

## Conductivity of Pedot-Pss with Gold and Silver Nanocomposites Modified Gold Electrodes for *Ganoderma boninense* DNA Detection

<sup>1</sup> Sabo Wada Dutse, <sup>2</sup> Nor Azah Yusof, <sup>3</sup> Haslina Ahmad

<sup>1</sup> Department of Science Laboratory Technology, Hussaini Adamu Federal Polytechnic, P.M.B 5004, Kazaure, Nigeria

<sup>2,3</sup> Department of Chemistry, Faculty of Science, Universiti Putra Malaysia, 43400 UPM Serdang, Malaysia

<sup>1</sup> Tel.: +2348035860235

E-mail: swdutse@yahoo.com, sabo.wada@hafedpoly.edu.ng

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**Abstract:** The conductivity of a designed electrochemical DNA biosensor was improved using gold and or silver nanoparticles. A gold electrode modified with a conductive nanocomposite of poly(3,4-ethylene dioxythiophen)-poly (styrenesulfonate) (Pedot-Pss) and gold or silver nano particles enhanced the conductivity of the electrode surface area. Bare and modified gold electrode surfaces were characterized using cyclic voltammetry (CV) technique in ethylenediaminetetraacetic acid (TE) supporting electrolyte. Immobilization of a 20-mer DNA probe was achieved by covalent attachment of the amine group of the capture probe to a carboxylic group of an activated 3,3'-dithiodipropionic acid layer using EDC/NHSS for Hybridization. The effect of hybridization temperature and time was optimized and the sensor demonstrated specific detection for the target concentration ranged between  $1.0 \times 10^{-15}$  M to  $1.0 \times 10^{-9}$  M with a detection limit of  $9.70 \times 10^{-19}$  M. Control experiments verified the specificity of the biosensor in the presence of mismatched DNA sequence. The DNA hybridization was monitored using a new ruthenium complex  $[\text{Ru}(\text{dppz})_2(\text{qtpy})\text{Cl}_2]$ ; dppz = dipyrrodo [3,2-a:2',3'-c] phenazine; qtpy=2,2',-4,4''-4'4'''-quarterpyridyl redox indicator.

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**Keywords:** Biosensor, Pedot-Pss, DNA, Nanocomposite, Ruthenium complex.

### 1. Introduction

The new ruthenium complex  $[\text{Ru}(\text{dppz})_2(\text{qtpy})\text{Cl}_2]$  has emerged as a novel redox marker for the selective detection of DNA probe (ssDNA) in solution via electrochemical sensor. Interaction of ruthenium complex with DNA has been studied by Photophysical techniques [1]. The ruthenium complex has a high affinity to DNA intercalation due to its interacting properties that

include its extended aromatic heterocyclic surface that extrude from its central core [2-3]. Fundamentally, conducting polymers have emerged as potential candidates for biosensors [4], and in this development Gerard, *et al.*, have reviewed the literature on applications of conducting polymers to biosensors [5]. However, this study was designed to investigate the effect of gold and or silver nanoparticles in improving signal enhancement of PEDOT-PSS modified gold electrode in DNA

biosensing. The use of nano-material was considered for its advantageous properties, such as hydrophilic, excellent biocompatibility, unique characteristics in the conjugation with biological recognition elements and multiplex capacity for signal transducer [6]. Ideally, nano-materials in DNA biosensors are used as substrates for DNA attachment and as signal amplifiers for hybridization [7]. Consequently, a considerable amount of literature that described electrode modification using nanoparticles and conducting polymers has been published. Conductivity of PEDOT-PSS aqueous solution film containing gold nanoparticles are reported to have improved with the addition of a liquid or solid organic compound, such as methyl sulfoxide (DMSO), glycerol, or sorbitol [8]. One observer has already drawn attention that the mechanism responsible for this increase in conductivity has been controversial [9]. Occasionally, carboxyl groups are incorporated in the attachment of these polymers when a particular electrochemical effect is needed such as that of DNA biosensing.

## 2. Methodology

### 2.1. Reagents and Solvents

Synthesized new ruthenium complex of  $[\text{Ru}(\text{dppz})_2(\text{qtpy})\text{Cl}_2]$ ; dppz = dipyrido [3,2-a: 2', 3' - c] phenzine; qpty = 2, 2', - 4, 4'', 4', 4''' - quaterpyridyl was obtained from the Department of Chemistry, Faculty of Science, Universiti Putra Malaysia. It was synthesized by Haslina Ahmad according to the literature [2] with slight experimental modification. All reagents and solvents were purchased from a standard company in Malaysia and are used without further purification. Deionized water from a Millipore Milli-Qpurifier was used throughout the experiments.

### 2.2. Preparation of Solutions

Stock solution of 25  $\mu\text{M}$  ruthenium complex  $[\text{Ru}(\text{dppz})_2(\text{qtpy})\text{Cl}_2] = [\text{Ru}(\text{dppz})]^{2+}$  was prepared in a mixture of 50 mM Tris-HCl, 20 mM NaCl (volume 90 %) and methanol (volume 10 %). A washing buffer and supporting electrolyte of 10mM Tris-HCl containing 1 mM EDTA (TE) (pH 7.15) were prepared. Another supporting electrolyte of a 5 mM Potassium Hexacyano ferrateIII ( $\text{K}_3\text{Fe}(\text{CN})_6$ ) was prepared in 10 mM Tris-HCl containing 1 mM EDTA TE (pH 7.15). An activation solution of 5 mM N-hydroxysulfosuccinimide (NHSS) sodium salt in a mixture of 2 mM 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide (EDC) was prepared in deionized water. More solution of 10 mM Tris-HCl containing 1 mM EDTA (TE) (pH 8.0) was prepared for the dilution of DNA oligonucleotide. Another solution of 1.0 mM  $\text{K}_3\text{Fe}(\text{CN})_6$  was prepared for the characterization of electrode active surface area and a

3 mM solution of 3, 3'-dithiopropionic acid (DPA) was formed in absolute ethanol as a linker between the modified electrode and probe DNA.

Oligomers 20-mers probe DNA modified at the 5' end with an amino ( $\text{NH}_2$ ) group (5'- $\text{NH}_2$ -CCT GCT GCG TTC TTCTTC AT-3'), 20-mers complementary target (5'-ATG AAG AAG AAC GCA GCA GG-3') and 20-mers single-mismatch (5'-ATG AAG AAG TAC GCA GCA GG-3') sequences were synthesized by First Based Laboratories Sdn Bhd, Selangor, Malaysia. DNA-oligonucleotide (100  $\mu\text{M}$ ) stock solutions and other dilute concentrations  $1 \times 10^{-9}\text{M}$  to  $1 \times 10^{-15}\text{M}$  of the DNA were prepared in trisaminomethane-HCl containing EDTA buffer solution (pH 8.0) and kept frozen. The DNA solution was defrosted when needed and returned immediately frozen when not in use. All other reagents used are of analytical grade.

### 2.3. Apparatus and Electrodes

Voltammetric measurements were obtained using  $\mu\text{Autolab}$  (Ecochemie, The Netherlands) Potentiostat incorporated with a General-Purpose Electrochemical System (GPES 4.9, Eco Chemie) software. The electrochemical cell was a three electrodes system of a Metrohm gold electrode as the working electrode, platinum wire as counter electrode, and Ag/AgCl/KCl (3.0M) as a reference electrode.

### 2.4. Pretreatment of Gold Electrode

The bare gold electrode (bare AuE) was polished using alumina slurry and sonicated in deionized water to remove stacked particles. It was then dipped in a concentrated sulphuric acid for 10 min and rinsed with a copious amount of deionized water. The electrode was further sonicated in absolute ethanol for 5 minutes, rinsed with deionized water and then in TE washing buffer respectively. It was subsequently dried under nitrogen gas (N-gas) flow for 10 min and kept for further drying at 25  $^{\circ}\text{C}$  for 45 min.

### 2.5. Gold Electrode Modification with PEDOT-PSS, PEDOT-PSS/AgNps and PEDOT-PSS/AuNps

The pretreated bare gold electrode (AuE) was drop coated on the gold electrode and exposed to oven dry at 70  $^{\circ}\text{C}$  for 15 h. This was to obtain a cured film of PEDOT-PSS on the gold electrode surface. The film surface was rinsed with deionized water and TE washing buffer to remove the unbound remnants on the film surface of the gold electrode. It was dried gently under N-gas flow for 10 min and further dried for 45 min at 25  $^{\circ}\text{C}$ . The PEDOT-PSS/AuE modified electrode was then obtained.

Some prepared PEDOT-PSS containing AgNPs solution was drop coated on pretreated bare gold electrode and the curing procedure performed as with PEDOT-PSS modification protocol. Then AgNPs/PEDOT-PSS/AuE modified electrode was obtained.

Another gold electrode modification with PEDOT-PSS and gold nanoparticles was obtained by immersing PEDOT-PSS modified gold electrode in a colloidal gold nanoparticles solution and allowed for 24 h to achieve a nanocomposite film on the surface of the PEDOT-PSS modified gold electrode. The film surface was rinsed with deionized water and TE washing buffer respectively to remove the unbound gold nanoparticles from the film surface. It was dried gently under N-gas flow for 10 min and further dried for 45 min at 25 °C.

## 2.6. Electrochemical Characterization for the Active Surface Area of Bare and Modified Gold Electrodes

Cyclic voltammetry (CV) experiments were performed in a solution of 1.0 mM  $K_3Fe(CN)_6$  prepared in double distilled water. The CV voltammograms of bare AuE, gold electrode modified with PEDOT-PSS, gold electrode modified with PEDOT-PSS/AuNPs, and gold electrode modified with PEDOT-PSS/AgNPs were carried out at scanned potential between -0.3 V to 0.7 V varying the scan rates using 50, 100, 150, 200, 250, 300 and 350 mV/s. The sensitivity effect of each modification was observed based on the peak current responses from the different scan rate.

## 2.7. Immobilization of Probe DNA on PEDOT-PSS with Gold Nanocomposite Modified Electrode

Layer of 3,3'-dithiopropionic acid (DPA) was formed on the AuNPs/PEDOT-PSS/AuE modified electrode surface by submerging the modified gold electrode into an ethanolic solution of 3 mM DPA and allowed for 45 min. The unbound DPA was removed by washing the layer with an absolute ethanol, rinsed with Millipore water and TE washing buffer respectively. The drying process was carried out in N-gas gentle flow for 10 min.

The layered DPA modified electrode (AuNPs/PEDOT-PSS/AuE) was activated by immersing in solution containing a mixture of 2 mM EDC and 5 mM NHSS for 1 hr. This was to activate the carboxylic group of the DPA for easy bonding with the amine group of the probe DNA sequence related to *G. boninense* as shown in Fig. 1. It was rinsed with TE washing buffer and dried via 10 minutes gentle flow of N-gas and further dried at 25 °C for 45 min. The 30  $\mu$ L of 100  $\mu$ M probe DNA aseptically accumulated on the EDC-activated

modified electrode surface for at least 12 hrs at room temperature. It was then washed with a washing TE-buffer solution to remove any unbound ssDNA. The probe-captured electrode was then denoted as ssDNA/AuNPs/PEDOT-PSS/AuE. The CV and DPV measurements were carried out utilizing the ruthenium  $[Ru(dppz)]^{2+}$  metal complex, using TE containing  $K_3Fe(CN)_6$  supporting electrolyte.

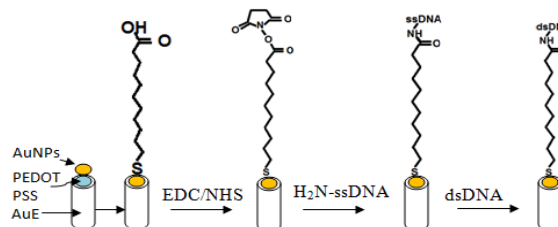


Fig. 1. Schematic representation of electrode modification and covalent attachment of DNA probe for hybridization events.

## 2.8. Effect of Hybridization Temperature and Time

Probe-modified electrode was hybridized with the complementary target DNA sequence related to *G. boninense*, by accumulating 30  $\mu$ L of 100  $\mu$ M target DNA containing 625  $\mu$ M ruthenium complex. The target DNA was heated (denatured) in a water bath at temperature of 35 °C, 45 °C and 55 °C each in 25 min, 45 min, and 55 min respectively. The respective denatured target DNA was casted on the probe-modified electrode with addition of 625  $\mu$ M ruthenium indicator, and then cooled gradually at room temperature for annealing. The electrode surface was then rinsed with TE washing buffer to remove any unbound target DNA and dried for 5 minutes under gentle flow of N-gas and further dried at 25 °C for 45 min. The protocol allowed the specific target sequence of the DNA to hybridize with the probe ssDNA and formed a double strand of the DNA (dsDNA) on the surface of the gold electrode.

The effects of the hybridization temperature and time was optimized on ssDNA/AuNPs/PEDOT-PSS/AuE sensor using CV voltammogram utilizing  $K_3Fe(CN)_6$  contained in TE supporting electrolytes with the ruthenium  $[Ru(dppz)]^{2+}$  complex as hybridization monitoring indicator.

## 2.9. Selectivity Study

A 30  $\mu$ L of 100  $\mu$ M target DNA was collected in a vial and heated in a water bath at 45 °C for 35 min to denature. It was quickly casted on the probe-modified electrode surface, and a 625  $\mu$ M ruthenium indicator was added, and then allowed to anneal for 90 min. The electrode surface was then

rinsed with TE washing buffer to remove any unbound target DNA. The hybridized DNA on the probe DNA modified electrode was dried for 5 min in a gentle flow of N-gas and further dried at 25 °C for 45 min. The protocol allowed for the specific target sequence of the DNA related to *G. boninense* to hybridize with the probe DNA. The hybridized DNA to the probe DNA was labeled dsDNA / AuNPs / PEDOT-PSS / AuE. Similar procedure was also followed to test the hybridization of mismatch target DNA sequence.

The CV voltammograms are obtained for the hybridization responses. The hybridizations were monitored using the ruthenium complex  $[\text{Ru}(\text{dppz})]^{2+}$  as the hybridization redox indicators in the  $\text{K}_3\text{Fe}(\text{CN})_6$  containing TE supporting electrolytes.

## 2.10. Effect of Target DNA Concentrations

The probe-modified electrode surface of ssDNA/AuNPs/PEDOT-PSS/AuE was hybridized with different prepared complementary target DNA sequences related to *G. boninense*. The target DNA concentrations were prepared in TE dilution buffer (pH 8.0) in the range  $1.00 \times 10^{-9}$  M to  $1.00 \times 10^{-15}$  M. Hybridization protocol was carried out by accumulating 30  $\mu\text{L}$  of the target concentration and subsequent addition of 625  $\mu\text{mol}$  ruthenium  $[\text{Ru}(\text{dppz})]^{2+}$  complex to the probe DNA. The hybridization was performed on probe DNA under temperature and time of 45 °C and 35 minutes, and then cooled gradually to room temperature for annealing. The electrode surface was then rinsed with the TE washing buffer to remove the unbound DNA and dried for 5 min under gentle flow of N-gas and further dried at 25 °C for 45 min.

The DPV measurements were performed for the hybridization responses of the prepared different concentrations in the  $\text{K}_3\text{Fe}(\text{CN})_6$  containing TE supporting electrolytes. The DNA hybridizations were monitored via the ruthenium metal complex  $[\text{Ru}(\text{dppz})]^{2+}$ .

## 3. Results and Discussion

### 3.1. Electrochemical Characterization of Bare and Modified Gold Electrode Active Surface Area

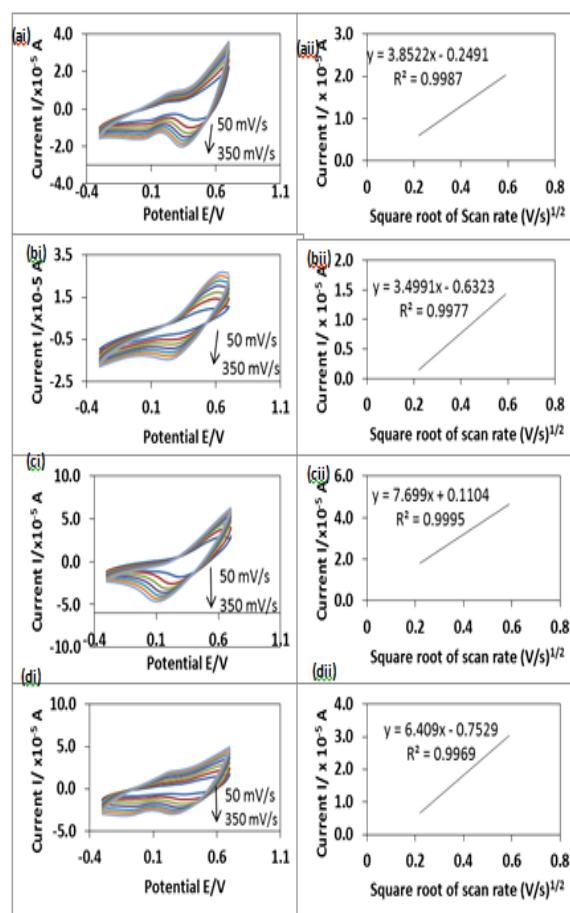
Cyclic voltammograms of bare, PEDOT-PSS / AuE, AuNPs / PEDOT-PSS / AuE, AgNPs / PEDOT-PSS / AuE modified gold electrodes in 1.0 mM  $\text{K}_3\text{Fe}(\text{CN})_6$  are shown in Fig. 2(ai-di). Peak current obtained is proportional to the square root of the scan rate, suggesting a sensitive diffusion - control electro chemical process [10]. Active surface area of the bare and modified electrodes can be

calculated according to the Randles-Sevcik formula [11].

$$i_p = (2.19 \times 10^5) n^2 A D^{\frac{1}{2}} C v^{\frac{1}{2}}, \quad (1)$$

where  $n$  is the number of electron participating in the redox reaction,  $A$  is the surface area of the electrode ( $\text{cm}^2$ ),  $D$  is the diffusion coefficient of the molecules in the solution ( $\text{cm}^2\text{s}^{-1}$ ),  $C$  is the concentration of  $\text{K}_3\text{Fe}(\text{CN})_6$  (M) and  $v$  is the scan rate ( $\text{V s}^{-1}$ ). From the slope of the plot of  $i_{pc}$  versus  $v^{1/2}$  (Fig. 2), the sensitive active surface area of the electrodes can be calculated.

For bare AuE, the active surface area was calculated to be  $0.052 \text{ cm}^2$ . There was a decrease in the effective surface area to  $0.047 \text{ cm}^2$  when bare AuE was modified with PEDOT-PSS. In further modification with gold nanoparticles, the effective surface area increased to  $0.104 \text{ cm}^2$  whereas the modification with silver nanoparticles had increased the active surface area to  $0.093 \text{ cm}^2$ . Therefore, further investigations are carried out using AuNPs/PEDOT-PSS/AuE modified electrode.

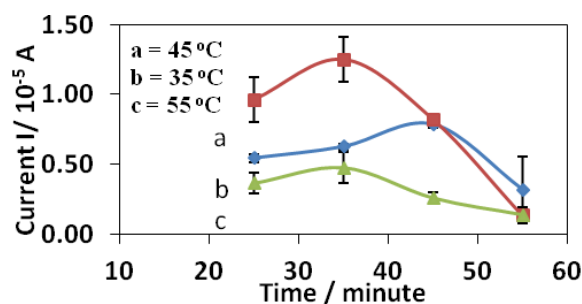


**Fig. 2.** Cyclic voltammograms and current versus square root of scan rates (50, 100, 150, 200, 250, 300, 350) mV/s of (a) bare AuE (b) PEDOT-PSS/AuE (c) AuNPs/PEDOT-PSS/AuE (d) AgNPs/PEDOT-PSS/AuE modifications in a 1.0 mM  $\text{K}_3\text{Fe}(\text{CN})_6$ .

### 3.2. Effect of Hybridization Temperature and Time

The efficiency of hybridization response is dependent on the hybridization temperature and time, these parameters are optimized by cyclic voltammetry (CV) technique. The plots of time versus current of the CV oxidation peaks [replicates (n=3)] are shown in Fig. 3. The developed biosensor show higher hybridization current at 45 °C and 35 min.

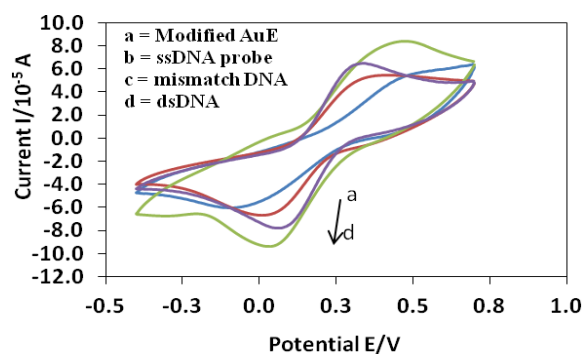
Based on the results obtained in Fig. 3, the optimum hybridization conditions are at 45 °C and 35 min, and are therefore chosen for further hybridization analysis.



**Fig. 3.** Reduction peaks for hybridization of dsDNA/AuNPs/PEDOT-PSS/AuE in 5 mM  $K_3Fe(CN)_6$  containing TE with  $[Ru(dppz)]^{2+}$  for target DNA of 100  $\mu M$  concentration.

### 3.3. Selectivity of the Developed Sensor

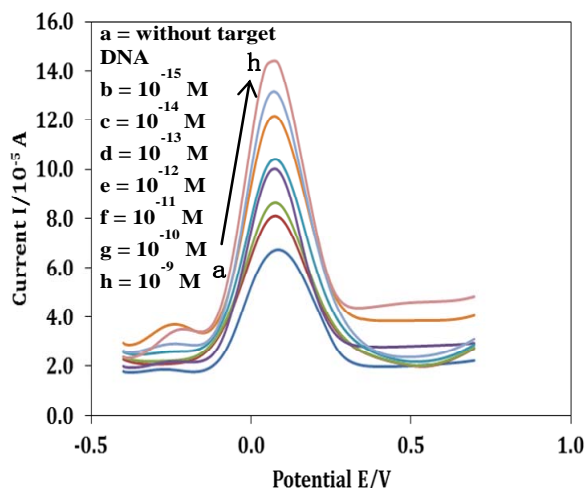
The selectivity of the developed DNA biosensor AuNPs/PEDOT-PSS/AuE was examined based on the electrochemical transduction of the probe ssDNA/AuNPs/PEDOT-PSS/AuE hybridized with target DNA sequence related to *G.boninense*. The hybridization events are monitored using ruthenium  $[Ru(dppz)]^{2+}$  complex at optimized hybridization temperature of 45 °C and time 35 min. The CV hybridization selectivity responses between capture probes, complementary and mismatch Target DNA sequences related to *G. boninense*, at 100  $\mu M$  concentrations are shown in Fig. 4.



**Fig. 4.** CV Selectivity of reduction peaks for hybridization of dsDNA/AuNPs/PEDOT-PSS/AuE in 5 mM  $K_3Fe(CN)_6$  containing TE with  $[Ru(dppz)]^{2+}$ .

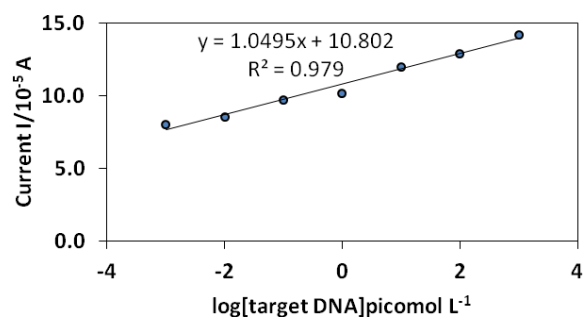
### 3.4. Effect of Different Concentrations of Target DNA

Differential pulse voltammogram (DPV) technique was used to detect different concentrations of target DNA sequence to examine the sensitivity of the developed biosensors AuNPs/PEDOT-PSS/AuE. The DNA hybridization was monitored by ruthenium  $[Ru(dppz)]^{2+}$  complex as indicator, results are shown in Fig. 5. The hybridization conditions applied are 45 °C and 35 min hybridization temperature and time. It is observed that the DPV peak current increases with the increased target DNA concentration.



**Fig. 5.** DPV of dsDNA/AuNPs/PEDOT-PSS/AuE in 5 mM  $K_3Fe(CN)_6$  containing TE with  $[Ru(dppz)]^{2+}$  of different target DNA concentrations (ranged  $1.0 \times 10^{-15}$  M to  $1.0 \times 10^{-9}$  M) at scan rate 100 mV/s.

Consequently, the variations of the DPV peaks current observed before and after hybridization are linearly related with logarithmic value of target DNA concentrations in the range of  $1 \times 10^{-9}$  M to  $1 \times 10^{-15}$  M. The correlation between the DPV peak current and the target DNA concentrations are clearly explained from a plot of peak current versus  $\log[\text{concentration}]$  of the target DNA shown in Fig. 6.



**Fig. 6.** Linear plot of current against  $\log[\text{con.}]$  of the target DNA of dsDNA / AuNPs / PEDOT-PS / AuE in 5 mM  $K_3Fe(CN)_6$  containing TE with  $[Ru(dppz)]^{2+}$  of different target DNA concentrations (ranged  $1.0 \times 10^{-15}$  M to  $1.0 \times 10^{-9}$  M) at scanrate 100 mV/s.

The detection limit (LoD) of the sensor was calculated as  $9.70 \times 10^{-19} \text{ mol L}^{-1}$  from a well-known equation ( $3.3 \sigma/S$ ), where  $\sigma$  is the standard deviation of the blank measurements ( $n=5$ ) and  $S$  is the slope of the linear regression equation of the plot of current versus  $\log[\text{target DNA}]$ .

#### 4. Conclusions

The application of nanoparticles with PEDOT-PSS improves the conductivity of modified gold electrode for *Ganoderma boninense* DNA detection. The selectivity of the developed biosensor has allowed for the recognition of labeled DNA and could differentiate between ssDNA and dsDNA of fully complemented and mismatched target DNA sequences. The new ruthenium ruthenium  $[\text{Ru}(\text{dppz})_2(\text{qtpy})\text{Cl}_2]$ ;  $\text{dppz} = \text{dipyrido}[3, 2\text{-}a:2', 3' \text{-}c]$  phenazine;  $\text{dpty} = 2, 2', -4, 4'', 4', 4''' - \text{quaterpyridyl}$  complex was found to be a good candidate as DNA intercalating indicator.

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