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Enhancing Electrochemical Signal based on One-Step Electrodeposition Ofpani/Ppy/Agnp/Gr Nanocomposite on Screen-Printed Gold Electrodefor Dna Diagnosis Detection

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ABSTRACT

This paper presents the development DNA sensors based on screen-printed gold electrode *(*SPGE*)* for specific and rapid detection of synthetic DNA diagnostic**.** For the objective of this work, one-stepelectrodeposition of polyaniline/polypyrrole/silver nanoparticle/graphene *(*PANI/PPY/AgNP/GR*)* was utilized as a new sensing material for enhancing electrochemical signal**.** The disposable SPGE and cost-effective DNA affinity biosensor based on the single-strand DNA probe tagged with anthraquinone *(*AQ*)* as redox indicator and detected the current signal with differential pulse voltammetry *(*DPV*)***.** Briefly, a pretreated SPGE was modified with PANI/PPY/AgNP/GR nanocomposite and immobilized by cross-linking with DNA-AQ probe**.** The methodology and its efficiency have been proved using cyclic voltammetry *(*CV*)***.** For hybridization between ssDNA-AQ probe and the synthetic target DNA on the SPGE surface was detected by monitoring the current signal from the electron transfer of AQ**.** Furthermore, SEM observations were performed to observe the surface changes of SPGE after electrochemical deposition**.** These DNA biosensor showed a good linear target DNA concentration range from 1.0×10^{-15} to 1.0×10^{-11} M with limit of detection at femtomolar**.** In addition, the disposable DNA sensor could also be regenerated easily and can be reused 45 times for hybridization studied**.** The developed DNA sensor was found rapid, cost-effective, good reusability for hybridization detection of the synthetic DNAdiagnostic and can be used as an excellent tool for future prospective clinical applications.

Keywords
DNA sensor, screen-printed gold electrode, electrodeposition, redox indicator

INTRODUCTION

Due to their rapid response, easy fabrication, high sensitivity and specificity, and reliability, electrochemical methods have attracted great attention for the detection of DNA sequences **[**1, 2, 3**].** The use of electrochemical DNA sensorshave been recently gained popularity and much important for detection of DNA sequence responsible for clinical, forensic, food industry, environment and agriculture **[**4**].** Moreover, nanomaterials have also accelerated the performance of electrochemical application by improving bio-compatibility, enhancing electron transfer that enhanced signal can be achieved **[**5, 6**].** Therefore, nanoparticle for DNA electrochemical biosensor applicationshave shown potential applications in genetic testing with high performances **[**7, 8, 9**].**

In recent years, miniaturized electrochemical DNA sensors are possible using commercial available screen-printed electrodes **(**SPE**)** modified through electrodeposition, electropolymerizationor functionalized with self-assembled monolayers **(**SAM**)** to create an organized and oriented layer for different applications **[**10, 11**].** SPE has been widely used in a number of biological systems and challenged the conventional three-electrode system due to its various advantages including ease of operation, simple fabrication, low power consumption, low cost, small size, disposability, easy mass-produced and scalable sensor for electrochemical detection, leading to its development in electrochemical DNA biosensor **[**12**].** The main advantage associated with the miniaturization of the electrochemical DNA sensors is the reduction of sample volume required, as low as a few microliters**.** Several materials were

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modified electrode surface to improve the performance of sensor**.**The incorporation of carbon-based nanomaterials, such as carbon nanotubes and graphene, as a modifier for the working electrode in the field of electroanalytical sensing has increased considerably nowadays due to their unique properties **[**13**].** Moreover, both label and label-free electrochemical DNA biosensor using SPE modified with nanomaterials was fabricated for detection DNA oligomers related to *Escherichia coli*DNA and others **[**14, 15, 16**].** For enhancing the electrochemical signal, a screen-printed gold electrode **(**SPGE**)** is alternative electrode for the fabrication of electrochemical DNA biosensor**.** The surface of SPGE can be easily modified that related to many analytes **[**17**].** This versatility, its miniaturized size, and the possibility of connecting it to portable instrumentation make its possible highly specific on-site determination of target DNA**.** Moreover, SPGE avoid the common problems likes a classical solid gold electrode, such as memory effects and tedious cleaning electrode surface**.** In addition to the evaluation of potential measurement and signal amplification schemes, the importance of the underlying electrode has also been investigated through the use of nanostructured gold electrodes**.** As a consequence, the idea in this research is to develop electrochemical DNA nanosensor based on the fabrication of the nanocomposite onto SPGE using one-step electrochemical deposition for detection of DNA**.**

In this present study, the development of a disposable, sensitive, cost-effective, fast response,and accurate electrochemical DNA biosensor based on a SPGE modified with nanocomposite of polyaniline/polypyrrole/silver nanoparticle/graphene **(**PANI/PPY/AgNP/GR**)** was prepared for increasing the current signal from the electron transfer of DNA hybridization**.** DNA probe tagged with anthraquinone **(**DNA-AQ**)** as an electrochemical indicator was immobilized on the modified SPGE for hybridization with target DNA**.** The signal generated is measured using differential pulse voltammetry **(**DPV**).** This nanocomposite modified SPGE exhibited a low detection limit and wide linear range as anelectrochemical DNA sensor and can be applied to detect hybridization event**.** Therefore, in future this method to be successfully used in the clinical laboratories**.**

MATERIALS AND METHODS

Materials

The sequences for the 11 bases of DNA probe tagged anthraquinone is 5′-AQ-GCT CCG TCC TC-NH_2-3′ that purified by reverse phase HPLC and its identity was verified by MALDI-TOF mass spectrometry**.** Both synthetic complementary and mismatched target DNA were synthesized and purified by Bioservice Unit, National Science and Technology Development Agency and Bio Design Co**.**, Ltd**.**, Thailand; their sequences are listed in **Table 1.** The reagents used were analytical grade**:** 11-mercapto-1-undecanol **(**11-MUL**)**, glutaraldehyde and silver nitrate were purchased from Sigma-Aldrich **(**Steinheim, Germany**).** Graphene nanosheets **(**surface area 500-800 m^2g^{-1}, particle diameter 3 μm**)** were obtained from Cheap Tubes Inc **(** Brattleboro, USA**).** Aniline and pyrrole solution were purchased from Merck **(**Germany**)** and purified before using**.** Milli-Q water **(**18 MΩ cm at 25^oC**)** was used to prepare all buffer solutions**.**

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Table 1 All synthetic DNA sequences used in this study.

Name	Sequences
Complementary DNA	5'-GAG GAC GGA GC-3'
Single-base mismatched DNA	5'-GAG GA**A** GGA GC-3'
	5'-GAG GA**T** GGA GC-3'
	5'-GAG GA**G** GGA GC-3'
Double-base mismatched DNA	5'-GAG GA**AA**GA GC-3'
	5'-GAG GA**TT**GA GC-3'

Underlined bases in target DNA sequences denote mismatches to DNA-AQ probe

For the apparatus, a Metrohm 910 PSTAT mini controlled by PSTAT software version 1.1was used to record all the electrochemical measurement**.** SPGEs were purchased from Metrohm Siam Co**.**, Ltd**.**, Thailand with dimensions of 34 × 10 × 1 mm **(**L × W × D**).** Each stripcontains three electrodes screen-printed on the same planar ceramic platform, consisting of a 4 mm diameter gold working electrode, Pt counter electrode and silver-based reference electrode**.**

Pre-treatment of SPGE

The SPGE should be pre-treatment before using with electrochemical method for increasing the electrical properties**.** The electrochemical pre-treatment of SPGE was carried out by applying potential in a potential range of -0**.**6 to 0**.**6 V with the scan rate 50 mVs^{-1}, 600 s in 0**.**05 mol L^{-1} sulfuric solution**.** After conditioning, the SPGE strip was rinsed with de-ionized water and placed in an electrochemical cell for voltammetric measurement, the electrode response remained stable with no baseline noise and low background currents**.**

One-step electrodeposition process of PANI/PPY/AgNP/GR nanocomposite modified SPGE

For a PANI/PPY/AgNP/GR nanocomposite modified gold surface of SPGE, 0**.**10 M aniline and 0**.**10 M pyrrole aqueous solution with a 2**.**0 mg mL^{-1} graphene and 0**.**20 M $AgNO_3$ were added into the electrodepositing solution **(**0**.**50 M H_2SO_4**)**, mixed with 0**.**25 M polyacrylic acid for getting a better stability with improved polymer properties**.** The electrodeposition was performed by cyclic voltammetry for 10 scans using the potential range from -0**.**4 to 1**.**0 with a scan rate of 50 mVs^{-1}**.**

Scanning Electron Microscopy and EDX of modified SPGE

SEM micrograph and EDX were obtained with JSM 5800 Quanta from JEOL, Japan, scanning electron microscope equipped with a link analytical system**.** The electron energy used was 20 keV**.** In this study, the glove box filled with Ar gas **(**99**.**99**%)** was coupled to the microscope for analysis at the potential value applied**.**

Immobilization of DNA-AQ probe

The PANI/PPY/AgNP/GR nanocomposite coated SPGE was cleaned by rinsing with distilled water and treated with glutaraldehyde **(**5**.**0 **%**v/v**)** in 10 mM phosphate buffer pH 7**.**00 at room temperature for 20 min to activate the aldehyde groups**.** Then 20 μL of 5**.**0 μM of DNA-AQ probe **(** prepared in phosphate buffer**)** was placed over the working electrodeand incubating

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overnight at 4°C in a humidified chamber. Finally, 1.0 mM of 11-mercapto-1-undecanol (11-MUL) solutions was dropped on the immobilized SPGE for 1 h to block any remaining pinholes, hence preventing any non-specific binding on the electrode surface.

Detection of the complementary and mismatched target DNA

Hybridization with target DNA was performed by dropping 20 µL of DNA solutions onto the modified SPGEs and incubated 30 min in humidity box at room temperature. Then, the non-hybridized DNAs were removed by washing with distilled water.

Differential pulse voltammetry measurement

The DNA hybridization measurement was studied using three electrode system of the SPGE, connected to 910 PSTAT mini controlled by PSTAT software version 1.1. The hybridization response (current) was the decrease of the oxidation peak of the electrochemical indicator AQ (tagged to the DNA probe) detected using differential pulse voltammetry (DPV). The DPV was operated from -0.5 to 1.0 V, with a scan rate of 50 mVs^{-1}, a step width of 100 ms, a step potential of 5.0 mV, the pulse width and pulse amplitude were 60 mV. The DPV was performed in a 20 mL batch plastic cell containing 100 mM sodium phosphate buffer pH 7.00 with potassium chloride.

RESULTS AND DISCUSSION

Pre-treatment of the SPCE

Initially, SPGE was subjected to a cleaning conditioning in order to remove the organic constituents of the metallic in ink, as well as some possible contaminants. Thus,the SPGE are often preconditioned by applying anodic potential in sulfuric solution for enhancing the electrochemical activities. Under an appropriate electrochemical pre-treatment condition for the SPGE, cyclic voltammetry (CV) test was carried outby using 5 mM potassium ferricyanide, $K_3Fe(CN)_6$ in 0.1 M of potassium chloride (KCl) at the potentials of -0.6 V to 0.5 V, and 0.05 V/s scan rate . According to**Figure 1**, after pre-treatment the SPGE exhibited discernable redox peaks for redox system. That means, this conditions can be improved the electrochemical activity of SPGE and the activation procedure in sulfuric solutionresulted in the good electrochemical characteristic.

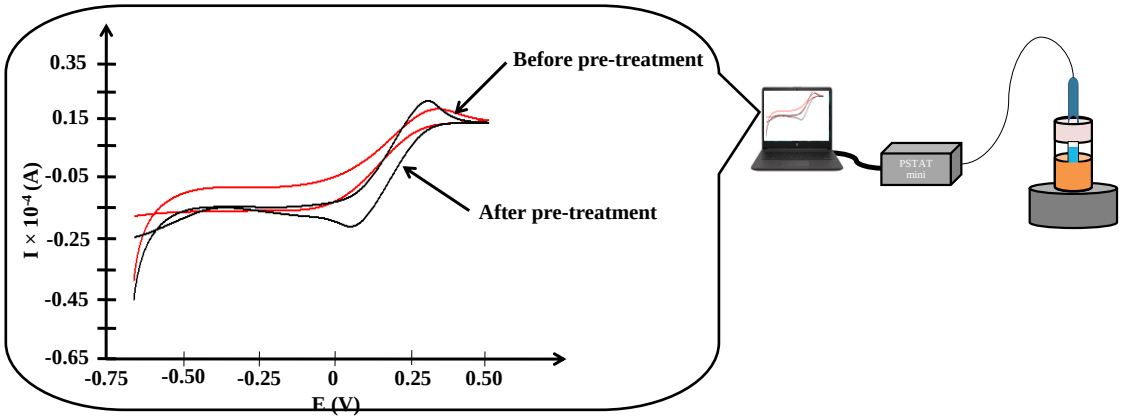

Figure 1 Cyclic voltammograms of SPGE with before and after electrochemical pre-treatment.

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Electrochemical characterization of the immobilization process

An electrochemical behavior of the modified SPGE surface studied by cyclic voltammetry using 5.0 mM $K_3Fe(CN)_6$ in 0.1 M KCl between -0.75 to 1.00 V at a scan rate of 0.1 Vs^{-1} as shown in **Figure 2.** The pre-treatment SPGE showed a voltammogram with oxidation and reduction peaks **(Figure 2(**a**)).** Both peaks were increased when PANI/PPY/AgNP/GR nanocomposite was deposited onto the gold surface **(Figure 2(**b**))** indicated that the PANI/PPY/AgNP/GR helped to increase the electrical conductivity**.** When glutaraldehyde **(**5.0 **%**v/v**)** in 10 mM sodium phosphate buffer pH 7.00 was used to activate the covalent bonding between the amine group of the DNA-AQ probe and the free amine group of PANI and PPY at room temperature for 20 min, the redox peaks of the electrode was decreased **(Figure 2(c)).** The response was further reduced when DNA-AQ probes were immobilized **(Figure 2(d)).** The modified gold surface was then react with ethanolamine pH 8.50 to occupy all the remained aldehyde groups of glutaraldehyde that were not bound to the probes**.** Finally, PANI/PPY/AgNP/GRnanocomposite modified SPGE was rinsed with 100 mM phosphate buffer pH 7.00 and then immersed in 1.0 mM of 11-MUL blocking solution for 60 min to cover any pinholes on the electrode surface**.** The cyclic voltammograms showed complete blockage of the redox species **(Figure 2(e)).**

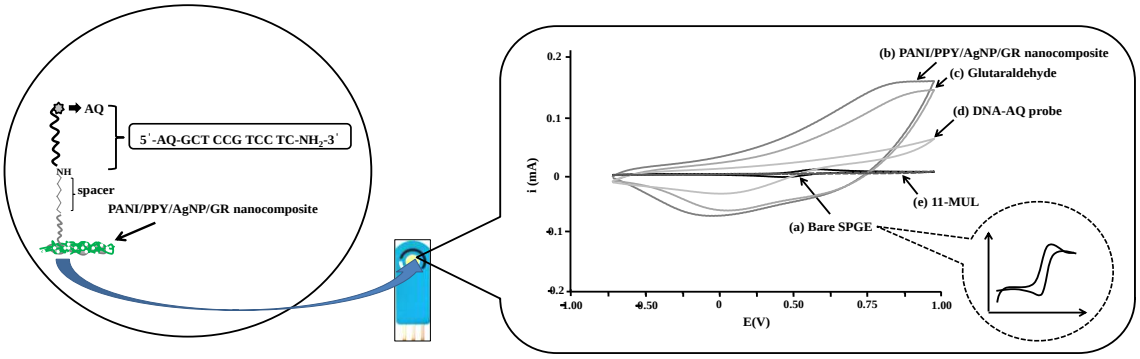

Figure 2Cyclic voltammograms behavior of the modified SPGE surface.

Surface Morphology with SEM and EDX

The PANI/PPY/AgNP/GRnanocomposite modified on SPGE was characterized using SEM and EDX**.** According to **Figure 3**, the morphology of PANI/PPY/AgNP/GR nanocomposite, graphene sheets were seen embedded within the PANI and PPY nanofiber with the silver nanoparticles decorated on the PANI and PPY nanofibers**.** The fibrous network structure of PANI film having a diameter of 40 - 70 nm whereas PPY film have a diameter of 50 - 80 nm that measured from an SEM image using electronic digital calliper**.** Silver nanoparticles were decorated on the surface of both PANI and PPY nanofibers with the particle size 50 - 90 nm**.**

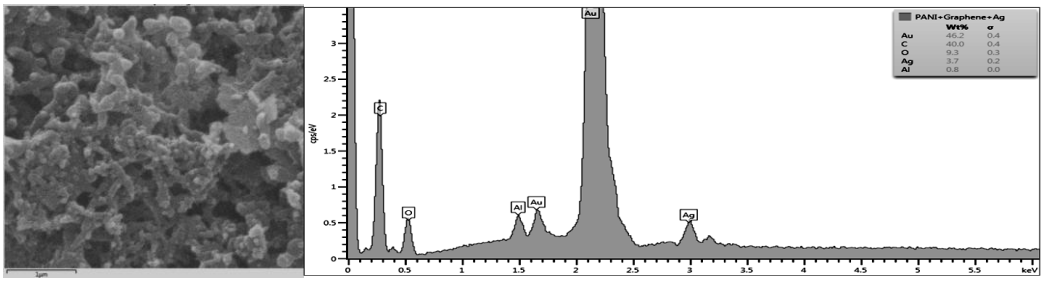

Figure 3 SEM image of PANI/PPY/AgNP/GRnanocomposite modified SPGE and EDX spectrum of silver nanoparticle on the surface layer of the modified SPGE at 20 keV.

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From EDX spectrum, which revealed the peak of silver **(**Ag**)** and confirmed the presence of silver element decorated on PANI and PPY nanofibers**.** Although to a lesser extent than expected, because the EDX spectra are highly influenced by the material underlying the first layers of oxidized material**.**

Reusability

The reusability of the modified SPGE was studied by analyzing the same concentration of complementary target DNA **(**1**.**0× 10^{-12} M**).** After hybridization, the regeneration step **(**dropped with 20 μL of 50 mM sodium hydroxide and 30 min of the incubation time**)** was included in the analysis cycle**.** The residual activity **(%)** of immobilized DNA-AQ probe to its target after regeneration was calculated and plotted against the number of hybridization**.** According to the result in **Figure4** found that, 45 times of the hybridization between DNA-AQ probe and target DNA, the average residual activity was 95 ± 3**% (**RSD = 3**.**2**%)** that revealing the biosensors good reusability**.** After 46 regeneration cycles, the average residual activity reduced to 90**%.** The gold electrode surface of the SPGE was then tested by cyclic voltammetry**.** A flat voltammogram similar to one obtained after electrode preparation was observed**.** This confirmed that the film on the electrode surface was not destroyed by the regeneration solution**.** The results indicated that the decrease of residual activity after being used several times**.**

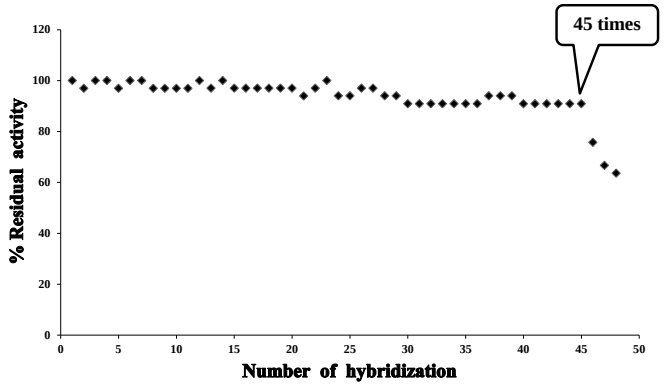

Figure 4The relationship between percentages of the residual activity and number of hybridization.

Specificity of DNA sensor

For evaluation of the specificity was examined using different synthetic DNA targets including complementary, single-base and double-base mismatched DNA of the same concentration at 1**.**0× 10^{-12} M**.** From the result, the hybridization between the complementary DNA and DNA-AQ probe provided the largest electrical signal difference compared with mismatched DNA as shown in **Figure 5.** These result indicated that the developed biosensor was able to discriminatecomplementary targets and mismatched strands**.**

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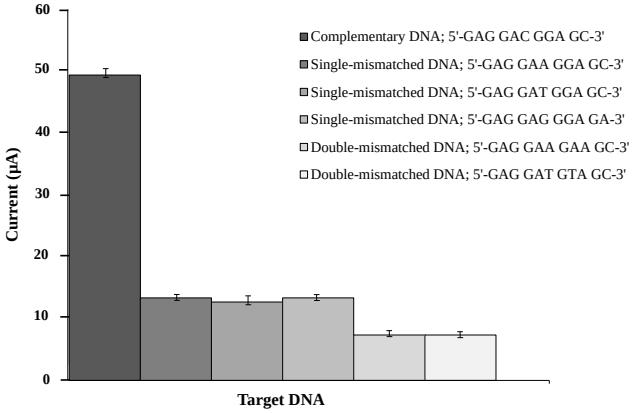

Figure 5Thedifferential electrochemical signal from the proposed DNA sensor with the complementary target DNA, single-base mismatched DNA and double-mismatched DNA.

DNA Hybridization between DNA-AQ probe and synthetic complementary DNA

Under the hybridization, the current response from the electron transfer of AQ was studied for DNA detection**.** Oxidation peak current from electron transfer of AQ to the electrode surface was measured using DPV**.** The batch system conditions were; complementary target DNA volume 20 µL in PBS pH 7.00 with 30 min for hybridization event**.** The regeneration solution for DNA dissociation can be done using 50 mM NaOH for 30 min**.** Under optimized conditions, this sensor showed a good linear response from1.0 × 10^{-15} to 1.0 × 10^{-11} M with limit of detection at femtomolaras shown by**Figure 6.**

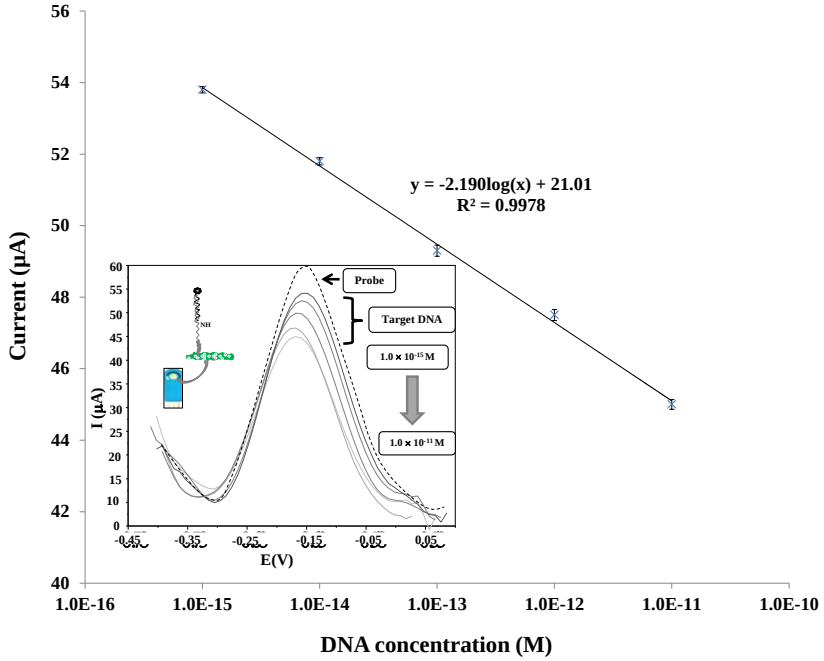

Figure 6The linear response range for hybridization reactionbetween DNA-AQ probe with various target DNAconcentrations, inset showsthe DPV anodic peak current of the DNA biosensor.

In the absence of the synthetic target DNA, single-stranded DNA-AQ probe can be closed to the electrode surface, resulting in the electron transfer between the AQ and the electrode can easily occur, that provided a high current**.** After hybridization, the hybrids between the probes and the target DNAs made the probe structure more rigid**.** Therefore, AQ at the end of the DNA probe moved further away from the electrode which can reduce the response**.** That means the high concentration of target will be reduce more of the signal, suggesting that the changes in DNA concentration and the voltammograms are related**.**

CONCLUSIONS

In conclusion, the strategy of developing a DNA biosensor employing PANI/PPY/AgNP/GRnanocomposite modified SPGE with easy and fast of the one-step electrodeposition process and a hybridization detection label with AQ has been successful in creating a DNA biosensor of improved analytical performance**.** The success was attributed to the immobilization of DNA-AQ probe on the modified SPGE, good electrical conductivity provided by usingPANI/PPY/AgNP/GRnanocompositeand less unspecific response from AQ as a hybridization electrochemical indicator**.**This sensor provided the good discriminatory confirmation of binding events**.** Moreover, PANI/PPY/AgNP/GRnanocomposite can be a potential matrix material for the fabrication of highly sensitive DNA biosensors**.** Thus, the developed DNA sensor with electrochemical detection methods employed in this work can be extended further to develop DNA-based biosensors for the detection of other applications in medical,agriculture and food industries**.**

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