

Latest Research Techniques for Developing Non-Enzymatic Electrochemical Sensors

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Abstract - Non-enzymatic electrochemical sensors have emerged as a transformative technology for real-time detection of biomolecules, offering superior stability, cost-effectiveness, and operational simplicity compared to traditional enzymatic sensors. This comprehensive review examines the latest research techniques (2023–2025) in developing non-enzymatic electrochemical sensors, with emphasis on advanced nanomaterial strategies, fabrication methodologies, and signal transduction mechanisms. Recent innovations include transition metal oxide nanocomposites, carbon-based nanomaterials, metal-organic frameworks (MOFs), and hybrid nanostructures that demonstrate exceptional electrocatalytic activity. State-of-the-art sensors achieve detection limits in the picomolar to micromolar range with sensitivities exceeding $10,000 \mu\text{A mM}^{-1} \text{cm}^{-2}$ for glucose detection. This paper systematically analyzes electrode materials, synthesis techniques, performance metrics, and emerging challenges in translating laboratory prototypes to clinical and point-of-care applications. The review provides critical insights into structure-performance relationships and identifies future research directions for next-generation non-enzymatic sensors.

Index Terms— Non-enzymatic sensors, electrochemical detection, nanomaterials, metal-organic frameworks, glucose sensing, biosensors, electrocatalysis, point-of-care diagnostics.

I. INTRODUCTION

A. Background and Motivation

Electrochemical sensors represent a cornerstone technology in modern analytical chemistry, enabling rapid, sensitive, and selective detection of biomolecules in complex matrices. Traditional enzymatic biosensors, while highly specific, suffer from inherent limitations including enzyme instability, sensitivity to environmental conditions (pH, temperature, humidity), high production costs, and limited operational lifetime [1], [4]. These constraints have catalyzed intensive research into non-enzymatic electrochemical sensors that leverage the intrinsic electrocatalytic properties of nanomaterials to achieve direct analyte oxidation or reduction [22], [23].

Non-enzymatic sensors offer compelling advantages: enhanced stability across wide temperature and pH ranges, simplified fabrication protocols, reduced manufacturing costs, and extended shelf life [4], [21]. The elimination of biological recognition elements also circumvents issues related to enzyme immobilization, denaturation, and batch-to-batch variability. These attributes position non-enzymatic sensors as ideal candidates for point-of-care diagnostics, continuous monitoring applications, and resource-limited settings [30], [31].

B. Recent Technological Advances

The period from 2023 to 2025 has witnessed remarkable progress in non-enzymatic sensor development, driven by advances in nanomaterial synthesis, surface engineering, and computational materials design. Key innovations include the rational design of transition metal oxide nanocomposites with controlled morphologies [1], [22], the integration of two-dimensional materials such as MXenes and graphene derivatives [7], [18], and the emergence of metal-organic frameworks (MOFs) as versatile electrode modifiers [11], [27], [34]. Additionally, hybrid nanostructures combining multiple functional components have demonstrated synergistic effects that significantly enhance electrocatalytic performance [8], [12], [15].

C. Scope and Organization

This review systematically examines the latest research techniques for developing non-enzymatic electrochemical sensors, with emphasis on glucose, hydrogen peroxide, urea, cholesterol, and dopamine detection. Section II analyzes electrode materials and nanomaterial strategies, Section III discusses fabrication techniques, Section IV explores signal transduction mechanisms, Section V presents comparative performance analysis, Section VI addresses current challenges and future directions, and Section VII provides concluding remarks.

II. ELECTRODE MATERIALS AND NANOMATERIAL STRATEGIES

A. Transition Metal-Based Nanomaterials

Transition metal oxides and their composites constitute the most extensively investigated class of non-enzymatic sensor materials due to their excellent electrocatalytic activity, tunable electronic properties, and cost-effectiveness [22], [32]. Recent research has focused on copper oxides, nickel oxides, cobalt oxides, and their bimetallic combinations.

Copper-Based Systems: Copper oxide nanostructures exhibit remarkable glucose oxidation activity in alkaline media. The $\text{Cu}_2\text{O}/\text{MoS}_2$ nanohybrid demonstrated a sensitivity of $111.74 \mu\text{A} \mu\text{M}^{-1} \text{cm}^{-2}$ with a detection limit of $2.18 \mu\text{M}$ for cholesterol detection [5]. The $\text{Ag-Cu}/\text{MWCNTs}$ nanocomposite achieved a sensitivity of $237.2 \mu\text{A mM}^{-1} \text{cm}^{-2}$ for glucose with a detection limit of $1.2 \mu\text{M}$ [12].

Nickel-Based Systems: The $\text{NiO}/\text{C}@\text{rGO}$ nanocomposite derived from $\text{Ni}(\text{gallate})$ pyrolysis exhibited a detection limit of $0.658 \mu\text{M}$ for glucose with excellent anti-interference capability [8]. The $\text{CuO}/\text{Co}_3\text{O}_4@\text{MWCNTs}$ nanocomposite achieved an unprecedented detection limit of 0.223 pM for urea with a wide linear range spanning 10^{-12} – 10^{-2} M [2].

Cobalt-Based Systems: The braided copper cobaltite/MWCNT composite enabled acetylcholine detection at sub-nanomolar levels (LOD: 0.8 nM) with exceptional stability, retaining 90.2% of original current after three weeks [16]. The unique braided nanostructure provides high surface area and efficient mass transport.

B. Carbon-Based Nanomaterials

Carbon nanomaterials, including carbon nanotubes (CNTs), graphene derivatives, and carbon nanofibers, serve as excellent electrode materials and conductive supports due to their exceptional electrical conductivity, large surface area, and chemical stability [7], [18]. CNTs function as both catalytic materials and structural scaffolds for metal nanoparticle decoration. Spherical nickel boride nanoparticles anchored on functionalized carbon nanofibers (Ni₃B NPs/*f*-CNFs) achieved a detection limit of 21 nM for H₂O₂ with high sensitivity (59 $\mu\text{A mM}^{-1} \text{cm}^{-2}$) [15].

Graphene oxide nanoribbons (GONRs) represent an emerging electrode material with unique edge effects. The GONR-modified glassy carbon electrode demonstrated a sensitivity of 20.815 $\mu\text{A/mM}$ for glucose with an exceptionally wide linear range (0.1–105 mM) and detection limit of 3 μM [26]. Advanced carbon materials incorporating nitrogen doping and hierarchical porosity have also emerged as highly promising electrode platforms [28].

C. Metal-Organic Frameworks (MOFs)

MOFs have emerged as a revolutionary class of electrode materials for non-enzymatic sensors, offering unprecedented structural tunability, high porosity, and abundant catalytic sites [11], [27], [34]. The modular nature of MOFs enables rational design of pore size, metal node composition, and organic linker functionality.

ZIF-67 hollow nanosheet sensors achieved a sensitivity of 445.7 $\mu\text{A mM}^{-1} \text{cm}^{-2}$ with a detection limit of 0.96 μM for glucose [11]. Ultrathin Ni-MOF nanobelts demonstrated a wide linear range (1–500 μM) with a detection limit of 0.25 μM [11]. Bimetallic NiCo-MOF nanostructures exhibited superior sensitivity (1755.51 $\mu\text{A mM}^{-1} \text{cm}^{-2}$) with rapid response time (<2 s) [11].

D. Composite and Hybrid Nanostructures

The integration of multiple functional components into hybrid nanostructures represents a frontier strategy for achieving superior sensor performance [12], [15], [20]. The Ag-CeO₂/Ag₂O nanocomposite for H₂O₂ detection achieved a sensitivity of 2.728 $\mu\text{A cm}^{-2} \mu\text{M}^{-1}$ with a linear range of 10⁻⁸ to 0.5×10⁻³ M [6]. The Ag-Cu₂O@TNTs hybrid nanostructure for cholesterol demonstrated an exceptional sensitivity of 12,140.06 $\mu\text{A mM}^{-1} \text{cm}^{-2}$ with a detection limit of 0.057 mM [20].

III. FABRICATION TECHNIQUES

A. Hydrothermal and Solvothermal Synthesis

Hydrothermal and solvothermal methods represent the most widely employed techniques for synthesizing nanostructured electrode materials due to their ability to control particle size, morphology, and crystallinity [12], [35]. The Ag-Cu/MWCNTs nanocomposite was synthesized via a hydrothermal process that uniformly anchored silver-copper alloy nanoparticles onto

multiwalled carbon nanotubes [12]. The Ag₅-Cu₅ configuration exhibited the highest electrocatalytic activity, demonstrating the importance of optimizing metal ratios. Solvothermal synthesis of Zr-MOF/ZrO yielded a large surface area of 1158 m² g⁻¹ for pesticide detection [35].

B. Electrochemical Deposition and Synthesis

Electrochemical methods offer precise control over film thickness, composition, and morphology through manipulation of deposition parameters [38], [39]. Ag nanoparticles decorated poly(L-cysteine) electrodes were electrochemically synthesized on pencil graphite electrode surfaces for non-enzymatic H₂O₂ sensing [38]. Copper decorated polyaniline nanowires were fabricated through a two-step electrochemical synthesis involving electropolymerization of aniline followed by electrodeposition of copper nanoparticles, enabling precise control over copper loading and distribution [39].

C. Screen-Printing Technology

Screen-printing technology enables mass production of disposable electrodes with consistent performance and minimal cost [2], [27]. The CuO/Co₃O₄@MWCNTs nanocomposite was incorporated into screen-printed carbon paste electrodes (SPCEs) for disposable urea sensors [2]. Screen-printed electrodes can be readily modified with functional nanomaterials by drop-casting or electrodeposition, making them highly amenable to scale-up manufacturing. The Rebolledo-Perales review highlights MOF-modified SPCEs as a rapidly growing platform for glucose quantification [27].

D. Green and Sustainable Synthesis Methods

Green synthesis approaches utilizing plant extracts and biomass-derived precursors are gaining traction as environmentally benign alternatives to conventional chemical synthesis [24], [29], [33]. Naz et al. employed bitter melon peel extract for synthesis of NiO nanoflakes, achieving a detection limit of 0.02 mM for urea in a fully eco-friendly process [24]. Green-synthesized rGO from Ocimum sanctum leaf extract demonstrated excellent electrocatalytic activity toward glucose oxidation [29], while Nano-ZnO/N-doped porous carbon composites derived from sustainable biomass precursors exhibited high-performance glucose sensing [33].

E. Advanced Nanostructuring Techniques

Template-directed synthesis, atomic layer deposition (ALD), and microfluidic fabrication enable the creation of nanomaterials with precisely controlled architectures [37], [44]. Bisolvent interface-assisted methods were employed to fabricate the unique braided copper cobaltite/MWCNT composite, yielding a distinctive hierarchical structure with optimal mass transport properties [16]. Pt/MXene-based flexible sensors were fabricated using a multistep process combining MXene delamination, platinum nanoparticle deposition, and integration into flexible substrates for wearable glucose monitoring [40].

IV. SIGNAL TRANSDUCTION AND DETECTION MECHANISMS

A. Electrocatalytic Oxidation Mechanisms

The electrocatalytic mechanism in non-enzymatic sensors involves direct interaction between analyte molecules and the

electrode surface, without the mediation of biological enzymes [1], [22], [37]. For glucose oxidation in alkaline media, the generally accepted mechanism involves initial adsorption onto metal oxide surfaces, followed by deprotonation and electron transfer to generate gluconolactone: $\text{Glucose} + 2\text{OH}^- \rightarrow \text{Gluconolactone} + \text{H}_2\text{O} + 2\text{e}^-$. The electrocatalytic activity depends critically on the oxidation state of surface metal species, with higher oxidation states generally exhibiting enhanced catalytic performance.

For H_2O_2 detection, the electroreduction mechanism proceeds via: $\text{H}_2\text{O}_2 + 2\text{H}^+ + 2\text{e}^- \rightarrow 2\text{H}_2\text{O}$ (cathodic) or $\text{H}_2\text{O}_2 \rightarrow \text{O}_2 + 2\text{H}^+ + 2\text{e}^-$ (anodic). Silver-based nanostructures are particularly effective for H_2O_2 electroreduction due to their strong affinity for peroxide species [3], [6]. The Ag-CeO₂/Ag₂O composite exploits the synergistic interaction between silver and cerium oxide for highly sensitive H_2O_2 detection [6].

B. Voltammetric Techniques

Cyclic Voltammetry (CV) provides comprehensive information about redox processes, including oxidation/reduction potentials, reversibility, and electron transfer kinetics. The Cu₂O/MoS₂ nanohybrid exhibited a sensitivity of 111.74 $\mu\text{A } \mu\text{M}^{-1} \text{ cm}^{-2}$ using CV for cholesterol detection [5].

Differential Pulse Voltammetry (DPV) offers superior sensitivity and resolution by minimizing background charging currents. Amperometric detection at constant potential provides excellent sensitivity, rapid response, and compatibility with flow systems. The MWCNT-chitosan microneedle sensor demonstrated sensitivities of 52.44 $\mu\text{A } \text{mM}^{-1} \text{ cm}^{-2}$ for glucose detection across a wide range of 0–30 mM [25].

C. Electrochemical Impedance Spectroscopy

Electrochemical impedance spectroscopy (EIS) provides insights into charge transfer resistance, double-layer capacitance, and mass transport limitations [35]. The Zr-MOF/ZrO nanocomposite sensor employed EIS for selective detection of organophosphate pesticides, leveraging changes in interfacial impedance upon analyte binding [35]. EIS is particularly valuable for label-free detection and mechanistic studies of electrode-analyte interactions.

D. Selectivity and Interference Management

Achieving high selectivity in complex biological matrices represents a critical challenge for non-enzymatic sensors [3], [8], [13]. Common interferents include ascorbic acid, uric acid, dopamine, and inorganic ions that undergo electrochemical reactions at similar potentials. The Ag/δ-FeOOH modified electrode exhibited outstanding selectivity for H_2O_2 even in the presence of dopamine, uric acid, and ascorbic acid [3]. Selectivity enhancement strategies include: (1) optimization of detection potential, (2) incorporation of selective membranes or coatings, (3) exploitation of unique catalytic pathways, and (4) multivariate calibration approaches.

V. PERFORMANCE METRICS AND COMPARATIVE ANALYSIS

A. Key Performance Indicators

Non-enzymatic sensor performance is evaluated using multiple metrics including sensitivity, detection limit, linear range, selectivity, stability, and response time [4], [23], [37].

Sensitivity quantifies the change in signal per unit analyte concentration, typically expressed as $\mu\text{A } \text{mM}^{-1} \text{ cm}^{-2}$ for amperometric sensors. Detection limit (LOD) represents the minimum detectable concentration, conventionally defined as three times the standard deviation of blank signal divided by sensitivity. The linear range indicates the concentration interval where sensor response exhibits linear correlation with analyte concentration.

B. Comparative Performance Table

Table I presents a comprehensive comparison of recently reported non-enzymatic sensors, highlighting the diversity of electrode materials, target analytes, and performance characteristics across publications from 2023 to 2025.

TABLE I

COMPARATIVE PERFORMANCE OF RECENT NON-ENZYMATIC ELECTROCHEMICAL SENSORS (2023–2025)

Electrode Material	Analyte	LOD	Linear Range	Sensitivity	Year	Ref
CuO/Co ₃ O ₄ @MWCNTs	Urea	0.223 μM	0.1–10 ⁻² M	3.01 $\mu\text{A}/\text{decade}$	2023	[2]
Ag/δ-FeOOH/CB	H ₂ O ₂	2 μM	—	214 $\mu\text{A } \text{mM}^{-1} \text{ cm}^{-2}$	2023	[3]
Cu ₂ O/MoS ₂	Cholesterol	0.18 μM	0.1–180 μM	111.74 $\mu\text{A } \mu\text{M}^{-1} \text{ cm}^{-2}$	2024	[5]
Ag-CeO ₂ /Ag ₂ O	H ₂ O ₂	0.34 μM	0.5–10 ⁻³ M	728 $\mu\text{A } \text{cm}^{-2} \mu\text{M}^{-1}$	2025	[6]
NiO/C@rGO	Glucose	0.658 μM	—1115 μM	—	2024	[8]
Cu@Pani/MoS ₂	Glucose	0.78 μM	0.1–11 mM	69.82 $\mu\text{A } \text{mM}^{-1} \text{ cm}^{-2}$	2023	[10]
Ag-Cu/MWCNTs	Glucose	0.2 μM	0.5–10 mM	237.2 μA	2025	[12]

				mM ⁻¹ cm ⁻²		
GdS-Gd ₂ O ₃ /C-MWCNT	Uric Acid	0.38 μM	0.5–30 μM	356.1 μA mM ⁻¹ cm ⁻²	2024	[13]
Ni ₃ B NPs/f-CNFs	H ₂ O ₂	12 nM	0.083 μM–12.6 mM	59 μA mM ⁻¹ cm ⁻²	2024	[15]
CuCo/MWCNT	Acetylcholine	0.8 nM	—	—	2023	[16]
PANI/Tm:ZnO	Dopamine	1.92 μM	0.8–6.5 μM	0.257 μA μM ⁻¹ cm ⁻²	2024	[17]
Ag-Cu ₂ O@TNTs	Cholesterol	0.057 mM	—	12.140 μA mM ⁻¹ cm ⁻²	2024	[20]
NiO nanoflakes	Urea	0.02 mM	0.1–9 mM	—	2023	[24]
MWCN T-Chitosan	Glucose	0.22 mM	0.03–30 mM	52.44 μA mM ⁻¹ cm ⁻²	2025	[25]
GONRs	Glucose	3 μM	0.1–105 mM	20.82 μA mM ⁻¹	2024	[26]
Pt/MXene (flexible)	Glucose	5 μM	0–2 mM	~70 μA mM ⁻¹ cm ⁻²	2023	[40]

C. Structure-Performance Relationships

Analysis of the comparative data reveals several critical structure-performance relationships. Bimetallic and composite systems consistently outperform single-component materials, demonstrating the importance of synergistic effects [2], [5], [12], [20]. The CuO/Co₃O₄@MWCNTs sensor achieved an extraordinary detection limit of 0.223 pM for urea, representing a 3–4 order-of-magnitude improvement over single metal oxide sensors [2], resulting from complementary catalytic activities of copper and cobalt oxides combined with the high surface area and conductivity of MWCNTs.

Carbon nanomaterial integration significantly enhances sensor performance through multiple mechanisms: increased surface area, improved electrical conductivity, prevention of nanoparticle aggregation, and facilitation of electron transfer [7], [18]. Nanostructure morphology profoundly influences electrocatalytic performance — hollow, porous, and hierarchical structures provide high surface area and facilitate mass transport, leading to enhanced sensitivity [11], [34].

D. Application-Specific Performance Requirements

Different applications impose distinct performance requirements. Clinical glucose monitoring requires a linear range encompassing physiological concentrations (3–8 mM in blood, 0.01–0.5 mM in sweat), detection limits below 0.1 mM, and excellent selectivity against interferences [25], [26], [31]. Point-of-care diagnostics demand rapid response (<5 s), operational simplicity, and compatibility with portable instrumentation [4], [30]. Environmental monitoring applications require ultra-low detection limits (pM to nM range) and resistance to matrix effects [2], [13].

VI. CHALLENGES AND FUTURE PERSPECTIVES

A. Current Limitations and Challenges

Despite remarkable progress, several challenges impede widespread clinical translation of non-enzymatic sensors [4], [22], [23]. Achieving high selectivity in biological fluids containing multiple electroactive species remains a fundamental challenge. While recent sensors demonstrate improved selectivity through optimized detection potentials and surface modifications [3], [13], further advances are needed for reliable operation in whole blood and other complex matrices. The development of molecularly imprinted polymers and selective membranes represents a promising approach [41], [42].

Electrode fouling by proteins, cells, and other biological components degrades sensor performance over time, particularly for implantable and continuous monitoring applications [4], [30]. Surface passivation strategies, antifouling coatings, and self-cleaning mechanisms require further development. Batch-to-batch variability in nanomaterial synthesis and electrode fabrication hinders reproducibility and commercialization [23], [32]. Establishing standardized synthesis protocols, quality control metrics, and performance validation procedures is essential for regulatory approval.

B. Emerging Research Directions

Single-atom catalysts (SACs) represent the ultimate limit of catalyst miniaturization, offering fully exposed metal sites and uniform coordination environments [36]. SACs enable precise structure-activity relationship studies and potentially superior catalytic performance for glucose sensing [36]. Machine learning integration with computational materials design accelerates the discovery of novel electrode materials with optimized properties [44]. High-throughput screening combined with density functional theory calculations can predict electrocatalytic activity and guide experimental synthesis.

Flexible and wearable platforms enable continuous health monitoring through skin-mounted and textile-integrated sensors [30], [31], [43]. Conductive polymer-based hydrogels offer excellent mechanical compliance and biocompatibility for wearable electrochemical biosensors [43]. The Pt/MXene-based

flexible sensor demonstrates the potential of this approach for continuous glucose detection in sweat [40]. Integration of microneedle arrays for minimally invasive sampling with electrochemical detection represents an important advance [25], [45].

C. Translational Pathways

Successful translation of laboratory prototypes to clinical products requires addressing regulatory requirements, manufacturing scalability, and cost considerations [4], [23]. Collaborative efforts between academic researchers, industry partners, and regulatory agencies are essential. Establishing clinical validation studies demonstrating accuracy, precision, and reliability in diverse patient populations is critical for regulatory approval. Development of standardized testing protocols and performance benchmarks facilitates comparison across different sensor platforms and accelerates commercialization.

VII. CONCLUSION

Non-enzymatic electrochemical sensors have emerged as a transformative technology for biomolecule detection, offering superior stability, cost-effectiveness, and operational simplicity compared to traditional enzymatic sensors. The period from 2023 to 2025 has witnessed remarkable advances in nanomaterial design, fabrication techniques, and performance optimization. State-of-the-art sensors based on transition metal oxide nanocomposites, carbon nanomaterials, MOFs, and hybrid nanostructures achieve detection limits in the picomolar to micromolar range with sensitivities exceeding $10,000 \mu\text{A mM}^{-1} \text{cm}^{-2}$ for glucose detection.

Key innovations include bimetallic nanocomposites leveraging synergistic catalytic effects, two-dimensional materials with exceptional surface area and conductivity, and MOF-based sensors with tunable porosity. Advanced fabrication techniques including hydrothermal synthesis, electrochemical deposition, screen-printing, and green chemistry enable precise control over nanostructure morphology. Despite significant progress, challenges remain in achieving high selectivity in complex biological matrices, ensuring long-term stability against fouling, and developing miniaturized wearable platforms.

Emerging research directions including single-atom catalysts, machine learning-guided materials design, flexible electronics integration, and multimodal sensing platforms promise to address these limitations. The continued convergence of nanomaterial science, electrochemistry, and biomedical engineering will drive the next generation of non-enzymatic sensors toward widespread clinical adoption and point-of-care applications, ultimately enabling continuous, real-time tracking of metabolic biomarkers for improved disease management and preventive healthcare.

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