

Research Article

Sustainable paper-based MIP-LIG sensor for antibiotic detection in milk

Muhammad Afiq Abdul Ghani^{1,2}, Muhammad Rizwan Mohamad Noor², Adilah Mohamed Nageib³, Anis Nurashikin Nordin^{1,2*}, Rosminazuin Ab Rahim^{1,2}, and Amanatuzzakiah Abdul Halim³

¹VLSI-MEMS Research Unit, International Islamic University Malaysia, 53100, Kuala Lumpur, Malaysia

²Department of Electrical and Computer Engineering, Kulliyah of Engineering, International Islamic University Malaysia, 53100, Kuala Lumpur, Malaysia

³Department of Chemical Engineering and Sustainability, Kulliyah of Engineering, International Islamic University Malaysia, 53100, Kuala Lumpur, Malaysia

*Corresponding author: anisnn@iiu.edu.my

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Abstract

Extensive use of sulfamethoxazole (SMX) in dairy farming leads to pollution of the environment and contamination of dairy products with antibiotic residues. Regular antibiotic testing in milk using electrochemical sensors can help to combat antimicrobial resistance and protect human health. To mitigate electronic waste, this green technology integrates Laser-Induced Graphene (LIG) and Molecularly Imprinted Polymers (MIPs) on a paper-based electrochemical sensor. The MIPs provide highly specific recognition cavities tailored for SMX. The LIG platform, manufactured on paper, provides a biodegradable, highly conductive, porous three-dimensional transducer that facilitates rapid electron transfer and signal amplification alternative to plastic. To fabricate the transducer, the paper substrate was pre-treated with a sodium tetraborate fire retardant, followed by direct laser scribing using a 660 mW diode laser to generate a robust, porous graphene network. Specific recognition cavities were engineered by synthesising MIPs via polymerisation at 60°C using SMX as the template. The sensor was assembled by drop-casting the purified MIP microparticles directly onto the working electrode. Electrochemical measurements were performed using differential pulse voltammetry (DPV) in 5 mM ferrocyanide with 0.1 M KCl. The paper-based MIP-LIG sensor exhibited linearity in the concentration range of 0.2–0.7 μM , with a sensitivity of 43.64 $\mu\text{A}/\mu\text{M}$, a limit of detection (LOD) of 0.11 μM , and a limit of quantification (LOQ) of 0.33 μM . When tested in spiked milk samples, the sensor maintained a linear response with a sensitivity of 11.11 $\mu\text{A}/\mu\text{M}$ and an LOD of 0.36 μM , demonstrating reliable performance in complex matrices. This work introduces a sustainable, low-cost, and high-performance sensing platform that bridges material innovation and public health, enabling rapid, on-site monitoring of antibiotic residues in food safety applications.

Keywords: electrochemical, paper, sulfamethoxazole, molecularly imprinted polymers, laser-induced graphene

Introduction

The persistent presence of antibiotic residues in food products, particularly milk, represents a growing and multifaceted threat to public health and global food security. Antibiotics such as sulfamethoxazole (SMX) are widely administered in dairy farming, leading to their accumulation in milk, often in trace concentrations ranging from nanograms to micrograms per litre [1, 2]. The consumption of milk containing these residues can not only lead to adverse health effects in humans but, more critically, it acts as a selective pressure that drives the evolution and proliferation of antimicrobial resistance (AMR) in bacteria, fungi, viruses, etc [1,

3, 4]. To mitigate associated risks, regulatory authorities such as the Food and Drug Administration (FDA) and European Medicines Agency (EMA) enforce maximum regulatory limits (MRLs) for antibiotic residues in food products in which maximum permissible concentration of sulfonamides in foodstuffs of animal origin is restricted to 100 $\mu\text{g}/\text{kg}$ [2]. Therefore, the effective and rapid detection of SMX in milk is not merely an issue of regulatory compliance or food quality control; it is one of the critical issues to public health.

Traditional analytical techniques for the detection of

antibiotic residues, such as high-performance liquid chromatography (HPLC), liquid chromatography-mass spectrometry (HPLC), liquid chromatography-mass spectrometry (LC-MS/MS), and immunoassays, have long been considered the gold standard in laboratory settings [1, 3, 5]. However, their practical application for routine SMX screening is severely restricted by several critical limitations. First, the complex biological matrix of raw milk, which is rich in proteins, lipids, and minerals, is notorious for causing severe matrix effects and equipment fouling in chromatographic systems. Consequently, these conventional methods necessitate exhaustive, multi-step sample preparation procedures such as protein precipitation, centrifugation, and solid-phase extraction prior to analysis. As this sample preparation is labour-intensive and requires specialised equipment, these methods can only be performed in fully equipped, centralised laboratories [3, 6]. Second, the highly perishable nature of dairy products demands rapid, real-time decision-making at the point of collection to prevent contaminated batches from mixing with clean silos. However, the reliance on highly trained personnel, combined with lengthy sample preparation and prolonged chromatographic run times, creates a significant diagnostic bottleneck.

This technological gap emphasises the urgent need for complementary analytical tools that can offer rapid, accessible preliminary screening at the point-of-care, rather than replacing current gold-standard methods. Electrochemical biosensors fulfil this exact role by successfully overcoming the logistical barriers of LC-MS/MS rather than vying with its ultra-trace analytical capabilities. By offering rapid response times, cost-effectiveness, portability, and user-friendly operation, electrochemical platforms provide a vital diagnostic layer which could offer early warning [3, 6].

To optimise the performance of these screening tools, the transducer material must offer exceptional electron transfer capabilities without compromising on manufacturing scalability. In this regard, Laser-Induced Graphene (LIG) represents a transformative advancement in carbon-based electrode fabrication. This technique involves the direct irradiation of a carbonaceous precursor, such as a paper substrate, with a laser to initiate a photothermal conversion process [7, 8]. The result is a three-dimensional, porous, and electrically conductive graphene network. This laser direct-writing method is simple, scalable, and remarkably cost-effective, eliminating the need for clean rooms, toxic solvents, or complex photolithographic masks [7, 8]. The resulting porous, three-dimensional framework provides a large accessible surface area with a rich network of defects, achieving low sheet resistances as low as $56.0 \Omega \text{ sq}^{-1}$ [7]. This morphology exposes abundant

active sites for subsequent functionalisation while promoting rapid analyte transport and fast electron transfer kinetics, making LIG an ideal transducer platform for high-performance biosensing [8].

However, the ultimate efficacy of a biosensor relies heavily on its ability to selectively recognise the target analyte within a complex, interference-heavy matrix like raw milk. While biological receptors such as antibodies or enzymes are highly specific, they are notoriously fragile and susceptible to environmental denaturation. To address this, Molecularly Imprinted Polymers (MIPs) offer a robust synthetic alternative. The foundational principle of MIP synthesis involves a polymerisation reaction in which functional monomers, such as methacrylic acid (MAA), which offers strong hydrogen-bonding compatibility with SMX [3, 4], self-assemble around a template molecule in the presence of a cross-linking agent and an initiator [6]. Once polymerisation is complete, the template molecule is extracted, leaving behind a stable polymer matrix with binding cavities precisely matched to the original target in size, shape, and chemical functionality [6] [9]. This biomimetic “lock-and-key” mechanism ensures high-affinity target recognition [4]. Compared to fragile biological receptors, these synthetic MIPs exhibit exceptional stability under extreme environmental conditions, powerful resistance to degradation, and remarkable reusability over multiple analytical cycles [4, 6].

Leveraging these inherent advantages, researchers are increasingly focused on developing highly selective, SMX-specific MIP platforms capable of bypassing exhaustive sample preparation. For instance, Frigoli et al. recently introduced a molecularly imprinted polymer-based thermal biosensor designed to detect SMX traces directly in dairy. By immobilizing MIPs on an aluminum chip and utilizing the heat-transfer method (HTM) to measure interfacial thermal resistance changes upon target binding, the sensor achieved an ultra-low detection limit of 261 pM [2]. Notably, this device successfully quantified SMX in milk samples without any sample pretreatment, highlighting how modern MIP-transducer pairings can completely overcome matrix fouling.

To further push the boundaries of sensitivity and reusability for closely related sulfonamide antibiotics, recent studies have optimized the polymer matrix and incorporated nanomaterials. Kong et al. designed a reusable, label-free MIP electrochemical sensor for sulfamethizole that could be synthesized *in situ* in under 30 minutes [10]. This sensor achieved a detection limit of 0.18 nM and retained over 90% of its initial signal even after being reused seven times [10].

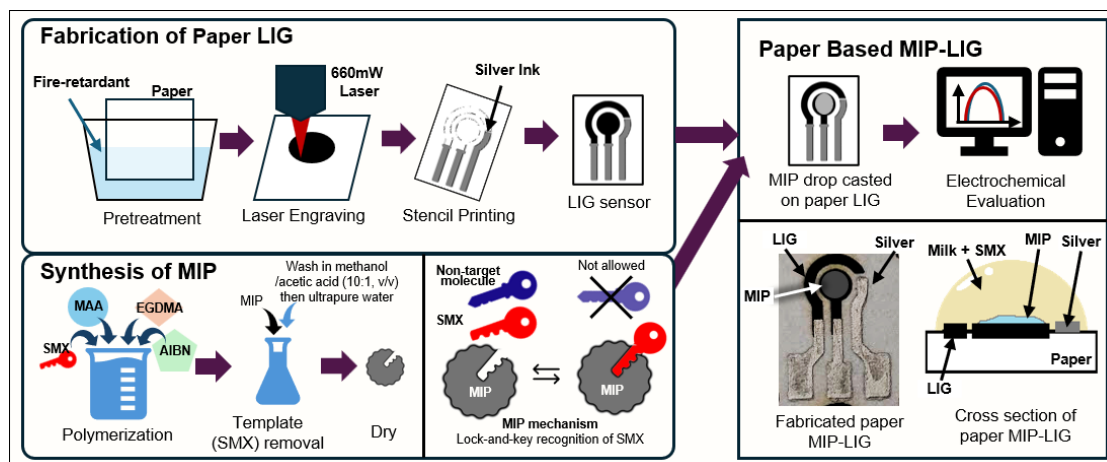


Figure 1. Overview of the experimental workflow for the fabrication and electrochemical evaluation of the paper-based molecularly imprinted polymer laser-induced graphene (MIP-LIG) sensor. The process includes fabrication of the paper-LIG electrode, synthesis and template removal of the MIP, deposition of the MIP onto the paper-LIG surface, and subsequent electrochemical detection of sulfamethoxazole (SMX) through selective molecular recognition.

Similarly, Elamin et al. addressed the inherently low conductivity of some polymer backbones by embedding copper nanoparticles into a molecularly imprinted overoxidized polypyrrole composite [11]. This combination allowed for the highly sensitive detection of sulfadiazine in raw milk samples with a detection limit of 0.31 nM, further demonstrating how advanced MIP composite strategies can operate flawlessly in challenging real-world dairy testing [11].

Rationale for experimental design

The core rationale of this study is to explore an integrated platform that integrates three distinct, complementary technologies: Laser-Induced Graphene (LIG), Molecularly Imprinted Polymers (MIPs), and a paper substrate. While the fundamental principles of laser-induced graphitization and polymerization are established, this experimental workflow was specifically engineered to optimize the physical and electrical interface between them. By utilizing the highly porous, defect-rich 3D network of the paper-LIG sensor to effectively anchor the MIP microparticles, the design creates a specialized transducer-receptor architecture that amplifies signal transduction while providing "lock-and-key" specificity for sulfamethoxazole (SMX). Consequently, the experimental parameters were systematically selected to merge individual fabrication techniques into a unified, low-cost, and matrix-resilient biosensing platform suitable for decentralised food safety screening, rather than to reinvent them.

Experimental work

The experimental workflow can be divided into three main parts: (i) fabrication and characterization

of paper-based LIG sensors, (ii) synthesis and characterization of Non-Imprinted Polymer (NIP) and Molecularly Imprinted Polymers (MIP) polymers to compare the performance of MIP, and (iii) integration of paper-based LIG with MIP, followed by sensor characterization as shown in **Figure 1**.

Reagents and materials

The reagents involved in the study comprise Sulfamethoxazole (SMX, template molecule), methacrylic acid (MAA, functional monomer), ethylene glycol dimethacrylate (EGDMA, cross-linking agent), and azobisisobutyronitrile (AIBN, radical initiator). Acetonitrile (used as the porogen), methanol, and glacial acetic acid, which were utilised for MIP synthesis, washing, and template extraction, were of analytical or high-performance liquid chromatography (HPLC) grade.

For the fabrication of the paper-based transducer, Whatman filter paper Grade 1 was utilised as the biodegradable substrate. Sodium tetraborate, employed as the fire-retardant pre-treatment. Silver conductive ink was used for defining the reference electrode and contact pads, while chitosan was utilised as a structural binding layer during the drop-casting of the MIP.

Electrochemical measurements were conducted using a redox probe solution composed of potassium ferrocyanide ($K_4[Fe(CN)_6]$) and potassium chloride (KCl). Ultrapure deionised (DI) water was utilised for all aqueous solution preparations and final washing steps. Finally, the fresh pasteurised milk (Dutch Lady, 100% Fresh Milk) used for the real-sample matrix analysis was purchased from a local

commercial supermarket. All chemical reagents were of analytical grade and used as received with no further purification unless otherwise specified.

Fabrication and characterisation of paper-based LIG sensors

Whatman filter paper was used as a biodegradable substrate. To prevent combustion during laser scribing, the papers were immersed in 200 mL of 0.1 M sodium tetraborate for 20 minutes and dried overnight at room temperature [12]. Sodium tetraborate functions as a fire retardant by releasing water when heated. This process absorbs heat, cools the affected material, and simultaneously forms a glassy boron oxide layer, which effectively restricts the movement of oxygen and heat. It interacts with cellulose chemically to promote char formation instead of flaming combustion, and this reaction would reduce volatile gas release and enhance thermal stability [14]. During laser-induced graphene fabrication on paper, this treatment prevents ignition and uncontrolled burning, which will facilitate controlled carbonisation and the production of uniform, conductive graphene tracks while preserving the substrate's integrity.

Engraving test and laser parameter optimisation

Engraving tests were performed on fire-retardant-treated papers using a Crealty Laser Falcon 2 Pro with a 22 W diode laser module. Laser power (660mW – 880mW) and speed (500–1000 mm/min, 100 mm/min steps) were varied at a fixed resolution of line per inch of 300. The parameter testing ranges were established based on preliminary visual and electrical screening. Scanning speeds under 500 mm/min will result in significant substrate ablation, even after the fire-retardant treatment. However, speeds over 1000 mm/min will cause incomplete photothermal conversion. Similarly, laser powers below 660 mW (corresponding to 3% of the maximum module output) failed to induce contiguous graphitisation, whereas powers exceeding 880 mW (4% output) negatively impacted the structural integrity of the cellulose matrix. To achieve precise thermal dosage control within these established boundaries, laser power was varied from 660 mW to 880 mW with fine 44 mW increments (0.2% module output increments), and scanning speed was varied from 500 to 1000 mm/min with 100 mm/min increments.

Next, the material test tool in Lightburn software was used for systematic evaluation. Sodium tetraborate-treated papers exhibited more consistent LIG formation. Two-point probe measurements identified parameter sets yielding resistance <100 Ω , which were applied for subsequent LIG fabrication. A rectangular LIG pattern (15 mm $\times$ 5 mm) was fabricated on sodium tetraborate-treated Whatman filter paper using the optimised

parameters (660 mW power, 500 mm/min scanning speed, and 300 LPI). This structure served as a preliminary test for evaluating the quality of LIG formation prior to electrode design, with the fabricated sample examined using scanning electron microscopy (SEM) to assess surface morphology and confirm the formation of porous graphene-like structures on the paper substrate.

Electrochemical characterisation

Electrochemical characterisation was performed using Cyclic Voltammetry (CV) and Electrochemical Impedance Spectroscopy (EIS) with a PalmSens4 potentiostat. A test was conducted using a 15 mm $\times$ 5 mm rectangular LIG electrode. Before testing, carbon electric paint was applied to the connection pads and baked at 65°C for 15 minutes to ensure reliable conductivity. A standard three-electrode setup was used for the rectangular electrode in a 5 mM ferrocyanide solution in 0.1 M KCl. Using the Randles-Sevcik equation as shown in Eq. (1), the Electrochemical Surface Area (ECSA) was determined from the peak current observed in the cyclic voltammetry.

$$i_p = 2.69 \times 10^5 A D^{\frac{1}{2}} n^{\frac{3}{2}} v^{\frac{1}{2}} C \quad (1)$$

where i_p is the peak current, A is the ECSA, D is the diffusion coefficient, n is the number of electrons participating in the charge-transfer process, v is the scan rate, and C is the concentration of the supporting electrolyte [15]. The equation above can be used to calculate the ECSA by using $D = 7.17 \times 10^{-6} \text{ cm}^2\text{s}^{-1}$ in 0.1 M KCl and $n = 1$ for $\text{Fe}(\text{CN})_6^{3-/4-}$ redox couple.

Synthesis and characterisation of NIP and MIP polymers

To rigorously validate the effectiveness of the molecular imprinting process, it is essential to synthesize and evaluate a Non-Imprinted Polymer (NIP) as a control matrix alongside the MIP. The NIP is synthesized using the exact same functional monomers, cross-linkers, and environmental conditions as the MIP, but strictly without the introduction of the target template molecule (SMX). Consequently, while it shares the same bulk chemical composition, the NIP fundamentally lacks the recognition cavities. Running this parallel control allows for the accurate quantification of non-specific binding.

MIPs were synthesised using sulfamethoxazole (SMX) as a template. The polymerisation mixture contained methacrylic acid (5 mmol), EGDMA (0.5 mmol), SMX (0.085 mmol), and AIBN (1.1 mmol) in 0.7 mL acetonitrile. After 5 minutes of ultrasonication, the solution was polymerised in an oil bath at 60°C for 20 hours. The resultant polymer underwent multiple methanol washes to eliminate

unreacted monomers and initiator remnants, with purification verified via UV-vis spectrophotometry at 285 nm. Then, the template was removed by washing the polymer four times with a methanol/acetic acid solution (10:1, v/v) for 10 minutes each, followed by two washes with ultrapure water, also for 10 minutes each. The complete removal of SMX was confirmed when the UV-vis wavelength reading reached 285 nm. It shall be noted that the formation of imprinted cavities was attributed to the specific recognition of the analyte. The final polymer was dried to constant weight at 60 °C [14]. Non-imprinted polymers (NIPs) were also synthesised using the same procedure without SMX, omitting the template removal step.

For electrode preparation, MIP and NIP microparticles were dispersed in ethanol (1 mg/mL) and dropcasted onto commercial screen-printed carbon electrodes (SPCEs, DRP-C11L-U75, Metrohm). To ensure firm adhesion of the microparticles, a chitosan solution (0.5% w/v in 0.2 M acetic acid) was drop-cast onto the dried electrode surface as a binding layer. Then, electrochemical performance was evaluated by differential pulse voltammetry (DPV) using a three-electrode system with the MIP- and NIP-modified electrodes as the working electrodes. Measurements were conducted in 5 mM ferrocyanide containing 0.1 M KCl as the supporting electrolyte. Calibration curves were obtained by recording DPV responses due to increasing SMX concentrations (0 – 1.0 μM , 0.1 μM increments), while the sensitivity, LOD, and LOQ were determined from the calibration plots.

Sensor assembly and integration of paper-based LIG with MIP

Laser-induced graphene (LIG) was fabricated on a paper substrate using the same diode laser engraver under optimised parameters to induce localised carbonisation. The LIG electrodes were patterned into a three-electrode configuration, comprising a working electrode, a counter electrode, and a reference electrode. The design was replicated from the design of the DRP-C11L-U75 by Metrohm. To enhance conductivity and ensure stable electrical contact, silver conductive ink was deposited onto the electrode contact pads via stencil printing. Then, the electrodes were dried in the oven at 65°C for 15–20 minutes to ensure proper adhesion. MIP microparticles were dispersed in ethanol (1 mg/mL), and 15 μL was dropcasted onto the working electrode of the paper-based LIG sensor.

Electrochemical setup and real sample preparation

Each electrochemical test utilised a 2 mL sample volume, and the electrochemical performance of the fabricated paper-based MIP-LIG electrodes was evaluated by differential pulse voltammetry (DPV)

using a three-electrode configuration. Measurements were carried out in a 5 mM ferrocyanide solution, which contained 0.1 M KCl as the supporting electrolyte. Calibration curves were constructed by recording DPV responses at successive sulfamethoxazole (SMX) concentrations ranging from 0.2 μM to 0.7 μM with 0.1 μM increments. The sensitivity, LOD, and LOQ were determined from the resulting calibration plots. Following the electrochemical characterisation in the model system, the study was extended to evaluate analytical performance using milk-based samples to assess real-matrix applicability.

A critical aspect of demonstrating a sensor's real-world applicability is its ability to perform in complex matrices accurately. Raw milk is a challenging sample matrix due to the presence of fats, proteins, and other biological molecules that can cause electrode fouling and generate interfering signals, which lead to false-positive or false-negative results [3]. However, this can be mitigated by introducing a supporting electrolyte such as KCl and ferrocyanide. The process for preparing the sample involved diluting fresh milk (Dutch Lady, 100% Fresh Milk) by combining 20% v/v of the milk with deionised water. Ferrocyanide and KCl were then added to the diluted milk to obtain a final concentration of 5 mM ferrocyanide and 0.1 M KCl. Then, electrochemical performance was evaluated by differential pulse voltammetry (DPV). DPV responses were measured with increasing SMX concentrations (ranging from 0.2 to 0.7 μM , with 0.1 μM steps) to generate calibration curves. Sensitivity, LOD, and LOQ were determined from the calibration plots.

Results and Discussion

Characterisation of paper-based LIG sensors

Figure 2 summarises the fabrication process and characterisation of the paper-based LIG sensors. The initial optimisation of laser parameters in **Figure 2(a)** was performed to identify suitable conditions for effective carbonisation without over-burning the cellulose substrate. The optimised parameters are necessary to produce uniform and well-defined LIG patterns. Based on the material test optimisation, the best condition was obtained at 22 W laser power (660 mW output), a scanning speed of 500 mm/min, and 300 LPI resolution, which yielded consistent carbonisation with minimal substrate damage. These parameters provided sufficient energy density to induce localised pyrolysis of cellulose, resulting in the formation of uniform and well-defined graphene patterns. When operating at lower powers, incomplete carbonisation led to insufficient conductivity, yet higher power could result in over-burning and compromise the structural integrity of

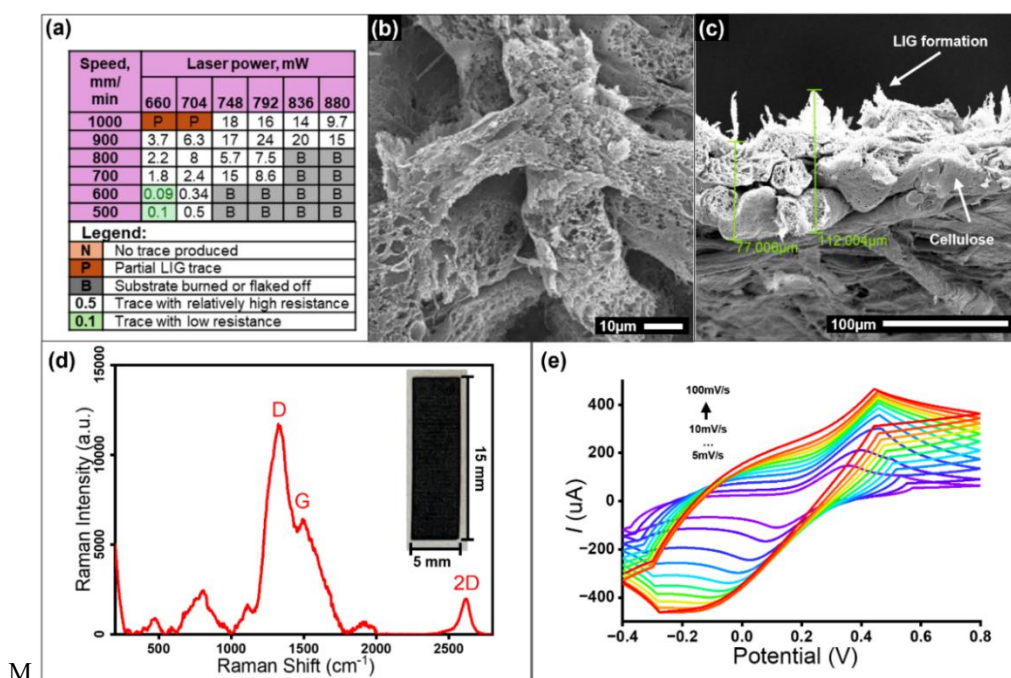


Figure 2. Characterisation and fabrication of paper-based laser-induced graphene. (a) Material test table to find optimum laser parameters for LIG (b) SEM image from a top view of paper LIG, (c) SEM images from a side view of paper LIG (d) Raman spectroscopy plot that shows the LIG presence on the paper and rectangular paper LIG for characterisation (15 mm $\times$ 5 mm) (e) Cyclic voltammetry was performed at scan rates ranging from 5 mV/s, 10mV/s to 100 mV/s, with increments of 10 mV/s in 5 mM ferrocyanide, 0.1 M KCl.

the paper fibres. The proper optimisation of settings is critical to maintain a balance between achieving effective graphitisation and preserving structural integrity. This process is required to produce LIG with high electrical conductivity and a consistent surface finish, which are prerequisites for subsequent electrochemical characterisation.

The morphological features of the fabricated LIG were examined using SEM. The top-view SEM image, as shown in **Figure 2 (b)** revealed a porous and interconnected graphene network formed on paper fibres, which provides a large electroactive surface area favourable for electron transfer. Meanwhile, the cross-sectional SEM image, as illustrated in **Figure 2(c)**, explained that the laser-induced carbonisation had penetrated through the fibre matrix to ensure good structural stability and conductivity.

Subsequently, the average size of the graphene structures was measured using ImageJ software by selecting ten random points per sample. The measurements showed that laser-induced graphene samples exhibit multiple lengths ranging from

70.605 - 96.187 μ m with an average length around 77.2 μ m.

The chemical composition and graphitic nature of the LIG were verified by Raman spectroscopy **Figure 2(d)**, where the presence of the characteristic D ($\sim 1324 \text{ cm}^{-1}$), G ($\sim 1525 \text{ cm}^{-1}$), and 2D peaks confirmed the formation of graphene-like structures. The intensity ratio (I_D/I_G) suggested the presence of defects, which are beneficial for enhancing surface reactivity in electrochemical sensing applications.

For electrochemical characterisation, rectangular LIG electrodes (15 mm $\times$ 5 mm) were fabricated (see **Figure 2(d)**) and tested in a standard three-electrode setup with Ag/AgCl as the reference electrode and Pt as the counter electrode. Cyclic voltammetry (CV) was performed at scan rates ranging from 5 to 100 mV/s in **Figure 2(e)**. A diffusion-controlled electrochemical process was demonstrated by the CV curves, which showed a linear increase in peak current as the scan rate increased. The ECSA was then calculated using Eq. (1) based on the CV data at different scan rates using the Randles-Sevcik equation. The calculated ECSA values are plotted in **Figure 3**.

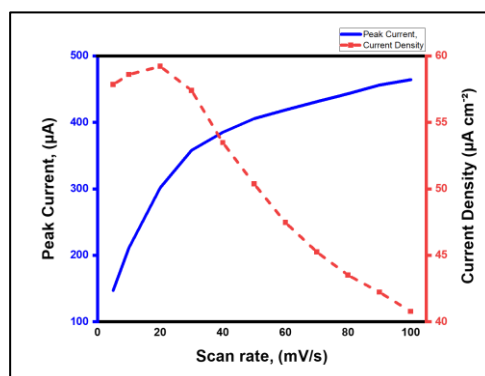


Figure 3. Scan rate mV/s relations against peak current (μA) as well as ESCA or current density ($\mu\text{A cm}^{-2}$)

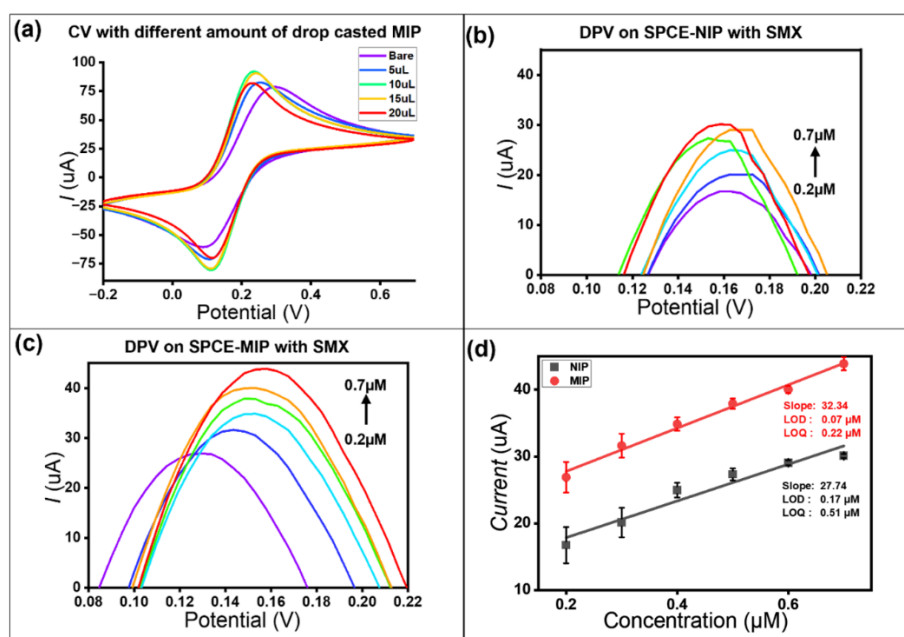


Figure 4. (a) CV -0.2V to 0.7V and scan rate 100mV/s for different volumes of MIP deposition in which sample b) DPV voltammogram at 100 mV/s scan rate for different concentration of SMX ranging from 0.2 μM to 0.7 μM with an increment of 0.1 μM in 5 mM ferrocyanide, 0.1 M KCl (c) DPV voltammogram at 100 mV/s scan rate for different concentration of SMX ranging from 0.2 μM to 0.7 μM with an increment of 0.1 μM in 5 mM ferrocyanide, 0.1 M KCl (d) The calibration curve of SMX between peak current of NIP and MIP on SPCE

The calculated ECSA confirmed that the LIG electrode possessed a substantial electroactive surface. A gradual decrease in ECSA was observed at higher scan rates, which is consistent with diffusion-controlled electrochemical behaviour typically reported for porous carbon-based electrodes. With its proven ability to facilitate efficient charge transfer and offer a stable electrochemical response, the porous LIG structure is confirmed to be an excellent candidate for further functionalisation and biosensing applications.

Electrochemical characterisation of NIP and MIP on SPCE

Electrochemical performance was first evaluated using cyclic voltammetry as shown in **Figure 4(a)**

within -0.2 V to 0.7 V at a scan rate of 100 mV/s. Increasing the deposition volume of MIP from 10 μL to 15 μL resulted in higher peak currents up to an optimal loading, beyond which a decrease was observed (20 μL), probably due to excessive polymer thickness that hindered charge transfer. The optimised volume of MIP deposition was found to be 10 μL . This optimisation confirmed that controlled MIP deposition is critical for achieving maximum electrochemical activity.

Differential pulse voltammetry (DPV) was used to determine the sensitivity and selectivity of the MIP and NIP-modified electrodes toward SMX. **Figures 4(b) and 4(c)** show the DPV responses for SMX concentrations ranging from 0.2 μM to 0.7 μM with

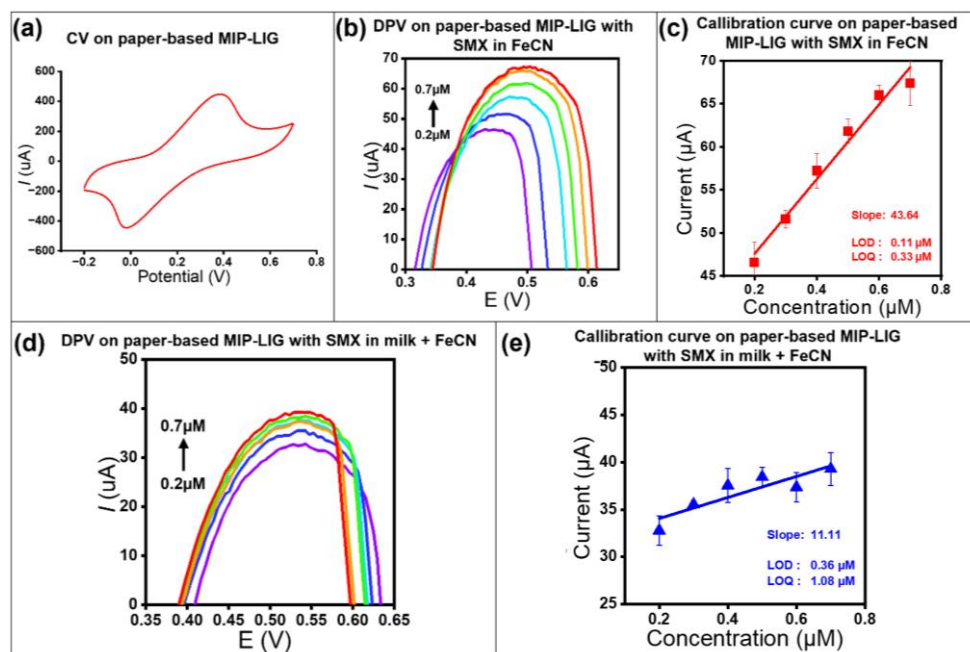


Figure 4. Paper-based MIP-LIG sensor fabrication and electrochemical characterisation. (a) CV of paper LIG with MIP in the potential range of -0.2 V to 0.7 V at a scan rate of 100 mV/s in 5 mM ferrocyanide, 0.1 M KCl. (b) DPV recorded in 5 mM ferrocyanide with 0.1 M KCl at a scan rate of 100 mV/s, for SMX concentrations ranging from 0.2 μM to 0.7 μM in increments of 0.1 μM. (c) The calibration curve of SMX in 5 mM ferrocyanide with 0.1 M KCl. (d) DPV recorded in fresh milk added with 5 mM ferrocyanide and 0.1 M KCl at a scan rate of 100 mV/s, for SMX concentrations ranging from 0.2 μM to 0.7 μM in increments of 0.1 μM. (e) The calibration curve of SMX in fresh milk added with 5 mM ferrocyanide and 0.1 M KCl.

an increment of 0.1 μM. A linear increase in peak current with SMX concentration was observed for both electrodes. However, the MIP electrode exhibited a steeper calibration slope compared to the NIP. The calibration plot in **Figure 4(d)** for the MIP-modified electrode yielded a regression equation

$$I_{pa} (\mu A) = a + bC \quad (R^2=0.99) \quad (2)$$

where b represents the sensitivity in $\mu A/\mu M$. With a calculated sensitivity of $32.34 \mu A/\mu M$, the MIP electrode demonstrated a greater response than the NIP electrode, which registered $27.74 \mu A/\mu M$. The LOD is $0.07 \mu M$ for the MIP, which is lower than that of the NIP at $0.17 \mu M$, more than twice the value recorded for the MIP. Similarly, the LOQ was $0.22 \mu M$ for MIP and $0.51 \mu M$ for NIP.

These results reveal that the MIP-modified LIG electrode provides superior sensitivity, but with slightly lower detection and quantification limits. The enhanced performance is attributed to the presence of imprinted recognition cavities, which improve analyte binding and facilitate efficient electron transfer at the electrode interface.

Paper-based MIP-LIG

The fabrication process, in which laser engraving was used to create conductive graphene electrodes,

is shown in **Figure 1**. Afterward, silver ink was applied using a stencil printing method to improve conductivity, establish reliable electrical connections, and form a reference electrode.

Electrochemical performance was first assessed using cyclic voltammetry (CV). As shown in **Figure 5(a)**, the CV of the paper LIG with MIP exhibited well-defined redox peaks in the potential range of -0.2 V to 0.7 V at a scan rate of 100 mV/s, and this suggests an efficient electron transfer at the electrode interface. Then, differential pulse voltammetry (DPV) was employed to evaluate the sensing performance. DPV responses are shown in **Figure 5(b)**, which were obtained in 5 mM ferrocyanide with 0.1 M KCl for SMX concentrations ranging from 0.2 μM to 0.7 μM. The peak current increased linearly with SMX concentration, demonstrating the effective binding of SMX to the MIP recognition sites. The corresponding calibration curve in **Figure 5(c)** is obtained with a calculated sensitivity of $43.64 \mu A/\mu M$, a LOD of $0.11 \mu M$, and a LOQ of $0.33 \mu M$.

To assess the applicability of the sensor in a complex real matrix, SMX detection was further evaluated in fresh milk spiked with 5 mM ferrocyanide and 0.1 M KCl. The DPV responses as shown in **Figure 5(d)** presented a clear increase in current with rising SMX concentrations (0.2 μM to

0.7 μM), although the peak currents were slightly lower compared to the buffer system, most probably due to matrix effects from proteins and fats present in milk. Nevertheless, the calibration curve (see **Figure 5(e)**) remained highly linear, and this indicates that the quantification of SMX in a real sample is reliable. The sensitivity in milk was determined to be 11.11 $\mu\text{A}/\mu\text{M}$, with an LOD of

0.36 μM , which remains within the range of relevant detection for practical applications. Overall, these results suggest that the paper-based MIP-LIG sensor exhibits excellent sensitivity for detecting SMX in both buffer and real sample matrices, justifying its potential as an affordable and portable platform for antibiotic monitoring in food safety and environmental applications.

Table 1. Comparison of the proposed paper-based MIP-LIG sensor with conventional methods and other recent sensors for sulfamethoxazole (SMX) detection

Detection Method	Category	Transducer/ Material	Matrix	Analysis Time	LOD (μM)	Key Advantages	Disadvantages
LC-MS /MS [17]	Conventional	Mass Spectrometry	Milk/ Food	2 - 15 min [18]	2×10^{-5} - 0.05	Ultra-high precision, multiclass multiplexing	High equipment cost; requires centralised infrastructure, complex sample preparation, and highly trained personnel.
HPLC [19]	Conventional	Ultraviolet (UV)	Milk/ Eggs	5-20 min	8×10^{-5}	Reliable, validated routine quantitative analysis	Expensive instrumentation; high consumption of organic solvents; strictly limited to laboratory settings.
Immuno-assay (LFIA) [20]	Conventional	Au /Ir@Zn/ Cu-MOF	Food	10-15 min	0.027	Rapid visual detection, portable on-site screening	Often semi-quantitative; relies on fragile biological antibodies prone to environmental degradation; susceptible to cross-reactivity.
Thermal Biosensor [2]	Emerging	MIP-based	Milk	~ 20 min	2.6×10^{-4}	High selectivity, low-cost thermal readout	Slower response time (~ 20 min); requires strict environmental temperature control/isolation to prevent signal drift.
DPV (This Work)	Emerging	Paper-based LIG with MIP	Buffer	~ 1 min	0.11	Bio-degradable, low-cost, portable, and real-matrix compatible	Higher LOD compared to conventional lab methods Slight susceptibility to matrix effects (fats/proteins), resulting in reduced sensitivity compared to buffer performance.
			Milk	~ 1 min	0.36		

Comparison with conventional methods and recent biosensors

To determine if the proposed paper-based MIP-LIG sensor is practically viable, its analytical performance was compared with traditional analytical methods and contemporary biosensors for SMX detection, as presented in **Table 1**. While conventional techniques such as LC-MS/MS [15] and HPLC [17] remain the gold standard for definitive quantitative analysis due to their unparalleled precision and ultra-trace limits of detection (ranging from 2 to 8×10^{-5} μM), they are fundamentally affordable only at centralised laboratories. These methods require expensive infrastructure, complex sample preparation with a high consumption of organic solvents, highly trained personnel, and extended analysis times (up to 20 minutes). On the other hand, although portable screening alternatives like lateral flow immunoassays (LFIA) [18] could provide rapid on-site detection, they rely on fragile biological antibodies that are highly susceptible to environmental degradation and cross-reactivity. In a similar vein, although novel MIP-based thermal biosensors [2] demonstrate superior selectivity, their real-world application is hampered by slower response times of approximately 20 minutes and the critical need for precise environmental temperature control to avoid signal instability.

In contrast, the proposed paper-based MIP-LIG sensor offers a highly complementary approach that directly addresses the critical gap in decentralised food safety monitoring. With a remarkably quick sample preparation time of about 1 minute and the adoption of an eco-friendly, inexpensive paper base, this electrochemical platform is attractive particularly for fieldwork applications. Although its limit of detection in milk matrices (0.36 μM) does not surpass the ultra-trace capabilities of LC-MS, it remains well within the regulatory limits of EMA which is below 0.4 μM . Ultimately, these advantages position the MIP-LIG sensor as a highly competitive, matrix-resilient, and sustainable early-warning alternative for point-of-care analysis, rather than a replacement for definitive laboratory instruments.

Conclusion

This study successfully demonstrates a paradigm shift in decentralised food safety monitoring by introducing a fully biodegradable, paper-based electrochemical sensor for the rapid detection of sulfamethoxazole. Rather than merely presenting an alternative to existing analytical methods, the primary novelty of this work lies in the synergistic architectural integration of Laser-Induced Graphene and Molecularly Imprinted Polymers. By directly anchoring SMX-specific synthetic recognition

cavities onto the highly porous, three-dimensional defect network of a paper-LIG transducer, this design achieves exceptional signal amplification while maintaining remarkable structural and environmental stability.

Consequently, this sensor overcomes the fragility of biological receptors and the complex sample preparation requirements that are typically associated with complex biological matrices. While it does not aim to replace the ultra-trace confirmatory capabilities of centralised LC-MS/MS instrumentation, it successfully addresses a critical diagnostic bottleneck in the dairy supply chain. Importantly, even if the sensitivity of the sensor at 0.36 μM for SMX detection in milk is lower than that of conventional analytical methods, its detection limits remain strictly compliant with the Maximum Residue Limits (MRLs) established by the European Medicines Agency (EMA) for SMX which is at 0.4 μM . With its matrix resilience, speed (approximately one minute), and extremely low cost, this initial screening tool allows for real-time decisions to be made right where care is provided. In conclusion, the successful deployment of this paper-based MIP-LIG platform in raw milk validates its potential as a highly sustainable, scalable, and eco-friendly early-warning system to combat antibiotic residue contamination and mitigate the proliferation of antimicrobial resistance.

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References

1. Singh, B., Bhat, A., Dutta, L., Pati, K. R., Korpan, Y., & Dahiya, I. (2023). Electrochemical Biosensors for the Detection of Antibiotics in Milk: Recent Trends and Future Perspectives. *Biosensors*, 13(9), 867.
2. Frigoli, M., Lowdon, J. W., Cleij, T. J., Diliën, H., Eersels, K., & van Grinsven, B. (2025). Detection of antibiotic sulfamethoxazole residues in milk using a molecularly imprinted polymer-based thermal biosensor. *Food Chemistry*, 476, 143525.
3. Kurç, Ö., & Türkmen, D. (2022). Molecularly imprinted polymers based surface plasmon

- resonance sensor for sulfamethoxazole detection. *Photonic Sensors*, 12(4), 220417.
4. Kang, M. S., Lee, J. H., & Kim, K. S. (2025). Small toxic molecule detection and elimination using molecularly imprinted polymers (MIPs). *Biosensors*, 15(6), 393.
5. Itumoh, E. J., Liu, Y., Cassie, E., Billington, C., & Raymond, O. (2025). An electrochemical aptamer-based sensor for detection of aminoglycosides in milk: addressing practical challenges. *The Analyst*, 150(18), 4111-4121.
6. Alberto, J., Rodríguez, C., Arévalo, F. J., & Granero, A. M. (2025). Design of an MIP-based electrochemical sensor for the determination of paracetamol in pharmaceutical samples. *Biosensors*, 15(8), 544.
7. Pinheiro, T., Silvestre, S., Coelho, J., Marques, A. C., Martins, R., Sales, M. G. F., & Fortunato, E. (2021). Laser-induced graphene on paper toward efficient fabrication of flexible, planar electrodes for electrochemical sensing. *Advanced Materials Interfaces*, 8(22).
8. Guo, Y., Zhang, C., Chen, Y., & Nie, Z. (2022). Research progress on the preparation and applications of laser-induced graphene technology. *Nanomaterials*, 12(14), 2336.
9. Xu, S., Xu, Z., & Liu, Z. (2022). Paper-based molecular-imprinting technology and its application. *Biosensors*, 12(8), 595.
10. Kong, J., Xu, X., Ma, Y., Miao, J., & Bian, X. (2023). Rapid and sensitive detection of sulfamethazole using a reusable molecularly imprinted electrochemical sensor. *Foods*, 12(8), 1693.
11. Elamin, M. B., Ali, S. M. A., Essousi, H., Chrouda, A., Alhaidari, L. M., Jaffrezic-Renault, N., & Barhoumi, H. (2023). An electrochemical sensor for sulfadiazine determination based on a copper nanoparticles/molecularly imprinted overoxidized polypyrrole composite. *Sensors*, 23(3), 1270.
12. Coelho, J., Correia, R. F., Silvestre, S., Pinheiro, T., Marques, A. C., Correia, M. R. P., Pinto, J. V., Fortunato, E., & Martins, R. (2023). Paper-based laser-induced graphene for sustainable and flexible micro supercapacitor applications. *Microchimica Acta*, 190(1), 1–10.
13. Hassan, M. N., Abdullah, T. S., Mou, M. B., & Towsif, H. R. (2024). Analysis of the flame retardancy effect of boron-containing compound on polyester-cotton blended fabric. *Heliyon*, 10, e34007.
14. Lin, M., Guo, X., Xu, Y., Zhang, X., & Hu, D. (2024). A top-down approach to the fabrication of flame-retardant wood aerogel with in situ-synthesized borax and zinc borate. *Materials*, 17(11), 2638.
15. Bhattacharya, G., Fishlock, S. J., Hussain, S., Choudhury, S., Xiang, A., Kandola, B., Pritam, A., Soin, N., Roy, S. S., & McLaughlin, J. A. (2022). Disposable paper-based biosensors: optimizing the electrochemical properties of laser-induced graphene. *ACS Applied Materials & Interfaces*, 14(27), 31109–31120.
16. Mohamed Nageib Amanatuzzakiah Abdul Halim, A., Nurashikin Nordin Fathilah Ali, A., Mohamed Nageib, A., Abdul Halim, A., Nurashikin Nordin, A., & Ali, F. (2025). Adsorption isotherm and kinetic analysis of sulfamethoxazole-imprinted polymers with two functional monomers using bulk polymerization. *Researchsquare*, 2025, 1-18.
17. Khaled, O., Ryad, L., Nagi, M., & Eissa, F. (2024). Multiclass method for detecting 41 antibiotic residues in bovine liver, muscle, and milk using LC-Q-Orbitrap-HRMS. *Journal of Food Composition and Analysis*, 132, 106299.
18. Ng, C. Y. J., Lai, N. P. Y., Ng, W. M., Siah, K. T. H., Gan, R. Y., & Zhong, L. L. D. (2024). Chemical structures, extraction and analysis technologies, and bioactivities of edible fungal polysaccharides from *Poria cocos*: An updated review. *International Journal of Biological Macromolecules*, 261, 129555.
19. Shaaban, H., Mostafa, A., Alqarni, A. M., Alsaman, M., Alkhalaf, M. A., Alrofaidi, M. A., Khzem, A. H. Al, & Alturki, M. S. (2026). Sustainable anisaldehyde-based natural deep eutectic solvent dispersive liquid–liquid microextraction for monitoring antibiotic residues in commercial milk and eggs: a comprehensive evaluation of greenness, practicality, analytical performance and innovation. *Foods*, 15(2), 258.
20. Lu, L., Wang, L., Jiang, K., Li, N., Li, X., Xu, Z., Wu, S., Madushika, L., Yuan, J., Ling, S., & Wang, S. (2026). Au/Ir@Zn/Cu-MOF-based lateral flow immunoassay employing high-affinity monoclonal antibody for sulfamethoxazole detection. *Food Chemistry: X*, 33, 103525.