



Electrochemical Biosensors Based on Bimetallic and Trimetallic Nanomaterials: Synthesis, Properties and Applications

Parul Verma¹, Shashank Sharma², Mohammed Tajammul Hussain³, Mohammed Faisal Ahmed³, Shilpi Khurana⁴, Sachin Saxena⁵, Akhilesh Singh⁶, Sajid Ali⁷, Kalpana Singh^{8,*}

¹Department of Applied Sciences and Humanities, Ajay Kumar Garg Engineering College, Ghaziabad, 201009, India

²Department of Chemistry, Dayalbagh Educational Institute (Deemed University), Agra, INDIA

³Saudi Chevron Phillips, Al Jubail 35722, SAUDI ARABIA

⁴Department of Chemistry, Deshbandhu College (University of Delhi), Kalkaji, New Delhi, 110019, INDIA

⁵Department of Chemistry, School of Basic Sciences & Research (SBSR), Sharda University, Greater Noida, INDIA

⁶Department of Chemistry, K.S. P.G. Saket College, Ayodhya, INDIA

⁷Department of Chemistry, Jamia Millia Islamia, New Delhi, 110025, India, INDIA

⁸Lloyd Institute of Management and Technology, Plot No.-11, Knowledge Park-II, Greater Noida, Uttar Pradesh, INDIA

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ABSTRACT:

Over the past few years, there has been significant progress in electrochemical biosensors, mainly due to the growing demand for quick and highly sensitive detection techniques across various sectors, including medical diagnostics and environmental analysis. This review critically evaluates the recent advancements in bimetallic and trimetallic nanomaterials used in electrochemical biosensing, highlighting their synthesis strategies, functional properties, and biomedical applications. The novelty of this review lies in its structured classification of biosensor systems based on synthesis routes, application fields and electrochemical techniques. Furthermore, this work identifies and discusses existing research gaps, including limited clinical validation, poor reporting standardization, insufficient biocompatibility data, industrial adoption and cost-effectiveness. The review addresses the present challenges, Life Cycle Assessment, biocompatibility and toxicity of glucose biosensors in therapeutics, along with the limitations and future potential of bimetallic and trimetallic biosensors concerning their stability, reproducibility, toxicity, industrial adoption, circular economy and environmental impact. It also discusses other innovative systems and the challenges of scaling and commercializing them.

1. Introduction

Electrochemical biosensors have garnered significant interest and have become essential instruments across various domains, including food safety, environmental surveillance and medical diagnostics. Adding cutting-edge nanomaterials to electrochemical biosensors is essential for improving their performance since they offer a large surface area, excellent conductivity and biocompatibility [1].

An exciting development in biosensors is incorporating nanoscale materials with distinct physical and chemical characteristics. The application of various nanoscale materials, each offering unique chemical and physical attributes, represents a novel and promising direction in bio-

sensor technology. The fundamental analytical properties of biosensors, such as sensitivity, linear detection range, repeatability, stability, response time and limit of detection (LOD), are enhanced by the use of nanomaterials. A notable increase in the transducer's sensitive surface and more efficient enzyme immobilization are guaranteed by the special qualities of nanomaterials, particularly their high surface-to-volume ratio. Nanomaterials also have high magnetic qualities, electrical conductivity, catalytic activity and other characteristics that are crucial for biosensors [2]. Moreover, nanomaterials' surface is easily modifiable with various chemical groups, which is necessary for the interaction of biomaterial with biosensors and other biotechnological assays [3]. Doped nanomaterials offer a versatile approach to producing highly efficient sensors [4]. The synthesis



of nanomaterials that combine with their targets to form coloured complexes that are visible to the unaided eye is a perspective approach [5]. Nanomaterials can also be used to separate molecules in complicated matrixes [4,6,7].

Modern biosensors can detect various pollutants, including heavy metals, which pose significant environmental concerns. Aquatic habitats and human health are at risk from these non-biodegradable metals, which can linger in soil and water bodies for years. Anthropogenic activities, such as mining, lead to the discharge of heavy metals, which are used in industries including electronics, paints, and electroplating. Heavy metals have the potential to down-regulate antioxidant metabolism and induce oxidative stress [8,9]. Furthermore, the World Health Organization (WHO) and the U.S. Environmental Protection Agency (US-EPA) were compelled to provide the stringent criteria for an admissible level of heavy metal content in drinking water due to the deadly diseases spread by heavy metals contaminated drinking water. Once inside the body, these heavy metals can lead to oxidative stress by producing more reactive oxygen species (ROS) and reducing the body's ability to use antioxidants. Overuse of agrochemicals, such as fertilizers and pesticides, can harm ecosystem balance and human health, endangering the environment.

Electrochemical biosensors are a versatile category of analytical instruments that can detect biological analytes with remarkable sensitivity and precision. In recent years, there has been a distinguished increase in research aimed at improving these devices through the use of nanomaterials, especially bimetallic and trimetallic nanocomposites. Abdullatif *et al.* [4] explores these materials, which provide combined properties such as exceptional electrocatalytic activity, enhanced conductivity and increased surface area that greatly boost the performance of biosensors. Bimetallic and trimetallic nanoparticles are increasingly employed to eliminate heavy metals from water environments, owing to their enhanced properties over monometallic counterparts. These nanoparticles, which incorporate two or three metals, improve adsorption capacity, catalytic performance and chemical stability [5]. Heavy metals like lead present a major environmental and public health issue due to their persistent nature and widespread occurrence [6]. International regulatory agencies have set emission standards to control lead levels in wastewater and drinking water, with the U.S. Environmental Protection Agency establishing a maximum contaminant level of 0.015 mg/L for lead in drinking water [7].

Despite the rapid advancements, much of the current literature either lacks clinical significance or overlooks essential factors like long-term biocompatibility, scalable production and toxicity. Previous reviews often list materials without offering a structured framework or synthesizing mechanistic patterns. Furthermore, the integration with wearable technologies and cost-effective data-driven methods like artificial intelligence is still largely unexplored. To address these gaps, this review presents a unified classification system based on synthesis methods, application areas, and electrochemical techniques. It also includes recent advancements (2015–April 2025), focusing on performance evaluation, biocompatibility and potential for real-world application. By doing so, this review seeks to offer a thorough and forward-thinking view on the design and use of bimetallic and trimetallic nanomaterials in future biosensors.

2. Objectives

In this review, bibliometric data were retrieved from published articles accessed on April 1, 2025, using different search engines/databases [Fig. 1] (Web of Science, Scopus, PubMed, Google Scholar) with keywords about biosensors. The specific search terms or keywords used were “glucose biosensor”, “gold nanoparticles”, “enzyme immobilization”, “LOD”, “nanocomposites electrode”, “bimetallic nanoparticles”, “electrodeposition”, “green synthesis”, “H₂O₂ detection”, “glucose sensing”, “cancer biomarkers”, “cyclic voltammetry”, “amperometry”, “nanoparticles”, “enzymatic sensors with gold”, “sensitivity”, “trimetallic catalysts”, “real-time implementation”, “cost-effectiveness”, “industry adoption” and “co-occurrence for electrochemical biosensors”. While preparing the review outlines, we screened and excluded unrelated and superficial literature. The articles related to the synthesis and applications of biosensors were carefully reviewed, and the relevant information gathered from these articles was compiled in this review.

3. Advances in nanomaterials for electrochemical biosensing

3.1 Primary/binary and ternary nanocomposites: The role of bimetallic and trimetallic nanomaterials has recently been observed in binary nanocomposites, ternary nanocomposites and quaternary nanocomposites [13]. Cerium and cadmium binary nanocomposites have been found to have good therapeutic properties [14]. These binary advanced nanomaterials are found to have better catalytic properties in determining organic analyte systems [15]. Further,

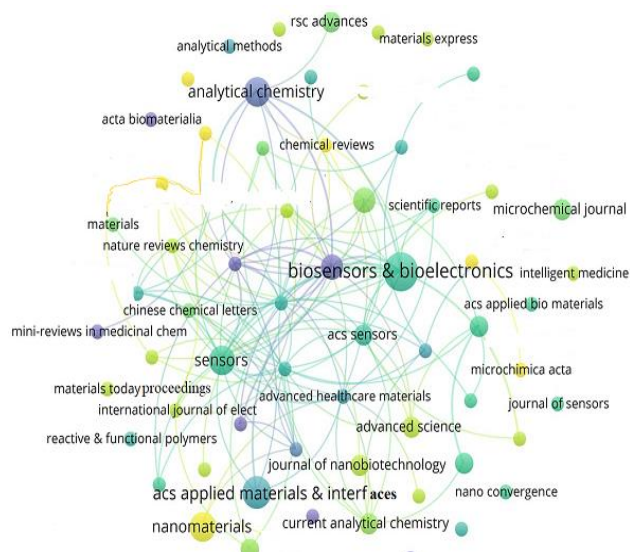


Fig. 1: A bibliometric map was generated using VOSviewer, showing keyword co-occurrence strength. Each colour-coded cluster signifies a thematic category such as green for materials (nanoparticles, electrodes), red for applications (glucose detection, O₂ sensing), and blue for techniques (CV, amperometry). The size and connectivity of nodes indicate the prominence of research and the strength of co-occurrence.

ternary nanocomposites containing reduced graphene oxide carbon nanotubes have provided high stiffness and resistance with large strength [16]. Controlled synthesis is observed in the case of CuO@Ni/polyaniline/MWCNT, where the nanocomposite was observed with high charge storage capacity, *i.e.* supercapacitance [17].

3.2 Nanoscale metal-organic frameworks (MOFs) and functionalized nanomaterials: Nanometal organic frameworks combine nanomaterials with a class of hybrid porous materials constructed by self-assembling metal ions and organic linkers. Ocular, neurological, cardiovascular diseases, pathogenic ailments and other chronic diseases can be detected using highly sensitive nanoscale metal-organic frameworks-based sensors. Functionalization, further changes the surface properties and interaction phenomenon towards the analyte system. The tunable properties of the nanocomposites comprising nanomaterials enhance and amplify the redox system's electrochemical signal. Electro-analytical techniques in tandem with the nanomaterials containing nanocomposites are listed in Table-1. Table-1 comprehensively summarizes the various functionalized nanomaterials used as biosensor platforms for disease diagnosis. The list includes MOFs, carbon nanomaterials, DNA-functionalised particles and gold nanoparticles. Surface functionalization is pivotal in improving these biosensors' selectivity, stability and sensitivity. MIL-101(Fe),

functionalized with β -cyclodextrin, is distinguished by its dual functionality in tumor targeting and drug delivery thanks to its large surface area and functional binding sites. The MOF-5/CoNiS₄ nano biosensor is very good at identifying the SARS-CoV-2 spike protein, essential for quick testing during pandemics [21]. The frequent mention of AuNPs underscores their well-established role in biosensor development due to their strong biocompatibility and easy functionalization. For example, AuNPs-FM DV biosensors have shown promising results against the foot-and-mouth disease virus and AuNPs mixed with dendrimer nanocomposites have successfully detected Alzheimer's disease. Also, materials made from two metals, like NiCo-based MOFs and Co-Cu nanocomposites, help detect genes and multiple biomarkers because the combination of their metal parts works well together. Overall, the observed trends indicate that MOF-based systems provide adjustable porosity and functionality for targeted applications. At the same time, gold and bimetallic nanoparticles are preferred for their excellent conductivity, biocompatibility and ease of integration into biosensing platforms.

3.3. Bimetallic nanomaterials: Bimetallic nanoparticles have garnered increased interest lately due to their unique physical characteristics such as a large surface area, mobility, quantum effects and a range of chemical, mechanical, thermal, optical, catalytic and magnetic properties [32]. These properties set bimetallic materials apart from their monometallic counterparts, offering enhanced performance that individual metals cannot achieve [33]. The use of both mono and bimetallic nanoparticles can benefit numerous industries, including biomedical, biosensors, imaging, nanomedicine, wastewater treatment, oil and gas, food/agriculture processing and gene/drug delivery [34]. Also, the antimicrobial and anticancer properties of bimetallic nanoparticles have been extensively researched [35]. The bimetallic nanoparticles are formed when two distinct metals react together under optimal conditions [36]. By combining noble and transition metals, a wide array of bimetallic nanoparticles can be produced, including those based on gold, silver, copper, nickel, iron, platinum or palladium.

3.4. Trimetallic nanomaterials: Trimetallic nanomaterials have been proposed to be constructed using a "triple core-shell structure," where one element forms the core, another element creates an interlayer around the core, and a third element forms a shell over the interlayer [35]. The size, shape and surface morphology of particles cannot be adjusted



TABLE-1
OVERVIEW OF ELECTROANALYTICAL TECHNIQUES COUPLED WITH NANOMATERIALS IN NANOCOMPOSITES

Functionalization	Nanomaterials	Biosensors	Disease diagnosis	References
Bicyclononyne (BCN)-functionalized β -cyclodextrin (β -CD)	MIL101(Fe)	Electrochemical biosensor	Tumor targeted drug delivery	[18]
	ZIF-8/P-DNA complex	Biosensor	Coronavirus (Covid-19)detection	[19]
Dibenzylcyclooctyne (DBCO)-functionalized DNA	UiO-66-N3 (Zr6O4OH4(C8H3O4-N3)6) nanoparticles with an azide functional group	Electrochemical biosensor	Intracellular gene regulation	[18]
	Bimetallic Ni-Co-based Metal-Organic Framework(MOF)	Electrochemical biosensor	Human immune deficiency virus-1 genedetection	[20]
	MOF-5/CoNi2S4 composite	Nanobiosensors	SARS-CoV-2 spike protein detection	[21]
Nucleic Acid-Functionalized Metal-Organic Framework	MOFs	Electrochemical Biosensor	Detection of Multiple tumor biomarkers	[22]
Aminofunctionalized iron-carboxylate metal organic frameworks	Iron-carboxylate	Biosensor	Drug delivery and biomedical applications	[23]
	Gold nanoparticles (AuNPs)	AuNPs-FMDV based biosensor	Foot and mouth disease virus	[25]
	Gold nanoparticle-poly(amidoamine) (PAMAM) dendrimer nanocomposites	Nanoparticle-based multiplex biosensor	Alzheimer's disease diagnosis	[24]
	Gold nanoparticles (AuNPs)	Biosensor	Alzheimer's disease (AD)detection	[26]
	Carbon Nanomaterial	Biosensors	Clinical Diagnosis	[27]
	Bimetallic Nanomaterials	Electrochemical Biosensor	Broad -spectrum clinical Applications	[28]
	Gold nanoparticles (AuNPs)	Biosensor	Detection of pathogenic bacteria	[29]
	Hollow nanoporous carbon framework decorated with bimetallic nanoparticles	Nanozyme based biosensor	Uric acid detection	[30]
Bimetallic nanostars	miRNA probe integrated biosensor	Circulating colorectal cancer biomarkers in clinical samples	[31]	

to achieve desired outcomes with the help of the second metal. However, it can enhance catalytic efficiency by transferring electron density from the initial metal to the surface catalytic structure [36].

Triple core-shell particles with important features have been created to alter the functions of bimetallic and trimetallic nanoparticles effectively. The physical combination of nanoparticles, like Ru/Pt in solution, shows more catalytic activity when compared to similar monometallic nanoparticles. Single monoatomic catalysts, such as Pd, Pt, and Au in nanoscale, have been replaced by bimetallic structures. According to studies, in an aqueous solution, Pd, Rh and Pt nanoparticles easily form a bimetallic structure with an Au core. Since Ag is less expensive than other materials, a comprehensive assessment of Ag-core nanoparticle production is currently available and it demonstrates notable individual catalyst activity.

4.0 Synthesis and fabrication strategies of biosensors:

4.1. Bimetallic nanocomposites in glucose sensing:

Potential carbon materials include graphene and carbon nanotubes (CNTs), which have been thoroughly investigated as electrode transducer materials due to their easy functionalization, lowering internal resistances of electrodes, remarkable biocompatibility, excellent conductivity, and large specific surface area [34]. Nanocomposites made from graphene, carbon nanotubes and polymer matrix can also prevent graphene layers from stacking, which reduces



TABLE-2
SUMMARY OF THE FABRICATION APPROACHES AND ANALYTICAL PERFORMANCES OF
GLUCOSE SENSORS BASED ON BIMETALLIC NANOCOMPOSITE MATERIALS

Bimetallic Nanocomposite Material	Linear Range Concentration of Glucose	Sensitivity	Detection Methods	LOD (μM)	References
Co_3O_4 -Ag NWs/Graphene and CA 0.98 [63]	3 μM –2000 μM	2.49 $\mu\text{A}\mu\text{M}^{-1}\text{cm}^{-2}$	CV and CA	0.98	[33]
Ni-Fe@polyaniline	10 μM –1 mM	-		0.5	[34]
Ce-Cu/Graphene/SWCNT	1–1000 μM -	-	CV and CA	0.095	[41]
CuSn/CNFsNanocomposites	0.1–9000 μM	-	CV and CA	-0.08	[42]
NCNTs/Co-Cu nanocomposites	0.05–2.5 mM	1027 $\mu\text{A mM}^{-1}\text{cm}^{-2}$	CV and CA	0.15	[43]
Cu-Ag bimetallic nanocomposites	0.01–30 mM	1340 $\mu\text{A mM}^{-1}\text{cm}^{-2}$	CV and CA	0.6	[44]
Cu-Co nanocomposites	1 μM –825 μM	567 $\mu\text{A mM}^{-1}\text{cm}^{-2}$	CV and CA	3	[45]
CuO-NiO nanocomposites	0.2 μM –1 mM	4022 $\mu\text{A mM}^{-1}\text{cm}^{-2}$	CV and CA	0.08	[46]
Ag-Ni@MWCNTs	1 μM –4 mM	1485 $\mu\text{A mM}^{-1}\text{cm}^{-2}$	CV and CA	0.06	[47]
Cu-Cu ₂ O carbon spheres -	0.3 μM –24.5 mM		CV and CA	0.06	[48]

carbon nanotube adsorption [33,35]. Li *et al.* [36] used electrospinning and thermal treatment methods to fabricate a range of bimetallic MCo (M = Cu, Fe, Ni, and Mn) nanoparticles incorporated into carbon nanofibers (CFs). By employing cyclic voltammetry (CV) and chronoamperometry, they assessed the electrochemical reactions for non-enzymatic glucose sensing. The investigation highlighted the catalytic abilities of the synthesized bimetallic nanoparticles, with CuCo-CFs demonstrating the highest efficacy, followed by FeCo-CFs, NiCo-CFs, Co-CFs, and MnCo-CFs (as shown in Figure 1A). Notably, CuCo-CFs displayed exceptional detection performance, even in human serum samples ($507 \mu\text{A cm}^{-2} \text{mM}^{-1}$), with a detection range of 0.02 to 11 mM, rapid detection within 2 sec, and advantages stemming from 3D network films and the synergistic effects of the Co(III)/Co(IV) and Cu(II)/Cu(III) redox couples. It exhibited outstanding reproducibility, long-term stability and resilience to interference from electroactive molecules.

Subsequently, Lakhdari *et al.* [33] developed a NiFe-polyaniline monohybrid electrode with notable sensing sensitivity ($1050 \mu\text{A mM}^{-1} \text{cm}^{-2}$), a broad linear range (from 10 μM to 1 mM), and a low limit of detection (LOD) of 0.5 μM (S/N = 3) [33]. Additionally, Zhuang *et al.* [91] created a nanocomposite material comprising Cu/Cu₂O@C, featuring a Cu/Cu₂O heterojunction [91]. They constructed a glucose sensor using the Cu/Cu₂O@C nanocomposite (Fig. 2B), which exhibited impressive sensing capabilities with high detection sensitivities of $621.12 \mu\text{A mM}^{-1} \text{cm}^{-2}$ and $372.67 \mu\text{A mM}^{-1} \text{cm}^{-2}$ across various

glucose concentration ranges (0.001–1.7 mM and 1.7–9.7 mM, respectively) [91].

Furthermore, Li *et al.* [37] employed carbon nanomaterials like graphene and single-walled carbon nanotubes, along with nanocomposites of copper and cerium bimetallic nanoparticles, to create a highly sensitive electrochemical dual-signal glucose sensor in the presence of dopamine (DA) and uric acid (UA) (Fig. 2c) [37]. This method effectively measured blood serum samples' glucose, DA and UA levels [Table-2]. Table-2 summarizes the methods used to fabricate glucose sensors based on bimetallic nanocomposite materials and their linear detection ranges, sensitivities and detection limits (LOD). These sensors have been assessed using cyclic voltammetry (CV) and chronoamperometry (CA), both well-known electrochemical methods. Among the materials listed, Cu–Ag bimetallic nanocomposites showed the highest sensitivity, achieving $1340 \mu\text{A mM}^{-1} \text{cm}^{-2}$, making them highly suitable for glucose sensing with a strong response. Moreover, CuO–Ni₃O₄ nanocomposites demonstrated the lowest LOD at 0.08 μM , highlighting their exceptional ability to detect trace amounts of glucose, which is particularly important for early diabetes detection. Notably, Ce–Cu/graphene/SWCNT composites also exhibited impressive performance with a low LOD of 0.095 μM due to the enhanced electron mobility offered by carbon-based materials. Including nanotube (CNTs) support in Co–Cu nanocomposites resulted in moderate sensitivity ($1027 \mu\text{A mM}^{-1} \text{cm}^{-2}$) with low detection limits, emphasizing the significance of support matrices in amplifying signals. A general pattern observed is that integrating carbon nanomaterials (like graphene and



CNTs) enhances electrical conductivity and provides a large surface area for immobilizing catalytic species. This synergy is crucial for improving the sensitivity and detection range of biosensors.

4.2 Signal amplification strategies using bimetallic nanoparticles: The development of a bimetallic-based signal

amplification matrix has recently received significant attention due to the wide range of catalytic and electrical capabilities of bimetallic materials [38-40]. To detect miRNA, Masud *et al.* [50] developed a cobalt-nickel bimetallic organic framework that was immobilized on a gold electrode. Subsequently, complementary DNA was attached to the modified electrode [50]. The sensor demon-

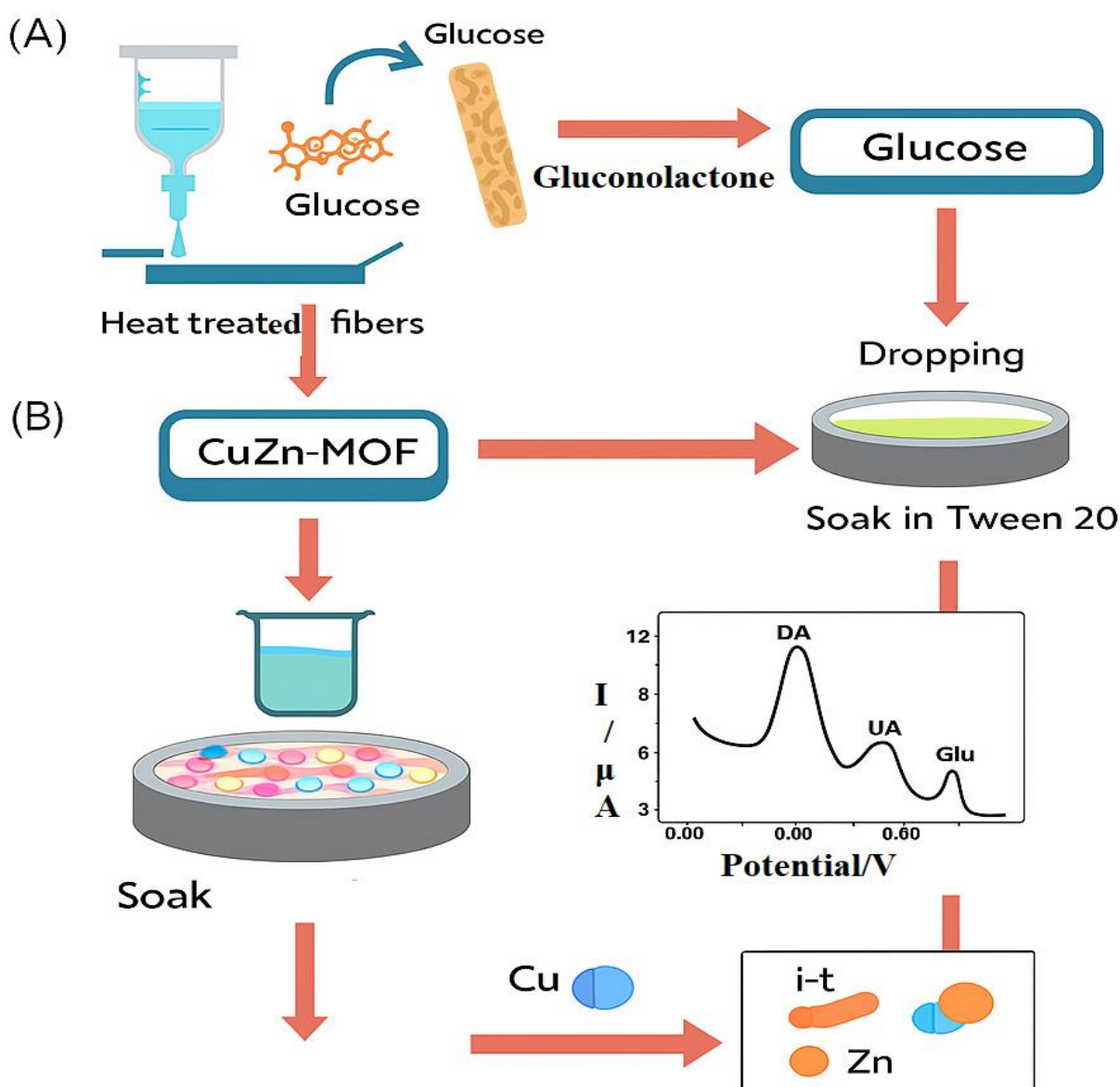


Fig. 2: Schematic overview of the synthesis, modification, and electrochemical testing of a CuZn-based metal-organic framework (CuZn-MOF) for glucose sensing. (A) Glucose is incorporated into electrospun fibers, followed by heat treatment to produce Glucoate-functionalized CuZn-MOF. (B) The synthesized CuZn-MOF is integrated onto a working electrode and soaked in surfactants to enhance stability. (C) The modified electrode is evaluated using differential pulse voltammetry (DPV) and *i-t* measurements to detect glucose alongside dopamine (DA) and uric acid (UA), with contributions from Cu and Zn ions enhancing sensor



strated an extremely low detection limit (LOD) of around 0.14 femtomolar (fM) [49]. Complementary DNA was then affixed to the altered electrode. The sensor's ultra-low LOD was approximately 0.14 fM. This approach has demonstrated encouraging results in identifying cancer biomarkers and has the potential to be applied to creating diverse biosensors with varying objectives. In other study, electrodeposition was used to develop unique mesoporous bimetallic films of alloys made of silver and gold (Au and Ag). The constructed electrode had more miRNA adsorption sites and improved signal amplification [49-50] [Fig. 2]. Bimetallic nanoparticles have also been utilized in sensors with support materials such as graphene, carbon nanotubes, and silica derivatives. Pt has super catalytic activity in the presence of H_2O_2 .

In contrast, Cu nanoparticles also have catalytic properties, so using Pt and Cu bimetallic nanocomposites, an ultrasensitive sandwich-type electrochemical immunosensor for prostate cancer detection was developed. In order to improve electrical conductivity and facilitate effective charge transfer, 2D rGO was added to address the low conductivity of $g-C_3N_4$. Primary antibodies have been demonstrated to be immobilized by the thionine-functionalized graphene oxide (GO) matrix loaded with gold [52-53]. In order to further improve electrical conductivity, thionine interacts with rGO and functions as an electron mediator. Early pancreatic cancer detection can be done with the use of bimetallic label-free immunosensors that employ the carbohydrate antigen (CA19-9) marker, which is often seen in trace amounts. Using a one-pot procedure, a bimetallic matrix of polythionine-Au (AuNPs@PThi) was created in order to generate materials with increased sensitivity through signal amplification.

4.3. Trimetallic nanoparticles for enhancing signals and Biomarkers: Recently, researchers have developed trimetallic materials to enhance electrocatalytic capabilities, offering advantages for signal amplification. Mao *et al.* introduced a signal amplification technique utilizing a trimetallic nanocomposite consisting of RuPdPt nanoalloy particles. This nanocomposite was incorporated into a matrix composed of rGO-TEPA-Thi-Au (reduced graphene oxide, tetraethylenepentamine, thionine and gold particles). The main antibody was immobilized on this trimetallic nanocomposite. The rGO-TEPA-Thi-Au-based nanocomposite facilitated easier targeting with biological molecules or metal nanoparticles. Moreover, the r-GO-TEPA matrix provided a larger surface area and increased amino groups.

Thionine, when adsorbed onto the r-GO-TEPA matrix, exhibits electroactive redox properties. A more efficient rGO-TEPA-Thi-Au nanocomposite was created in which positively charged thionine molecules were adsorbed with negatively charged gold nanoparticles (Au). Moreover, the use of a trimetallic compound produced stronger signal amplification. On COOH-terminated graphene trimetallic AuPdPt nanoparticles have been electrodeposited by Barman *et al.* [48] EDC-NHS chemistry was then used to connect anti-PSA and anti-CEA antibodies to various templates. L-proline was used to create trimetallic hollow dendritic AuPtAg nanocrystals in a single pot. The large surface area facilitates higher sensitivity than the hollow dendritic trimetallic nanocrystals for antibody binding. To identify MCP-1, the monocyte chemoattractant protein,

Trimetallic nanocomposites for biomarker detection: Table-3 examines the use of trimetallic nanocomposites in identifying various clinically significant biomarkers. These cutting-edge materials incorporate three metal elements, enhancing their electrocatalytic capabilities, conductivity and surface reactivity, reducing detection limits and wider linear ranges. The COOH-NH₂-rGO/AuPtPd nanocomposite is an auspicious system that achieves a detection limit of 0.2 nM for prostate-specific antigen (PSA), indicating strong potential for early cancer diagnosis. Similarly, AuPdPt nanocomposites integrated into miRNA probe biosensors showed outstanding sensitivity in detecting circulating colorectal cancer biomarkers, with a limit of detection (LOD) of 2 nM. For glucose detection, PtAuPd nanocomposites offered a wide detection range (0.005-9 mM) and a low LOD (0.13 μ M), highlighting their effectiveness in dynamic glucose sensing environments. Furthermore, AuAgPt alloy nanoparticles and AuPdPt-functionalised nanodots demonstrated excellent performance detecting various analytes, including dopamine, ascorbic acid, and uric acid, with multiple LODs in the nanomolar range. The superior performance of these trimetallic systems can be attributed to the synergistic catalytic effects and enhanced electron transfer kinetics facilitated by the presence of multiple metal components. Compared to monometallic or bimetallic systems, these nanocomposites consistently exhibit broader linear ranges, lower LODs, and greater selectivity, positioning them as leaders in developing next-generation biosensors.

4.4. Surface engineering and antibody-Pd@Pt NP conjugation: Zhao *et al.* [31] developed antibody-nanoparticle conjugates. The suspension liquid is separated into



TABLE-3
TRIMETALLIC NANOCOMPOSITES USED IN THE DETECTION OF VARIOUS BIOMARKERS

Description	Nanocomposite	Linear range	Limit of detection (LOD)	Ref.
H ₂ O ₂ and prostate-specific antigen (PSA) detection	COOH-NH ₂ -rGO/AgPtPd nanocomposite	The linear range of the prostate-specific antigen is between 4 fg mL ⁻¹ and 300 ng mL ⁻¹	H ₂ O ₂ , and its detection limit is 0.2 nM and 4 fg mL ⁻¹ , in that order.	[54]
Finding semaphorin 3E (Sema 3E) The identification of glucose	The nanocomposite of trimetallic CuAuPd nanowire networks has a	100 fg mL ⁻¹ to 10 ng mL ⁻¹	LOD of 1.5 fg mL ⁻¹ (S/N = 3)	[55]
The detection of glucose with a limit of detection	utilizing PtAuPd trimetallic nanocomposite	ranges from 0.005 to 9 mM,	(LOD) of 0.13 μM (Signal-to-Noise ratio = 3).	[56]
Detection of acetaminophen (AP), dopamine (DA), ascorbic acid (AA), and tryptophan (TP)	The trimetallic nanocomposite of (Au/Ag/Pd) NPs/EPGr	The linear range for AA, DA, AP, and TP were, in order, 5–650 μM, 1–700 μM, 5–700 μM, and 1–600 μM.	The limit of detection (LOD) for ascorbic acid (AA), dopamine (DA), acetaminophen (AP), and tyrosine (TP) was determined to be 0.24 ± 0.03, 0.02 ± 0.01, 0.12 ± 0.04, and 0.03 ± 0.01 μM, respectively.	[57]
There was a detection of serum human epididymis protein 4 (HE4).	AgPtCo trimetallic nanodendrites (NDs) and magnetic nanocomposites (Fe ₃ O ₄ @SiO ₂ @Au MNCs)	The linear range extended from 0.001 to 50 ng mL ⁻¹ .	The limit of detection (LOD) is 0.487 pg mL ⁻¹ .	[58]
Dynamic tracking of malignant cells' generation of hydrogen peroxide	Trimetallic AuPtAg nanoalloy with poly(diallyl dimethylammonium chloride)-capped reduced graphene oxide	The linear range spans from 0.05 μM to 5.5 mM.	The limit of detection (LOD) is 1.2 nM	[59]
Heart troponin I detection	Trimetallic alloyed AuPtPd porous fluffy-like nano dendrites (AuPtPd FNDs)	Linear range of 0.01–100.0 ng mL ⁻¹	The limit of detection (LOD) is 3 pg mL ⁻¹ .	[60]
Detection of breast cancer	AuPtPd trimetallic nanocomposites combined with decreased graphene oxide	Linear range from 0.005 μM to 6.5 mM	LOD of 2 nM	[61]

two portions and then potassium carbonate (0.02 mol/L) is added to solutions A) 8.20 ~ 8.25 and B) 8.10 ~ 8.15 to correct the pH. These were then continuously mixed at room temperature for an hour. Next, an analogue incubator was used to incubate each of A and B for 30 min at room temperature after 10.0% BSA was added to each temperature in an analogue incubator for 30 min. The conjugates underwent 8 min of centrifugation at 12,000 rpm. Then, they were washed five times with 0.01 M phosphate-buffered saline at pH 7.4, which contained 1.0% BSA. Ultimately, they were suspended again in 200 μL of 0.01 M phosphate buffered saline at pH 7.4, now with 2.0% BSA and 3.0% sucrose. In this context, the resulting conjugates are referred to as Ab-Pd@Pt NPs. The findings are shown in the supplementary information (Fig. 3b) Active absorption forms the Ab-Pd@Pt NPs. In this process, the pH is marginally higher than the isoelectric point of each antibody, and the passive absorption process forms the Ab-Pd@Pt

NPs. As a result, the negatively charged NPs will firmly attach to positively charged groups connected to an antibody macromolecule [20].

5.0 Applications of biosensors

5.1 Detection of disease biomarkers: Carcinogenesis is a hereditary process that starts at the cell level and travels along a convoluted pathway, eventually throwing off the homeostatic balance by changing the rate at which cells divide and die [62]. With advancements in technology, the following factors make cancer treatment difficult: (i) due to proto-oncogene mutation, cancer cells and proteins proliferate; (ii) growth inhibition signals are rejected; (iii) evade apoptosis or activate anti-apoptotic genes in cells [63]. It is crucial to monitor disease-specific biomarkers for early cancer diagnosis. Biomarkers are traits or biomolecules that are over-expressed during the onset of carcinogenesis, either by the tumour cell itself or by the

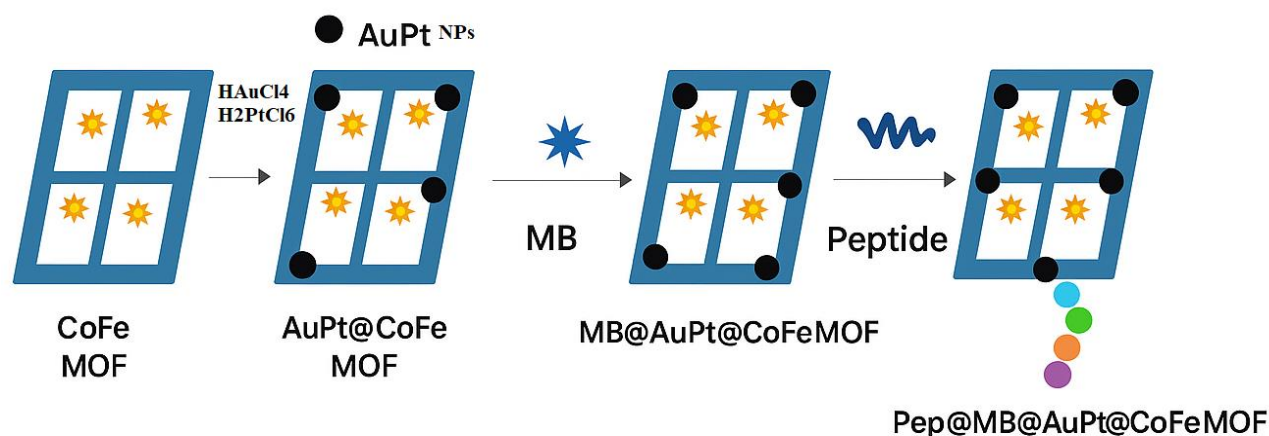


Fig. 3: Stepwise schematic illustration of the synthesis and surface functionalization of a CoFe MOF-based nanoplatform. Initially, AuPt nanoparticles (AuPt NPs) are anchored onto the CoFe MOF framework to form AuPt@CoFe MOF. Subsequently, methylene blue (MB) is introduced for electrochemical signaling, followed by the conjugation of a specific peptide, yielding the final construct Pep@MB@AuPt@CoFe MOF for targeted biosensing applications . . Source :Redrawn with modification from [1]

immune system reacting to the illness [64-65]. Biomarkers may be employed to evaluate the body's reactions to a particular disease-control therapy regimen [66]. To identify malignancies and ascertain prognostic factors, a wide array of biomarkers encompassing genetic, proteomic, glycomic and other factors are extensively researched [67]. Utilizing noninvasive and cost-effective cancer screening techniques is imperative for evaluating numerous biomarkers in bodily fluids like serum, blood, urine, saliva, tears and sputum [68]. Consequently, despite clinical samples and analyses being in the early stages, the electrochemical-based detection of biomarkers has emerged as an effective means for early cancer diagnosis. MNPs and MNPCs are the most common types of materials used for electrode modification. For biomolecule conjugation, MNPs offer enhanced surficial stability, enhanced biocompatibility and enhanced binding affinity [62]. The surface immobilisation of biomolecules (antibody, peptide or aptamer) to create immune or aptasensors is contingent upon the biomolecule's functioning and the kind of nanostructured electrode modifiers employed. These two require compatibility with one another [69]. For instance, by activating the thiol group (SH)-Au link, AuNPs enable thiol-functionalized antibodies and aptamers to be attached across the electrode surface. This is a key approach followed in most electrochemical bio-affinity sensor preparations [70]. AuNPs play a crucial role in creating bioaffinity electrochemical sensors. They activate a specific group called thiol (SH), forming a link with AuNPs. This link allows thiol-functionalized antibodies and aptamers to attach to the electrode surface.

This approach is commonly used in preparing electrochemical bioaffinity sensors. Bio-recognition elements (BRCs) such as aptamers, peptides and antibodies, bind to nanostructured electrode materials [71-75]. These BRCs can efficiently capture antigens or biomarkers, facilitating the creation of bio-affinity electrochemical biosensors [76-78].

5.2 Applications in point of care testing devices: A point-of-care testing (POCT) device allows medical professionals and individuals to rapidly and precisely diagnose diseases and monitor health problems. Also, widespread POCT deployment would pave the way for the provision of personalized health care [80]. This will help identify β -amyloids early in Alzheimer's disease patients, senescent β -cells in type I diabetic patients, and cancer biomarkers [81]. As a result, ECB research has concentrated on creating biosensor prototypes that may be quickly and easily analyzed using mobile or other handy electronic systems [82]. The portable potentiostat, EmStat3 and electrochemical biosensors (ECBs) that use magnetic nanoparticles (MNPs) or magnetic nanoparticle composites (MNPCs) generally show the compatibility between MNPs and BRCs can significantly enhance the stability of the developed ECBs. Fig. 4a depicts the sensor construction process along with its 2019-nCoV detection procedure. Initially, thiolated capture probes were immobilized on the surface of Au@Fe₃O₄ NPs (Premix A) for sandwich construction. In essence, the host-guest complex made use of an rGO system, AuNPs, CX8, TB and LP (label probe). The auxiliary probe (AP) was incorporated into the immobilized host-guest system

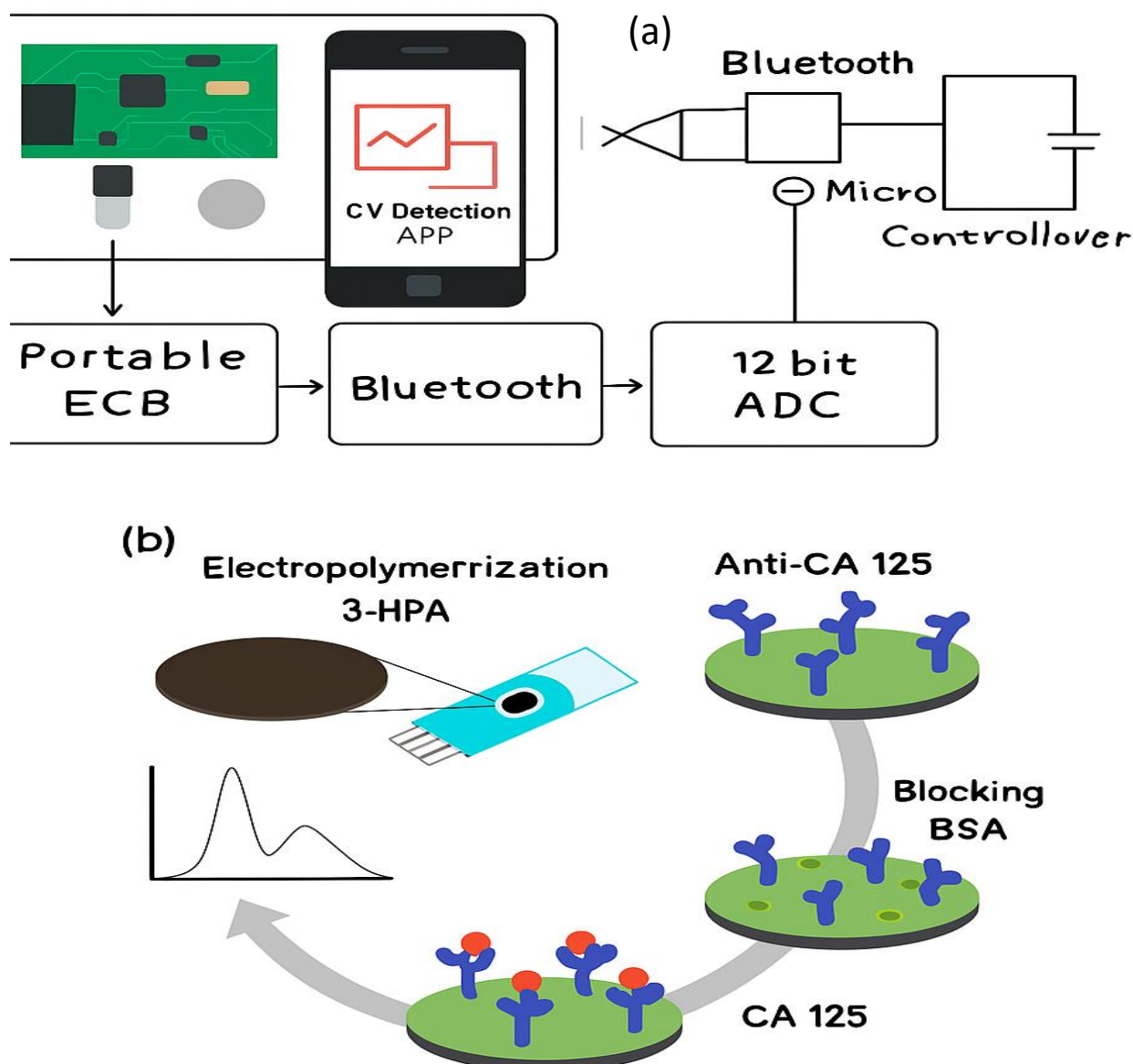


Fig. 4: Schematic illustration demonstrating the potential of point-of-care testing (POCT) using a portable electrochemical biosensor (ECB) system. (a) A compact ECB device integrated with a smartphone via Bluetooth for glucose detection using cyclic voltammetry (CV), showcasing the internal components including ADC and microcontroller. (b) Stepwise functionalization process of a biosensor for CA 125 detection: electropolymerization with 3-HPA, antibody immobilization, blocking with BSA, and final antigen binding, enabling sensitive biomarker identification [64].

for final adjustments (Premix B). The 2019-nCoV RNA was then incubated for 1 h with Premix A and 2 h with Premix B. When Premix A and B were combined, the 2019-Mag-biosensors with magnetic nanoparticles (MNPs) had a wider linear range, higher sensitivity, and more stability than those without MNPs. However, it is crucial to carefully select MNPs that are compatible with specific bio-recognition elements (BRCs).

SPCE*/AuNP-MnO₂/Immunosensor LSV showed noticeably higher DPV current signals than when Premix B was used alone. Reverse transcription real-time polymerase chain reaction (RT-qPCR) was not as effective as this sensor in detecting 2019-CoV in patients in recovery or those with active COVID-19. Furthermore, it may be incorporated into a plug-and-play smartphone point-of-care testing (POCT) system [83-84] (Fig. 4A). Moreover,



Castro *et al.* [64] (Fig. 4B) also reported the development of label-free immunosensor strips for the sensitive detection of CA125 cancer biomarkers from human blood samples [64,87].

5.3 Detecting hydrogen peroxide with bimetallic nanoporous gold electrodes: Khan *et al.* [88] reported the development of a bimetallic nanoporous Pt-Au composite electrode designed for detecting H_2O_2 , particularly when albumin is present, for biofouling situations. The Pt-Ag alloy was electro-deposited onto a gold electrode to create the composite electrode, which was then dealloyed and heated to a high temperature. As compared to planar Pt or NPG, the NP-Pt (Au) electrode demonstrated quicker kinetics for H_2O_2 reduction. Cyclic voltammetry revealed that the bimetallic NPG electrode needed a significantly lower overpotential to reduce H_2O_2 . Albumin, a typical biofouling agent, did not significantly reduce the electrode's ability to detect H_2O_2 . H_2O_2 may be detected due to the unique porous structure of the bimetallic nanoporous electrode and biofouling conditions may occur [84,85,95]. Other methods have been used for H_2O_2 biosensing besides Pt-NPG, for the electrochemical reduction of H_2O_2 . Ke *et al.* [89] reported a 3D NPG/Ni foam hybrid electrode for the electrochemical deposited with an Au-Sn alloy, which was then dealloyed. The electrode's durability in detecting H_2O_2 in an acidic medium was enhanced, and the NPG increased the stability of the Ni foam in an acidic solution [87]. It was also detected using a NPG electrode, which showed high sensitivity in alkaline circumstances. In this instance, the composite electrode was created using a one-pot method. As an additional illustration, a freestanding NPG was created utilizing the ALD technique, permitting the detection of H_2O_2 with sensitivity and selectivity [88]. Several figures of merit for recently used bimetallic NPG electrodes for H_2O_2 electrochemical detection.

5.4. Trimetallic nanoparticles for a next-generation glucose monitoring device: Metal nanoparticles have been the focus of extensive research in recent years due to their distinctive physical and chemical characteristics, which can be tailored to specific needs. Noble metals like Pt [89], Au, Ag and Pd [88] have been prominently utilized in the creation of sensitive glucose sensors, alongside other nanoparticles being explored [89]. However, since they are frequently costly, these nanomaterials are not widely available. Furthermore, single metal nanoparticles don't exhibit the high levels of specificity, selectivity and sensitivity required for ongoing glucose monitoring. In order to

address these drawbacks, numerous studies have created nanoporous heterogeneous structures that enhance the catalytic efficiency of the sensors by combining multiple nanomaterial structures, resulting in bimetallic and trimetallic nanomaterials that can be used for the detection of glucose. Thus, several investigations have been conducted to create trimetallic nanoporous materials by partially substituting base metals or carbon nanoparticles for noble metals. These structures are low-cost and exhibit excellent catalytic efficiency and extended shelf life [90-93]. Furthermore, increased nanocomposite dispersion increases the catalytic efficiency and conductivity over the sensor's given surface area. Moreover, due to the unique characteristics of the individual metals involved, trimetallic nanostructures exhibit distinctive and enhanced properties [94-96].

6.0 Biocompatibility and Toxicity of nanomaterial based biosensors for medical Applications

6.1 Real-world implementation and biocompatibility considerations: Liu *et al.* [96] developed a portable sensor for real-time detection of breath acetone using In_2O_3 -Pt core-shell nanowires, which operate at 320 °C and are resistant to humidity. Another sensor was introduced with enhanced temperature and response times, maintaining high stability over 150 days. Broek *et al.* [97] described a selective isoprene breath sensor featuring an alumina powder filter, offering quick response and a low detection limit. Park *et al.* [99] employed Co_3O_4 -polyoxometalate yolk-shell metalloidal nanoparticles for isoprene detection, achieving rapid response and low detection limits even in humid conditions. A gas sensor utilizing BNP technology for diabetes monitoring is capable of detecting both acetone and formaldehyde at the same time, even when other gases are present in the breath [98] (Table-4). This sensor boasts low detection limits between 30 and 45 ppb, which are on par with current commercial gas sensors. SnO_2 nanosheets were created using a hydrosolvothermal process and were enhanced with Pd, Au and Au-Pd BNPs, ensuring they are reusable, dependable and resistant to humidity and other breath components. The SnO_2 decorated with AuPd surpassed those with single metal decorations due to chemical sensitization, Pd's electrical sensitisation and the synergistic effects of AuPd bimetal.

6.2. Cost-effectiveness and regulatory challenges: The expenses related to the development, production and implementation of biosensor technology can be substantial,



TABLE 4
NANOMATERIALS-ENABLED BIOSENSORS IN ENVIRONMENTAL MONITORING AND REAL-WORLD APPLICATION

Application Area	Type	Nanomaterial/Technology Used	Purpose/Detection Target	Reference
Plastic Pollution	Electrochemical Impedance Spectroscopy (EIS)	Cyanobacterial EPSs on gold electrode	Identifying microplastics (0.1 μm –1 mm) in water systems	[100]
	Surface Acoustic Wave (SAW) Sensor	Lithium niobate	Enhanced detection of polystyrene nanoplastics	[101]
	Microwave Sensors	Split-ring resonator + coplanar waveguide	Tracking microscale plastic pollution; real-time monitoring of cell-size changes	[102]
	Electrochemical Sensor	Screen-Printed Carbon Electrodes (SPEs)	Detecting hydroquinone, BPA, and catechol in water	[103]
	Hyperspectral Sensors	Narrow SWIR + specialized absorbance bands	Differentiating types of plastic pollution	[103]
Eutrophication	Optical Diode Sensor	Paired Emitter-Detector Diodes (PEDD)	Enhanced turbidity sensing; cost-effective eutrophication monitoring	[104]
	Microbial Fuel Cell (MFC) Sensor	Sensor Ceramic-based autonomous device	Detection of dissolved oxygen, nitrate, and algal bloom conditions	[105]
Climate Change & PV Monitoring	Opto-chemical Sensor	Fluorescent dye in fluoro resin membrane	Tracking acetic acid in PV modules; pH sensor pH sensor resistant to moisture and heat	[107]
	Thermo-sensor	ZnO-coated fiber-optic nanostructure	High-sensitivity temperature monitoring in supercapacitors (30°C–90°C)	[108]
	Integrated Sensor	Si Solar cell + stress/temperature sensors	Monitoring temperature and mechanical stress in solar panels	[106]
Medical Diagnostics	Plasmonic Sensor	Graphene + TiO ₂ composite	Detecting early-stage cancer cells from various organs	[109-110]
	SERS Biosensor	Gold-coated polystyrene nanospheres	Non-invasive breast cancer detection from human tears	[111]
	Microfluidic Nano-biosensor	Integrated microfluidics platform	Detecting breast cancer via extracellular vesicle microRNA	[112]
	SPR-based Sensor	Surface Plasmon Resonance (SPR) Platform	Identifying miR-15a for rheumatoid arthritis	[113]
	Electrochemical Sensor	Polyhydrogel film	Electrochemical detection of osteoarthritis	[113]
Tissue Engineering	Enzyme Biosensor	Platinum wire	Monitoring implant integrity in brain tissue	[114]
	Amperometric Sensor	Various bio-analyte detection methods	Detecting dopamine, ascorbic acid, glucose, oxygen, and NO in implants	[247]
Viral & Bacterial Detection	Opto-microfluidic Sensor	Quantum Dots + Microfluidics	COVID-19 detection; enhanced fluorescence for H5/H9 influenza detection	[115]
	Electrochemical Immunosensor	TNF- α -specific electrode	Monitoring wound healing, inflammation	[116]
	Immunological Sensor	Gold-based screen-printed electrode	Detecting of <i>E. coli</i> in drinking water	[117]
Food Safety	Electrochemical Sensor	Gold-DNA conjugated electrode	Identification of pork (<i>Sus scrofa</i> mtDNA) in food	[118]
	Fluorescence Biosensor	Carboxylated Porphyrin compounds	Detecting aflatoxin B1 in pharmaceutical products	[119]
Glucose Monitoring	Non-enzymatic Sensor	CuO nanoleaves on glassy carbon electrode	Glucose detection with excellent sensitivity and stability	[120]
	Electrochemical Sensor	Cu nanoflowers on GO nanofibers	Catalytic detection of glucose to gluconic acid	[121]
Precision Agriculture	Smart Nano-biosensor	Nanobionics and signaling sensors	Real-time plant health monitoring; GPS-enabled for remote agriculture management	[123]
	Phytohormone Sensors	Nanomaterials for hormone flow detection	Identifying stress and disease in crops for smart farming	[122]



limiting its widespread adoption. Achieving cost-effective biosensor production while maintaining high performance and quality is a challenging endeavor. Also, the scalability, especially in terms of large-scale manufacturing and deployment, presents logistical and economic hurdles. In Japan and China, scientists are working on biosensors designed to identify cancer cells in the bloodstream, enhance air quality and track athletes' heart rates, body temperatures and hydration levels [124]. Meanwhile, in Australia, these biosensors are capable of detecting endangered species within their natural environments, supporting conservation initiatives [135]. Across Africa, portable biosensors facilitate the swift identification of diseases such as malaria, HIV and Ebola, allowing for early diagnosis and treatment, which leads to improved health outcomes. In Europe, wearable biosensors are utilized to monitor an athlete's heart rate, body temperature and hydration levels, offering crucial data to enhance performance and prevent injuries [125].

The deployment of biosensors across different sectors raises ethical concerns related to privacy, data protection, and informed consent. Striking a balance between the benefits of biosensing technology and the protection of individual rights and autonomy is a challenging task that necessitates robust ethical guidelines. Tackling these particular limitations requires collaboration across disciplines, technological advancements and continuous research efforts. Successfully addressing these challenges will unlock the full potential of biosensors, enabling their seamless integration into various domains and driving transformative advancements in healthcare, environmental monitoring, and beyond.

7.0 Life Cycle Assessment and Industrial Adoption

The global nanotechnology market is projected to expand by 34% by 2030 [126,127], with a significant rise in new journals and research papers since 2000, a trend expected to persist due to emerging applications [128]. As environmental awareness grows, researchers must evaluate materials' functionality and their production's ecological consequences. Life cycle assessment (LCA) is a valuable tool for evaluating the environmental impacts of manufacturing processes, product use phases, and end-of-life scenarios. However, there is a notable gap in the literature regarding the LCA of fabrication methods. Several studies have explored the LCA of various fabrication techniques, identifying major environmental pollutants. For instance, Nturos

et al. [136] investigated the LCA of different synthetic routes for ZIF-8 nanomaterials, examining five representative fabrication methods, including using various solvents. Their findings revealed that DMF and methanol were significant environmental pollutants, contributing to over 85% of the total environmental impacts in some fabrication routes.

Furthermore, power consumption during synthesis emerged as a critical ecological concern, accounting for up to 13% of the total environmental impacts. The study suggests employing alternative, clean, and renewable energy sources to optimize energy consumption impacts. The most significant impact on water consumption was noted. However, using life cycle assessments (LCA) faces challenges like missing data on life cycle inventories, insufficient details on how synthetic processes work and a lack of scientific studies on recycling nanotechnology materials. The increasing number of publications, companies and applications of nanomaterials calls for heightened environmental awareness [129]. While physical fabrication techniques are considered environmentally friendly, there is a scarcity of studies that quantify and validate this claim.

Biosensors made from valuable metal combinations are very sensitive and selective. Still, their use in industry is limited because of several problems *viz.* the high cost of precious metals, complicated production processes and poor recycling options. Teeba & Abd Ali investigated issues such as scalability limitations, delays in regulatory approvals, and the necessity to meet standards like ISO 10993 (biocompatibility) and ISO 13485 (medical device manufacturing) further hinder their commercialization [130]. For practical application, biosensor platforms need to prove their analytical reliability, economic feasibility, manufacturability and environmental safety. Future research should focus on eco-friendly synthesis methods, standardization protocols, and industrial pilot trials to bridge the gap between laboratory research and market readiness. Although biosensors made from noble metal-based bimetallic and trimetallic materials exhibit excellent sensitivity and selectivity, their broader industrial use is currently limited by several challenges: the high expense of precious metals, intricate multistep synthesis processes and inadequate recycling systems.



8.0. Biocompatibility and Toxicity of Nanomaterials in Biosensors

Nanomaterials play a crucial role in enhancing the functionality of biosensors, especially when analysing molecules such as bacteria, viruses, and DNA. Researchers are investigating the stability, encapsulation, and storage methods of 2D materials to find effective solutions. Polyether compounds can be employed for *in-situ* encasement and grafting of nanomaterials, while enzymatic biosensors based on platinum wire are useful for assessing the integrity of tissue transplants. Gold nanoparticles are extensively used in electrochemical biosensors as carriers for signalling molecules or probes [131]. Despite this, the cytotoxicity of nanomaterials has not been thoroughly examined in most research, pointing to potential risks to human cells. The cytotoxicity of nanocomposites is largely influenced by their chemical and physical properties, which are determined by the synthesis process and the reagents or precursors used. The graphene-zirconia composite in this study was produced using an eco-friendly method, ensuring high biocompatibility [132]. Nanobiosensors are designed to support and enhance environmental management practices and regulatory frameworks, but they might also release organic compounds that could contaminate the natural environment. The proper use of nanobiosensors carries risks to both ecological systems and human health, potentially resulting in legal issues [133]. Implementing nano-biosensing systems for environmental monitoring may result in the emission of some carbon-based organic molecules, including synthetic materials such as pesticides and polychlorinated biphenyls (PCBs), which can hurt the environment. For environmental monitoring, it is essential to set up an efficient process for managing, addressing and getting rid of wastes or organic pollutants in the nanomaterials or chemicals that comprise NBS [134].

9.0. Conclusion (research challenges, future trends and specific recommendations)

The advancement of bimetallic and trimetallic nanomaterials based biosensors has played a crucial role in creating highly sensitive and selective electrochemical biosensors. A primary issue is these nanomaterials' sustainable and consistent production, which frequently involves hazardous substances, costly precursors, and intricate processes. Although trimetallic nanoparticles offer improved performance due to synergistic effects, they are still not widely used in eco-friendly synthesis methods. The transition of these

biosensors from lab prototypes to clinical or industrial use is impeded by challenges such as limited long-term stability, interference from biological matrices and the necessity for antifouling strategies. Regulatory hurdles and the high cost of noble metals further complicate commercialization efforts. Emerging trends, including the adoption of green chemistry principles in nanoparticle synthesis, will influence the future of this field. Artificial intelligence, particularly machine learning, is expected to transform biosensor performance by improving data analysis, pattern recognition, and time decision-making hybrid and multifunctional sensing platforms and integration of these nanomaterials into wearable or point-of-care devices is anticipated. To bridge the gap between research and application, future research should focus on developing standardised and scalable synthesis methods for trimetallic nanoparticles, emphasising environmental sustainability. Working together, material scientists, doctors and regulatory experts will be essential in creating biosensors that fulfil medical requirements and follow regulations. Promoting small-scale testing and partnerships with industries will be necessary to confirm how well these systems work, how easy they are to make and whether they are affordable.

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