Ultra-sensitive selective detection of HopQ protein as a biomarker for *Helicobacter pylori* bacteria by an electrochemical voltammetric sensor

Hussamaldeen Jaradat 1,* Ammar Al-Hamry 1, Mohammed Ibbini 2, Najla Fourati 3 and Olfa Kanoun 1,*

1 Measurement and Sensor Technology, Chemnitz University of Technology, 09126 Chemnitz, Germany; hujar@hrz.tu-chemnitz.de; ammar.al-hamry@etit.tu-chemnitz.de; Olfa.Kanoun@etit.tu-chemnitz.de
2 Department of Biomedical Engineering, Jordan Uni. of Science and Technology, 22110 Irbid, Jordan; mohib@just.edu.jo
3 CNAM, SATIE, UMR CNRS 8029, 292 rue Saint Martin, 75003, Paris, France.; fourati@cnam.fr
* Correspondence: Olfa.Kanoun@etit.tu-chemnitz.de; hujar@hrz.tu-chemnitz.de

Abstract:

**Background** *Helicobacter pylori* (*H. pylori*) is a highly contagious pathogenic bacterium that can cause gastrointestinal ulcers and may gradually lead to gastric cancer. *H. pylori* expresses the outer membrane HopQ protein at the earliest stages of infection. Therefore, HopQ is a highly reliable candidate as a biomarker for *H. pylori* detection in saliva samples.

**Materials and Methods:** An *H. pylori* immunosensor is developed based on detecting HopQ as a biomarker in saliva by a screen-printed carbon electrode (SPCE) modified with MWCNT-COOH decorated with gold nanoparticles (AuNP). The HopQ antibodies are grafted on the SPCE/MWCNT/AuNP surface using EDC/S-NHS chemistry. The sensor performance is investigated by various methods and *H. pylori* detection performance in spiked saliva samples is evaluated by square wave voltammetry.

**Results:** The sensor is suitable for HopQ detection with high sensitivity and excellent linearity in the 10 pg/mL - 100 ng/mL range and with a 10 pg/ml limit of detection. The sensor was tested in saliva at 10 ng/mL and returned an 107.6% recovery. The dissociation constant $K_d$ for HopQ/HopQ antibody interaction, estimated from Hill’s model, is calculated with a value of an order of $4.605 \times 10^{-10}$ mg/mL.
Conclusions: Due to the strategical choice of biomarker, the utilization of nanocomposite material to enhance the SPCE electrical performance, the intrinsic selectivity of the antibody-antigen interaction, and effective immobilization, the fabricated platform shows high selectivity, good stability, reproducibility, and cost-effectiveness for early H. pylori detection. Additionally, we provide insight into possible future aspects the researchers are recommended to focus on.

Keywords: Immunosensor; H. pylori; HopQ; Saliva; biosensor; nanotechnology; CNT; biomedical engineering

1. Introduction

*Helicobacter pylori* (*H. pylori*), the gram-negative pathogenic bacteria, is hosted by more than 50% of people worldwide [1], [2]. According to the World Health Organization (WHO) and the International Agency for Research on Cancer (IARC), *H. pylori* is considered a class I carcinogen [3]–[6]. In 2005, B. Marshall and R. Warren received the Nobel Prize in Physiology or Medicine for the discovery of *H. pylori* bacteria in 1982 [7]. *H. pylori* attacks the gastric mucosa layer of the human stomach and can be prevalent in the gastrointestinal (GI) tract. Eventually, *H. pylori* infection is a causative agent of chronic gastritis, ulcers, and gastric cancer that can eventually lead to death. This widely spread and highly contagious bacteria utilizes virulent factors as mechanisms for gastric colonization [8], [9]. *H. pylori* colonization causes gastric diseases that, synergically, with its consequent host’s inflammatory response and dietary/lifestyle factors, can lead to cancer [10]. Most *H. pylori* transmission occurs through individual-individual interaction or contaminated mediums such as communal spaces, contaminated food, and water. The exact routes for *H. pylori* transmission are still unproven, but saliva is one of the body fluids that contain *H. pylori*, proving the oral-oral transmission [11]–[13].

Several conventional approaches are available for detecting *H. pylori* at the stage of infection, including invasive and non-invasive techniques, but these techniques can
experience several limitations, such as increased time consumption, high cost, and limited shelf-life [14]–[18]. In addition, testing for children and some incapable adult cases can be very challenging, especially in cases of invasive testing. Thus, the advent of biosensors as an analytical tool in the clinical and environmental detection of virulent microbiomes that cause diseases plays an intriguingly important role, particularly in biomarker-based environmental contamination monitoring and cancer-related disease diagnosis [16], [18].

Immunosensing-based biosensors are fundamentally based on transducing the highly specific immunoreaction between antibodies (receiver) and their (partially) complementary antigen/protein/peptide/hapten (analyte) at the surface of the electrode. Electrochemical sensing is inherently very sensitive to chemical events at an infinitesimally small scale. Therefore, electrochemical biosensors are very sensitive to surface changes giving rise to a very low limit of detection (LOD), high sensitivity, specificity, and selectivity. The electrochemical biosensor field has reached a milestone, driven by the highly enhanced sensitivity, excellent selectivity, lower detection limits, detection ranges, shelf-life, simplified sample preparation, and cost affordability [19]–[25]. Scaling down the sensitivity and LOD has proven to beat the fundamental limitations imposed by classical methods, and therefore, the biosensor’s sensitivity is expected to be the main driving force for future investigations [26]–[31].

Regarding the H. pylori recognition element, antibodies are considered the gold standard biomarkers for specific and selective recognition. The choice of the biomarker for any medical complication can be partially decided by the reliability of using it as an indicator for diseases at a specific stage or contamination monitoring. H. pylori uses its outer membrane proteins, such as HopQ, to facilitate the transfer of its pathogenic factor, such as CagA, to the host cells, which exploit the family of the cell adhesion molecules family related to the carcinoembryonic antigen [8], [9], [32], [33]. Therefore, HopQ, as an outer membrane protein, can be effectively utilized as a biomarker for reliable, selective,
and specific non-invasive detection of *H. pylori* bacterial infection through body fluids like saliva [4], [12], [13], [16], [34]–[41]. Detection in saliva is very promising to be as minimally invasive as possible and convenient to perform for almost all patients ages [34]. In addition, monitoring *H. pylori* contamination in mediums such as water and food can prevent the spread of *H. pylori* and will be one more step toward fighting this contagious carcinogen bacteria and help curb its antimicrobial resistance development [33], [42].

Biomarker-based biosensors need to be one-time use (disposable), cost-affordable, simple, accurate, repeatable, and sensitive. Screen-printed carbon electrodes (SPCEs) are famous for their simple designs, low cost, and mass production, making them suitable for developing disposable biosensing platforms. Electrochemical SPCEs are usually designed with a 3-electrode configuration, working electrode (WE), counter electrode (CE), and reference electrode (RE). WE and CE are graphitic carbon-based, while the RE is Ag/AgCl. Electrochemical immunosensing relies on monitoring charge transfer between the redox couple and the WE surface. Therefore, WE of SPCE are modified with nanomaterials to upstream the graphite electrodes [43]–[47].

Nanomaterials are increasingly used in biosensors due to their unique physicochemical properties and high surface area-to-volume ratio. Biosensors combined with nanotechnology allow variable surface modification that boosts and upscales the loading capacity of the sensing platform [22]–[25], [48]–[50]. Amongst several nanomaterials, carbon-based nanomaterials have been broadly investigated and studied, driven by their unique properties such as high conductivity, mechanical stability, and biocompatibility. Carbon nanotubes (CNTs) are one of the most unique nanostructured carbon materials, primarily due to their unique wrapped-like 2D structure that enables their electronic ballistic transfer capability. CNTs are considered one-dimensional needle-looking hollow cylindrical graphitic carbon nanostructures with Sp² atoms arrangements.
CNTs chirality has several arrangements, theoretically considered as a rolled-up graphene sheet(s) structure. Among zigzag and single-walled CNTs (SWCNTs), Multi-walled CNTs (MWCNTs) have superior qualifications such as simple, low-cost mass production, chemical inertness, and stability[51]–[53]. MWCNTs are excellent as surface modifying-material for electrochemical sensing due to their ability to upstream sensitivity by enhancing electrical conduction through their network forming on electrodes surface and its ballistic electronic transfer. In addition, MWCNTs, due to their multi concentric multi cylindrical structure, allow for different chemical functionalized of the outer layers with groups such as carboxyl group (MWCNT-COOH), while the inner cylinders preserve the electrical properties. MWCNT-COOH offers the capability to chemically immobilize antibodies through amid bonding between MWCNT carboxyl end and antibody amino group which is necessary for immobilization of HopQ antibodies on the electrode surface [51], [52], [54]–[56]. Amongst metallic nanomaterials, gold nanoparticles (AuNPs) are the most widely used metallic nanoparticles, especially for protein stabilization. AuNPs biocompatibility plays an important role in preserving the biological activity of antibodies and proteins [57]. Biomolecules’ weaker electrical conductivity hampers electronic exchange between redox couple and the electrode surface. Therefore, the use of AuNPs greatly enhances surface conductivity, which upstream sensors’ sensitivity, enlarges sensing surface area, which increases immobilization of antibodies leading to a broader dynamic range of detection, and enhances antibody-antigen interaction, which reflects on enhancing selectivity [46], [47], [57]. Herein, the focus is on harnessing the aforementioned outstanding properties of MWCNT-COOH and AuNPs to realize an electrochemical-based biosensor to detect *H. pylori*’s HopQ protein.

A few electrochemical immunosensors have been developed to detect *H. pylori* in humans based on virulent biomarkers, mostly in stool or blood samples. However, *H. pylori* transmission routes are still elusive; for example, humans can get infected by using
contaminated water and food, interacting with people, and using contaminated tools and facilities [16]. Therefore, efforts for *H. pylori* detection are expected to focus on infection detection in less invasive mediums, such as saliva, and a broader range of environmental media, such as water or food. Researchers reported electrochemical sensors for *H. pylori* detection based on antibody-antigen interaction for human infection. All the used biomarkers are virulent factors the bacteria inject into infected tissue after infection. However, HopQ protein is utilized by *H. pylori* as adhesion to the GI epithelial tissue exists as an OMP. Therefore, relying on it is more advantageous to use as a biomarker than other biomarkers that appear after infection. Therefore, there is still an imminent need for *H. pylori* sensors for early-stage infection diagnosis for humans and to track and monitor *H. pylori* in the environment, water supplies, fruits, and vegetables [16]–[18], [50], [58], [59].

The main aim of this study is to develop, fabricate and realize a simple and cost-effective SPCE immune-biosensor to apply in saliva samples to achieve a sample-collection convenient, minimally invasive, and early-stage detection of *H. pylori* within a short time. Briefly, the WE’s surface of commercially available SPCE is firstly pretreated with 2-(N-morpholino)ethanesulfonic acid (MES) to enhance surface properties [60], followed by MWCNT-COOH deposition to increase the electrodes’ surface area and provide a scaffold to attach HopQ antibodies as recognition elements covalently. Then, AuNPs colloid is prepared and used to boost electrical properties further and enhance antibody stability [23], [49], [50], [61]. Then the HopQ antibody is covalently immobilized using EDC/NHS chemistry, followed by blocking non-specific binding sites. To optimize antibody immobilization, as the most crucial step in sensor development, synergically, the MES pretreatment, nanocomposite surface modification, and antibody immobilization chemistry led to targeting the detection of *H. pylori* at a very low detection limit for the HopQ as an *H. pylori* biomarker. Several characterizations are performed to optimize, confirm, and realize the sensing platform, such as cyclic voltammetry (CV), electrochemical impedance...
spectroscopy (EIS), square wave voltammetry (SWV), energy dispersive spectroscopy (EDX), and scanning electron microscopy (SEM). Several performance analytical studies are carried out, such as dissociation constant calculation, reproducibility, selectivity, shelf-life, cross-reactivity, and recovery study in artificial saliva sample. It is worth noting that the HopQ/HopQ-Ab dissociation constant has never been reported to the author’s knowledge.

This paper is structured into four sections. Materials and methods are mentioned and explained in section 2. Section 3 illustrates the results of the experiments, summarizes the findings, and discusses them based on theoretical background. Section 4 concludes the work while highlighting possible future directions in detecting *H. pylori*.

2. Materials and Methods

2.1. Apparatus

Electrochemical characterization/pretreatment is performed using palmsens4 potentiostat (*PalmSens BV, GA Houten, The Netherlands*). Scanning electron microscopy (SEM) spectroscopy is performed using FEI Nova NanoSEM 200 microscope. The SoCorex Acura® XS 826 precision Micropipettes 0.5-10 μl is purchased from (*SoCorex Isba SA, Ecublens, Switzerland*). Polyester-substrate-based electrodes (ItalSens IS-C) are purchased from (*PalmSens BV, GA Houten, The Netherlands*). All other lightweight equipment and tools, including glassware and pH electrodes, were obtained from the local *Technical University Chemnitz Chemical Storage, Chemnitz, Germany*. All graphs are produced using OriginPro, Version 2021b. (*OriginLab Corporation, Northampton, MA, USA*).

2.2. Reagents
HopQ protein and its antibody are purchased from Biotrend Chemikalien GmbH, Köln, Germany. HopQ protein is especially sequenced by Biotrend company for this experiment.

MES acid is obtained from Thermo Fisher (Kandel) GmbH, Kandel, Germany. All other chemical reagents are obtained from Sigma-Aldrich Chemie GmbH, Taufkirchen, Germany, including, Artificial saliva, 2-Mercaptoethanol, Sulfo-N-Hydroxysulfo Succinimide sodium salt (S-NHS), Gold (III) chloride tri-hydrate (HAuCl₄·3H₂O), tri-sodium citrate dehydrate, N-(3-Dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride (EDC) 98%, disodium hydrogen phosphate, potassium dihydrogen phosphate, phosphate buffer saline (PBS), K₃Fe(CN)₆, K₄Fe(CN)₆, potassium chloride, MWCNT's, polyethyleneimine (Mn 60,000 g/mol) and gold standard solution for inductively coupled plasma (ICP) of (1000 mg/L Au), and Bovine serum albumin (BSA), all without any further purification or treatment.

2.3. Immunosensor preparation

2.3.1. Activation and pretreatment of SPCE

The bare SPEC is pretreated with MES buffer (0.01 M) electroactively with CV from -1 to 1 V at a scan rate of 50 mV for 10 cycles[60]. Electrochemical cleaning is needed to help remove external contaminants and loosely attached structures on the surface material like printing ink polymers and any other chemical content, which can enhance the attachment of nanocomposites on the solid structure at later fabrication stages to boost electrical conductivity [55], [62]–[65].

2.3.2. Nanocomposite preparation and surface modification

The MWCNT dispersion is prepared to produce a concentration of 0.05% wt. in isopropanol by sonication with 35% amplitude for 90 minutes [66]. A biocompatible AuNP colloidal suspension is synthesized using the approach of photochemical-assisted synthesis with the help of branched poly (ethylene imine) (PEI, Mn = 60,000 g.mol⁻¹) as a
stabilizing agent. Thus, a colloidal solution suspension (0.03 M Au) of positively charged AuNPs is prepared [67], [68].

For simplicity in fabrication, the WE of the SPCE is modified with a simple drop-casting of nanomaterials solutions. The first step is the deposition of the MWCNT-COOH. In this step, 3 µL of the MWCNT dispersion was evenly drop-casted in a 0.75 µL step to ensure more even distribution of MWCNT on the electrode surface and to avoid concentric coffee-ring-like distribution [69], [70]. The electrode is left to dry, followed by drop-casting 2 µL of the water-based AuNP’s colloidal dispersion in 0.5 µL steps. The electrode is rinsed with DI water between steps and gently derided with the N₂ stream.

2.3.3. Immobilization of the HopQ-Ab on WE

Before proceeding further with functionalization, the electrode is thoroughly washed with DI water and MES acidic buffer (pH = 6) [60]. The originally supplied antibody stock concentration was 0.75 mg/ml, which then is aliquoted in 10 µL aliquots immediately after receiving it. Aliquots are diluted to reach a 10 µg/mL concentration in an acidic buffer medium (10 mM PBS, pH = 6). To immobilize HopQ-Ab, the MWCNT-COOH is activated with the versatile EDC/S-NHS chemistry. Briefly, a 5 µL of 4:1 molar ratio of the EDC/S-NHS solution in PBS buffer (pH = 6) is dropped on the WE surface. The EDC/S-NHS was left on the WE for 1 hour for conjugation in a dim atmosphere [71]. Afterward, 7.5 µL of HopQ-Ab in PBS is dropped on the WE surface and left for conjugation for 45 minutes, followed by dropping 10 µL of 1% BSA in PBS on the WE to block non-specific binding sites.

At this stage, the sensor’s interface layer is prepared, and the platform is ready to be used in detection. The electrodes are stored in a liquid buffer (10 mM PBS) with a pH of pH = 6) at 4° C for further use and investigation.
2.4. Electrode characterization

2.4.1. Electrochemical measurements

The sensor is characterized by CV and EIS through the main development steps, such as deposition of nanomaterials, immobilization of HopQ-Ab and BSA, and detection of HopQ protein. CV is performed in the -0.6 ~ +1.0 V range with a scan rate of 100 mV/s. Standard CV study for the fabricated sensor is performed at different scan rates from 10 ~ 100 mV/s. EIS was performed in the frequency range from 0.1 ~ 10k Hz with 10 mV amplitude around the open circuit potential (OCP) voltage. All measurements have been performed in 5 mM of K₃[Fe(CN)₆]/K₄[Fe(CN)₆] in 10 mM PBS solution with pH = 7.4 at room temperature.

2.4.2. Surface characterization by scanning electron microscopy

SEM surface characterization is performed using FEI Nova NanoSEM 200 microscope to assess the surface morphology changes on the WE surface throughout CNT/AuNP and HopQ-Ab/BSA deposition.

2.5. Electrochemical measurements and detection of HopQ

Square wave voltammetry (SWV) measurements were performed in triplicates to evaluate HopQ detection performance. The parameters used are voltage range from -0.3 ~ 0.6 V with an amplitude of 0.1 V at 10 mV steps with 10 Hz frequency. The peak current difference between the SWV measurement with a sample containing the analyte and an analyte-free sample is recorded and plotted against the logarithm of the analyte concentration expressed in ng/mL. A 7.5 µL of HopQ-spiked buffer samples are placed on the WE area and left for conjugation for 15 minutes. Then all SWV measurements were performed using 5 mM of K₃[Fe(CN)₆]/K₄[Fe(CN)₆] in 10 mM PBS buffer solution with pH =
7.4 at room temperature to obtain the calibration curve. Analytical performance analysis of artificial saliva spiked sample is performed with 10 ng/mL HopQ. The sensor is incubated in HopQ spiked artificial saliva for 15 minutes, and the SWV response is recorded.

3. Results

3.1. Electrochemical measurements and detection of HopQ

3.1.1. Electrochemical measurements

The CV is performed along the electrode’s fabrication steps to confirm the sensing platform’s proof of concept. Figure 1A shows how the peak current of the CV voltammograms gets higher with surface modification using MWCNT and AuNP with respect to a bare SPCE. Additionally, the CV peak separation voltage got narrower with nanocomposite modification, for which peak current and peak separation confirm faster kinetics at the electrode surface which helps to increase sensor sensitivity and detection range [22]. After adding HopQ-Ab, the CV peak current is greatly diminished, and voltage separation between the current’s peaks ($\Delta E_p$) considerably widened, implying a slower charge transfer kinetics which suggests more steric exclusion hindrance at the electrode surface. This surface blocking is further confirmed by adding BSA and an excess concentration of HopQ protein, $I_{pa}$ and $I_{pc}$ are furtherly lowered, and $\Delta E_p$ gets more dilated. This expected behavior supports the blocking effect of the bio-elements immobilized on the surface.

The developed electrode is studied for several scan rates from 10-100 mV/s in the potential range from $-0.6 \text{ V} \sim +1.0\text{V}$ in 10 mM PBS with a pH of 7.4, which contains a 5 mM potassium ferrocyanide/ferricyanide redox probe. The voltammograms in Figure 1B notably demonstrate the increase in cathodic and anodic peak currents as the scan rate increases indicating a thinner diffusion layer at the electrode surface and faster diffusion charge transfer with the bare electrode surface. The voltammograms show that the peak current at the anodic (oxidation) and the cathodic (reduction)
potentials ($\Delta E_p$) increased linearly versus the square root of the voltage scan rate, demonstrating the diffusion-controlled mechanism linearity on the electrode surface, Figure 1C. The regression coefficient ($R^2$) for $I_{pa}$ and $I_{pc}$ versus square root of scan rate are 0.999 and 0.992 for HopQ-Ab/AuNP/MWCNT/SPCE electrode Figure 1C.

3.1.2. Electrochemical impedance studies

EIS measurements were investigated to follow up on the electrode’s surface modifications. The corresponding impedance spectrograms are presented in Figure 1D. EIS curves are fitted with the Rundle circuit model Figure 1D. The fitting results, shown in Table 1, demonstrate the enormous drop in the charge transfer resistance ($R_{ct}$) value after the deposition of nanoparticles on the WE’s surface with respect to the bare graphite electrode. Oppositely, the $R_{ct}$ value has increased approximately 11 folds after immobilization of HopQ-Ab and BSA adsorption due to the weak electrical activity of biomolecules. This colossal increase in $R_{ct}$ supports the evidence for successful immobilization and surface blocking. These conclusions are also supported by SEM spectroscopy in Figure 2A-C, in which a complete blocking of the surface is evident through the lower surface conductivity, even after increasing the incident beam intensity to 20kv [72]. [73]

The value of CPE of the fitted EIS complex data indicates a considerable increase after the deposition of nanomaterials on the bare electrode, indicating a massive increase in surface area and more rough surface morphology. Nevertheless, the CPE value decreases after the immobilization of HopQ-Ab and blocking with BSA, indicating the additional spacing added by immobilizing BSA and HopQ-Ab [73].

3.2. Immunosensor Analytical Performance

SWV technique is utilized to evaluate the sensing process performance of the immunosensor. Figure 3A exhibits the reduction in redox peak current vs. the concentration of HopQ protein. The study is
carried out in 5mM of K$_3$[Fe(CN)$_6$]/K$_4$[Fe(CN)$_6$] dissolved in 10 mM PBS solution with pH = 7.4 at room temperature. Measurement showed that peak current linearly decreased with an increase in HopQ concentration, mainly due to steric exclusion hindrance at the surface and the expected negative charge of the protein at this pH.

To quantify the immunosensor response, we plotted the relative variation of current intensity $(I_0-I)/I_0$, where $I_0$ is the current measured after HopQ antibodies grafting and I is the current value obtained after each HopQ concentration. The resulting corresponding calibration curve is plotted in Figure 3B. The LOD of the designed immunosensor was equal to 10pg/ml. The sensitivity, calculated from the slope at the origin, was equal to 11.77 µA/Log [HopQ, ng/mL].

In the context of antibody-antigen interactions, Hill’s model describes the dissociation constant (Kd) of the antibody-antigen complex. The dissociation constant measures the strength of the interaction between the antibody and antigen, with a lower value indicating a stronger interaction. A measure of the dissociation constant Kd is one of the most important factors of the immunorecognition process in immunosensors. It offers information on the affinity between the receiver antibody immobilized on the sensor’s surface and the protein. In this work, the Kd value is calculated by fitting the experimental data using the Hill model according to the below equation:

$$ S(C) = \frac{A \times C^a}{K_d^a + C^a} $$

(1)

Where:

- $S(C)$ is the normalized current variation $(I_0-I)/I_0$
- $a$ is Hill’s coefficient,
- $A$ is an empirical constant, and
- $K_d$ is the dissociation constant. Fitting the measurement data according to Hill Model, the dissociation constant is $K_d = 4.605 \times 10^{-10}$ mg/mL. The value range of
the reported $K_d$ is very low, indicating a solid attachment in this recognition system, which is expected for antibody-antigen immunoreaction [74].

3.3. Selectivity and cross-reactivity

3.3.1. Selectivity

The selectivity and cross-reactivity evaluation of the fabricated sensing platform is studied in the presence of an excess concentration of various interferants (10 times the highest concentration in the detection range). Several interferants, such as CagA, AFP, BSA, dopamine, and estrogen, have been used. The selectivity of the platform is studied against CagA because it is an $H. pylori$ protein, Afp because it is from humans, and BSA, which has been used in electrode fabrication. The peak of the SWV after incubation at 15 minutes did not return a noticeable difference in the current response from the blank for this selectivity test, Figure 4A.

3.3.2. Cross-reactivity

The cross-reactivity investigation is conducted in the presence of 5 ng/mL HopQ protein. Figure 4B shows the cross-reactivity of the fabricated immunosensor platform of 5 ng/mL HopQ in excess concentrations of different interferants. The results show that a maximum of 14% variation in current is observed for interferants in the presence of HopQ. No significant changes are noticed in the SWV peak currents compared to the one measurement of HopQ only. Therefore, the developed platform showed excellent specificity.

3.4. Reproducibility

The reproducibility of the sensing platform has been studied by independently preparing five electrodes. The reproducibility is observed by comparing the performance of the equally prepared five electrodes with 5 ng/mL HopQ concentration. Figure 4C depicts...
the current response of the five different electrodes, and it is evident that it varies slightly, which indicates excellent reproducibility of the immunosensor.

3.5. Shelf-life studies and comparison with other platforms

The shelf-life stability of the prepared immunosensor stored at 4 °C is investigated by checking the peak current every 1 week at different time intervals. The SWV peak current difference was reduced by only 15% after 4 weeks, which illustrates how promising such immunosensor platforms can be in terms of shelf-life when the monolithic fabrication process is utilized.

3.6 Artificial saliva samples

The developed sensing platform is tested with HopQ-spiked artificial saliva samples. The recovery percentage of 10 ng/mL of HopQ-spiked artificial saliva triplicate is calculated using the standard method. The results demonstrate a 107.6% recovery of the expected peak current, which supports the accuracy of the developed biosensor and its usability for saliva real sample analysis in the future. Also, when the biosensor was incubated with artificial saliva only (without the HopQ biomarker), SWV data showed no significant change in the SWV peak current.

4. Conclusions

For the detection of H. pylori, we proposed a biosensor based on the detection of HopQ protein as an H. pylori OMP biomarker. This immunosensor is based on a polyester-substrate SPCE pretreated with MES acid modified with MWCNT-COOH adorned with AuNPs, then covalently immobilized HopQ-Ab layer as recognition layer and the non-specific binding sites blocked by BSA. The fabrication method of the detecting layer is optimized starting from nanocomposite deposition by simple drop-casting to upstream
electrode conductivity and laying a platform to immobilize HopQ-Ab, then the covalent immobilization of HopQ-Ab is optimized. Electrochemical methods like SWV, CV, and EIS are used to characterize and assess the performance and effectiveness of the HopQ immunosensor. The developed and optimized biosensor has a relatively wide detection range of 10 pg/mL to 100 ng/mL, with excellent linearity and LOD of 10 pg/ml. Such performance measures indicate how simple and yet optimized fabrication can perform superiorly. The biosensor is used in artificial saliva samples with good recovery. The use of HopQ as a biomarker for detecting H. pylori is novel in terms of being expressed by H. pylori at an early stage of a bacterial infection in saliva or in contaminated water samples and in terms of being an OMP expressed by H. pylori. In addition, this work reports the Hill’s dissociation constant of HopQ for the first time. The developed and optimized biosensor demonstrates high selectivity, good stability, reproducibility, and cost-effectiveness.

Besides, it is obvious how strategies and optimizations used in this investigation have improved the developed immunosensor, especially in terms of linear range and LOD. The linear range for the prepared electrode is 10 pg/mL - 100 ng/mL, which is wider than the reported literature with a LOD of 10 pg/mL; Table 2 sets forth a brief comparison of the reported immunosensors.

[31], [75]–[78] Reliable detection of H. pylori at very low concentrations still embraces several challenges related to sensing setups, such as minimal sample preparation for complex body fluid or contaminated water samples, incubation time, and the needed measurement routine. Therefore, future enhancements are highly favored in the direction of monolithically fabricated detection setups that minimize the needed human intervention in the detection process. On the other hand, more intelligent software
could be developed to enhance the processing power of the produced information, such as using self-calibration algorithms.


**Funding:** The authors thank the German Academic Exchange Service (DAAD) for financial support.

**Acknowledgments:** We thank the Professorship of Solid Surfaces Analysis, Chemnitz University of Technology (Prof. Christoph Tegenkamp and Doreen Dentel), for the access to SEM spectroscopy.

**Conflicts of Interest:** The authors report no conflicts of interest in this work.

**References:**


