developed GCE/AuNP/NiMOF/AuND sandwich probe can only be proven if its specificity is towards the target analyte at a given potential when tested against co-existing interferent species. Therefore, a variety of interferent molecules (e.g., L-histidine, sucrose, β -alanine, urea, glucose, caffeine) were chosen along with our target analyte FA to be tested against the sensor probe in the similar experimental conditions. **Figure 3.14A** demonstrates a comparative histogram of the sensor selectivity against a range of interferents with comparison to our target analyte. An equation (**Equation ii**) to determine the coefficient of selectivity (K_{sel}) was used for the co-existing molecules. The obtained values have been tabulated in **Table 3.3**. The values were found to be significantly low ($K_{\text{sel}} \ll 1$), with p-value < 0.0001 emphasizing an extremely fair selectivity of the developed GCE/AuNP/NiMOF/AuND probe for FA.

$$K_{sel} = \text{Signal}_{\text{interfering molecule}} / \text{Signal}_{\text{FA}} \dots \text{Equation ii}$$

where K_{sel} stands for the coefficient of selectivity, $(\text{Signal})_{\text{interfering molecules}}$ is the current response of the probe corresponding to specific interferant, and $(\text{Signal})_{\text{FA}}$ is the response obtained from probe after being tested for FA.

The passivity of the developed GCE/AuNP/NiMOF/AuND sensor probe resulted to no considerable signal output generation against only the interferants molecules at the specific potential.

Table 3.3. Table discussing K_{sel} of the sensor against a range of analytes.

Co-existing molecules	Current (μA)	K_{sel}	Five different electrodes were fabricated with the same methodology and tested discretely against the same concentration (10^{-3} M) of FA to verify
PBS	0.047	0.059	
L-histidine	0.051	0.065	
sucrose	0.080	0.102	
beta alanine	0.099	0.126	
Urea	0.057	0.072	
Glucose	0.093	0.118	
caffeine	0.094	0.120	
Folic acid	0.783	1	

the electrode-to-electrode reproducibility where negligible variations were observed (**RSD** <3.5). The stability of the surface was due to direct electrodeposition of the structural architecture and no involvement of any biorecognition elements (antibodies, enzymes, aptamers, etc.) during its fabrication imparted the GCE/AuNP/NiMOF/AuND sensing probe with good stability (**Figure 3.14B**).

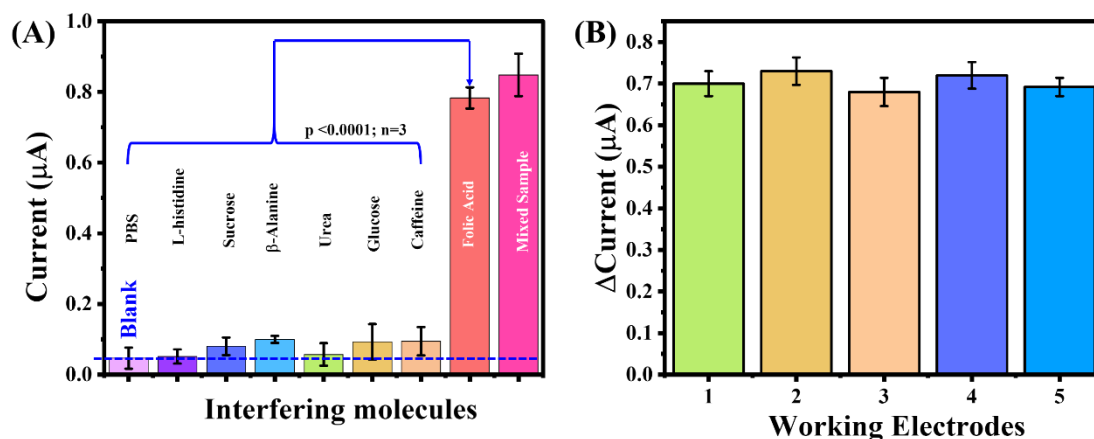


Figure 3.14. (A) Interference study for the developed sensing probe against a range of competitive possible interferents, (B) The probe reproducibility was checked across a range of different electrodes fabricated under similar circumstances.

Further FA was measured using the GCE/AuNP/NiMOF/AuND probe in serum samples and *in vitro* microenvironment in order to evaluate the practicability of our developed sensing matrix as discussed in the upcoming section.

3.3.5. Real sample analysis

i. Serum-sample based study

FA is an important biomolecule in regulating cellular pathways, and diverse metabolic roles⁴². Hence, its quantitative analysis in serum poses enormous clinical significance. The serum-based study was conducted by testing the developed sensing probe against different concentrations of FA-spiked serum samples. The spike and test recovery model were used to evaluate the sensor performance. Initially, the procured serum sample was equilibrated in PBS, following which it was further spiked with different known concentrations of FA and current response was documented. The experiments were conducted in triplicates under optimized experimental settings.

The FA recovery percentage was calculated using equation, as stated below,

$$\% \text{ Recovery} = (S_{FA} - B_{FA}) / (SS_{FA}) * 100 \quad \dots \text{Equation iii}$$

where, $[S]_{FA}$ and $[B]_{FA}$ correspond to the analytical response of GCE/AuNP/NiMOF/AuND nanohybrid sensing probe carried out in serum samples spiked with FA and blank sample, respectively; $[SS]_{FA}$ refer to the response generated in standard buffer (PBS in this case) with same concentration of FA.

The current response pertaining to different concentrations of FA in serum has been represented through a calibration curve reported in **Figure 4B (red curve)** and compared with the standard calibration curve for FA in PBS. There was an incremental shift in the current magnitude with increasing concentration of the target analyte, with the current recovery ranging between 95.11-98.17%. The mathematical representation of signal outcome can be denoted by the equation, $\Delta I (\mu A) = 0.850 (\pm 0.038) + 0.071 (\pm 0.005) \log \text{Conc. [FA (M)]}$. The sensor efficiency was found commendable even in serum samples with a correlation coefficient of 0.96. The comparable results with the standard calibration plot vouch for sensor efficiency however, slight deviations in current magnitude could be an impact of handling or minor ion mobility changes in matrix. The different serum constituents however did not impact much on the experimental data therefore, making it a suitable sensing probe for precise FA monitoring, evoking its future commercial aspects.

Further, *in vitro* studies were attempted for the sensing probe to re-affirm its utility in real cellular microenvironment.

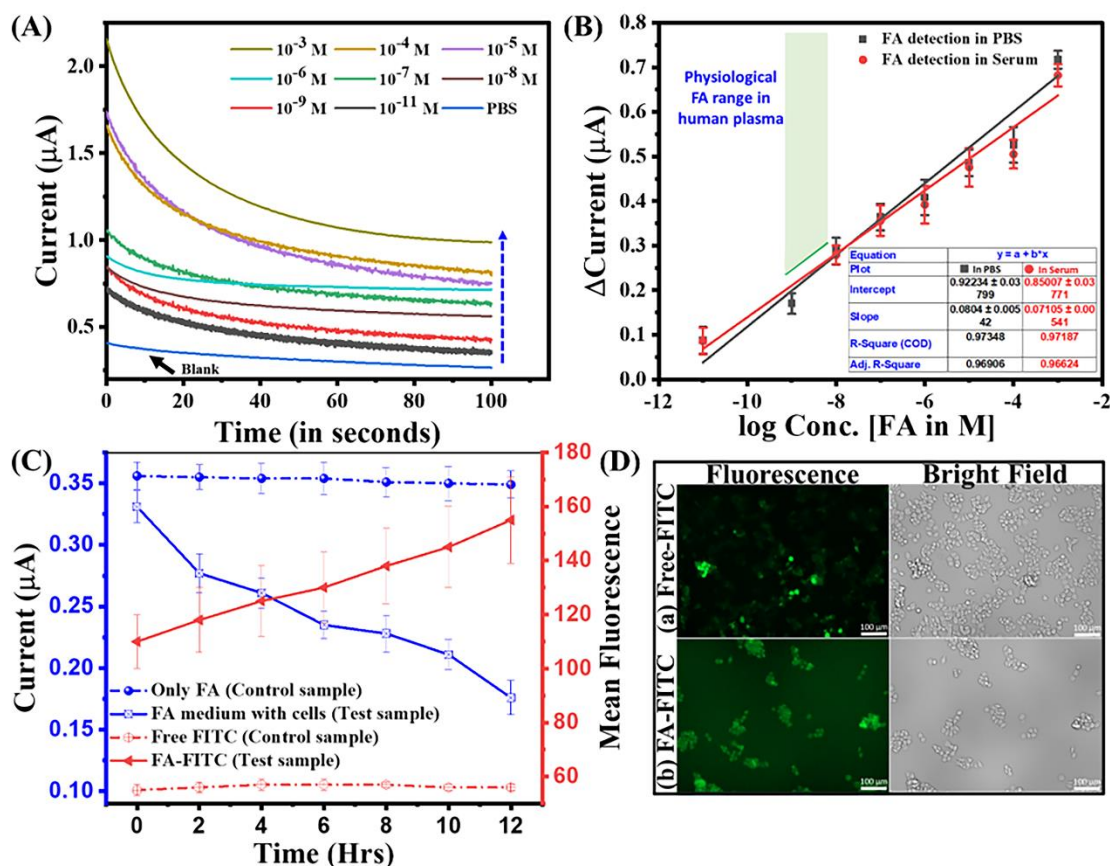


Figure 3.15. (A) Chronoamperometric response curve of the final sensing probe against varying concentrations of FA, ranging from 1×10^{-11} M to 1×10^{-3} M; (B) Calibration curves for FA detection in standard PBS buffer and serum sample with an R^2 value of 0.969 and 0.966, respectively; (C) A comparative FA uptake analysis in *in vitro* cellular microenvironment by developed sensor (blue curves) and its validation through standard fluorescence technique (red curves). Fluorescence intensity and current output was mapped over time for both control and test samples; (D) Fluorescence (left panel) and bright field images (right panel) comparing the uptake of free FITC (control sample) and FA-FITC conjugates (test sample) by MCF-7 cells: (a) Cells incubated with free FITC show minimal and non-specific fluorescence, (b) Cells incubated with FA-FITC conjugates display enhanced and widespread fluorescence, suggesting active and specific uptake of the conjugate. Bright field images confirm the presence of cells in both conditions (images represented at 100 μ m scale).

ii. In vitro sensing using MCF-7 cell lines and its validation through fluorescence imaging

Certain types of cancer cells are known for their folic acid receptor (FAR) overexpression characteristics, one being MCF-7 cells as well⁴⁷. In our study, we attempted to recreate the

cancer cellular uptake microenvironment for FA, where MCF-7 cell lines were grown in controlled systems and the media concentration of FA was recorded (**Figure 3.15C**). The rationale behind using cancer cells as the test system instead of a normal cell system is to govern FA cellular uptake in a shorter time frame, taking aid of its FAR overexpression. MCF-7 cells of the same cellular confluency growing in different wells of a well plate was inoculated with the same concentration (10^{-4} M) of FA. It is important to note that the cells were cultured in a FA-free media to avoid any false positive results. A time dependent study was conducted, where the cells uptake FA naturally and its concentration in media decreases over time which was determined over a span of 0 hours to 12 hours through the developed sensor. We also performed a negative control experiment, where free FA was injected in a cell free media. Fresh test samples (i.e., 1 ml FA inoculated media in cellular microenvironment) were procured at every 3 hours and tested for the presence of FA. With subsequent readings, a decline in FA concentration within cellular microenvironment was noted affirming the successful FA uptake by growing MCF-7 cells as depicted in **blue solid curve** of **Figure 3.15C**. No change in current response was noted for the control test samples (**blue dotted curve**) as there was no cells to uptake FA. To validate the results through a standard method, a time dependent FA uptake study was conducted in the MCF-7 cells through detailed fluorescence-based analysis via live cell imaging⁴⁸. **Figure 3.15C** depicts fluorescence and bright field images demonstrating MCF-7 cells' uptake of FA-FITC conjugates (test sample) and free FITC (control sample). In this, FA-FITC conjugate uptake was monitored in MCF-7 cells as a function of time from 0 to 12 hours through continuous live cell imaging. There was a constant increase in fluorescence intensity over time in FA-FITC sample, starting from around 112 at 0 hours and increasing to around 155 at 12 hours (**red solid curve**). This increase shows active uptake of the FA-FITC conjugate by MCF-7 cells. In contrast, the fluorescence intensity of the control group (free FITC) remains relatively low and consistent over the same period (**red dotted curve**),

demonstrating minimal or no uptake in the absence of folic acid conjugation. The results were recorded at multiple zones and in triplicate. The mapped data results of the live cell imaging and sensor makes the discussion more lucid, where the graphs are inversely directing the whole phenomena. With a decline in the media/extracellular FA concentrations, there is an enhancement in the fluorescent intensity corresponding to increased FA-FITC conjugate uptake within the cells with each passing hour. Thus, the study re-confirmed three important inferences as follows: (i) the MCF-7 cells actively uptake spiked FA from cellular microenvironment; (ii) the sensor is capable of detecting cellular FA uptake over a period of time with even in the slightest change of concentrations; (iii) the system is highly stable in such complex cellular microenvironment with highly precise estimation of FA in extracellular premises.

4. Conclusion

The work focusses on the development of a novel sandwich sensing probe for monitoring a physiologically significant vitamin (FA) through electrochemical route. The sandwich probe was fabricated by optimization of process parameters to replace existing tedious MOF synthesis process by a simpler electrochemical approach, followed by its conjugation with Au nanostructures. It was further characterized through AFM, SEM, EDX and electrochemical methods to deploy against detection of the target analyte in serum and cellular microenvironment. The inter-junction of Au based surfaces with a highly catalytic Ni-centered MOF resulted into a hybrid catalytic surface with superior charge transfer characteristics and analytical performance. The calibration study yielded a linear slope within a FA concentration range of 1×10^{-11} and 1×10^{-3} M and a LOD of $0.48 \pm 0.02 \times 10^{-11}$ M. A wide LDR is of great significance since dietary supplements can raise the blood FA concentration in μM . Thus, the developed sensor is capable of detecting FA in both cases i.e. FA deficiency or FA accumulation. The engineered sensor probe displayed a commendable response time of less

than 2.1 seconds, attributing to its excellent sensitivity towards FA determination. When subjected to significantly higher concentrations of interfering species prevalent in the biological microenvironment, it could easily selectively detect FA with $K_{\text{sel}} \ll 1$. The results were also quite promising in real sample matrices wherein the outcomes were in consistency with the standard conditions. The proposed sensor displays good current recovery percentages and therefore, could be an optimal promising platform for sensitive detection of FA in serum as well as other biological samples. To our knowledge, the devised sandwich model sensor is one of its kind incorporating easy fabrication steps, remarkable charge transfer characteristics and a wide detection range for analytes like FA covering its clinical range and beyond. The developed system can be further deployed for future integrations within multiplexing setups for a simultaneous and rapid detection of diverse analytes.

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Chapter IV

*Ni/Co stacked MOFs as a nano-electronic transducer
integrated with smartphone for nitrite sensing in
environmental water samples*

Shubhangi et al., 2024 (under revision)

1. Introduction

Sensitive detection of nitrite (NO_2^-), a heavily talked about environmental menace, is important due to its detrimental effects on humans and other living beings¹. Anthropogenic activities (use of nitrogenous pesticides) and discharge of industrial effluents in water bodies has resulted in the surge of its concentration in ground water too². It also finds its widespread usage in food preservation industries where it prevents food spoilage by species such as *Clostridium botulinum*³. Nevertheless, its highest exposure to humans remains through ingesting its high dosage through the medium of food and water. Some of the untreated foods such as beets, potatoes and tomatoes inherently contain low concentrations of NO_2^- ³. When at high concentrations, it combines with haemoglobin in blood restricting oxygen supply to cells, a phenomenon called as the ‘blue baby syndrome’ in newborns⁴, making drinking water nitrite concentration an important concern in infants. Its known conversion from nitrite to nitrosamines, a known carcinogen, necessitates its sensitive detection in water and food in current world. Lethal concentrations of NO_2^- in drinking water as per WHO guidelines falls above $65\mu\text{M}$, forcing industry to adapt newer methods to estimate nitrite in water bodies since a longtime⁵. There have been different methods reported for NO_2^- detection such as capillary electrophoresis⁶, spectrophotometry⁷, chromatography⁸, chemiluminescence⁹ and sequential injection¹⁰. It is also important to understand that most of the listed methods however are expensive, require hefty machinery, derivatization, large solution processing time and profound expertise, limiting their deployment for on-site detection. An easier, faster and cheaper approach is the electrochemical estimation which is now being explored for its sensitive sensing in wide matrices^{11,12}. Unmodified electrodes can detect NO_2^- but suffer from large overpotential, demanding suitable electrode modifications for their commercial sensing utility¹³.

Metal organic frameworks (MOFs) are evolving 2D and 3D microporous materials which have recently found their utility in several applications including the trending roles in electrochemical sensing matrices¹⁴. They find their purpose as surface area enhancing moieties enabling functionalization along with the catalytic roles often played by their metal centers¹⁴. Their roles advance with integration of metal nanoparticles (NPs) by facilitating reduction in their agglomeration and increment in NP stability¹⁵. However, the current challenges for MOFs still resides in their poor stability within a range of solvent and media systems, complex synthesis routes involving high temperature and compromised charge transfer characteristics¹⁵. Again, as sensor matrix component, MOFs often lead to compromised conductivity, poor limit of detection (LOD), limited linear dynamic range (LDR) and low sensitivity. To address these problems, they are often adorned with quantum dots or NPs to elevate sensor performance and sensitivity towards the target analyte^{16,17}. Specifically in case of our analyte of interest, the usage of AuNP was imperative with the fact that Au binds with nitrite, thereby oxidizing and sensing it at lower potential values, as suggested by numerous reports^{18,19}.

The current work attempts at exploiting the porous architecture of MOFs with catalytic metal centers (Ni-MOF stacked upon Co-MOF) to accommodate and adhere AuNP, yielding synergistic outcomes for effective NO_2^- oxidative sensing. The work focusses on the fabrication of the above versatile system over the surface of a glassy carbon electrode (GCE) followed by its extensive physical and electrochemical characterization and thereafter utility as a sensing matrix against NO_2^- in real water samples collected from pond and local community supply. The sensor has been further integrated with a smart-phone platform providing an on-site NO_2^- estimation tool giving its concentration in nM in no time. This is the first report on stacking two highly catalytic metal-centered MOFs and integration of smart-phone interface for automated and user-friendly sensing of NO_2^- (**Figure 4.1**).

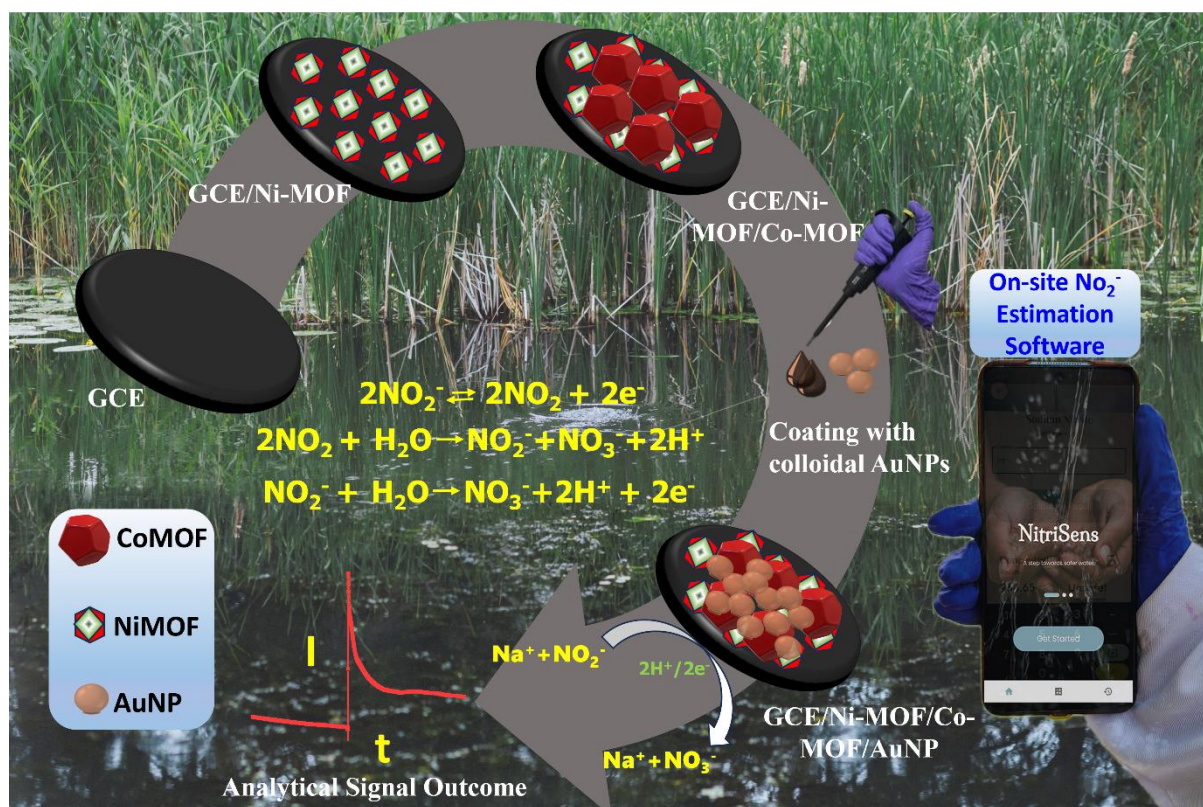


Figure 4.1. A schematic representation of the fabrication strategy adopted to develop the nitrite sensing and thereby its estimation module involving a smartphone-integration.

2. Materials and Methods

2.1. Chemicals and Instruments

All chemicals and reagents used in the study are of the analytical grade, in their purest form. ZoBell's electrolytic solution (ZS) ($\text{K}_3[\text{Fe}(\text{CN})_6]/\text{K}_4[\text{Fe}(\text{CN})_6]$), 5mM; pH-7 was prepared using Potassium hexacyanoferrate (III) ($\text{K}_3\text{Fe}(\text{CN})_6$) and potassium hexacyanoferrate (II) ($\text{K}_4\text{Fe}(\text{CN})_6$) were purchased from Himedia Pvt. Ltd., India. NaNO_2 was obtained from Sisco Research Pvt. Ltd. (SRL), India. All standard solutions, like phosphate buffer saline (PBS) and ZS were prepared in-house using double-distilled water which was obtained from a Milli-Q water purifier (water resistance 18.2 M Ω). PBS was prepared using sodium monophosphate (NaH_2PO_4), sodium bisphosphate (Na_2HPO_4), and sodium chloride (NaCl), bought from SRL,

India. The MOF precursor solutions were prepared using the nickel nitrate hexahydrate ($\text{Ni}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$), cobalt nitrate hexahydrate ($\text{Co}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$), and 2-methyl-imidazole (MeIm) in dimethyl formamide (DMF), all procured from SRL, India. AuNP preparation involved usage of chloroauric acid (HAuCl_4) and trisodium citrate which were purchased from SRL, India. Tap water was obtained from the local water supply pipeline available to nearby household and pond water samples were obtained from local pond present within premises of Indian Institute of Technology (BHU).

All physical characterization tools were availed at the Central Instrument Facility Centre (CIFIC), IIT (BHU). HR-SEM results were obtained using the Nova Nano SEM 450 by FEI, EDS by EDAX Inc., and the EVO - Scanning Electron Microscope MA15 / 18, CARL ZEISS Microscopy Ltd. and the XPS using the K-Alpha, by Thermo-Fisher Scientific. For all the electrochemical measurements, an electrochemical workstation (Palm Sens 4.0, Houten, Netherlands) was deployed. It comprised of a three-electrode system with Ag/AgCl as the reference, Pt wire as the counter and GCE as the working electrode in the current set of experiments.

2.2. Fabrication of the GCE/NiMOF/CoMOF/AuNP sensing platform

The GCE was initially pre-treated as per the standard protocol to remove all impurities²⁰. Then, it was dipped into the standard Ni-MOF precursor solution comprising of 50 mM $\text{Ni}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ and 75 mM of 2-MeIm dissolved and stirred in 10 ml DMF solvent system. The deposition of the Ni-MOF was done using chronoamperometry (CA) at a deposition potential of -1.4 V vs. Ag/AgCl for a duration of 400 seconds, using optimized CA parameters. Then, the obtained probe was rinsed and dried for few minutes to further coat it with the next layer of the Co-MOF. The Co-MOF precursor solution was prepared likewise as the former MOF by dissolving 50 mM $\text{Co}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ and 75 mM of 2-MeIm in 10 ml DMF and stirring at room temperature for 5 minutes. After preparing the precursor solutions, the modified

GCE/Ni-MOF electrode was dipped into this solution and electrodeposited at an optimized potential of -1.4 V vs. Ag/AgCl to obtain the Co-MOF over it. It is important to understand that optimization of the deposition time is also an important aspect to obtain the Co-MOF with the desired charge transfer characteristics and morphology, which has been discussed in detail in the upcoming section.

On further rinsing of the GCE/Ni-MOF/Co-MOF with distilled water post deposition, the final layer of the probe, i.e. colloidal AuNP prepared through the standard citrate method²¹, was coated onto the sensor surface. 4 μ l of the synthesized colloidal AuNP were coated onto the GCE/Ni-MOF/Co-MOF surface and allowed to dry at room temperature for 15 minutes. Then, upon drying, the final surface was mildly rinsed with distilled water to obtain the desired sensing surface. The electrode was stored in desiccator till further usage. The confirmation of the metal centers in the stacked MOFs and the gold in the AuNP layer was first concluded through the EDX analysis, mapping, and XPS results. Further, the confirmation of metallic presence in the sensor matrix was validated through electrochemical estimation in respective electrolytic solutions. Thereafter, individual layers of the final sensing surface have also been fabricated and evaluated in order to determine the effectiveness of each surface.

2.3. Electrochemical Evaluation of the GCE/NiMOF/CoMOF/AuNP sensing platform

The electrochemical characterization of the final sensing matrix was done by dipping the electrodes in ZS solution, undergoing analysis through various electrochemical measurements such as cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS). The measurement parameters have been mentioned appropriately at places of relevant discussions. The results of EIS were acquired at an open-circuit potential with a frequency between 10 to 104 Hz. The LSV and chronoamperometric signals were recorded between a potential range of +0.4 V to +1.2 V vs. Ag/AgCl and +0.95 V vs. Ag/AgCl, respectively to procure the outcomes for the control studies and the standard calibration plots.

3. Results and Discussion

3.1. The deposition of the stacked MOF over the electrode surface, followed by AuNP functionalization

One of the most promising methods for fabricating MOF is electrodeposition, and in this method, optimizing voltage and deposition time is crucial²². At first, the potential and deposition time optimization for the electrodeposited MOFs is a crucial aspect to start the discussion with. The deposition of the Ni-MOF was done referring our previous work with minor changes like alterations in deposition parameters²². The deposition time optimization was done varying it between 20-400 seconds, as shown in **Figure 4.2A**. It is interesting to note that the charge transfer characteristics of the layer rises at 150 seconds but drops back a little and finally rises again at 400 seconds (**Figure 4.2B**). We checked the formed nanostructures for their catalytic potential, however, the most conductive nanostructures formed at 150 seconds were not able to catalyse our target nitrite anions. Therefore, we moved further and found that the ones formed at 400 seconds were the most catalytic species facilitating maximal nitrite conversion and its thereby, detection. Hence, we chose 400 seconds as the optimum time point for Ni-MOF synthesis for catalytic nitrite sensing matrix fabrication.

This was followed by a controlled deposition of the Co-MOF detailed in the current study which is one of its kind. For utilizing the developed electrodeposited MOF for purpose of sensor probe conductivity enhancement and thereby, electro-oxidation of the target analyte, it is important to understand the parameters to obtain maximum conductance of the deposited layer. The time duration for deposition of Co-MOF was varied from 100 seconds to 1500 seconds (**Figure 4.2C**). It was interesting to note that with an increase in the electrodeposition time from 100 to 600 seconds, there was a gradual rise in the conductivity of the modified probe however, it became almost constant after 600 seconds till 1500 seconds, as shown in **Figure 4.2D**. Therefore, the deposition time of 600 seconds was chosen as the optimum time

to obtain the most conductive form of Co-MOF. There have been previous reports on the electro-deposition of Co-MOF (ZIF-67), through various routes with higher deposition potentials and long deposition times (**Table 4.1**). It is important to note that this study reports the improved process of Co-MOF electrodeposition in terms of lower potential and shorter deposition time with its strikingly enhanced conductivity posing its utility in molecular sensing applications. It was further investigated that the nanostructures formed at 600 seconds were catalytically potent against our target species. Furthermore, stacking both metallic MOFs on top of one another improved the composites' catalytic properties and overall sensing attributes, as discussed in succeeding sections. Integration with AuNP on the system was done by first synthesising the AuNP through the standard citrate method followed by its coating onto the stacked MOF surface²¹. In brief, 100 ml of 1 mM HAuCl₄ was brought to boil and stirred for 30 minutes until 5 ml of 78 mM of trisodium citrate solution was added to it and stirred for another 15 minutes to obtain the wine-red coloured colloidal NP solution. To validate the formation of all the desired surface components and the synchronous activity of the Ni-MOF/Co-MOF/AuNP probe, it was meticulously characterized through a range of physical and electrochemical characterizations tools.

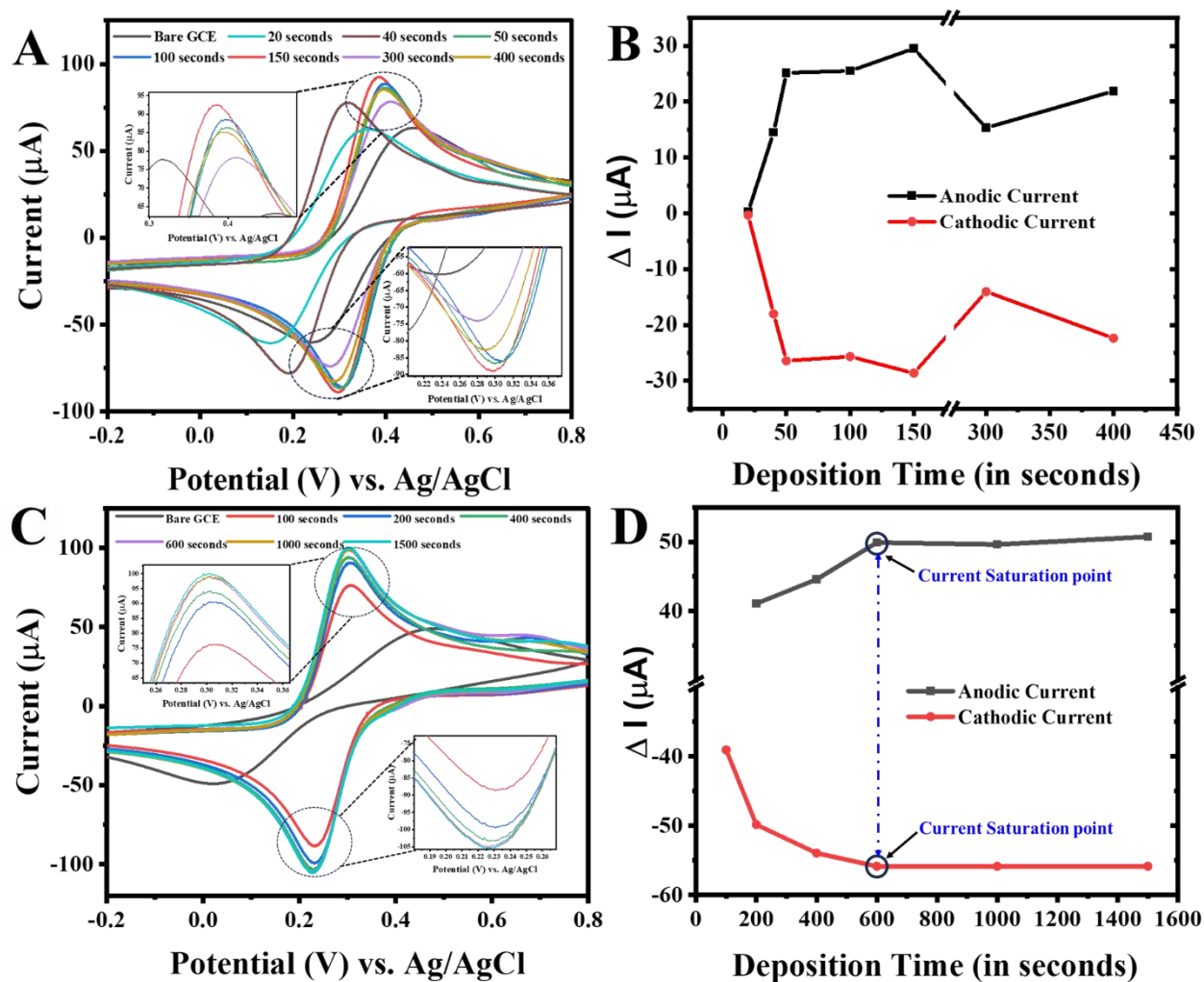


Figure 4.2. CV curves for optimization of time-dependent deposition of (A) Ni-MOF (B) optimization plot for anodic and cathodic currents obtained at various time points (20-400 seconds) in case of Ni-MOF; (C) Time-dependent deposition of Co-MOF (D) optimization plot for anodic and cathodic currents obtained at various time points in case of Co-MOF (100-1500 seconds).

Table 4.1: Co-MOF deposition parameters comparison with previous reports.

S.No.	MOF composite	Method of fabrication	Precursor salt and its concentration	Precursor linker and its concentration	Solvent system	Reaction conditions	Total time	Reference
1.	ZIF-67	Electrochemical	Co(NO ₃) ₂ ·6H ₂ O (0.1 mol/L)	2-MeIm (0.8 mol/L)	Methanol/water (95:5)	Two electrode system WE: Cu mesh CE: Graphite plate V= 5.0 V	200 seconds	²³
2.	GO-ZIF67	Hybrid	Co(NO ₃) ₂ (0.05 M)	2-methylimidazole (5.5 g/20 mL H ₂ O)	water	Hybrid method where Co(OH) ₂ was electrodeposited at -1.0 V (vs. SCE) followed by reaction in at RT, 6 hours	6 hour 30 minutes	²⁴
3.	ZIF-67	Electrochemical	Co foil	2-methylimidazole (0.12–0.24 mol per dm ⁻³) Mediator:MT BAMS: (0.06–0.12 mol per dm ⁻³)	DMF/H ₂ O (3:4)	Two metal foil electrodes (Co), V=2.5V	60–300 minutes	²⁵
4.	ZIF-67	Solvothermal method at RT	Co(NO ₃) ₂ ·6H ₂ O (2.9 g)	2-methylimidazole (6.5 g)	Methanol	RT synthesis followed by separate electrophoretic deposition on FTO coated glass substrate in electric fields ranging from 20 to 60 V·cm ⁻¹	Not specified	²⁶
5.	ZIF-67	Hybrid method	Co(NO ₃) ₂ ·6 H ₂ O (0.5M)	2-Methylimidazole (5 g) was dissolved in deionized water (20 mL)	Water	Electrodeposition of Co(OH) ₂ nanosheets at -1 V (vs. SCE) for 10 min at (25±1) °C, dried then dipped in 2-MeIm solution for 15 minutes.	10 minutes+12 hours+15 minutes	²⁷
6.	ZIF-67	Solvothermal at RT	(Co(NO ₃) ₂ ·6 H ₂ O, 3.492g)	2-methylimidazole (3.94 g) was added to the 60 ml mixed solution	ethanol and methanol (V:V =1:1)	Stirring for 20 hours at RT, washing and drying	20 hours	²⁸
7.	ZIF-67	Electrodeposition (2 step)	Co(CH ₃ COO) ₂ (20 mM)	2-methylimidazole (1M)	.1 M Na ₂ SO ₄	cobalt-modified FTO electrode was prepared on Au electrode at -1.0 V, followed by deposition of ZIF-67 at 0.5 V in the electrolytic solution.	30 minutes	²⁹
8.	ZIF-67	Hybrid	0.1 M Co(NO ₃) ₂	2-MeIm (0.5, 1.0, or 2.0 M) was prepared in a mixed solvent of 5 mL H ₂ O and 5 mL EtOH	Ethanol and water (V:V=1:1)	Cobalt hydroxide (Co(OH) ₂) was directly deposited on CP at -0.95 V vs. Ag/AgCl, followed by drying and immersion in 1-MeIm solution	30 minutes+12 hours+12 hours	³⁰
9.	ZIF-67	Electrodeposition	Co(NO ₃) ₂ ·6H ₂ O (0.145 mol/L)	2-MeIm (0.145 mol/L)	methanol/water solution (10:1 in volume)	Stainless steel mesh was used as the WE and two graphitic plates as counter electrodes (separated by 2 cm) V= -15.0 V	30 minutes	³¹
10.	ZIF-67	Electrodeposition	Co(NO ₃) ₂ ·6H ₂ O (0.5 Mm)	2-MeIm (0.75 mol/L)	DMF solution (10 ml)	GCE directly electrodeposited with ZIF-67 with Pt wire CE. V= -1.4 V	600 seconds	Our work

3.2. Physical Characterization of the developed 3D stacked MOF sensing probe

Physical characterization tools such as SEM, EDX-mapping and XPS analysis were used to initially confirm the formation of the MOFs and their successful stacking over the probe surface.

3.2.1. SEM and EDX Analysis

SEM is a powerful tool to initially deduce the surface morphology of the formed nanostructures over the surface of the probe. A cleaned electrode surface was deposited with the stacked Ni-MOF and Co-MOFs one over the other, with the deposition parameters same as discussed in the preceding section. **Figure 4.3A, B, C** shows the SEM micrograph of the electrode/Ni-MOF/Co-MOF at various degree of magnifications. **Figure 4.3A-inset** shows only Ni-MOF grown over the electrode surface. Dense structures were seen closely packed on the surface of the electrode stating the parameters of deposition apt for formation of Ni-MOF and Co-MOFs. To understand the distinct morphology of the Co-MOFs, a separate deposition of only the Co-MOFs on a clean electrode was examined. The electrode/Co-MOF was found with distinct cuboidal morphology³² (orange encircled structure in **Figure 4.3D**) formed on the electrode, magnified Co-MOF morphology shown in **Figure 4.3D (inset i & ii)**. Some spaces on the electrode surface also exhibited clusters of Co-MOFs, as shown in **Figure 4.3E**. The curve shown in **Figure 4.3E** exhibits the normalized particle size distribution curve plotted for a wide area, stating most Co-MOFs to be present between 300 to 400 nm in size range. Hence, the SEM analysis confirmed the uniform deposition of both MOFs onto the surface of the electrode as well as the morphology of Co-MOF deposited through such simple electrodeposition approach for the first time.

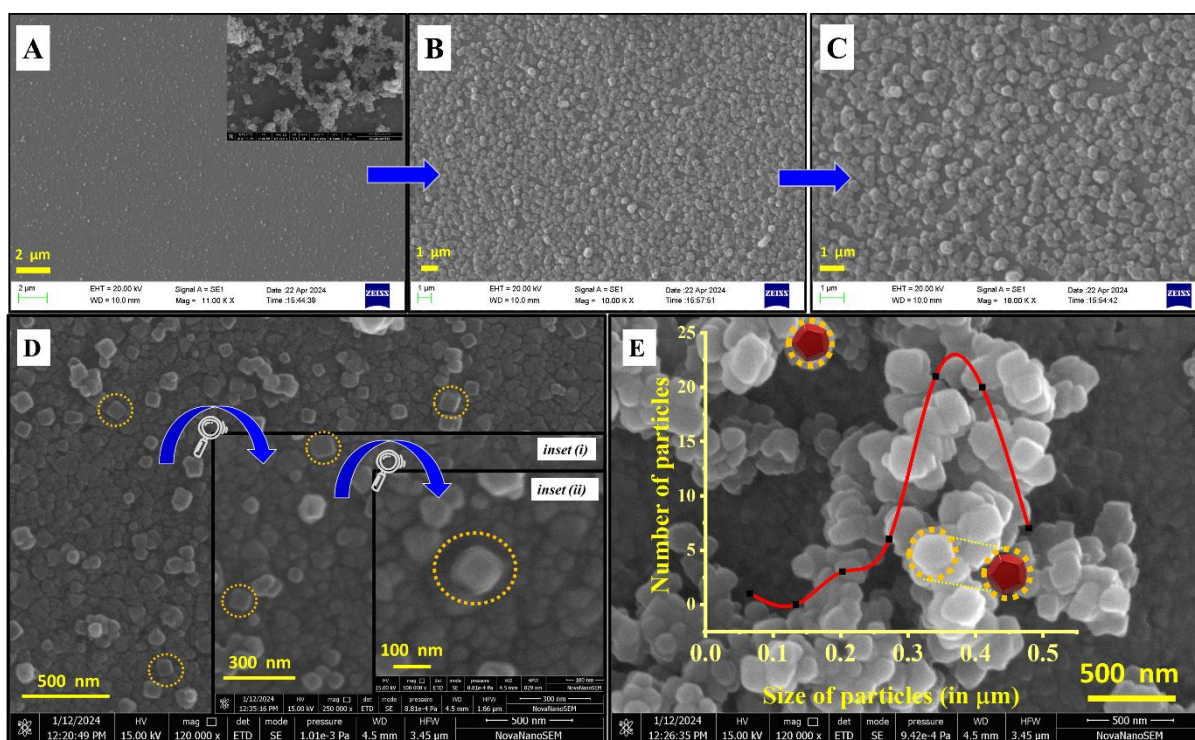


Figure 4.3. (A), (B) and (C) SEM micrographs of electrode/Ni-MOF/Co-MOF at varying magnifications; (A-inset) is the SEM micrograph for only Ni-MOF synthesized through the electrochemical route; (D) Co-MOF at varying magnifications (inset i,ii); (inset-ii) shows single crystal of Co-MOF encircled in orange dotted line (E) clusters of Co-MOF formed on the electrode surface. The red background curve shows the normal size distribution obtained from SEM images using Image J software. On analysis, the average particle size was found to be between 300 to 400 nm.

To further deduce the elemental composition of the stacked MOFs and final probe, EDX-elemental mapping of electrode/Ni-MOF/Co-MOF interface and electrode/Ni-MOF/Co-MOF/AuNP surfaces was done. **Figure 4.4A** shows the EDX elemental composition of the developed sensor matrix comprising the stacked MOFs, with peaks corresponding to both the elements being clearly noticeable in electrode/Ni-MOF/Co-MOF. The atomic percentage composition of individual elements viz. Co is 1.86% and Ni is 0.59% in the case (**Figure 4.4A**) which in the final probe i.e. electrode/Ni-MOF/Co-MOF/AuNP remains comparable with an additional presence of Au due to the introduction of AuNP in the layer (**Figure 4.4B**). The Au component can be clearly concluded due to the introductory 8% rise in its composition within final matrix, confirming the successful fabrication on the electrode. For lucid comparative

understanding, elemental composition of the structures has been tabulated in their respective spectra reports as inset tables. This data has been reconfirmed through the mapping outcomes as shown in **Figure 4.4C**. The individual elemental maps have been clearly shown where **Figure 4.4C-I** is the merged micrograph while **4.4C-II**, **4.4C-III**, **4.4C-IV**, **4.4C-V**, **4.4C-VI** micrographs correspond to the Co (**red**), Ni (**green**), C (**white**), N (**yellow**) and O (**magenta**) matrix constituents, respectively. The mapping results for only Co-MOF have been shown in **Figure 4.5**.

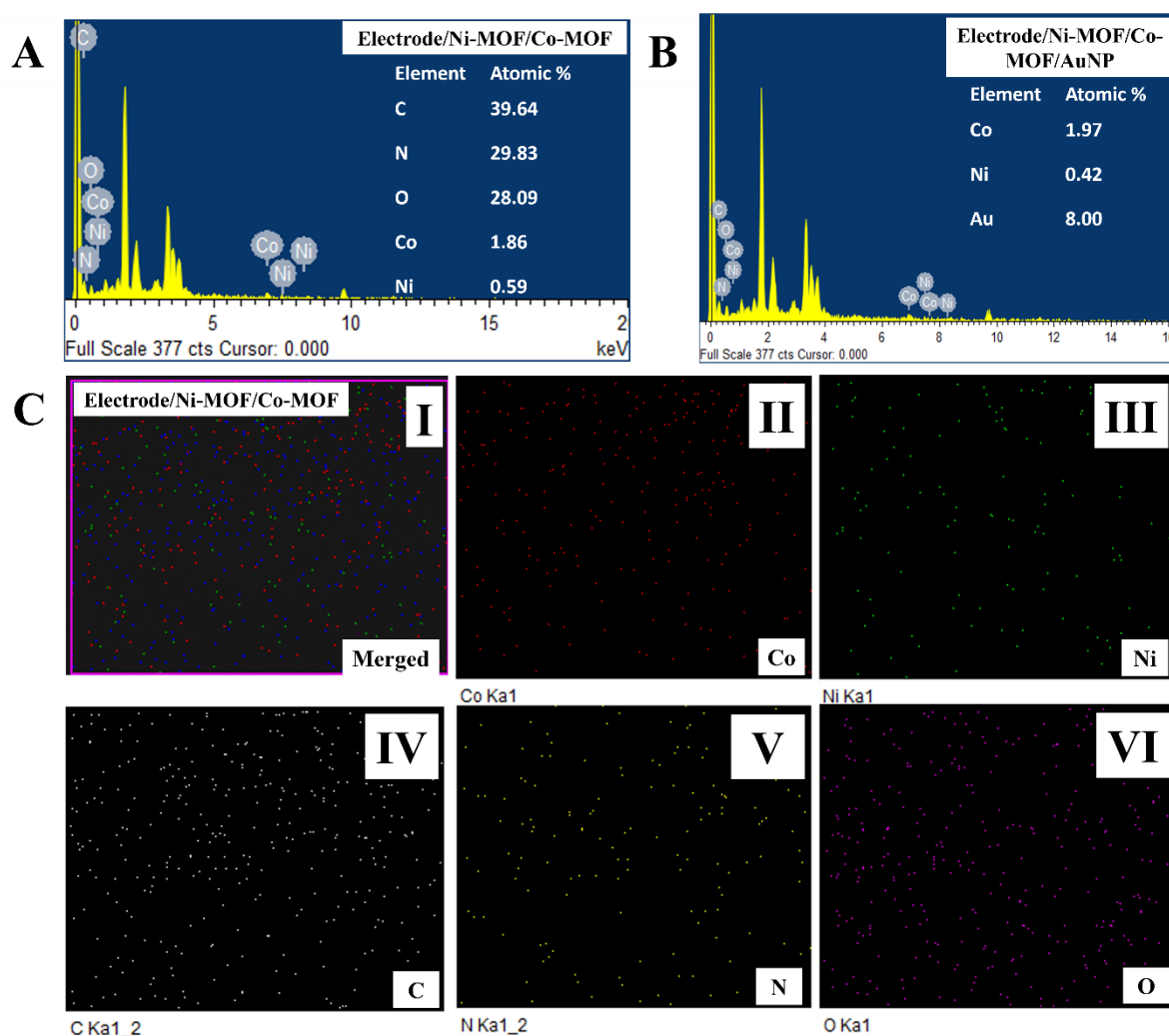


Figure 4.4. (A) and (B) are EDX analysis corresponding to electrode/Ni-MOF/Co-MOF interface and electrode/Ni-MOF/Co-MOF/AuNP surfaces, respectively and (C) is the EDX-mapping results corresponding to the individual elements present in an area comprising of Ni-MOF/Co-MOF interfacial surface where, **C(I)** is the merged micrograph corresponding to electrode/Ni-MOF/Co-MOF; **C(II)** red coloured micrograph for Co; **C(III)** green coloured micrograph for Ni; **C(IV)** white coloured micrograph corresponding to C; **C(V)** yellow

micrograph for N; and C(VI) magenta coloured micrograph corresponding to O. **Note:** To increase visualization of O micrograph, the colour coding in its individual graph has been changed to magenta.

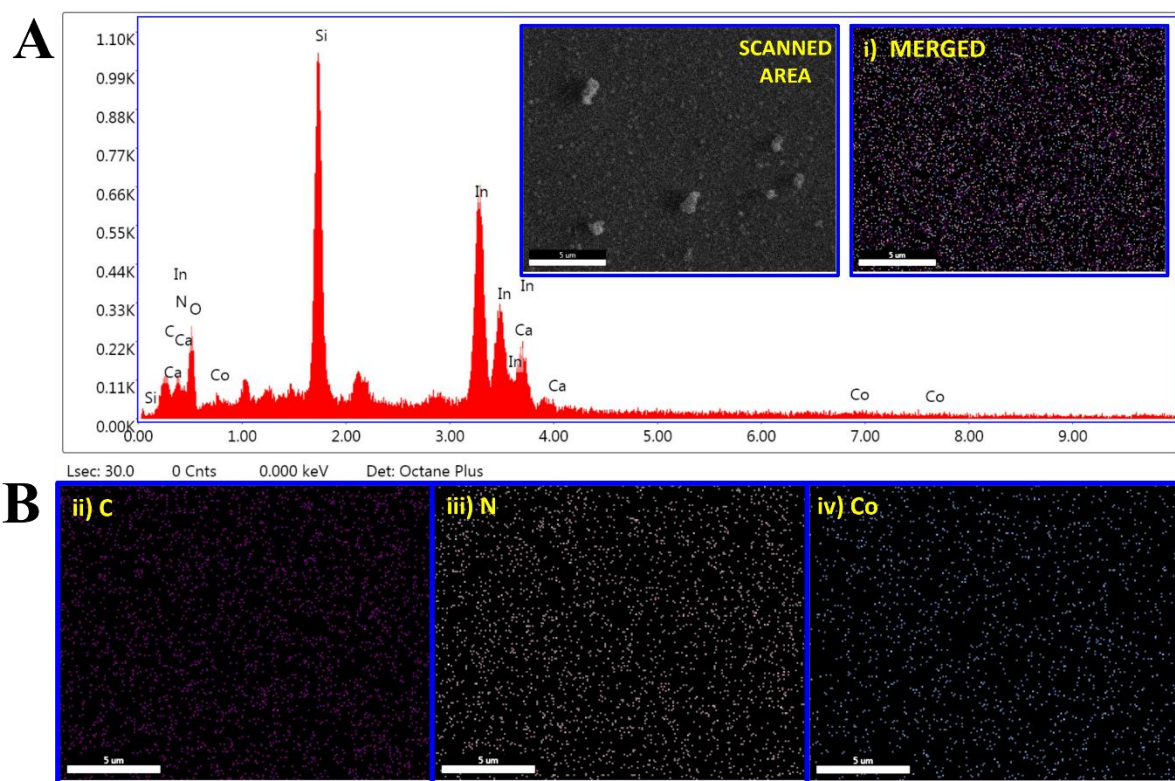


Figure 4.5. Mapping results for only the Co-MOF layer, where (A) is the EDX spectra for constituting elements in the matrix. **Note:** the presence of In, Ca and Si in the spectrum is due to the glass coated with ITO used for the MOF fabrication. (B) (i) is the merged micrograph for all MOF elements; (ii) C/carbon elemental micrograph represented in purple color (iii) N/nitrogen elemental mapping results represented in light blue color; and (iv) Co/cobalt micrograph represented in yellow color

3.2.2. XPS Analysis

To further validate the elemental composition and elucidate the metal oxidation states on the fabricated surface, the probe underwent XPS examination. XPS spectra stating the presence of C1s, N1s, Ni 2p_{3/2}, Co 2p_{3/2}, and Au 4f, in stacked MOFs layered with colloidal AuNP within electrode/Ni-MOF/Co-MOF/AuNP are depicted in **Figure 4.6**. The XPS spectra of the bare electrode (**Figure 4.6A**) clearly states the absence of all elements of interest, which on the deposition of the first layer of Ni-MOF brings noticeable changes. The appearance of the C 1s peak due to the presence of the C=C and C-N (**Figure 4.6B-I**) was also further complimented

by the presence of N 1s spectrum with a distinct peak at binding energy (B.E). value of 398.38 e.V. (**Figure 4.6B-II**) due to their presence in the MOF backbone, as part of the linker molecule^{33,34}. **Figure 4.6B** also demarcates the appearance of the 4 distinct peaks for Ni 2p3 (**Figure 4.6B-III**). While its two peaks are at a B.E of 855 and 874.4 e.V. which correspond to the $\text{Ni}^{2+}(2p_{3/2})$ and $\text{Ni}^{2+}(2p_{1/2})$ peaks respectively, the adjoining ones at 860 and 879.4 e.V. correspond to their respective satellite peaks^{34,35}. It is important to observe that the comparative analysis in bare electrode surface yields no peaks in the same region, stating them to be purely related to Ni^{2+} metal center in a successfully deposited Ni-MOFs. The intense peaks corresponding to $\text{Ni}^{2+}(2p_{3/2})$ and $\text{Ni}^{2+}(2p_{1/2})$ establish the presence of Ni core levels in its Ni^{+2} state within the MOF when bonded to imidazole linker moiety. The difference between the core level Ni peaks (19.4 e.V.) and the satellite peaks (19.4 e.V.) remained comparable^{35,36}. There are clearly no peaks corresponding to Co and Au due to their absence in the matrix at the current stage (**Figure 4.6B IV, V**). The C 1s and N 1s peaks remain the same in the 3rd layer (**Figure 4.6C**) too due to their obvious presence in the framework by virtue of the linker moiety (refer **Figure 4.6C I, II**). On further examination of the XPS spectra for the advancing layers, the preceding elements (**Figure 4.6C I, II, III**) were accompanied with 4 sharp peaks at 780, 784.8, 796.3, and 802.2 e.V. where, the first and third B.E. values corresponded to the $\text{Co}^{2+}(2p_{3/2})$ and $\text{Co}^{2+}(2p_{1/2})$ peaks while the other two were the adjoining satellite peaks (**Figure 4.6C-IV**)^{37,38}. It is to note again that the negative control for the same at the previous electrode surfaces yielded no peaks at the same B.E. values which confirmed the notion for peak generation only due to successful Co-MOF synthesis on the surface³⁸. On scanning for the final electrode surface viz., electrode/Ni-MOF/Co-MOF/AuNP, Au 4f reveals two representative doublet peaks at 83.66 and 87.48 e.V. on deconvolution which corresponds to characteristic peaks of metallic Au^0 on its surface due to coating with the colloidal gold particles³⁹ (**Figure 4.6D-V**). These peaks are present in only the final surface while being absent in the previous

ones (**Figure 4.6 A,B,C-V**) lucidly indicating them, being gold NP originated ones. It is interesting to observe that on subsequent layering of electrode/Ni-MOF with Co-MOF and AuNP, the intensity of the Ni 2p3 peaks decreases also suggesting proper layering of the succeeding surfaces over it. The XPS analysis, thus, evidenced the presence of both metals as well as non-metals in their desired states on the nanoimprinted surface.

The study re-affirmed us on the successful deposition of the matrix components onto the electrode surface which was further verified through the electrochemical elemental verification, as discussed in the upcoming section.

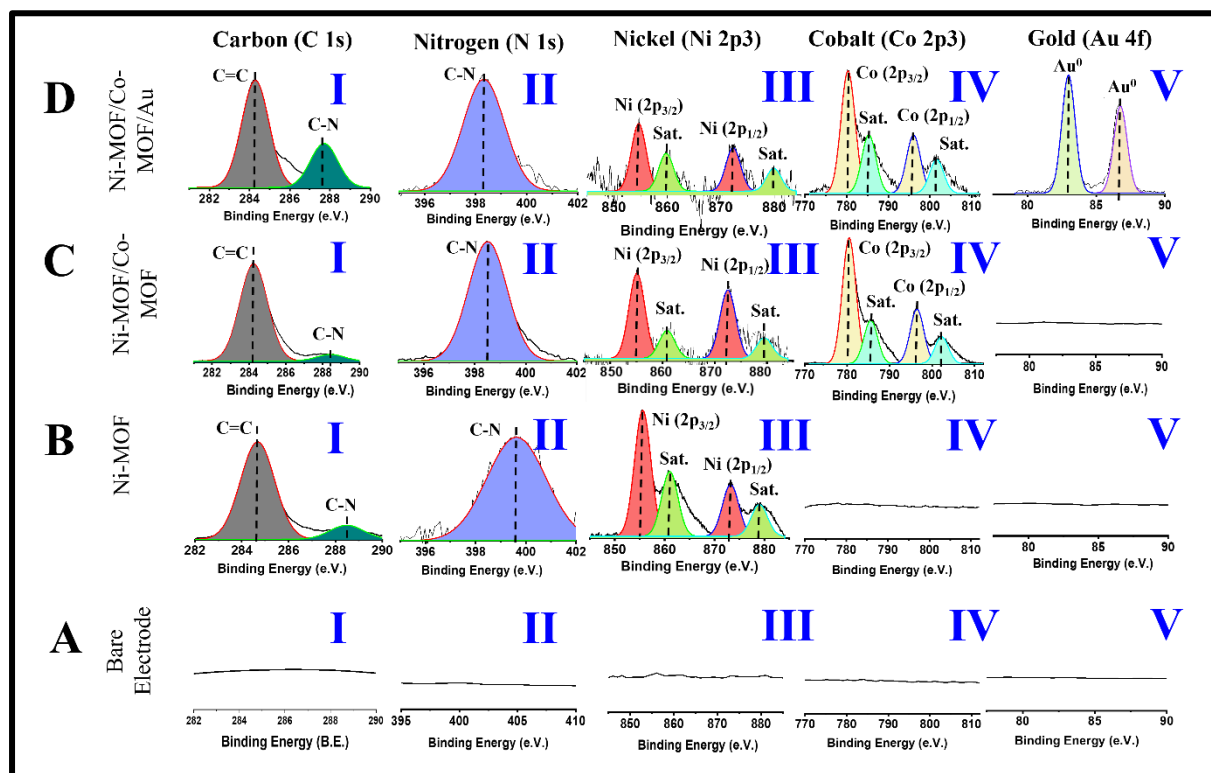


Figure 4.6. XPS analysis of the (A) Bare electrode surface; (B) Electrode/Ni-MOF; (C) Electrode/Ni-MOF/Co-MOF; and the final electrode surface (D) Electrode/Ni-MOF/Co-MOF/AuNP in comparison with all preceding probe layers. On stacking the MOFs and coating it with colloidal AuNP, both metal peaks are retained with an additional appearance of peak pertaining to Au^0 .

3.3. Electrochemical characterization

3.3.1. Catalytic metal elemental validation on the GCE/Ni-MOF/Co-MOF/AuNP sensor probe

Following physical characterization investigations that verified the presence of gold metal in the NPs and the metal core of stacked MOFs, electrochemical characterization experiments were conducted to further validate the metal element. Three separate set of experiments were performed using different electrolytic solutions to validate the presence of Co, Ni and Au metal centers in the composite matrix fabricated. In the very first step, Ni and Co metal centers were determined step-by-step, through electrodeposition of the nanostructures followed by dipping the modified probe into 0.1 M NaOH solution and scanning between -0.4 V vs. Ag/AgCl to +0.5 V vs. Ag/AgCl in case of Ni-MOF (**Figure 4.7A**) and -0.4 V vs. Ag/AgCl to +0.4 V vs. Ag/AgCl, in the case of Co-MOF (**Figure 4.7B**). On deposition and scanning the probe for testing of 'Ni' metal center at the primary stage, the characteristic redox peaks pertaining to Ni^{2+} were observed at +0.32 V vs. Ag/AgCl and -0.33 V vs. Ag/AgCl. Comparable redox potentials values for Ni^{2+} have been reported in previous articles too, with similar profiling solvent, validating the desirable state of Ni^{2+} . On further modification of the GCE/Ni-MOF probe by stacking it with Co-MOF, along with the previous noted peaks for Ni redox, an additional enhanced peak at +0.32 V for Co^{+2} was also observed in the oxidation window, whereas, the reduction peak intensity at -0.33 V was further enhanced in magnitude due to the superimposing of the reduction peak for both the metals at the same potential^{40,41}. Additionally, a similar set of experiment was performed to determine the presence of 'Au' metal in the top coat of final probe comprising colloidal AuNP (**Figure 4.7C**). As anticipated, a redox peak at around +1.2 V vs. Ag/AgCl and +1.05 V vs. Ag/AgCl was seen when dipping the electrode in 0.5 M H_2SO_4 scanned within a potential window of +0.8 V vs. Ag/AgCl to +1.4 V vs. Ag/AgCl⁴². A control experiment where the unmodified probe was dipped in the same electrolytic solutions within the same potential range was performed, which yielded no results. The stability of the individual probe layers and electron transfer mechanisms were validated through a scan rate study as well. The anodic (I_{pa}) and cathodic (I_{pc}) peak currents were found

directly proportional to the scan rates, with the correlation coefficient values of 0.99 and 0.97 for 'Ni' in Ni-MOF (**inset Figure 4.7A**), 0.99 and 0.98 for 'Co' in Co-MOFs (**inset Figure 4.7B**), and 0.99 and 0.99 for 'Au' in AuNP (**Figure 4.7D**), respectively. This demonstrates the stable surface adsorption/adhesion phenomena with stability even at higher scan rates, making the engineered probe ideal for sensing applications. Thus, the set of physical and electrochemical characterization studies conclusively validated the presence, composition and overall morphology of the constituting metallic nanostructures in the final GCE/Ni-MOF/Co-MOF/AuNP sensing probe.

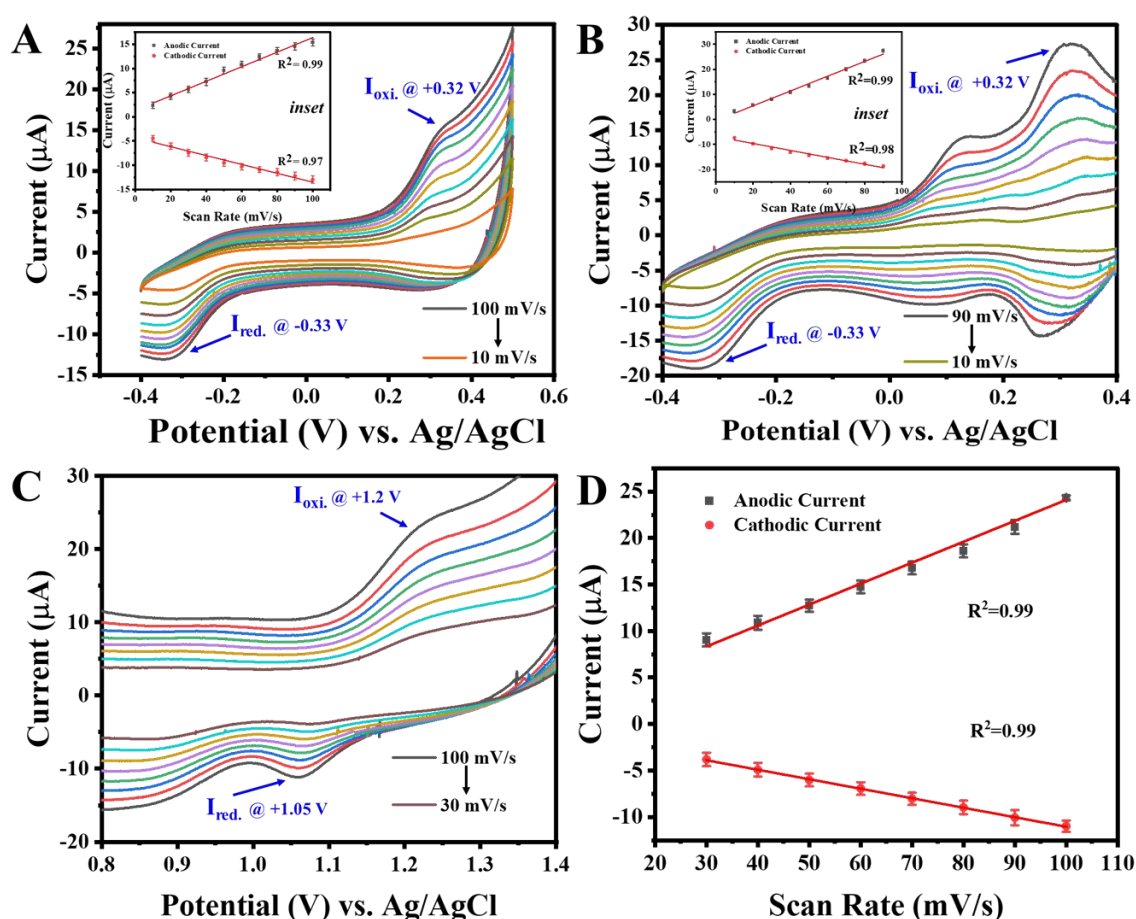


Figure 4.7. Electrochemical estimation of probe elements where (A) is the CV curve corresponding to redox behaviour of Ni-MOF in the presence of 0.1 M NaOH at different scan rates ranging from 10-100 mV/s ($n=5$), **inset** depicts the corresponding fitting curve for the redox values; (B) is the CV curve corresponding to redox behaviour of Co-MOF in the presence of 0.1 M NaOH at different scan rates ranging from 10-90 mV/s ($n=5$), **inset** shows the corresponding fitting curve for the redox values. (C) is the CV curve corresponding to

redox behaviour of colloidal AuNP in the presence of 0.5 M H₂SO₄ at different scan rates ranging from 30-100 mV/s (n=5), and (D) is its corresponding fitting curve.

3.3.2. Electrochemical performance of the stacked MOFs in the sensor probe

After confirmation of the desirable metals on the surface of the developed matrix, electrochemical examination was conducted to understand its charge transfer behaviour and electrocatalytic capabilities. The electrochemical examination of the developed surface was done through dipping the probe in ZS and scanning it at a scan rate of 50 mV/s (n=5) at room temperature within a potential window of -0.15 V vs. Ag/AgCl to +0.75 V vs. Ag/AgCl. Sharp redox peaks were observed for all surfaces within the potential range for the (K₃[Fe(CN)₆]/K₄[Fe(CN)₆]) redox species. Redox peaks were observed at +0.45 V vs. Ag/AgCl and +0.065 V vs. Ag/AgCl in correspondence to the intrinsic behaviour of ZS at the bare GCE surface (**black curve**). A significant shift at a lower potential and higher current magnitude in the peak was observed for the same ZS species at +0.25 V vs. Ag/AgCl and +0.20 V vs. Ag/AgCl for GCE/Ni-MOF (**red curve**). Similar enhanced peaks at the lower potential and higher current magnitude pertaining to GCE/Co-MOF (**blue curve**) and GCE/Ni-MOF/Co-MOF (**green curve**), were further obtained on consecutive fabrication steps, as shown in **Figure 4.8A** (n=5). The lower potential shift and higher current magnitude cumulatively demarcate the layer's catalytic as well as charge transfer characteristics. Comparative histograms depict the cathodic and anodic responses of the electrode surfaces on successive modifications (**Figure 4.8A-inset**). The modified surface, i.e., GCE/Ni-MOF/Co-MOF comprising of the stacked MOFs exhibited the highest charge transfer characteristics and electrocatalytic behaviour when compared to the individual surfaces with the single metal MOFs. The proficiency of the stacked MOFs to conduct the electrons more efficiently can be attributed to the conductive metal synergies facilitating seamless electron transfer⁴³. The charge transfer characteristics of individual surfaces could be a function of their respective surface

areas on the electrode, as calculated through the below **Randles Sevcik equation**⁴⁴ (equation i),

$$I_p = (2.69 \times 10^5) n^{3/2} A C D^{1/2} v^{1/2} \dots\dots\dots \text{Equation i}$$

where, surface area of the electrode is denoted by A (in cm^2), the anodic peak current is depicted through I_p in terms of ampere, v is the scan rate in V s^{-1} , the number of electrons transferred in the redox process are represented by n , concentration of the transferred redox species is stated by C in mol cm^{-3} , and the diffusion coefficient is denoted by D which is in $\text{cm}^2 \text{s}^{-1}$.

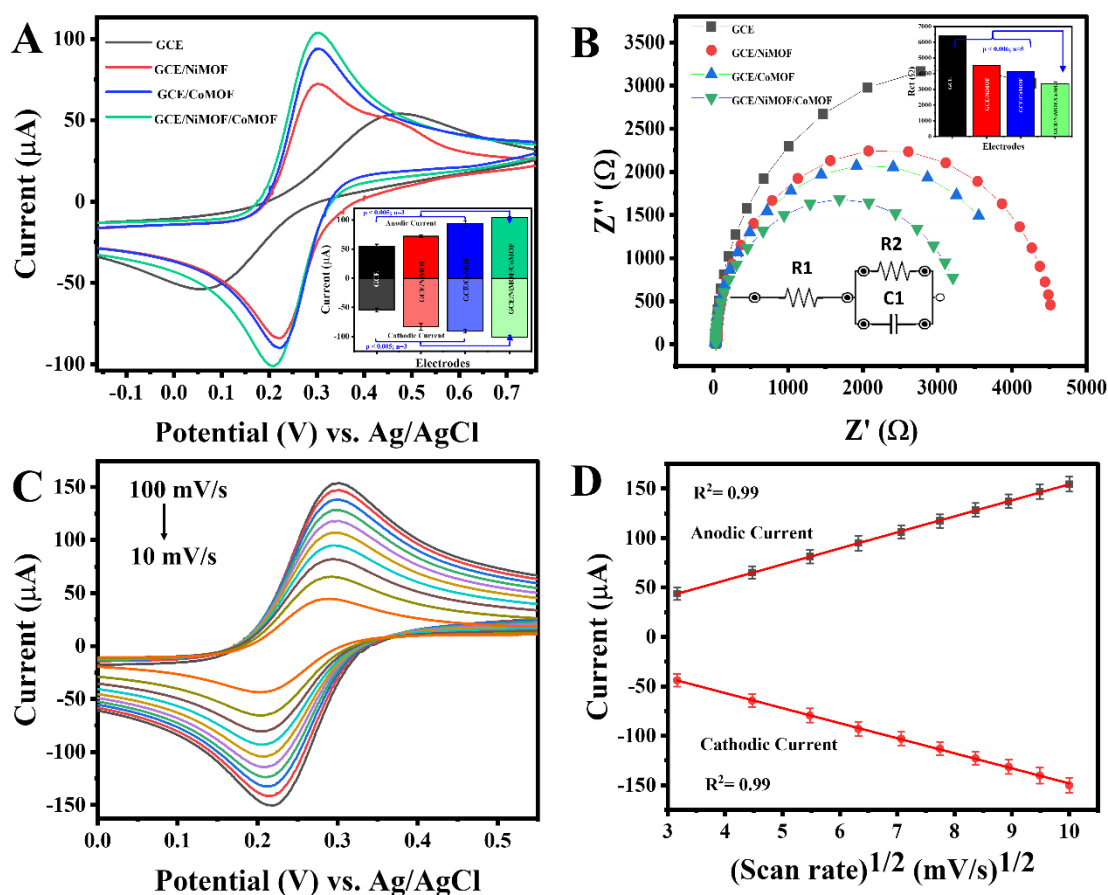


Figure 4.8. (A) CV response of bare GCE (black curve), GCE/Ni-MOF (red curve), GCE/Co-MOF (blue curve), and GCE/Ni-MOF/Co-MOF (green curve) in ZS (5 mM; pH-7, scan rate-50 mV/s; $n=5$); inset depicts comparative histogram of the anodic and cathodic current responses corresponding to each surface, with an amplified response for the GCE/Ni-MOF/Co-MOF electrode. (B) EIS response in the form of Nyquist plots for bare GCE (black curve), GCE/Ni-MOF (red curve), GCE/Co-MOF (blue curve), and GCE/Ni-MOF/Co-MOF (green curve) in ZS (5 mM; pH-7; $n=5$) with its equivalent circuit model where R_1 denotes the solution resistance, R_2 denotes the charge transfer resistance (R_{ct}), and C_1 is the double layer capacitance; inset shows a comparative histogram displays the R_{ct} values of each surface. (C) Scan rate study of GCE/Ni-MOF/Co-MOF in ZS solution at varying scan rates ranging between 10-100 mV/s; (D) obtained linear plot corresponding to current vs. $(\text{scan rate})^{1/2}$.

The calculated effective surface area of each surface, viz., GCE, GCE/Ni-MOF, GCE/Co-MOF, GCE/Ni-MOF/Co-MOF was $66.6 \times 10^{-3} \text{ cm}^2$, $89.27 \times 10^{-3} \text{ cm}^2$, $116.13 \times 10^{-3} \text{ cm}^2$, and $127.95 \times 10^{-3} \text{ cm}^2$, respectively. These results are in agreement to our electro-characterization results that the electrocatalytic potential of the layers increase with successive layering of the matrix with individual MOFs and is maximum in case of the stacked MOFs. The area of the GCE/Ni-MOF/Co-MOF increases by 1.92, 1.43, and 1.10 times when compared to bare GCE, GCE/Ni-MOF, and GCE/Co-MOF, respectively. The CV results were further validated using the EIS study (**Figure 4.8B**). Upon fitting the obtained values to the Randles circuit the Nyquist plots were determined for the respective surfaces. Briefly, the Randles circuit is an equivalent electrical circuit which comprises of a resistance (R_1), which actually is a resistance of the solution, coupled in series with double layer capacitance (C_1) and faradaic charge transfer resistance (R_{ct}/R_2) connected to each other in parallel. The R_{ct}/R_2 calculated through the Nyquist plots were $6393 \pm 4.55 \ \Omega$, $4509 \pm 1.33 \ \Omega$, $4154 \pm 7.54 \ \Omega$, and $3364 \pm 2.7 \ \Omega$, respectively for bare GCE (**black curve**), GCE/Ni-MOF (**red curve**), GCE/Co-MOF (**blue curve**), and GCE/Ni-MOF/Co-MOF (**green curve**) electrodes, respectively. The results of the EIS study verify the outcomes of the CV results indicating that the GCE/Ni-MOF/Co-MOF has the lowest resistance and therefore the greatest charge transfer characteristics (**Figure 4.8B-inset**). Hence, it can now be checked for its catalytic potential against the target analyte for establishing it as a sensing platform. In the meantime, the robustness of the platform was also assessed using a small yet crucial control experiment, where the developed probe was scanned at varying scan rates (from 100 mV s^{-1} to 10 mV s^{-1}) within a potential window of 0.0 V vs. Ag/AgCl to +0.5 V vs. Ag/AgCl in the same electrolytic solution (5mM ZS). The peak oxidative and reductive currents (I_{pa} and I_{pc}) were found to be proportionate to the square root of the scan rates with a correlation coefficient of 0.99 in both the cases evoking the exceptional stability of the probe even at higher and lower scan rates in a diffusion-controlled manner, as

shown in **Figure 4.8C and 4.8D**. The electrochemical investigations so far state the potential usability of the sensor probe for forthcoming applications.

3.4. Analytical Feasibility of the Developed Surface

To check the feasibility of the developed system, NO_2^- was used as the target analyte, whose detection importance has been stated through an elaborate discussion made in the introductory section. On thorough survey of the developed sensors against NO_2^- , it was understood that the Au core particles engage optimally to form a stable Au-N bond with 'N' of the nitrite anions along with providing a huge surface area for NO_2^- adhesion^{4,17,45–47}. To reap benefit of this established notion, we introduced a layer of colloidal AuNP onto the matrix aiding towards increased sensing efficiency of the developed surface, making the final surface as GCE/Ni-MOF/Co-MOF/AuNP. This developed system was subjected to rigorous testing against the analyte where preliminary testing involved control studies. At first, each electrode surface was tested against NO_2^- to ascertain the contribution of each metallic architecture. LSV was performed for the bare GCE within a range of +0.5 V vs. Ag/AgCl to +1.0 V vs. Ag/AgCl in a 0.1 M PBS solution containing 0.1 M NO_2^- (n=3). A slight oxidative current was recorded at approximately +1.0 V vs. Ag/AgCl with no distinct peaks in the scanned window (**Figure 4.9A, black curve**) since unmodified bare electrodes are known to detect NO_2^- but suffer from large overpotential, demanding suitable electrode modifications for their commercial sensing utility¹³. A clear shift in the peaks towards the lower potential with improved current magnitude was seen on subsequent surface modifications, proving the catalytic ability of the modified surfaces against NO_2^- with a distinct peak at +0.95 V vs. Ag/AgCl. The anodic peak was constantly enhanced on addition of each consecutive layer one over the other, investigated in similar experimental conditions (**Figure 4.9A**). Because of the Ni-Co synergy and the interaction between Au and the analyte species, the final layer showed the highest current value in the presence of the analyte. When compared to the unaltered bare GCE surface, there was

approximately 4.83-fold increase in signal responsiveness against the oxidative irreversible conversion of NO_2^- to nitrates. A comparative histogram was plotted to understand the quantitative rise in signal output (**Figure 4.9B**), pictorially stating the same. To eliminate the possibility of signal interference by PBS, the bare GCE probe was subjected to pristine PBS solution, yet yielded no response (**olive curve, Figure 4.9A**).

To further validate that the signal outcomes were a result of the target analyte only, a set of control experiments were performed. The first control test was meant to understand the response of the GCE/Ni-MOF/Co-MOF/AuNP probe against the target analyte. For the test, relatively higher concentrations of NO_2^- solutions (0.1 M to 0.5 M) in 0.1 M PBS were prepared and tested against GCE/Ni-MOF/Co-MOF/AuNP via LSV (**Figure 4.9C**). A linear trend was obtained in the standard plot with gradual increase in the concentrations of NO_2^- with a correlation coefficient of 0.97 (**Figure 4.9D**). The obtained regression equation can be stated as,

$$\Delta I (\mu\text{A}) = (1248.64 \pm 226.63) + 12179 (\pm 931.72) \times \text{Conc. } [\text{NO}_2^- (\text{M})].$$

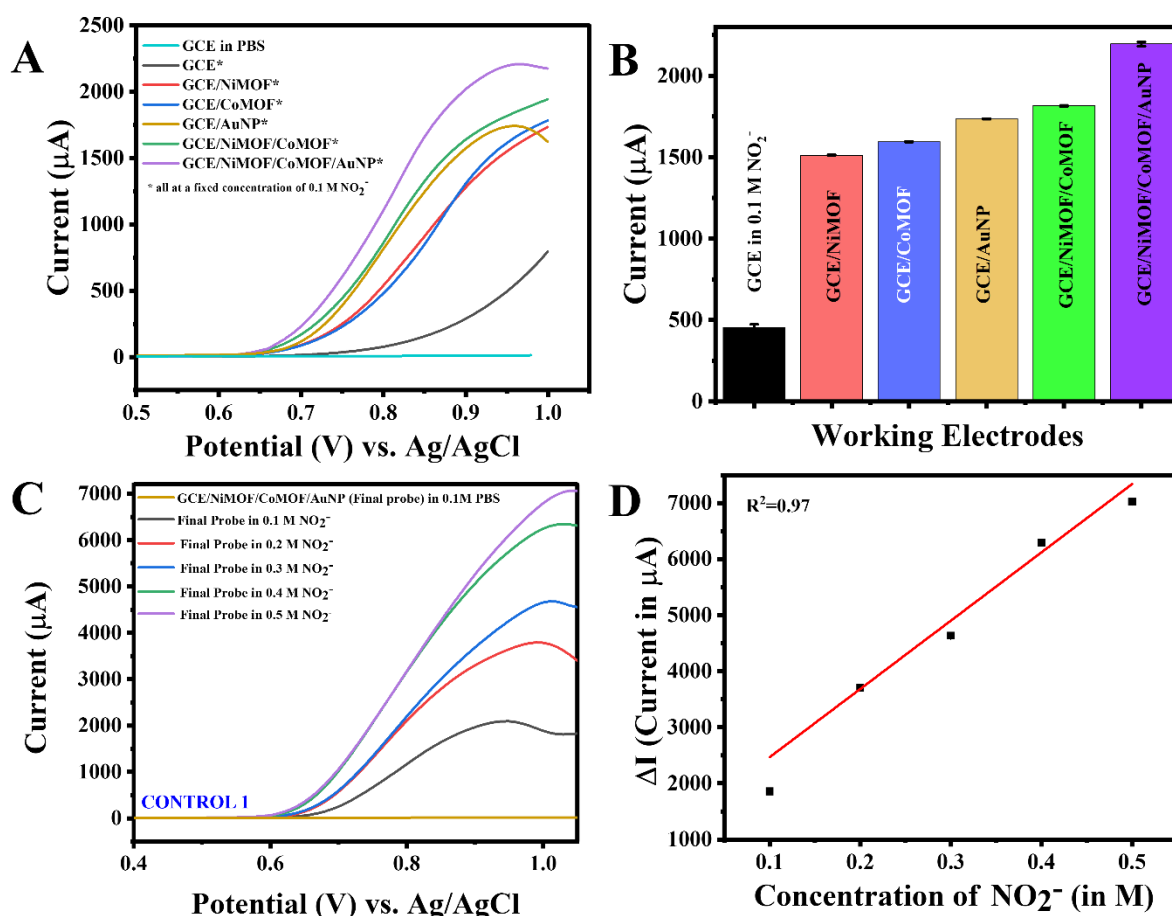


Figure 4.9 (A) Layer by layer analysis of the sensor matrix components against the target analyte, i.e. 0.1 M NO_2^- . (B) Comparative histogram for the analysis (C) Concentration dependent analysis of the final probe, i.e. GCE/Ni-MOF/Co-MOF/AuNP against higher concentrations of the analyte (D) Corresponding linear regression plot for the probe against the concentrations.

Another control study, i.e. the scan-rate dependent study was conducted in the presence of 0.1 M NO_2^- . The experiment comprised of ranging the scan rates from 10 mV/s to 100 mV/s in 0.1 M NO_2^- solution, where a rise in anodic current response was recorded with an increase in square root of the scan rate ($n=3$) with a correlation coefficient of 0.98 (Figure 4.10, Figure 4.10-inset). Both the control experiments conclusively proved the robustness, stability and direct sensing capabilities of the platform for NO_2^- .

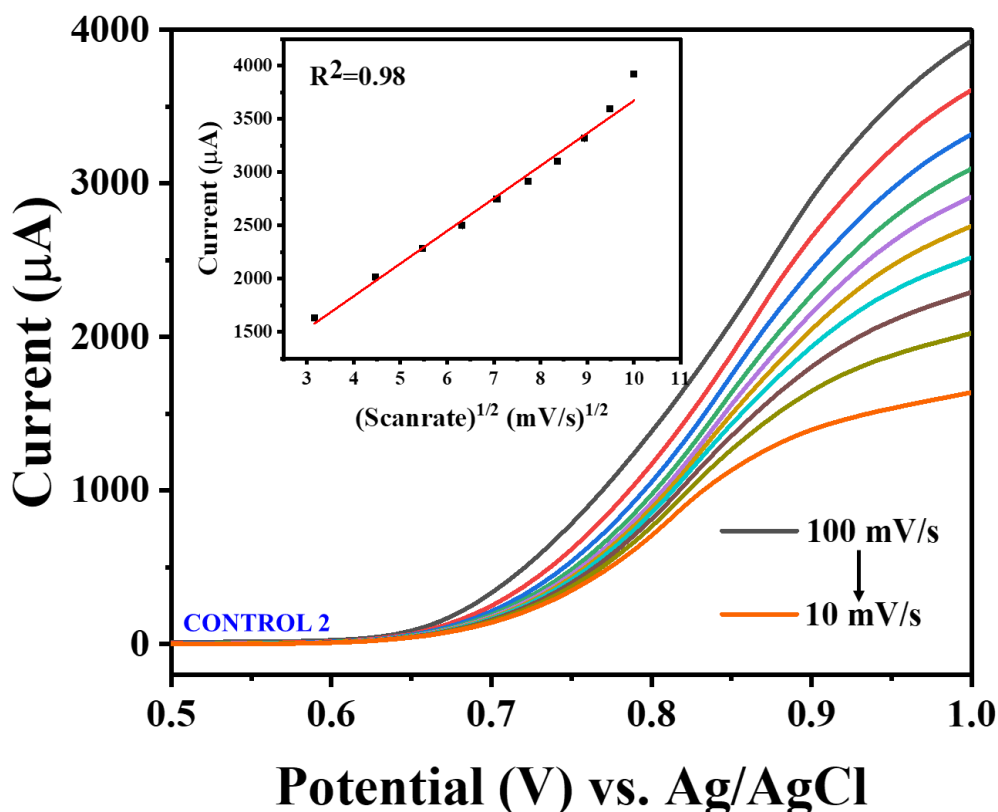


Figure 4.10. Scan rate study of the final probe in 0.1 M NO₂⁻, inset depicting the anodic current versus square root of scan rate.

The analytical abilities of the GCE/Ni-MOF/Co-MOF/AuNP was further examined by deployment of chronoamperometry (**Figure 4.11A**). At first, the response of the final probe was recorded at +1.0 V vs. Ag/AgCl in PBS (**blank red curve**). Further the interface was investigated against a dose-dependent study of NO₂⁻ at the same potential and the signal response was noted. With rise in the concentration of NO₂⁻, the shift from the blank reading was widened, generating a noticeable 'ΔI' in each case. Two linear calibration plots were obtained in the dosage range, one from 0.01 to 10 μM (R²=0.91) and the other from 100 to 1000 μM (R²=0.97), covering an overall wide linear range from 0.01 to 1000 μM, having a detection limit (LOD) of 4.2 nM, as suggestive by the chronoamperometric data (**Figure 4.11B**). Appearance of such ranges can be attributed to the differential saturation of analyte

onto the electrode surface with rapid interaction at the lower concentrations, generating steeper slope⁴⁸. The LOD was calculated using the **equation ii** which is

$$LOD = \frac{3\sigma_b}{m} \quad \text{.....Equation ii}$$

where σ_b represents the blank sample's standard deviation, and m is the calibration plot's slope. The regression equations for the lower and higher concentrations ranges were obtained from the standard curves, represented as follows:

Regression equation 1: (0.01 to 10 μM)

$$I (\mu\text{A}) = 0.688 (\pm 0.09) + 0.071 (\pm 0.015) \times [\text{Conc. of NO}_2^- (\mu\text{M})].$$

Regression equation 2: (100 to 1000 μM)

$$I (\mu\text{A}) = 1.096 (\pm 0.234) + 0.00623 (\pm 4.16) \times [\text{Conc. of NO}_2^- (\mu\text{M})].$$

3.5. Response Time Analysis for the GCE/Ni-MOF/Co-MOF/AuNP Sensor

Response time, a crucial parameter in sensor performance analysis is defined by the time needed for the sensing interface to generate a signal response after the analyte is spiked. For determining the same in case of our platform, a chronoamperometric run was performed by dipping the sensing probe in 0.1 M PBS to obtain a stable signal (**Figure 4.11C, point a**). Following which, a known concentration of NO_2^- was injected into the electrolytic solution (**point b**). Interestingly, a sharp rise in the current (**point c**) was observed between 317.20 seconds and 318 seconds, followed by observance of a current saturation point (**point d**). The signal was obtained at +1.0 V (same as the calibration study in previous section) and the response time was found to be 0.80 seconds (318-317.20), highlighting the ultra-swift sensing capability of the platform. **Figure 4.11C** depicts the real-time response generated by testing the sensor against 1000 μM of NO_2^- . We also examined the sensor response against different concentrations of NO_2^- , and the sensor exhibited commendable response with an average response time of <1.5 seconds in all cases.

The developed sensor has been extensively evaluated in terms of various governing parameters and compared to the latest ones in the literature, as represented in **Table 4.2 (at the end of the chapter)**. Most of them either lag behind in covering the permissible limit of WHO, or have tedious steps of fabrication with elongated fabrication period. The engineered surface is highly resilient and beneficial in multiple ways with the biggest advantage being a simple fabrication approach and a rapid detection of NO_2^- , covering the WHO permissible limits and beyond.

3.6. Selectivity, Reproducibility, and Stability of the GCE/Ni-MOF/Co-MOF/AuNP Sensor Probe

Selectivity is one of the most important characteristics that a sensor possesses. For an electrochemical sensor, it is an important trait of it to detect only the target analyte in the effective potential window scanned. To understand the same, a range of possible ions and related compounds were tested against the signal probe. Interferents such as Cl^- , CO_3^{2-} , SO_4^{2-} , H_2PO_4^- , Sucrose, D-Glucose, Lactate Monohydrate, Sodium Nitrate, L-histidine, L-methionine, K^+ were tested against a possible response generation at a concentration as high as 1 M, but yielded no result at the NO_2^- signature oxidation potential of +1.0 V vs. Ag/AgCl (**Figure 4.11D**). The study proved the efficacy of the sensor selectively to the target analyte.

The coefficient of selectivity (K_{sel}) of the coexisting molecules was determined using **equation iii**, found between 0.026 to 0.119, for which their respective individual values have been stated in **Table 4.3**.

$$K_{\text{sel}} = \text{Signal}_{\text{interfering molecule}} / \text{Signal}_{\text{NO}_2^-} \quad \text{..... Equation iii}$$

Table 4.3. Co-efficient of selectivity for possible interferent species present in the real samples.

Co-existing molecules	Current (μA)	$K_{\text{sel.}}$
Cl^-	0.41	0.050
CO_3^{2-}	0.607	0.074
SO_4^{2-}	0.979	0.119
H_2PO_4^-	0.336	0.041
Sucrose	0.371	0.045
D-Glucose	0.266	0.032
Lactate Monohydrate	0.255	0.031
Sodium Nitrate	0.371	0.045
L-histidine	0.21	0.026
L-methionine	0.805	0.098
K^+	0.979	0.119
NO_2^-	8.16	1

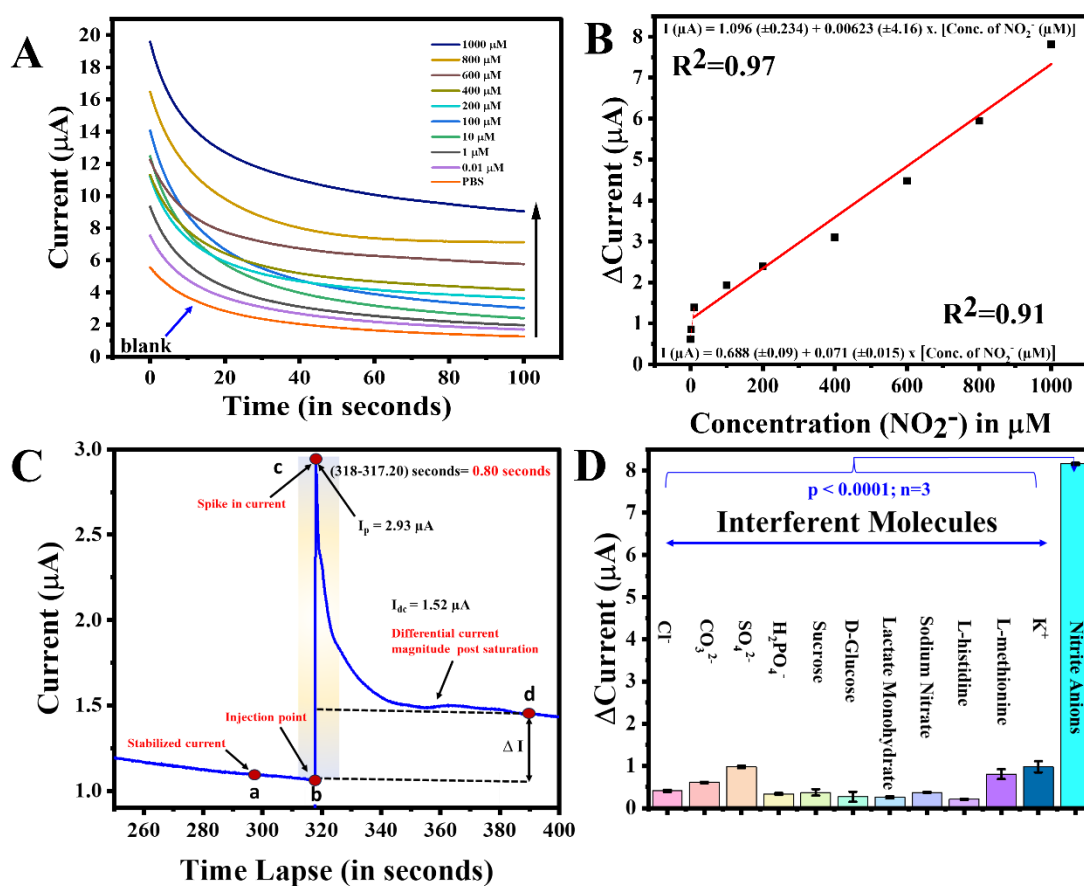


Figure 4.11. (A) Dose dependent study for varying concentrations of NO_2^- (B) Calibration plot (C) Response time analysis (D) Interference study of the sensor against a range of possible interferences likely to be present in the real sample premises

In further course of action, an independent study governing the reproducibility of the response at five different electrodes in a sample solution containing 1000 μM of the target analyte was registered (**Figure 4.12A**). A negligible deviation in the current response was observed across all tested electrodes (RSD 3.7 %, $n=3$), which can be a function of handling errors or mild changes in testing conditions such as environmental factors.

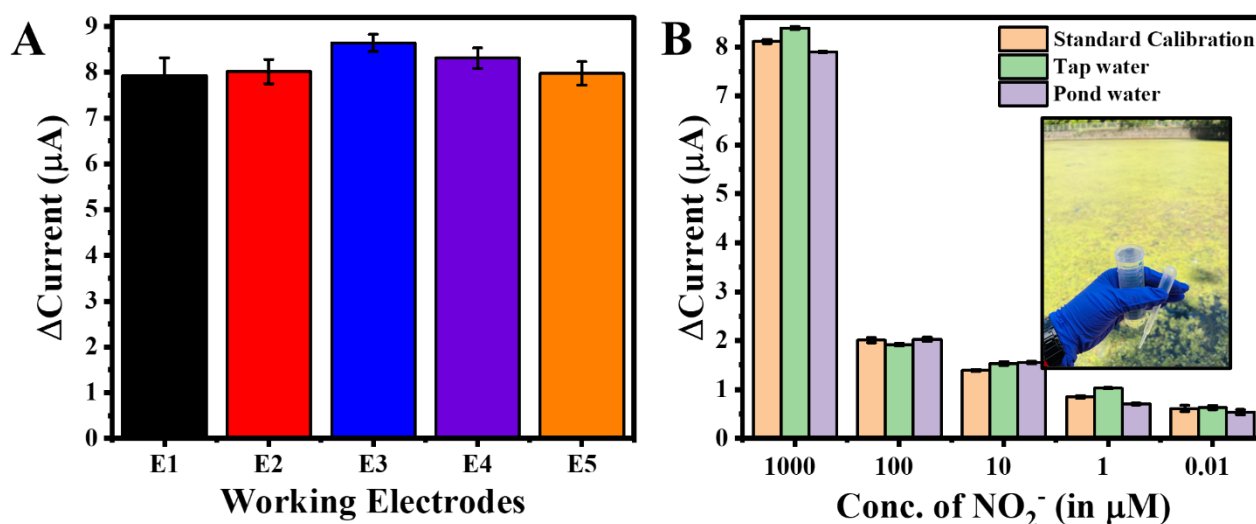


Figure 4.12. (A) Probe reproducibility analysis in a range of electrodes. (B) Real sample study of the sensor involving tap water and pond water.

3.7. Real Sample Analysis

NO_2^- a common fertilizer for plants and a food preservative can often be exploited or misused, cascading generation of carcinogens and result into ill-health⁴⁶. In view of this, the developed sensor was tested for NO_2^- in its natural premises such as tap water and pond water to understand its practical applicability. A standard spike and recovery route was adopted for validating NO_2^- concentrations in spiked real water samples in the concentration range of 0.01 to 1000 μM under optimized experimental settings. Chronoamperometric response was recorded in a dose-dependent manner as a function of NO_2^- in PBS-equilibrated water samples and then compared to the standard calibration plot obtained in **Section 3.4**, as represented through bar graphs in **Figure 4.12B**. The figure depicts the histogram-based analysis of the real

samples results adjacent to the standard calibration curve, evoking optimal sensor behaviour even in real-sample matrices. The recoveries were calculated at each tested concentration of NO_2^- using **equation iv**, and was found between 95.32 to 108 % in tap water (RSD%= 3.60, n=5) and 93 to 100.89% in pond water, respectively (RSD%= 4.80, n=5).

$$\% \text{ Recovery} = (S_{\text{NO}_2^-} - B_{\text{NO}_2^-}) / (SS_{\text{NO}_2^-}) * 100 \quad \dots \text{Equation iv}$$

where, $[S]$ and $[B]$ are the analytical responses of the GCE/Ni-MOF/Co-MOF/AuNP sensing platform in PBS spiked with real samples and blank PBS, respectively while $[SS]$ correspond to response of the standard buffer with the same concentration of NO_2^- , under consideration.

It is worthy to mention that the designed sensing matrix, i.e., GCE/Ni-MOF/Co-MOF/AuNP, is capable to detect NO_2^- in a wide range, even covering the recommended permissible limits of it ($<65 \mu\text{M}$) in drinking water and beyond, as per the guidelines of the WHO^{45,49}. The discussed findings strongly instigate that the fabricated engineered sensing surface comprising of two stacked MOFs is an interesting and potent platform capable of estimating NO_2^- , revealing its future commercial prospects.

3.8. Integration of Sensor with a Smartphone Interface for Field Deployment

A conventional system utilizes multi-stepped analysis and conclusive comments by an expert on the basis of a generated current response by a CA curve obtained at a specific potential to quantitatively estimate NO_2^- in a setup. To minimize these steps and remove possibility of errors caused by manual interventions, an automated data analysis and display smartphone application (**NitriSens app**) has been developed to create a user-friendly experience. It was a specially designed application for the purpose of quantitative estimation of NO_2^- in water samples. The app was programmed using Flutterflow software assisted by an in-built editor utilizing Dart language. The app development cycle has been briefly stated in **Figure 4.13** (step 1-10), as a flowchart, involving all crucial steps such as ideation and multi-iterative optimizations.



Figure 4.13. The ‘NitriSens’ software’s developmental cycle.

The NO_2^- app namely, ‘NitriSens’, was built over an android platform for developing a hand-held device where the calibration plot had already been fed, allowing real-time information of the NO_2^- value on a given real sample (**Figure 4.14**). The results are displayed with a caution message, stating the NO_2^- levels in the sample as ‘SAFE’ or ‘UNSAFE’, giving a hassle-free and easily understandable response output. The results can also be re-visited by the user in future by referring to the History section of the app post saving the particular entry (steps **(A-E)** briefly demonstrated in **Figure 4.14**). A drive link with all information pertaining to the app including the software code components can be accessed via a link mentioned below.

Google drive link here:

https://drive.google.com/drive/folders/1IRbZeTk1YBU_2Ec4KE16ZiG1n-ZqPriY

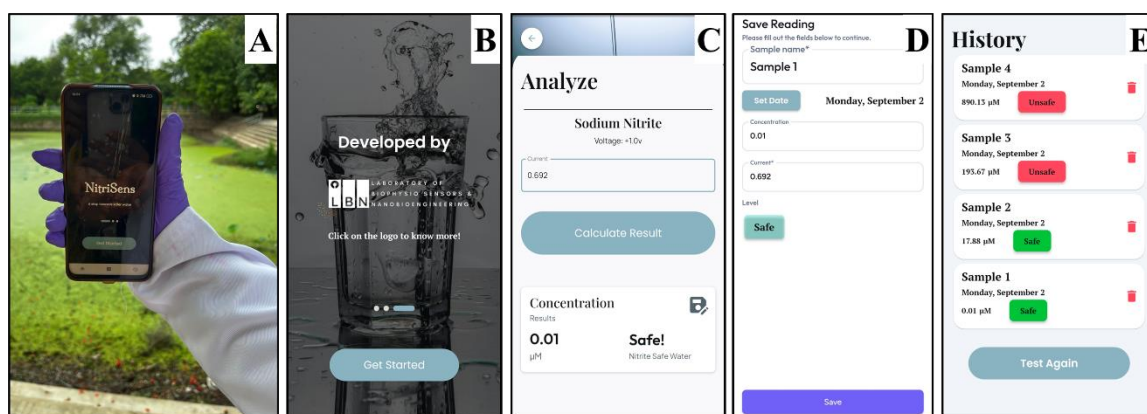


Figure 4.14. Brief demonstration of the software user-interface. (A) The software user interface (primary page); (B) Brief introduction (C) Analysis window where the current value is to be fed by the user, followed by clicking on 'Calculate result' button; (D) Display of results in form of quantitative NO_2^- estimation, with clear information about safe/unsafe NO_2^- levels in the test sample; (VI) Saved history of the results obtained, for future reference.

4. Conclusion

In this study, a novel approach was adopted to fabricate Co-MOF at a low potential in a short fabrication time, as low as 600 seconds. The developed Co-MOF was then assembled as a crucial component within a sensing matrix by stacking over a Ni-MOF surface. The surface was further conjugated with AuNP to engineer the final sensing probe as GCE/Ni-MOF/Co-MOF/AuNP. The sensor probe surface was studied extensively using a plethora of physical and electrochemical characterization methods ranging from SEM-EDX to XPS, followed by electrochemical validation studies. Then, the sensor analytics were established deploying CV, EIS, and chronoamperometry. The developed sensor is interesting attributed to its simple steps of fabrication, a wide LDR and LOD against NO_2^- , a lighting fast response time of <1.5 seconds, and commendable selectivity towards the analyte even in the presence of interfering species. This is the first report of its kind where such a stacked MOF-based platform has been deployed for NO_2^- sensing purpose along with a smartphone interface giving real-time qualitative and quantitative concentration outputs using only current as an input. Such a

handheld analytical platform could be a valuable asset for futuristic field-deployable sensing applications for a range of molecules, including NO_2^- , driving human and environmental well-being.

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S.No.	Fabricated probe surface	Technique used	Fabrication strategy	Number of wet chemistry steps involved	Total fabrication time	Fabrication temp	LDR	LOD	Response Time	Real Sample used	Ref.
1.	Ni-HHTP@MWCNTs	DPV	A composite comprising of HHTP, Ni(OAc) ₂ ·4H ₂ O and MWCNTs was prepared through the hydrothermal method which was later coated on a GCE surface to form the final sensing probe.	1	16 hours,	85 °C	1-10000 μM	0.95 μM	N.R.	Tap and river water	⁵⁰
2	CdO/SWCNTs/1-3-MBIB/CPE	CA, SWV	A mixture of ionic liquid binder, liquid paraffin, CdO/SWCNTs and pure graphite powder was prepared and homogenized. Then, it was packed within a glass tube to form the CdO/SWCNTs/1-3MBIB/CPE.	0	3 hours	RT	0.1-900 μM	0.07 μM	N.R.	Sausage, pickled vegetables	⁵¹
3.	GCE/MoS ₂ /AuNPs	CA	GCE/MoS ₂ /AuNPs were functionalized on a clean GCE surface. MoS ₂ was dropcasted initially on the GCE surface followed by electrodeposition of AuNPs.	0	>2 hours	RT	10-2100 μM	0.09 μM	N.R.	river and drinking water	⁴⁶
4.	Fc-ECG/SPE	DPV	Initially, Fc(Cys) ₂ was dissolved in acetonitrile. Similarly, Fc-ECG stock solution was prepared by dissolving in Milli-Q water. The sensor was prepared by	0	30 minutes	RT	1.0–50 $\mu\text{mol}\cdot\text{L}^{-1}$	0.3 $\mu\text{mol}\cdot\text{L}^{-1}$	>5 minutes	Pickle juice	⁵²

			dropcasting these materials onto SPE surface								
5.	Na/K doped carbon-nitride	LSV	A cleaned GCE electrode was dropcasted with calculated volumes of homogeneous solutions of Na/K-doped carbon nitride	3	8.5 hours	70 ° C followed by 550 ° C	10 μM to 2 mM	1.9 μM	N.R.	Water and food	¹³
6.	GO-CS-AuNPs/GCE	CA	The GCE surface was initially drop coated with a solution of CS-GO and allowed to dry at RT. It was followed by electrodeposition of the surface with AuNPs to form the final sensor probe.	2	>23 for CS-GO synthesis followed by 30 min. for AuNP deposition	80 °C for the hummer’s method	0.9 μM to 18.9 μM	0.3 μM	N.R.	N.R.	⁴
7.	Fe ₂ O ₃ /rGO	DPV	A dispersed mixture of Fe ₂ O ₃ /rGO composite was dropped onto GCE electrode surface and dried on RT to fabricate the final sensing surface.	2	>10.5 hrs	170 °C followed by 80 °C	5.0 × 10 ⁻⁸ to 7.8 × 10 ⁻⁴ M	1.5 × 10 ⁻⁸ M	N.R.	Tap water	²
8.	ERGOs/AuNP	DPV	SPCE was used as a WE. It was adorned with electrodeposited ERGO/AuNPs by scanning potential between 0 V to -1.5V for 15 cycles in a solution containing 1:1 v/v GO and [AuCl ₄] ⁻ solution.	0	>2 hrs	RT	1–6000 μM	0.13 μM	N.R.	Packaged drinking water, processed meats, aquatic products	⁴⁷
MOF -BASED NITRITE SENSORS											
9.	Pd/NH ₂ -MIL-101(Cr)	SWV and CV	SPCE was chosen as the working electrode which was dropcoated with Pd/NH ₂ -MIL-101(Cr) dispersion mixture.		>31 hours	160 °C for MOF synthesis followed by 200 °C for activation.	5 to 150 nM	1.3 nM	N.R.	Sausage and pickle samples.	¹⁵

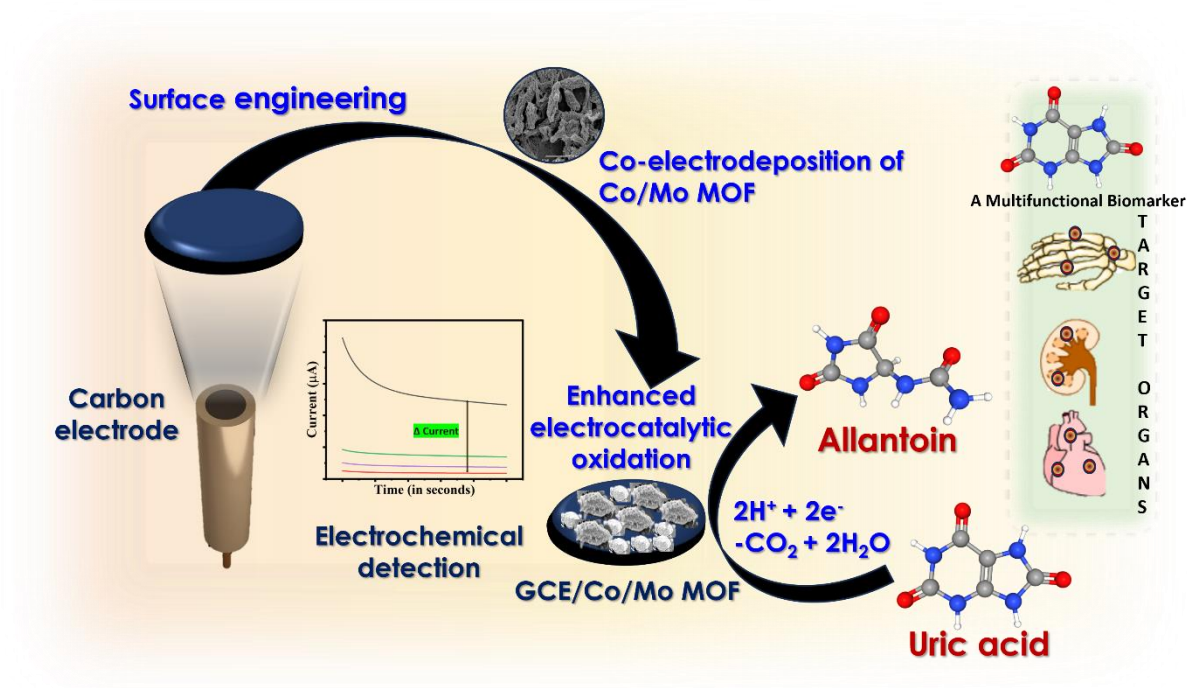
10.	Cu-MOF/Au	CA	A GCE working electrode was dropcoated with Cu-MOF aqueous suspension. It was followed by potentiostatic electrodeposition of AuNPs.	1	>32 hours	RT synthesis of Cu-MOF followed by drying at 80 °C	0.1–4000 and 4000–10000 μ M	82 nM	N.R.	River water	¹⁷
11.	GQD-PCN-222 (Zr-based MOF)	CA	Fluorine-doped tin oxide conducting glass substrates were chosen as the WE. GQD-PCN-222 thin films were prepared by dispersing initial sample pellets into acetone followed by dropcasting on the WE surface.	1	>7 days	Pellet drying at 80 °C	40–18 000 μ M	6.4 μ M	N.R.	N.R.	¹⁶
12.	Zr-MOF	CA	A fluorine doped tin oxide conducting glass substrate WE was dropped with a thin-film of MOF/Ag composite dispersed in acetone. The deposition procedure was iterated thrice to fabricate the final sensing probe.	2	>66 hours on material preparation followed by film deposition on substrate	Synthesis at 60 °C followed by post synthetic drying at 80 °C.	Upto 2.0 mM	9.1 μ M	N.R.	Tapwater, lake water, and rain water	⁵³
13.	Iridium-decorated UiO-66	CA	The nodes of the UiO-66 were introduced with iridium sites through solvothermal approach. Further, a thin film of the dispersed Ir-UiO-66 in acetone was casted onto the surface of FTO substrate to form the WE.	2	>20 hrs	Varying temperatures ranging from 25 °C to 130 °C	0 mM to 0.2 mM	0.41 μ M	60 s	N.R.	⁴⁹

14.	Cu-MOF/Au@Pt/GCE	CA	The prepared Cu-MOF was dispersed onto the surface on a GCE WE and dried under UV lamp. Further, the modified electrode was electrodeposited with Au@P NPs by CV to obtain the final WE.	2	>20 hrs for Cu-MOF preparation	RT	0.0001–12 mM	72 nM	5s	Ham sausage sample	⁴⁵
15.	NiPc-MOFs	DPV, CA	Ni phthalocyanine MOF synthesized through the solvothermal route was casted onto a GCE surface sealed with nafion.	1	>12 hrs for MOF-nanosheet synthesis	70 °C	0.01 mM to 11 500 mM	2.3 µM	6 s	Tap water	⁵⁴
16.	Ni-MOF/ Co-MOF/AuNPs	LSV, CA	<i>Ni-MOF and Co-MOFs were synthesized through the electrochemical approach and stacked over one another. The system was further functionalized with AuNPs.</i>	1	<i>1000 s for MOF deposition and 15 minutes for AuNP drying.</i>	RT	<i>0.01-1 µM to 10-1000 µM</i>	<i>4.2 ± 0.0003 nM</i>	<i><1.5 s for a range of concentrartions</i>	<i>Pond water, Tap water</i>	<i>Our work</i>

Abbreviations: MWCNTs: Multiwalled Carbon Nanotubes; DPV: Differential Pulse Voltammetry; CdO: Cadmium oxide; SWV: Square wave voltammetry; MoS₂: Molybdenum oxide; AuNPs: Gold nanoparticle; CA: chronoamperometry; SPE: Screen Printed Electrode; rGO: reduced Graphene oxide; CV: cyclic voltammetry; LSV: linear sweep voltammetry; GCE: Glassy carbon electrode

Chapter V

Engineering Cobalt/Molybdenum Bimetallic MOF for Ultra-swift Detection of Uric Acid



1. *Shubhangi et al.*, 2024, *IEEE Xplore*, 10.1109/BioSensors61405.2024.10712685
2. *Shubhangi et al.*, 2024, (under review)

1. Introduction

MOF, a class of self-assembled coordination complex-based porous materials, comprise of metal centre coordinated with organic linker moieties to form two or three-dimensional structures¹. They have been designated as materials with high specific surface area, tunable mesoporosity, diverse catalytic metal centers linked to organic ligands with good adsorption affinities and superior redox behaviour². These porous materials have found their utility in innumerable applications ranging from gas storage to catalysis and now to analytical applications like sensors and drug delivery systems³. However, the major challenges with working on these materials lies primarily with their wide band gap resulting into compromised electrochemical conductivity, heavily aggregated morphology and high resistance to diffusion¹. Such phenomena have usually been observed in MOFs synthesized through the conventional solvothermal route which are now being replaced by newer synthesis protocols involving microwave, sonochemical and electrochemical methods³. MOFs have been projected as plausible solutions towards replacing enzyme-based sensors due to their catalytic metal centers imparting them with electrocatalytic potential⁴. Co-based MOFs have been recently reported as sensitive electrode matrix components for detection of analytes like UA⁵.

Uric acid, a bio-compound also known as 2,6,8-trihydroxypurine, is often linked to various biophysiological pathways like purine (adenine and guanine) metabolism and decomposition. An abnormally high concentration or low concentration of UA is regarded as a causative factor for diseases like gout, hypertension, hyperuricemia, cardiovascular and chronic renal diseases, and oxidative stress. The clinical range of UA lies between 0.13-0.46 mM in blood serum and 1.49-4.46 mM in urine samples within healthy individuals. Therefore, a timely check on its physiological concentration holds enormous clinical significance in disease diagnosis. UA is routinely detected through enzyme-based assays or alternatively through spectrometry and chromatography-based methods⁶. However, electrochemical non-enzymatic methods hold

great promise for sensing UA due to faster response time, greater selectivity and cheaper operations. Although there always lies a scope for an alteration in sensor components which can improvise sensitivity and contribute towards realistic applications of the sensors.

In this work, we have attempted to fabricate a novel combination of Mo and Co-based bimetallic MOF within a sensor matrix for the selective and sensitive detection of UA through the electrochemical approach. The novelty lies in conjugation of two highly conductive metal centers conjugated with methyl imidazole linker moiety. The usual method of growing MOFs through the solvothermal approach followed by coating it onto a bare electrode surface is a complex operation which involves usage of binder components which may reduce the current response. We have tried to eliminate such problems by *in situ* growth of MOF onto the carbon electrode through a one-step electrodeposition procedure followed by testing it against our target analyte. The high conductivity and enormous surface area of the fabricated MOF matrix eliminates the need for further addition of successive conductive or catalytic components onto the surface, making it a simpler and effective solution for UA detection.

2. Materials and Methods

2.1. Chemicals and Instruments

Cobalt nitrate hexahydrate ($\text{Co}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$), Dimethyl formamide (DMF), 2-methyl imidazole (2-MeIM), Potassium ferricyanide ($\text{K}_3\text{Fe}(\text{CN})_6$), Potassium ferrocyanide ($\text{K}_4\text{Fe}(\text{CN})_6 \cdot 3\text{H}_2\text{O}$), Potassium chloride (KCl), Sodium chloride (NaCl), Sodium phosphate dibasic (Na_2HPO_4), Sodium phosphate monobasic (NaH_2PO_4), were procured from SRL research laboratory, India. Molybdenum trioxide (MoO_3) was procured from HiMedia Laboratories Pvt. Ltd. Distilled water (Milli-Q) (18 M Ω) was used throughout the experiment. The phosphate-buffered saline (PBS; 5 mM; pH 7.4) was prepared using a standard protocol.

2.2. Electrochemical Sensor Probe Characterization and Analytics

SEM and FTIR spectroscopy measurements were performed on JCM-6000 Plus BenchTop Sem Neoscope and Nicolet iS5, respectively availed at the Central Instrument Facility of IIT (BHU). All electrochemical operations required for facilitation of UA oxidation were performed on an electrochemical work station (Palm Sense BV, Houten, The Netherlands) connected to a three-electrode system constituting a bare glassy carbon electrode (GCE) as the working electrode, an Ag/AgCl as the reference electrode and, platinum wire as the counter electrode, respectively. CV was used for the electrochemical probe characterization and scan rate study by scanning between -0.2 V to +1.0 V vs. Ag/AgCl at a scan rate of 0.05 V/s in $[\text{Fe}(\text{CN})_6]^{3-}/[\text{Fe}(\text{CN})_6]^{4-}$ (ZoBell's) solution. ZoBell's solution was prepared through a pre-existing standard protocol⁷⁻⁹.

2.3. Preparation and preliminary physical characterization of the Co/Mo MOF probe

GCE surface was cleaned and pretreated as per the standard protocol¹⁰. A solution containing 40 mM $(\text{Co}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O})$, 40 mM MoO_3 , and 80 mM 2-MeIM were dissolved in 20 ml DMF and was stirred for 30 minutes to obtain the MOF precursor solution. The GCE electrode was then dipped into the precursor solution and scanned at a potential of -1.5 V for 500 seconds using chronoamperometry to deposit the Co/Mo MOF. The potential was optimised by performing experiments at varying potential points ranging from -0.4 V to -1.5 V. Similarly, the time for electrodeposition was optimised by performing the co-electrodeposition at fixed potential (-1.5 V) but at different time spans such as 100s, 200s, 300s, 500s and 700s. Post electrodeposition, the fabricated electrode was rinsed with Milli-Q water and cured for 10 minutes at RT to obtain the final sensing probe.

2.4. Physical and electrochemical characterization of the nanoengineered Co/Mo MOF sensing probe

The probe was then subjected to physical characterisations like SEM and FTIR to confirm the formation of the bimetallic Co/Mo MOF. To confirm the presence of functional groups and binding of target metal center with the linker molecule, FTIR analysis was also done. The probe was then subjected to electrochemical measurements to deduce its electron transfer capabilities, as discussed in the upcoming sections.

2.5. Real Sample Analysis

The real-life application of the developed nanoengineered bimetallic sensing probe GCE/Co/Mo MOF was validated on synthetic serum samples. For the effective validation study on detection of UA, a conventional spike and recovery model was used. The synthetic serum was equilibrated with PBS by 10 times dilution and then spiked with varying concentrations of UA. This is a vital step to maintain the molar strength of the real matrix when compared with the standards to prove the sensor's performance. The recorded output current was plotted and compared with the calibration curve obtained from an initial concentration-dependent study on UA in standard buffer solutions.

3. Results and Discussion

3.1. Electrochemical nanoengineering and characterization of the GCE/Co/ sensing probe

An optimized synthesis of AuND was done through a simple yet robust electroimprinting technique to electrodeposit the Co/Mo bimetallic MOF. It was then characterized physically as well as electrochemically to validate its formation. Various physical techniques were adopted at first stage to confirm the probe deposition of MOF layer such as SEM and FTIR analysis.

3.1.1. SEM and FTIR studies

The probe was subjected to physical characterisations like SEM and FTIR to confirm the formation of the bimetallic Co/Mo MOF, as seen in **Figure 5.1**. Below the magnification of 5 μm , spindle-shaped particles were seen evenly distributed on the entire topography of the electrode flanked with 2D spherical structures suggestive of desirable MOF-like patterns (**Figure 5.1-I**). To confirm the presence of functional groups and binding of target metal center with the linker molecule, FTIR analysis was also done. Characteristic peaks corresponding to 2-MeIM moiety were seen between 690 to 1631 cm^{-1} suggesting its successful incorporation with the metal centers (**Figure 5.1-II**).

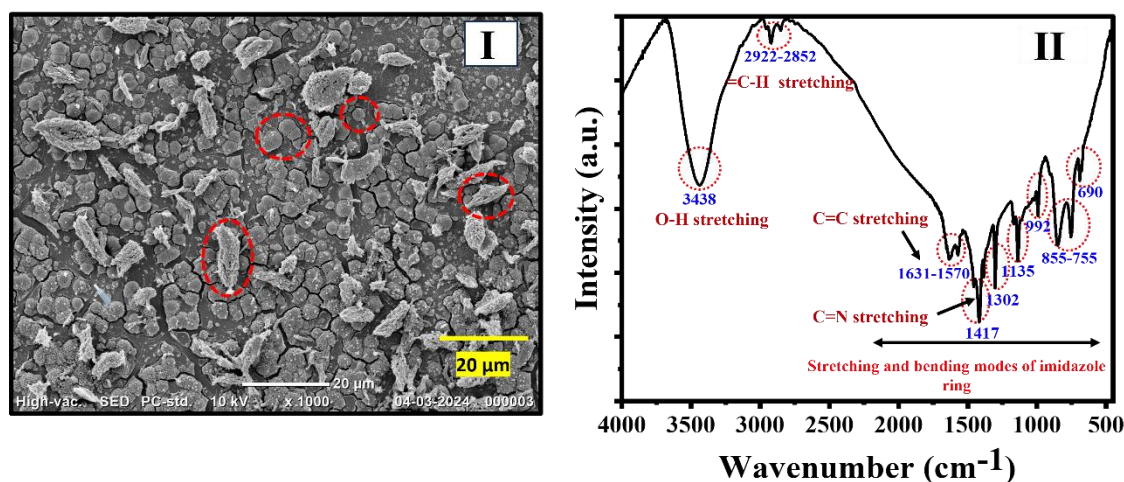


Figure 5.1. (I) SEM micrograph of final fabricated electrode surface, i.e., Co/Mo MOF where red circles show spherical as well as spindle-shaped morphology of developed MOF particles. (II) FTIR spectrum of electrode/Co/Mo MOF.

3.1.2. Electrochemical characterization of GCE/ Co/Mo MOF sensing probe

The developed final nanoengineered sensing surface GCE/Co/Mo MOF was electrochemically characterized through the CV measurements. Initially, the unmodified electrode surface was scanned in ZoBell's solution to obtain a characteristic peak of the ($\text{K}_3[\text{Fe}(\text{CN})_6]/\text{K}_4[\text{Fe}(\text{CN})_6]$) redox couple at +0.4V. The operations were performed sequentially for both unmodified and modified GCE surfaces i.e., GCE, GCE/ Co/Mo MOF. At the first step, CV was performed in a potential range of -0.2 V to +1.0 V vs. Ag/AgCl with a scan rate of 50 mV/s in a 5 mM

ZoBell's solution. **Figure 5.2-I** represents the CV signals for both surfaces where GCE is denoted through a black curve and GCE/Co/Mo MOF is represented by a red curve. Further the anodic and cathodic peaks currents have been represented through histogram in **Figure 5.2-I (inset)**. The final probe's surface charge transfer characteristics via quantification of the effective surface area was done using the Randles-Sevcik's equation

$$I_p = (2.69 \times 10^5) n^{3/2} A C D^{1/2} v^{1/2} \dots\dots\dots \text{Equation i}$$

where, I_p stands for the highest current output (in amperes), n denotes the no. of electrons involved in redox reaction (here $n = 1$), A stands for the electrodes' effective surface area (in cm^2), C is the electroactive species' concentration (in mole cm^{-3}), D demarcates the diffusion coefficient (in $\text{cm}^2 \text{s}^{-1}$) with its value being constant, i.e., $7.6 \times 10^{-6} \text{ cm}^2 \text{s}^{-1}$ for aqueous ferrocyanide, and v denotes the scan rate (in Vs^{-1}).

The effective surface area of GCE and GCE/Co/Mo MOF was found to be $6.15 \times 10^{-5} \text{ cm}^2$ and $9.1 \times 10^{-5} \text{ cm}^2$, respectively. There was an increase in the effective surface area of the probe by 1.48 times post modification with the bimetallic MOF component. Additionally, scan rate study for the final modified surface was performed to understand the mobility of ions and its stability by scanning within a potential window of -0.2 V and +1.0 V using CV with a varying scan rate (10-100 mV/s). The peak currents obtained at each scan rate were further plotted with current against the square root of scan rate in **Figure 5.2-II**, with the linear coefficients as 0.91 and 0.98 for the anodic and cathodic currents, respectively. The plotting results demonstrate a linear behaviour of the curve which clearly suggests that the electrode/electrolyte interface is a diffusion-controlled process. The linearity obtained in this study also confirms the stable fabrication of final sensing surface (**Figure 5.2-II-inset**).

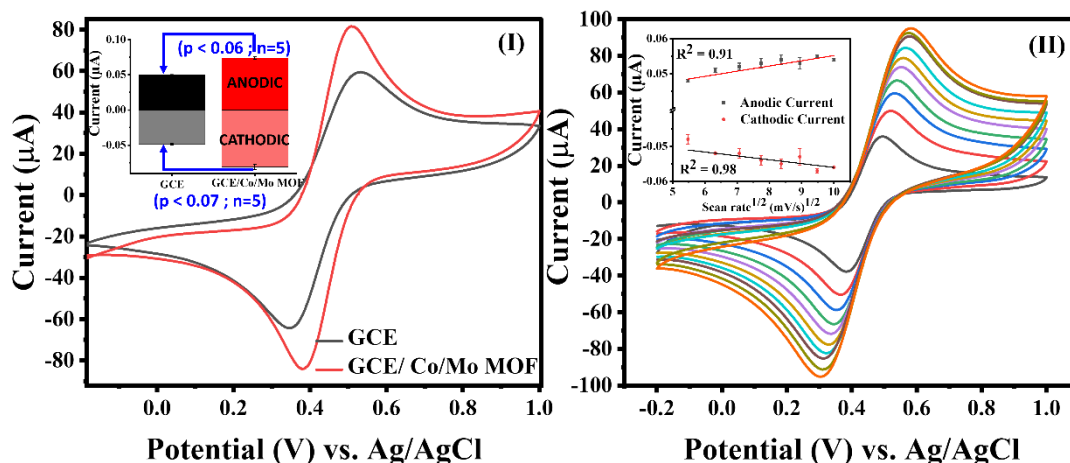


Figure 5.2. (I) CV response of the modified surface at a scan rate of 50 mV/s in 5 mM ZoBell's solution (I-inset) Histogram representing the anodic and cathodic peak currents of the modified probe surface. (II) CV response of the final sensing probe GCE/Co/Mo MOF with varying scan rates (10-100 mV/s) in 5 mM ZoBell's solution (II-inset) Linear fit of the anodic and cathodic current vs. square root of scan rate.

MOFs have always been known to hamper the conductivity of the sensor probe but in our case, interestingly, when the GCE surface was electrochemically deposited with Co/Mo bimetallic MOF through the novel route, a rise in conductivity was noted. Such electrodeposited MOF-dependent conductivity enhancement has been also observed earlier for different MOFs¹¹. The great charge conducting capabilities of the nanoimprinted probe illuminates its enormous possibility in futures sensing applications.

3.2. Analytical performance of GCE/ Co/Mo MOF sensing probe

A set of comparative and control experiments were conducted to undermine the role of the MOF layer in the catalytic oxidation of UA. The obtained results can be seen through the generated data below. In the first set of control experiment, the data was generated for both unmodified and MOF-modified electrode surfaces against 20 mM UA concentration through LSV, by scanning between the potential window off +0.1 to +0.9 V vs. Ag/AgCl (**Figure 5.3-A**). Since UA is an electroactive molecule, a distinct peak was obtained at around +0.65 V vs. Ag/AgCl for 20 mM UA, even in the case of a bare electrode surface, which gets shifted to a

lower potential (+0.55 V) generating almost a double current response when oxidized by the MOF-modified probe.

Therefore, this engineered nanohybrid sensor probe brings out the inherent characteristics of MOF metal center (Co and Mo) for deployment in UA sensing application which may lead to its ultrasensitive detection without the need for more components in the sensing matrix.

The efficacy of the electrochemically developed sensing probe, GCE/ Co/Mo was analysed for its application by utilizing UA as a test molecule. The GCE/ Co/Mo nanoengineered sensing probe was subjected to a series of control experiments to establish that the increment in current magnitude with increase in potential was a resultant of direct and precise electron transfer with UA. In the first control experiment, a study was conducted at relatively higher concentrations of UA i.e., in 0.1 M to 0.5 M UA. In case of the blank reading, negligible intensity UA peak was seen, however, subsequently, current rose with increasing UA concentrations between 0.1 to 0.5 M (**Figure 5.3-C**). The curve shows a linear trend signifying concentration dependent signal, with a correlation coefficient of 0.98 (**Figure 5.3-D**). The regression equation obtained from curve is as follows: $\Delta I (\mu A) = -8.277 (\pm 3.453) + 2.757 (\pm 0.167) \times \text{Conc. [UA (M)]}$.

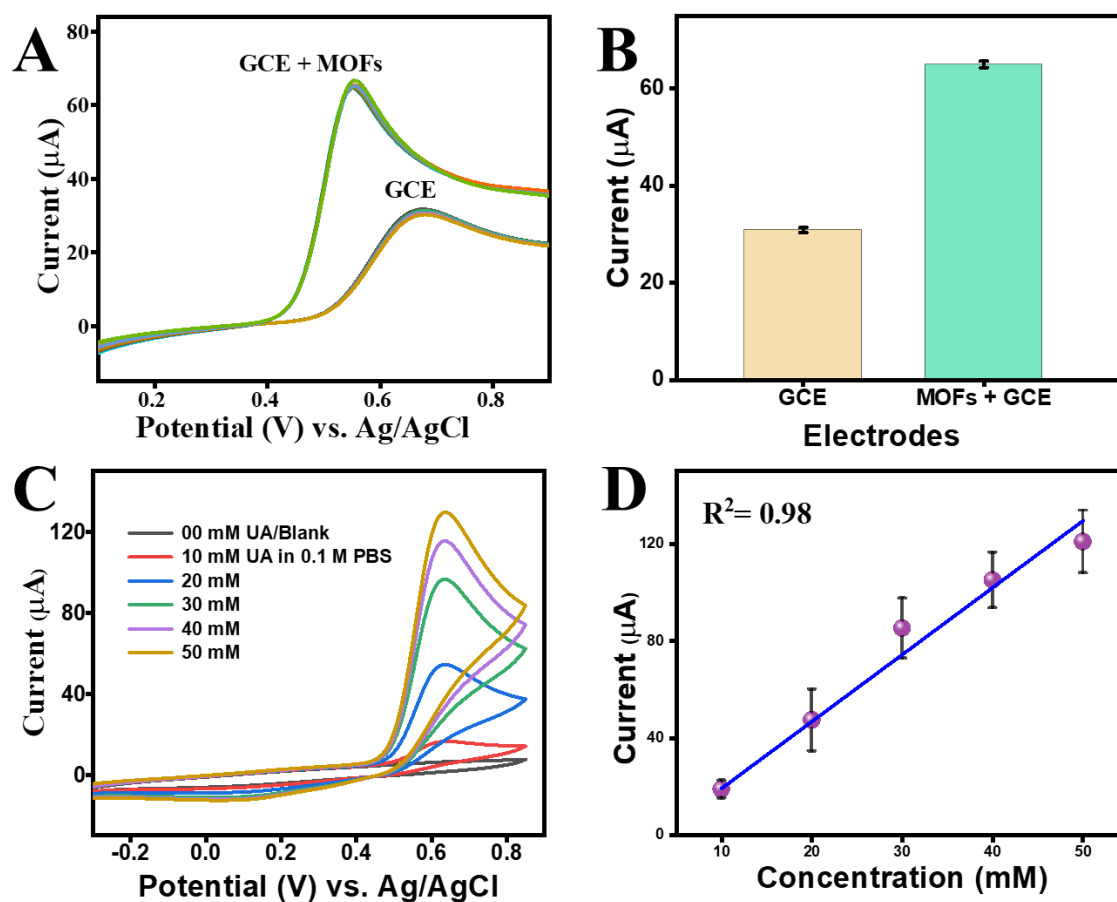


Figure 5.3. (A) A comparative LSV curve corresponding to unmodified and modified (GCE/Co/Mo MOF) surface against PBS and 0.2 M UA dissolved in PBS. (B) Corresponding histogram generated from the layer-by-layer study, plotting the cathodic current response generated by the LSV curve measurements. (C) Concentration-dependent control analysis of GCE/Co/Mo probe in PBS (black curve), and in 0.1 M (red curve), 0.2 M (blue curve) and 0.3 M (green curve), 0.4 M (light purple curve) and 0.5 M (yellow curve) UA to validate that peak is exclusively due to UA; (D) Corresponding calibration curve at varying concentrations ranging from 0.1 M to 0.5 M UA in PBS with correlation coefficients of 0.98.

In the second control test, the charge transfer behaviour and stability of the analyte towards the GCE/Co/Mo bimetallic probe was analysed. It comprised of scan rate dependent study through an alteration in the scan rates between 10 and 100 mV/s in the presence of 10 mM UA (Figure 5.4A). With an increment in scan rates, the cathodic peak currents also increased linearly. The engineered nanohybrid probe demonstrates diffusion-control charge transfer behaviour and excellent surface stability as the cathodic peak current was found to be proportional to the square root of scan rate possessing a correlation coefficient of 0.94 (Figure

5.4B). Therefore, both the control studies inferred that the designed GCE/Co/Mo engineered sensing probe is highly stable and can effectually sense UA.

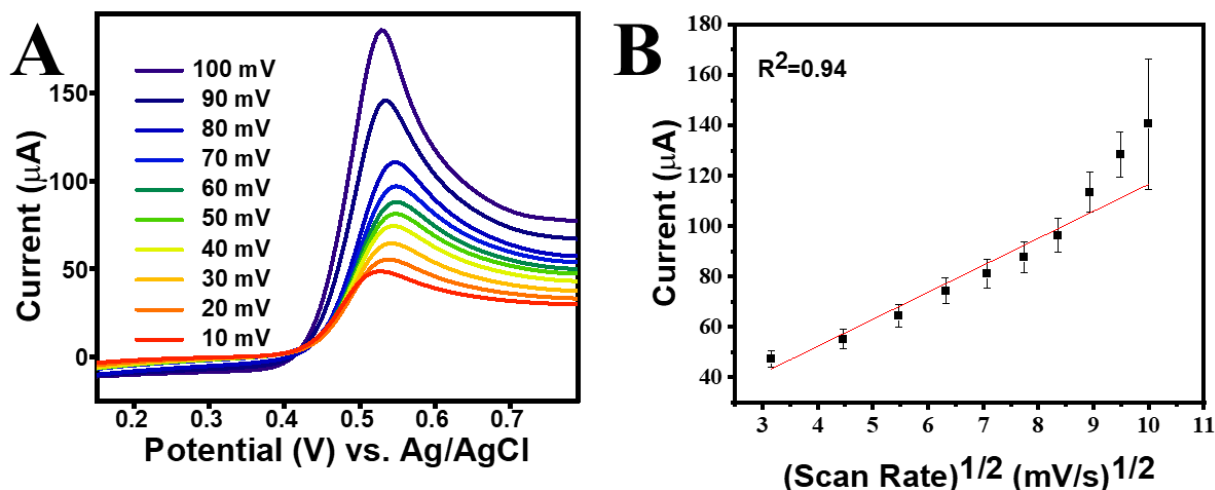


Figure 5.4. (A) Scan rate dependent analysis of GCE/Co/Mo bimetallic engineered probe in 0.1M UA at a scan rate between 10 and 100 mV/s; As scan speeds increased, peak current rose with a small shift to the negative side; (B) corresponding current at different scan rates showed linearity with a correlation coefficient of 0.94.

The sensing capabilities of the probe were further investigated using DPV to find the minimal concentration since DPV is considered as a highly sensitive technique. The GCE/Co/Mo probe was evaluated for varying concentrations of UA and the signal output was found to be increasing with an increase in UA levels (**Figure 5.5A**). On the basis of the amperometry data, the calibration curve showcased a linearity within the concentration range of 1×10^{-2} to 1×10^{-9} M (**Figure 5.5B**). The LOD of UA was calculated as $0.34 (\pm 0.05) \times 10^{-10}$ M using **equation ii**

$$\begin{aligned}
 LOD &= \frac{3SD_b}{Slope} = \frac{3SD_b}{\frac{dy}{dx}} = \frac{3SD_b}{\frac{dy}{d\ln x} \times \frac{d\ln x}{dx}} = \frac{3SD_b}{\frac{dy}{2.303(d\log x)} \times \frac{1}{x}} = \frac{3 \times 2.303 \times SD_b \times x}{\frac{dy}{d\log x}} \\
 &= \frac{3 \times 2.303 \times SD_b \times x}{Slope \text{ of semilog plot}}
 \end{aligned}$$

.....Equation ii

where σ_b represents the blank sample's standard deviation, and m is the calibration plot's slope

The linear increment in DPV current responses with rise in UA concentrations in between 1×10^{-7} to 1×10^{-9} M can be summed up through the regression equations shown below:

Regression equation 1: For lower concentration range

$$I (\mu\text{A}) = 6.447 (\pm 1.047) + 0.660 (\pm 0.117) \times [\log \text{Conc. of UA (M)}].$$

Regression equation 2: For higher concentration range

$$I (\mu\text{A}) = 46.116 (\pm 2.561) + 8.645 (\pm 0.791) \times [\log \text{Conc. of UA (M)}].$$

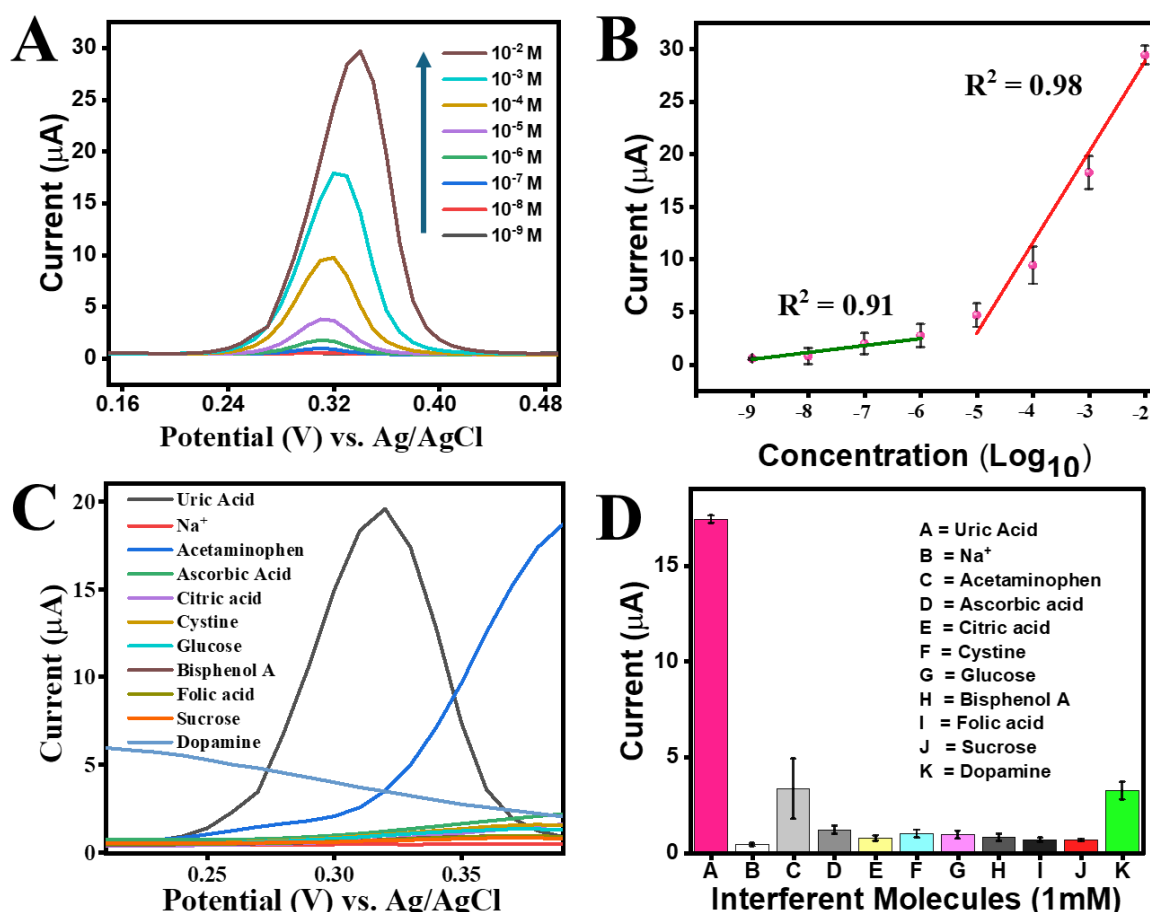


Figure 5.5. (A) The DPV response of GCE/Co/Mo bimetallic MOF-based sensor probe in the range of 1×10^{-2} to 1×10^{-9} M UA in PBS to find the minimal concentration of UA; (B) Corresponding calibration curve of varying concentrations of UA in PBS with a correlation coefficient of 0.94 for 10^{-7} to 10^{-9} M UA as 0.91 and 10^{-2} to 10^{-5} M UA is 0.98, respectively; (C) Interference analysis study (D) Interference study histogram.

3.3. Selectivity assay

The commercial applications of the developed GCE/Co/Mo nano-engineered probe requires proving its selectivity towards the specific target analyte present amongst many co-existing interferant molecules within a complex matrix. Therefore, we chose various co-existing molecules such as sodium ions (Na^+), acetaminophen, ascorbic acid, citric acid, cysteine, glucose, bisphenol A, folic acid, sucrose, dopamine along with our target analyte UA, to examine nanohybrid sensing probe's selectivity. **Figure 5.5-C** highlights the signal response of the co-existing molecules which displayed minimal signals at around +0.32 V vs. Ag/AgCl. **equation iii** was used to determine the coefficient of selectivity (K_{sel}) of the co-existing molecules, with their respective values stated in **Table 5.1**. The values were found to be significantly low ($K_{\text{sel.}} \ll 1$), with p-value <0.02 emphasizing good selectivity of the developed GCE/Co/Mo bimetallic MOF probe for UA analysis.

$$K_{\text{sel.}} = (\text{Signal})_{\text{Interfering molecules}} / (\text{Signal})_{\text{UA}} \quad \dots \text{Equation iii}$$

where $K_{\text{sel.}}$ denotes the coefficient of selectivity, $(\text{Signal})_{\text{interfering molecules}}$ are the current corresponding to interferants, and $(\text{Signal})_{\text{H}_2\text{O}_2}$ is the response generated by the nanoprobe after being treated with UA.

Co-existing molecules	Current Signal (μA)	$K_{\text{sel.}}$
<i>Na^+</i>	0.409	0.023
<i>Acetaminophen</i>	3.324	0.190
<i>Ascorbic Acid</i>	1.183	0.067
<i>Cysteine</i>	0.978	0.056
<i>Citric Acid</i>	0.743	0.042
<i>Glucose</i>	0.925	0.053
<i>Bisphenol A</i>	0.784	0.044
<i>Folic Acid</i>	0.409	0.023
<i>Dopamine</i>	3.228	0.185
<i>Uric Acid</i>	17.423	1

Table 5.1. Coefficient of selectivity (K_{sel}) of the co-existing interferant molecules

No significant response was recorded for the co-existing interferants in our experiments due to the electrochemical passivity of developed GCE/Co/Mo probe towards these molecules. Another reason could be the analytical potential range, which forbidden other electroactive species from experiencing the redox reaction.

4. Conclusion

We have developed a bimetallic MOF based nanocomposite (GCE/Co/Mo MOF) through the electrochemical route for the electro-catalytic oxidation and thereby sensing of UA. Co/Mo-MOF was electrochemically deposited on the GCE surface using a one-step approach where a stable catalytic layer suffices the need for UA detection. Physical characterization tools like SEM and FTIR were adopted for confirming the formation of the target MOF. These methods were also supplemented by electrochemical techniques such as CV, DPV and CA to characterize as well as understand the analytical potential of developed probe. The synergistic effect of Co/Mo MOF results in an electroconductive surface facilitating enhanced oxidation of the target analyte. The developed nanocomposite was used to detect UA concentration electrochemically in the known clinical ranges. The future prospects could also be integration of the proposed matrix in wearable and patch-based sensors which would however require advance analyses involving the toxicity aspects of these materials. This is a preliminary study which may further help in exploring applicability of such MOFs in integration with other catalytic surfaces for the detection of a range of clinically significant analytes.

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Chapter VI

Conclusion and Future Prospects

1. Major shortcomings of MOFs in sensor development

Despite their high specific surface area and high porosity, the traditional MOFs possess drawbacks when used as opto-electrochemical sensors. Here, some of the challenges related to MOFs and new approaches to improve their performance in commercial prospects are discussed as follows.

I) Low electrical conductivity: The potential of conventional MOFs as electrode materials in electrochemical sensors for practical applications is severely constrained by their weak electrical conductivity and low electron carrier mobility. To address this issue, various approaches and tactics have been used to generate MOF materials with remarkable electrical conductivity and high carrier mobility^{1,2}. For example, to create conductive MOF materials, MOF-based nanocomposites have been produced with carbon-based materials (graphene, carbon dots, SWNTs, etc.) and various metallic/metal oxide nanostructures (AuNPs, AgNPs, Fe₃O₄, CuO, etc.) to make novel nanohybrid systems³⁻⁵. In addition, redox-active linkers, conductive metal nodes, and conducting molecules are doped or encapsulated with the MOFs⁶.

II) Poor analyte specificity: The selectivity for a single analyte is lacking in conventional MOF-based sensors, irrespective of the transduction mechanism. However, several approaches have been recently developed to boost the selectivity of MOFs for the recognition of specific analytes. These include the development of functional MOFs or combining MOFs with specific recognition molecules such as aptamers, antibodies, enzymes, molecularly imprinted polymers (MIP), and bioconjugation pairs⁷⁻⁹. Such conjugative fabrication strategies can be adopted to enhance MOF-based sensor specificity towards analytes.

III) Instability of MOFs: Although many MOFs contain divalent metals had outstanding porosity, their instability prevents them from being used in extreme conditions with moisture, water, or that in the acidic or basic media. As a result, the stability issue is now being addressed by the scientific community to produce more robust framework structures so as to enable

further use and commercialisation of such developed sensors. The catalytic roles of MOFs in a sensing matrix are often hampered by chemical and thermal instability along with blockage of metal sites by the linkers¹⁰. Most of the MOFs use carboxylate linkers for the framework structures which are usually considered hard bases. So, instead of using divalent metal ions, usage of high-valence metal ions results in the formation of stable MOFs. These MOFs have proven to show exceptional stability in highly humid environments as well as in extreme pH conditions^{11,12}. In addition to this, adding hydrophilic groups to the organic linkers may also enhance the structural integrity of MOFs.

IV) Toxicity and lack of biocompatibility of MOFs: For most of the conventional MOF materials, the synthesis pathways include toxic solvent systems such as DMF due to the compromised solubility of the precursors. These render to the low biocompatibility of the MOF-based materials. However, in recent times greener synthetic approaches are being adopted where biocompatible solvents are being used or solvent-less synthesis is being performed as already discussed in the **Section 3**. Additionally, the modulation of the MOF size into nano-MOF (nMOF) has been proven to drastically decrease the cytotoxicity of MOF making them suitable for biosensor applications¹³.

V) Low limit of detection: The major hindrance towards commercialization of MOF-based biosensors lies in their poor LOD which makes them commercially non-viable. For surfaces involving MOF as a sensor matrix component, high LOD is unsuitable for some analytes existing in trace concentrations within real samples¹⁴. This thereby defies the sole purpose of sensor development where most nanomaterial-based advanced commercial sensors work in the range of picomolar and femtomolar concentrations. The remedial approach would be development nanohybrids comprising of MOFs and high-performance nanostructures that assist in improved electron transfer at the probe-analyte interface.

VI) Processability: Being an overlooked problem in MOFs, this arises due to the inherent nature of powdered reticular materials that restricts their application in unprocessed form at bulk¹⁵. The steps involved in MOF processing and thereby application-based commercial synthesis complexities makes its usage less viable in upscaling of these materials.

2. Conclusions and Future Prospects

Phenomenal progress has been seen recently in the field of biomolecular detection using MOFs. They have been profoundly integrated in detection systems for their usage in different transducer environments ranging from mechanical, optical, electrochemical to opto-electrochemical hybrids.

The **first chapter** highlights the literature review on introduction of biosensors, its types, different transduction systems and the role of nanomaterials. It then converges to our material of interest, i.e., MOFs where we discuss about different unique properties ranging from mechanical to catalytic to opto-electronic ones. Then, the different methods that can be employed for their synthesis have been discussed such as dry-gel conversion, diffusion, electrochemical, solvothermal, mechanochemical, microwave-assisted, sonochemical and ionothermal synthesis. Based on various literatures, it has been found for all above listed methods that each one has certain restricted usage with some of them involving high energy consumption, unconventional equipment or lengthy reaction period. From some methods it was inferred that use of unwanted anions, with the introduction of metal salts, makes the process control cumbersome and increases cost of production considerably. For methods like solvothermal the advantage lies in rendering chemical and structural stability along with morphological symmetry to the developed MOF structure whereas, the challenge is in optimizing of temperature conditions for desirable morphological characteristics. Methods like electrochemical synthesis are a fast, clean, cost-effective method favourable for fabrication of films rather than particles with an associated advantage of lesser errors since are devoid of

human interventions. However, it can be concluded that the choice of method for MOF fabrication entirely depends upon one's choice of material and its further applicability. We have elaborately discussed on several biosensing strategies involving MOFs-based sensing matrices for detection of free radicals, metal ions, small and macro molecules and cells.

MOFs, though not being good conductors, are still extensively being used in electrochemical sensing methods due to their other merits like high surface area which imparts greater chelation with metal ions like Au^{3+} , Pd^{2+} . These metals not only improve their conductivity rather also widen their usage as nanozymes or catalysts. More research should be promoted in this field on doping MOFs with electroactive species to widen their applicability in this area. MOFs involving d-block metal centres like Cu and Co have been widely used in such case without the need for extensive doping too due to their inherent catalytic properties. This opens new window for label-free biosensors with excellent repeatability and low LOD values. The luminescence characteristics of MOFs can also be accredited to the excitation of direct organic ligands causing ligand-to-ligand charge transfer (LLCT). Lanthanum-based MOFs involving mostly Eu^{3+} are now trending as MOFs hence such systems can be implemented fluorescence-based diagnostics in future. MOFs have also been known to possess commendable ECL activity with distinct emission peaks. The luminescence can be generated by the metal center (antenna effect) where a ligand-to-metal or vice-versa charge transfer can lead to luminescence. Emission MOFs need to be researched more for their usage as theranostic agents with improvement in their *in vivo* compatibility. Future work should be directed towards exploring and engineering MOF platforms for attachment of functional molecules which can act as moieties for macromolecular sensing without using sophisticated and multistep functionalization methods. Additional efforts are also required for use of these systems in miniaturized platforms and point-of-care diagnostics.

The **second segment of the chapter** talks about the evolutionary progress that has been reported in recent times for detection of biomarkers using MOFs. They have been explored vividly and integrated strategically in sensing matrices within a range of transduction environments. The article focusses only over the electrochemical transduction systems on MOF-based disease diagnosis tools where the segregation of study is primarily done on the basis of multiple diseases. Each disease segment has been briefed with recent advances in MOF-based probe fabrication strategies involving elaborate discussions on sensor fabrication and sensing parameters. It has been found that the selection of MOFs in specific sensing applications relies primarily on the suitability of the metal center. Ni, Co and Cu based MOFs have been opted more in recent past due to their proven catalytic potential and electroactive properties. Bimetallic MOFs and their use in sensor matrices are evolving as a new prospect where relatively simpler probe fabrication can generate enhanced signal amplification which was being previously achieved through incorporation of multiple matrix layers. Ni-Co bimetallic MOFs are the prominent ones in the category, however, more research in the field of other bimetallic MOFs with different metal centers can further be developed. MOFs, though being not very good conductors, are enormously being used in electrochemical transduction matrices by virtue of their high surface area which endows them with greater chelation ability with other metal ions. An attempt towards bimetallic MOFs and their further doping with electroactive species can be another exciting area for research. The authors ponder if trimetallic MOFs could also be an option in near future. Also, a range of MOF synthesis approaches discussed in all segments of the article are suggestive of a notion that solvothermal still remains the most adopted method. However, its major challenge would still be elimination of toxic synthetic solvents like DMF and methanol by the greener ones, decreasing dependency on high temperature conditions. Sustainable MOF synthesis methods like microwave-assisted or dry-gel conversion methods are rare in reports when it comes to development of biosensors. Label-

free detection is another promising aspect of MOFs that is being heavily explored. Direct functionalization of MOF surface with target specific molecules could envision future work for development of miniaturized platforms and point-of-care diagnostics. However, the commercial bottleneck would still be the development of envisioned MOF-based POCTs due to challenges arising from high cost, scattered literature and knowledge gap on sustainable MOF development strategies. The major drawbacks in the commercial prospects of these materials are compromised conductivity, poor analyte specificity, instability in a range of solvents, toxicity leading to poor biocompatibility and low detection limits which need to be tackled. For diseases included in the article, a lot of progress has been done which have been tabulated in each section. The future work could be directed towards similar diagnostic solutions for other disease categories too like pulmonary disorders which the authors could not find much reports on.

In the **second** chapter, a novel 3D AuND/Ni-MOF/Hyd nanohybrid sensor surface was developed which was precisely directed towards the non-enzymatic sensing of hydrogen peroxide. The electrodeposition variables were keenly optimized for construction of the matrix with superior analytical performance. The addition of successive layers developed into an interesting hybrid module with enormous surface area and charge transfer characteristics favoring sensing applications. The nanohybrid sensing probe displayed a commendable response time of 5.02 ± 0.42 seconds, attributing to its excellent sensitivity towards H_2O_2 determination within the detection range of 1×10^{-8} and 1×10^{-15} M and a LOD of $0.34 (\pm 0.05) \times 10^{-15}$ M.

The **third** chapter focusses on the development of a novel sandwich sensing probe for monitoring a physiologically significant vitamin (FA) through electrochemical route. The sandwich probe was fabricated by optimization of process parameters to replace existing tedious MOF synthesis process by a simpler electrochemical approach, followed by its

conjugation with Au nanostructures above and beneath it. The calibration study yielded a linear slope within a FA concentration range of 1×10^{-11} and 1×10^{-3} M and a LOD of $0.48 \pm 0.02 \times 10^{-11}$ M. The engineered sensor probe displayed a commendable response time of less than 2.1 seconds, attributing to its excellent sensitivity towards FA determination.

In the **next** study (chapter 4), a novel approach was adopted to fabricate Co-MOF at a low potential in a short fabrication time, as low as 600 seconds. The developed Co-MOF was then assembled as a crucial component within a sensing matrix by stacking over a Ni-MOF surface. The surface was further conjugated with AuNP to engineer the final sensing probe as GCE/Ni-MOF/Co-MOF/AuNP. This is the first report of its kind where such a stacked MOF-based platform has been deployed for NO_2^- sensing purpose along with a smartphone interface giving real-time qualitative and quantitative concentration outputs using only current as an input.

The **last** chapter discloses the method for development of a bimetallic MOF based nanocomposite (GCE/Co/Mo MOF) through the electrochemical route for the electro-catalytic oxidation and thereby sensing of UA. The sensor was capable of detecting UA in the detection range of 1×10^{-2} to 1×10^{-9} M. The future prospects of the study could also be integration of the proposed matrix in wearable and patch-based sensors which would however require advance analyses involving the toxicity aspects of these materials.

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Annexure

Annexure 1

List of publications from thesis and associated works

1. **Shubhangi**, Rohini Kumari, S.K. Rai, and **Pranjal Chandra***, *Electrochemical Assembly of Nickel Metal Organic Framework-Decorated Nanoimprinted Gold Dendrites as Peroxidase Mimic for High-Performance Hydrogen Peroxide Sensing*, *ACS Appl. Nano Mater.* 2024, 7, 1, 1388–1401.
2. **Shubhangi**, Indrani Nandi, S.K. Rai, **Pranjal Chandra***, *MOF-based nanocomposites as transduction matrices for optical and electrochemical sensing*, *Talanta*, 2024, 266 (2), 125124.
3. **Shubhangi**, Ruchita Chaudhari, S.K. Rai, **Pranjal Chandra***, *Engineered Cobalt/Molybdenum Bimetallic MOF as Electrochemical Signal Transducer for Uric Acid Detection*, 2024, *IEEE BioSensors Conference (BioSensors)*, Cambridge, United Kingdom, 2024, pp. 1-4, doi: 10.1109/BioSensors61405.2024.10712685.
4. **Shubhangi**, Divya, S.K. Rai, **Pranjal Chandra***, *Shifting Paradigm in Electrochemical Biosensing Matrices Comprising Metal Organic Frameworks and their Composites in Disease Diagnosis*, *WIREs Nanomedicine and Nanobiotechnology*, 2024; 16:e1980. <https://doi.org/10.1002/wnan.1980>.
5. **Shubhangi**, Rohini Kumari, Kajal Kacchawaha, Sumit K Singh, S.K. Rai, **Pranjal Chandra***, *Pristine Ni-MOF sandwiched between 1D and 3D engineered Au sensor for in vitro Folic acid sensing*, 2024, (*ACS Anal. Chem.: In Press*; <https://doi.org/10.1021/acs.analchem.4c03564>).
6. **Shubhangi**, Rohini Kumari, Arkya Jyoti Ghosh, S.K. Rai, **Pranjal Chandra***, *Layering pristine Ni and Co MOFs to develop transduction matrix for sensing Sodium Nitrite in water samples*, 2024.(under revision).
7. **Shubhangi**, Ratul Paul, S.K. Rai, **Pranjal Chandra***, *Bimetallic MOFs as trend-shifters in electrochemical sensing modules*, 2024. (manuscript under preparation).
8. **Shubhangi**, Niharika, Smridhi, Bhadra, **Pranjal Chandra***, *Deciphering the functional potential of dopants in 2D material matrix in sensors*, 2024. (manuscript under preparation).
9. **Shubhangi**, Amisha Kushwaha, Ratul Paul, **Pranjal Chandra***, *Engineering Cobalt/Molybdenum Bimetallic MOF for Ultraswift detection of Uric Acid*, 2024, (under Review).

10. **Shubhangi***, Marshal Dhayal, Substrate assisted formation of ZIF-8 crystals for tunable size and morphological characteristics: Investigating the role of temperature, method and solvent on the size of ZIF-8 crystals, In: Kumar, V., Dubey, B.K., D. Yadav, K. (eds) *Technological Advancements in Waste Management: Challenges and Opportunities*. TAWMCO 2023. *Lecture Notes in Civil Engineering*, vol 542. **Springer, Singapore**. https://doi.org/10.1007/978-981-97-6024-4_22.
11. Rohini Kumari, **Shubhangi**, Daphika S Dkhar, **Pranjal Chandra***, Metallic Dendrites: How Far Can We Go?, *ACS Anal. Chem.*, 2024 (submitted).
12. Divya, **Shubhangi**, **Pranjal Chandra***, Electrochemical Synthesis of Pristine MOF for High Performance Sensing of Caffeine in Beverages, 2024. (under review).
13. Divya, **Shubhangi**, **Pranjal Chandra***, Electrochemical Synthesis of Engineered Bimetallic Pristine MOF-MoS₂ Nanocomposite for Sensing Application, 2024, **IEEE BioSensors Conference (BioSensors)**, Cambridge, United Kingdom, 2024, pp. 01-04, doi: 10.1109/BioSensors61405.2024.10712708.
14. Ankur Singh, **Shubhangi**, **Pranjal Chandra***, Molecularly Imprinted Polymers as Emerging Engineered Platforms for Precision Molecular Sensing (under review).
15. Marshal Dhayal, **Shubhangi**, Juhi Jaiswal, Electrodeposited folic acid platinum electrode for rapid detection of human hepatocellular carcinoma cells, *Electroanalysis*, 2023, e202200482 (<https://doi.org/10.1002/elan.202200482>).
16. Rohini Kumari, Aditi Sammi, **Shubhangi**, Ananya Srivastava, U P Azad, **Pranjal Chandra***, Emerging 3D Nanomaterials as Electrocatalysts for Water-Splitting Reactions, *International Journal of Hydrogen Energy*, 2024, 74, 214-231.

Patent during thesis

Authors: Pranjal Chandra, **Shubhangi**, Rohini Kumari, S.K. Rai.

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Electrochemical Assembly of Nickel Metal Organic Framework-Decorated Nanoimprinted Gold Dendrites as Peroxidase Mimic for High-Performance Hydrogen Peroxide Sensing

Shubhangi, Rohini Kumari, S.K. Rai, and Pranjal Chandra*



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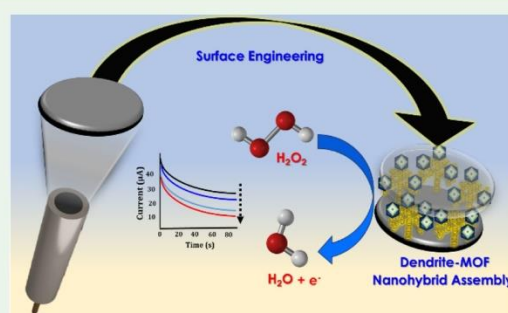
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ABSTRACT: Hybrid nanomaterials with distinct properties and morphologies when clubbed within a sensor matrix can generate a synergistic effect on molecular sensing. In this work, creation of such nanohybrid platform has been attempted for rapid detection of hydrogen peroxide (H_2O_2), which has tremendous role in area of medical diagnostics. Conventionally, the peroxidase (POD) enzyme catalyzes H_2O_2 ; however, it is prone to inherent chemical and thermal instabilities reducing the overall stability and shelf life of sensor probe. A possible solution for this problem has been attempted in this work where a nonenzymatic peroxidase mimic nanohybrid probe comprising gold nanodendrites (AuND), nickel metal organic framework (Ni-MOF), and hydrazine has been synergistically deployed for rapid detection of H_2O_2 . The developed sensor probe has been rigorously characterized through various characterization techniques, including scanning probe microscopy (SPM), scanning electron microscopy (SEM), energy dispersive X-ray analysis (EDX-mapping), Fourier transform infrared spectroscopy (FTIR), X-ray diffraction analysis (XRD), X-ray photoelectron spectroscopy (XPS), cyclic voltammetry (CV), and electrochemical impedance spectroscopy (EIS). The probe demonstrated impressive analytical performance, possessing a linear dynamic range (LDR) between 1×10^{-8} M and 1×10^{-15} M and a limit of detection (LOD) of $0.34 (\pm 0.05) \times 10^{-15}$ M. The probe's average response time with changing H_2O_2 concentrations was 5.02 ± 0.42 s, making it an agile sensing platform for H_2O_2 detection. The nanohybrid probe displayed minimal response toward interferants such as superoxide radicals, ascorbic acid, cysteine, glucose, alanine, and citric acid, which usually coexist in a real sample matrix. In order to investigate the real-life applicability of the developed sensor probe, a real sample analysis involving synthetic serum was adopted, which yielded a current recovery between 90.20 and 94.14%. The probe fabrication time and on-chip synthesis procedure are facile, making it a robust and efficient sensing platform for H_2O_2 free radicals in clinical settings.

KEYWORDS: electrodeposition, hydrogen peroxide, nanodendrites, MOF, surface engineering, nanohybrid systems



1. INTRODUCTION

H_2O_2 , an intermediate in various biological reactions and signaling pathways, is difficult to detect in natural premises due to its instability. However, its excessive concentration can pose threat to cellular health by damaging vital proteins and DNA due to the generation of reactive oxygen species. In view of the importance of H_2O_2 , a number of analytical techniques such as colorimetry, fluorescence,¹ gas chromatography, high-performance liquid chromatography (HPLC),² and HPLC-UV³ have been used. Techniques like chromatography and colorimetry are highly skill-dependent ones with necessary preprocessing requirements incurring higher costs and labor for the target specimen detection.⁴ Whereas, techniques such as fluorescence can have hindered outcomes due to involvement of interferants. Therefore, to avoid such limitations, electrochemical sensing approach could be a plausible solution due to

its rapid response, ease of procedure, sensitivity, and low cost.^{5,6}

Enzymatic sensors are widely being used nowadays for sensitive and selective sensing of H_2O_2 . POD is a naturally occurring enzyme found in many plants as well as in a few animal cells. This enzyme is known to be highly selective toward combining with H_2O_2 and methyl or ethyl peroxides.⁷ Horseradish peroxidase, a heme-containing enzyme of this class, is known to utilize H_2O_2 in order to oxidize a vivid

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Pristine NiMOF Sandwiched between 1D and 3D Engineered Au Particles on a Dendritic Platform for Ultraswift Folic Acid Sensing in Cellular Microenvironments

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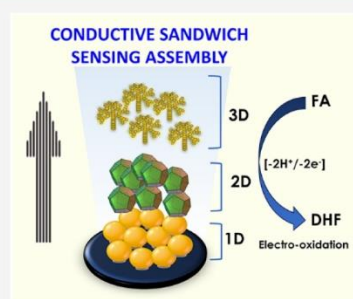
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Supporting Information

ABSTRACT: Catalytic metal-organic frameworks (MOFs)-based sensor matrices can act synergistically with Au metallic nanostructures to generate amplified signal readouts by causing the electro-oxidation of the target analyte. Folic acid (FA), an essential water-soluble vitamin and a precursor for enzymes, requires timely and precise monitoring in the serum of individuals with varying clinical diagnoses. An attempt has been made in this direction through our work, where the rapid detection of FA through its oxidation at metal centers from hybrid nanomaterials is deployed for signal generation. A nonenzymatic, nonimmunometric approach involving a sandwich model, comprising NiMOF layered between gold nanoparticles (AuNPs) and gold nano-dendrites (AuNDs) incorporated within a sensor matrix, has been deployed for this purpose. The probe displayed great analytical performance with a linear dynamic range (LDR) from 1×10^{-11} M to 1×10^{-3} M and a limit of detection (LOD) of 0.43×10^{-11} M. The probe's average response time with respect to changes in FA

concentration was recorded as less than 2.1 s, making it a rapid sensing platform for FA detection. The real-life applicability of the developed sensor was tested in serum, followed by analysis in a breast cancer cellular microenvironment, which yielded a current recovery between 95.11 and 98.17%. The *in vitro* analysis was further validated through live-cell imaging using the standard method of fluorescence. The shorter fabrication time of the developed sensor compared to existing ones makes it a facile and efficient sensing platform for FA detection in clinical settings. This study represents the first report on the conjunction of 1D, 2D, and 3D materials as a sensing matrix for molecular detection applications.



INTRODUCTION

Folic acid (FA), commonly known as pteroylglutamic acid, is an essential water-soluble vitamin (vitamin B9) of the B-complex category. Its primary essentiality revolves around important bodily functions, serving as a precursor for various coenzymes such as ferroheme and contributing to the synthesis of purines and pyrimidines. Consequently, it plays a vital role in the repair, methylation, and synthesis of DNA.^{1,2} It is a synthetic substitute for vitamin B9 or folacin, chemically known as (2S)-2-[[4-[(2-amino-4-oxo-1H-pteridin-6-yl)-methylamino]benzoyl]amino] pentanedioic acid. It gained prominence at the beginning of the century as understanding of its deficiency grew.^{3,4} A decrease in its body fluid concentration ($34.4 \pm 10.4 \times 10^{-9}$ M) has been linked to various diseases such as mental deconvolution, leucopenia, gigantocytic anemia, and increased possibilities for heart attacks and cancer.⁵ While its supplementation reduces the occurrence of neural tube defects such as spinal bifida in women and decreases carcinogenesis due to its involvement in nucleotide synthesis, the overexpression of its receptors on cancerous cells makes them classic tumor markers due to the increased metabolic FA uptake by these cells.^{6–8} There have been conflicting reports on its role in suppressing as well as

increasing carcinogenesis in clinical subjects with varying clinical diagnoses.^{9–11} This makes its judicious supplementation and quantitative detection through selective and sensitive sensing modules the need of the hour. While it is naturally synthesized in green leafy plants, algae and microbes such as yeast and bacteria, its deficiency is coped up in humans through dietary or pharmaceutical supplementation.¹²

FA detection has been practiced through various methods including the traditional spectrophotometry,¹³ colorimetry,¹⁴ mass spectroscopy,¹⁵ and electrophoretic¹⁶ methods along with sophisticated methods like high-performance liquid chromatography,¹⁷ flow injection chemiluminescence,¹⁸ ELISA,¹⁹ and many others. The major drawback of these methods is that they require expensive setups and expertise in operations and are time-consuming. To overcome these challenges, newer and more sensitive tools such as electro-

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MOF-based nanocomposites as transduction matrices for optical and electrochemical sensing

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Molecular detection

ABSTRACT

Metal Organic Frameworks (MOFs), a class of crystalline microporous materials have been into research limelight lately due to their commendable physio-chemical properties and easy fabrication methods. They have enormous surface area which can be a working ground for innumerable molecule adhesions and site for potential sensor matrices. Their biocompatibility makes them valuable for *in vitro* detection systems but a compromised conductivity requires a lot of surface engineering of these molecules for their usage in electrochemical biosensors. However, they are not just restricted to a single type of transduction system rather can also be modified to achieve feat as optical (colorimetry, luminescence) and electro-luminescent biosensors. This review emphasizes on recent advancements in the area of MOF-based biosensors with focus on various MOF synthesis methods and their general properties along with selective attention to electrochemical, optical and opto-electrochemical hybrid biosensors. It also summarizes MOF-based biosensors for monitoring free radicals, metal ions, small molecules, macromolecules and cells in a wide range of real matrices. Extensive tables have been included for understanding recent trends in the field of MOF-composite probe fabrication. The article sums up the future scope of these materials in the field of biosensors and enlightens the reader with recent trends for future research scope.

1. Introduction

MOFs are a diverse class of multifunctional, crystalline and porous materials with their evolution dating back to late 1990s [1]. These porous materials often referred to as ‘coordination polymers’, are formed through coordination of organic linker molecules with metal ions by their self-assembly [2,3]. They find their applications in a plethora of fields ranging from gas storage [4–7], gas separation [8], catalysis [9–12] to drug delivery, theranostics and sensing [13–17]. For example, Cu-based MOFs are often used as nanozymes like proteases, peroxidases and laccases [18–20]. The applications of these MOFs are supported by their commendable properties such as uniform and tunable pore sizes, large accessible surface area, high thermal and chemical stabilities [1]. Some MOFs have intrinsic properties like low toxicity, inherent stability and good biocompatibility such as Zr-based MOFs [21]. However, for expansion and enrichment of their properties, further, these MOFs are often decorated with functional species to form

composite materials with synergistic or improved features [22–24]. For example, Wen et al. in their recent work improved the electron-transfer efficiency of ZIF-8 by conjugating it with graphene for fabrication of a pesticide biosensor matrix [25]. Similarly, in a work by Lai et al. [26], a porphyrin-based MOF was doped with Mn^{2+} ions to selectively detect glutathione. In another study by Kulandaivel et al. [27], copper-based MOF nanozymes after being doped with Cu^{2+} ions, were found to be catalytic with oxidoreductase activity which helped in enhanced colorimetric paper-based detection of Cr^{6+} . Apart from doping, the key to MOF’s reticular design lies with binding of metal nodes to different linker molecules yielding MOF structures of varying pore sizes and morphology. There is liberty for precise control of preferred topology by change in the molecular building blocks [28]. The guest accessible voids can be exploited as per needs for the development of optical, electro-mechanical, electrochemical, chemicapacitor sensors, and sensor arrays. For sensing applications, the target moiety’s counter molecule can be either encapsulated or adsorbed on the MOF surface making its physical

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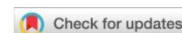
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ADVANCED REVIEW



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Shifting paradigm in electrochemical biosensing matrices comprising metal organic frameworks and their composites in disease diagnosis

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Email: pranjal.bce@iitbhu.ac.in**Funding information**

Indian Institute of Technology (BHU) Varanasi; Ministry of Education; Prime Minister's Research Fellowship (PMRF) by Ministry of Education, Government of India

Edited by: Dipanjan Pan, Associate Editor and Gregory M. Lanza, Co-Editor-in-Chief**Abstract**

Metal Organic Frameworks (MOFs) are an evolving category of crystalline microporous materials that have grabbed the research interest for quite some time due to their admirable physio-chemical properties and easy fabrication methods. Their enormous surface area can be a working ground for innumerable molecular adhesions and site for potential sensor matrices. They have been explored in the last decade for incorporation in electrochemical sensor matrices as diagnostic solutions for a plethora of diseases. This review emphasizes on some of the recent advancements in the area of MOF-based electrochemical biosensors with focus on various important diseases and their significance in upgrading the sensor performance. It summarizes MOF-based biosensors for monitoring biomarkers relevant to diabetes, viral and bacterial sepsis infections, neurological disorders, cardiovascular diseases, and cancer in a wide range of real matrices. The discussion has been supplemented with extensive tables elaborating recent trends in the field of MOF-composite probe fabrication strategies with their respective sensing parameters. The article sums up the future scope of these materials in the field of biosensors and enlightens the reader with recent trends for future research scope.

This article is categorized under:

Diagnostic Tools > Biosensing

Diagnostic Tools > Diagnostic Nanodevices

KEYWORDS

diagnosis, disease, electrochemical biosensors, metal organic frameworks, nanobioengineering

1 | INTRODUCTION

In recent times, the convergence of nanotechnology, materials science, and bioengineering has led to revolutionary progress in the advancement of biosensors, heralding a new age of accurate diagnosis and personalized healthcare (Borse et al., 2022; Chandra & Prakash, 2020; Huang, Zhu, & Kianfar, 2021). The increasing worldwide prevalence of infectious and fatal diseases, including those that can be transmitted and others that cannot, highlights the dire need for diagnostic methods that are simple, fast, accurate, and affordable (Mahato et al., 2016). Conventional

Engineered Cobalt/Molybdenum Bimetallic MOF as Electrochemical Signal Transducer for Uric Acid Detection

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Abstract—

Electrochemical signal transduction systems involving metal organic frameworks (MOFs) are new buzz words in sensing due to their astounding functional capabilities and catalytic potential. In this work, creation of one such novel sensing platform comprising of cobalt/molybdenum (Co/Mo) bimetallic MOF has been attempted for the sensitive detection of uric acid (UA) in clinical ranges. Conventionally, UA is detected through an enzyme called uricase which converts UA to allantoin. However, the enzyme-based diagnostic solutions are prone to chemical and thermal instabilities making the process cumbersome and tedious. To eliminate such challenges, the developed non-enzymatic detection system uses a highly catalytic bimetallic sensing module which electrochemically facilitates conversion of UA to allantoin, and yields electrochemical signal outcomes. The developed sensing probe was characterized through physical and electrochemical techniques such as scanning electron microscopy (SEM), Fourier transform infrared spectroscopy (FTIR) and, cyclic voltammetry (CV). Here, UA has been used as the sample target analyte to validate the efficacy of the bimetallic system using differential pulse voltammetry (DPV) and chronoamperometry (CA). The preliminary results hold immense promise for the developed system in application for sensing a wide range of analytes through electrochemical methods in clinical settings.

Keywords—metal organic framework; uric acid; nanocomposite; electrochemical; biosensing

I. INTRODUCTION

MOF, a class of self-assembled coordination complex-based porous materials, comprise of metal centre coordinated with organic linker moieties to form two or three-dimensional structures [1]. They have been designated as materials with high specific surface area, tunable mesoporosity, diverse catalytic metal centers linked to organic ligands with good adsorption affinities and superior redox behaviour [2]. These porous materials

have found their utility in innumerable applications ranging from gas storage to catalysis and now to analytical applications like sensors and drug delivery systems [3]. However, the major challenges with working on these materials lies primarily with their wide band gap resulting into compromised electrochemical conductivity, heavily aggregated morphology and high resistance to diffusion [1]. Such phenomena have usually been observed in MOFs synthesized through the conventional solvothermal route which are now being replaced by newer synthesis protocols involving microwave, sonochemical and electrochemical methods [3]. MOFs have been projected as plausible solutions towards replacing enzyme-based sensors due to their catalytic metal centers imparting them with electrocatalytic potential [4]. Co-based MOFs have been recently reported as sensitive electrode matrix components for detection of analytes like UA [5].

Uric acid, a bio-compound also known as 2,6,8-trihydroxypurine, is often linked to various biophysiological pathways like purine (adenine and guanine) metabolism and decomposition [1],[5]. An abnormally high concentration or low concentration of UA is regarded as a causative factor for diseases like gout, hypertension, hyperuricemia, cardiovascular and chronic renal diseases, and oxidative stress [4],[6]. The clinical range of UA lies between 0.13-0.46 mM in blood serum and 1.49-4.46 mM in urine samples within healthy individuals [7],[8]. Therefore, a timely check on its physiological concentration holds enormous clinical significance in disease diagnosis. UA is routinely detected through enzyme-based assays or alternatively through spectrometry and chromatography-based methods [6]. However, electrochemical non-enzymatic methods hold great promise for sensing UA due to faster response time, greater selectivity and cheaper operations. Although there always lies a scope for an alteration in sensor components which can improvise sensitivity and contribute towards realistic applications of the sensors.

In this work, we have attempted to fabricate a novel combination of Mo and Co-based bimetallic MOF within a sensor matrix for the selective and sensitive detection of UA through the electrochemical approach. The novelty lies in conjugation of two highly conductive metal centers conjugated with methyl imidazole linker moiety. The

Bimetallic Copper/Zinc Metal Organic Framework-MoS₂ Nanohybrid based Electrochemical Sensor

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Abstract— Acetaminophen is a globally used antipyretic analgesic drug to relieve pain. The excess usage of acetaminophen leads to various health implications including cardiovascular ailments, asthma, liver and kidney damage. Bimetallic MOFs are emerging materials in the field of electrochemical sensing domain utilizing the synergistic effect of both the metal ions present within. In this study, we report a sensing matrix comprising of an electrochemically fabricated novel bimetallic MOF (CoZn) conjugated with MoS₂ nanosheets to form an electroconductive nanocomposite. Layer-by-layer characterization of CoZn-MOF/MoS₂ modified electrode surface was done through different electrochemical analytical techniques like CV and EIS. The composite (GCE/CoZn-MOF/MoS₂) can find its applications in sensing a plethora of analytes based on the catalytic potential of the metal nodes. In this work we have attempted the application of the developed nanocomposite probe in the electrochemical oxidation and thereby detection of acetaminophen. The developed nanocomposite was able to detect acetaminophen with enhancement in signal, proving the improved electroconductivity of the surface due to synergistic effect of CoZn-MOF and MoS₂.

Keywords—metal organic framework; bimetallic; nanocomposite; electrochemical; sensing

I. INTRODUCTION

Metal-organic frameworks (MOFs), often referred to 'porous coordinate polymers', are a class of recently developed porous materials that are multifunctional, crystalline compounds made up of clusters of metal ions coordinated to organic linkers to form one, two, or three-dimensional structures [1]. The high specific surface area, adjustable pore size, diversity in metal centres, organic ligands, and easy tunability in their morphology has contributed to their current significance in sensing applications [2][3]. The eminence of these structures have resulted to their potential applications in a number of industries, including batteries, supercapacitors, gas separation, adsorption, energy storage, and electrochemical sensors [4]. Contrary to this, a high electron transfer energy barrier of these materials when

synthesized through a variety of routes, including the most common solvothermal one makes their usage challenging in electrochemical biosensing [5]. A novel way to tackle this problem could be incorporation of a second metal ion in the MOF's lattice nodes or doping with a highly conductive metal within the MOF cavity or the sensor matrix. The first approach is a lesser transversed route that can be explored to achieve the synergistic effect of metal nodes in the developed bimetallic MOF. This could possibly be induced by lattice distortions, interfacial electronic coupling interactions, and increased porosity through combinational effects between distinct metal ions. Bimetallic MOFs are useful for catalyzing electron transfer-driven reactions because of their exposed active sites and great stability [6][7]. The common method of MOF synthesis can be replaced by electrodeposition-based methods which have been explored in recent times [8]. A range of bimetallic MOFs based on Ni/Cu catalytic bimetals [9], Cu/Co [10] for glucose, Fe/Ni for dopamine [11], Co/Ni for miRNA 126 [12], Cu/Ce for malathion [13], Mn/Fe for organophosphates [14], Ag/Zn for HCV-RNA [14] have been reported for a range of target analytes belonging to varied molecular strata. These research findings preach that bimetallic MOF are becoming one of the major areas which need to be explored more to utilize the potential lies in bimetallic MOFs for electrochemical sensing.

Acetaminophen is an antipyretic analgesic medication that is extensively used globally to relieve mild to moderate pain brought on by post-operative treatments, arthritis, headaches, and back pain [15][16]. Furthermore, it lowers fever in cases of viral and bacterial illnesses. An appropriate dosage of acetaminophen has no negative side effects; however, exceeding beyond this limit might result in a build-up of toxic acetaminophen metabolites, which can induce a variety of adverse effects, including skin rashes, pancreatic inflammation, hepatotoxicity, and nephrotoxicity. Specifically, the latter two effects may cause harm to the human body's kidneys and liver [17]. Numerous techniques, including titrimetry, UV-vis spectrophotometry, spectrofluorometry, and high-resolution liquid chromatography, have been used to measure acetaminophen in biological fluids, wastewater, and pharmaceutical formulations [18][19]. The evaluations become more challenging because of high expenses, prolonged processing time, and complex sample preparation requirements.

Annexure 2

Conference presentations

1. **Shubhangi**, Rohini Kumari, S.K. Rai, **Pranjal Chandra***. **Title:** *Rapid High-Performance Sensing of Hydrogen Peroxide using Nickel MOF adorned Au dendrites in electrochemical settings* in ETID-2024: National Conference on Emerging Trends in Implants and Diagnostics. Organized by Center of Excellence, at **NIPER-Ahmedabad**, India. Date: 1-2 February, 2024. **(BEST POSTER PRESENTATION)**
2. **Shubhangi**, Rohini Kumari, S.K. Rai, **Pranjal Chandra***. **Title:** *Electrochemically Nanoengineered MOF- dendrite Composite as Rapid Sensing Platform for Hydrogen Peroxide*, in the National Conference on Technological Advancements in Waste Management: Challenges and Opportunities-2023, at **IIT (ISM) Dhanbad**, India. Date: 4-5 December, 2023. **(BEST POSTER PRESENTATION)**
3. **Shubhangi**, Ruchita Chaudhari, S.K. Rai, **Pranjal Chandra***, **Title:** *Engineered Cobalt/Molybdenum Bimetallic MOF as Electrochemical Signal Transducer for Uric Acid Detection*, in the IEEE BioSensors Conference (BioSensors 2024), at **Cambridge, United Kingdom**, Date: 28-30 July, 2024. (Awarded with the **IEEE BIOSENSORS WISE TRAVEL GRANT 2024**, by the IEEE SENSORS COUNCIL and **DST-SERB Travel Support Grant**, Govt. of India).
4. **Shubhangi**, Rohini Kumari, S.K. Rai, **Pranjal Chandra***. **Title:** *Conjugation of MOF with Dendrites: A Novel Platform Exhibiting Peroxidase Mimicking Activity in the 3rd International Conference on Electrochemical Science and Technology-2024*, organized by the CSIR-National Physical Laboratory, New Delhi & The Electrochemical Society, India Indian Institute of Science Campus, Bengaluru, at **CSIR-NPL, New Delhi**, India. Date: 18-20 September 2024.
5. **Shubhangi**, Marshal Dhayal, **Sanjay Kumar Rai***, **Title:** *Rapid Quantification of HepG2 Cells aided by Electrochemically Deposited Folic Acid on Platinum Electrode*, in the NATIONAL SYMPOSIUM ON ELECTROCHEMICAL SCIENCE AND TECHNOLOGY [NSEST - 2023] by THE ELECTROCHEMICAL SOCIETY OF INDIA, Indian Institute of Science Campus, Bengaluru and International Advanced Research Centre for Powder Metallurgy and New Materials (ARCI), **Hyderabad**, Date: September, 2023.

6. Presented my work at the 6th edition of World Nanotechnology Conference (**hybrid mode**) held at **Orlando**, USA, Date: **April 24-26, 2023**.
7. Scientific Writing Workshop by ACS Publications in collaboration with Indian Institute of Technology (BHU), Varanasi. Date: **29th June, 2023**.
8. Presented talk on our work titled, "*Optimizing conditions for solvothermal syntheses to obtain precise hexagonal crystals of ZIF*", in **AFMFP- Advanced Functional Materials: Future Perspectives**, held and organized by Dr BR Ambedkar National Institute of Technology, Jalandhar, India. Date: **6-8 August, 2022**.
9. Attended webinar on "The development and application of Trupath biosensors to the study of GPCR pharmacology and functional selectivity, by Dr Reid Olsen. Date: **15th July, 2021**.
10. Attended the **4th Annual Drug Discovery & Development Virtual Conference**. Date: **24th February, 2021**.
11. Attended seminar on "Trends in Publishing" by Dr. Harry Blom, Vice President - Journals, Development, Policy & Strategy, **Springer Nature**. Date: **16th June 2020**.
12. Attended the "**Immuno-Oncology & Cancer Biology Virtual Conference**", Global Event. Date: **29th July 2020**.
13. Attended the science webinar on "Deciphering the cell cycle: The role of cell cycle control in cancer." By the **Science/AAAS Custom Publishing Office**. Date: **8th July 2020**.
14. Attended the webinar on "Exploring and Preventing cell culture contamination", organized by **ThermoFischer Scientific**. Date: **15th July 2020**.
15. Attended the webinar on "Understanding Genomics and Genetic Testing in Cancer Immunotherapy", by the **Cancer Research Institute**. Date: **24th June 2020**.



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