



Therapeutic drug monitoring of meropenem in human serum using a sensitive four-electrode electrochemical biosensor for critically ill patients in intensive care wards

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ARTICLE INFO

Keywords:

Drug monitoring
Electrochemical biosensor
Impedance
Meropenem
Sepsis

ABSTRACT

Therapeutic drug monitoring of antibiotics in critically ill patients with sepsis remains challenging due to highly variable pharmacokinetics, increasing the risk of under- or overdosing. This paper presents a novel single-use tetrapolar electrochemical impedance spectroscopy (EIS) biosensor for the rapid detection of meropenem in human serum. The sensor is based on a gold microband electrode functionalised with a self-assembled monolayer, protein G and anti-meropenem antibodies to enable selective antigen capture and impedance-based quantification. The biosensor demonstrated sensitive detection of meropenem over a range of 1–100 µg/mL in phosphate-buffered saline and within clinically relevant concentrations (8–16 µg/mL) in spiked human serum, with the lowest measured concentration of 1 µg/mL. Binding specificity was validated using tandem mass spectrometry and immunocapture liquid chromatography-mass spectrometry. The total assay time was under 15 min. This platform represents a promising approach for rapid, bedside-compatible monitoring of meropenem. While the impedance results demonstrate the potential of the biosensor for therapeutic drug monitoring, further validation is required to establish its specificity, robustness, and performance in clinically relevant samples. Additional studies are also needed to evaluate its potential role in supporting antibiotic dose optimisation in sepsis management and intensive care settings.

1. Introduction

Sepsis remains a leading cause of morbidity and mortality worldwide (Singer et al., 2026; Rudd et al., 2020; Vincent et al., 2020). Even in well-resourced healthcare systems, mortality rates reach 20%–30%, with even higher rates observed in frail or immunosuppressed patients. Among survivors, approximately 40% experience long-term complications, including recurrent infections, physical weakness, cognitive impairment and psychological effects (Iwashyna et al., 2010). The economic burden is substantial, with an estimated annual direct cost of two billion Sterling to the UK National Health Service (<https://www.gov.uk/government/news/new-action-to-reduce-sepsis>. Last accessed 13 April).

A cornerstone of sepsis management is timely and appropriate

antibiotic therapy. However, standard practice is reliant on fixed antibiotic dosing, but this is inadequate for critically ill patients whose antibiotic pharmacokinetics often deviate markedly from the norm (Novy et al., 2023). These variations arise from multiple physiological changes during critical illness, including vascular leak with increased volume of distribution, reduced protein binding, impaired hepatic metabolism and fluctuating renal clearance. Consequently, many receive subtherapeutic dosing that risks treatment failure and development of antimicrobial resistance, while others receive excessive dosing associated with toxicity (Roberts et al., 2014) (Hagel et al., 2022).

Apart from a few antibiotic classes (aminoglycosides and glycopeptides), therapeutic drug monitoring (TDM) of serum antibiotic levels is generally unavailable in most hospitals. This applies particularly to β-lactam antibiotics, such as penicillins and carbapenems, the most used

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<https://doi.org/10.1016/j.bios.2026.119218>

Received 24 April 2026; Received in revised form 3 September 2026; Accepted 9 September 2026

Available online 16 September 2026

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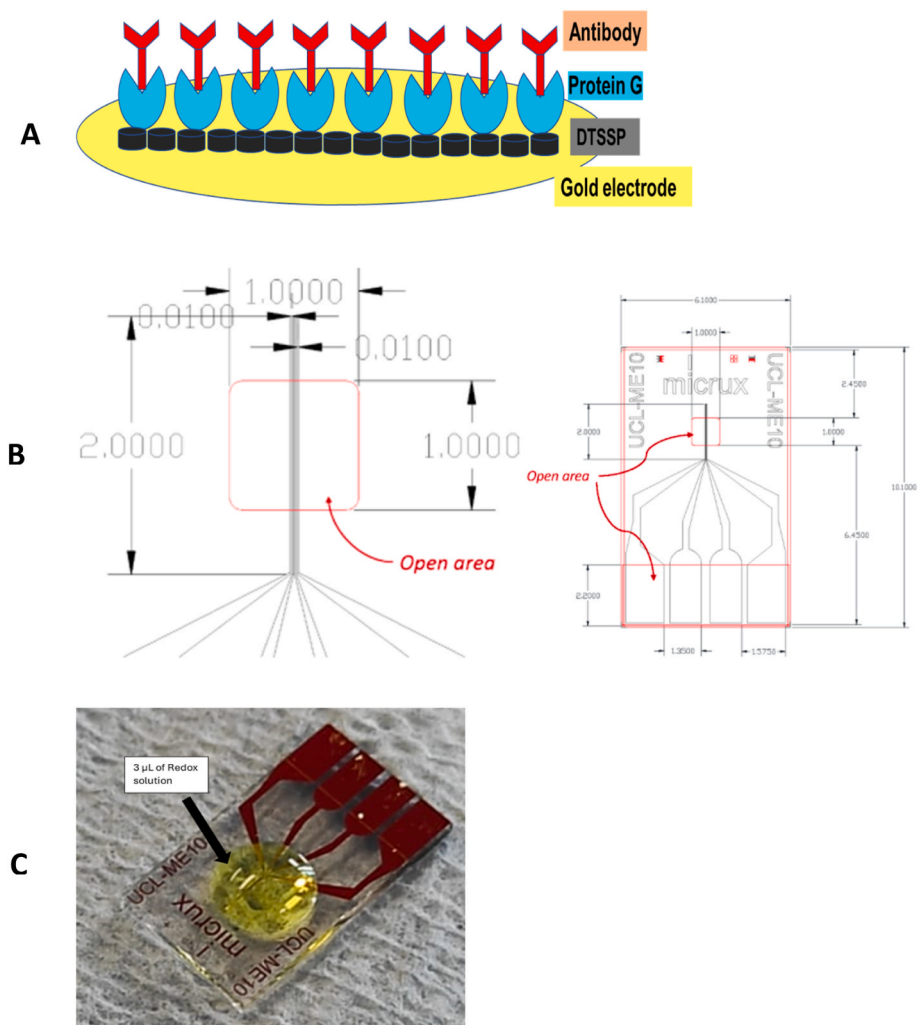


Fig. 1. Electrochemical impedance spectroscopy (EIS) biosensor (A) Schematic cross-sectional view of the biosensor with biolayers used for functionalising the biosensor. Bottom to top: gold on glass substrate, SAM layer (DTSSP), protein G and antibody. (B) Geometry and dimensions of the electrode. (C) Ready-for-measurement biosensor with the redox on top. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

agents in hospital practice (Williams et al., 2023). In the few hospitals where TDM of β -lactam antibiotics is performed as a laboratory service, results are often not available on the same day or even on a daily basis. However, antibiotic dosage optimisation should be time-critical to improve outcomes and avoid toxicity. A key solution is to develop rapid and reliable near-bedside measurement techniques.

Electrochemical biosensors principally work on electrochemical reactions that cause electron movement driven by the interaction of the analyte with the bioreceptor on the transducer surface, producing measurable changes in current, impedance, potential and capacitance. The electrical signals in response to the chemical change are proportional to the analyte concentration (Naresh and Lee, 2021) (Gundogdu et al., 2023).

In this study, initial efforts focused on measuring meropenem, a β -lactam antibiotic commonly used in critically ill patients, with the aim of subsequent expansion to other antibiotics. The development of a novel single-use tetrapolar electrochemical impedance spectroscopy (EIS) biosensor for the detection of meropenem is presented, enabling quantifiable monitoring of meropenem levels in human fluids.

2. Methods

2.1. Materials

The biosensor was developed on a microelectrode array manufactured by MicruX Technologies (Asturias, Spain) and composed of four gold microband electrodes fabricated using standard microfabrication technology on a p-doped silicon substrate (Fig. 1). The gold surface was coated with an organic polymer passivation layer, leaving an open window of 1 mm $\times$ 1 mm that exposed a 1 mm length of each gold microband electrode - the active area. The microelectrode array was connected to a printed circuit board via four gold-plated pins and then to a Solartron 1260 impedance gain-phase analyser (Amtek, formerly Solartron Analytical, Farnborough, U.K.). Phosphate buffer saline (PBS), 3,3'-dithiobis (sulfosuccinimidyl propionate) (DTSSP), and protein G were from Thermo Fisher (Basingstoke, U.K.). Human serum, Type AB (HCV, HIV, HBs Ag-free) was from Fisher (Loughborough, U.K.). Ethanolamine, potassium hexacyanoferrate (II) trihydrate (455989) and potassium hexacyanoferrate (III) (244023) were from Sigma-Aldrich (Gillingham, U.K.). Anti-meropenem antibody (abx170011) was from Abxexa (Milton, U.K.) and meropenem from ACS Dobfar S.p.A. (Tri-biano, Italy).

2.2. Development of the biosensor

Four gold microbands were fabricated on a glass substrate and connected to the appropriate Bayonet Neill–Concelman (BNC) socket of the impedance analyser via pogo pins attached to the drop cell connector carrying the electrode for impedance measurements. The first electrode was the working electrode (WE), connected to the impedance analyser's signal generator output. The fourth electrode was the counter electrode (CE), connected to the instrument's current input BNC socket. The second and third electrodes were reference electrodes (RE) and were connected to the instrument's V1 Hi and V1 Lo BNCs, respectively. A shield was used to protect the measurements from external electromagnetic noise.

The gold microband electrodes were washed with 5 mL of 100% ethanol, followed by de-ionised water and PBS to remove any dielectric material from the sensor surface. The self-assembled monolayer of cross-linker DTSSP was attached to the cleaned microband electrodes by dissociative chemisorption, forming a self-assembled monolayer (SAM). Protein G and antibodies were added layer by layer, with 45 min of incubation in PBS between layers, and washed to remove excess unbound substrate after each incubation. DTSSP attaches protein G via the interaction between the N-hydroxysulfosuccinimide (sulpho-NHS) ester and amino groups. Ethanolamine was used to saturate any unreacted sulpho-NHS ester groups on DTSSP after protein G binding, before addition of the antibody. A range of anti-meropenem antibody concentrations was evaluated to determine the optimum concentration with 100 µg/mL identified as optimal and used in all subsequent experiments.

2.3. Electrodes

The electrode array was designed to build on the authors' previous work with the tau biosensor (Wang et al., 2016), which used a four-microband printed-circuit board electrode (Fig. 1). In the current study, a four-microband array was designed using computer-aided design (CAD) on a glass substrate to reduce the cost per unit and allow sensors to be disposable (Bayford et al., 2022).

2.4. Electrochemical impedance spectroscopy (EIS)

At each stage of biosensor assembly, impedance measurements were recorded in 5 mM $K_4Fe(CN)_6/K_3Fe(CN)_6$ (potassium ferro/ferricyanide) (1:1 ratio) in PBS at pH 7 using SMarT software (Ametek Inc., USA) on the Solartron analyser.

A sinusoidal signal of 5 mV amplitude was applied between WE and CE electrodes (Fig. 1) over a frequency range of 100–100,000 Hz to produce EIS measurements. The fully built biosensor was subjected to successive incubations with meropenem in PBS (1, 5, 10, 50 and 100 µg/mL) for 15 min at room temperature. After each reaction, the biosensor electrodes were flood-washed with 5 mL of de-ionised water and PBS, and impedance was recorded in the presence of a redox mediator. EIS parameters R_s (solution resistance), R_{ct} (charge-transfer resistance or interfacial resistance) and C_p (constant phase element, an equivalent model of double-layer capacitance) were generated by fitting the data to Randles equivalent circuit model using Zview software (Solartron Analytical, U.K.).

2.5. Cyclic voltammetry

Cyclic voltammetry (CV) was performed in 50 mM sulphuric acid for electrode regeneration and in 5 mM $K_4Fe(CN)_6/K_3Fe(CN)_6$ (1:1 ratio) in PBS at pH 7 for the biosensor's layer-by-layer construction. For electrode regeneration, the potential was cycled from -1.0 V to $+1.3$ V at 100 mVs^{-1} , using an external Ag/AgCl reference electrode for 30 cycles to remove surface analytes and/or contaminants. For biosensor construction, scans were conducted between 0.0 V and $+1.0$ V.

2.6. LC-MS/MS for confirmation of antibody binding

Immunocapture liquid chromatography tandem mass spectrometry (IC-LC-MS/MS) was used to validate the specific binding of the anti-meropenem antibody to meropenem, given its small size. A $10\text{ }\mu\text{g/mL}$ solution of meropenem was prepared in water to identify product ions and optimise dwell time, collision energy and voltages. MS analysis was performed using a Shimadzu Prominence ultra-high-performance liquid chromatography system connected to an 8060 triple quadrupole mass spectrometry (Shimadzu, Milton Keynes, U.K.) comprising a degasser, HPLC pumps, a column oven and an autosampler. Meropenem separation was achieved using a binary gradient elution with solvent A as 0.1% aqueous formic acid and solvent B as 0.1% formic acid in acetonitrile. The gradient profile was set as follows: 0 min at 10% B, increasing to 90% B over 10 min. Eluates were detected using a Shimadzu 8060 triple quadrupole mass spectrometer equipped with an electrospray ionisation (ESI) source. Three precursor-to-product ion transitions were monitored: $m/z\ 384 \rightarrow 68$, $384 \rightarrow 141$, and $384 \rightarrow 114$, with collision energies of -43 , -19 , and -27 , respectively.

2.6.1. Method for the immunocapture IC-LC-MS technique

Dynabeads™ magnetic beads were used to bind the anti-meropenem antibody, followed by meropenem to form a Dynabead-antibody-antigen complex. The magnetic Dynabead was then dissociated, and the Ab-Ag complex analysed. To establish consistent results of meropenem binding to the anti-meropenem antibody on the biosensor, the immunoprecipitation step allowed incubation of meropenem with the anti-meropenem antibody for 15 min.

3. Results and discussion

3.1. Functionalisation of bilayers on the electrode surface confirmed by EIS and CV

A biosensor was built on the tetrapolar gold microbands (Fig. 1) on a glass substrate, with the anti-meropenem antibody used to detect the meropenem antibiotic. The biosensor development followed a similar coupling chemistry protocol to anchor the antibodies in the correct orientation to capture the analyte on the fully developed biosensor, as developed in the authors' previous work (Wang et al., 2016).

Tetrapolar impedance was employed as polarisation effects are minimised when current-injecting and voltage-measuring electrodes are separated, a limitation that significantly affects bipolar impedance systems (Veil et al., 2025) (Schwan, 1957). It thus enables measurement of only the electrical properties of the biological sample. With the elimination of the polarisation effect in a tetrapolar system, the density of morphological tissues is measured more sensitively and accurately, along with morphological information, making it better suited for bio-impedance measurements (Schwan, 1957) (Grimnes and Martinsen, 2008) (Geddes, 1997).

The biosensor was built stepwise, functionalising all four microbands on the gold electrode with the relevant biomolecules, beginning with the development of a SAM layer. This was done by coupling the cross-linked polymer DTSSP to the gold surface, binding to gold by chemisorption and forming the self-assembled monolayer as explained in Section 2.2. The use of crosslinking SAM is necessary as direct immobilisation of proteins on the gold surface can lead to loss of structure and conformational changes in antibodies with random orientation (Kim et al., 2008), making binding less effective (Huang et al., 2022). Poor immobilisation can lead to a loss of biosensor sensitivity (Barlen et al., 2009). Protein G was used in the biosensor construction because of its ability to specifically bind to the Fc region of the antibody (Zhang et al., 2020). This then binds covalently to the sulpho-NHS ester group of the previously assembled DTSSP monolayer, which in turn binds to the gold surface through thiol bonds.

EIS is a popular, non-destructive, steady-state technique that can

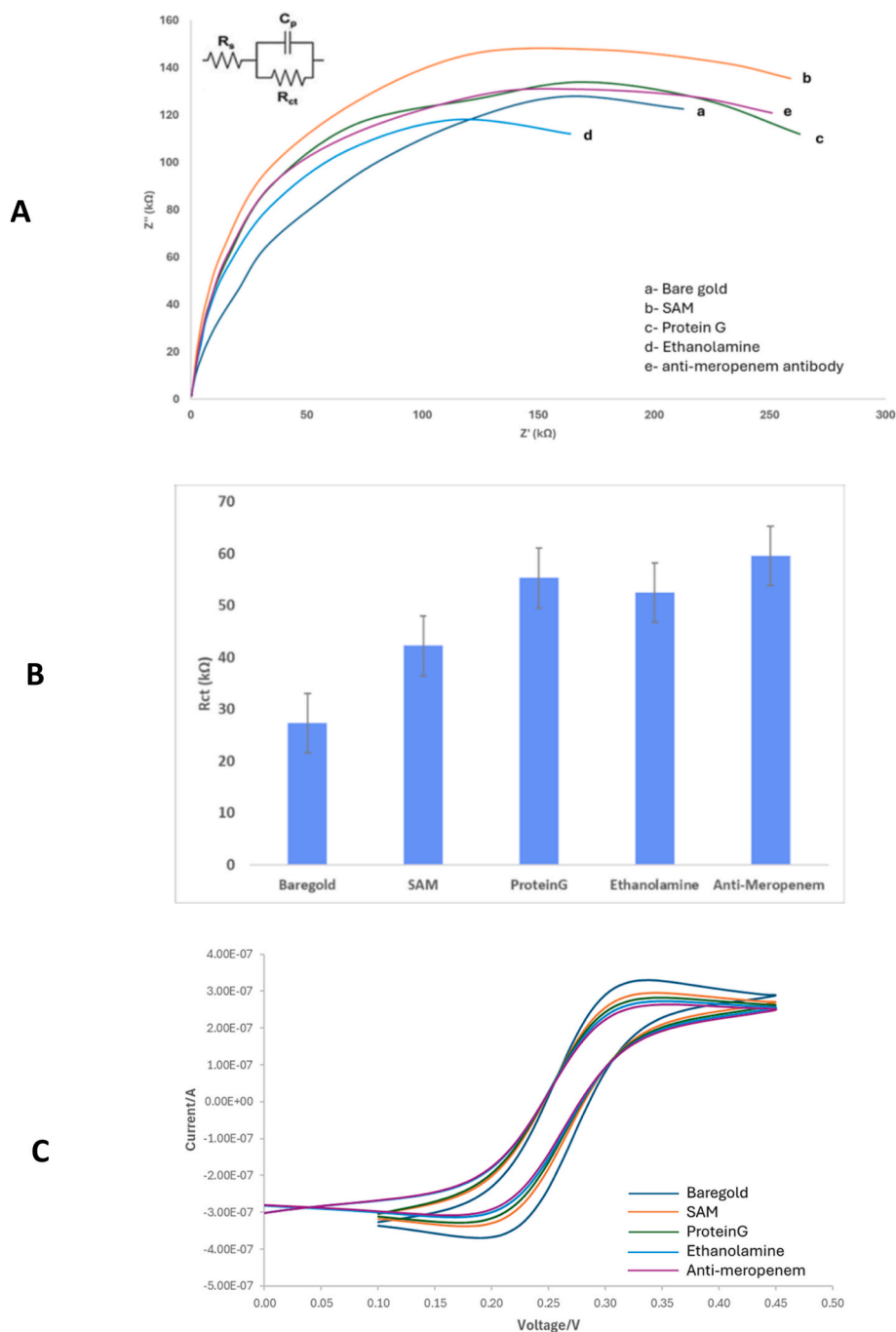


Fig. 2. Construction and assembly of the biosensor. (A) Fully constructed biosensor layers verified by EIS. The Nyquist plot was obtained by plotting the imaginary component of impedance (Z'') against the real part of impedance (Z'). The impedance data were recorded for each constructed layer: (a) bare gold, (b) SAM, (c) protein G, (d) ethanolamine, and (e) anti-meropenem in 5 mM $K_4Fe(CN)_6/K_3Fe(CN)_6$ (1:1; w/w) in PBS at an AC probe of 5 mV and over a frequency range of 100 Hz–100 kHz. The Randles equivalent circuit model (inset A) used to fit the data is presented with the Nyquist plots. (B) Interfacial charge transfer (R_{ct} in $k\Omega$) varies with each functionalised layer on the biosensor surface. $N = 3$. (C) Characterisation of layer-by-layer construction of the biosensor verified by cyclic voltammetry. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

effectively detect small changes on the electrode electrolyte interface occurring in response to the electrochemical interaction of the electrolyte with the electrode (Jin et al., 2020) (Wang et al., 2021). EIS converts biological signals into observable impedance by analysing changes in resistance and capacitance on the electrode surface due to chemical interactions on the electrode interface by immobilisation of biomolecules (DNA, enzyme, antibodies) (Jin et al., 2020). R_{ct} was chosen as the parameter for interpreting the signal change, offering the advantage of directly monitoring the reaction. Faradaic impedance

measured using a redox mediator (Rushworth et al., 2013) (Wang et al., 2016) often provides a more prominent signal change (larger ΔR_{ct}), specifically while observing small binding events or detecting low concentrations, which can contribute to enhanced sensitivity (Zhang et al., 2025).

The biosensor response to the deposited bilayers is recorded as changes across a range of frequencies. For each layer of biosensor assembly, impedance was measured using 5 mM $K_4Fe(CN)_6/K_3Fe(CN)_6$ (1:1; w/w) in PBS across 100 Hz–100 kHz. The impedance data, when

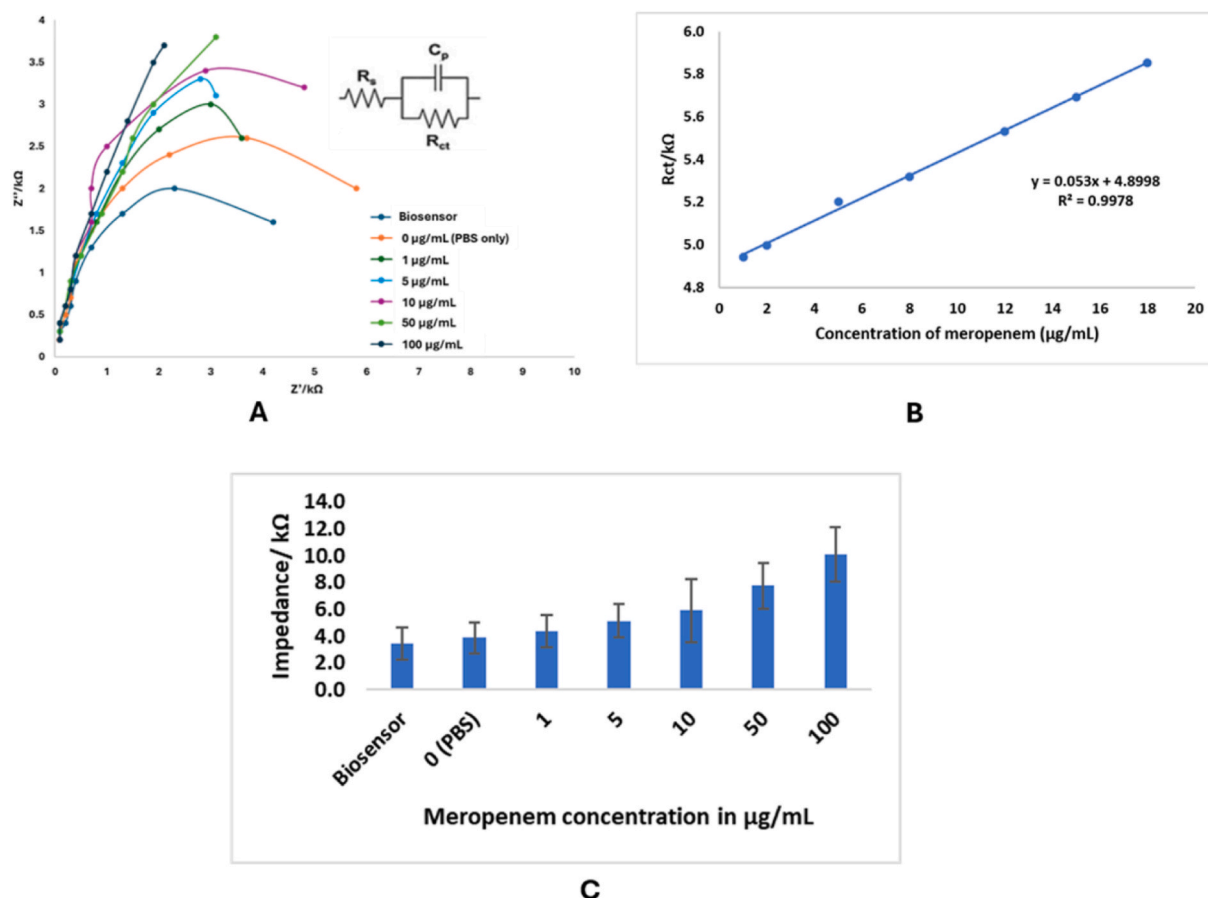


Fig. 3. (A) Biosensor was optimised for concentrations of meropenem ranging from 1 to 100 $\mu\text{g/mL}$ measured by EIS, with 0 indicating the functionalised biosensor with anti-meropenem antibody and PBS only measures PBS without any meropenem. The EIS measurement of the successive incubations of meropenem in PBS on a fully constructed biosensor in 5 mM redox in PBS at an AC probe of 5 mV and over a frequency range of 100 Hz–100 kHz. Temperature range 16–20°C. Each incubation was followed by rinsing of electrodes in di-H₂O and PBS before each impedance measurement. The Nyquist plot was obtained by plotting the imaginary component of impedance (Z'') against the real part of impedance (Z') after fitting the EIS data into a Randles equivalent circuit (shown in the inset). (B) R_{ct} vs concentration of meropenem ranging from 1 to 18 $\mu\text{g/mL}$. $\text{LoQ} = 4.95 \mu\text{g/mL}$ calculated using the formula $10 \times \text{stdev} / \text{slope}$; $\text{SD} = 0.02622$, $\text{Slope} = 0.053$ (standard deviation calculated using ANOVA regression curve and slope calculated from the graph equation of line). (C) Interfacial charge transfer (R_{ct} in $k\Omega$) varying with each concentration of meropenem-spiked human serum on the biosensor surface; $N = 3$.

fitted in the Randles equivalent circuit (shown in the inset Fig. 2A), produce a stereotypical semicircular plot describing the interface characterisation, with immobilisation of the bilayers on the biosensor accounting for surface resistance and capacitance. The changes in resistance (Z') and capacitance (Z'') are presented by the Nyquist plot (Fig. 2A).

Fig. 2A represents changes in impedance with each immobilised layer modifying the surface of the gold electrode, namely DTSSP, protein G, ethanolamine, and the antibody (anti-meropenem) presented as a Nyquist plot. The EIS data, when fitted in Randles equivalent circuit model, showed a change in impedance with the addition of each layer. Ethanolamine was used to block unreacted active sites and prevent non-specific binding (Al- et al., 2024). The addition of each layer during functionalisation of the biosensor increases impedance (Fig. 2B). The successful modification of the biosensor surface with each immobilised bilayer, showing a decrease in current with the addition of each layer, was confirmed by cyclic voltammetry (Fig. 2C). The decrease in current confirmed the successful construction of the layers on the electrode, as adding layers increased impedance, allowing less current to flow through. CV confirmed successful biosensor construction, showing characteristic oxidation and reduction peaks in $[\text{Fe}(\text{CN})_6]^{3-/4-}$ redox solution for anti-meropenem. The surface modification led to reduced electron-transfer kinetics, as evidenced by a decrease in current and a smaller peak. The final layer in the biosensor construction was the

antibody (Ab-Au), which showed the lowest current. This was due to surface insulation caused by the biolayer addition, resulting in a decrease in interfacial charge transfer. The successful surface modification of the gold electrode was confirmed by both CV and EIS. The increase in the interfacial charge transfer R_{ct} in EIS, brought about due to increased obstruction in the flow of ions, was supported by the drop in current value (I) in CV oxidation/reduction peaks.

3.2. Detection of meropenem in PBS

The range and sensitivity of the constructed biosensor were tested by measuring impedance on incubation with increasing concentrations of meropenem ranging from 1 to 100 $\mu\text{g/mL}$. The optimum frequency range and incubation time for binding efficiency were tested at each meropenem concentration. The biosensor response was monitored by changes in impedance values with potassium ferro/ferricyanide $[\text{Fe}(\text{CN})_6]^{3-/4-}$ as the redox probe in less than 15 min.

This range was tested to confirm the biosensor's ability to detect low and high concentrations, with a lowest concentration of 1 $\mu\text{g/mL}$ to be tested on the biosensor. The EIS data were fitted to the Randles equivalent circuit and plotted as a Nyquist plot in Fig. 3 to show the biosensor response to increasing concentrations of meropenem.

The impedance plots in Fig. 3 show an increasing trend in impedance with increasing meropenem concentration as the analyte binds to the

Table 1

EIS parameters obtained from Randles equivalent circuit of the biosensor for meropenem detection.

Meropenem ($\mu\text{g/mL}$) on ^a Biosensor	R_s (Ω)	C_p (F)	R_{ct} (Ω)
0	47.2	8.8E-08	5853
PBS only	52.1	7.6E-08	6500
1	55.8	7.3E-08	6715
5	58.4	7.4E-08	7526
10	55.6	7.8E-08	10231
50	54.9	6.7E-08	4814
100	54.1	7.3E-08	6198

* 0 = Anti-meropenem (100 $\mu\text{g/mL}$) without meropenem added to the biosensor for detection.

^a Biosensor = Gold + SAM + Protein G + Ethanolamine + Anti-meropenem.

biosensor surface. The impedance appeared to increase between 1 and 10 $\mu\text{g/mL}$, rising from 6.7 to 10.2 k Ω (Table 1). No further increase in impedance was observed at higher meropenem concentrations, which may suggest that the available antibodies became saturated and were insufficient to bind additional meropenem molecules. A similar phenomenon has been reported in previous studies, where electrochemical impedance plateaued or even decreased despite increasing target analyte concentrations, consistent with the Langmuir adsorption isotherm model (Sopoušek et al., 2021) (Daniels and Pourmand, 2007).

Table 1 presents EIS parameters for meropenem concentrations obtained after fitting EIS data to the Randles equivalent circuit model.

3.3. Detection of meropenem in human serum

Fully functionalised biosensors were tested for specificity and selectivity by exposing them to various concentrations of meropenem (1–100 $\mu\text{g/mL}$) spiked into commercial human serum. The biosensor could successfully detect meropenem down to a concentration of 1 $\mu\text{g/mL}$ (Fig. 4). A trend of increasing impedance was observed with increasing meropenem concentrations, while no significant response was observed for commercial human serum without meropenem. Cole-Cole plots were similar to those with meropenem in PBS (Fig. 3). This indicated the biosensor's ability to detect meropenem down to 1 $\mu\text{g/mL}$ in human serum, confirming that human serum did not compromise the biosensor's specificity nor sensitivity. Impedance increases with increasing meropenem concentration, reaching a maximum when the electrode surface is saturated with analyte, plateauing and then reducing.

Although the biosensor response for meropenem in human serum was lower than that for meropenem in PBS, the relative increase in R_{ct} for the tested concentration range followed a similar trend. A calibration curve constructed for these concentrations had an R^2 value of 0.97, indicating a strong linear relationship between concentration and response, with a calculated signal-to-noise ratio (SNR) of 1.4. The lower R_{ct} value for meropenem spiked into human serum may be due to

competitive binding of other serum components to the anti-meropenem antibody.

The functionalised biosensor could potentially detect meropenem within a concentration range of 1–100 $\mu\text{g/mL}$ in human serum, which includes the therapeutic range of 8–16 $\mu\text{g/mL}$ (Chiriac et al., 2023) (Fransson et al., 2021).

Personalising antibiotic therapy is an essential approach towards augmented antibiotic efficacy (Ates et al., 2020) (Sjövall et al., 2023). As a time-dependent drug, the therapeutic success of meropenem is associated with the percentage of time the antibiotic level exceeds the minimum inhibitory concentration (MIC) at the site of infection (Nezarat et al., 2020; Steffens et al., 2021; Saad et al., 2023; Wang et al., 2024). As a standard, the dosing interval during which the antibiotic concentration remains above MIC ($T > \text{MIC}$) should be 40%, and up to 100% for patients with more resistant bacterial infections (Maimongkol et al., 2022) (Angelini et al., 2023). Meropenem has a high bactericidal effect at 4x MIC, with no additional bactericidal activity at higher levels but an increasing risk of toxicity, especially at levels $>8x$ MIC (Wang et al., 2024). A clinical trial concluded that 8–16 mg/L is the desired therapeutic serum level, 16–24 mg/L is moderately high, and concentrations above 24 mg/L are potentially harmful (Chiriac et al., 2023). Another study concluded that trough concentrations >8 mg/L could achieve the MIC target (Fransson et al., 2021).

Clinically, biosensor-assisted drug monitoring may have potential, with further technological development and clinical validation, to support both near-real-time monitoring (e.g. intermittent blood sampling) and continuous monitoring (e.g. intravascular sensors) of critically ill patients. However, the clinical utility, specificity, and feasibility of such approaches remain to be established in prospective clinical studies before they can be considered for routine use in disease management or antibiotic dose optimisation. This would help overcome the problem of slow turnaround times with conventional diagnostic methods (Frigoli et al., 2024) (Mishi et al., 2023).

3.4. IC-LC-MS/MS study confirms binding of anti-meropenem antibody to meropenem

An alternative technique, immunocapture-LC-MS/MS, was employed to validate the specific binding of anti-meropenem antibody to meropenem. LC-MS/MS techniques are generally employed to determine meropenem levels in patient cerebrospinal fluid and serum samples (Jin et al., 2024) (Berska et al., 2024). Due to meropenem's small molecular weight (383.46 g/mol), conventional immunoassay techniques such as Western blotting are unable to confirm its specific binding to anti-meropenem antibodies, as these interactions fall below the detection limits of this assay. ELISA was not used as an alternative assay because of the lengthy, time-consuming nature of assay development. Additionally, commercially available ELISA kits for meropenem detection do not use the same anti-meropenem antibody as the

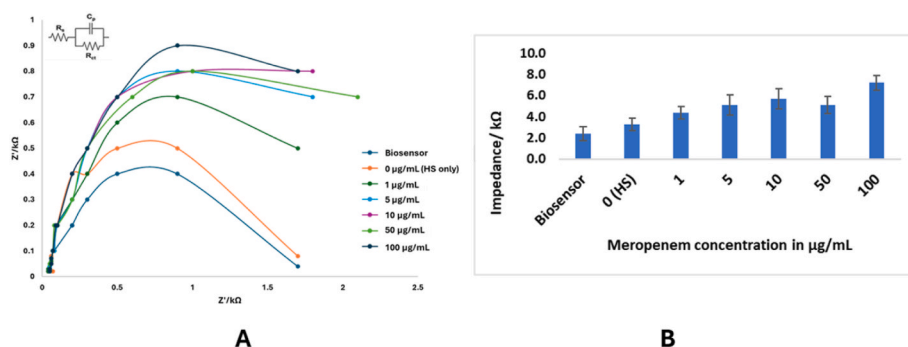
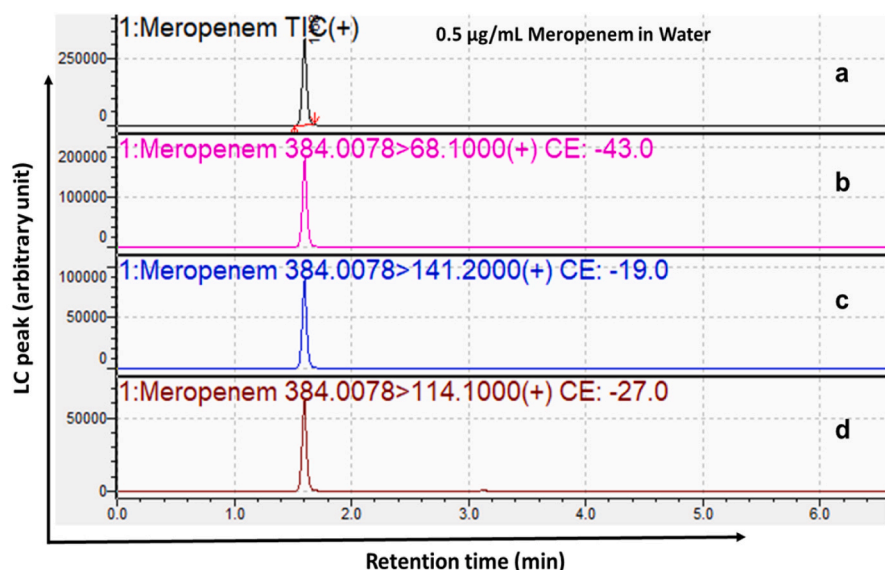
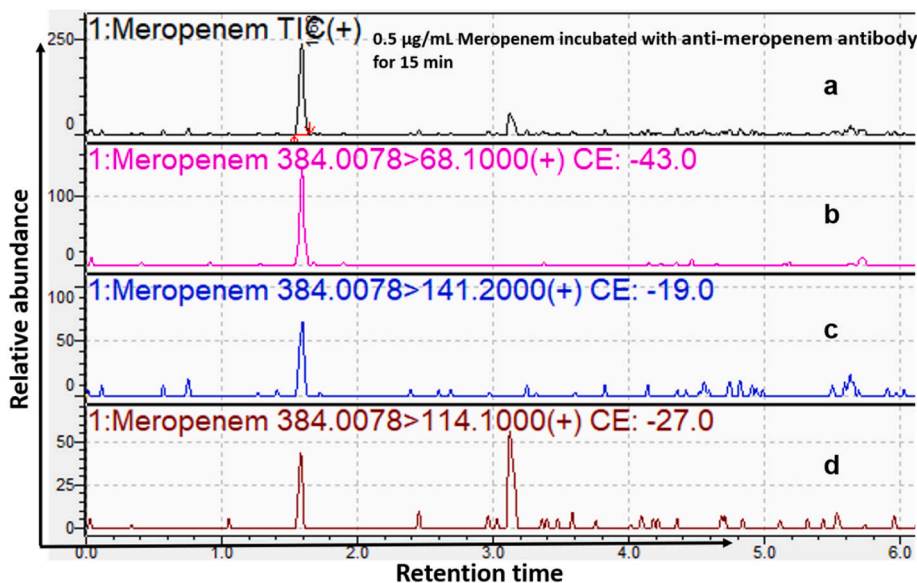


Fig. 4. (A) Biosensor response to varying concentrations of meropenem-spiked human serum and blank human serum. (B) Interfacial charge transfer (R_{ct} in k Ω) varying with each concentration of meropenem-spiked human serum on the biosensor surface; $N = 3$.



A



B

Fig. 5. Abundance vs retention time for (A) 0.5 µg/mL of meropenem in water. (B) Abundance vs retention time for 0.5 µg/mL of meropenem incubated with anti-meropenem antibody bound to magnetic beads for 15 min and confirmed binding of meropenem to anti-meropenem antibody through the Ab-Ag complex using the immunocapture LCMS/MS technique. Meropenem-bound anti-meropenem antibody complex was analysed using LC-MS/MS.

biosensor. As a result, this study reports for the first time the use of immunocapture-LCMS to validate the specific binding of an anti-meropenem antibody to meropenem.

Meropenem in water had a retention time of 1.5 min. This was highly reproducible across runs and concentrations, confirming the effectiveness of the optimisation steps. LC-MS/MS was performed on pure meropenem in water to establish its retention time, free of protein or other molecular interference, before analysing meropenem in commercial human serum samples.

The MS/MS ionisation generated daughter ion fragments m/z 68.1, 141.2 and 114.1, which are frequently used as quantifier ions to determine meropenem levels in patients' CSF and serum samples (Jin et al.,

2024; Berska et al., 2024; Parker et al., 2019). The total ion chromatogram and multiple reaction monitoring (MRM) chromatogram in Fig. 5 A (a-d) display the fragment ion transitions m/z 384.0 > 68.1, 384.0 > 141.2 and 384.0 > 114.1 with chromatograms b-d showing a single HPLC peak at a retention time of 1.5 min, attributable to meropenem. The chromatogram (b) for the m/z 384.0 → 68.1 fragment ion shows the highest HPLC peak, indicating its greatest relative abundance among the fragments formed during ionisation, and can therefore be used as the quantitative ion, while the less abundant ions in chromatograms (c) and (d) act as qualifier ions to confirm the presence of meropenem. Meropenem was incubated with anti-meropenem antibody bound to Dyna-beads for 15 min to imitate the meropenem-antibody binding time on

the biosensor. A retention time of 1.5 min was observed for meropenem captured with antibody-bound Dynabeads, matching that of the reference standard. The MRM chromatograms for transitions m/z 384 $\rightarrow$ 68.1, 384 $\rightarrow$ 141.2, and 384 $\rightarrow$ 114.1 each showed a single peak at this retention time across different antibody incubation periods, with consistent relative fragment abundances confirming effective ionisation and successful antibody-mediated capture. Fragment abundances remained stable, confirming the specificity of the immunocapture method and the reliability of MS in detecting meropenem concentrations spiked into serum.

The HPLC peaks showed consistent retention time and m/z fragmentation when meropenem was captured using an anti-meropenem antibody when incubated for 15 min (Fig. 5 B). This was an attempt to establish the consistent binding of anti-meropenem antibody to meropenem on the biosensor and for the LC-MS/MS analysis. The amount of antibody typically bound to the magnetic beads determines how much meropenem can be captured, and thus the incubation time must be optimised. This approach would be useful when determining meropenem levels in patients' blood samples by using the optimised incubation time for the IC-LCMS/MS.

4. Conclusion

A novel tetrapolar electrochemical biosensor for the detection of meropenem was developed based on the authors' previous work. The platform shows promise as a rapid and sensitive point-of-care device for the detection of meropenem within the clinically relevant range (1–100 $\mu\text{g/mL}$) in under 15 min. Binding specificity was confirmed using immunocapture followed by LC-MS/MS analysis, and meropenem was quantified in both PBS and human serum, with minimum matrix interference observed. The initial results suggest that the biosensor may have potential for rapid therapeutic drug monitoring of meropenem. Several limitations should be acknowledged in the current study. First, the findings are preliminary and represent an initial proof of concept for the developed biosensor platform. The biosensor has not yet been validated using clinical samples, and therefore, its performance under clinically relevant conditions remains to be established. In particular, the analytical performance, specificity, sensitivity, reproducibility, and robustness of the sensor require further assessment using complex biological matrices, where potential matrix effects may influence the measured response.

A further limitation is that the long-term operational stability, shelf-life, and batch-to-batch reproducibility of the biosensor have not yet been fully established. These factors are important for determining the reliability and practicality of the platform for routine clinical use. In addition, although the platform demonstrated potential for biomarker detection, its performance under real-time measurement conditions and its ability to provide consistent measurements over extended periods require further investigation.

Future studies should therefore focus on comprehensive analytical and clinical validation, including assessment of sensitivity, selectivity, accuracy, reproducibility, stability, and performance in clinically relevant samples. Such studies will be necessary to determine whether the developed platform can be translated from proof-of-concept research into a reliable system for real-time diagnostic and therapeutic monitoring.

CRedit authorship contribution statement

Richard H. Bayford: Writing – review & editing, Writing – original draft, Supervision, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization. **Neha Nitika Nathaniel:** Writing – review & editing, Writing – original draft, Validation, Methodology, Investigation. **Ajit J. Shah:** Writing – review & editing, Writing – original draft, Supervision, Methodology, Formal analysis. **Sandra Appiah:** Writing – review & editing, Writing – original draft,

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

This work was supported by the National Institute for Health Research, University College London Hospitals under grant no: BRC655/HEI/AD/110410 and by EPSRC under grant no.: EP/T001240.

Data availability

The data that has been used is confidential.

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