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The Study of Sensing Elements Parameters Optimization for Developed Biosensor of SARS-CoV-2 Detection

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ABSTRACT

New advancements in developing sensitive and selective biosensors have demonstrated outstanding potential for Deoxyribonucleic Acid (DNA biosensors). The detection mode of DNA biosensors primary depends on a particular DNA hybridization that precisely occurs on the surface of the physical transducer that can only be detected using high-performance assays due to slight current changes. The analytical performance (sensitivity) of the DNA biosensor is conclusively rely on the confluence constructing of the sensing surface, which must be optimized. Thus, in this study, the sensing elements of the developed biosensors were optimized for detecting RNA of SARS-CoV-2. This optimization included concentration of nanomaterials (carbon quantum dots), probe density (concentration of DNA probe) and concentration of linker (APTES). It was observed that 0.15 % V/V of concentration CQD, 0.1µM of DNA probe and 36% V/V of APTES were the optimum parameters which provided their maximum response during electrical measurements and increased the sensitivity of the developed biosensor for SARS-CoV-2 detection

Keywords: DNA biosensor, DNA hybridization, parameters optimization, sensitivity, SARS-CoV-2

1. INTRODUCTION

Over the last decade, there has been a surge in interest in designing biosensors for nucleic acid detection, thanks to nucleic acids immense range of chemical and biological activities. This biosensor also holds much potential for acquiring the detection of specific sequences in a rapid, more accessible, and more affordable manner than the typical one. For nucleic acids-based biosensors, the bioreceptor used as biorecognition elements are oligonucleotides that can be DNA, RNA, or PNA [1]. Contrary to enzymes or antibodies, the nucleic acid biorecognition strands may be synthesized and regenerated for several uses [2]. The construction of duplex hybrid double-stranded nucleic acids from two complementary strands, known as the hybridization process, is a crucial component of nucleic acid biosensor technology [3]. These devices combine the nucleic acid-based identification layer's specificity with different transducer systems that report detection limits as low as sub-nanomolar. These devices can

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distinguish between target sequences that are one base mismatched and complementary target sequences[4].
The hybridization of the DNA biosensor can generally be detected indirectly using a redox label (label-based) and directly (label-free). There will be chemical changes in the redox label as recognized DNA duplex for the label-based DNA biosensor, increasing the current signal. As for the direct detection technique (label-free), the detection can be measured based on the change in conductivity or capacitance as a DNA duplex is formed [5]. Albeit label-based assay approaches attain the highest sensitivity, removing the labeling stages streamlines the output, allowing the device to be performed efficiently and simply. In the context of label-free procedures or direct detection methods, the transduction process converts the biological interaction, specifically the hybridization event occurring between the immobilized probe and its complementary target sequence, into a measurable response (current, capacitance, impedance), which corresponds to the amount of analyte in sample. Label-free approaches minimize analysis time and cost and are devoid of the labels' adverse effects, including instability and steric hindrance[6].

The conformation changes resulting from the interaction of complementary nucleic acid occur in a slight current range and can only be detected using high-performance devices. In order to obtain a high performances sensor, the sensing elements of a particular biosensor need to be optimized to enhance both sensitivity and specificity in the detection process. This can be achieved by employing nanomaterial, including carbon quantum dots (CQD) as the conducting elements to increase the device's conductivity. CQD is not only utilized to immobilize the high density of immobilization of DNA probe, but it may also be employed as a potent amplifier to boost hybridization signal transduction[7]. The optimization of the concentration of CQDs has been carried out as one of the optimization parameters for the development of biosensors and has been reported [8]. DNA biosensor performance relies heavily on how well the nucleic acid probe is immobilized onto the transducer surface. During the immobilization phase, the DNA probe should achieve a clear orientation that facilitates access to the target. The state of the immobilized probes on the solid surface may differ from that of the bulk solution depending on the immobilization method. Depending on the kind of physical transducer, different methods can be used to adhere the DNA probe to the surface. These include a carboxylated modified DNA probe for self-assembly onto an amine-terminated transducer (Al-IDE APTES silanization) by covalent linkage to the IDE surface via functional amide-based-monolayer. As in surface-based hybridization assays, conditions for interfacial sensing elements (probe concentration, APTES concentration) have to optimized. In this study, the optimization of sensing elements was carried out for detection of RNA of SARS-CoV-2 virus. The parameters involve in this study were the DNA probe concentration, concentration of nanomaterial (CQD) and the concentration of APTES. The major steps of functionalization for this biosensor were CQD modification, silanization using APTES, immobilization of DNA probe and hybridization of RNA target. The I-V characteristics were performed for every step of modification and functionalization as well as for the parameter optimization to obtain an optimum current for each of the optimization steps.

2. MATERIAL AND METHODS

2.1 Chemical, Reagents and Instruments

The analytical reagents grade used, such as sulphuric acid (H_2SO_4), hydrogen peroxide (H_2O_2), (3-Aminopropyl) triethoxysilane (APTES), and carbon quantum dots (CQDs), were purchased from Sigma Aldrich USA. DEPC treated water obtained from 1st base (Singapore) was used as diluting and washing reagent throughout this experiment. The 30-base synthetic oligonucleotides, including a modified 5-end DNA probe and RNA target, were designed as the method reported by [9] and were commercially synthesized from Integrated DNA Technologies (United States), as shown in **Table 1**. The commercial aluminum interdigitated electrode (IDE)

was obtained from Silterra (M) Sdn Bhd, Kedah. Lastly, the I-V characterization measurements were carried out using (Keithley 2450), Kickstart software, and a probe station, as shown in **Figure 1.**

Table 1: 30 mer oligonucleotide sequences for SARS-CoV-2 including carboxylate modified 5'end DNA probe and complementary RNA targets

Probes/Targets	Nucleotides sequences
DNA probe	5' COOH-C6-CTG AAG CGC TGG GGG CAA ATT GTG CAA TTT 3'
Complementary RNA (target)	5' AAA UUG CAC AAU UUG CCC CCA GCG CUU CAG 3'

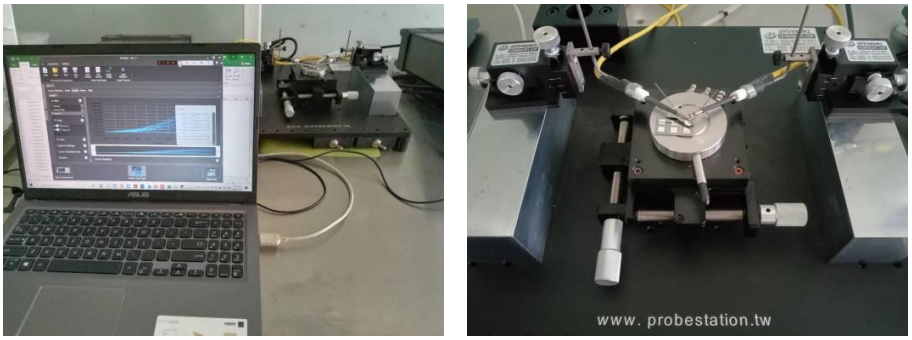

Figure 1: I-V measurement by Keithley 2450 equip with Kick start software and probe station with Al-IDE biosensor.

2.2 Surface Modification of IDE (Modification, Silanization, Immobilization and Hybridization)

Before modification, the active area of bare IDE was activated using piranha solution for 2 minutes, rinsed with DEPC water and left dry. This created a hydroxyl group that changed the surface from being hydrophobic to being hydrophilic. Next, the (2.0 µL, 0.2% v/v) of CQDs was adsorbed by drop-casting onto the IDE surface and left to incubate for 10 minutes. The concentration of CQDs were optimized using different concentration (0.0125%, 0.025%, 0.05%, 0.1% and 0.2%). After that, the CQD-modified IDE surface was functionalized with APTES. 2.0 µL of 24% v/v of APTES was dropped on the CQD-modified IDE and left for 10 minutes. The concentration of APTES was optimized using different concentration (1%,12%,24%,36%,48%, 60%, 72%, 84%, 96%). The silanization reaction occurs through the hydrolysis and condensation reaction of APTES with the surface hydroxyl group, resulting in a covalent Si-O-Si bond and amine-terminated surface. Next, the immobilization of the bioreceptor was carried out using 2.0µL, 1µM of DNA probe and left incubated for 20 minutes until dried. The optimization of the immobilization step was completed by varying the concentration of the DNA probe (1 pM-100 µM). Next, for the hybridization of the target, about 2.0µL of 1.0µM of RNA target was dropped and left incubated for 20 minutes until it dried. The sensitivity value of developed biosensor was calculated using calibration graph by varying the concentration of RNA target (1 aM-1 pM). The I-V measuring technique was employed to quantify changes in the nanoampere range resulting from several processes, including modification, silanization, immobilization, and hybridization. **Figure 2** shows the mechanism process of sensing surface IDE.

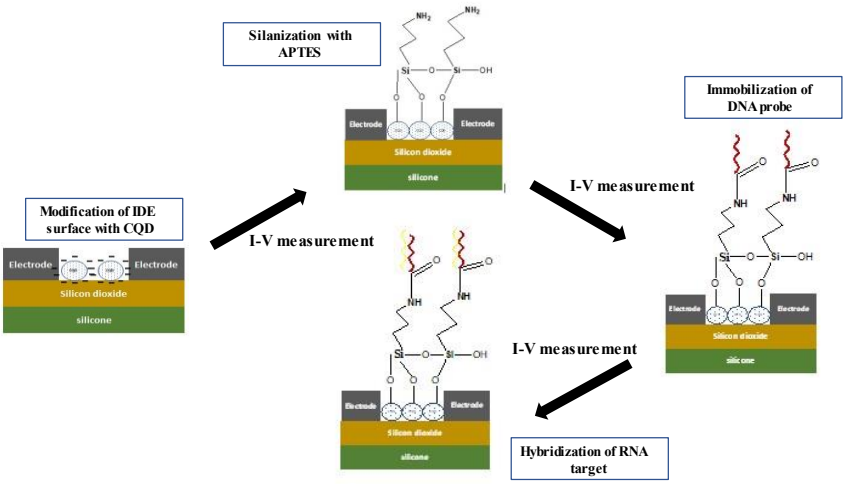

Figure 2: Mechanism of biosensor modification

3. RESULTS AND DISCUSSION

3.1 I-V Measurement of Surface Modification

Figure 3 depicts the I-V measurement for bare and each modification of AI IDE to detect SARS-CoV-2 RNA before optimization. The bare AI IDE current at 1V shows 2.15 x 10^{-11} A. After modification with CQD, the current keeps increasing to 1.03 x10^{-10} A because the conductivity of the transducer was enhanced as the electron density increased [10]. The application of silanization using APTES has resulted in a notable enhancement in the current, which has now reached a value of 4.01x10^{-9}A. APTES possesses a significant number of amine groups that are positively charged, thereby serving as a linker for the immobilization of the DNA probe. This immobilization process leads to an augmentation in the current. Following the immobilization of the DNA probe, a consistent rise in current was observed, reaching a value of 7.03x 10^{-8} A. The rise of current after the immobilization of DNA was due to the negative charge in the phosphate backbone of nucleic acid[11]. After hybridization with RNA target, the current keeps increasing to 2.70 x10^{-7}A. The current signal's amplified after hybridization is due to the formation of duplex DNA-RNA that raises the density of the negative charge (phosphate backbone), which subsequently increases the current signal [12].

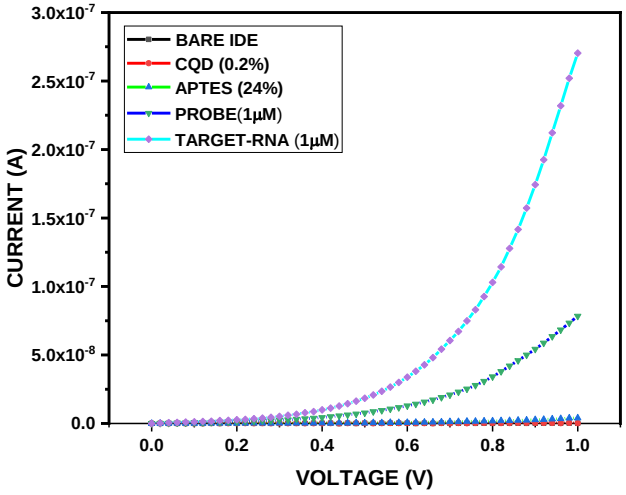

Figure 3: Graph of current vs. voltage for each step of modification of Al-IDE (modification, silanization, immobilization, hybridization)

3.2 Parameters Optimization of Sensing Elements

3.2.1 Effect Concentration of CQD Towards Current

The concentration of CQD is a vital control factor as far as the electrode conditions are concerned. The use of CQD as an electrode modifier can significantly increase the sensitivity of analyte determination. The effect of CQD concentration on the IDE current is shown in **Figure 4**. The result clearly shows that the current of IDE increased with the increased concentration of CQDs from 0.0125%v/v to 0.15%v/v. This is due to the increase in the negative charge (oxygen-containing group), which results in the creation of an electrical double layer on the surface of the IDE. This double layer has the potential to reduce resistance and thus enhance the conductivity of the IDE.[13], [14]. The current intensity reached a plateau at 0.2%v/v CQD, which can be attributed to the saturation of the CQD on the electrode. Therefore, 0.15% v/v was selected as an optimal concentration of CQD for further experiments.

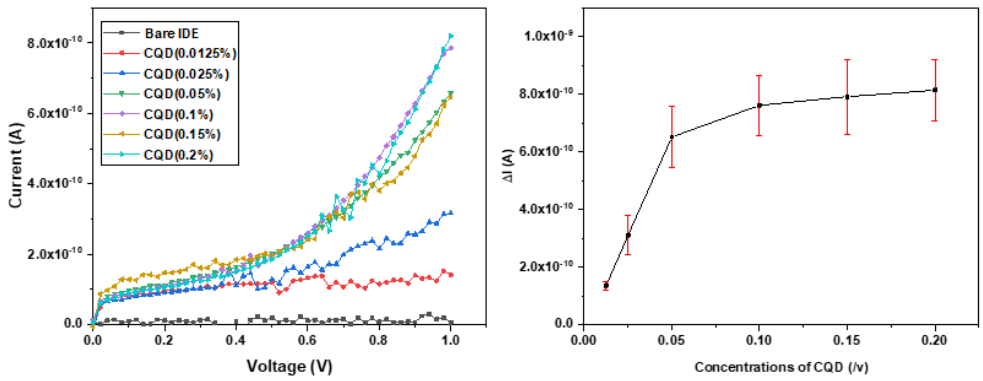

Figure 4: Graph current vs. voltage for different concentrations of CQD towards the IDE current.

3.2.2 Effect Concentration of APTES Towards Current

The amount of APTES is a critical factor in achieving the optimal adhesion of the bioreceptor to the substrate. The chemical composition of APTES comprises an amine functional group (-NH_2) and hydrolyzable groups (-$OCH_2CH_3)_3$, enabling its attachment to the surface of modified IDE. During the process of hydrolysis, the hydrolyzable groups (-OCH2CH3)3 in the structure of APTES were transformed into hydroxyl (-OH) groups. Following the condensation procedure, the surface of the modified IDE was subjected to the attachment of APTES through the utilization of silanol bridging mode. During this particular stage, the NH_2 groups of APTES remained accessible in the tails for the purpose of immobilizing the DNA probe. In contrast, the hydrolyzable groups were covalently attached to the hydroxyl (-OH) groups present on the modified interdigitated electrode (IDE) surface[15]. **Figure 5** shows the current formation when the various APTES concentrations were dropped onto the modified IDE surface. As the percentage of APTES increases from 1% v/v to 36% v/v, the current increases, yet, upon adding a higher concentration of APTES (48-98%v/v), the current obtained decreases. This is due to the lack of the hydrolyzable groups in the APTES at the higher percentage of APTES, therefore preventing the binding of APTES on the modified IDE surface. The more concentrated APTES will lower the current production due to the formation of the linker on the modified IDE, causing current passivation and impeding the electron movement. The reduction in the percentage of APTES results in an increased surface area conducive to electron transfer, as well as a higher concentration of amino groups on the modified IDE surface. These characteristics are indicative of a favorable biosensor platform [16]. Thus, 36%v/v of APTES was chosen as the optimum parameter and was used for subsequent experiments.

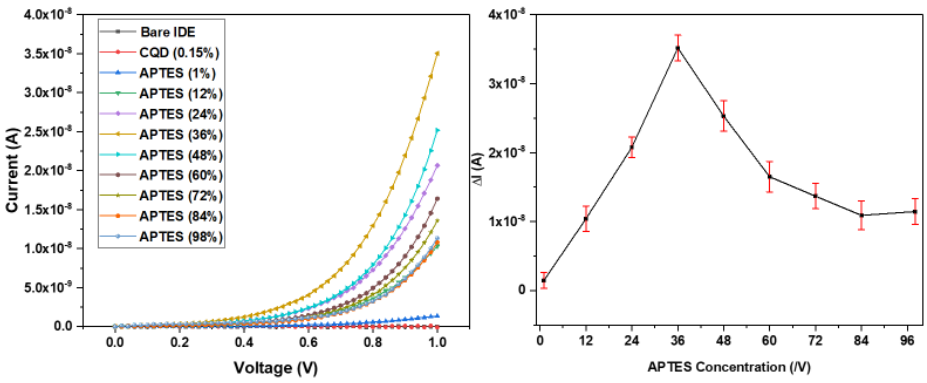

Figure 5: Graph current vs. voltage for different concentrations of APTES towards the IDE current

3.2.3 Effect Concentration of DNA Probe Towards Current

Figure 6 demonstrates that DNA probe concentration affects modified IDE current response after 20 minutes of incubation. The current response increases as the concentration of DNA increases from 10 pM to 1µM due to the high-density DNA probe loading on the surface of the modified silanize IDE. Nevertheless, as the concentration of the DNA probe ranges from 10 µM to 100 µM, there is a drop in current production. This can be attributed to the increased electrostatic repulsion and crowding of the immobilized DNA probe, resulting in a reduction of the current. Therefore, 1 µM concentration of DNA was selected as an optimum parameter that was used to optimize the other parameter for other experiments.

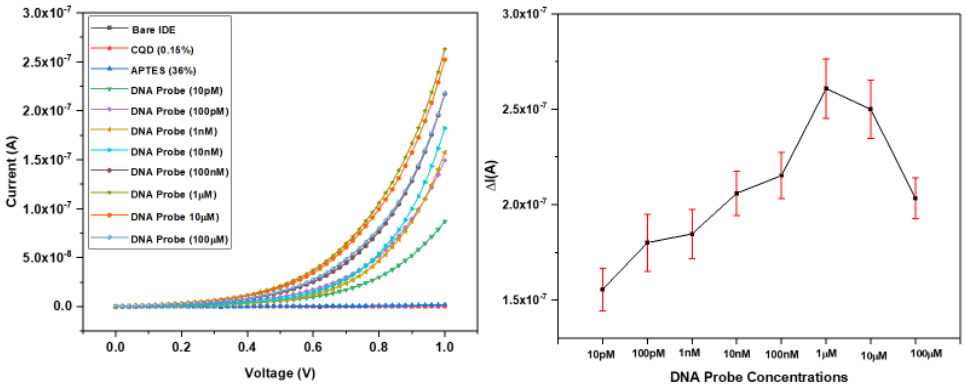

Figure 6: Graph current vs. voltage for different concentrations of DNA probe towards the IDE current

3.3 Detection of RNA Target for Various Concentration

The constructed DNA biosensor's analytical capability was investigated utilizing different amount of target RNA under optimal and non-optimal experimental circumstances. As shown in **Figure 7a**, the current output increased gradually with increasing concentration of the target RNA (1 aM to 1 pM) for both optimal and non-optimal conditions, respectively. However, the current response of RNA target at optimal conditions was increased significantly compared to the at non-optimal conditions due to the greatest hybridization of RNA target and DNA probe after parameter optimizations. **Figure 7b** depicts the sensitivity value achieve for the both conditions. The developed DNA biosensor after parameter optimization has the highest sensitivity with a value of 1.488 x10^{-3} A pM^{-1} cm^{-2}, compared to the before optimization which was 1.584 x 10^{-4} A pM^{-1} cm^{-2}.

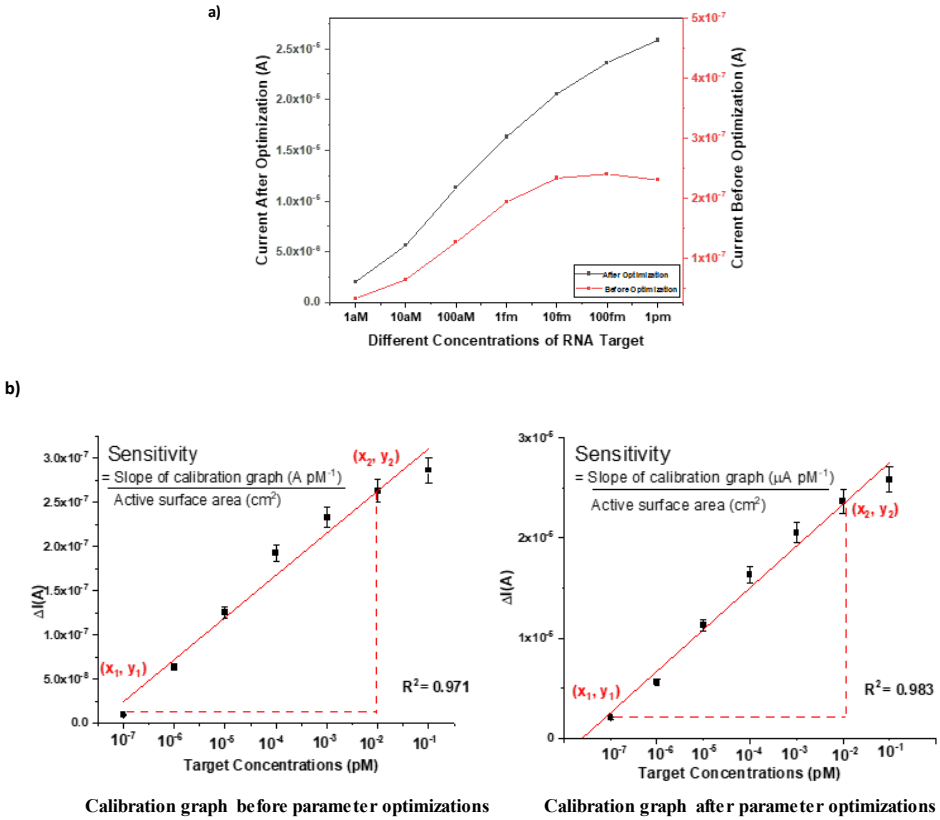

Figure 7: Calibration Graph before and after parameter optimization

4. CONCLUSION

In conclusion, this paper described the biosensor development for SARS-CoV-2 RNA and its parameter optimization of the sensing elements. I-V measurements were performed for each modification and parameter optimization, respectively. The optimum condition for the parameter of sensing elements were 0.15% for the concentration of CQD, 36% V/V for the concentration of APTES, and 1.0 µM for the concentration of DNA probe. These optimum conditions of sensing elements have improved the biosensor's sensitivity which was 1.488 x 10^{-3} A pM^{-1} cm^{-2} for detecting the virus without involving the labelling steps that subsequently make this label-free assay very convenient to utilize for various applications.

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