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# The Effect of Gold & Carbon Screen-Printed Electrode (SPE) for Biosensing Application by Electrochemical Method

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**Abstract:** This study primarily characterizes the aptamer-based biosensor as a straightforward and sensitive device that operates on the principle of aptamer-antigen interaction. The characteristics of the aptamer-based biosensor are examined by observing morphologies, charge transfer, impedance, potential, and current on the carbon and gold screen-printed electrode (SPE) using electrochemical techniques and scanning electron microscopy (SEM). This work presents the results of cyclic voltammetry conducted on both carbon and gold screen-printed electrodes (SPEs), the analysis of immobilization of five biochemical solutions (Aptamer resuspension buffer, aptamer folding buffer, aptamer reducing buffer, thiol-modified aptamer, and BSA), and chronoamperometry of different concentrations of hCG antigens. The experiments were performed using a Metrohm Autolab Potentiostat on both gold and carbon SPEs. The polarization resistance (Rp) significantly decreases at each fabrication stage, indicating that the SPE remained stable and conducive for use as a biosensor due to redox reactions (2.41k Ω, 79.8 Ω, 24.5 Ω). The polarization resistance (Rp) of an electrochemical process is inversely proportional to the current flow. Changes in the diffuse layers of the analyte on the working electrode result in variations in the currents. The detection of hCG antigens within a wide range of concentrations, as low as 0.1 ng/ml, was successfully achieved. Based on the data obtained, the electrochemical method demonstrated the high specificity and sensitivity of the aptamer-based gold screen-printed electrode in comparison to carbon.

**Keywords**: Screen-Printed Electrode (SPE), Biosensing, Electrochemical Method

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## 1. Introduction

Prostate cancer (PCa) is the most common cancer diagnosed in males globally, and it remains the leading cause of cancer-related deaths [1]. In 2020, worldwide statistics reported 1,414,259 cases of prostate cancer, with 375,304 resulting in fatalities [2]. In Malaysia, there were 2,146 reported cases of prostate cancer in the same year. Prostate cancer cells can travel through the bloodstream and form deposits, particularly in the bones, including the spine, a process known as metastasis or "change of location." Unfortunately, some diseases, such as cancer, chronic respiratory conditions, and diabetes, are challenging to diagnose in their early stages with current healthcare technologies [3]. In 2018, cancer claimed the lives of 9.6 million people, according to the World Health Organization (WHO).

The primary methods for early detection of prostate cancer involve digital rectal examination (DRE) and blood testing for prostate-specific antigen (PSA) levels [4]. DRE, with an estimated accuracy of around 59 percent, is considered a fundamental technique for prostate cancer screening and early diagnosis. It often detects tumors that other tests may miss.

In recent decades, sensors have found widespread use across various fields, including medical diagnosis [5]. To address the challenge of prostate cancer detection, a chronoamperometric technique using both carbon and gold electrodes has been developed [6]. The hCG antigen will serve as the analyte for identifying prostate cancer in patients. Scanning electron microscopy (SEM) will be employed to examine the microstructure of the carbon electrodes, and electrochemical results will be analyzed using Autolab with NOVA software.

The primary medium for this project involves the use of carbon and gold on Screen Printed Electrodes (SPE), achieved through an electrodeposition step. A critical requirement for analytical methods in evaluating this sample directly is maintaining it in its natural state without alterations. SPE is well-suited for creating sensors that can be incorporated into modern mobile devices [7-9]. The hCG aptamer is deposited on the carbon and gold electrodes of the Screen-Printed Electrode. The detection of prostate cancer will rely on the potential difference generated by the electrode's activity, monitored by AutoLab.

## 2. Materials and Methods

Twenty-seven-point-twenty-five micrograms (27.25 µg) of aptamer were swiftly vortexed for 10 seconds to ensure complete dissolution in the buffer after being diluted with 1 ml of aptamer resuspension buffer. Subsequently, 50 µl of the stock solution was diluted with 450 µl of Aptamer Folding Buffer, and the mixture was heated for 5 minutes at 85–95°C. After allowing the folded aptamer to cool to room temperature for 15 minutes and incubating it for an additional 10 minutes, it was diluted with aptamer reducing buffer in a 1:1 ratio of equal volumes. For optimal aptamer performance, the aptamer was then diluted with a phosphate-buffered saline solution containing 1mM $MgCl_2$ and used immediately. Finally, hCG antigens were diluted with TRIS buffer to achieve different concentrations using an acid-base formula:

$$\mathbf{M_a V_a = M_b V_b} \qquad \mathbf{Eq}.\ 1$$

$M_a$ represents the initial concentration, and $V_b$ represents the initial volume of the stock solution, we can calculate the final concentration and volume needed for the experiment [13]. For example, in the case of hCG antigens, 20 µl of the stock solution was diluted with 480 µl of TRIS buffer to achieve a final concentration of 1000 ng/mL of hCG antigen, and 500 µl of this solution was used in the experiment. This process was repeated for various concentrations, including 0.1 ng/mL, 1 ng/mL, 10 ng/mL, and 100 ng/mL

The surface of the screen-printed electrode (SPE) was treated by dipping it in a 0.1 M solution of sulfuric acid (H2SO4) to remove any contaminants. To assess the electrode's electrochemical behavior using cyclic voltammetry (CV) and connect it to a Metrohm Autolab Potentiostat, both SPEs were

immersed in a 1 mM solution of Potassium Ferrocyanide ($K_4[Fe(CN)_6]\cdot 3H_2O$). This was followed by a 30-minute incubation period, after which the SPEs were washed with deionized water, and 20 µl of aptamer was applied to the working electrode. The SPE was then connected to the potentiostat after incubating for 12 hours.

Another 20 µl of a 0.1 ng/ml hCG solution was added to the SPE to hybridize with the immobilized aptamer probe. The aptamer and active hCG mixture were incubated for an additional hour at room temperature. The hybridization procedure was repeated with hCG concentrations ranging from 1 ng/ml to 1000 ng/ml, each lasting an hour. The aptamer concentration, referred to as the hCG-apt conjugate, was synthesized and stored at 4°C for subsequent use.

Electrochemical impedance spectroscopy (EIS) was used to assess all different hCG antigen concentrations before being examined by chrono-amperometric measurement at 0.9 V for 0.1 seconds. Finally, the deposition on the SPE surface before and after the immobilization stage was examined using a scanning electron microscope (SEM)

## 3. Results and Discussion

### 3.1 Electrochemical Characterization of The Aptamer-based Biosensor Array

To achieve a smooth surface and remove any contamination from the SPE's surface, we used 0.1 M sulfuric acid (H2SO4) for cyclic voltammetry (CV) cleaning. Simultaneously, we employed potassium ferrocyanide ($K_4[Fe(CN)_6]\bullet 3H_2O$) to analyze the electrochemical behavior of the electrode using the CV technique. It is common practice to illustrate CV by reducing ferricyanide to ferrocyanide. In this one-electron redox reaction, the ferricyanide ion ($Fe(CN)_6^{3-}$) functions as an oxidant, while the ferrocyanide ion ($Fe(CN)_6^{4-}$) acts as a reductant.

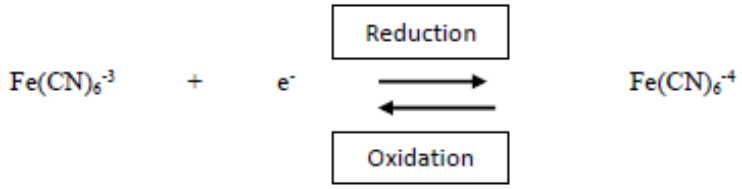



**Figure 1: The electrochemical behavior of 1 mM potassium ferrocyanide ($K_4[Fe(CN)_6]\bullet 3H_2O$) measured at a scan rate of 0.1 V/s for 8 full cycles. The potential is scanned in the opposite direction to complete the cycles, resulting in a negative scan that returns to the starting potential.**

Figure 1 also presents the measurement of the current, which initiates flowing in the negative direction of potential at $4.76 \times 10^{-10}$ A and peaks at 0.048 V. The direction of the potential sweep is then reversed at -0.4 V, initiating the oxidation of the ferrocyanide ion ($Fe(CN)_6^{4-}$) at -0.027 V. Consequently, the peak potential difference is determined by comparing the peak potentials for oxidation and reduction [10].

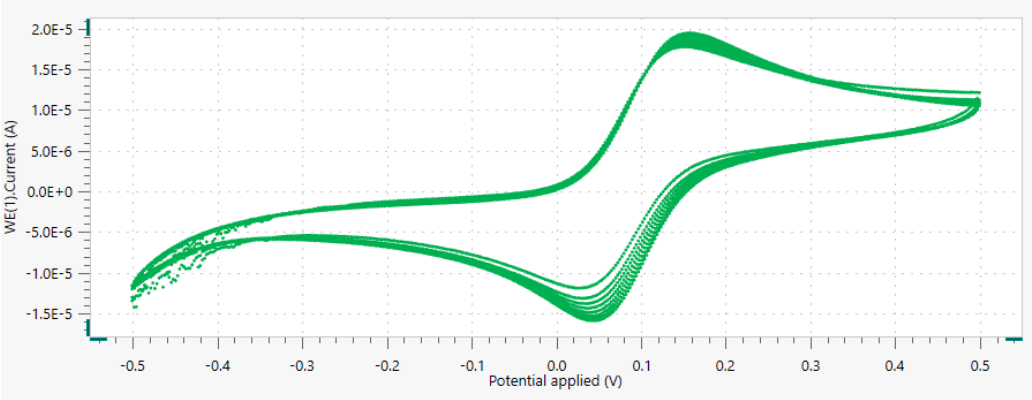
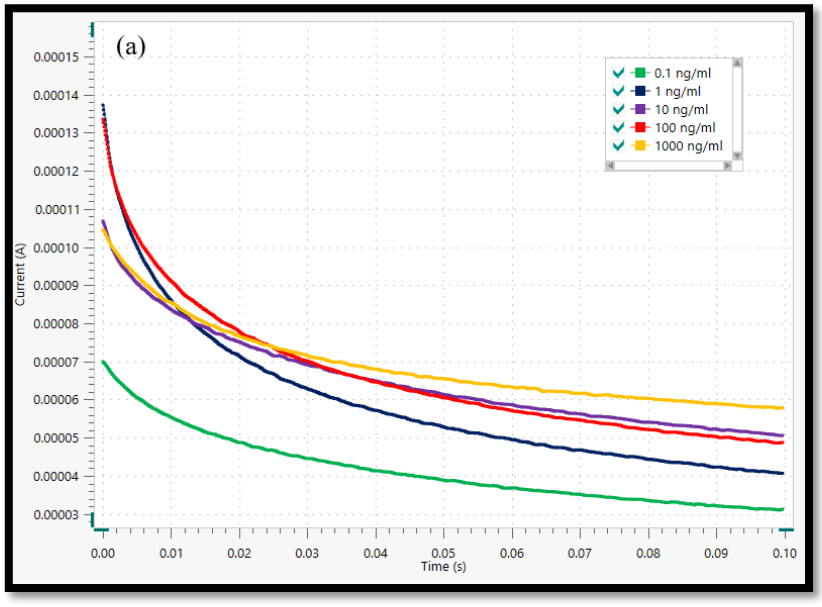


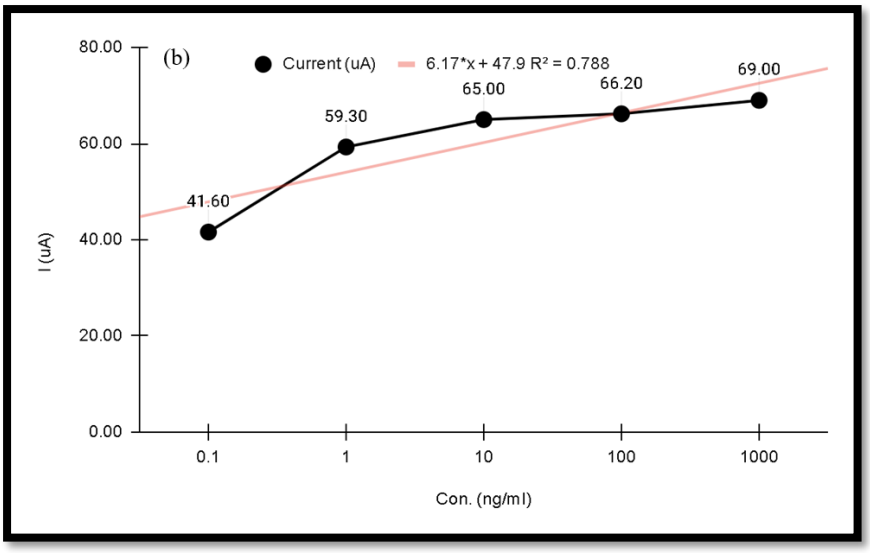

**Figure 1: (a) Before immobilization; (b) After immobilized**

## 3.2 Immobilization Stage

Chronoamperometry measurements were performed with the working potential set at 0.9 V for enhanced accuracy and sensitivity to examine the electrochemical behavior of a TRIS-buffered solution containing various concentrations of hCG antigens. Figure 2(a) illustrates the temporal dependence of cathodic current at different hCG antigen concentrations, which is redox reaction dependent. The absence of electrochemical processes and the absence of net current flow at the working electrode surface cause the initial current decline after applying voltage to the electrode. Figure 2(b) presents a linear graph with current responses (A) plotted against hCG antigen concentrations (ng/ml) along with a regression equation, $y = 6.17x + 47.9$, and a correlation coefficient of $R^2 = 0.788$. The electrode's surface redox reaction was activated with increasing hCG antigen amounts, leading to an increase in current. Therefore, the current is inversely proportional to the analyte concentration [11]. The currents change when the diffuse layers of the analyte on the working electrode change.




(a)




(b)

**Figure 2: (a) Time dependencies of the cathode current at fixed voltage of 0.9V for different concentrations of hCG (b) calibration curve for hCG antigens at varied concentrations.**

## 3.3 Immunoassay Stage

Electrochemical impedance spectroscopy (EIS) was utilized to perform a quantitative analysis of hCG antigens after describing each production stage. Analytes can be identified by measuring how much the charge transfer resistance (Rp) changes when an aptamer-antigen complex forms on the SPE. EIS was conducted on the SPE using immobilized aptamers dipped in various concentrations of hCG antigens (0.1-1000 ng/ml), as depicted in Figure 3. Figure 3 displays nearly perfect semicircular Nyquist plots, indicating that charge transfer on the electrode is not restricted by diffusion and that the diameter is significantly influenced by the concentration of hCG antigen [12].

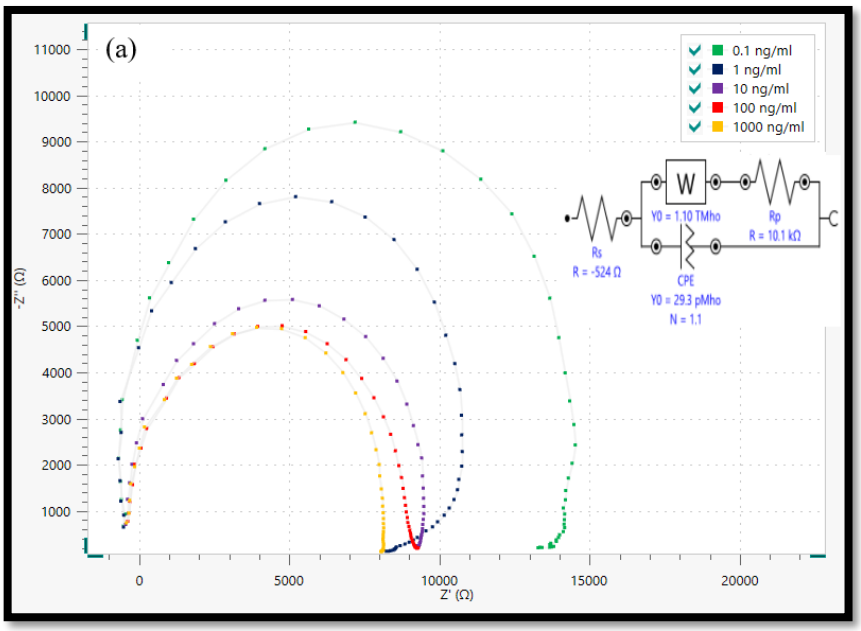



**Figure 3: Nyquist plots of hCG-aptamer SPE with different concentrations of hCG antigens (green) 0.1, (blue) 1, (purple) 10, (red) 100, (yellow) 1000, ng/ml**

### 3.4 Surface Morphology

High-resolution images of both the bare and immobilized SPE surfaces were compared using a scanning electron microscope (SEM). The surface of the bare SPE appeared rough before the deposition of five biological solutions, as shown in Figure 4(a). However, these four biological solutions successfully deposited on the electrode's surface, resulting in a tiny granular structure, as depicted in Figure 4(b). With the immobilization of an antibody, the impedance of the immunosensor increased, indicating that the antibody had adhered to the electrode interface [14].

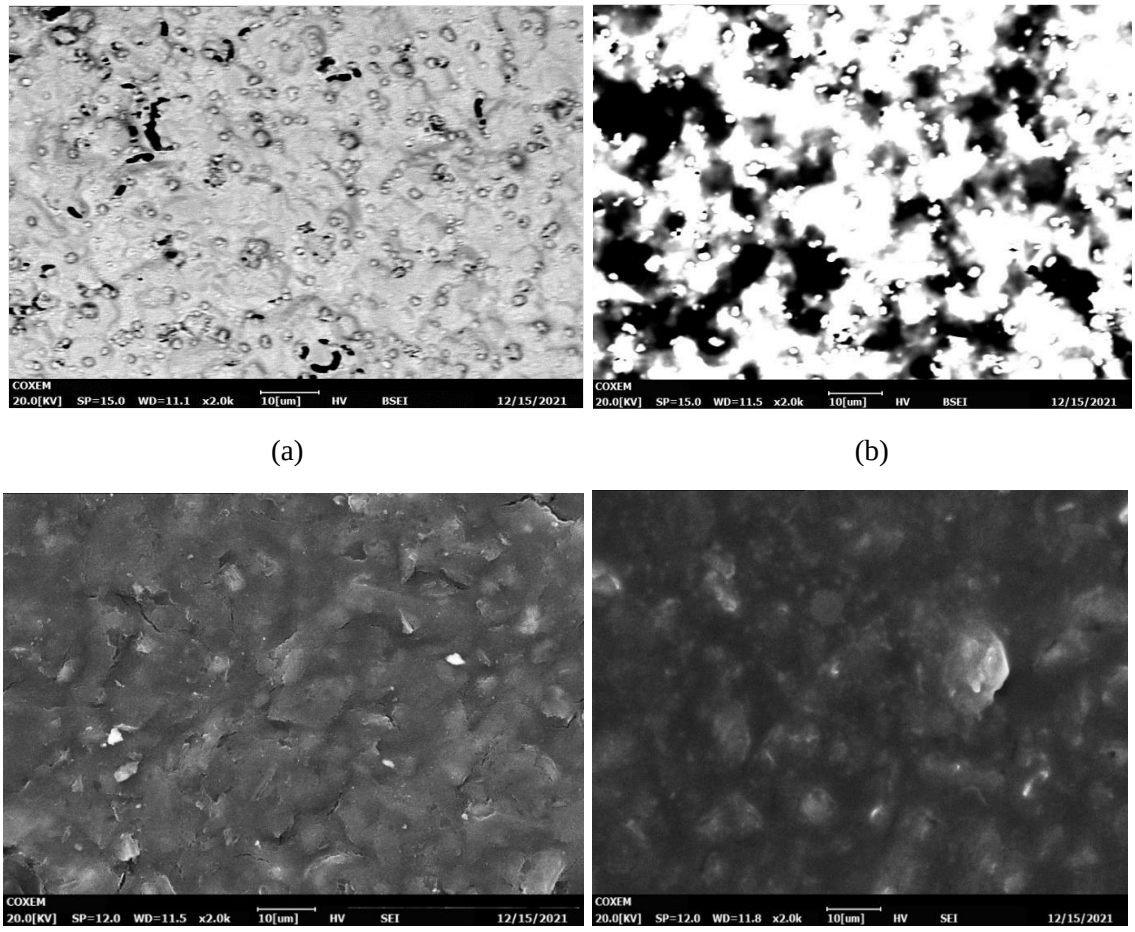



**Figure 4: (a) Gold SPE before immobilization; (b) Gold SPE after immobilization; (c) Carbon SPE before immobilization; (d) Carbon SPE after immobilization**

## 4. Conclusion

According to the experiment's findings, the reduction and oxidation peaks for the gold SPE employing iron (Fe) are at 0.048 V and 0.151 V, respectively. Calculating the difference between the oxidation peak potential and the reduction peak potential yields a peak potential difference of 0.103 V. The study demonstrates that one promising technique for rapid detection analysis is the electrochemical biosensor based on aptamers. Additionally, thermogravimetric analysis (TGA) can be used to analyze the presence of functional moieties on the carbon SPE surface as the temperature increases. Atomic Force Microscopy (AFM) can also be employed to examine and determine the surface texture and roughness at each production stage. Next, the process of hCG antigens hybridizing with aptamers must be confirmed to validate the biochemical response occurring on the surface.

To characterize the aptamer layers mounted on the surface with hCG antigen binding, the total internal reflection ellipsometry (TIRE) technique can be used [15]. Furthermore, due to their higher conductivity compared to carbon electrodes, gold screen-printed electrodes can be employed for detection analyses with increased sensitivity and specificity.

**Acknowledgement**

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**Appendix A (Optional)**

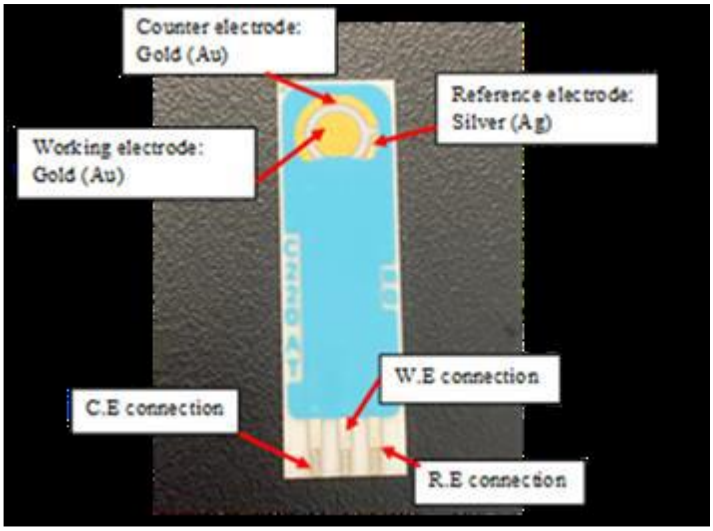


**Figure 5: Dropsens gold screen-printed electrode (SPE)**

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