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Electrochemical biosensors for vancomycin monitoring in blood: advances, strategies, and future perspectives

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Vancomycin is a critical antibiotic for treating life-threatening infections caused by resistant bacteria. Due to its narrow therapeutic window and complex pharmacokinetics, regular monitoring of vancomycin levels is essential to prevent toxicity and minimize the risk of resistance development. Traditional methods for vancomycin analysis, such as chromatographic and immunoassay techniques, are often time-consuming, expensive, and sometimes lack sensitivity and specificity. Recent research has explored electrochemical biosensors as a promising alternative for rapid, cost-effective, and highly sensitive vancomycin detection. These biosensors leverage various electrode modifications and molecular recognition elements, including aptamers, graphene, gold nanoparticles, and molecularly imprinted polymers, to enhance selectivity and sensitivity. This review provides a comprehensive overview of the electrochemical methods for vancomycin detection in human and animal blood samples. It highlights different sensor designs, their advantages and limitations, and compares single-use and reusable biosensors. By analyzing the effectiveness and practicality of each approach, this review aims to guide future advancements in electrochemical biosensing for therapeutic drug monitoring.

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1 Background

Vancomycin is a glycopeptide antibiotic that exerts its antibacterial effect by inhibiting cell wall synthesis in Gram-positive bacteria, ultimately leading to bacterial death. It has been approved by the U.S. Food and Drug Administration (FDA) for the treatment of various infections, including those caused by *Staphylococcus* species such as methicillin-resistant *Staphylococcus aureus* (MRSA), as well as *Staphylococcus* enterocolitis, *Clostridioides difficile*-associated diarrhea, and pseudomembranous colitis.[1–3] Due to its narrow therapeutic window and complex pharmacokinetics, careful monitoring of vancomycin serum levels is essential. Approximately 55% of vancomycin is bound to plasma proteins, mainly albumin and immunoglobulin A (IgA), which influences the drug's free (active) concentration. Alterations in these protein levels, often seen in conditions such as malnutrition, intestinal malabsorption syndromes, nephrotic syndrome, severe burns, and end-stage liver disease, can significantly affect vancomycin pharmacokinetics.[4,5] Likewise, decreased IgA levels, as observed in Bruton agammaglobulinemia or resulting from co-administration of medications like cyclosporine, penicillamine, or antiepileptics, can further influence vancomycin distribution and clearance.[6,7] Impaired renal function also contributes to elevated serum concentrations due to reduced excretion. On the other hand, increased IgA levels, commonly seen in HIV infection, rheumatoid arthritis, liver cirrhosis, and age-related macular degeneration, may bind more vancomycin, reduce its free fraction, and potentially compromise therapeutic efficacy, thereby raising the risk of treatment failure and antibiotic resistance.[8–12]

The recommended therapeutic trough concentration of vancomycin ranges between 10–20 μg mL^{-1}, depending on the clinical indication.[9] However, maintaining concentration within this range presents a significant challenge. Trough levels exceeding 15 μg mL^{-1} are associated with a markedly increased risk of nephrotoxicity, which affects approximately 5–43% of patients, typically manifesting between the 4th and 17th day of therapy.[13] On the other hand, subtherapeutic levels can facilitate the development of vancomycin-resistant bacterial strains, presenting serious clinical complications.[14] Notably, the financial costs associated with managing vancomycin-induced nephrotoxicity or antibiotic resistance far surpass those of routine therapeutic drug monitoring.[4]

Currently, vancomycin levels are measured using chromatographic techniques, immunoassays, and, more recently, electrochemical methods.[15] Chromatographic techniques, such as liquid chromatography, are highly specific and sensitive, with detection limits as low as 0.1 μg mL; but their high cost and operational complexity limit their clinical use.[16] Immunoassays

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are widely adopted in clinical laboratories due to their simplicity and cost-efficiency; however, they offer lower specificity and have a higher detection threshold of approximately 5 μg mL^{-1}.[17] Recently, electrochemical biosensors have emerged as a promising alternative, offering rapid, cost-effective, and sensitive detection of various drugs.

Despite the growing interest in electrochemical sensing technologies, there is a lack of comprehensive reviews that address the electrochemical application for vancomycin detection in blood samples. Thus, this review aims to bridge this gap by systematically evaluating current electrochemical detection strategies, analyzing their performance, advantages, and limitations, and emphasizing their potential to serve as reliable alternatives to conventional therapeutic monitoring tools.

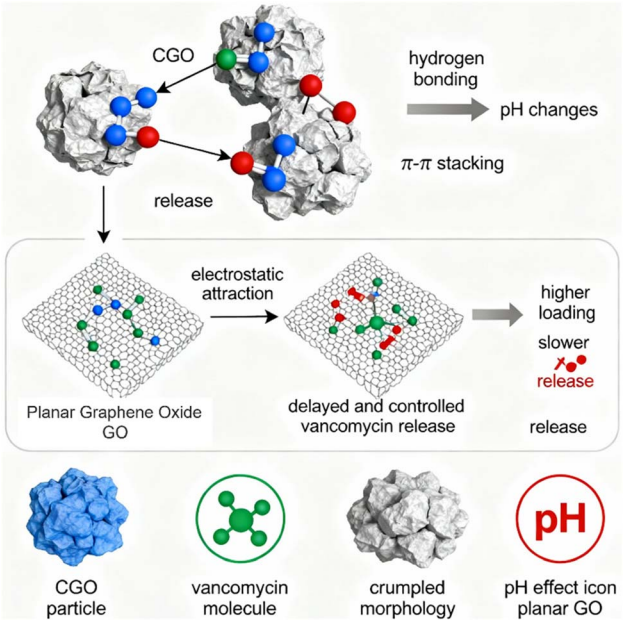
2 Electrochemical analysis of vancomycin in blood

Electrochemical biosensors are based on redox (oxidation-reduction) reactions that enable electron or ion transfer *via* a conductive transducer. These sensors are mainly categorized as either amperometric or voltammetric, and both detect target analytes by measuring electrical current. A typical electrochemical biosensor comprises three essential electrodes: the working electrode (WE), which directly interacts with and detects the target molecule; the counter (or auxiliary) electrode (CE), which completes the electrical circuit and allows current flow; and the reference electrode (RE), which maintains a stable and known potential to ensure accurate measurements. The key difference between amperometric and voltammetric biosensors lies in how the potential is applied. In amperometric biosensors, a constant potential is applied over time to drive the redox reaction. In contrast, voltammetric biosensors apply a varying potential, enabling more detailed analysis of redox behavior. This variation is achieved using techniques such as cyclic voltammetry, square wave voltammetry, differential pulse voltammetry, and anodic stripping voltammetry. Amperometric biosensors are widely used for detecting small-molecule metabolites such as glucose and other sugars, often through enzyme-based recognition mechanisms. On the other hand, voltammetric biosensors are more commonly applied to detect larger biomolecules, including proteins, nucleic acids, and biomarkers, using affinity-based recognition strategies.[18–20]

2.1 Glassy carbon-based biosensors

2.1.1 Graphene oxide-glassy carbon-based biosensors. Graphene (G)-based working electrodes are widely employed in the electrochemical analysis of vancomycin owing to their high electrical conductivity end rich electrochemical active sites, resulting in fast electron transfer and high sensitivity.[21] Graphene mainly interacts with vancomycin through π–π interactions and hydrogen bonding,[22] Scheme 1.

Researchers have produced graphene-modified, carbon nanotube-modified, and carbon black-modified electrodes based on glassy carbon electrodes for the electrochemical analysis of vancomycin. Electrode fabrication involves



Scheme 1 Controlled, pH-responsive release of vancomycin from crumpled graphene oxide nanocarriers illustrating drug loading interactions (hydrogen bonding, π–π stacking) and delayed release kinetics enabled by the crumpled structure.

dispersing reduced graphene oxide (CR-GO), graphitized multiwalled carbon nanotube (G-CNT), or carbon black powder (CB) separately in *N*, *N*-dimethylformamide (DMF), followed by sonication in an ultrasonic bath. They have also used graphite rod electrodes, an auxiliary electrode, and Ag/AgCl as a reference one. The Sama 500 electrochemical analyzer, which is used for the cyclic voltammetry technique, has shown the following: the peak current of graphene glassy carbon (GR-GC), produced from dipping into CR-GO, is higher than CNT-GC, produced from dipping into G-CNT, and CB-GC electrodes, produced from dipping into CB, with a potential of 100 mVs^{-1} in 0.1 M phosphate buffer (pH 7.0) containing 50.0 μM. The ratio of intensities is GR-GC, 0.31; CB, 0.10; CNT, 0.11,[21] as illustrated in Fig. 1.

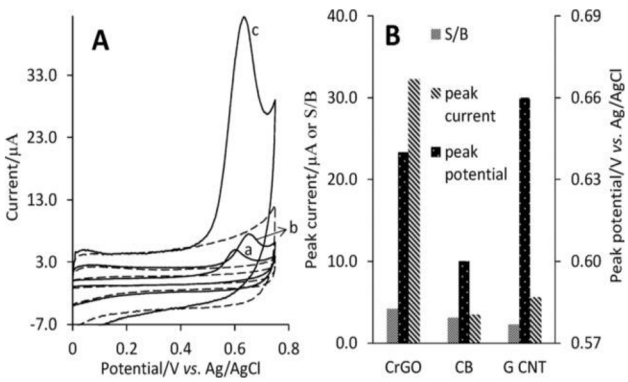
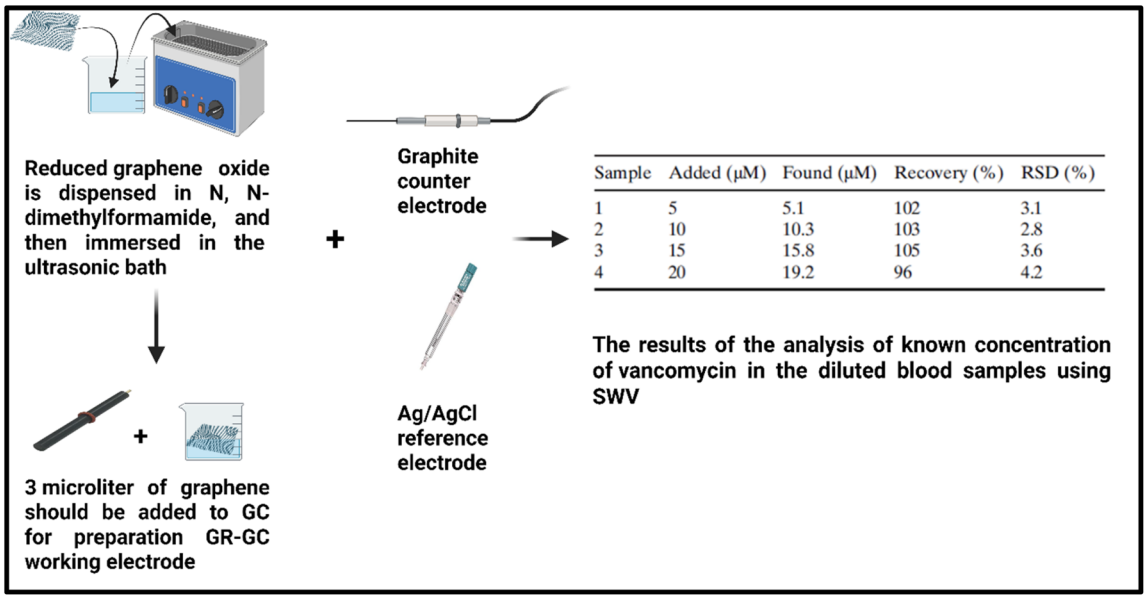


Fig. 1 (A) CVs for vancomycin at different electrodes and (B) peak potential, peak current, and S/B ratio for each electrode.[21]



Sample	Added (μM)	Found (μM)	Recovery (%)	RSD (%)
1	5	5.1	102	3.1
2	10	10.3	103	2.8
3	15	15.8	105	3.6
4	20	19.2	96	4.2

Fig. 2 Fabrication of GR-GC, working electrode-based biosensor for the electrochemical detection of vancomycin in serum, and obtained SWV values for the GR-GC working electrode in detecting vancomycin in diluted blood.[21]

Square wave voltammetry (SWV) was used for better resolution and sensitivity, and 3 μL of *N*, *N*-dimethylformamide (DMF) graphene oxide dispersion was drop-casted onto a GC electrode, and the solvent was allowed to evaporate during preparation for optimal analytical performance. The plasma samples of healthy people were diluted 50 times using 0.1 M phosphate buffer (pH 7.0). Using SWV, the values are obtained as shown in Fig. 2. Specifically, a sensitivity of 0.8 μA μM^{-1}, a limit of detection of 0.2 μM, and a recovery of 102% for 5 μM vancomycin were obtained using a GR-GC electrode. Furthermore, the technique showed strong selectivity for 20 μM vancomycin, even in the presence of high concentrations of blood components, due to

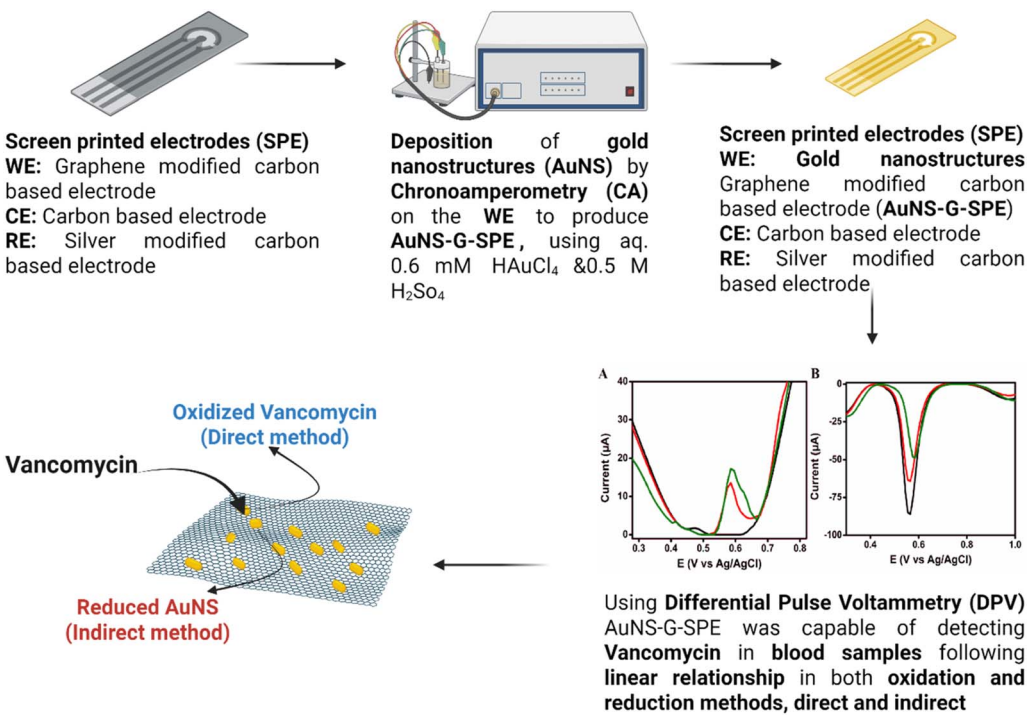
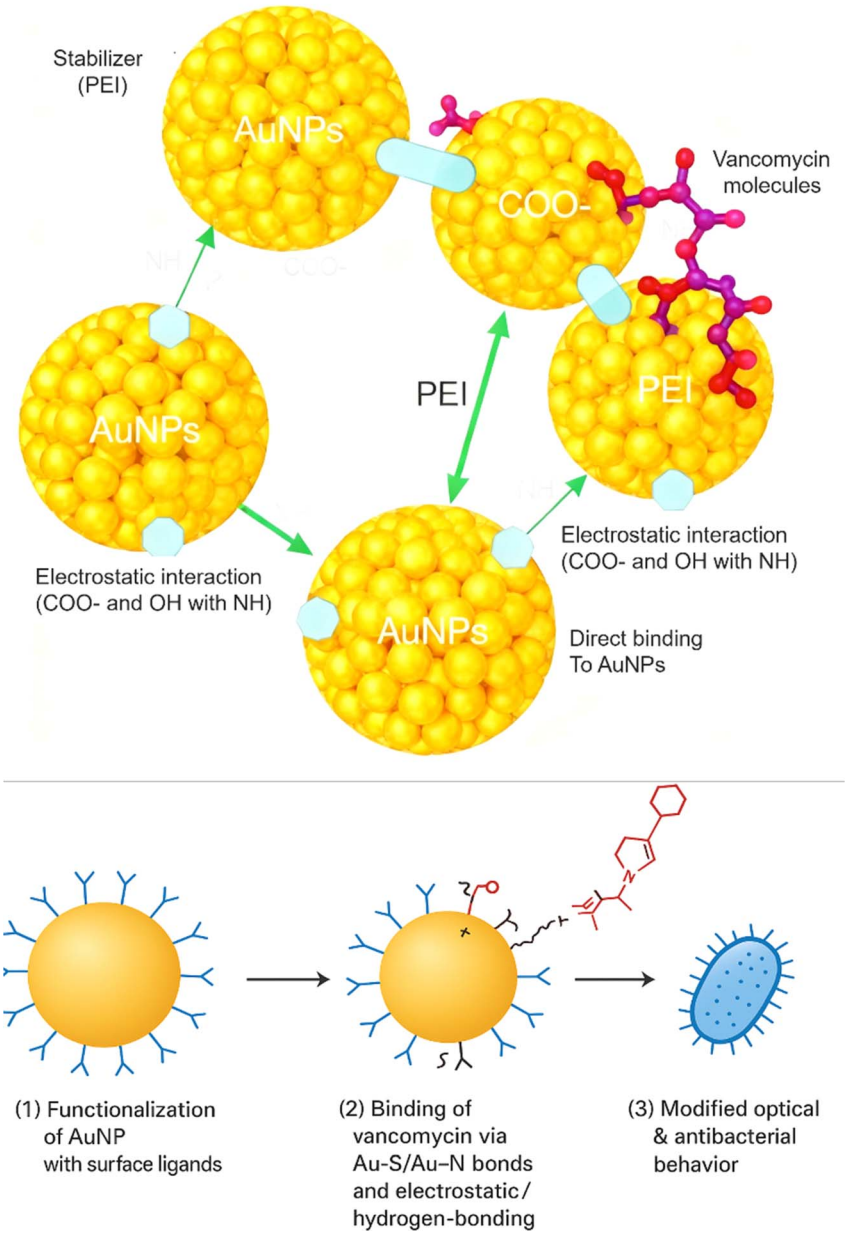


Fig. 3 Summary of AuNS-G-SPE, working electrode-based biosensor fabrication and electrochemical detection using DPV for vancomycin with direct (A) and indirect (B) detection approaches, using blank serum (black), and vancomycin spiked serum in 10 μM (red), and 50 μM (green), with the mechanisms for direct and indirect detection.[25]

high surface wettability. The GR-GC electrode has demonstrated high sensitivity for detecting low concentrations of vancomycin, offering a simple, selective, cost-effective, and rapid detection method.[21]

2.1.2 Metal–organic frameworks-glassy carbon based biosensors. Metal–organic frameworks (MOFs) were also employed as electroactive signal probes for detecting the target compound. However, due to the limited dispersion of MOFs in water, researchers have incorporated poly (acrylic acid) (PAA) to enhance their properties. Not only does PAA improve MOF dispersion, but it also has affinity towards vancomycin and increases its conductivity. This PAA-Cu–MOF composite was used to modify a glassy carbon electrode, providing a high surface area and rich active sites tailored for vancomycin adsorption. For MOF electrode fabrication, copper benzene tricarboxylic acid (HKUST-1) was selected as the MOF material. Through hydrothermal modification with PAA, a functionalized composite (P-HKUST-1) is synthesized. Vancomycin detection occurs through complex formation between vancomycin and P-HKUST-1, which leads to a decrease in the current peak. The electrochemical system utilized a glassy carbon electrode with potassium ferricyanide/potassium ferrocyanide ($Fe(CN)_6^{3-/4-}$) as redox species, operating within a potential window of 0.4 to 1.0 V *via* DPV. To minimize matrix effects, sheep blood samples were diluted 10 times before analysis. The method is tested in water and demonstrates a detection limit as low as 1 nM with



Scheme 2 Schematic illustration of vancomycin binding to gold nanoparticles (AuNPs) *via* Au–S and Au–N bonds, electrostatic forces, and hydrogen bonding, resulting in vancomycin-coated AuNPs with altered optical properties and enhanced antibacterial activity.

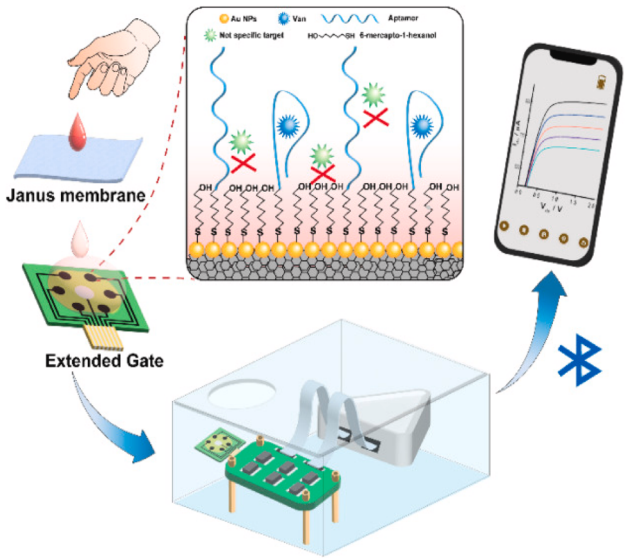
a relative standard deviation of ±4.27% and a sensitivity of 496.429 μA μM^{-1} cm^{-2}. The method was also tested in the presence of metal ions, and selectivity is affected due to the presence of magnesium ions, which decrease the binding affinity of vancomycin. Moreover, the technique was checked using a known concentration of vancomycin in a urine sample, and it was noticed that there was less than 5% variation from the concentrations of the calibration curve. The technique has proved to be selective and maintains performance even in the presence of vancomycin analogues. However, a notable limitation is that the electrodes begin to degrade after six weeks.[23]

2.2 Graphene-based biosensors

2.2.1 Gold nanostructure-graphene-based biosensors. Feier *et al.* investigated the electrochemical detection of vancomycin at varying concentrations in human serum using a sensing platform based on graphene and gold nanostructures (AuNSs) on a carbon-based electrode. The selection of gold is owing to its synergistic effect for oxidation of vancomycin and the binding ability of the glycosidic part or amino group of vancomycin with,[24] Schematic 2. Their approach employs differential pulse voltammetry biosensors. In the oxidation-based (direct) method, vancomycin interacts directly with AuNSs, whereas the reduction-based (indirect) method relies on the ability of vancomycin to reduce gold cations. To enhance sensitivity, a screen-printed graphene electrode is used to facilitate vancomycin adsorption, thereby improving interaction with gold and AuNSs. Prior to analysis, the blood sample is treated with 70% $HClO_4$ to precipitate proteins, followed by centrifugation for 10 minutes. The resulting supernatant is then diluted with H_2SO_4 to adjust the pH to 1, optimizing the electrochemical activity of vancomycin during detection,[25] as illustrated in Fig. 3 and Scheme 2.

This method shows excellent selectivity, effectively distinguishing vancomycin even in the presence of multiple antibiotics and pharmaceuticals. It also demonstrates high sensitivity, achieving detection limits of approximately 0.29 μM for the direct oxidation method and 0.5 μM for the indirect reduction method. Recovery rates were 97.38% and 104.54% in oxidation, and 107.06% and 103.70% in reduction, for vancomycin concentrations of 10 μM and 50 μM, respectively. Selectivity was further validated using a mixture containing vancomycin and eight other antibiotics, confirming the platform's specificity in both detection modes.[25]

2.2.2 Aptamer-graphene-based portable wireless EG-FET. A recent study reported the development of an extended-gate field-effect transistor (EG-FET) biosensor that integrates a multi-doped graphene electrode as the sensing interface with a commercial FET serving as the signal transducer. The device employs laser-induced graphene (LIG) as the base electrode material, which is further modified with MnO_2 nanoparticles to enhance its electrical conductivity and surface area. Subsequently, gold nanoparticles (AuNPs) are deposited onto the LIG surface to improve electron transfer and provide favorable anchoring sites for sulfhydryl-modified vancomycin-specific aptamers. These aptamers self-assemble into an ordered monolayer *via* Au–S (gold–thiol) coordination, enabling highly specific binding of vancomycin molecules as the core sensing mechanism. The resulting EG-FET sensor demonstrates a broad linear detection range from 1 nM to 100 μM and an exceptionally low detection limit of 0.187 nM. To facilitate point-of-care testing, the system was further adapted into a portable, wireless sensing platform integrated with a Janus membrane for rapid serum separation, allowing direct and efficient detection of vancomycin in whole blood samples Scheme 3.[26]



Scheme 3 Compact EG-FET-based sensor system for quick detection of vancomycin,[26] with permission from Elsevier, copyright 2025.

2.3 Gold-based biosensors

2.3.1 Aptamer-gold-based biosensors. Initially, the optimization of the aptamer sequence length has been accomplished using methylene blue only as a reporter. The efficacy was measured using known concentrations of vancomycin. The main electrochemical technique utilized was CV. The 3 trunc aptamer (red curve in Fig. 4) shows a strong and sensitive response with a low dissociation constant (K_D) of 18.1 ± 1.6 μM, indicating high binding affinity. Further studies confirmed that the 3 trunc makes the electrochemical method reversible.[27]

In a later study, the aptamer-based sensor employed a single-stranded oligonucleotide aptamer as the recognition element. The system consisted of a three-electrode setup: a gold WE modified with a thiolated aptamer, a platinum wire as the CE, and an Ag/AgCl RE. This was employed using two redox reporters. The detection mechanism utilized the two redox reporters, ferrocene (Fc) as the reference and methylene blue (MB), attached to the aptamer, as the primary signal reporter. The main electrochemical technique utilized was SWV. Upon binding vancomycin to the aptamer, the aptamer undergoes a conformational change that causes the MB redox label to move away from the electrode surface, resulting in a measurable decrease in current. As the vancomycin concentration increases, the signal from MB diminishes accordingly. This sensing

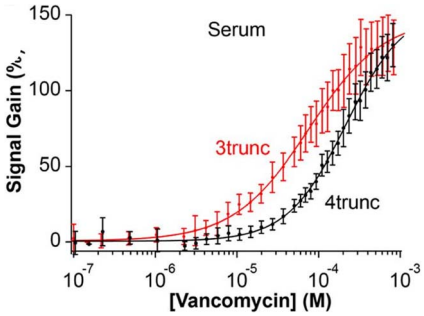


Fig. 4 The efficacy of truncated aptamers was determined *via* SWV in serum. The *y*-axis represents the signal gain, which is the change in the signal relative to measurements in the absence of vancomycin.[27]

strategy has shown high sensitivity and specificity for vancomycin detection in serum samples, with a recovery error of ±30% across a concentration range of 0.1 μM to 6.3 μM. The method is rapid, selective, well-suited for clinical applications, and the ratio of the two detectors is stable with time,[28] as illustrated in Fig. 5. The improvement in sensitivity was mainly attributed to the use of two redox reporters, improving measurement precision and reliability in electrochemical aptamer sensors.

A more recent study[29] employed a DNA aptamer immobilized on a gold electrode to specifically detect the free (non-protein-bound) fraction of vancomycin, the pharmacologically active form. The working electrodes, fabricated from gold, were modified with self-assembled monolayers of vancomycin-specific aptamers labeled with 3′-methylene blue (MB) and 5′-hexylthiol groups. Additionally, 6-mercaptohexanol was co-adsorbed to facilitate optimal surface packing and electron transfer, forming a well-organized electrochemical aptamer-based (E-AB) sensing interface. Square-wave voltammetry (SWV) was used as the primary electrochemical detection technique. The biosensor demonstrated reliable operation across the therapeutically relevant concentration range of 0.1–15 mg L^{-1}, achieving a detection limit of approximately 69 nM. It exhibited excellent sensitivity, precision, and accuracy, achieving 95% agreement within ±15% relative standard deviation when benchmarked against standard immunoassay methods. Despite utilizing only a single redox reporter, the system achieved superior analytical sensitivity, which was primarily attributed to the use of a multiplexed detection setup comprising three gold-based working electrodes that enhanced the overall signal response.[29]

2.3.2 Aptamer-gold-based microneedle biosensor. The microneedle biosensor offers a significant advantage of continuous monitoring of the concentration of vancomycin with minimal invasiveness. It is specifically designed to detect vancomycin in interstitial fluid (ISF) rather than plasma, thereby preventing large protein molecules and cell accumulation on the electrodes, which can interfere with measurements. Researchers,[30] have developed a dopamine-conjugated

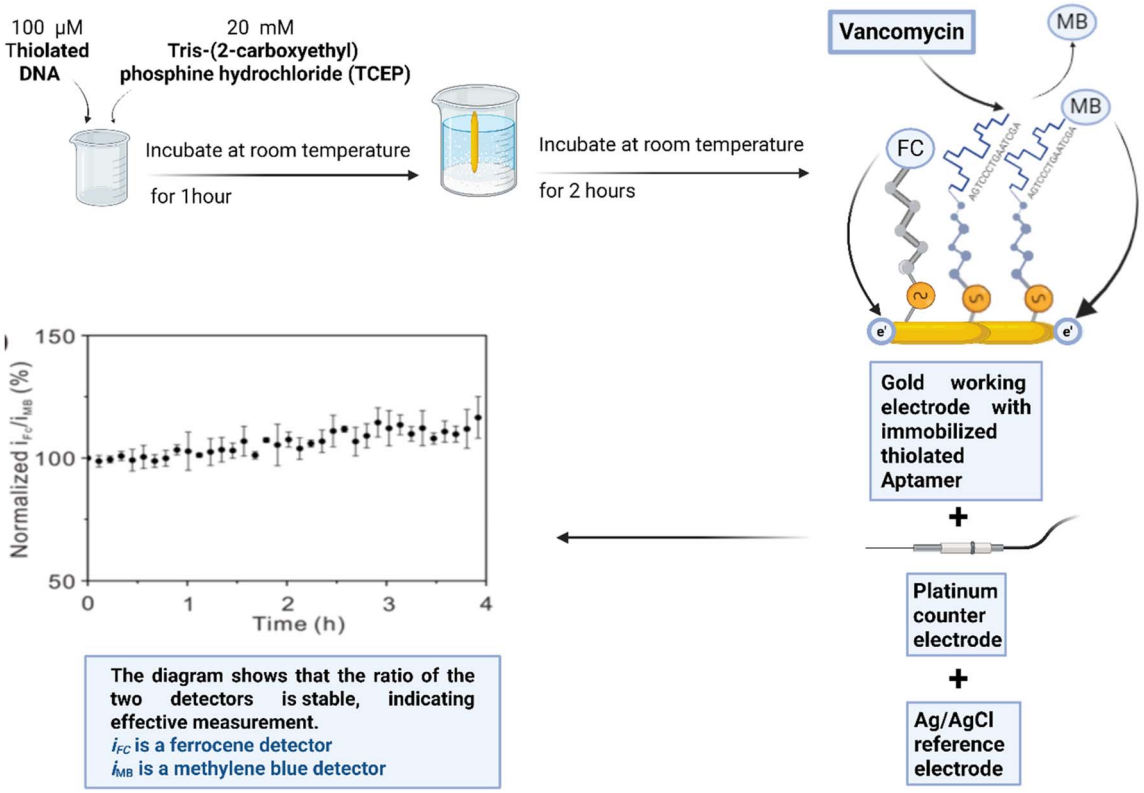


Fig. 5 The fabrication of MB-labeled aptamer crosslinked gold electrode, WE, for electrochemical analysis of vancomycin, showing the stability of electrodes during the analysis.[28]

hyaluronic acid (DAHA)-based hydrogel microneedle (HMN) integrated with flexible (flex) electrodes (HMN-flex) for vancomycin detection in ISF. The DAHA hydrogel facilitates ISF extraction, allowing only small molecules such as vancomycin to reach the electrodes. The three-electrode system consists of a silver/silver chloride (Ag/AgCl) reference electrode and two gold electrodes, serving as the CE and WE. The WE are functionalized with a thiolated and crosslinked to an aptamer labeled with methylene blue (MB).

The detection mechanism follows a conformational change-based sensing approach, similar to the previous aptamer-based method but with a different signal response. Upon insertion into the skin, the HMN swells, allowing vancomycin to diffuse into the hydrogel. Two scenarios arise:

(1) In the presence of vancomycin, binding to the aptamer brings MB closer to the working electrode, leading to an enhanced electrochemical signal.

(2) In the absence of vancomycin, no conformational change occurs, and MB remains distant from the electrode, resulting in a lower signal output.

To evaluate the biosensor's performance, researchers administered two different vancomycin doses (45 mg kg^{-1} and 15 mg kg^{-1}) to rats. The HMN-flex sensor effectively differentiates between the two concentrations *in vivo*, demonstrating its potential for real-time electrochemical monitoring. Besides that, researchers used to measure the concentration of vancomycin in serum along with the analysis in ISF, and they observed that they exhibit similar behavior, as in the serum the concentration of vancomycin is high after administration then significantly declines due to metabolism, and in ISF concentration of vancomycin increases when it distributes through the body then decline,[30] as illustrated in Fig. 6.

Further evaluation of the specificity of the vancomycin sensor against tobramycin, doxorubicin, and common chemical constituents found in interstitial fluid, was done and results showed that the sensor responds only to vancomycin, confirming its high specificity. Building on the hyaluronic acid (HA)-based hydrogel method, a modified approach was developed to enable simultaneous monitoring of both vancomycin and blood pH. Methacrylated hyaluronic acid (MeHA) was synthesized by reacting HA with methacrylic anhydride (MAA). This MeHA was then mixed with *N*, *N*′-methylenebisacrylamide (MBA) and a photo initiator (PI), poured into a polydimethylsiloxane (PDMS) mold, and allowed to dry. To introduce pH sensitivity, phenol red (PR) was incorporated into the hydrogel. In addition to its simplicity, low cost, and capability for real-time vancomycin detection, the biosensor is also enabled to track pH, which is useful for monitoring the effect of the treatment. This approach demonstrated high accuracy with pH measurement variance within 0.054 ± 0.09 compared to standard blood measurements and with a vancomycin detection threshold of 5 μM.[30]

In a separate study, a similar strategy was employed but with embedding electrochemical aptamer sensors within stainless-steel microneedles for minimally invasive monitoring. The working electrode was formed by crosslinking MB-labeled aptamers, while gold wires coated with perfluoroalkoxy (PFA) were cut at one end for electrical connection and beveled at a 45–60° angle at the other to create microneedle tips. A platinum wire and an Ag/AgCl wire, both PFA-insulated, served as the counter and reference electrodes, respectively, and were also embedded in the needle. When tested in undiluted bovine blood, the sensor accurately detected 29 μM of vancomycin in a 30 μM sample. Additionally, it successfully detected

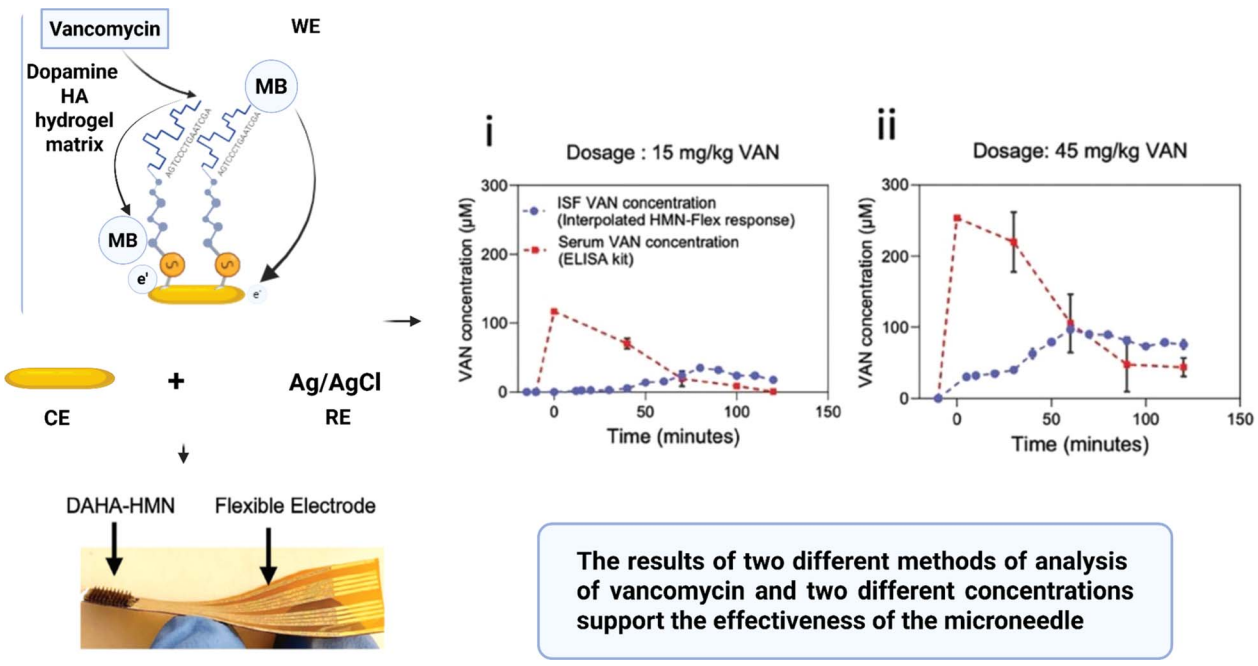


Fig. 6 The electrochemical analysis of vancomycin in the ISF using gold flexible (flex) electrodes (HMN-flex), with an aptamer cross-linked MB labeled surface embedded on dopamine conjugated HA-based hydrogel.[30]

vancomycin in porcine skin, demonstrating feasibility for real-time, *in vivo* monitoring. However, further refinement is needed to reduce signal noise and enhance measurement precision.[31]

2.4 Graphite-based biosensor

2.4.1 MIP-graphite-based biosensor. An electrochemical sensor for selective vancomycin detection using a graphite electrode modified with molecularly imprinted polymers (MIPs), which serve as artificial recognition elements that mimic biological receptors, was also developed.[31] The MIP synthesis involves combining a functional monomer, which binds to the target analyte, a crosslinking monomer, a cross-linking regulator, a redox initiator, and vancomycin (as the template molecule) in a solvent mixture of distilled water and *N*, *N*-dimethylformamide (DMF). This mixture is introduced into a test tube containing initiator-treated graphite, bubbled with inert gas for 30 minutes, and irradiated with a xenon lamp for another 30 minutes to initiate polymerization. Afterwards, the vancomycin template is extracted, and the mixture is centrifuged to remove residuals, followed by vacuum drying of the MIP.

To prepare the MIP-graphite paste, the dried MIP particles are blended with silicone oil to form a uniform paste using a mortar and pestle, as shown in Fig. 7. The sensor employs a ceramic-based platform composed of aluminum oxide and platinum wiring and integrates a three-electrode system: a platinum CE, an Ag/AgCl (RE), prepared using conductive ink, and a graphite paste working electrode.

DPV is used for detection due to its high sensitivity, with optimized parameters: an initial potential (E_s) of 0.0 V, terminal potential (E_e) of 0.9 V, pulse time of 10 ms, pulse amplitude of 50 mV, step potential (E_{step}) of 5 mV, scan rate of 10 mV s^{-1}, and a current range of 10 μA. As shown in the figure, the concentrations of vancomycin in the blood and saline are almost similar. This method demonstrates high selectivity, rapid detection, low sample volume requirements, and ease of use. It offers a cost-effective alternative to traditional immunoassays and chromatographic techniques, especially suitable for resource-limited settings. However, its main drawback is that the sensor is designed for single use.[32]

In a later study, further enhancement of the biosensor sensitivity was achieved using glassy carbon electrodes to fabricate MIP on them. The method is considered fast and extremely sensitive, as shown in Table 1, with a detection limit of 2.808 pM. To prepare the MIP, the glassy carbon electrode (GCE) was sonicated in a methanol and double-distilled water mixture (1 : 1 v/v) for 15 minutes to clean. It was then polished with alumina slurry on a polishing pad, washed, and air-dried. Vancomycin was coated onto TiO_2 nanoparticles dispersed in phosphate buffer saline (pH 7.4, 100 mM) by stirring at 125 rpm at room temperature for 30 minutes. Unbound vancomycin was

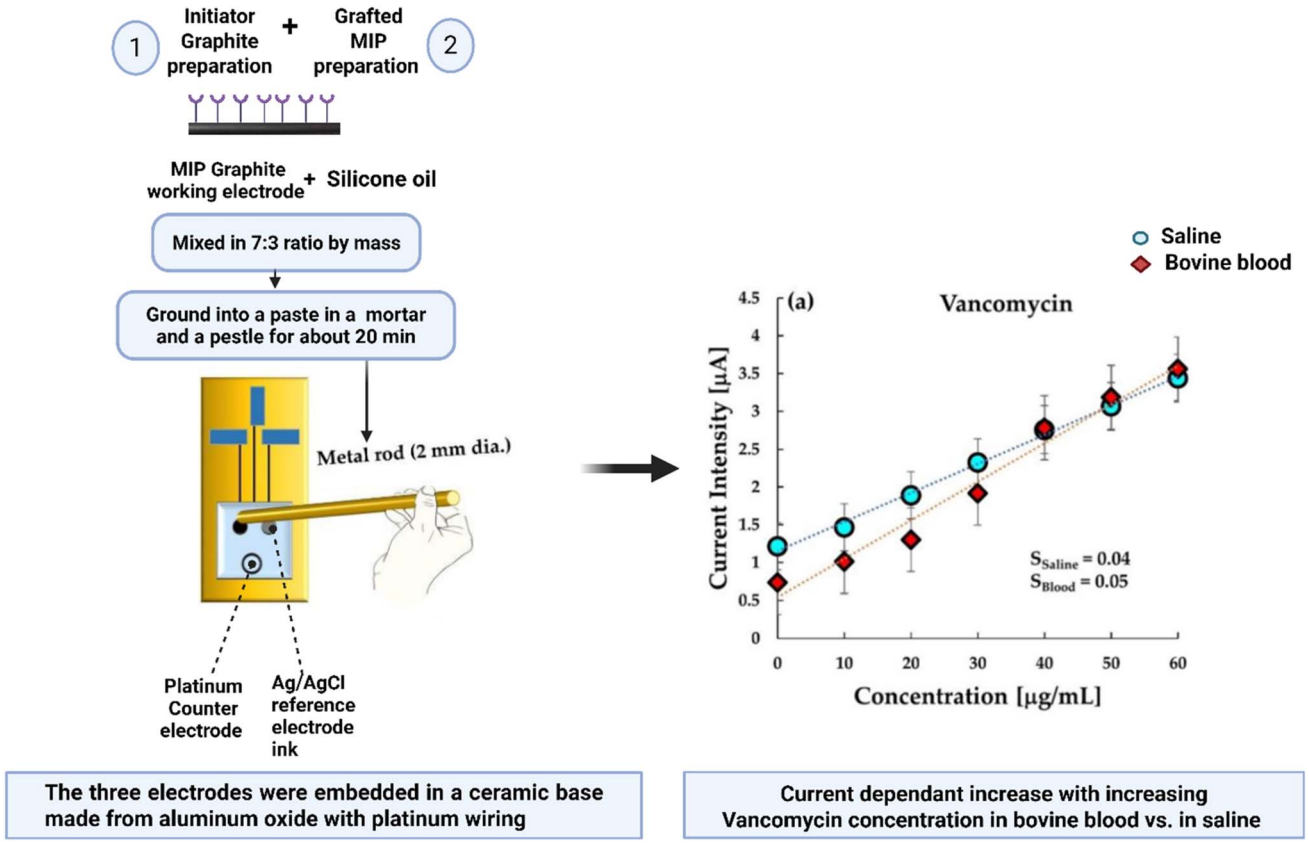


Fig. 7 The electrochemical analysis of vancomycin in blood and saline using a graphite electrode with polymeric imprinted cross-linked molecules after its mixing with silicon oil in a ceramic-based biosensor.[29]

Table 1 The table illustrates the efficacy of the measurement of vancomycin in the serum and water using DPV

Sample	Spiked amount (pM)	Found amount (pM)	Recovery (%)	RSD (%)	Bias (%)
Serum	25	25.4	101.6	25.4	101.6
Serum	75	75.6	100.79	75.6	100.79
Tap water	50	50.5	101.05	50.5	101.05
Tap water	75	76.2	101.57	76.2	101.57

removed by rinsing with deionized water. Further, the vancomycin-coated TiO_2 nanoparticles were added to a 1% alginate solute on and stirred vigorously for 30 minutes, and A 2.0 μL drop of the alginate-TiO_2-VAN mixture was cast onto the cleaned GCE surface, followed by a 10 μL drop of $CaCl_2$ solution to cross-link and form a stable alginate gel film. The main drawback was that the sensor was designed for single use, as well as in the previous MIP-based biosensor.[33]

Eguchi *et al.* also reported the fabrication of an MIP on an indium-doped tin oxide (ITO) electrode using UV-initiated graft polymerization. The polymer layer was prepared with MAA as the functional monomer, acrylamide (AAm) and methylenebisacrylamide (MBAA) as crosslinkers, and allylamine carboxypropionate-3-ferrocene (ACPF) as a redox-active monomer. Vancomycin served as the template during copolymerization, which was carried out in a water/DMF solvent system under argon. Following polymerization, the bulk polymer was removed by sonication and washing, and the template was extracted using a 1 M NaCl solution, leaving behind a thin, covalently grafted MIP film on the electrode surface. This film enabled direct and reagentless electrochemical detection *via* DPV. In terms of performance, the ACPF-containing MIP-ITO electrode exhibited a linear response to vancomycin concentrations between 0 and 40 μM (within the therapeutic range), with sensitivities of 17.4 ± 0.6 mA M^{-1} in phosphate-buffered saline and 19.2 ± 1.9 mA M^{-1} in whole blood. The electrode also demonstrated high selectivity for vancomycin over the structurally related glycopeptide teicoplanin, supporting its potential for multiple uses as well as real-time therapeutic drug monitoring in complex biological samples such as whole blood.[34]

2.5 Carbon-based biosensors

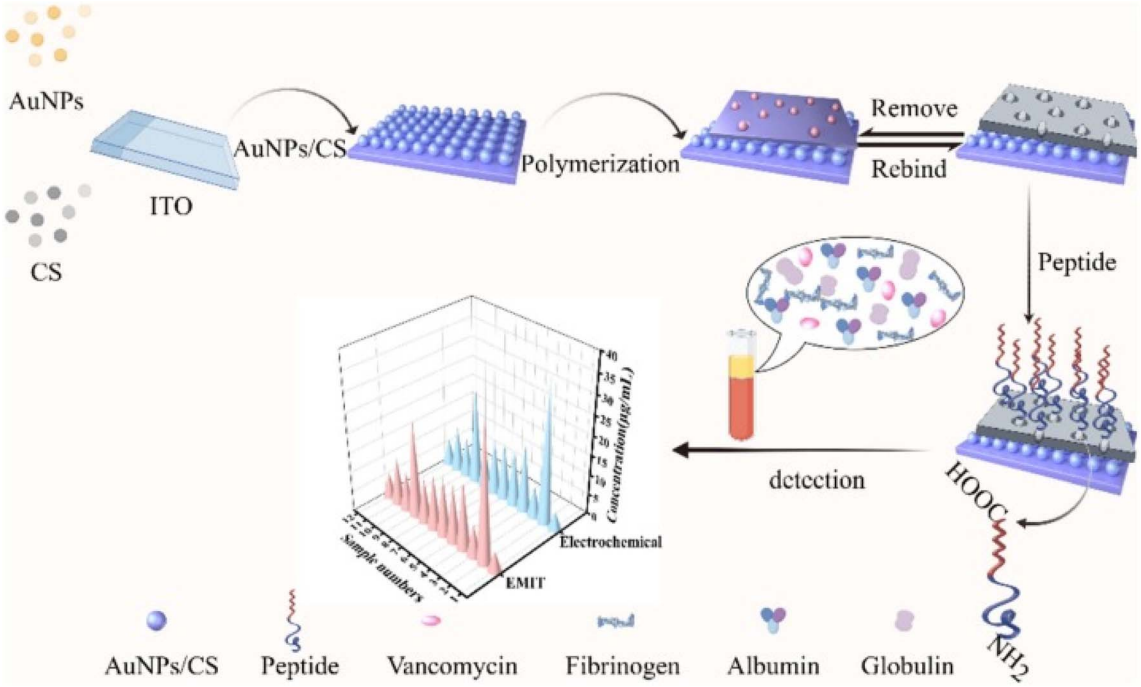
2.5.1 Aptamer-carbon-based biosensors. Carbon-based electrodes have emerged as versatile, cost-effective platforms for electrochemical biosensing. In one approach, laboratory-printed carbon electrodes (C-PEs) were modified with cauliflower-shaped gold nanostructures (AuNSs) to enhance electrochemical conductivity and provide a high density of active sites for aptamer immobilization. Vancomycin-specific aptamers were anchored onto the AuNS surface *via* thiol–gold linkages, while short-chain alkanethiols were employed as blocking agents to minimize nonspecific adsorption. The biosensor operated on a label-free detection principle using electrochemical impedance spectroscopy (EIS), where the binding of vancomycin to the surface-immobilized aptamer increased the interfacial charge-transfer resistance. This aptasensor demonstrated a broad linear detection range from 50 nM to 1000 nM and an exceptionally low limit of detection (LOD) of 1.721 nM. It also exhibited high selectivity against common interferents present in human serum and milk, highlighting its suitability for rapid, disposable, and sensitive on-site vancomycin analysis in both clinical diagnostics and food safety applications.[35]

A more recent study[36] introduced a dual-recognition electrochemical biosensor that integrates antibiotic-based and aptamer-based recognition mechanisms for the sensitive and rapid quantification of vancomycin or vancomycin-susceptible bacteria in biological fluids. The platform utilized a screen-printed carbon electrode (SPCE) functionalized through a layer-by-layer assembly process. First, bovine serum albumin (BSA) was drop-cast onto the SPCE (5 μL, 2 mg mL^{-1}) to generate an amine-rich surface. Vancomycin molecules were then covalently coupled to BSA using EDC/NHS cross-linking, forming stable amide bonds. Unreacted sites were blocked with ethanolamine to prevent nonspecific adsorption. For bacterial recognition, species-specific aptamers were subsequently immobilized onto the surface. Cyclic voltammetry (CV) was used to confirm each surface modification step (BSA, vancomycin, ethanolamine).

The immobilized vancomycin enabled selective binding either to the D-Ala–D-Ala residues of Gram-positive bacterial cell walls or directly to vancomycin molecules in plasma. This design established a dual recognition system, combining antibiotic–target and aptamer–target interactions, that significantly enhanced detection reliability. Binding events at the electrode interface induced measurable changes in charge-transfer resistance (EIS) and peak current (DPV). Specifically, EIS quantified overall binding events, while DPV differentiated target identity, producing a complementary and highly selective dual-mode detection scheme. The biosensor achieved excellent reproducibility, with relative standard deviations (RSD) below 10–13%, and completed detection and identification within 45 minutes, even in untreated complex matrices such as milk and serum. This system effectively merges covalent antibiotic immobilization with aptamer-mediated specificity, achieving ultrasensitive, rapid, and multiplexed vancomycin and bacterial detection suitable for clinical and food safety monitoring Scheme 4.[36]

3 Discussion

Electrochemical biosensing techniques for vancomycin detection have advanced significantly in recent years, offering



Scheme 4 Diagrammatic representation of a Peptide/MIPDA/AuNPs/CS-modified electrode used for the electrochemical sensing of vancomycin, with permission from Elsevier, copyright 2025.

a diverse array of strategies tailored to different analytical needs. These methods include amperometric, voltammetric (*e.g.*, differential pulse voltammetry DPV, SWV, CV, potentiometric, and EIS-based sensors, each with distinct operational principles and performance characteristics. Amperometric sensors are widely used in platforms such as aptamer-based, molecularly imprinted polymer (MIP)-based, and metal–organic framework (MOF)-based biosensors. These sensors measure the current generated by redox reactions at a fixed potential, which is directly proportional to analyte concentration. Their key strengths are rapid response times and quantitative outputs, making them highly suited for continuous, real-time monitoring. However, a major limitation is the presence of background currents, which can obscure low-level signals, especially in complex biological matrices such as serum or plasma.[37] For using the EIS-based electrochemical detection, it is necessary to note that the highest sensitivity and stability were obtained with the aptamer-based C-PE.[35]

Voltammetric techniques, including DPV, SWV, and CV, enhance both sensitivity and selectivity compared to amperometric or potentiometric approaches. These methods rely on modulating the applied potential while analyzing the shape, amplitude, and position of resulting redox peaks. For instance, DPV minimizes the capacitive background signal, enabling ultra-low detection limits, even down to a few colony-forming units per milliliter (CFU mL^{-1}) for bacterial targets and pharmaceutical analytes in clinical samples. SWV offers faster measurements with higher sensitivity by improving signal-to-noise ratios, while CV is primarily used for electrode surface characterization and monitoring modification processes in sensor fabrication. Potentiometric biosensors, which detect changes in electrode potential under zero current flow, are highly selective for ionic species but face challenges related to interference from competing ions and limited adaptability for miniaturized devices in point-of-care applications.[38,39] Overall, the sensitivity and specificity of vancomycin detection depend not only on the affinity between vancomycin and the electrode surface but also on the performance characteristics of the chosen electrochemical technique.

The long-term stability of biosensors, particularly those based on MIPs and MOFs, has recently gained significant attention due to its importance for reliable, real-world applications. In MIP synthesis, two primary fabrication strategies are employed: bulk polymerization and graft polymerization, each with unique implications for sensor performance. Bulk polymerization is a classical approach wherein functional monomers, cross-linkers, and template molecules are polymerized in a bulk solution, producing a solid polymer block. This block is later ground and sieved to form polymer particles containing template-shaped cavities. This method is straightforward and easily scalable, making it attractive for producing large quantities of MIP material. However, it often results in heterogeneous particle sizes and non-uniform binding site distributions, which can compromise sensor selectivity, stability, and reproducibility. Furthermore, template removal is typically labor-intensive, requiring extensive washing or Soxhlet extraction. Traditional bulk polymerization usually employs organic solvents to initiate polymer growth.[32] Recently, greener, water-based bulk polymerization techniques have emerged, offering environmentally friendly alternatives while retaining

Table 2 Comprehensive summary of electrochemical biosensors for vancomycin detection in blood, serum, or ISF

Sensor type	Recognition element	Electrode/ substrate	Technique	Matrix tested	Linear range	LOD	Key findings/strengths	Clinical validation	Limitations	Ref.
Glass carbon (GR-GC) based	Graphene	Glass carbon (GC)	SWV	Plasma samples	Therapeutic range (μM)	5 μM	Proof-of-concept	Plasma samples	Plasma samples of healthy people were diluted 50 times	21
Gold NS-graphene-based	Gold nanostr-ucture (NS)	Graphene	DPV	Human serum	Clinical range	0.29 μM	Recovery rates were 97.38% and 104.54% in oxidation, and 107.06% and 103.70% in reduction, for vancomycin	Human serum	Confirmed platform's specificity using a mixture containing vancomycin and eight other antibiotics	24 and 25
Electrochemical aptamer-based (E-AB) sensor	DNA aptamer (structure-switching)	Gold microelectrode	SWV	Human plasma (finger-prick)	Therapeutic range (μM)	Low nM	Foundational work enabling calibration-free E-AB sensing	*Ex vivo* plasma	Proof-of-concept only; limited real-world testing; single-use configuration	41
MIP-based biosensor	MIP	Graphite	DPV	Human serum	Therapeutic range	2.808 pM	Proof-of-concept	Serum	Designed for single use	33
E-AB clinical validation	3′-MB/5′-hexylthiol aptamer	Gold electrode array	SWV	Human serum	0.1–15 mg L^{-1}	~69 nM	95% agreement with immunoassays; robust analytical validation	Yes (clinical samples)	Multiplexed setup increases fabrication cost; not yet miniaturized for POC use	29
Microneedle E-AB	Structure-switching aptamer	Goid-based stainless steel microneedle	SWV	Whole blood (bovine)	Clinical range	29 μM	Wearable continuous monitoring concept demonstrated	*Ex vivo*/on-body	Limited to *ex vivo* testing; long-term stability and biofouling unaddressed	31
Multiplexed E-AB	Vancomycin aptamer	3D-printed electrode array	SWV/EIS	Human serum	Therapeutic range	Low nM	Multipoint sensing improved reproducibility and throughput	Yes	Complex 3D printing setup; device-to-device variability not fully optimized	42
Integrated microfluidic E-AB	Vancomycin aptamer	Gold-based-integrated microelectrode chip	SWV/ amperometry	Complex fluids	Clinical range	0.28 μM	Demonstrated continuous operation and microfluidic integration	Proof-of-concept	Prototype only; animal ISF	30
			FET		1 nM–100 μM	0.187 nM				26

Table 2 (*Contd.*)

Sensor type	Recognition element	Electrode/substrate	Technique	Matrix tested	Linear range	LOD	Key findings/strengths	Clinical validation	Limitations	Ref.
EG-FET wireless aptasensor	Sulfhydryl-modified aptamer	MnO_2/AuNPs on laser-induced graphene		Whole blood (janus membrane separation)			Portable wireless operation; ultra-low detection limit	Direct blood testing	Requires specialized janus membrane prep; potential sample clogging	
Label-free EIS aptasensor	Vancomycin aptamer	Carbon electrode w per Au nanostructures	EIS	Human serum, milk	50–1000 nM	1.721 nM	Highly sensitive, disposable and low-cost platform	Spiked samples	Lacks direct patient testing; shelf-life not assessed	35
Disposable aptasensor	High-affinity aptamer	Printed carbon electrodes	EIS/SWV	Serum	Clinical range	Low nM	Designed for portable, low-cost testing (POCT focus)	Serum validation	Limited operational lifetime; one-time use only	43

functional performance. These strategies yield well-defined nanoparticles ideal for composite electrode construction. For example, adding TiO_2 nanoparticles to an alginate polymer matrix enhanced sensitivity, but the bulk polymerization method still fell short in terms of long-term stability and sensor reusability.[33]

Graft polymerization, in contrast, involves initiating polymerization directly on a substrate surface such as an electrode or nanoparticle, forming a covalently attached MIP layer. Methods such as UV-induced radical polymerization on initiator-coated indium tin oxide (ITO) electrodes produce thin, uniform polymer films with controlled thickness. The surface-bound MIPs enable better accessibility to binding sites and improved electron transfer, both of which are crucial for electrochemical biosensing. Template removal is simpler, typically achieved by washing, and the modified electrode can be reused multiple times without significant loss of performance. This approach enables reagentless, real-time sensing with superior stability and sensitivity compared to bulk-polymerized systems.[34] Thus, graft polymerization is ideal for direct fabrication of robust sensor interfaces, whereas bulk polymerization is best suited for producing free MIP particles that later require integration into electrode assemblies.

MOF-based electrochemical sensors have emerged as highly promising platforms due to their exceptionally high surface area, chemical tunability, and ability to function as efficient electron transfer mediators. These properties make MOFs particularly well-suited for detecting vancomycin in complex biological fluids. By combining nanomaterial engineering with selective bio-recognition strategies such as aptamer or antibody immobilization, MOF-based sensors achieve high sensitivity and selectivity, with detection limits reported in the low nanomolar to picomolar range. For enhanced stability, future designs should focus on immobilizing MOFs directly onto electrode surfaces using solvothermal or hydrothermal synthesis techniques, followed by functionalization with vancomycin-specific recognition elements.[40] A comparative evaluation of electrochemical sensors *versus* traditional analytical methods is summarized in Table 2. While HPLC and immunoassays remain the gold standards for regulatory validation due to their unmatched accuracy, they are limited by high costs, labor-intensive procedures, and slow turnaround times, making them impractical for therapeutic drug monitoring in urgent care settings. Electrochemical biosensors, on the other hand, offer portability, cost-effectiveness, and rapid response times, though with some trade-offs in absolute quantitation and multiplexing capacity. These advantages position electrochemical platforms, particularly aptamer-based sensors, as the future of real-time, ultra-sensitive, and user-friendly vancomycin detection across clinical, food safety, and environmental applications. In summary, advances in electrode design, polymerization strategies, and nanomaterial integration are driving electrochemical biosensors toward replacing traditional methods for vancomycin monitoring. While conventional assays remain indispensable for confirmation and regulatory compliance, electrochemical platforms offer transformative potential for decentralized, point-of-care diagnostics.

4 Conclusion and future perspective

Vancomycin therapy presents a significant challenge due to its narrow therapeutic window and the high variability in pharmacokinetics among patients, influenced by co-administered drugs and underlying diseases. Conventional methods, such as chromatographic and immunoassay techniques, are commonly used for vancomycin monitoring. However, these methods are often costly, time-consuming, and may lack specificity and sensitivity. Recent advancements in electrochemical biosensors have demonstrated promising efficacy for vancomycin detection. These biosensors offer faster analysis times, improved sensitivity, and the potential for real-time monitoring with minimal invasiveness.

To summarize, electrochemical-based biosensors for vancomycin detection offer advantages such as low cost, simplicity, and rapid analysis compared to HPLC and immunoassay methods. MIP and MOF-based biosensors demonstrate sensitivity and selectivity that are either higher or comparable to those of HPLC and immunoassay methods, as summarized in Table 2. However, further exploration for optimizing the regeneration of MIP-based biosensors and improving the stability of MOF-based biosensors should be conducted. Additionally, further optimization is essential to ensure their clinical applicability and to establish them as viable alternatives to conventional laboratory-based methods.

To facilitate the clinical adoption of electrochemical biosensors, several key areas require further research and development:

- Enhancing stability and reproducibility to ensure long-term sensor performance and reliability.
- Miniaturization and integration into portable or wearable devices for real-time, point-of-care monitoring.
- Addressing regulatory requirements to meet the standards necessary for hospital implementation.
- Expanding multiplexing capabilities to allow simultaneous detection of vancomycin alongside other critical biomarkers.

The successful commercialization of these biosensors will transform vancomycin monitoring, reduce hospitalization time and costs, minimize the need for frequent blood sampling, and ultimately improve patient health outcomes. Additionally, real-time monitoring with minimally invasive biosensors can significantly reduce the risk of infection transmission in hospital settings, making them a valuable tool for personalized medicine.

Author contributions

Aya M. Gaber: literature review, writing – original draft. Hadir M. Emara: writing – original draft, visualization, and editing. Nageh K. Allam: conceptualization, supervision, revision, and editing.

Conflicts of interest

The authors declare no competing interests.

Data availability

No datasets or software were used in this review.

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