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# A disposable and cost-effective electrochemical DNA sensor using nanocomposite modified screen-printed gold electrode

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**Abstract.** This research involved the preparation of an electrochemical biosensor using a disposable screen-printed gold electrode (SPGE) for the DNA hybridization detection. An electrochemical DNA biosensor was successfully fabricated based on DNA probe tagged with methylene blue (MB) as redox hybridization indicator, was immobilized on the nanocomposite modified electrode. The modified SPGE was characterized by using cyclic voltammetry (CV) and scanning electron microscopy (SEM) with energy dispersion x-ray spectroscopy (EDS). The current signal of target DNA hybridization was monitored using differential pulse voltammetry (DPV). These DNA biosensor showed a good current response with the complementary target DNA concentration range from  $1.0 \times 10^{-11}$  to  $1.0 \times 10^{-7}$  M. This fabricated genosensor could also be regenerated easily and can be reused 36 times for hybridization studied.

## 1. Introduction

Affinity DNA biosensor has been conjugated between the biological material of DNA or PNA probe and transducers. For DNA electrochemical biosensor has widely reported in many fields due to its high sensitivity, specificity, easy to use, low cost, compatible with microfabrication and direct convert to the hybridization events into the electrical signal. In this technique, the stability of the immobilized single-stranded probe on the electrode surface as well as its accessibility toward the target DNA played an important role in the performance of the DNA sensor [1,2]. Moreover, nanomaterials have also accelerated the performance of electrochemical application by improving bio-compatibility, enhancing electron transfer that enhanced signal can be achieved [3]. Therefore, the advantages of nanomaterial modified electrode surface can be enhanced the signal due to high surface area and strong adsorption ability.

In recent years, screen-printed gold electrode (SPGE) has been widely used and challenged the conventional three-electrode system which consists of a reference electrode, a counter electrode and a working electrode. The advantages of SPGE including ease of operation, simple fabrication, low cost, small size, disposability, reusability and easy mass-produced, leading to its development in electrochemical DNA biosensor. 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 [4]. The surface of SPGE can be easily modified that related to many analytes. 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. Furthermore, SPGE avoid the common problems

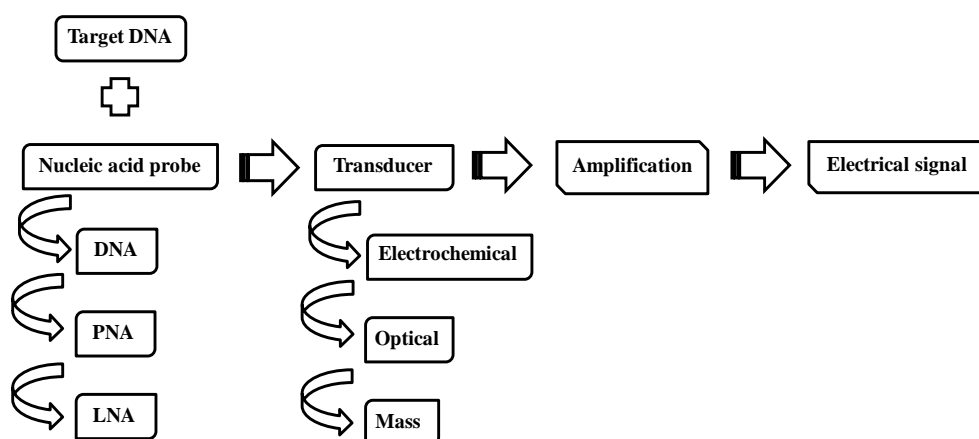


likes a classical solid gold electrode, such as memory effects and tedious cleaning electrode surface. The development of SPGE and their applications for electrochemical DNA hybridization detection have attracted and extensive attention such as detection of acrylamide in potato fries [5], swine flu (H1N1) infection in human [6] and heavy metals detection [7].

In this paper, we describe the development of a sensitive, cost-effective, fast response, and accurate electrochemical DNA biosensor using a SPGE modified with polyaniline-graphene-silver (PANI-Grap-Ag) nanocomposite for the detection of DNA(MB)-DNA hybridization. DNA probe tagged with methylene blue (MB) as an electrochemical indicator was immobilized on the modified SPGE for hybridization with the complementary target DNA. The signal generated is measured using differential pulse voltammetry (DPV). That means these fabricated DNA sensor was interrogated using electrochemical transducer and scanning electron microscopy (SEM) with energy dispersion X-ray spectroscopy (EDS) as characterization techniques.

## 2. Theoretical Background

DNA biosensor is one method that has attracted much attention for DNA hybridization detection [8]. The sensor generally composed of ss-DNA probes immobilized on a transducer surface that are able to form duplex with the target DNAs. The hybridization event is then converted into a measurable signal by a transducer as shown in Fig 1. The detection of DNA hybridization in a biosensor can either be direct or indirect [9]. In the indirect approach, after the hybridization event the signal is determined from indicator molecules such as enzymes, electroactive compounds or nanoparticles. The indicator is tagged either directly on the target DNAs prior to the hybridization (competitive method) or with the secondary target after the hybridization on the sensing surface (sandwich method). The tagged DNA probe or target DNA can then generate a hybridization signal that can be used to obtain the amount of DNA. In this approach, the signal generation is extremely sensitive but it requires several steps, is expensive, time-consuming, and real-time measurement is also not possible. In contrast, for a direct detection approach the signal comes from the change of the physical properties at the electrode-solution interface. Therefore, the technique is more attractive since it can provide a fast response, low-cost and can be monitored in real time.



**Figure 1.** DNA affinity biosensor.

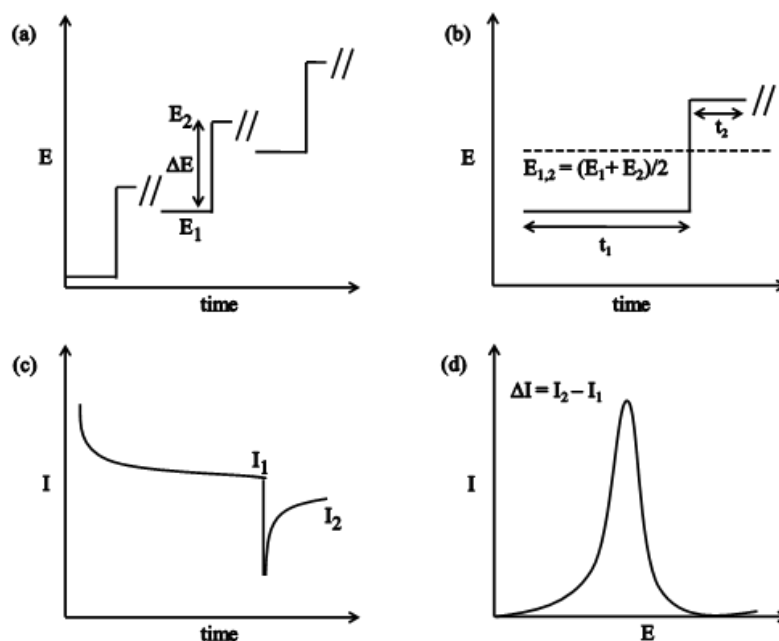
In the DNA hybridization sensors, DNA probes are typically short oligonucleotides that are able to hybridize with the specific (complementary) target DNA sequence to form a double-stranded hybrid [10]. A longer probe often exhibits unfavorable hybridization specificity due to intramolecular hydrogen bonding and consequent formation of the non-reaction hairpin structure. For the end-labels, such as thiols, disulfides, amines, or biotin are incorporated with immobilize DNA probe to electrode surface. A long flexibility spacer of hydrocarbon is usually added to provide sufficient accessibility for

surface attachment. Although, DNA probe is usually use in DNA biosensor, it still has some issues with the specificity, sensitivity and stability under various conditions [11].

Electrochemical detection of DNA hybridization is one strategy being explored because an electrochemical device provides a high sensitivity, rapid response, is easy to use, low cost and can be miniaturized. The transduction relies on the conversion of a base-pair recognition event into a useful electrical signal. Electrochemical methods for direct DNA detection are such as voltammetry, impedance spectroscopy and capacitance measurement.

The common principle of the voltammetric measurement is that involving the application of a potential to an electrode and the monitoring of the current signal flowing through the cell. It is considered as active technique because the applied potential force a change in the concentration of an electroactive species at the electrode surface due to electrochemically reducing or oxidizing.

In this work, the transducer that conversing the chemical reaction to electrical sinal is Pulse voltammetry. This technique can be measured the current while making pulse changes in the applied working electrode potential. The most extensively used method of pulse voltammetry is differential pulse voltammetry or DPV which is based on the application of successive double potential pulse [12] as shown in Fig 2.



**Figure 2.** The step of differential pulse voltammetry. (a) Potential-time function; after each double pulse, the initial equilibrium conditions are restored (b) technique parameter (c) current samples and (d) DPV signal.

In this technique two potential pulses of amplitude  $E_1$  and  $E_2$  and length  $t_1$  and  $t_2$ , respectively are first applied with  $t_1 \gg t_2$  and  $\Delta E = E_2 - E_1$  (Fig. 2(a)-(b)). The potential is scanned in the negative ( $\Delta E < 0$ ) or positive direction ( $\Delta E > 0$ ) in such a way that a delay between each pair of pulses is introduced in order for the equilibrium to be re-established. In this potentiostatic technique the difference current responses  $I_{DPV}$  or  $\Delta I = I_2(t_1 + t_2) - I_1(t_1)$  is plotted versus  $E$ , referred to as differential pulse voltammogram [13] (Fig. 2(c)-(d)). That means, the difference between current measurement at these points for each pulse is determined and plotted against the base potential. The resulting voltammogram consists of a current peak, the height of the peak is directly proportional to the concentration of analyte.

### 3. Materials and Methods

#### Materials

The sequences for the 12 bases of DNA probe tagged methylene blue is 5'-MB-TTT TTT TTT TTT-NH<sub>2</sub>-3' that purified by reverse phase HPLC and its identity was verified by MALDI-TOF mass spectrometry. The synthetic complementary target DNA with 12 bases (5'-AAA AAA AAA AAA-3') was purchased from the Bioservice Unit, National Science and Technology Development Agency and BioDesign Co., Ltd., Thailand. The blocking thiol of 11 carbon length, 11-mercapto-1-undecanol (11-MUL) was purchased from Aldrich (Steinheim, Germany). Silver nitrate was purchased from Aldrich (Steinheim, Germany). Graphene nanosheets (4-5 layers, thickness of 10 nm, surface area 500-800 m<sup>2</sup>g<sup>-1</sup>, particle diameter 3 μm) were obtained from Cheap Tubes Inc (Brattleboro, USA). Aniline solution was purchased from Merck (Germany) and purified using the normal distillation method and keep at 4 degree celsius in the refrigerator. All aqueous solutions were prepared with analytical reagent grade chemicals and de-ionized water (Milli-Q, Merck). For SPGE consists of gold working electrode (4 mm diameter), Pt counter electrode and Ag/AgCl reference electrode.

#### Methods

##### SPGE pre-treatment

The electrochemical pre-treatment of SPGE was carried out by applying potential at 1.2 V in the saturated Na<sub>2</sub>CO<sub>3</sub> with the scan rate 5 mVs<sup>-1</sup>, 600 s. After the activation, the SPGE strip was rinsed with de-ionized water and placed in an electrochemical cell for voltammetric measurement.

##### PANI-Grap-Ag nanocomposited modified SPGE

For a PANI-Grap-Ag nanocomposite modified gold surface, a 2.0 mg mL<sup>-1</sup> graphene and 0.20 M AgNO<sub>3</sub> with 0.10 M aniline aqueous solution were added into the electrodeposition solution (0.50 M H<sub>2</sub>SO<sub>4</sub>), mixed with 0.25 M polyacrylic acid (PAA) to get a better stability with improved polymer properties [14]. The electrodeposition was performed by cyclic voltammetry for 10 scans using the potential range from -0.4 to 1.0 V vs. Ag/AgCl with a scan rate of 50 mVs<sup>-1</sup>.

##### Immobilization of DNA-MB probe

The PANI-Grap-Ag coated SPGE was cleaned by rinsing with distilled water for 3 times and treated with 5.0 % (v/v) glutaraldehyde 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-MB probe was placed on the modified electrode for 24 h in the refrigerator (4 °C). Finally, the immobilized SPGE was immersed in 1.0 mM of 11-mercaptoundecanol (11-MUL) solutions for 1 h to block any remaining pinholes, hence preventing any non-specific binding on the electrode surface.

##### Surface morphology characterization

The surface morphology of PANI-Grap-Ag nanomaterial modified SPGE was characterized using SEM and EDS. Both SEM image and EDS spectrum were characterized with a JSM 5800 Quanta from JEOL, Japan.

##### Electrochemical measurement

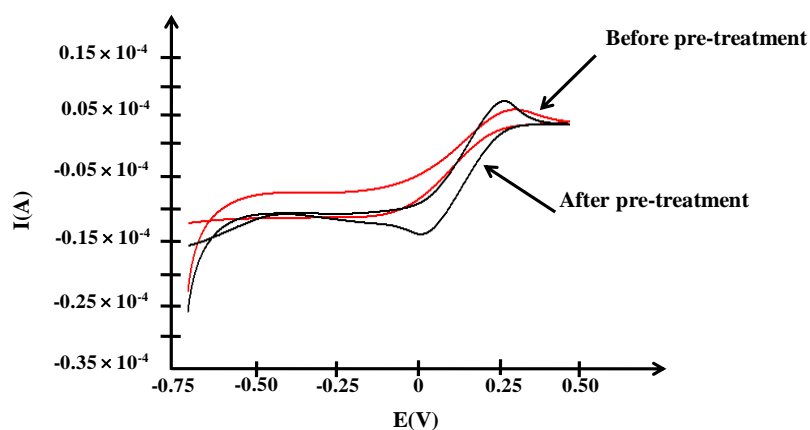
The hybridization behavior measurement was studied using three electrode system of the SPGE, connected to 910 PSTAT Mini (Metrohm Applikon, Utrecht, The Netherlands) controlled by PSTAT Software Version 1.1. The hybridization response was the decrease of the oxidation peak of the electrochemical indicator MB (tagged to the DNA probe) detected using DPV. The DPV was operated from -1.2 to -0.3 V, with a scan rate of 50 mVs<sup>-1</sup>, 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 batch vessel containing 100 mM sodium phosphate buffer pH 7.00 with 100 mM potassium chloride.

### 4. Results and Discussion

#### Pre-treatment of the SPCE

SPGE are often preconditioned by applying anodic potential in electrolyte solution (saturated Na<sub>2</sub>CO<sub>3</sub>) to enhance the electrochemical activities. Under an appropriate electrochemical pre-treatment

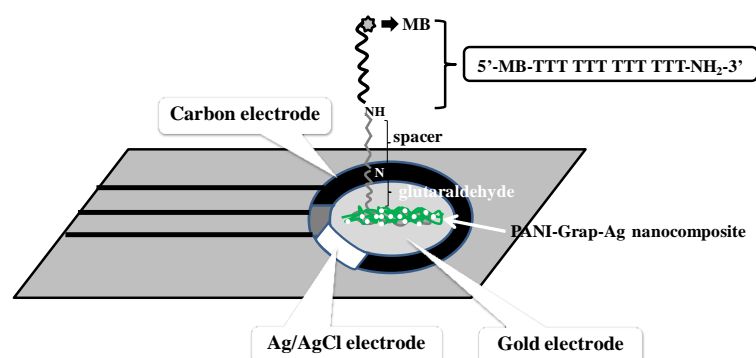
condition for the SPGE, we studied the cyclic voltammetric behavior using redox system. As shown in Fig 3, after pre-treatment the SPGE exhibited discernable redox peaks for potassium hexacyanoferrate (III)-(II) system. That means, this conditions can be improved the electrochemical activity of SPGE and the activation procedure in  $\text{Na}_2\text{CO}_3$  solution resulted in the good electrochemical characteristic.



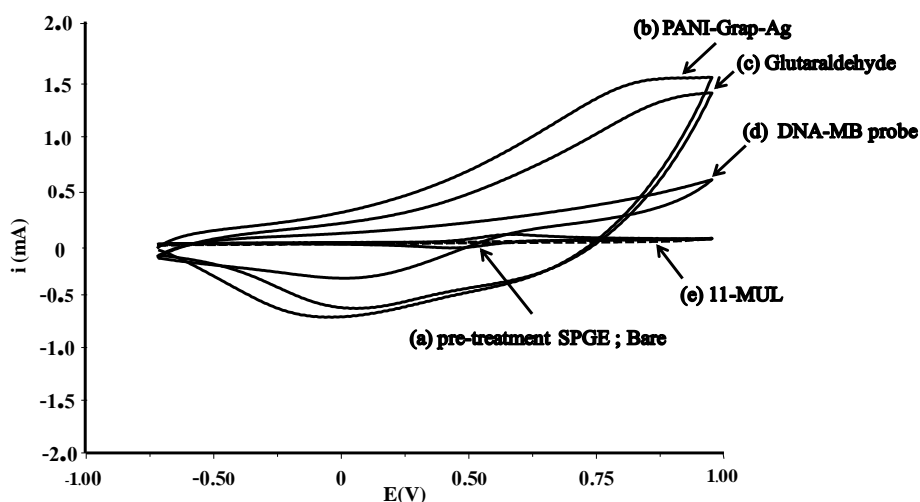
**Figure 3.** Cyclic voltammograms for 5 mM hexacyanoferrate (III)-(II) with before and after pre-treatment SPGE at the scan rate  $5 \text{ mVs}^{-1}$ , 600 s.

#### Electrochemical characterization of the immobilization step

PANI-Grap-Ag nanocomposite modified gold surface in Fig 4 and Fig 5 shows an electrochemical behavior of the modified SPGE surface studied by cyclic voltammetry using  $5.0 \text{ mM K}_3\text{Fe(CN)}_6$  in  $0.1 \text{ M KCl}$  between  $-0.3$  to  $0.7 \text{ V}$  at a scan rate of  $0.1 \text{ Vs}^{-1}$  vs a  $\text{Ag/AgCl}$  reference electrode. The regenerated gold surface showed a voltammogram with oxidation and reduction peaks (Fig 5(a)). Both peaks increase when PANI-Grap-Ag nanocomposite was deposited onto the gold surface (Fig 5(b)) indicated that the PANI-Grap-Ag helped to increase the electrical conductivity. When  $5.0 \%$  (v/v) glutaraldehyde in  $10 \text{ mM}$  sodium phosphate buffer pH 7.00 was used to activate the covalent bonding between the amine group of the DNA-MB probe and the free amine group of PANI at room temperature for 20 min, the redox peaks of the electrode decrease (Fig 5(c)). The response was further reduced when DNA-MB probes were immobilized (Fig 5(d)). The modified electrode 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-Grap-Ag nanocomposite modified SPGE was rinsed with  $100 \text{ mM}$  phosphate buffer pH 7.00 and then immersed in  $1.0 \text{ mM}$  of 11-MUL solution for 60 min to cover any pinholes on the electrode surface. The cyclic voltammogram showed complete blockage of the redox species (Fig 5(e)).



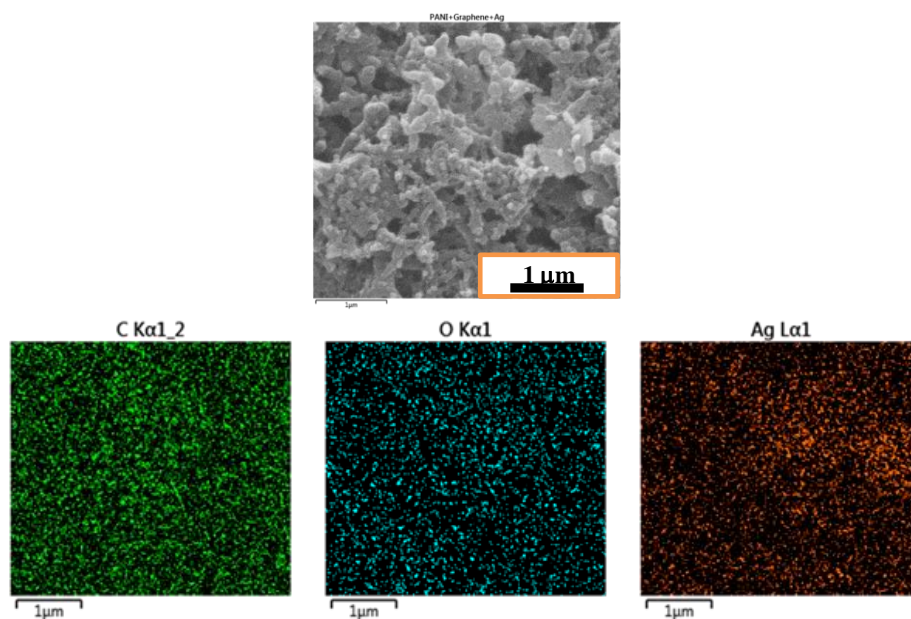
**Figure 4.** The PANI-Grap-Ag nanocomposite modified SPGE.



**Figure 5.** Cyclic voltammograms behavior obtained in 5.0 mM  $K_3[Fe(CN)_6]$  with 0.10 M KCl solution; bare SPGE (a) then electrodeposited with PANI-Grap-Ag nanocomposite (b) and crosslinked with glutaraldehyde (c) for immobilization with DNA-MB probe (d) and blocked with 11-MUL (e).

### Surface Morphology with SEM and EDX

The PANI-Grap-Ag nanocomposite modified on SPGE was characterized using SEM and EDX. Figure 6, show the morphology of PANI-Grap-Ag nanocomposite, graphene sheets were seen embedded within the PANI nanofiber with the silver nanoparticles decorated on the PANI nanofibers. The PANI Film has a fibrous network structure having a diameter of 40 - 70 nm that measured from an SEM image using electronic digital calliper. Silver nanoparticles were decorated on the surface of the PANI nanofibers with the particle size 50 - 90 nm. From EDX spectrum, which revealed the peak of silver (Ag) and confirmed the presence of silver element decorated on PANI nanofiber.



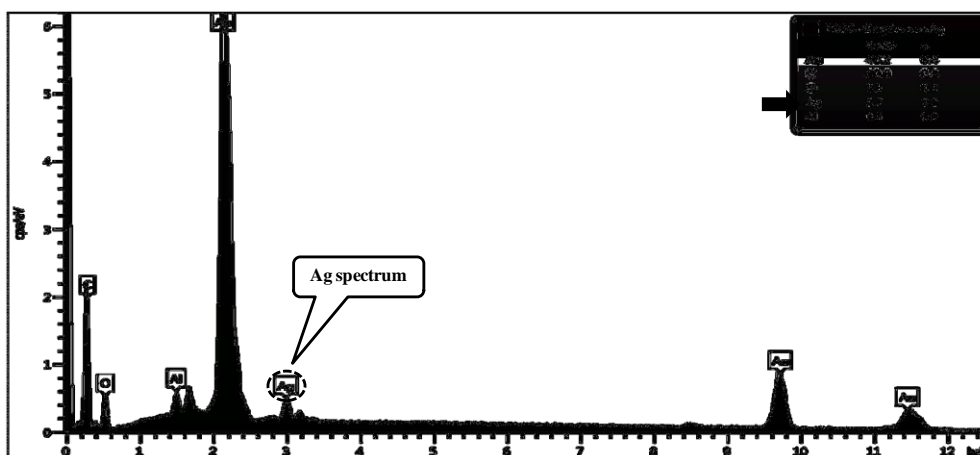


Figure 6. SEM images and EDX spectrum of PANI-Grap-Ag nanocomposite modified SPGE.

### Reusability

The reusability of PANI-Grap-Ag modified SPGE was tested by analyzing the same concentration of target DNA ( $1 \times 10^{-9}$  M). After hybridization, the regeneration step (dropped with 20  $\mu$ L of 0.05 M sodium hydroxide with 30 min of the incubation time) was included in the analysis cycle. The residual activity (%) of immobilized DNA-MB probe to its target after regeneration was calculated. The residual activity (%) was plotted against the number of hybridization. Figure 7, 36 times of the hybridization between DNA-MB probe and target DNA, the average residual activity was  $95 \pm 3\%$  (RSD = 4%). After 37 regeneration cycles, the average residual activity reduced to 90%. The gold electrode surface 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.

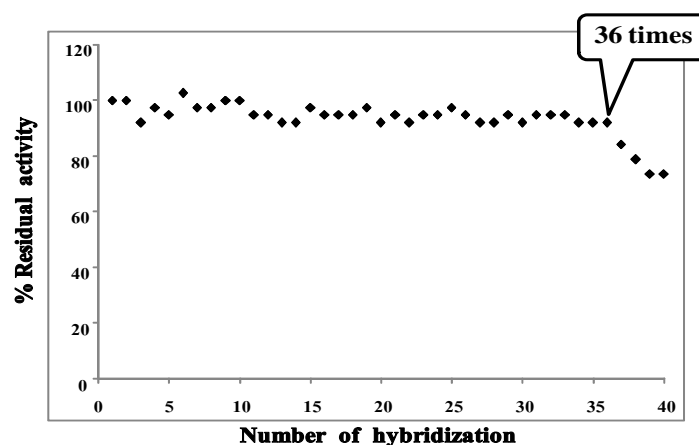


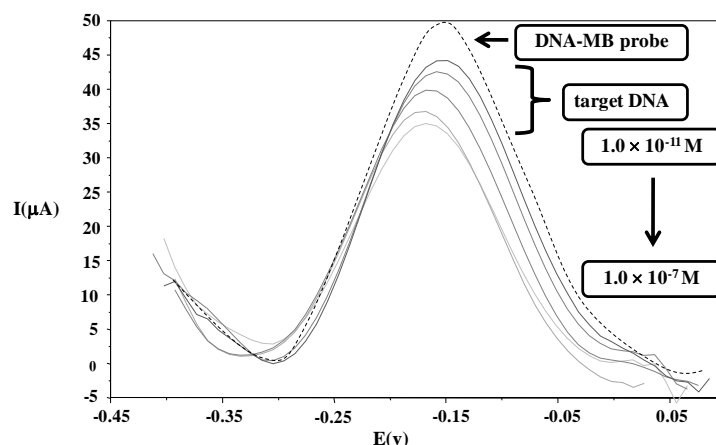
Figure 7. The relationship between percentage of the residual activity and number of hybridization.

### Hybridization study between DNA-MB probe and synthetic complementary DNA

Under the hybridization, the current response from the electron transfer of MB was studied for DNA detection. Oxidation peak current from electron transfer of MB to the electrode surface was measured using DPV. The batch system conditions were; complementary target DNA volume 20  $\mu$ L in PBS pH 7.00 with 30 min for hybridization event. The regeneration solution for dissociation DNA can be done using 50 mM NaOH for 10 min. The DPV response between potential (volt) and current ( $\mu$ A)



that obtained from five concentrations of the complementary target DNA from  $1.0 \times 10^{-11}$  to  $1.0 \times 10^{-7}$  M is shown in Fig 8. In the absence of the synthetic target DNA, single-stranded DNA-MB probe can be closed to the electrode surface, resulting in the electron transfer between the MB 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, the MB at the end of the DNA probe moved further away from the electrode resulting in the decrease of 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.



**Figure 8.** Differential pulse voltammograms of the hybridization between DNA-MB probe and synthetic complementary DNA.

## 5. Conclusions

This work shows a successfully developed PANI-Grap-Ag nanocomposite modified a disposable SPGE for hybridization detection. The SPGE was initially pre-treatment with saturated sodium carbonate for enhancing electrochemical activities. From the cyclic voltammetry study, the nanocomposite modified electrode provides a larger current over bare SPGE. The DNA-MB probe immobilization and hybridization on SPGE were investigated using DPV method. This sensor is simple and cost-effective electrochemical biosensor for detection of DNA. Our going research will be focused on using other conducting materials to enhance the electrochemical signal that means using the electro-natural product to modified the SPE.

## Acknowledgements

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