

Review

Electrochemical Hemoglobin Biosensors for Point-of-Care Diagnostics

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Abstract

Hemoglobin (Hb) and its glycosylated variant (HbA1c) are essential clinical biomarkers for diagnosing anemia, acute hemorrhage, and long-term glycemic control. The growing demand for decentralized point-of-care (POC) testing has accelerated the development of rapid, inexpensive, and portable electrochemical sensing platforms. These technologies exploit the intrinsic redox activity of the heme prosthetic group, yet achieving efficient direct electron transfer (DET) remains a fundamental challenge because the electroactive iron center is deeply embedded within the globin structure. This review critically examines recent advances in electrochemical Hb sensing, tracing the evolution of electrode architectures from conventional carbon substrates to nanostructured materials, including graphene, MXenes, metal–organic frameworks (MOFs), and molecularly imprinted polymers (MIPs). We compare the advantages and limitations of non-enzymatic biomimetic platforms and affinity-based sensing strategies, including aptamer- and antibody-based biosensors, with emphasis on electron-transfer efficiency, molecular selectivity, analytical performance, and suitability for POC implementation. Beyond analytical performance, we evaluate the principal barriers to clinical translation, including biofouling, whole-blood matrix effects, viscosity-dependent mass transport, manufacturing scalability, and the persistent gap between validation in synthetic media and performance in clinical samples. Finally, we discuss emerging applications in wearable menstrual health monitoring and ingestible gastrointestinal bleeding sensors, highlighting the integration of advanced electrochemical materials with miniaturized electronics as a pathway toward practical, consumer-oriented diagnostics.

Keywords: hemoglobin biosensors; electrochemical biosensing; direct electron transfer; point-of-care diagnostics; molecularly imprinted polymers



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1. Introduction

Hemoglobin (Hb), a tetrameric hemeprotein found in erythrocytes, serves as the primary vehicle for oxygen transport from the lungs to peripheral tissues [1]. Beyond its fundamental physiological role, the concentration of Hb in whole blood is a critical diagnostic indicator for a spectrum of pathological conditions [2–4]. Maintaining Hb levels within a narrow physiological range is vital as deviations often signal underlying health issues, most notably anemia, which affects over two billion people globally [4]. Rapid Hb monitoring is indispensable in emergency medicine for detecting acute hemorrhage and managing surgical patients [5].

Total Hb and glycated hemoglobin (HbA1c) serve distinct yet complementary roles in clinical diagnostics. Total Hb quantification provides an immediate snapshot of systemic oxygen carrying capacity, serving as a critical indicator for acute hemorrhage, surgical monitoring, and chronic anemia. In contrast, HbA1c represents the non-enzymatic glycation of the N-terminal valine residue on the β -chain of hemoglobin, occurring proportionally to ambient plasma glucose levels over the erythrocyte lifespan, which is about 120 days [3,6]. Clinically, HbA1c provides diagnostic advantages over transient glycemic markers such as fasting plasma glucose (FPG) or the oral glucose tolerance test (OGTT). While FPG and OGTT reflect acute, short-term glucose fluctuations heavily influenced by recent dietary intake, stress, physical activity, and acute illness, HbA1c yields a retrospective, weighted average of glycemic exposure over the preceding 2 to 3 months [7]. Furthermore, HbA1c assays do not require patient fasting, exhibit lower pre-analytical variability, and demonstrate superior biological stability. Standard diagnostic benchmarks establish an HbA1c level of $<5.7\%$ (39 mmol/mol) as normal, $5.7\text{--}6.4\%$ (39–47 mmol/mol) as prediabetes, and $\geq 6.5\%$ (48 mmol/mol) as diagnostic for diabetes mellitus [6,8]. Integrating decentralized, rapid electrochemical detection for both total Hb and HbA1c onto point-of-care (POC) test strips is therefore critical for managing emergency acute blood loss alongside long-term chronic metabolic monitoring.

Traditionally, the “gold standard” for Hb quantification is Drabkin’s method, a spectrophotometric approach that converts Hb to cyanmethemoglobin [9]. While it is a highly accurate method, this technique relies on using toxic reagents such as potassium cyanide, bulky benchtop equipment, skilled laboratory personnel, and time-consuming sample preparation, hindering its utility in POC or resource-limited settings [10]. The shift toward POC diagnostics demands a transition from bulky laboratory equipment to portable, low-cost, and high-throughput platforms that can deliver rapid results from a single measurement [11]. Unlike optical spectrophotometry and colorimetric test strips, which suffer from severe signal attenuation in whole blood due to erythrocyte light scattering and background chromophore interference, electrochemical detection operated independently of optical clarity [10]. This physical indifference to sample turbidity and opacity allows direct, undiluted measurements in complex biofluids, while benefiting from low operational power consumption in the micro to milli-watt range and rapid miniaturization into handheld devices and wearables [12]. Nevertheless, translating these platforms from benchtop electrochemistry to robust POC applications requires overcoming key electrochemical bottlenecks such as non-specific protein biofouling at the electrode interface, mass transport limitation in high-viscosity bodily fluids (whole blood), and bioreceptor calibration drift over time. Addressing these interfacial engineering challenges remains central to advancing next-generation electrochemical Hb sensors [13].

Recent innovations in material science, specifically electrode modification with carbon nanotubes, graphene, and metallic nanoparticles, have significantly enhanced electron transfer kinetics between the Hb redox centers and the electrode surface [14,15].

To provide a clear analytical framework, this review categorizes the recent landscape of electrochemical Hb sensors into two primary operational paradigms: (1) non-enzymatic, biomimetic platforms that leverage direct electron transfer (DET) across advanced two-dimensional (2D) nanomaterials (MXenes), metal–organic frameworks (MOFs), and molecularly imprinted polymers (MIPs), and (2) affinity-based biosensors, including aptamer-based field effect transistors and miniaturized impedance immunosensors. We evaluate the progress in electrode functionalization, electron-transfer kinetics, real-sample matrix performance, and the development of “lab-on-a-chip” devices, providing a comprehensive overview of how these advancements are bridging the gap between benchtop research and commercial POC applications.

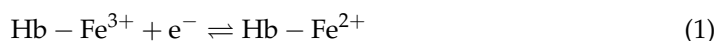
2. Fundamentals of Hemoglobin Chemistry

2.1. Direct Electron Transfer of Hb and Structural Challenges

Achieving Direct Electron Transfer (DET) between hemoglobin and an unmodified electrode surface is inherently challenging due to the protein's structural architecture [16]. Hemoglobin is a large, globular, tetrameric protein (~64.5 kDa) consisting of two α and two β polypeptide chains. Each subunit encapsulates a redox-active iron protoporphyrin (IX) heme group [17]. This polypeptide shell insulates the heme, which makes the $\text{Fe}^{2+}/\text{Fe}^{3+}$ redox centers sterically hindered from the electrode [18]. The primary obstacle to DET is spatial insulation. The electroactive $\text{Fe}^{2+}/\text{Fe}^{3+}$ redox centers are deeply embedded within the hydrophobic pockets of the globin chains, at a distance that severely restricts tunneling rates [19]. According to electron tunneling theory, the rate of electron transfer decreases exponentially as the distance from the electrode increases [20,21]. Furthermore, when native Hb adsorbs directly onto bare inorganic electrode surfaces such as glassy carbon [22] or gold [23], it frequently undergoes irreversible conformational changes or denaturation. This structural flattening orientationally misaligns or further buries the heme groups, leading to the passivation of the electrode surface and a negligible electrochemical response [23].

2.2. Electron Transfer Kinetics and pH Dependence

The intrinsic electrochemical activity of Hb relies on the reversible, single-electron reduction and oxidation of its heme iron center [18].



The kinetics and thermodynamics of this redox couple are profoundly influenced by the pH of the microenvironment. This dependency arises because the electron transfer process is coupled with a proton-coupled electron transfer (PCET) to maintain charge neutrality within the hydrophobic pocket [24–26]. This phenomenon is common in many other biological systems, such as bacterial cells, that contain structures similar to hemoglobin [27].

The relationship between the formal potential ($E^{\circ'}$) of the Hb redox couple and the solution pH can be quantitatively described by the Nernst equation at 25 °C [28].

$$E^{\circ'} = E^{\circ} - \frac{2.303RT}{nF} \log \frac{[\text{Red}]}{[\text{Ox}]} \quad (2)$$

When accounting for a coupled transfer of m protons and n electrons, the formal potential shifts as a function of pH according to the simplified relationship:

$$E^{\circ'} = E^{\circ} - \frac{2.303mRT}{nF} \text{pH} \quad (3)$$

Given that $R = 8.314 \text{ J mol}^{-1} \text{ K}^{-1}$, $F = 96,485 \text{ C mol}^{-1}$, and the reduction of each heme group involves a single electron ($n = 1$), a system demonstrating an equal number of electrons and protons ($m = 1$) will yield a theoretical Nernstian slope described below:

$$\frac{2.303RT}{F} = 0.05916 \frac{\text{V}}{\text{pH}} = 59.2 \frac{\text{mV}}{\text{pH}} \quad (4)$$

Experimental observations of Hb immobilized on biocompatible matrices typically yield slopes near -59 mV/pH , confirming that the reduction of Hb-Fe^{3+} to Hb-Fe^{2+} is accompanied by the transfer of one proton ($m = 1$) [14,25,29]. Deviations from this slope usually indicate alterations in the microenvironment's ionic strength or partial structural

changes in the globin shell that affect the pK_a of the amino acid residues, such as distal histidine near the heme pocket [30].

2.3. Evolution of Electrode Materials

To overcome the kinetic limitations of DET, recent works have shifted away from traditional substrates to more advanced, nanostructured surfaces. In earlier research, bare glassy carbon electrodes (GCEs) were used with a suitable binder [23]. Toh et al. used Nafion films to bind the red blood cells firmly, employing the sandwich design shown in Figure 1A [23]. While this simple design provided successful detection limits and linear response for red blood cells at concentrations between 0.0003 mg/mL and 0.145 mg/mL, at higher red blood cell concentrations (>1.45 mg/mL), it led to concentration saturation and rapid electrode fouling as quantified in Figure 1B [23]. Since then, research also advanced to incorporate gold macrostructures and self-assembled monolayers (SAMs) in combination with carbon, which improved electron-transfer kinetics by providing a biomimetic surface and reducing interference from dissolved oxygen in the buffer [31]. Moreover, GCEs were also modified with lipid-protected gold nanoparticles to obtain more stability of hemoglobin and biocompatibility [22]. This work was then expanded to incorporate nanomaterial-modified surfaces, such as graphene, multiwalled carbon nanotubes (MWCNTs), mesoporous carbon, and metal nanoparticles, which gave much more favorable responses towards Hb detection [14–16]. The electron-transfer kinetics with these systems are known to be highly favorable so far, due to their high surface-to-volume ratio, which increases the loading capacity of Hb. Nanomaterial could act as conducting wires, penetrating the surface of the globulin structure.

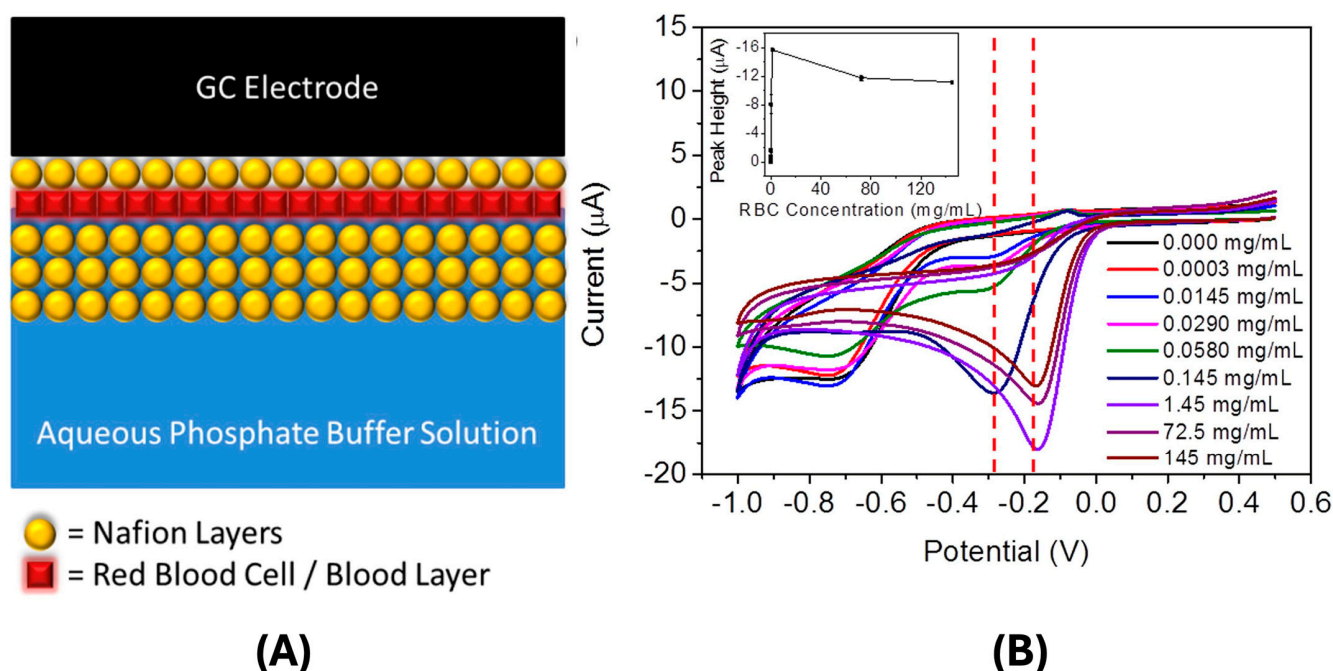


Figure 1. (A). Electrode structure of glassy carbon electrode (GCE) with red blood cells sandwiched with Nafion binder. (B) Cyclic voltammograms (from +0.5 V to −1.0 V) that were obtained in aqueous 50 mM phosphate buffer at pH 3.5 for the electrochemical reduction of red blood cells at a scan rate of 0.1 Vs^{-1} . Reduction peak height corresponds to different concentrations of RBC. Concentration saturation was observed to start at 1.45 mg/mL. Reproduced with permission from ref. [23].

The transition to nanomaterial-modified surfaces represents the foundation of modern POC hemoglobin sensors. Carbon nanotubes (CNTs), carboxylic acid functionalized multi-

walled carbon nanotubes (FMWCNTs), and graphene offer high electrical conductivity and unique geometries. These properties allow them to act as nanoscale molecular wires or structural bridges, effectively narrowing the tunneling distance to the buried heme pocket [14]. Concurrently, metal nanoparticles such as Au or Ag NPs provide a high-surface-area, biocompatible matrix that preserves the native conformation of Hb while facilitating rapid, direct electron shuttling [32]. By exploiting the direct electron-transfer properties of Hb using these materials, Hong et al. and Xu et al. have successfully modeled and predicted interfacial electron-transfer kinetics, enabling a deeper understanding of the system's thermodynamic behavior [14,15]. Ultimately, this material evolution is what enables the high sensitivity, low detection limits, and rapid response times strictly required for POC clinical testing.

3. Transduction and Signal Amplification Strategies

To translate the redox behavior of hemoglobin into a robust, quantifiable signal with the intention of using the systems in future applications in POC microfluidics, two distinct analytical methodologies have been explored: non-enzymatic (biomimetic) sensing and enzymatic/affinity-based biosensing. Regardless of the recognition pathway, the structural architecture must be engineered to amplify the resulting faradaic currents while minimizing background capacitive noise.

3.1. Non-Enzymatic Sensors

Non-enzymatic platforms bypass the vulnerability of natural biomolecules to environmental variation by utilizing synthetic, biomimetic materials that display inherent electrocatalytic activity or facilitate DET from hemoglobin [33,34]. Non-enzymatic sensors are advantageous over enzymatic sensors because they can operate under broader conditions than physiological conditions, exhibit greater stability over time, and are more reusable [35,36].

3.1.1. MXenes

MXene-based platforms address one of the principal limitations of earlier carbon nanomaterials by simultaneously providing high electrical conductivity and hydrophilic surface terminations ($-\text{OH}$, -2F , and $=\text{O}/-\text{O}$) that facilitate biocompatible protein immobilization. Two-dimensional transition metal carbides/nitrides, such as accordion-like multilayered MXenes ($\text{Ti}_3\text{C}_2\text{T}_x$), create a protective microenvironment that hosts immobilized Hb without inducing structural denaturation [37]. In these systems, Hb has primarily been incorporated as an electroactive or biocatalytic element to interrogate interfacial charge transport and enable downstream sensing such as electrocatalytic nitrite reduction [38]. The open, conductive architecture of $\text{Ti}_3\text{C}_2\text{T}_x$ significantly lowers interfacial charge-transfer resistance at the buried heme center compared to conventional graphene or TiO_2 nanorod scaffolds. For instance, Liu et al. demonstrated that Hb immobilized onto pristine $\text{Ti}_3\text{C}_2\text{T}_x$ achieved a heterogeneous electron transfer rate constant (k_s) exceeding 3.6 s^{-1} during electrocatalytic biosensing [37]. This represents an order of magnitude increase over MWCNT, which typically exhibited k_s values between 0.05 and 0.99 s^{-1} due to unfavorable steric orientation and sluggish interfacial kinetics [38].

While these studies highlight how MXene scaffolds enhance DET to immobilized heme centers, they primarily demonstrate biocatalytic signal transduction rather than direct clinical quantification of Hb as the target analyte. The high electron transfer kinetics and preservation of native heme electroactivity provide a compelling physical foundation for adopting MXene architectures in future direct Hb and HbA1c POC sensors.

3.1.2. Metal–Organic Frameworks (MOFs)

Porous crystalline Metal–Organic Frameworks (MOFs) provide a fundamentally different advantage from conductive carbon nanomaterials. Their primary strength lies in their exceptionally high surface area and tunable pore architecture, which increase biomolecule loading and facilitate analyte diffusion rather than maximizing intrinsic electrical conductivity [39]. This structural flexibility enables integration with catalytic nanoparticles or molecular recognition layers. By coordinating transition-metal nodes such as Fe, Co, or Cu with organic linkers, MOFs mimic the active-site structure of metalloproteins [40]. However, many MOFs require combination with conductive materials to overcome their relatively limited electrical conductivity, meaning they often function as part of hybrid sensing architectures rather than standalone transducers. Therefore, MOFs are often selected to maximize analyte loading rather than electron-transfer efficiency, making them complementary to conductive nanomaterials instead of direct replacements. They can act as pre-concentration matrices that entrap Hb molecules while allowing rapid electrolyte mass transport, lowering overpotentials.

3.1.3. Molecularly Imprinted Polymers (MIPs)

Molecularly imprinted polymers (MIPs) are often referred to as “plastic antibodies” and represent a breakthrough in label-free, reagent-less POC hemoglobin testing. They are fabricated by polymerizing functional monomers such as pyrrole, amino phenylboronic acid, or dopamine around a template molecule [41]. Once the template is extracted using surfactant or chemical washes, stable, three-dimensional nanocavities are left behind that match the precise shape, size, and functional group orientation of the target molecule [42]. Compared with antibody-based recognition, MIPs offer substantially greater chemical robustness, lower production costs, and improved shelf stability, making them particularly attractive for disposable POC devices. Their synthetic recognition cavities also eliminate many of the storage challenges associated with biological receptors.

For clinical tracking where distinguishing specific variants is critical, such as in glycated hemoglobin (HbA1c) analysis, Zhan et al. used boronic acid-based functional monomers such as 3-aminophenylboronic acid. The boronic acid groups form a reversible, covalent cyclic ester bond with the cis-diol groups of the glucose moiety on HbA1c, providing an extra layer of thermodynamic selectivity in complex whole-blood matrices [43]. When integrated onto transducers via electro-polymerization (eMIPs), the polymer film thickness can be controlled with nanometer precision via cyclic voltammetry or potentiostatic pulses [42]. Rebinding of the bulky Hb molecule into these cavities typically blocks the mass transport of a redox probe such as $[\text{Fe}(\text{CN})_6]^{3-/4-}$ to the electrode surface, resulting in a quantifiable decrease in peak faradaic current [44]. Therefore, molecular imprinting introduces its own limitations. Rebinding of large protein targets such as hemoglobin can be diffusion-limited, and incomplete template removal or heterogeneous cavity formation may reduce reproducibility.

To address the steric hindrance and slow electron-transfer kinetics typically encountered when detecting large macromolecular analytes like hemoglobin, recent strategies coupling MIPs with plasmonic gold interfaces, conductive nanostructures, or MOFs have demonstrated ultra-low, picomolar detection limits, making them highly suitable for fingerprick blood volumes [45]. Mandani et al. developed an ultrasensitive non-enzymatic electrochemical sensor by modifying a gold electrode with a silver-based MOF encapsulated within mesoporous carbon (Ag-MOF@MC), followed by the electro-polymerization of a hemoglobin-imprinted nanopolymer layer (Ag-MOF@MC-MIPs). In this architecture, the Ag-MOF@MC core functions as a highly conductive transduction platform that accelerates heterogeneous electron transfer, while the customized biomimetic cavities of the MIP

selectively isolate Hb from complex whole blood matrices [45]. By utilizing differential pulse voltammetry (DPV) to monitor the resulting electrochemical response, Mandani et al. demonstrated a synergistic sensing platform that combines a conductive Ag-MOF scaffold with molecular imprinting to address both electron-transfer kinetics and molecular selectivity. This non-enzymatic assembly achieved a linear range from 0.2 pM to 1000 nM, with a detection limit of 0.09 pM. This strategy highlights how integrating conductive transition-metal MOFs with synthetic affinity matrices can deliver sub picomolar sensitivity and long-term storage stability, which is essential for robust POC development.

Therefore, MIPs offer one of the strongest pathways toward inexpensive disposable sensors, provided reproducible fabrication can be achieved. Although hybrid MIP nanocomposites have achieved extremely low reported detection limits, reproducible fabrication and large-scale manufacturing remain important challenges for clinical translation.

3.2. Enzymatic and Affinity Biosensors

While biomimetic surfaces offer chemical stability, clinical diagnostic applications often require molecular specificity to distinguish hemoglobin variants or quantify target proteins in complex matrices, such as whole blood. To achieve this specificity, researchers utilize the intrinsic property of the protein itself to make affinity-based biological receptors such as aptamers and antibodies.

3.2.1. Enzymatic Sensors—Immobilized Hb as a Biocatalytic Model

Beyond its role as a clinical biomarker, immobilized Hb is widely utilized as an enzymatic platform to drive the electrocatalytic reduction of hydrogen peroxide (H_2O_2) and other small molecules [46,47]. In these catalytic configurations, Hb acts as the immobilized recognition and biocatalytic element. These systems harness the intrinsic pseudoperoxidase activity of hemoglobin's heme center to catalyze the reduction of H_2O_2 to water [46,47]. The widely accepted electrocatalytic mechanism proceeds via a multi-step pathway where the native iron center is oxidized by H_2O_2 to form a high-valent oxo-iron intermediate ($\text{Fe}^{4+}\text{-O}$), which is subsequently reduced back to its resting state (Fe^{3+}) via a sequential interfacial electron transfer [48]. The resulting cathodic current generated at a fixed operating potential is directly proportional to the concentration of H_2O_2 or other small molecule analytes such as herceptin in the sample [46,47].

While these sensors are designed to detect small molecules rather than quantify hemoglobin itself, evaluating their electrocatalytic behavior provides essential insights into interfacial material design. Specifically, they serve as robust benchmark platforms for assessing whether advanced electrode architectures, nanomaterial matrices, and protective scaffolds can facilitate DET while preserving the native structural integrity of the heme core. However, dependence on hydrogen peroxide introduces additional reagents, increases assay complexity, and may reduce suitability for simple POC devices compared with reagent-free direct electrochemical detection.

3.2.2. Aptamer-Based Sensors

Aptamer-based sensors occupy an intermediate position between synthetic MIPs and antibody-based assays. They generally offer higher molecular specificity than MIPs while avoiding many of the stability and production challenges associated with antibodies. Their programmable sequence design also enables discrimination between closely related analytes such as Hb and HbA1c. Electrochemical aptamer-based sensors utilize short, synthetic single-stranded DNA or RNA oligonucleotides to bind to hemoglobin with high affinity (K_d), typically in the low nanomolar to picomolar range, with representative Hb- and HbA1c-specific aptamer architectures depicted in Figure 2. Aptasensors offer many

advantages for POC devices, including lowering the manufacturing costs, superior thermal stability, and simple chemical modification protocols for electrode anchoring [44,49].

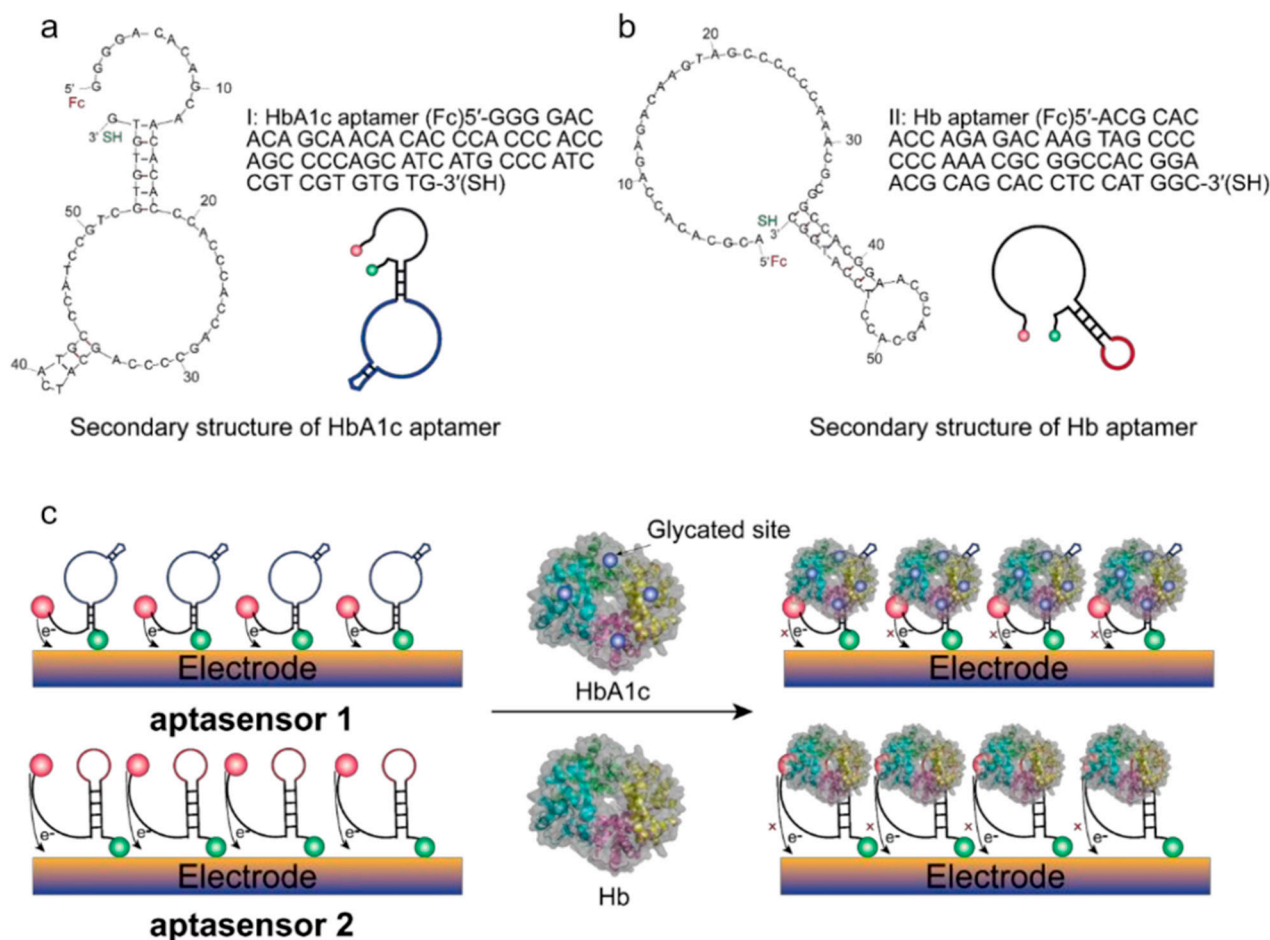


Figure 2. Representative structures of (a) Hb aptamer, (b) HbA1c aptamer, (c) schematic representation of the construction of aptasensors through sulphhydryl-gold self-assembly on screen printed electrodes and the sensing mechanism of the functionalized aptasensors for hemoglobin (Hb) and glycated hemoglobin (HbA1c). Reproduced with permission from ref. [49].

In a typical electrochemical aptamer configuration, the aptamer is covalently immobilized onto a gold or carbon electrode surface via a thiol or amine linkage at one terminus while the distal end is modified with a small molecule redox reporter such as methylene blue (MB) or ferrocene (Fc) [49–51]. The primary signal transduction pathways are divided into two mechanisms: signal-off architecture and signal-on architecture. In signal-off architecture, the flexible aptamer chain allows the redox reporter to approach the electrode surface closely, facilitating rapid interfacial charge transfer and yielding a faradic current at baseline. Upon introduction of the bulky hemoglobin target, the aptamer undergoes target-induced conformational folding or a structural transition to encapsulate the protein. This steric rearrangement pushes the redox tag away from the collector surface, creating an electronic tunneling barrier that decreases the peak faradaic current measured by DPV or square wave voltammetry (SWV) [50,51]. Alternatively, in signal-on architecture, sensors can be designed where the aptamer is initially hybridized to a complementary DNA strand, locking the redox reporter far from the interface. Target binding forces the displacement of the complementary strand, allowing the aptamer to fold closer to the electrode surface, structurally driving a sharp increase in the current output upon target capture [52].

For advanced POC clinical diagnostics such as differentiating HbA1c from total hemoglobin for long-term diabetes management, Lin et al. utilized aptamers engineered to selectively identify the specific glycosylated N-terminal valine residue on the β -chain of the hemoglobin tetramer. This approach resulted in achieving high resolution without cross-reactivity from non-glycosylated variants [53]. Expanding on these conformational properties beyond traditional voltametric interfaces, Hao et al. developed an ultra-sensitive, label-free graphene field-effect transistor (GFET) aptasensor engineered for rapid Hb quantification in undiluted clinical biofluids [54]. In this architecture, an amino-functionalized Hb aptamer (G15) was anchored to a pristine chemical vapor deposition (CVD)-grown graphene channel via a bifunctional pyrene linker. Polyethylenimine (PEI) was utilized as a low-cost insulating nanolayer to mitigate background noise. In the baseline state, the single-stranded aptamer remains flexible and extended; however, upon binding the bulky, weakly negative hemoglobin target at physiological pH, the resulting structural rearrangement pulls the target into the immediate vicinity of the graphene channel. This close-range encapsulation induces an interfacial charge-redistribution that modulates the source-drain current (I_{ds}) within 6 to 8 min. By bypassing standard diffusional redox probes, this platform achieved femtomolar detection limits (14.2 fM) in raw serum and 11.9 fM in undiluted urine while maintaining structural resilience across 100 continuous bending cycles on flexible PET substrates [54]. This further highlights the potential of transistor-integrated aptasensors for wearable clinical screening. Although aptamer platforms provide an attractive balance between specificity and stability, the analytical performance depends strongly on aptamer orientation, surface density, and conformational switching efficiency after immobilization. These variables complicate device optimization and contribute to variability between sensing platforms, even when identical recognition sequences are employed.

3.2.3. Immunosensors and Antibody-Based Sensors

Immunosensors are widely regarded as the benchmark for analytical specificity because antibody–antigen recognition is well established clinically. Their principal advantage lies in reliable target discrimination within complex biological matrices. Electrochemical immunosensors rely on the classical, high-affinity lock and key binding kinetics between immobilized monoclonal or polyclonal antibodies and specific epitope regions on the hemoglobin structure [43,55]. Due to their exceptional molecular recognition capabilities, antibody-based sensors serve as a clinical standard for isolating specific target variants within complex whole-blood matrices without requiring extensive pre-separation steps [43]. The signal transduction of Hb immunosensors generally utilized one of the two primary architectural configurations. In label-free or direct configuration, capture antibodies are immobilized directly onto a conductive nanostructured matrix such as AuNPs or carbon ink. Target Hb binding physically blocks the mass transport of a diffusional redox probe such as $[\text{Fe}(\text{CN})_6]^{3-/4-}$ [56]. This steric hindrance results in a concentration-dependent decrease in peak current monitored via electrochemical impedance spectroscopy (EIS) or DPV.

In another configuration, the sandwich assay, the target Hb is captured by a primary surface antibody, followed by the binding of a secondary signaling antibody. The secondary antibody is conjugated to an enzyme label, such as horseradish peroxidase (HRP) or alkaline phosphatase (ALP), which acts as a catalytic amplifier [57,58]. Upon adding an enzymatic substrate, a chemical amplification cycle generated robust faradaic currents, providing high signal-to-noise ratios from finger prick sample volumes that are $<10\ \mu\text{L}$. This multi-step sandwich immunoassays often require additional incubation and washing steps, reducing their suitability for rapid point-of-care testing despite excellent analytical performance.

Translating these benchtop architectures into untethered, swallowable capsules for *in vivo* diagnostics introduces severe instrumentation bottlenecks, particularly regarding the power consumption, cost, and physical footprint of multi-channel potentiostats. Addressing these engineering limitations, Demirhan et al. designed a low-cost, miniaturized EIS device, with the motive for a future application of ingestible label-free detection of hemoglobin as an early diagnostic marker for gastrointestinal bleeding (GIB). The electrode modification steps and the working principle used in this study are illustrated in Figure 3 [59]. To bypass the bulky, power-hungry analog-to-digital converters (ADCs) and field-programmable gate arrays (FPGAs) typical of benchtop impedance analyzers, the authors implemented a hardware-efficient architecture pairing a direct-digital synthesizer (DDS) with a square-wave-based analog lock-in amplifier (LIA).

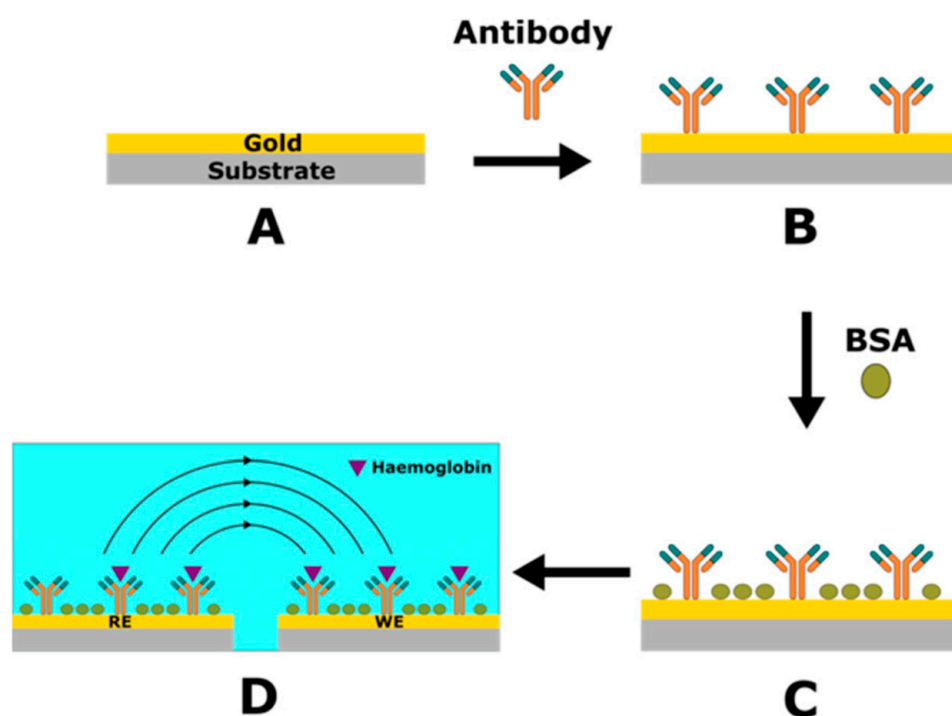


Figure 3. Schematic representation of the development of the immunosensors where (A) unmodified electrodes, (B) were modified with antibodies via dropcasting and incubated overnight, (C) followed by the addition of Bovin serum albumin (BSA) to block the empty spaces on the gold electrode surface. (D) Impedance spectroscopy was carried out in a flow-cell. Reproduced with permission from ref. [59].

When tested in a standard 5 mM $[\text{Fe}(\text{CN})_6]^{3-/4-}$ redox solution using screen-printed gold electrodes functionalized via physical adsorption with anti-human Hb antibodies, the device matched the performance of commercial benchtop potentiostats. It achieved a linear range of 5 $\mu\text{g/mL}$ to 60 $\mu\text{g/mL}$ and a limit of detection (LOD) of 13.3 $\mu\text{g/mL}$ based on shifts in charge-transfer resistance R_{ct} . The sensor also effectively responded to 1 mg mL^{-1} and 10 $\mu\text{g mL}^{-1}$ for Hb in simulated gastrointestinal fluid. Figure 4 illustrates the electrochemical impedance response of the portable sensing platform, showing representative Nyquist plots and calibration curves that demonstrate quantitative hemoglobin detection and analytical agreement with a commercial benchtop potentiostat. All analytical measurements were performed using an external benchtop flow-cell system.

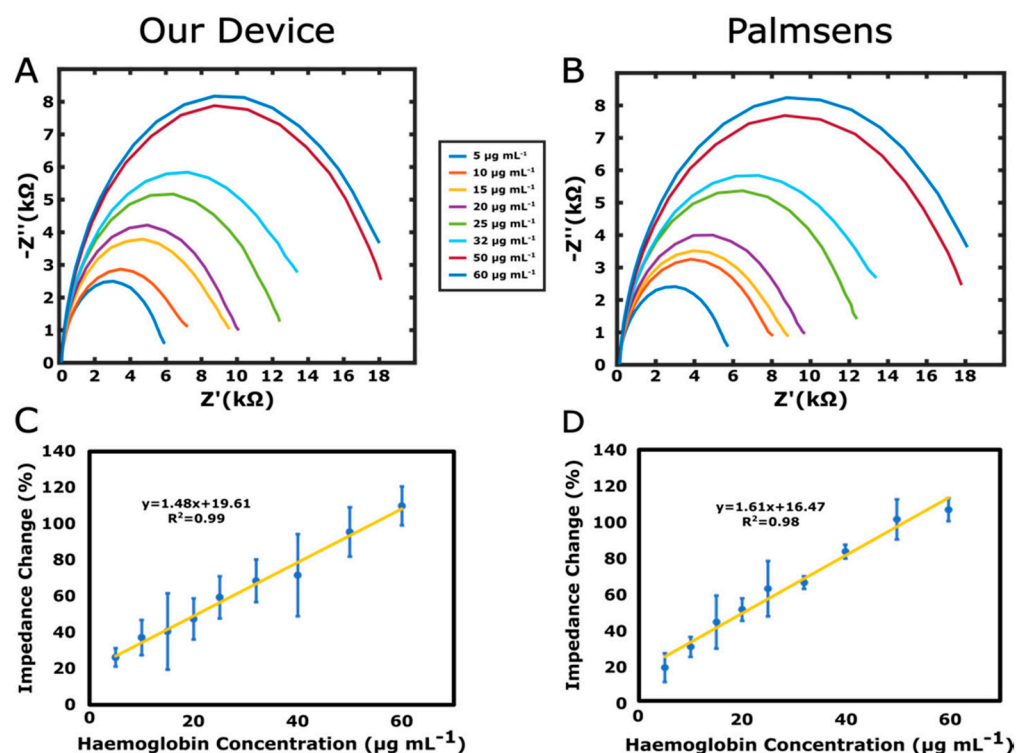


Figure 4. Electrochemical impedance spectroscopy (EIS)-based hemoglobin quantification. (A,B) Representative Nyquist plots obtained using a portable electrochemical analyzer and a commercial reference potentiostat, respectively. (C,D) Corresponding calibration curves demonstrating quantitative hemoglobin detection over the evaluated concentration range. Reproduced with permission from Ref. [59].

The authors showed that the device demonstrated feasibility for future POC screening of gastrointestinal bleeding by showing dimensional feasibility via assembling a stacked two-board PCB, coin cells, and a trimmed electrode inside a swallowable capsule shell; the ingestible pill itself remains a conceptual packaging prototype. Key operational features including autonomous microfluidic fluid sampling, onboard wireless telemetry, and in vivo gastrointestinal validation remain future engineering milestones. Moreover, the improved specificity achieved on the electrode surface in this system is offset by greater manufacturing complexity, higher production costs, and limited storage stability compared with synthetic recognition elements.

Therefore, no single electrochemical sensing strategy simultaneously maximizes sensitivity, selectivity, operational stability, manufacturing simplicity, and clinical practicality, as illustrated in Table 1. Conductive nanomaterials primarily improve electron-transfer kinetics, MOFs enhance analyte loading and mass transport, MIPs provide durable synthetic molecular recognition, aptamers offer programmable target specificity, and antibodies continue to provide the highest established clinical selectivity. Increasingly, recent sensor designs combine these complementary strengths within hybrid architectures rather than relying on a single material platform. This convergence suggests that future advances are more likely to arise from rational integration of transduction materials with robust molecular recognition strategies than from incremental improvements to individual sensing components alone. Despite greater complexity and cost, antibody-based sensors continue to offer the strongest clinical precedent for highly selective hemoglobin detection.

Table 1. Comparison of major electrochemical hemoglobin sensing platforms.

Platform	Strength	Limitation
Mxene	Fast DET	Oxidation stability
MOF	High loading	Poor conductivity
MIP	Robust recognition	Fabrication reproducibility
Aptamer	High specificity	Immobilization variability
Antibody	Clinical validation	Cost and stability

4. Challenges

Recent innovations in electrode functionalization and signal amplification have yielded good analytical performance, as depicted in Table 2. However, achieving sub-picomolar detection limits and fast interfacial charge transfer kinetics across diverse architectures and translating these benchtop systems into robust commercial POC tools (i.e., wearable patches or ingestible capsules) present significant physical, chemical, and engineering hurdles. This review reveals that the metrics reported in laboratory buffers often degrade when sensors are exposed to raw undiluted clinical biofluids.

Table 2. Comparative analysis of recent electrochemical hemoglobin biosensing platforms.

Platform	Method	Linear Range	Limit of Detection	Key Performance	Sample Matrix	Ref.
Nafion/RBC sandwich in GCE	CV	0.0003–0.145 mg/mL	0.1 µg/mL	Early baseline proof-of-concept	Intact RBC	[23]
MWCNTs/AuNPs complex	Amperometry (CA), CV	0.05–2.5 mM	0.01 mM	Overcomes steric electron tunneling barrier ($K_s \sim 0.99 \text{ s}^{-1}$)	Whole blood buffer	[14]
Silver MOF-mesoporous carbon and MIP	DPV	0.2 pM–1000 nM	0.09 pM	Picomolar selectivity	Spiked blood serum	[45]
PEI/Graphene GFET	Field effect transistor (I_{ds})	10 fM–100 pM	14.2 fM (Serum) 11.9 fM (Urine)	Label free, femtomolar detection in 6–8 min	Raw undiluted serum and urine	[54]
Ferrocene aptasensor	SWV	0.1–50 nM	0.03 nM	Selective detection of HbA1c	Diluted blood samples	[49]
Anti-Hb monoclonal antibody on gold SPE/ingestible	EIS	5–60 µg/mL	13.3 µg/mL	Low-cost pill hardware	Simulated GI fluid	[59]

4.1. The Matrix Effect and Viscosity of the Medium

One of the primary challenges in analyzing hemoglobin directly in complex biological matrices such as whole blood is the presence of electroactive, low-molecular-weight endogenous compounds. Co-existing molecules such as ascorbic acid, uric acid, and acetaminophen undergo direct electrochemical oxidation at potentials that heavily overlap with the redox windows of native hemoglobin heme centers, which leads to poor specificity

and false positive current signals [23]. Furthermore, non-specific macromolecular adsorption presents a significant hurdle. Background proteins like human serum albumin (HAS) rapidly crowd the electrode surface, creating an insulating layer that reduces active capture sites and blocks diffusional redox probes like ferricyanide [60]. To mitigate this matrix effect, current architectures incorporate protective anti-fouling coatings such as Nafion perfluorinated membranes, polyurethane layers, or biomimetic zwitterionic hydrogels, which physically or electrostatically exclude these interfering molecules while allowing small target analytes to pass through [61].

Additionally, the physical properties of blood result in significant differences in mass transport compared to standard laboratory aqueous buffers. Whole blood is highly viscous, and its consistency varies with a patient's hematocrit level. The higher viscosity of raw whole blood drastically reduces the rate of mass transport toward the electrode surface [29]. The variations between blood can distort calibration curves and may yield inaccurate estimations of hemoglobin levels in patients' blood analysis.

4.2. Discrepancy Between Synthetic and Clinical Matrices and Biofouling

A persistent challenge in the sensor literature is the performance gap between idealized laboratory validation and true clinical performance. A large percentage of proof-of-concept studies validate their sensors using commercial, purified hemoglobin dissolved in synthetic phosphate-buffered saline (PBS) or artificial serum matrices. These simulated environments fail to capture the cellular complexity, ionic variations, and organic compounds of authentic human biofluids. In real blood samples, hemoglobin is intracellularly compartmentalized within erythrocytes. Therefore, electrochemical POC sensors must either integrate an active, on-chip chemical lysing agent or apply high-frequency electric fields to burst cells before electrochemical analysis can proceed [62].

Furthermore, electrode biofouling remains a major limitation for sensors designed to operate in raw undiluted blood samples without extensive sample preparation. Upon contact with metal or carbon, the immediate activation of the coagulation cascade triggers fibrin deposition and platelet adhesion directly onto the bioelectrode interface. This biofouling can permanently passivate the surface, leading to a sharp loss in sensor sensitivity and poor reproducibility [63]. For wearable or ingestible continuous monitoring systems, the clotting behavior of blood makes the real-time monitoring almost impossible without integrating anti-clotting surface modification techniques such as immobilizing active heparin strands or creating hydrophilic textures that resist cell attachment [64].

4.3. Commercialization and Scalability Barriers

Beyond the chemical microenvironment, bridging the gap from benchtop to FDA-approved consumer products introduces key industrial hurdles [65]. Manual drop-casting methods and electrode preparation in laboratory settings suffer from high batch-to-batch variation (RSD > 15%), non-uniform film thickness, and coffee-ring aggregation [64]. Industrial scaling requires transitioning to high-throughput, reproducible manufacturing such as gravure coating or screen-printed carbon electrode assembly [66,67]. This demands reformulating functional nanomaterial pastes, such as CNTs, with specific binders and volatile solvents that preserve intrinsic electron transfer kinetics without passivating active catalytic sites [67]. Reagent stability remains another fundamental hurdle that needs to be addressed before commercialization. Natural biological recognition elements, such as antibodies and enzymes, are highly vulnerable to irreversible denaturation [65,68]. Furthermore, their purification process requires extensive specialized skills, equipment, and time, which makes the commercialization of the devices that use these sensors at an affordable price to the general public challenging [35,69].

Commercial POC test strips often operate under a strict “drop-and-read” paradigm from minimal finger-prick blood volumes (<5 μL) without requiring manual pre-centrifugation or liquid-handling steps [70]. Consumer devices are generally restricted from incorporating toxic, liquid-phase diffusional redox mediators such as ferricyanide [59]. To meet POC accuracy standards, it is necessary to have robust, matrix-matched calibration algorithms that are capable of dynamically compensating for patient-to-patient hematocrit variations and ambient temperature fluctuations in samples [67,70].

5. Future Perspectives and Emerging Paradigms

While electrochemical hemoglobin sensors have traditionally focused on centralized hospital diagnostics for anemia and acute hemorrhage, emerging wearable technologies and miniaturized electronics are expanding their utility toward continuous, non-invasive health monitoring. An emerging application lies in menstrual cycle monitoring and gynecological health, with the objective of quantifying the menstrual blood loss to screen for conditions such as menorrhagia, uterine fibroids, and endometriosis [71,72]. Historically, menstrual tracking has relied on self-reporting, subjective, qualitative logs, such as self-reporting and counting saturated pads and tampons, leading to delayed and inaccurate diagnosis. Recent advances in flexible electronics and microfluidics have enabled the integration of electrochemical and paper-based sensors directly into wearable hygiene products such as sanitary pads, tampons, and menstrual cups, as conceptualized in Figure 5 [73].

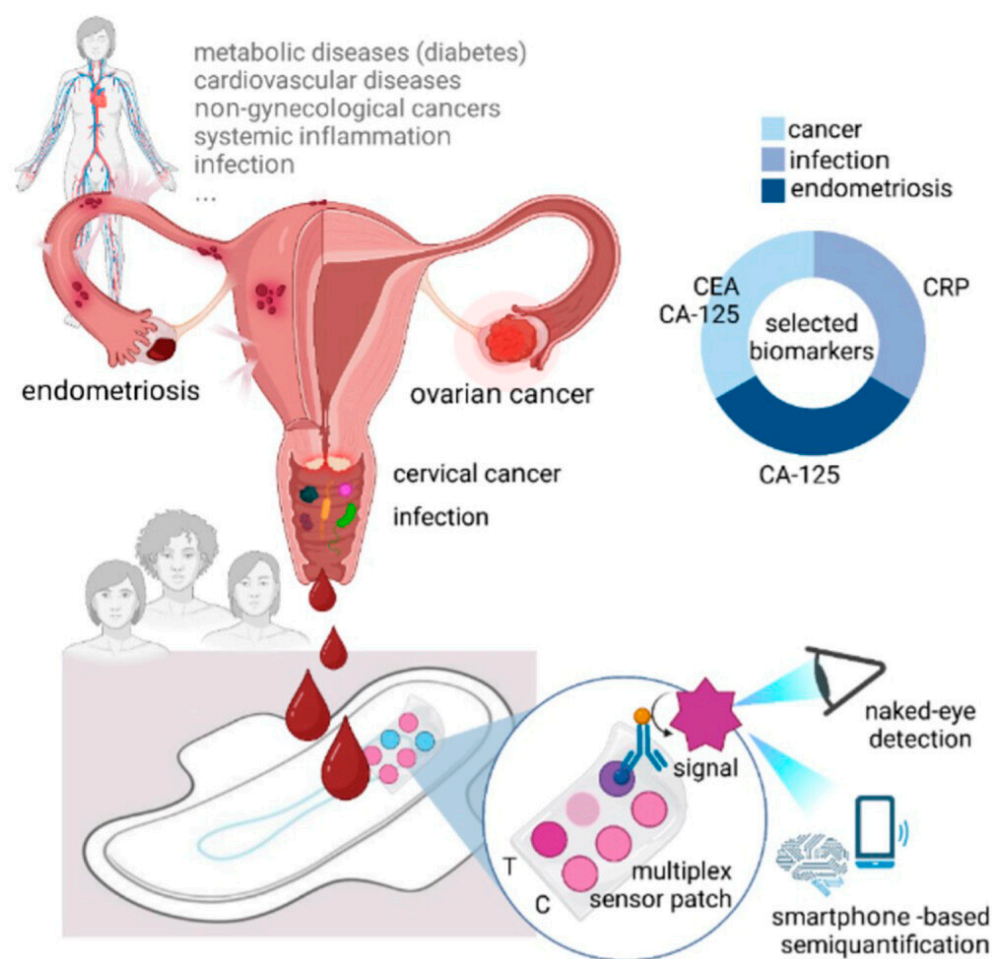


Figure 5. Schematic representation of the concept of menstrual blood biosensor incorporated into a hygienic pad, enabling the direct, readable, quantitative analysis of biomarkers in menstrual blood. Reproduced with permission from ref. [73].

Transitioning to objective electrochemical tracking represents a major paradigm shift. The core analytical strategy relies on linking localized electrochemical hemoglobin concentrations directly to total menstrual blood loss. By pairing a real-time electrochemical Hb sensor with volumetric flow or capacitive level sensors, an on-chip algorithm can calculate cumulative hemoglobin discharge over a bleeding phase, converting local concentration spikes into total clinical blood loss. A primary bioanalytical hurdle is that menstrual effluent is not simply systemic blood. It is a highly heterogeneous mixture that undergoes pH fluctuations between pH 4.5 and 7. Additionally, high-molecular-weight mucins resist capillary flow and rapidly foul the electrodes. These limitations should be considered when developing such sensors and anti-fouling coatings, as well as chemical lysing agents, which should be integrated accordingly to efficiently release hemoglobin from intact erythrocytes without clogging microchannels.

Translating these sensors into single-use formats can incorporate flexible, screen-printed paper microfluidic channels [74]. The capillary-driven flow of the effluent naturally guides the sample across an integrated pad onto the electrode array, eliminating the need for an active pump. Reusable medical-grade silicone menstrual cups or tampons are ideal for hosting miniature, solid-state electronics. By using highly stable MIPs in the cup's base, these devices can track cumulative volumetric statistics and track changes in analyte concentration over multiple cycles for continuous monitoring periods [75].

Another emerging application is the detection of gastrointestinal bleeding, where upper and lower gastrointestinal hemorrhage often require invasive endoscopy for definitive diagnosis. Ingestible electrochemical sensors offer the potential for continuous, minimally invasive monitoring that could complement conventional diagnostic workflows. Swallowable, untethered smart capsules featuring integrated electrochemical sensors represent a transformative alternative for real-time GI monitoring [59]. To achieve hardware miniaturization, multi-frequency EIS can be implemented on ultra-low-power custom-printed circuits ($33 \times 20 \text{ mm}^2$), by combining a direct digital synthesizer with an analog lock-in amplifier, to replace power-hungry benchtop ADCs, and an FPGA [59].

Point-of-Care Platforms and Digital Health Integration

The future of commercial hemoglobin sensing relies on integrating advanced electrochemical transducers with multiplexed analytical panels and modern digital health infrastructure. Pairing Hb quantification with concurrent detection of inflammation or gynecological markers, such as CRP and CA-125, on a single paper-based lateral flow or screen-printed array enables holistic, single-drop health screening [73,76]. Smartphone-enabled readout algorithms evaluate an amperometric decay or impedance profiles in real time ($<5 \text{ min}$), dynamically processing signals to compensate for patient-specific hematocrit, ambient temperature, and matrix variations [57]. These modified working electrode biointerfaces can be directly connected to consumer hardware. By pairing a sensor array with a miniaturized Bluetooth Low Energy or Near Field Communication module, real-time faradaic currents are converted into clinical-grade data, enabling seamless synchronization with a smartphone application and cloud-connected mobile health dashboards [77,78].

By bridging the gap between advanced electrochemical materials, bio-antifouling interfaces, and consumer-centric hardware design, the next generation of POC hemoglobin sensors will transcend traditional diagnostic testing, transforming complex, centralized laboratory assays and tests into continuous, everyday robust pathways for personalized and preventative healthcare. The convergence of hemoglobin sensing with wearable menstrual health monitors and ingestible capsules for gastrointestinal bleeding detection illustrates how this field is expanding beyond centralized diagnostics toward continuous, consumer facing health monitoring. Realizing this potential will depend on hybrid architectures

that combine the electron transfer advantages of nanostructured conductive scaffolds with the selectivity of molecular recognition elements, packaged within low power, wirelessly connected hardware suitable for everyday use.

Future progress is unlikely to arise from lower limits of detection alone. Instead, advances will depend on integrating anti-fouling materials, manufacturable sensing architectures, low-power electronics, and clinically validated machine-learning calibration algorithms into complete diagnostic ecosystems. Realizing this convergence, rather than pursuing incremental gains in any single performance metric, will determine whether electrochemical hemoglobin sensing moves from proof-of-concept devices to platforms that clinicians and consumers can rely on outside the laboratory.

6. Conclusions

Electrochemical hemoglobin sensing has progressed from early red blood cell sandwich assays on bare GCEs to a diverse toolkit of two-dimensional nanomaterials, MOFs, MIPs, and affinity-based recognition elements, each addressing the same underlying obstacle: the steric burial of the heme iron center that limits direct electron transfer. As summarized in Tables 1 and 2, no single platform yet satisfies every requirement for POC deployment simultaneously. Conductive nanomaterials such as MXenes and carbon nanotube composites deliver fast interfacial electron transfer kinetics, MOFs and MIPs extend analyte loading and selectivity at comparatively low cost, and aptamer and antibody-based architectures remain the benchmark for clinical specificity, particularly when distinguishing HbA1c from total hemoglobin. The central translational gap identified throughout this review is not sensitivity, since several architectures already achieve sub nanomolar or femtomolar detection limits in buffer, but robustness in the biological matrices that POC devices must actually encounter: whole blood viscosity and hematocrit variation, non-specific protein fouling, coagulation at the electrode interface, and the broader disconnect between synthetic phosphate-buffered saline-based validation and real clinical samples. Closing this gap will require anti-fouling interfaces, matrix-matched calibration, and manufacturing processes capable of reproducible, high-throughput electrode fabrication rather than further incremental gains in laboratory scale sensitivity alone.

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